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COMMERCIAL ORGANIC ANALYSIS;

A TREATISE ON THE

PROPERTIES, PROXIMATE ANALYTICAL EXAMINATION, AND MODES OF ASSAYING THE VARIOUS ORGANIC CHEMICALS AND PRODUCTS EMPLOYED IN THE ARTS, MANUFACTURES, MEDICINE, &c.;

WITH CONCISE METHODS FOR

THE DETECTION AND DETERMINATION OF THEIR IMPURITIES, ADULTERATIONS, AND PRODUCTS OF DECOMPOSITION

BY

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PUBLIC ANALYST FOR THE WEST RIDING OF YORKSHIRE, THE CITY OF SHEFFIELD, &c.

Second Edition, Revised and Enlarged

VOLUME IV.

PROTEIDS AND ALBUMINOUS PRINCIPLES, PROTEOÏDS OR ALBUMINOÏDS.

LONDON
J. & A. CHURCHILL
7 GREAT MARLBOROUGH STREET
1898

PREFACE TO VOLUME IV.

THE production of the Second Edition of my "COMMERCIAL ORGANIC ANALYSIS" has extended over fourteen years. With VOLUME IV., now issued, the work comes to an end. The earlier Volumes have long been out of print, but I felt it undesirable to attempt their revision until the work was finished, and the time required to effect this has greatly exceeded my expectations. I much regret that it is now impossible for me to undertake the enormous labour which would be necessary for the thorough revision of the earlier Volumes, and hence I am reluctantly compelled to leave the work to which I have devoted the best years of my life to a certain extent incomplete. I hope, however, to publish an Appendix to each Volume, which will contain the more important of the facts and processes which have accumulated in such enormous numbers of late years.

The hiatus occasioned by my inability to undertake personally the systematic revision of the earlier Volumes is fortunately minimised by the fact that my friend Dr Henry Leffmann, of Philadelphia, is now engaged in partially revising them. Volume I., as

edited by him, will be published by the time these lines are read, and Volume II. will follow very shortly.

I may here repeat that I am fully conscious that much of the matter of VOLUME IV. is scarcely such as might be expected to be contained in a work purporting to treat of Commercial Analysis, but I have thought it better to include all facts possessing for me an analytical or practical interest, believing that what I find useful myself will also be of value or interest to others.

I am indebted to Dr James Edmunds, Mr Otto Hehner, Mr F. W. Richardson, Mr H. Droop Richmond, Dr S. Rideal, Dr H. Clifton Sorby, Mr E. C. C. Stanford, Dr W. J. Sykes, and other friends for perusal and correction of certain of the articles, and express my sincere thanks for the services they have rendered me in this connection. I have also to thank Dr T. E. Thorpe for his courtesy in communicating the details of the method of analysing altered milk now employed in the Government Laboratory.

I desire likewise to acknowledge the zealous assistance rendered by Mr Alfred B. Searle and Mr Arnold R. Tankard in the investigation in my laboratory of many of the tests and processes described, and to express my obligation to the latter for his collaboration generally in the production of the Volume.

ALFRED H. ALLEN.

SHEFFIELD, 27*th August* 1898.

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PROTEIDS AND ALBUMINOUS PRINCIPLES.

Under the generic name of Proteids or Albuminoids are classed a number of highly complex compounds which form the chief part of the solid constituents of blood, muscles, nerves, glands, and other organs of animals, and which occur in smaller quantities in almost every part of plants. No proteid has hitherto been synthetically prepared from a non-albuminoid substance, though certain derived albuminoids are obtainable by the action of reagents on pre-formed proteids.

Although the term albuminoid is frequently used as synonymous with proteid, there is of late years a tendency to draw a distinction between them, and to confine the former term to substances like gelatin and keratin, which are distinguished from other proteids by well-marked differences of chemical composition and physiological significance.

The name albuminoid of course has reference to the similarity of the bodies described under it to *albumin*, the characteristic constituent of white of egg, while the name proteid originated in Mulder's so-called *protein*, a substance which he supposed to be the common basis of all albuminoids.

As a class, the proteids are white or yellowish non-volatile solids, usually but not invariably amorphous. They are unchangeable in a dry condition, but putrefy readily when moist or in solution. Some of the proteids are soluble in water, especially in presence of certain neutral salts, but they are nearly all insoluble in alcohol, and invariably so in ether, chloroform, petroleum-spirit, and carbon disulphide. The proteids are mostly precipitated from their solutions by a great number of reagents, including certain salts of the light metals, salts and hydroxides of the heavy metals, mineral acids, certain organic acids, &c., &c. The proteids are decomposed by treatment with strong mineral acids, ammonia, leucine, tyrosine,

and various other bodies being products of the reaction.[1] Strong caustic alkalies produce a somewhat similar decomposition.

The proteids are eminently colloid. Their solutions are lævo-rotatory. When heated in neutral or slightly acid solution, many of the proteids become coagulated and separate as a white insoluble precipitate. The same change is produced by prolonged treatment of certain solid or dissolved proteids with an excess of strong alcohol, and in some cases by the action of peculiar ferments.

The chemical affinities of the proteids are very feeble, but certain compounds with acids and bases are obtainable.

The physical and chemical characters of the proteids are described at greater length in the sequel.

CLASSIFICATION OF THE PROTEIDS.

The proteids proper may be distinguished as (*a*) *simple proteids*, of which *albumin* is the type, and (*b*) *compound proteids*, composed of simple proteids in union with some non-proteid body.

The further classification of the proteids is attended with great difficulties, owing to the slight differences existing between them and the readiness with which their behaviour is modified by the action or mere presence of foreign bodies.

R. H. Chittenden (*Digestive Proteolysis*, 1894) arranges the proteids according to their behaviour with solvents as follows:—

Simple Proteids.

A. Proteids soluble in water:—
   - *a.* Aqueous solutions coagulated by heat or long contact with alcohol:—*Albumins.*
   - *b.* Solutions not coagulated by boiling or alcohol:—*Peptones; Proto-proteoses; Deutero-proteoses.*

B. Proteids insoluble in water; soluble in salt solutions:—
   - *a.* More or less coagulable by heat:—*Globulins.*
     1. Soluble in both dilute and saturated salt solutions:—*Vitellins.*
     2. Soluble in dilute salt (NaCl) solutions, but precipitated on saturation with salt:—*Myosins* (*e.g.*, serum-globulin (incompletely precipitated), myo-globulin, fibrinogen, cell-globulins).
   - *b.* Not coagulable by heat; soluble in dilute salt solution; precipitated by saturation with salt:—*Hetero-proteoses.*

[1] Collagen and gelatin, by similar treatment, fail to yield any tyrosine, or more than traces of other aromatic compound, but give glycocine instead, in addition to leucine and other products of the decomposition of proteids.

C. Proteids insoluble in water; insoluble in salt solutions; soluble in dilute alcohol :—

a. Gliadins (constituent of gluten) :—*Zeïn* (proteid of maize).

D. Proteids insoluble in water, salt solutions, and alcohol; soluble in dilute acids or alkalies :—

a. Coagulable by heat when suspended in a neutral fluid: —*Acid-albumins, Alkali-albumins.*

b. Not coagulable by heat when suspended in a neutral liquid :—*Antialbumids, Dysproteoses, Glutenins.*

E. Proteids insoluble in water, salt solutions, alcohol, dilute acids, and dilute alkalies; soluble in strong acids or alkalies, pepsin-hydrochloric acid, and alkaline solutions of trypsin :— *Coagulated proteids, Fibrin.*

Compound Proteids.

A. Compounds of a simple proteid with a ferruginous pigment: —*Hæmoglobin, Oxyhæmoglobin, Methæmoglobin,* &c.

B. Compounds of simple proteids with members of the carbohydrate group. Insoluble in water; soluble in very dilute alkalies :—*Mucins; Mucinoid Bodies.*

C. Compounds of simple proteids with phosphorised bodies yielding metaphosphoric acid by decomposition. Insoluble in water and in pepsin-hydrochloric acid, but more or less soluble in alkalies :—*Nucleins.*

D. Compounds of proteids with nuclein :—*Nucleo-albumins*; as the casein of milk and nucleo-albumins of cell-protoplasm and cell-nuclei, &c.

Further information respecting the more important of the compound proteids will be found in the sections on the Proteids of Milk, Blood, &c.

Animal Proteids.—The following system of classification of animal proteids (simple) is that adopted by W. D. Halliburton (*Text-book of Chemical Physiology and Pathology*, 1891).

I. Albumins.—Proteids soluble in water and in dilute saline solutions, and not precipitated by saturating these solutions with common salt or magnesium sulphate, but precipitated by ammonium sulphate. Their solutions are coagulated by heat. The chief members of the class are :—

Egg-albumin (see page 33); *Serum-albumin* (page 41); *Cell-albumin*; *Lact-albumin*; and *Muscle-albumin.*

II. Globulins.—The proteids of this class are insoluble in water, but soluble in dilute solutions of neutral salts, and are precipitated in an uncoagulated condition by saturating these solu-

tions with common salt, or by magnesium or ammonium sulphate. Globulins are coagulated by heating their solutions, and at a lower temperature than the albumins. The chief members of the globulin class are :—

Fibrinogen, which is the main constituent of blood-clots, and forms coagulated fibrin when the blood is withdrawn from the body (page 37); *Serum-globulin*, occurring with fibrinogen in blood-plasma ; *Globin*, the proteid constituent of hæmoglobin, the red colouring matter of blood ; *Myosinogen* and *Myoglobulin*, in muscle ; *Crystallin*, a proteid existing in the crystalline lens of the eye ; and *Vitellin* (page 35), a proteid in yolk of egg, which differs from other globulins in being but imperfectly precipitated by saturating its solutions with neutral salts. *Snake poison* contains a globulin having well-marked toxic properties. (See an interesting article on toxic proteids, *Pharm. Jour.*, [3], xxi. 293, 333.)

III. Derived Albumins.—These compounds result from the action of very dilute acids or alkalies on albumins or globulins. They are insoluble in water and in 1 per cent. solution of common salt, but dissolve in very dilute hydrochloric acid (0·1 per cent.) in the cold, and are also soluble in dilute caustic alkalies. Their solutions are not coagulated by heat, but are precipitated by saturation with common salt, or with magnesium or ammonium sulphate.

Acid-albumin or *Syntonin* is produced when a small quantity of acetic or hydrochloric acid is added to white of egg or blood-serum. On heating the fluid to 70° no coagulation takes place, as would occur if the albumin existed unchanged, while the liquid is found to have increased in optical activity to about $[a]_D = -72°$. On cautiously neutralising the cooled liquid, the whole of the proteid matter is separated as a white, flocculent or gelatinous precipitate, which is insoluble in water. The precipitate is very readily soluble in excess of alkali or lime-water, but is precipitated unchanged on neutralisation. It is also soluble in dilute solutions of alkaline carbonates, but is insoluble in a *strong* solution of common salt. On adding chloride of calcium, sulphate of magnesium, or chloride of sodium to the solution of acid-albumin in lime-water or dilute alkaline carbonate, and boiling the liquid, the proteid is precipitated, in combination with hydrochloric acid if chlorides were present or employed for precipitation. Suspended in water and heated to 70° C., it yields coagulated albumin.

Acid-albumin results also from the solution of myosin and other globulins in very dilute acid.

Alkali-albumin or *Albuminate.*—If white of egg or blood-serum be treated with very dilute alkali, no coagulation occurs on heating the fluid to 70° C., but on exactly neutralising the liquid the

whole of the proteid is thrown down, the precipitate being readily soluble in excess of acid. The precipitate is apparently identical with that produced by neutralising acid-albumin. Alkali-albumin is coagulated by boiling or treatment with excess of strong alcohol. Its solution in a little dilute alkali is precipitated by cold alcohol, but the proteid dissolves in the spirit when hot. This behaviour distinguishes alkali-albuminates from acid-albumin. Very similar, if not identical, products are obtained by treating other proteid matters with caustic alkalies. Serum-albumin gives with alkali a product having a specific rotation of $-86°$; egg-albumin, of $-47°$; and coagulated albumin, of $-58{\cdot}8°$.

Casein, the characteristic proteid of milk, has many of the characters of alkali-albumin, but a portion of the sulphur is replaced by phosphorus (see page 91), and it not improbably has the constitution of a nucleo-albumin.

IV. Proteoses.—The bodies included in this class are intermediate products in the hydration of the proteids of the former groups, and have characters intermediate between these proteids and the peptones, which are the final products of proteid nature resulting from the action of ferments on the coagulable proteids. They are also formed by heating such proteids with water, or (more readily) with dilute mineral acids. The proteoses are not coagulable by heat, but are mostly precipitated by certain neutral salts, and by alcohol, which does not, however, coagulate them. They all give a rose-red coloration with Fehling's solution, and are precipitated in the cold by nitric acid, the precipitate dissolving on application of heat and reappearing as the liquid cools.

The proteoses may be subdivided into albumoses, globuloses, caseoses, &c., according to the nature of the original proteid from which they are derived. Of these, the albumoses have been the most studied. These are converted by the further action of digestive ferments into peptones, the variety which yields hemi-peptone being called *hemialbumose*, and that which gives anti-peptone being similarly termed *antialbumose*. The albumoses may be otherwise subdivided according to their solubilities into proto-albumose, hetero-albumose, and deutero-albumose (see page 18).

V. Peptones.—Peptones are the ultimate products of proteid nature formed by the action of proteolytic ferments on the bodies of the preceding groups. If the hydrolysis be pushed further, the proteid is split into simpler bodies of non-proteid character. The peptones are very soluble in water, are not precipitated by neutral salts or by nitric acid, and are not coagulated by heat. They are unaffected or only imperfectly precipitated by many of the general reagents for proteids, but give a rose-colour with Fehling's solution,

and are completely precipitated, but not coagulated, by excess of absolute alcohol. The peptones are readily diffusible as compared with other proteids.

The peptones are distinguished as *hemipeptone*, which yields leucine and tyrosine as the further result of pancreatic digestion; and *antipeptone*, which is not decomposed in this way, yields no tyrosine on treatment with sulphuric acid, and does not give Millon's reaction (compare page 11).

VI. Insoluble Proteids.—This class includes a number of bodies not grouped above, but which resemble each other in their extreme insolubility in various reagents. Among them are:—

Proteid coagulated by heat or alcohol, having identical characters whatever its origin. *Fibrin*, formed from fibrinogen, *myosin* from myosinogen, and *casein* from caseinogen are coagulated proteids, the coagulation of which has been produced by ferments. *Antialbumid* and *lardacein*[1] are other examples of insoluble proteids.

Composition of the Proteids.

All simple proteids are composed of carbon, hydrogen, nitrogen, and oxygen in very similar proportions. Gelatin differs from the true proteids in containing little or no sulphur, while hæmoglobin and hæmatin contain iron in addition to the five essential elements of the proteids; nuclein and casein contain phosphorus; while in turacin[2] a notable quantity of copper is present.

The composition of the simple proteids varies within comparatively narrow limits, the following being the extremes according to Hoppe-Seyler:—

Carbon,	from	51·5 to 54·5	per cent.
Hydrogen,	„	6·9 „ 7·3	„
Nitrogen,	„	15·2 „ 17·0	„
Oxygen,	„	20·9 „ 23·5	„
Sulphur,	„	0·3 „ 2·0	„

[1] Antialbumid is formed more or less in all digestions. (See page 8.)

Lardacein or Amyloid is a body produced in the liver and other organs in certain diseases. It resembles coagulated albumin in being undissolved by water, saline solutions, or dilute acids or alkalies; but differs from it by completely resisting the action of the gastric juice. Its most remarkable reaction is the reddish or reddish-brown colour it assumes when treated with iodine, and the violet or blue colour it takes with iodine and sulphuric acid. This behaviour has led to the name "amyloid," but the body is in no way related to the starches, and dissolves in concentrated hydrochloric acid with formation of acid-albumin but no trace of any sugar.

[2] Turacin is the remarkable colouring matter discovered by A. H. Church in the red feathers of certain species of the turaco or plantain-eater

A valuable table by R. H. Chittenden, showing the ultimate composition of the more prominent proteids and albuminoids occurring in nature, will be found at the end of the volume.

The figures obtained by the ultimate analysis of the proteids lead to extremely complex formulæ, none of which can be said to have been fully established as even empirically correct. Still less have their molecular weights and chemical constitution been ascertained.

The difficulty attending the study of the proteids is enhanced by their great molecular complexity and tendency to change. It is further increased by their amorphous and colloidal character, their non-volatility, and the weak and indefinite character of such compounds as they form. At the same time, they retain certain neutral salts with great tenacity, and hence it is almost impossible to obtain some proteids free from more or less mineral matter.

The percentage composition of albumin corresponds closely with the empirical formula, $C_{77}H_{120}N_{20}O_{26}S$, but its constitutional formula is only very imperfectly known. The properties of albumin and other proteids compel the study of their constitution to be limited almost entirely to that of the products of their decomposition. Thus Nasse and also Schützenberger found that by heating the proteids for a prolonged period in sealed tubes with baryta-water, ammonia and carbon dioxide were formed in the same proportions as if urea were similarly treated. In addition, certain volatile products were formed, among which acetic acid, indole and pyrrol were the most prominent; while the fixed residue contained a mixture of various amido-acids. Of these, *tyrosine* belongs to the aromatic series, while *leucine* is an amido-acid of the acetic series (Vol. III. Part iii.), and the leuceïnes are amido-acids of the acrylic series.[1] Both leucines and leuceïnes are produced by the splitting up of the so-called gluco-proteins, bodies of a sweet taste and the general formula $C_nH_{2n}N_2O_3$ (in which n is 10 or 12). Hence, according to Schützenberger, albumin is a ureïde, in which the urea is combined with gluco-proteins which on hydration split into amido-acids. (Compare page 67.)

("Cape Lory"). These feathers contain on the average 0·14 grain of copper in organic combination. The colouring matter has not been met with in the feathers of birds of other families. Turacin contains C, 53·69; H, 4·60; N, 6·96; O, 27·74; and copper, 7·01 per cent. An interesting account of turacin and its allies will be found in the *Pharm. Jour.*, [3], xxiv. 128.

[1] Lysine, $C_6H_{10}(NH)_2O_2$, a diamido-caproic acid, and *lysatine*, $C_6H_{13}N_3O_2$, a body allied to creatine, are characteristic products of the treatment of proteids with hydrochloric acid and stannous chloride. It is probable that these bases are also formed in the treatment of proteids with baryta-water.

Pflüger observed, and Loew and Bokorny have confirmed the fact, that living proteid matter in the cells of various algæ reduces silver from a dilute alkaline solution of the nitrate, but that dead protoplasm or proteid does not give the reaction. Hence it is suggested that a compound of aldehydic nature exists in living protoplasm. By the reaction of formic aldehyde with ammonia, aspartic aldehyde may be supposed to be formed, a body which, though unknown, has the same composition as leucine. By its repeated polymerisation in presence of hydrogen and a sulphur compound, with elimination of water, the following equation may be realised :—$6C_{12}H_{17}N_3O_4 + H_2S + 6H_2 = C_{72}H_{112}N_{18}SO_{22} + 2H_2O$.

Latham has suggested that living proteid may be composed of a chain of cyanhydrins or cyan-alcohols united to a benzene-nucleus. (*Lancet*, 1888, ii. 751.)

Grimaux (*Compt. rend.*, xciii. 771) has proposed to define proteids as "nitrogenised colloids, which by hydrolysis split up into amidated acids, carbon dioxide, and ammonia." On heating aspartic anhydride to 130° with urea for two hours, Grimaux obtained a thick mass, completely soluble in boiling water to a gummy, highly colloid liquid, yielding highly gelatinous precipitates with acids, alkaline salts, magnesium and aluminium sulphates, solutions of iron, copper, and mercury, and with tannin. The jelly yielded by acetic acid dried up to a substance resembling dried albumin. It softened in boiling water without undergoing solution, but dissolved in caustic potash to a liquid giving a violet coloration with cupric sulphate. The same reaction is yielded by aspartic anhydride alone.

All the simple proteids, at least, give evidence of the presence of two distinct groups or radicals, called by Kühne the *anti-* and *hemi-* groups respectively. These two groups, or their representatives, can be separated, in part, at any rate, by the action of dilute (3 per cent.) sulphuric acid at 100° C. After a few hours' treatment in this manner of coagulated egg-albumin, one-half the proteid passes into solution, while there remains a homogeneous mass of antialbumid, resembling silica, insoluble in dilute acid but readily soluble in dilute solution of sodium carbonate. Anti-albumid is only slightly soluble in gastric juice, but is readily attacked by alkaline solutions of trypsin, with conversion into a soluble peptone known as antipeptone; but the change goes no further. In the sulphuric acid solution are found representatives of the hemi-group; namely, albumose, originally known as one body, hemialbumose, together with more or less hemipeptone, leucine, tyrosine, &c. The members of the hemi-group are readily converted by the action of trypsin into leucine, tyrosine, and other crystallisable products.

ANALYTICAL REACTIONS OF PROTEIDS.

The leading characters of the proteids have already been summarised (page 1). Their properties are described in greater detail in the following sections, and in the articles on the proteids of eggs, blood, urine, milk, commercial peptones, &c.

The proteids as a class are recognised with tolerable facility, except the peptones and proteoses, which are not affected by many of the general precipitants of proteids. The following are the chief reactions of analytical value for the proteids.

1. On heating, proteids decompose with evolution of ammonia, and give an odour of burning animal matter. Smaller quantities may be recognised by the ammonia evolved when heated with soda-lime. Sulphur is present in all true proteids, and may be detected by igniting the substance with alkali-metal carbonate and nitre, dissolving the product in dilute hydrochloric acid, and testing the clear solution with barium chloride, when a white precipitate of barium sulphate will be produced if the substance tested contained sulphur. The sulphur of proteids may also be detected by boiling the substance with a strong solution of caustic soda and a few drops of lead acetate, when black lead sulphide will be produced.

The foregoing tests merely prove the presence of nitrogen and sulphur, and are not conclusive evidence of the presence of proteids. For the more certain recognition of proteids, the following tests may be employed.

2. For the detection of solid proteids the colour-tests described on page 11 are very serviceable, especially reactions 2, 3, 5, and 7. These tests are also available for the detection of proteids in solution, but if very dilute it is desirable to treat the liquid with a suitable precipitant, and apply the test to the precipitate obtained.

3. The solutions of proteids have a lævo-rotatory action on polarised light. The optical activity is modified by free acids and by alkalies, but not by neutral salts. (See also page 28.)

4. The neutral or faintly acid solutions of most proteids (not peptones or proteoses) are coagulated when heated to boiling for a few minutes. Very small amounts of free alkali suffice to prevent the reaction, and it is retarded or prevented by any considerable quantity of free acid. Coagulation of proteids (except peptones and proteoses) may also be effected by addition of excess of strong alcohol. The conditions affecting the coagulation of proteids are described at length on pages 14 and 16.

5. Neutral or alkaline solutions of many proteids (not peptones) are precipitated when cautiously acidulated with a dilute mineral acid (especially nitric acid), the precipitate being soluble in excess

with formation of acid-albumin. The best way of applying the reaction is described on page 52. Acetic acid, tartaric acid, lactic acid, and orthophosphoric acid do not precipitate proteids, but precipitation is caused by metaphosphoric acid.[1]

Phospho-tungstic and phospho-molybdic acids also precipitate most of the proteids very perfectly.

6. The proteids are thrown down more or less perfectly by saturating their neutral solutions with various neutral salts, ammonium sulphate being the most general and perfect of such precipitants. Precipitation by neutral salts is utilised for the separation of various proteids, as described on page 18.

7. Most salts of the heavy metals separate proteids from their solutions as insoluble compounds (albuminates) of the proteid with the oxide of the metal added. Silver nitrate, basic lead acetate (avoiding excess), cupric sulphate, ferric acetate, mercuric acetate, and mercuric chloride are very perfect precipitants of proteids (compare pp. 29, 30). On treating the precipitates with sulphuretted hydrogen, the proteids are recovered unchanged. Most proteids are likewise completely precipitated by boiling their solutions for a few minutes with the hydrated oxide of copper, lead, or zinc.

8. Most proteids are thrown down completely by acidulating their solutions with hydrochloric acid, and adding a solution of potassium mercuric iodide (Tanret's reagent, see page 57).

9. If the solution of a proteid be rendered distinctly acid with acetic acid, and potassium ferrocyanide added, a white flocculent precipitate is produced which is said to consist of a definite compound of the proteid with ferrocyanide.[2] The reaction is delicate, and answers with all varieties of proteid except deutero-proteose and peptones. These bodies and gelatin are not precipitated. Recently-prepared hydroferrocyanic acid has been recommended in place of acetic acid and a ferrocyanide. A thiocyanate gives a similar reaction. (Compare page 54.)

10. Picric acid precipitates proteids very completely from solutions acidulated with acetic acid. In the latter case, mucin is also thrown down, but not otherwise. The precipitate produced by deutero-proteose and peptones dissolves on heating the liquid.

[1] According to Liebermann (*Ber.*, xxi. 598) the precipitate produced by metaphosphoric acid in solutions of albumin closely resembles the phosphorised constituent of cell-nuclei called nuclein.

[2] 1612 parts of albumin are stated to combine or react with 211 parts of potassium ferrocyanide, but the form in which the ferrocyanide exists in the alleged "compound" is very doubtful. The molecular weight of $K_4FeCy_6,3H_2O$ is 422 (= 211 × 2), and that of $FeCy_6$, 212.

The application of picric acid to the detection of proteids in urine is described fully on page 54.

11. Phenol and tannin also precipitate proteids from solutions acidulated with acetic acid. *Almén's tannin reagent*, which precipitates proteids (including peptone) very perfectly, is prepared by dissolving 4 grammes of tannic acid in 190 c.c. of 50 per cent. alcohol, and adding 8 c.c. of acetic acid of 25 per cent. strength.

Colour-Reactions of Proteids.

Proteids give certain colour-reactions which, though also produced by gelatin and other allied bodies, appear to be related to their constitution, and are often valuable as a means of detecting them. The following are the more important of this class of reactions.

1. Solid proteids are coloured deep yellow by a solution of iodine.

2. Fuming nitric acid destroys solid proteids with evolution of nitrogen, but if treated in the solid state with somewhat weaker acid (*e.g.*, sp. gr. 1·2 to 1·25) they acquire a bright yellow colour. The same reaction is produced if the solution of a proteid be boiled for some time with strong nitric acid. The colour is attributable to the formation of a yellow substance of indefinite composition known as xanthoproteic acid. It is soluble in ammonia and fixed caustic alkalies with orange-red or brownish-red colour.

Gelatin and keratin behave like proteids with strong nitric acid.

3. When a solution of a proteid is treated with Millon's reagent, a white precipitate is formed which turns brick-red on boiling, the supernatant liquid also becoming red after a time. Solid proteids become red when boiled with Millon's reagent. The test is useful, but a similar reaction is yielded by gelatin, keratin, taurin, and allied bodies containing an aromatic group in the molecule.

Millon's reagent is prepared by treating metallic mercury with an equal weight of nitric acid of 1·4 specific gravity (or 1 c.c. of mercury to 10 c.c. of nitric acid). When the action slackens, a gentle heat may be applied till complete solution is effected. The solution is then diluted with twice its measure of cold water, allowed to stand for some hours, and decanted from the deposit which forms. The liquid thus prepared is a solution of mercurous nitrate, holding nitrous acid in solution, to the presence of which its action is partly due. Hence the reagent answers best when freshly prepared.

Barfoed substitutes for Millon's reagent a neutral solution of mercuric nitrate, which gives a yellow coloration when heated with

proteids; and on then adding a drop or two of yellow, fuming nitric acid, and again heating, a bright red or brownish-red coloration is produced.

4. When heated with concentrated hydrochloric acid, solid proteids dissolve with blue coloration, changing to violet and brown. This reaction is known as *Liebermann's test.*

5. Cold concentrated sulphuric acid affords no characteristic reaction with proteids, but on heating charring occurs. If, however, a proteid be dissolved in glacial acetic acid, and the resultant solution be treated with concentrated sulphuric acid, a fine violet coloration is produced, and the liquid appears faintly fluorescent. If sufficiently concentrated, the liquid will exhibit a spectrum very similar to that of urobilin (Vol. III. Part iii. page 407), having a well-marked absorption-band between *b* and F. The foregoing test is due to Adamkiewitz (*Ber.*, viii. 161). It is yielded by all proteids, including peptones, and is also produced by pepsin, ptyalin, and analogous bodies; but tyrosine, leucine, asparagine, glutamic acid, and other crystallisable derivatives of the proteids do not give it. The reaction is so delicate that a solution of white of egg in 1000 parts of water yields the coloration. Nitric acid greatly diminishes the delicacy of the test, but sodium chloride somewhat enhances it.

6. If some cane-sugar be dissolved in the not too dilute solution of a proteid, and the liquid be then cautiously poured on to some strong sulphuric acid, so as to avoid admixture of the two fluids, a fine violet-purple coloration will be developed at the junction of the two strata. This reaction may be conveniently employed for the microscopic detection of albuminoids. Strong phosphoric acid may be advantageously substituted for the sulphuric acid.

7. If a few drops of a dilute solution of copper sulphate be added to the solution of an albumin or globulin, a precipitate of copper albuminate will be produced. On addition of excess of caustic soda or potash to the liquid, the precipitate will dissolve with production of a fine violet coloration. If ammonia be substituted for a fixed alkali, a blue solution will be obtained. The test may be varied by dissolving the solid proteid in a strong solution of caustic soda, or by adding the alkali to a previously-prepared solution of the proteid. If to the strongly alkaline liquid obtained in either of these ways a few drops of dilute copper sulphate solution be added, the same violet coloration is produced. This does not undergo any change on heating the solution to boiling, unless glucose or other reducing body be simultaneously present. Fehling's solution may be substituted for cupric sulphate in applying the above test, but in either case an excess of

the reagent must be carefully avoided, or the violet coloration due to the proteid will be masked by the blue colour of the copper solution.

Proto-albumose and hetero-albumose differ from the albumins and globulins in giving a precipitate with copper sulphate which dissolves in caustic alkali with reddish-violet or rose-red colour. Deutero-albumose and peptones give no precipitate with copper sulphate alone, and on subsequently adding caustic soda the colour produced is rose-red; while if ammonia be substituted a reddish-violet solution is obtained.

The foregoing reaction is due to Piotrowski, but is generally known as the *biuret test*. It is also produced by gelatin, and, according to H. Schiff, by all bodies which contain two $CO.NH_2$ groups combined in the molecule with one or more CO.NH groups. (See abst. *Jour. Chem. Soc.*, 1896, i. 635.)

In practice, the biuret reaction has often to be applied in presence of neutral salts. Its indications are not affected by sodium chloride. In presence of magnesium sulphate a white precipitate of magnesium hydroxide is produced by the alkali, and should be filtered off before adding the copper solution. In presence of ammonium sulphate a large amount of fixed alkali must be added to obtain the violet coloration. Hence A. Bömer substitutes zinc sulphate for ammonium sulphate as a precipitant of proteids other than peptones.

The foregoing colour-reactions appear to depend on the presence of certain aromatic radicals in the proteid molecule. By the putrefaction of proteids various compounds of the aromatic series are formed, and these may be arranged in three classes:—(1) The phenol group, including tyrosine, aromatic hydroxy-acids, phenol, and cresol; (2) the phenyl group, which contains phenyl-acetic and phenyl-propionic acids; and (3) the indole group, of which indole, skatole, and skatoxyl-carboxylic acid are the chief members. E. Salkowski (*Zeit. physiol. Chem.*, xii. 215; abst. *Jour. Chem. Soc.*, 1888, p. 508) has tried various colour-reactions on the above-named putrefaction-products, with the following results:—

a. Millon's reaction does not occur in presence of sodium chloride. It is yielded only by group 1 of the putrefaction-products. Gelatin also yields the reaction faintly, but the purest gelatin yields traces of phenol on putrefaction.

b. The xanthoproteic reaction doubtless depends on the formation of nitro-derivatives. The bodies of group 1 give the reaction strongly, and those of group 3 less strongly, but fairly well, except indole, which gives only a faint coloration unless fuming nitric acid is used. The bodies of group 2 give a negative or

extremely faint reaction with the xanthoproteic test. The xanthoproteic reaction may be used for the approximate determination of peptone, and perhaps of albumin. Gelatin and gelatin-peptone yield no coloration with nitric acid, a fact which gives the xanthoproteic reaction an advantage over the biuret test, as in the latter case the shade as well as the intensity of the colour varies considerably.

Liebermann's colour-reaction with strong hydrochloric acid is not yielded by any of the decomposition-products named above.

Pickering considers that the xanthoproteic and Millon's reactions are probably due to the presence of a hydroxybenzene nucleus in the proteid molecule, and that the reactions of Liebermann and Adamkiewitz depend on the aromatic portion of the molecule, while the brilliant red colour given with a solution of alloxan observed by Krasser probably depends on the presence of an amido-group.

J. W. Pickering (abst. *Jour. Chem. Soc.*, 1893, i. 615) finds that cobalt salts with caustic alkali give distinctive colour-reactions with proteids. Native proteids give a heliotrope-purple, proteoses and peptones a red-brown. Gelatin, keratin, and various other organic substances related to proteids give similar colours. Gelatin gives a play of colours in the spectral order, as also do alloxan, allantoin, and biuret. Pickering thinks these reactions, as also those produced by nickel and copper salts, are due to the presence of the group CO.NH in the molecule. The precipitates produced in proteid solutions by mercuric chloride, silver nitrate, and sulpho-salicylic, phospho-tungstic, and phospho-molybdic acids yield the typical proteid colour-reactions. The nucleo-albumins behave like coagulable proteids, not as peptones, in their colour-reactions.

C. Reichl (abst. *Jour. Chem. Soc.*, 1889, p. 1092; 1890, p. 1350) has described a number of colour-reactions obtained by treating proteids with an alcoholic solution of benzaldehyde or other aromatic aldehyde in presence of dilute sulphuric acid and ferric chloride. He concludes that the colours are due to the skatole-group in proteids.

Coagulation of Proteids.

In studying the proteids it is important to distinguish between mere precipitation and true coagulation. Thus, on saturating a neutral solution of any proteid but the peptones with ammonium sulphate, the proteid is precipitated perfectly, but will redissolve when the greater part of the precipitant has been removed by washing. On the other hand, a true coagulated proteid, as

obtained by boiling the solution, by prolonged treatment with alcohol, or by the action of certain precipitants, cannot be redissolved by any method not involving chemical change. True coagulation of certain proteids is also produced by peculiar ferments, the curdling of milk by rennet and of blood when removed from the body being due to this cause.

Heat Coagulation.—One of the most characteristic of the properties of albumin and other soluble proteids is that of undergoing coagulation when their neutral solutions are heated. Thus the solution of white of egg in water becomes cloudy when heated to about 60° C., and on increasing the temperature to 72° or 73° the albumin coagulates and becomes wholly insoluble.

The temperature at which the coagulation of proteids occurs is modified by the dilution of the liquid and the time taken to raise it to the coagulating point. Haycraft and Duggan maintain (*Brit. Med. Jour.*, 1890, p. 167 ; abst. *Pharm. Jour.*, [3], xx. 604) that Halliburton's observation of the fractional coagulation of blood-serum does not prove the presence of several proteids of different coagulation-points, since the coagulation of part of the albumin would diminish the acidity and increase the dilution in which the remainder was left, necessitating for this a higher temperature for coagulation (compare page 42).

According to A. Gautier, on coagulating 100 grammes of egg-albumin by heat, alkali is set free in quantity sufficient to neutralise 1·53 gramme of H_2SO_4. The purest albumin invariably yields about 0·5 per cent. of ash, which usually consists of calcium phosphate, sodium chloride, and sodium sulphate. Gautier considers it probable that these salts exist in the unaltered albumin as calcium chloride and sulphate, and as sodium phosphate (*Bul. Soc. Chim.*, xliii. 596 ; abst. *Jour. Chem. Soc.*, 1885, p. 1082).

The coagulation of proteids by heat receives practical application in the use of albumin in calico-printing, and is one of the simplest and best tests for the presence of albumin, &c., in urine. The best method of applying the test is described on page 51.

Alcohol Coagulation.—Alcohol, added in moderate quantity, precipitates albumins and globulins from solution in a form soluble in pure water ; but if the alcohol be added in large excess, and the resultant precipitate be left for some time in contact with strong alcohol, true coagulated proteid is formed, indistinguishable from that produced by heat. Albumoses and peptones are precipitated but not coagulated by such treatment (page 18).

When employing the coagulation of proteids by alcohol for their gravimetric determination the liquid should be mixed with four times its measure of methylated spirit, and allowed to stand

for some hours. The precipitate is then separated, washed in succession with methylated spirit, absolute alcohol, ether, and warm water, dried at 100° C., and weighed. An alternative plan is to neutralise the liquid with acetic acid, add ten measures of methylated spirit, boil, and treat the precipitate as before.

Coagulation by Ferments.—By contact with peculiar ferments certain proteids are completely coagulated. The coagulation of the fibrinogen of blood outside the body, with formation of insoluble fibrin; the curdling of the caseinogen of milk by rennet, with formation of insoluble casein; and the coagulation of the myosinogen of muscle after death, with formation of insoluble myosin, are the best-known examples of such changes. It is held by Sidney Martin and others that the formation of gluten on kneading wheaten flour with water is also due to the action of a soluble ferment.

Dry proteids, when heated to a temperature of 110° C., become insoluble and indistinguishable from the product obtained in the wet way.

Coagulated Proteid has identical properties, from whatever source it may have been obtained, and whether coagulation has been effected by heat or by alcohol.

Coagulated proteids are wholly insoluble in water, alcohol, and other neutral solvents, and are dissolved only with great difficulty by ammonia. In acetic acid they swell up and gradually dissolve. They are insoluble in very dilute hydrochloric acid (0·1 per cent.) either cold or warm; but if pepsin be also present, at a temperature of 40° to 60° C., they dissolve as acid-albumin, and are subsequently changed to peptones.

Coagulated proteids dissolve in strong hydrochloric acid, and with caustic alkalies they yield albuminates.

Coagulated proteids are coloured yellow by iodine, by strong nitric acid, and by boiling with Millon's reagent. When dissolved in strong caustic alkali, the solution gives the biuret reaction (page 12).

Detection and Distinction of Proteids.

Of the foregoing methods of precipitating proteids, coagulation by heat is one of the most serviceable. As qualitative tests, nitric acid, picric acid, potassium ferrocyanide, and Tanret's reagent are among the best.[1] The best methods of applying them are described on page 52 *et seq.* For separating proteids with a view of subsequently weighing the precipitate or determining the

[1] The relative delicacy of the principal precipitants of urinary albumin is discussed on page 58.

contained nitrogen, the methods described on pages 29 and 30 are available.

The table on page 49, which exhibits the chief reactions of solutions of the proteids liable to occur in urine, may be conveniently put to more general use.

The systematic method on page 18 may be employed for the identification of dissolved proteids, and for their separation when co-occurring. The table assumes the simultaneous presence of all the more important soluble proteids, but in practice the method of analysis may be much simplified when the history of the proteid solution is known. Thus caseinogen can occur only in milk and milk-products, egg-albumin is peculiar to eggs, and fibrinogen and myosinogen to blood- and muscle-serum respectively. One of the most complex cases liable to occur in practice is when it is desired to separate the various proteids which may be present in pathological urine. These include serum-albumin, serum-globulin, possibly globin from blood, and the several modifications of albumoses and peptones.

The method in the table may readily be made quantitative, by determining the nitrogen in the various precipitates by Kjeldahl's process.

W. Kühne (abst. *Jour. Chem. Soc.*, 1893, i. 233) has pointed out various precautions which should be observed when separating albumoses from peptones by ammonium sulphate. A large volume of the saturated solution should be employed, besides adding the powdered salt as long as it dissolves. The last traces of albumose are more completely precipitated if the liquid be made alkaline. To remove the excess of salt from the filtrate it should be concentrated, the crystals removed, and excess of barium carbonate added. Dialysis does not sharply differentiate between peptones and albumoses, the latter being more or less diffusible. Hetero-albumose is the least diffusible, its neutral saline solutions being precipitated on dialysis, so that no albumose passes through the membrane unless the liquid be made ammoniacal. A precipitate formed on dialysis is not necessarily of proteid nature. If hard water be used it may consist of calcium sulphate.

H. Bömer has recommended the use of zinc sulphate instead of ammonium sulphate for precipitating albumoses. It answers equally well, and the nitrogen in the precipitate can be at once determined by Kjeldahl's process. The zinc can be conveniently removed from the filtrate by cautious addition of barium sulphide ; but its presence does not interfere with the detection of peptones by the xanthoproteic and biuret tests.

It will be observed that the peptones are not affected by many

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Ascertain the reaction of the liquid to litmus. If alkaline, cautiously neutralise with dilute acetic acid, and remove any precipitate of *alkali-albumin* by filtration. If the liquid be acid, render it exactly neutral to litmus by cautious addition of dilute caustic soda, and filter off any precipitate of *acid-albumin*. The neutralised or originally neutral liquid is filtered and divided into two portions, A and B.

A. Render the neutral or neutralised solution slightly but distinctly acid to litmus by dilute acetic acid (compare page 51), boil for a few minutes, and filter if any coagulation occurs.

PRECIPITATE may contain *albumins* and *globulins*, which may be separated by saturating another portion of the solution with magnesium sulphate, as in B.

FILTRATE may contain albumoses,[1] and peptones. Allow to cool, add powdered ammonium sulphate as long as the salt continues to dissolve on stirring, and filter.

PRECIPITATE consists of albumoses. Wash with a saturated aqueous solution of ammonium or zinc sulphate. Redissolve the washed precipitate in a minimum quantity of water. Faintly acidulate the solution with acetic acid, saturate it with common salt, and filter.

FILTRATE contains *peptones* only, recognisable by the xanthoproteic and biuret reactions, page 13.

PRECIPITATE consists of *primary albumoses*. Wash with saturated brine, redissolve by adding water, and dialyse the solution.

FILTRATE may contain *deutero-albumose*, precipitated by saturating the solution with ammonium sulphate, and recognisable by other reactions described on page 63.

PRECIPITATE consists of *hetero-albumose*, which may be further identified by reactions on page 62.

FILTRATE may contain *proto-albumose*, precipitable by excess of alcohol (10 : 1).

B. Saturate the neutral liquid with magnesium sulphate, by adding the powdered salt as long as solution occurs on stirring, and filter.

PRECIPITATE may contain globulins, proto- and hetero-albumose, and possibly derived albumins previously retained in solution by neutral salts. Wash the precipitate with a saturated solution of magnesium sulphate, and then dissolve in cold water. Divide the solution into four portions.

a. Add fibrin ferment. *Fibrinogen* is coagulated as fibrin.
b. Add myosin ferment. *Myosinogen* is coagulated as myosin.
c. Add rennet ferment. *Caseinogen* is coagulated as casein.
d. Heat liquid carefully, filtering off at 60° C. any *fibrinogen* and *myosinogen*; at 63° *myoglobulin*, and at 75° *paraglobulin* and *cell-globulin*. Add acetic acid to the hot liquid.

PRECIPITATE consists of *caseinogen*, which, if dissolved in a minimum of lime-water, is coagulated by rennet ferment.

FILTRATE. Boil to remove traces of coagulable proteids, and separate proteoses, if present, as in A.

FILTRATE. Remove the excess of salts by dialysis, and shake the dialysed liquid with ether.[2]

PRECIPITATE consists of *egg-albumin*.

FILTRATE. Acidulate slightly with acetic acid and boil.

PRECIPITATE consists of *serum-albumin*, or, possibly, of *vitellin*.

FILTRATE. Cool and saturate with ammonium or zinc sulphate.

PRECIPITATE consists of *deutero-albumose*.

FILTRATE contains *peptones*.

[1] Traces of primary albumoses are liable to be formed by the action of the boiling acidulated liquid on albumins and globulins. In cases of importance a sharper separation can be effected by treating the liquid with ten times its volume of strong alcohol, which precipitates all the proteids. The precipitate is rinsed off the filter with absolute alcohol, and left in contact with the alcohol for five to ten weeks. This treatment coagulates the albumins and globulins without affecting the albumoses or peptones. The supernatant alcohol is poured off, the remainder evaporated at a temperature not exceeding 40° C., and the residue treated with water, which dissolves the albumoses and peptones, leaving the albumins and globulins insoluble.

[2] When, from the history of the proteid solution, egg-albumin is known to be absent, this stage may be advantageously omitted.

of the reagents which precipitate other proteids. Thus they are not coagulated by heat or alcohol, and are not precipitated by ammonium sulphate or ferric acetate. On the other hand, they are completely precipitated by tannin, and less perfectly by phospho-tungstic acid.

Determination of Proteids.

The determination of proteids is effected in different manners according to the nature of the substance containing them. The chief general methods are based on the following principles :—

a. Determination of the proteid nitrogen, and calculation of the proteid from the amount found.

b. Observation of the optical rotation of the solution.

c. Precipitation or coagulation of the dissolved proteid, and determination of the precipitated proteid by direct weighing, measurement, observation of the specific gravity, or determination of the contained nitrogen.

Determination of the Nitrogen in Proteids.

In many cases, the amount of proteid or albuminoid matter present in a substance may be deduced with considerable accuracy from a determination of the nitrogen. The method, of course, is inapplicable in presence of other nitrogenised substances, unless these can be removed or the nitrogen contained in them estimated or allowed for.

The proportion of nitrogen contained in the different proteids ranges from 15·2 to 17·0 per cent. The nitrogen in a proteid or mixture of proteids of unknown nature may be safely assumed to be not far from 15·8 per cent., and hence the amount of proteid may be found by multiplying the nitrogen by $\frac{100}{15·8} = 6·33$.

Some chemists employ the factor 6·25, and others as high a multiplier as 6·37 ; but 6·33 is very generally adopted. Where the greatest possible accuracy is not aimed at, the factor 6·3 will answer every purpose.

Dumas' Method.—The determination of the nitrogen of proteids may be accurately effected by Dumas' method of combustion with copper oxide, absorption of the carbon dioxide by soda-lime or solution of caustic potash, and measurement, with due precautions, of the residual nitrogen gas.

Soda-lime Process.—Another equally reliable method of determining the nitrogen of proteids is that of ignition with soda-lime, the ammonia formed being determined by titration with standard acid, or precipitated as ammonium chloroplatinate. Some investigators have thrown doubt on the accuracy of the soda-lime process, and unless due precautions be taken it is undoubtedly liable to give

results below the truth. The chief sources of error are the dissociation of ammonia from the employment of too high a temperature, and the presence of atmospheric air in the combustion-tube.

If *nitrates* or other oxidised compounds of nitrogen be present, the results are usually in excess of the true nitrogen of the proteids, but notably less than the total amount of nitrogen present. Greatly improved results are obtainable by adding sugar to the soda-lime, by previously expelling the atmospheric air, and by similar devices; but the following modified method, due to J. Ruffle (*Jour. Chem. Soc.*, xxxix. 87), is capable of yielding the total nitrogen in the form of ammonia, no matter whether it exist in the substance as a nitrate, nitrite, nitro-compound, ammonia, proteid, amide, or other form.

From 1 to 1½ gramme of the substance to be analysed is mixed with 1½ gramme of a mixture in equal proportions of flowers of sulphur and finely-powdered granulated sugar; 20 grammes weight of crystallised sodium thiosulphate (hyposulphite) is powdered and thoroughly mixed with an equal weight of specially-prepared soda-lime.[1] About 5 grammes weight of the latter mixture is introduced into a combustion-tube 22 inches in length and ⅝ inch in internal diameter. This is followed by another 30 grammes of the soda-lime and thiosulphate mixed with the weighed portion of the substance and its previously added complement of sulphur and sugar. The remaining 5 grammes of mixed soda-lime and thiosulphate are then introduced, and the tube filled with about 18 grammes of unmixed granular soda-lime, which is followed by a plug of asbestos. The combustion is then made in the usual way, care being taken that the unmixed soda-lime is brought to a red heat before the remaining contents of the tube are heated, and that it is maintained at a red heat throughout the operation.

Johnson and Jenkins have obtained very satisfactory estimations of nitrogen in proteids by combustion with a soda-lime prepared by mixing equal measures of dry sodium carbonate and slaked lime. They consider the product thus made superior to the usual kind in several important respects.

The ammonia produced by the combustion with soda-lime may be condensed in cold water or dilute standard acid, and titrated in the usual way, either litmus, cochineal, lacmoid, or methyl-orange being employed as an indicator.

[1] The soda-lime is made by dissolving 160 grammes of caustic soda in an equal weight of hot water, pouring into the solution 56 grammes of finely powdered quick-lime made from marble, and stirring well till the slaking is complete. The mixture is then carefully dried, finely powdered, and carefully preserved.

Each cubic centimetre of normal acid neutralised by the product of the combustion represents ·08862 gramme of proteid in the substance analysed.

Sulphuric Acid Process.—By far the most convenient method of determining the nitrogen of the proteids and allied substances is to employ one of the modifications of Kjeldahl's process. This method is based on the fact that proteids, in common with the great majority of other nitrogenised organic substances, are decomposed when strongly heated with concentrated sulphuric acid, by which treatment the whole of the nitrogen is converted into ammonia. Certain intermediate products (*e.g.*, leucine, tyrosine, glycocine) are first formed, but by further heating these are decomposed with formation of ammonia.

The carbon and hydrogen of the organic matter are oxidised by the sulphuric acid to carbon dioxide and water, and hence much sulphur dioxide is evolved. This process of oxidation is materially assisted by the addition of such substances as potassium permanganate, manganese dioxide, &c., but the use of these powerful oxidising agents is unnecessary, and under certain conditions not well understood they are liable to occasion loss by oxidising the ammonia itself. A safer and equally satisfactory plan is to add a little mercury, mercuric oxide, cupric oxide, or cupric sulphate to the mixture. These reagents act as carriers of oxygen, and materially reduce the time required for the treatment. To ensure complete conversion and shorten the time of treatment, it is important to employ as high a temperature as possible, and this condition is effected by adding potassium sulphate to the contents of the flask, as first recommended by Gunning. When the conversion into ammonia is complete, the amount formed is usually determined by rendering the liquid alkaline, and distilling. The ammonia volatilised is absorbed by a known measure of standard acid, and the amount deduced from the volume neutralised. Instead of distilling off the ammonia, it may be decomposed by alkaline hypobromite, and the evolved nitrogen measured.

The Kjeldahl process in its various modifications has been very fully examined and reported on by the American Association of Official Agricultural Chemists. Bernard Dyer, who has had a wide experience of the process, has also examined it critically, and the following details are largely taken from his description (*Trans. Chem. Soc.*, 1895, p. 811) of the application of the method to the determination of the nitrogen in feeding-stuffs and fertilisers. The process is equally applicable to the determination of the nitrogen in horn-shavings, bone-dust, gelatin, and other proteid or albuminous substances containing no oxidised compounds of nitrogen. By

a slight modification to be subsequently described the process is applicable in presence of nitrates.[1]

A quantity of the substance varying in weight from 0·5 to 5·0 grammes or even more, according to its richness in nitrogen, is introduced into a round-bottomed flask of hard Jena glass, and treated with about 20 c.c. of strong sulphuric acid, with the addition of a single drop of mercury. The flask is closed with a loosely fitting balloon stopper made by blowing a bulb on a piece of quill-tubing and drawing out and sealing the stem. The flask is adjusted obliquely over a gas flame and heated, gently at first, until the initial vigorous action has ceased. The heat is then gradually increased, so that the liquid boils briskly. In about fifteen minutes 10 grammes of potassium sulphate should be added, and the boiling continued until the contents of the flask are clear and colourless, which generally occurs within about half an hour, or, in the case of very refractory substances, within an hour. The sulphuric acid condenses on the internal projection of the balloon stopper, and falls back, so that there is little loss of acid by volatilisation. The contents of the digesting flask are then washed out into a spacious distilling flask, also of Jena glass, which is connected by a doubly perforated cork with any convenient condensing apparatus; the second perforation bearing a tapped funnel through which an excess of caustic soda solution is added, and also a little sodium sulphide to decompose any nitrogen compounds of mercury that may have been formed. If mercury be not used, the sodium sulphide may be dispensed with. Some granulated zinc is put in the flask to prevent bumping, and the products of distillation are collected in a measured quantity of standard acid, the ammonia which distils over being determined by titration in the usual way, using methyl-orange or cochineal as the indicator of neutrality. It is desirable to allow the steam charged with ammonia to pass directly into the acid, which may be conveniently contained in a flask standing in a tank of running water. The means of communication between the distilling flask and the receiving flask should be a block-tin tube bent in the form of an arch; this

[1] Dyer, Dafert, Arnold and Wedermeyer, and the author have independently proved the applicability of the Kjeldahl process to a great number of organic compounds. These include uric acid, caffeine, asparagine, alkaloids (atropine, morphine, quinine, strychnine), indigotin, aniline, diphenylamine, β-naphthylamine, orthobenzoic sulphinide, pyridine, benzidine, nitroso-dimethylaniline, and potassium ferrocyanide, ferricyanide and cyanide (the last two not very perfectly). Nitro-derivatives, such as picric acid and dinitrobenzene, gave good results by Jodlbauer's modification, and azobenzene with the addition of salicylic acid and zinc-dust. Hydrazine derivatives failed to yield the whole of their nitrogen as ammonia.

rising perpendicularly from the cork of the distilling flask to a height of 15 or 18 inches before turning over. With the apparatus arranged in this way, there is no danger of the passing over of soda-spray with the steam, and the use of any form of "spray trap" is unnecessary. The other end of the tube is united by a cork to a pear-shaped adapter having a large expansion, and terminating in a narrow end which dips into an Erlenmeyer flask in which the acid is contained. The pear-shaped expansion allows

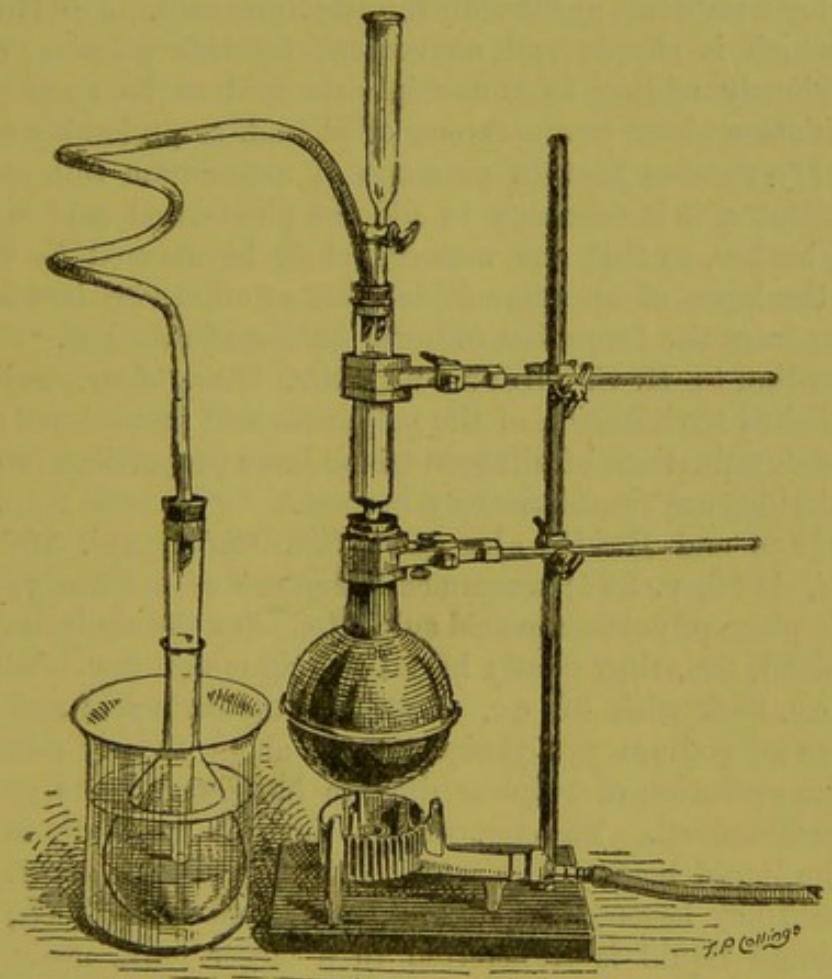


Fig. 1.—Distillation apparatus for Kjeldahl's process.

for the variations of pressure during distillation, and is sufficient to prevent any regurgitation of the acid into the distilling flask.

In conducting the distillation of the alkaline liquid the author greatly prefers a copper flask to one of glass. A convenient arrangement, employed by C. G. Moor, is shown in fig. 1. If a glass ball and some broken glass or glass beads be placed in the wide tube connected with the flask, the ammoniacal steam will be thoroughly washed, and all chance of spirting prevented. W. J. Sykes finds a common oil-can of tinned iron very satisfactory.

It is, of course, essential that the reagents employed should be practically free from nitrogen, but it is desirable to make a blank

experiment from time to time to ascertain the correction to be made for the unavoidable traces of nitrogen apt to be present. Dyer finds that a sensible error is caused by the action of the steam or standard acid on the glass, and hence he recommends the employment of a tin tube as a condenser. The whole correction due to this cause and to traces of impurities in the reagents should not be greater than would correspond to 0·0005 gramme of nitrogen.

In the presence of oxidised compounds of nitrogen, it is necessary to employ a reducing agent with the sulphuric acid. Jodlbauer's plan, which is simple and convenient for this purpose, consists in previously adding to the sulphuric acid to be employed for the oxidation about two grammes of phenol, or preferably salicylic acid. Dyer states that in presence of ammonium salts, together with nitrates, it is necessary to add the phenolated acid suddenly from a beaker, so that the material shall be covered by the acid before the lapse of an appreciable time, as otherwise loss is liable to occur from the formation of lower oxides of nitrogen.

According to H. C. Sherman (*Amer. Chem. Jour.*, xvii. 567) no published modification of the sulphuric acid process will give accurate determinations of nitrogen where large proportions of nitrates and chlorides are simultaneously present.

Riviere and Bailhache (*Bul. Soc. Chim.*, xvi. 806; abst. *Analyst*, 1896, p. 267) recommend the use of sodium pyrophosphate in place of potassium acid sulphate. For the analysis of horn, dried blood, &c., they gently heat 0·5 gramme of the substance in a 250 c.c. flask with 20 c.c. of strong sulphuric acid and 1 to 2 grammes of sodium pyrophosphate for about twenty minutes, or until the evolution of sulphur dioxide lessens and the pyrophosphate is dissolved. The temperature is then gradually increased until the liquid boils. The reaction is regarded as complete when the liquid has become limpid and nearly colourless. The cooled and diluted liquid is transferred to a larger flask, made faintly alkaline with soda, 3 grammes of hydrated magnesia added, the solution made up to about 500 c.c., and boiled for eighty to ninety minutes to ensure complete evolution of the ammonia, which is absorbed and titrated in the usual way. By this method, Riviere and Bailhache obtained from 0·05 to 0·02 per cent. more nitrogen than by the soda-lime or unmodified Kjeldahl process.

In cases where great accuracy is less important than economy of time, it is convenient to decompose the ammonia formed by hypobromite, and measure the nitrogen evolved, instead of distilling with alkali and titrating the distillate. Such a plan has been employed by the author with great satisfaction for the determination of the total nitrogen of urine, his mode of operating, which is

generally applicable with a few evident modifications, being as follows[1]:—

Twenty-five c.c. of the urine to be examined should be treated in a porcelain basin with 10 c.c. of strong sulphuric acid, and the liquid kept gently boiling until the volume is reduced to about 10 c.c. and white fumes of sulphuric acid are evolved.[2] The liquid is then allowed to cool, and carefully transferred to a pear-shaped flask, the basin being rinsed with a few drops of water. The flask is placed in an inclined position, to prevent loss by spurting, and the contents kept in gentle ebullition. If excessive frothing occur, it may be moderated by adding a small fragment of paraffin-wax (candle). When the frothing has ceased, about 5 grammes of potassium sulphate should be added and the flask heated strongly until the liquid is colourless or only a very pale yellow.[3] The contents of the flask are then allowed to become cold, when about 20 c.c. of water is added very cautiously and a few drops at a time, agitating the liquid by a rotatory movement between each fresh addition. A highly concentrated solution of caustic soda, made by dissolving the alkali in about an equal weight of water, is now added gradually with constant agitation, until the sulphuric acid is nearly neutralised. This point may be ascertained by means of litmus-paper, or a few drops of litmus or phenol-phthalein solution may be added to the contents of the flask.

The neutralised liquid, which will measure about 80 c.c., is next diluted to exactly 100 c.c. with water, and thoroughly mixed by agitation. Ten c.c. of the solution, representing 2·5 c.c. of the

[1] A process on the same lines has been described by Petit and Monfet (*Jour. Pharm. und Chem.*, 1893, page 297), but their method of manipulating is different in many respects from that employed by the author. Both modifications are liable to give results below the truth.

[2] As a rule, the quantity of sulphuric acid prescribed is amply sufficient for the decomposition of the solids of 25 c.c. of urine. In the case of highly saccharine urine, however, the sugar chars and forms a black pasty mass, which cannot be readily transferred to the flask. In such a case, a further addition of sulphuric acid (5 to 10 c.c.) should be made, and the heating continued till the greater part of the carbonaceous matter is oxidised. It is important in all cases to avoid the use of an excessive amount of sulphuric acid, or so large an amount of soda must be employed to neutralise it, and so large a volume of water added to retain the salts in solution, that the measure of the neutralised liquid cannot be kept within 100 c.c., or indeed within any reasonable limits. On the other hand, less than 10 c.c. of acid is an inconveniently small volume to heat and manipulate. Hence it is desirable to adhere to the quantities of urine and acid prescribed in the text, and take an aliquot part of the neutralised liquid for treatment with hypobromite.

[3] No addition of a compound of mercury or copper is admissible. In the former case, compounds are formed which do not evolve the whole of their

original urine, is now treated with the alkaline hypobromite reagent used for the determination of urea (Vol. III. Part iii. page 266).[1] Any of the forms of apparatus devised for that purpose may be employed, but by far the most convenient is, however, that shown in fig. 2. Ten c.c. of the neutralised solution from the sulphuric acid treatment being placed in the flask, 25 c.c. of the hypobromite reagent should be poured into the separator, and the connections made as shown in the figure.[2] The nitrometer should be filled to the tap. The apparatus being adjusted, and the clip at the top of the nitrometer-cup having been momentarily opened to equalise the pressure in the cup with that in the flask, the tap of the nitrometer is opened, and the hypobromite solution then allowed to flow gradually into the flask. After adding about 10 c.c., the separator-tap should be closed and the flask agitated. A further addition of hypobromite is then made, the flask again agitated, and this treatment repeated until no further evolution of nitrogen takes place. As a rule, 10 c.c. of the reagent is sufficient to complete the reaction, which occurs very promptly and completely.[3] The flask is now allowed

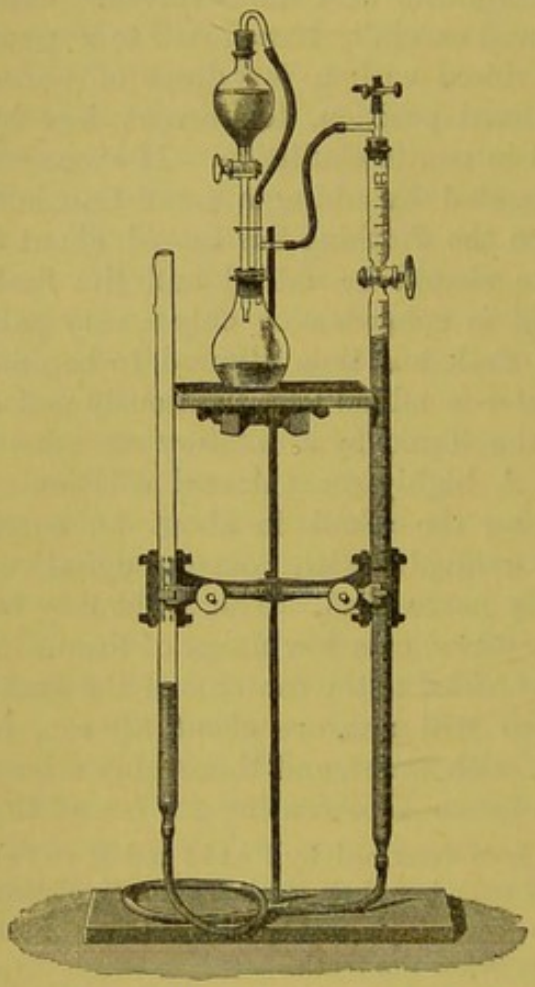


Fig. 2.—Nitrogen-evolution apparatus.

nitrogen on subsequent treatment with hypobromite, and in the latter case oxygen is evolved and the results wholly vitiated.

[1] The reagent is prepared by dissolving 100 grammes of good caustic soda in 250 c.c. of water, and thoroughly cooling the liquid. From 20 to 25 c.c. measure of bromine is then added and the resultant liquid kept in a cool place.

[2] If preferred, the hypobromite reagent may be placed in the flask and the neutralised solution added to it. In this case, a layer of water should be floated on the liquid in the separator, so as to rinse it completely into the flask.

[3] A number of experiments made in the author's laboratory by G. B. Brook show that the process gives good results with solutions of pure

to acquire the temperature of the room, when the liquid in the nitrometer-tube is brought to the same level with that in the reservoir-tube, and the volume of nitrogen read off. If less than 20 c.c. of gas has been evolved, the process may be advantageously repeated on 20 c.c. or more of the neutralised liquid from the sulphuric acid treatment.

From the volume of nitrogen evolved the corresponding weight and its equivalent in proteids may be readily calculated.[1]

As the process is not capable of yielding rigidly accurate results, the usual corrections for temperature, pressure, and tension of aqueous vapour may be conveniently omitted. The weight of nitrogen in milligrammes may be found by multiplying the volume of gas (in c.c.) evolved by seven and dividing by six.[2] This rule is based on the fact that a volume of 24 c.c. of moist nitrogen, measured at the ordinary pressure (762 mm. = 30 inches) and temperature (16° C.), corresponds to :—

0·028	gramme of	Nitrogen ;
0·034	,,	Ammonia ;
0·132	,,	Ammonium sulphate ;
0·060	,,	Urea ;
0·084	,,	Uric acid ;
0·164	,,	Albumin or other Proteid ; or
0·153	,,	Gelatin.

ammonium sulphate, and that the reaction is fairly complete unless the dilution is excessive.

[1] If v be the number of cubic centimetres of nitrogen evolved, the weight in milligrammes, W, may be ascertained by the following formula, in which p represents the barometric pressure in millimetres ; w, the tension of aqueous vapour at the temperature at which the gas was measured ; and t, the temperature in centigrade degrees :—

$$W = \frac{v \times (p - w)}{273 + t} \times 1{\cdot}251.$$

[2] The grammes of nitrogen contained in 100 c.c. of urine can be calculated by the following equation, in which G represents the number of c.c. of gas evolved, and U the volume (in c.c.) of the original urine represented by the neutralised liquid used :—

$$N = \frac{G \times 28 \times 100}{U \times 24 \times 1000} = \frac{G \times 7}{U \times 60}.$$

Thus if the gas evolved from a measure of the neutralised liquid corresponding to 5 c.c. of the original urine measured 38·2 c.c., the sample contained 0·891 gramme of nitrogen per 100 c.c.

$$N = \frac{38{\cdot}2 \times 7}{5 \times 60} = \frac{267{\cdot}4}{300} = 0{\cdot}891.$$

This figure, multiplied by 4·375, will give the grains of nitrogen per fluid ounce of the urine ; or, if divided by the specific gravity of the sample (water = 1·000), the actual percentage by weight of nitrogen contained in the urine will be obtained.

Another convenient way of avoiding troublesome calculations is to treat a known weight of pure ammonium sulphate (0·132 or 0·264 gramme) with the hypobromite reagent, and compare the volume of nitrogen gas obtained with that evolved from the substance under experiment.

Optical Determination of Proteids.

When in tolerably concentrated solution, in definite form, and in the absence of other optically active bodies, proteids may be determined by their action on polarised light. All proteids are strongly lævo-rotatory, the activity varying with the individual, but being unaffected by the co-occurring salts. The following are the specific rotations of various proteids for the sodium ray, $[\alpha]_D$, according to the best observations :—

Proteid.	$[\alpha]_D$ =°.	Observer.
Egg-albumin,	−33·5	Hoppe-Seyler.
,,	−38·1	Haas.
Serum-albumin,	−56	Hoppe-Seyler.
,,	−60	Starke.
Serum-globulin,	−59·7	Haas.
Sodium albuminate (alkali-albumin),	−55	Haas.
Casein (in weak $MgSO_4$ solution),	−80	Hoppe-Seyler.
Syntonin (acid-albumin), from egg-albumin,	−55	Haas.
Syntonin (from myosin),	−72	Hoppe-Seyler.
Various albumoses,	−70 to −80	Kühne and Chittenden.
Fibrinogen,	−52·5	Mittelbach.
,,	−43	Hermann.

A method of examining blood-serum by this means has been described by Fredericq (*Comp. rend.*, xciii. 465). The polarimeter may also be employed for determining the albumin of urine. No clarification of the liquid with alumina or basic lead acetate is permissible, but lime-water, sodium carbonate, or acetic acid may be used, with due regard to obvious conditions of acidity or alkalinity. Where the proportion of albumin is small, the polarimetric determination is inapplicable.

Determination of Proteids by Precipitation.

The proteids are precipitated from their solutions by a great variety of reagents, and many of these have been proposed for their detection and determination. By a judicious selection of precipitants, the separation of certain proteids can be effected.

These methods of separation are described on page 17 *et seq.*, while the behaviour of proteids with the various precipitants is detailed in the table on page 49.

The precipitants of proteids may be conveniently divided into those which yield a precipitate which can be directly weighed or measured, and those which give precipitates in which the amount of proteid must be deduced by determining the nitrogen. Even when a precipitant of the first class has been employed, the determination of the contained nitrogen is often preferable to the weighing or measurement of the precipitate. In all cases where it is desired to determine a proteid by weighing a precipitate, it is advisable to ignite the precipitate after weighing, and deduct the weight of the ash from the total weight.

Occasionally it is convenient or desirable to estimate proteids by adding a moderate excess of acetic acid, evaporating the liquid to dryness at 100° C., pulverising the residue, and exhausting it by successive treatments with boiling alcohol, ether, and water. Except traces of casein, all proteid matters will remain insoluble.

When existent in solution, proteids may be determined by one of the following methods, due regard being paid to the presence of other substances liable to interfere with the accuracy of the results.

The liquid is brought into a faintly acid condition by cautious addition of dilute acetic acid or caustic soda, and is then heated and kept in ebullition for a few minutes. Any proteids will be coagulated, and after standing a short time may be filtered off.[1] If the coagulation is not perfect, a *few drops* of nitric or acetic acid may be added to the hot liquid, but excess must be carefully avoided. The precipitate may be collected on a weighed filter, washed, dried at 110° C., and weighed. In accurate estimations it should then be ignited, and the residual ash deducted from the total weight of the precipitate. A preferable plan is to treat the filter with the adhering precipitate by the sulphuric acid process (page 21) and deduce the amount of proteid from the ammonia formed.

A neutral solution of ferric acetate is an excellent precipitant for proteids, and may be successfully used whenever addition of acetic acid and subsequent boiling has failed to effect separation. The reagent is employed in excess, and the liquid rapidly boiled, when all iron and proteid may be filtered off, and the albumin in the precipitate deduced from the nitrogen found by Kjeldahl's process.

[1] The use of a filter-pump or similar contrivance greatly facilitates the filtration and subsequent washing and drying.

Dehmel adds cupric sulphate to the solution of the proteid, exactly neutralises the liquid with soda, and heats to boiling. The precipitation is very complete, and the liquid filters readily. After being washed, the precipitate is treated by Kjeldahl's method.

Ritthausen substitutes freshly precipitated cupric hydroxide[1] for cupric sulphate and soda, and the value of his method has been fully confirmed by other chemists. The faintly acid or exactly neutral liquid is treated with some of the reagent and raised to incipient ebullition (compare Proteids of Milk).

Stützer has confirmed the value of cupric hydroxide as a precipitant for the soluble proteids of vegetables, but points out that the method is apt to give faulty results if the liquid contain much leucine, solanine, tannin, &c., together with much phosphates, as in such cases cupric phosphate is apt to be formed with liberation of alkali, which retains some of the copper-albumin compounds in solution. To avoid loss from this cause he boils 1 gramme of the substance with 100 c.c. of nearly absolute alcohol and 1 c.c. of acetic acid. The liquid is filtered after cooling, and the solid matter washed with alcohol. The residue is then heated with 100 c.c. of water, allowed to cool to 30° or 40°, and the dissolved albumin precipitated by 0·3 to 0·4 gramme of cupric hydroxide. The precipitate is filtered off, washed, and the contained nitrogen determined and calculated to soluble albumin. The insoluble albumin is deduced from the nitrogen contained in the residue insoluble in water.

Hofmeister employs lead hydroxide instead of copper hydroxide, taking care previously to add a little lead acetate in cases where sulphates or chlorides are present.

All these and many similar reagents precipitate proteid bodies very completely, but in some cases they have a tendency to carry down other nitrogenous matters as well. Hence Schultze and Barbieri recommend that trial-estimations should be made by using tannin, cupric hydroxide, ferric acetate, &c., the least amount of proteids obtained being regarded as the correct result; care of course being taken that no proteid substance remains in the filtered

[1] To prepare the reagent, 100 grammes weight of crystallised copper sulphate is dissolved in 5 litres of water containing 2½ c.c. of glycerin per litre, and sufficient caustic soda added to the cold liquid to render it faintly alkaline. The precipitate is thoroughly washed with cold water containing 0·5 c.c. of glycerin per litre, and then mixed with such an amount of water containing 10 per cent. of glycerin that the mixture can be readily sucked up in a pipette. In this condition the reagent can be readily kept without change.

liquid. This can be ascertained with certainty by testing it with acetic acid and potassium ferrocyanide.

A useful method of approximately estimating the coagulable proteids of urine, &c., is to determine the specific gravity of the liquid before and after coagulating the albumin by boiling. A quantity of the urine is filtered and acidulated with acetic acid as already described. The specific gravity is then observed as accurately as possible, the temperature being carefully noted. The liquid is next boiled, passed through a dry filter, and the specific gravity of the filtrate taken after cooling it to the original temperature. It is desirable to reserve a portion of the unboiled urine, and immerse it and the de-albuminised liquid in the same bath of water, so as to ensure exact equality of temperature. The diminution in the specific gravity by boiling (water being taken as 1000), multiplied by 0·4, gives the number of grammes of coagulable proteids per 100 c.c. of urine or other albuminous liquid.[1] (See H. Záhor, *Zeit. physiol. Chem.*, xii. 484; abst. *Jour. Chem. Soc.*, 1888, p. 1227.)

Instead of drying the albuminous precipitate, Bornhardt proposes to wash the coagulated albumin through the filter into a specific gravity bottle, fill up with water, and weigh the bottle in the usual manner. The weight of albumin (x) can be ascertained by the formula :—

$$x = \frac{d \times 1{\cdot}314}{0{\cdot}314};$$

where d is the difference between the weight of the bottle when full of water and when filled with coagulated albumin and water.

For rapid comparative estimations of albumin it is probable that this method would be useful.

PROTEIDS OF EGGS.[2]

According to W. D. Halliburton, the egg of the hen contains, on an average, in 1000 parts :—Shell, 106·9; white, 604·2; and, yolk, 288·9.

Egg shells consist of a keratinoid substance infiltrated with calcium carbonate and traces of calcium phosphate and magnesium carbonate.

[1] Thus if the original urine had a density of 1026, which was reduced by boiling to 1020, the difference, 6, multiplied by 0·4, equals 2·4 grammes of albumin per 100 c.c. of the sample.

[2] The author is indebted to Dr W. J. Sykes for perusal and criticism of this section.

White of Egg.

The white of eggs consists of a semi-fluid material of alkaline reaction, contained in a net-work of firmer fibrous substance, which latter is insoluble in hot or cold water, dilute acetic acid, or solution of common salt. According to Lehmann, the interstitial semi-fluid substance, or white of egg proper, contains from 82 to 88 per cent. of water and an average of 13·3 per cent. of solids. Of this, 12·2 per cent. consists of proteids, 0·5 per cent. of glucose, 0·66 of ash, with traces of cholesterin, lecithin, fat, alkaline soaps, &c.[1] Hence white of egg is a nearly pure solution of proteids, the principal of which is that commonly called egg-albumin.[2] A variety of globulin is also present in small quantity and may be separated from the albumin by treating the solution with dilute acetic acid or carbon dioxide, or by saturating it with common salt or magnesium sulphate (compare page 40). Peptones and albumoses appear to be absent from fresh eggs, but make their appearance in increasing amount as the egg becomes stale. According to E. Salkowski (abst. *Jour. Chem. Soc.*, 1894, i. 214), if a solution of hen's egg albumin be carefully neutralised by dilute acetic acid, and the liquid precipitated by boiling, a hitherto unobserved albumose is found in the filtrate, and may be precipitated by concentrating the liquid and adding absolute alcohol.

By saturating solutions of white of egg with salts, and applying heat-coagulation fractionally, Corin and Berand (*Arch. de Biologie*, ix. i.) obtained *α-oviglobulin* precipitated at 57·5° C.; *β-oviglobulin*, precipitated at 67°; and *ovalbumins* α, β, and γ, precipitated respectively at 72°, 76°, and 82° C.

According to Béchamp, the white of hen's egg contains three varieties of proteid, called by him *primovalbumin*, *secondovalbumin*, and *leucozymase*. These bodies are present in the proportions 5 : 4 : 1 respectively, and have specific rotations of −34°, −53°, and −78° for the transition-tint. These figures agree fairly with

[1] Poleck and Weber (*Poggendorff's Annalen*, lxxix. 155; lxxxi. 91) found the ash of white of egg to contain :—K_2O, 27 to 28 per cent.; Na_2O, 23 to 32; CaO, 1·7 to 2·9; MgO, 1·6 to 3·7; Fe_2O_3, 0·4 to 0·5; Cl, 25 to 28; P_2O_5, 3·7 to 4·8; SO_3, 1·3 to 2·6; SiO_2, 0·2 to 2·0; and CO_2, 7 to 9 per cent. Nicklés found a trace of fluorine.

[2] The term "albumen" should be limited to its original signification, namely, the white of egg; the word "albumin" being applied to the most characteristic constituent thereof, and extended to other analogous substances contained in blood-serum, &c. The terms albumen and albumin will then have the same relation to each other as benzol and benzene. In pronouncing the word albumin, it is correct to accentuate the penultimate, but of late years extensive custom has justified the accentuation of the first syllable.

the rotation of $-40°$ to $-43°$ observed for the mixed solids of white of egg.

White of egg, when evaporated to dryness at 60° C., yields from 12 to 13 per cent. of solid albuminous residue, having a density of about 1·314 and losing 4 per cent. of water on further heating to 140°, without thereby becoming insoluble. The residue yields about 7 per cent. of ash on ignition, consisting chiefly of sodium chloride and carbonate, with calcium phosphate.

Egg-albumin.—When white of egg is beaten up thoroughly with water, the albumin and salts pass into solution, while the insoluble membranous matter may be strained off. The albumin may be partially separated from the soluble salts by dialysis,[1] or by precipitating the liquid with basic lead acetate, decomposing the precipitate by carbonic acid, and removing the last traces of lead by sulphuretted hydrogen. On very cautiously warming the liquid to 60° C. incipient coagulation occurs, and the first flakes of albumin carry down with them every trace of lead sulphide, leaving the liquid perfectly colourless. On evaporating the solution at a temperature below 40° C., and completing the desiccation in shallow trays, the albumin is obtained in the form of transparent, pale yellow, horny scales, which may be reduced to a yellowish-white powder. In the solid state it may be kept without change, but the solution readily putrefies.

Albumin so obtained has a specific gravity of 1·262, and is tasteless, odourless, and neutral in reaction. It dissolves slowly in pure water, but more readily in presence of a little neutral salt or a trace of free alkali. The solution is glairy.

The albumin of blood-serum closely resembles egg-albumin, but the two bodies are not identical. The differences between them are detailed on page 47.

The coagulation of albumin is fully described on page 14.

If very dilute and added in small quantity only, the majority of mineral acids do not precipitate cold albumin solutions; but larger proportions of acid precipitate the albumin completely. Nitric acid acts most strongly, while the precipitate produced by hydrochloric acid is soluble in excess, and on diluting the resultant solution with water a precipitate is produced which, when separated and freed from the mother-liquor, dissolves in water and exhibits the reactions of acid-albumin (page 4).

[1] Albumin obtained by dialysing white of egg or blood-serum always retains about 1 per cent. of mineral matter. An ash-free product has been obtained from white of egg by Hofmeister, but from its method of preparation it cannot be regarded as unchanged albumin (see E. Harnack, *Ber.*, xxiii. 3745).

Cold solutions of egg-albumin are not precipitated under ordinary circumstances by carbonic, acetic, tartaric, or orthophosphoric acid, but in presence of a certain proportion of chloride of sodium, or other neutral salt, precipitation ensues. Hence common salt will precipitate a solution of albumin in acetic acid, the precipitate being soluble in pure water if heating has been avoided.

According to F. Blenn (*Zeit. Anal. Chem.*, 1896, xxii. 127; abst. *Jour. Chem. Soc.*, 1896, i. 658), if white of egg be diluted with water, the precipitated globulins filtered off, and a little formaldehyde added to the filtrate, the albumin will be found to have lost its power of coagulating, and to have undergone conversion into a substance exhibiting reactions distinct from those of any known proteid.

Yolk of Egg.

The solids of egg-yolk consist chiefly of a mixture of proteids, together with a considerable proportion of fat. Other constituents are small quantities of colouring matter, glucose, cholesterin, and a very considerable proportion of lecithin (Vol. III. Part iii.). According to Gobley, yolk of egg has the following composition:—

	per cent.		per cent.
Vitellin, . . .	15·8	Cholesterin, ...	0·4
Nuclein, . . .	1·5	Fats,[1]	20·3
Cerebrin, . . .	0·3	Colouring matters, . .	0·5
Lecithin, . . .	7·2	Salts, ...	1·0
Glycerol-phosphoric acid,	1·2	Water, ...	51·8

[1] Paladino and Toso (abst. *Analyst*, 1896, page 161) state that egg-fat is used in ointments. They find an iodine-number of 81·2 to 81·6. The KHO required for saponification is 18·6. Crystals of cholesterin often separate from the fat.

E. Spaeth (abst. *Analyst*, 1896, page 233) gives the following as the analytical characters of the fat of egg-yolk:—

Specific gravity at 100° C. (water at 15° being 1), .	0·881
Iodine-number of fat, ...	68·48
Reichert-Meissl value, ...	0·66
Refractive-index at 25° C. (on Zeiss' scale), . .	68·5
Melting-point of fatty acids, ...	36° C.
Iodine-number of fatty acids, ...	72·6

Spaeth proposes to employ these data for ascertaining whether pastry and other flour-products have been coloured by yolk of egg, or by saffron, picric acid, &c. It would be a simpler and more certain plan to examine the material directly for such colouring matters.

Of these constituents, the fats, cholesterin and glycerol-phosphoric acid are extracted by ether.

F. Jean (*Monit. Scient.*, 1892, p. 561) gives the following as the average composition of three specimens of egg-yolk :—

Water (loss at 110°), ...	52·6
Fatty matter (extract by petroleum-spirit), .	28·0
Vitellin (including aqueous extract), . .	18·0
Ash, ...	1·4

The predominating proteid of egg-yolk is a globulin called vitellin. Albumin is also present in small quantities, together with nuclein, with which last body the iron of egg-yolk is in combination. The proteids found by Valenciennes and Fremy in the yolk of the eggs of fishes, and called by them ichthin, ichthulin, and emydin, probably consisted of mixtures of vitellin with nuclein and lecithin.

Vitellin is insoluble in water, and is obtained as a white granular residue on extracting egg-yolk with large quantities of ether. It closely resembles myosin, the globulin of muscle, and may be purified by similar means. It differs, however, from other globulins in being soluble in a saturated solution of common salt. Vitellin may be purified by repeatedly dissolving it in a 10 per cent. solution of common salt and precipitating by excess of water. The neutral solution of vitellin in very dilute brine coagulates when heated to 70 to 75° C. Vitellin appears to exist in egg-yolk in combination or intimate association with the phosphorised bodies lecithin and nuclein.

Nuclein closely resembles mucin (p. 64) in its physical characters, but contains a notable proportion (1·89 to 2·28 per cent.) of phosphorus, and no sulphur. It is probable that numerous varieties of nuclein exist, all being compounds of simple proteids with nucleic acid.

Plant-vitellin, extracted by dilute solution of common salt from the seeds of oats, maize, peas, white mustard, &c., agrees in all its characters with egg-vitellin.

Analysis of Commercial Yolk of Egg.—Salted yolks of eggs, either alone or mixed with borax, are largely employed for dressing hides in the tawing process.

For the analysis of such products, F. Jean (*Monit. Scient.*, 1892, p. 561; abst. *Jour. Soc. Chem. Ind.*, 1892, p. 941) treats 10 grammes of the sample with a few drops of acetic acid, and evaporates the mixture slowly, with occasional stirring, at a temperature of 50° to 60° C. The drying is completed at 110°, and the residue weighed as *dry extract*, the loss being taken as *water*. The solids

are powdered and extracted in a Soxhlet-tube with hot petroleum-spirit, the solution being evaporated and the residual fatty matter weighed. The residue insoluble in petroleum-spirit is freed from the solvent by a current of air and then extracted with boiling distilled water, and the solution thus obtained evaporated. The total ash may be determined in the usual manner, but in presence of borax it is difficult to avoid loss of chlorine. Hence, for the determination of the chlorides Jean recommends that the aqueous extract of the sample should be treated with tannin, and an aliquot part of the filtered liquid concentrated, acidified with nitric acid, and precipitated with silver nitrate. In another portion of the clarified aqueous extract the sulphates, borates, &c., may be determined. Jean gives the following examples of analyses made by the above method :—

Analytical Data.	A.	B.	C.	D.	E.
Water, ...	58·54	48·910	52·694	46·60	50·76
Ash, ...	18·50	17·468	18·740	16·91	15·13
Oil petroleum extract), . .	14·23	18·840	15·550	18·52	19·78
Vitellin (insoluble), . . .	14·23	13·840	11·460	13·78	12·87
Aqueous extract, ...	10·34	0·942	1·556	1·24	1·46
	100·00	100·000	100·000	100·00	100·00

Saline Matters.	A.	B.	C.	D.	E.
Normal ash, ...	1·10	1·112	1·07	1·112	1·112
Sodium chloride, ...	16·71	14·850	17·80	14·420	13·080
Boric acid, &c., ...	0·78	1·500	17·80	1·478	0·938

Jean calculates the proportion of *pure yolk* in the samples from the fat, on the assumption that the normal proportion of this is constant at 28 per cent. The *added salts* are deduced from the excess over the normal ash corresponding to the pure yolk present. The *added water* is the excess over the quantity corresponding to the fat (that is, $\frac{52·6}{28}$ of the petroleum-spirit extract). The "*albumin in excess*" is the difference between the "vitellin" found and that corresponding to the fat present, on the assumption that the ratio between them is constant. In this manner, Jean deduced the following as the composition of the samples of egg-yolk in question.

	A.	B.	C.	D.	E.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Pure yolk, ...	50·80	67·000	55·00	65·00	70·00
Albumin in excess, . . .	1·19	0·890	3·01	3·13	1·63
Added salts, ...	17·40	14·782	18·74	15·80	14·01
Added water, ...	30·52	17·328	23·25	15·98	13·76

In the opinion of the author, the results of Jean can only be regarded as rough approximations.

PROTEIDS OF BLOOD-PLASMA.

The blood of mammalia is a viscous liquid of alkaline reaction and yellowish colour, having the blood-corpuscles in suspension. By suitable means, the coagulation which generally ensues very shortly after the blood is withdrawn from a living animal may be delayed, so as to allow a separation of the liquid or plasma from the blood-corpuscles. This is best effected by the addition of a suitable quantity of a neutral salt, such as sodium chloride or magnesium sulphate. Thus, if recently-drawn blood be mixed with an equal measure of a 10 per cent. solution of common salt, or with one-fourth of its measure of a saturated solution of magnesium sulphate, coagulation is delayed indefinitely, and the liquid may be filtered from the suspended corpuscles. The filtrate or "salted plasma," when diluted with a considerable quantity of water, gives a coagulum of fibrin when treated with fibrin-ferment.

In 1000 parts of blood, about 60 to 65 parts consist of plasma, and the remainder of corpuscles. Like the blood itself, the plasma readily coagulates, giving a clot consisting essentially of fibrin, and a yellowish liquid called *serum*, which contains albumin and globulin.

According to Gamgee, 1000 parts of *plasma* contain :—Water, 902·90 ; fibrinogen, 4·05 ; other proteids (albumin and globulin), 78·84 ; extractives, including fat, 5·66 ; and inorganic salts, 8·55 per cent. Hence the plasma contains approximately 10 per cent. of solid matter, of which about 8 per cent. is of proteid nature.

Fibrinogen is the proteid to which the coagulation of blood is due. For its preparation, Hammarsten recommends that blood-plasma or lymph should be mixed with an equal measure of a saturated solution of common salt, which completely precipitates

fibrinogen, and allows of its separation from the accompanying globulin and albumin. The precipitate is washed rapidly with half-saturated brine, dissolved in a 6 to 8 per cent. solution of common salt, and the fibrinogen again precipitated by adding an equal measure of saturated brine. The precipitate finally obtained dissolves on addition of water, owing to the adhering salt.

Thus obtained, fibrinogen has the general properties of a globulin. Thus it is insoluble in pure water, but soluble in water containing oxygen, and in dilute solutions of neutral salts, but is precipitated from such solutions either by increasing the concentration beyond a certain point (as practised in the method of its preparation), or by removing the salt by dialysis.

The coagulation of fibrinogen has been proved to be due to an unorganised proteid ferment. In the presence of minute quantities of neutral salts, especially sodium chloride and calcium sulphate, this fibrin-ferment[1] coagulates fibrinogen to insoluble fibrin, but in the absence of the ferment no such change occurs.

In the spontaneous coagulation of blood, the clot consists of red and white corpuscles entangled in the coagulated fibrin; but by appropriate means (see next page) the clot may be obtained nearly free from corpuscles. The coagulation of blood occurs very readily at the temperature of the blood, but more slowly at a moderate heat, and not at all at very low temperatures. Carbonic acid retards or prevents coagulation, while the presence of air facilitates it, as does any kind of agitation. Free phosphoric acid, lactic acid, or acetic acid prevents coagulation, as do also caustic and carbonated alkalies.

Fibrinogen is coagulated by exposing its solution to the comparatively low temperature of 56° C., the clot formed possessing a peculiar sticky character. Both by heat- and by ferment-coagulation of fibrinogen there is formed a small quantity of a globulin coagulating at 65°.

Fibrinogen is not precipitated by carbon dioxide gas except from very dilute solutions. Thus if blood-plasma be diluted with fifteen measures of water, and the liquid saturated with carbon dioxide, only serum-globulin is precipitated, but if the filtered liquid be then further diluted, and carbon dioxide again passed,

[1] *Fibrin-ferment*, first discovered by A. Schmidt, may be prepared by mixing blood-serum with ten to fifteen measures of absolute alcohol, and leaving the liquid at rest for six or eight weeks. The precipitate of proteids is then filtered off, washed with absolute alcohol, dried over sulphuric acid, and powdered. A filtered cold-water extract of the product will contain the ferment, and will cause the coagulation of fibrinogen existing in blood or blood-plasma, lymph, pericardial fluid, &c. A similar coagulation is occasioned by the myosinogen of muscle, by yeast, and by pieces of many fresh tissues.

the fibrinogen is precipitated, though not very perfectly. This behaviour, the low coagulating power, the action of fibrin-ferment, and its non-precipitability by half-saturated brine, allow of the separation of fibrinogen from blood-globulin and albumin.

The specific rotation of fibrinogen is stated by Hermann to be $-43°$ for the sodium ray, but the opalescent character of its solutions renders the observation difficult and uncertain.

Fibrin may be obtained by allowing blood-plasma to coagulate, or less abundantly by the coagulation of lymph or by the addition of fibrin-ferment to other solution of fibrinogen.

In a less pure state, fibrin may be obtained by stirring fresh blood with a bundle of twigs, when the coagulum adheres to the twigs, and may be stripped off and washed till colourless, and then treated in succession with alcohol and ether to remove fat and cholesterin.

Fibrin differs from all other proteids in possessing a filamentous structure and remarkable elasticity. It is insoluble in water at ordinary temperatures, but passes into solution under high pressure, with total change of characters.

Suspended in water and heated to 70°, fibrin shrinks, loses its elasticity, and yields a body similar to coagulated albumin.

When treated with very dilute hydrochloric acid (0·2 per cent.) in the cold, fibrin does not dissolve, but swells up into a transparent mass, resuming its original appearance on neutralising the acid. At 50° to 60° it dissolves in the acid, forming acid-albumin and albumoses. With dilute alkalies, fibrin swells less than with acids, but dissolves more readily. The swollen fibrin, washed free from alkali, is coagulated by heat.

A solution of hydrogen peroxide is decomposed by contact with fibrin with evolution of oxygen.

When treated with a 5 to 10 per cent. solution of nitre or common salt at about 40° C. fibrin swells up and gradually dissolves to a solution which is precipitated by acetic acid and coagulated by heat, and contains a body of the globulin class.

Fibrin is readily acted on and dissolved by digestive ferments. According to Halliburton, an acid solution of pepsin, or an alkaline solution of trypsin, first produces two globulins, coagulating respectively at 56° and 75° C., while by further action albumoses and peptones are formed.

For the determination of fibrin in blood, a known quantity of the sample should be vigorously whipped with a bundle of twigs or fine glass rods, the coagulum washed thoroughly with water, and then with alcohol (and ether), dried at 100° C., and weighed. It is then ignited, and the weight of the ash deducted. Instead

of weighing the clot, it may be conveniently treated by one of the modifications of Kjeldahl's process (page 21). Fibrin contains 16·91 per cent. of nitrogen.

In liquids like the pericardial fluid, which do not spontaneously coagulate, the fibrin must be precipitated by adding a small quantity of blood-serum, or a solution of fibrin-ferment.

Serum-Globulin.—On diluting blood-serum with about twenty measures of water, a slight precipitation of the contained globulin occurs, since that proteid is not soluble in saline liquids below a certain concentration.[1] If carbon dioxide be passed through the diluted serum a further precipitation of globulin occurs, but the precipitation is always far from complete. A slight further precipitation occurs on then adding a few drops of very dilute acetic acid.

Complete precipitation of the globulin can be effected by saturating the serum with magnesium sulphate, which has the advantage over other salts of leaving the accompanying serum-albumin wholly in solution. Addition of an equal measure of a saturated solution of ammonium sulphate is stated to effect an equally sharp separation. The saturation with magnesium sulphate is best effected by treating the serum with powdered crystals of the salt, at the ordinary temperature, with frequent agitation, during several hours. The precipitate is washed with a saturated solution of magnesium sulphate, and then treated on the filter with cold water, in which it dissolves, but is again thrown down on saturating the liquid with magnesium sulphate. This purified precipitate may be collected on a weighed filter and dried at 120° C. for some hours. This treatment renders the globulin insoluble, and the salt may then be washed away, and the filter with the adhering proteid redried and weighed. A simpler and preferable plan is to treat the unwashed and moist precipitate by one of the modifications of Kjeldahl's process (page 21). Serum-globulin contains on the average 15·85 per cent. of nitrogen. Neutral solutions of serum-globulin in dilute saline liquids coagulate at about 75° C., and the specific rotation is stated by Haas to be −59·75° for the sodium ray.

In solutions containing not less than 1 per cent. of serum-globulin, the proteid may be detected by pouring the liquid on to a saturated solution of magnesium sulphate, when a ring of precipitate will be formed at the junction of the two liquids.

The detection of serum-globulin in morbid urine is described on page 60.

[1] A similar precipitation of globulin occurs on removing the salts by dialysis.

Hammarsten recognises three varieties of serum-globulin, namely :—*Plasma-globulin*, pre-existent in the blood-plasma ; *cell-globulin*, resulting from the disintegration of the white corpuscles, and a *globulin* arising from the splitting of the fibrinogen molecule.

The globulin of serous exudations appears to be purely plasma-globulin. The so-called *para-albumin* of ovarian tumours is simply serum-globulin. *Myosinogen* is a globulin contained in muscle. Its coagulation, with conversion into insoluble myosin by the action of the muscle-ferment, is the cause of the change after death known as *rigor mortis*. Myosinogen may be obtained pure by soaking lean meat in cold water to remove the soluble matters, treating the residue with a 10 per cent. solution of common salt, diluting the resultant solution with much water, redissolving the precipitate in salt solution, and reprecipitating the myosinogen by copious dilution.

Serum-albumin. Seralbumin. This modification of albumin occurs normally in the serum of blood and in muscles, and in lymph and chyle. It exists to a limited extent in normal milk, and more abundantly in colostrum.[1] Serum-albumin also occurs, together with globulin, in albuminous urine.

Serum-albumin may be obtained pure by saturating blood-serum with magnesium sulphate as already described (page 40). The precipitate of globulin is filtered off, and the filtrate saturated with sodium sulphate. This latter salt does not alone precipitate serum-albumin, but the double sulphate of sodium and magnesium formed in the liquid throws it down very completely. On filtering off the precipitate and treating it with water, the albumin dissolves, and by subjecting the solution to dialysis the salts may be removed and the albumin obtained pure. It is, however, impracticable to obtain a product with less than 0·3 to 0·5 per cent. of ash.

When it is merely desired to determine the amount of albumin in the filtrate from the globulin precipitate, and not to isolate it, the solution may be rendered slightly acid (see page 29) and boiled, the coagulated albumin being filtered off, and weighed or treated by Kjeldahl's process.

From the experiments of Kauder, it appears that more than one modification of albumin exists in blood-serum. This conclusion is indicated by the results of fractional coagulation by heat, whereby products of somewhat different elementary composition were obtained. Halliburton has confirmed this view, and gives the following table (*Chemical Physiology and Pathology*), showing

[1] It is not certain that the albumin of milk is identical with that of blood-serum.

the temperatures of coagulation of the albumin in the blood-serum of different animals :—

Blood of	Temperature of Heat-Coagulation.				
	Fibrinogen.	Serum-Globulin.	Serum-Albumin.		
			α.	β.	γ.
Man,	56° C.	75° C.	73° C.	77° C.	85° C.
Monkey,	56°	75°	72°	77°	83°
Dog,	56°	75°	73°	78°	84°
Cat,	56°	75°	73°	77°	84°
Rabbit,	56°	75°	73°	77°	84°
Pig,	56°	75°	72°	77°	84°
Horse,	56°	75°	...	77°	84°
Ox,	56°	75°	...	77°	84°
Sheep,	56°	75°	...	77°	84°
Hen,	56°	75°	72-3°	78°	86°
Dove,	56°	75°	73°	77°	85°
Newt,	56°	75°	73°	...	...
Toad,	56°	75°	75°	...	...
Frog,	56°	75°	73°	...	...
Lizard,	56°	75°	74°	...	...
Perch,	56°	75°	73°	...	...
Roach,	56°	75°	73°	...	...

It will be observed that the coagulation-temperatures of the fibrinogen and serum-globulin are constant for all animals of which the blood was examined. In the blood of most mammals and birds the albumin could be differentiated into three varieties, distinguished by the temperature of their coagulation, but in the blood of certain herbivora only two of these varieties could be detected. In the blood of reptiles and fishes only one variety of serum-albumin appears to exist.

The homogeneous nature ascribed by Halliburton to the serum-albumin of cold-blooded animals, as compared with the complex nature of that of the higher animals, is a strong argument in favour of the actual co-existence of several modifications in the latter case ; since the alteration of the dilution and acidity of the liquid, to which Haycroft and Duggan (see page 15) ascribe the partial precipitation observed by Halliburton, would affect the serum of all animals alike.

The following table, also taken from Halliburton's *Chemical Physiology and Pathology*, shows the percentage amounts of proteids present in the blood-serum of various animals.

The total proteids were determined by weighing the precipitate formed when alcohol was added to the serum. The globulin was estimated by Hammarsten's method, the difference between the two results giving the amount of albumin. It will be observed that the proportions of total proteids are generally smaller in the case of cold-blooded animals than in the serum of mammals, the difference being chiefly due to the smaller quantity of albumin.

Animal.	Proteids in the Blood-Serum.			Observer.
	Total Proteids Per Cent.	Serum-Globulin.	Serum-Albumin.	
Man, . .	7·62	3·10	4·52	Hammarsten.
Horse, .	7·25	4·56	2·67	Hammarsten.
Ox, . .	7·50	4·17	3·33	Hammarsten.
Rabbit, .	7·52	1·78	4·43	Hammarsten.
Pigeon, .	5·01	1·32	3·69	Halliburton.
Hen, . .	4·14	2·90	1·24	Halliburton.
Tortoise, .	4·76	2·82	1·94	Halliburton.
Lizard, .	5·16	3·33	1·83	Halliburton.
Terrapin, .	5·25	4·66	0·69	Howells.
Snake, .	5·32	4·95	0·37	Wolfenden.
Frog, . .	2·54	2·18	0·36	May.
Newt, . .	3·74	3·31	0·43	Halliburton.
Eel, . .	6·73	5·28	1·45	Halliburton.
Dogfish, .	1·62	1·17	0·45	Halliburton.

The proportion of fibrinogen in blood-plasma averages 0·25, and appears rarely to exceed 0·4 per cent. Hence it bears but a small proportion to the other proteids.

No albumoses or peptones can be detected in normal blood under any circumstances.

Hæmoglobin, the red colouring matter of the blood-corpuscles, is described in the sequel.

The following is an outline-method for the proximate analysis of *blood*.

<table>
<tr><td colspan="5">Treat the freshly-drawn blood with an equal measure of a 10 per cent. solution of common salt and filter.</td></tr>
<tr><td rowspan="5">RESIDUE contains red and white corpuscles, blood crystals, &c.</td><td colspan="4">FILTRATE. Add an equal measure, or rather less, of saturated brine, and filter.</td></tr>
<tr><td rowspan="4">PRECIPITATE consists of fibrinogen.</td><td colspan="3">FILTRATE. Saturate with powdered magnesium sulphate and filter.</td></tr>
<tr><td rowspan="3">PRECIPITATE consists of serum-globulin.</td><td colspan="2">FILTRATE. Heat to 73° C. and filter.</td></tr>
<tr><td rowspan="2">PRECIPITATE consists of serum-albumin α.</td><td>FILTRATE. Heat to 77° and filter.</td></tr>
<tr><td>PRECIPITATE consists of serum-albumin β.</td><td>FILTRATE contains serum-albumin γ, precipitated on heating to 84° C.</td></tr>
</table>

The following method, taken, with a few minor alterations, from Halliburton's *Chemical Physiology and Pathology*, may be employed for the separation of the proteids of *plasma*:—

<table>
<tr><td colspan="3">Saturate the plasma or other fluid with powdered ammonium sulphate and filter.</td></tr>
<tr><td colspan="2">PRECIPITATE consists of proteids. Wash with a saturated solution of ammonium sulphate. Redissolve the precipitate on the filter by the addition of water and saturate the solution with solid magnesium sulphate. Filter.</td><td rowspan="3">FILTRATE contains the other constituents of plasma, e.g., lecithin, cholesterin, saponified fats, urea, uric acid, sugar, salts &c.</td></tr>
<tr><td colspan="2">PRECIPITATE consists of fibrinogen and serum-globulin. Wash with a saturated solution of magnesium sulphate. Then add water, when the proteids redissolve owing to the presence of the adherent salt. Heat this solution to 56° C. The fibrinogen is precipitated as a heat-coagulum at 56°, the serum-globulin remaining in solution. Or add an equal volume of a saturated solution of NaCl and filter.</td></tr>
<tr><td>PRECIPITATE consists of fibrinogen.</td><td>FILTRATE contains serum-globulin.</td></tr>
<tr><td colspan="2"></td><td>FILTRATE contains albumins, which may be precipitated and separated by fractional heat-coagulation. Or saturate the solution with sodium sulphate. A precipitate is produced, which consists of albumins, and this may then be redissolved in water and separated by heat-coagulation.</td></tr>
</table>

Instead of weighing the precipitated proteids, it is usually more convenient to treat them by one of the modifications of Kjeldahl's process.

COMMERCIAL ALBUMIN.

Albumin is now very largely employed for various purposes. Its chief application is for fixing dyes and pigments in printing calico. It is also used in photography, confectionery, and pharmacy. The employment of albumin or blood-serum for refining sugar is obsolete. The alkaline earths (*e.g.*, slaked lime) form with albumin solution a useful cement, which, when dry, sets as hard as stone.

Commercial albumin is obtained chiefly from two sources, eggs and the serum of blood.[1] Serum-albumin may be employed for printing all but the very finest and brightest colours. It is cheaper

[1] J. Hofmeier (*Jour. Soc. Chem. Ind.*, 1883, p. 174) has described a process which is essentially a manufacture of a soluble albumin from refuse protein matters, such as meat, meat powder, meat-extract residues, fresh or dried blood-fibrin, casein, and vegetable albuminous matters. The proteid is brought into solution by dilute acid and heat, or by prolonged boiling with dilute caustic soda. If acid has been employed, the solution is subsequently neutralised sufficiently to prevent precipitation. The product can be evaporated at 35° to a hard residue, which dissolves slowly in water like ordinary serum-albumin, and the solution, with proper additions, may be employed in the dye-bath in a similar manner. By heat or steaming the albumin becomes coagulated in the ordinary way. By using casein or vegetable proteid as the source of the albumin, products can be obtained nearly as light in colour as egg-albumin, and not inferior to it in fixing power.

than egg-albumin, and has a greater thickening power. For photographic purposes egg-albumin is preferable (see below).

Fish-albumin is met with occasionally, and may be recognised by its fishy odour.

Blood- or Serum-albumin is obtained by separating the serum from the clot of perfectly fresh blood.[1] The liquid, which contains from 7 to 8 per cent. of proteids, is evaporated in shallow trays at a temperature not exceeding 50° C., when the albumin is obtained in brittle scales or transparent flakes of a greyish, yellowish, reddish, brown, or black colour. The qualities of serum-albumin made by leading firms are "refined," "prime," "No. 1," "No. 2," and "black." "Refined albumin" is made from highly rectified serum, and is of a dirty yellow colour, and, like "prime," is employed for printing delicate colours. No. 1 is darker in colour and less valued, though suitable for all ordinary printing purposes. No. 2 quality is made from the second draining of the serum from the blood, which, after the clear top serum has been syphoned off, is more or less tinged with red, and consequently only fit for printing dark colours; as a rule, it also contains some insoluble matter, which is objectionable. "Black albumin," or "dried blood," is obtained from the last portions of serum, and is almost black in colour. It is not used in calico-printing, but finds applications in sugar-refineries and in turkey-red dyeing.

Egg-albumin is obtained in a solid state by cautiously evaporating the white of eggs at a temperature below 50° C. It is generally transparent, and of a light yellow colour. It should be free from blisters, which are often present in partially coagulated samples. Egg-albumin is more valuable than serum-albumin, and consequently is more liable to adulteration. Two genuine qualities are recognised in commerce.

Albumin is also sold in solution, which should have a specific gravity of about 1·102. The solution frequently contains about 2 per cent. of zinc salt, added as a thickener and preservative.

Commercial albumin of good quality is transparent in thin flakes, and free from unpleasant taste or odour of putrefaction. On treatment with cold water, with constant stirring, it should dissolve readily.[2]

[1] If not perfectly fresh the blood cannot be used for the preparation of albumin. The clot, consisting chiefly of fibrin and blood-corpuscles, is dried, roasted, and used as manure.

[2] For practical purposes, the albumin is best dissolved in warm water, of a *maximum* temperature of 45° to 50° C.; the albumin should be added gradually, and the liquid constantly stirred. The water should on no account be added to the albumin. The liquid, after straining through a fine silk sieve, is

Commercial albumin is often adulterated with gum, dextrin, sugar, flour, &c. For its examination, 5 grammes of the powdered sample should be treated with 50 c.c. of cold water, with frequent stirring, until all soluble matter is dissolved. Pure and good samples leave no residue. A few drops of acetic acid should be added, and any undissolved matter filtered off through silk or fine muslin. It may consist of coagulated albumin, casein, starch, or membranous matter. The *casein* may be dissolved out by treatment with very dilute caustic soda, and precipitated by exactly neutralising the solution with acetic acid. The aqueous solution of the sample is boiled, when the *albumin* is thrown down as a flocculent precipitate, which may be filtered off, washed, and weighed; or treated by Kjeldahl's process, and the albumin deduced from the ammonia obtained (p. 21). The filtrate should be treated with acetic acid and potassium ferrocyanide to make sure that no proteid remains in solution.[1] Its absence being proved, tannin may be added to precipitate *gelatin*, the liquid filtered, and the filtrate concentrated to a small bulk and treated with alcohol to precipitate any *gum* or *dextrin*. *Sugar*, if present, will remain in solution in the alcoholic liquid, and may be detected by boiling off the alcohol, heating with hydrochloric acid, and testing the liquid by Fehling's solution. Sugar might also be extracted by treating the original solid sample with alcohol.

Ziegler's method of assaying commercial albumin is to dissolve 20 grammes of the sample in 100 c.c. of cold water, strain through a sieve, and add 10 c.c. of the clarified liquid to a boiling 20 per cent. solution of alum. After noting the appearance and volume of the coagulum, it is washed, dried, and weighed. De Koninck (*Jour. Chem. Soc.*, xxv. 1129) finds that the process gives a precipitate containing not more than one per cent. of alumina, and that it is sufficiently accurate for the purposes of the calico-printer. With pure albumin very good results are obtainable, and their accuracy is not affected by the presence of dextrin, but gum arabic prevents the precipitation of albumin to a very notable extent.

The proportion of *ash* left on the ignition of commercial albumin cannot be readily ascertained by direct treatment of the sample, owing to the fusible nature of the sodium carbonate

usually mixed with a small proportion of ammonia, turpentine oil, &c., in order to prevent frothing and make it work smoothly. Turpentine also tends to prevent putrefaction, but an addition of about 1 per cent. of arsenious oxide is the best preservative.

[1] Any organic precipitate here produced will probably consist of casein. Zinc, if present, will be thrown down at this stage as a white ferrocyanide.

and other salts of which the ash is mainly composed. The author finds that the difficulty may be obviated by treating a weighed quantity of the sample in a porcelain crucible with nitric acid of 1·42 specific gravity and two or three drops of strong sulphuric acid. On heating gently, the albumin dissolves to a clear yellow liquid, which may be evaporated to dryness without trouble, giving a residue which readily burns and leaves an ash of tolerably high melting point. Operating in this manner, samples of commercial albumin examined in the author's laboratory gave the following percentages of "sulphated ash."

	Sulphated Ash.
Egg-albumin, No. 1 . . .	7·4 per cent.
„ No. 2 . . .	7·0 „
Blood-albumin, Refined . . .	9·1 „
„ Prime . . .	8·5 „
„ No. 1 . . .	9·2 „
„ No. 2 . . .	8·9 „
„ No. 3 . . .	9·7 „
„ Black . . .	6·2 „

In each case the ash was white, except that yielded by the black albumin. This gave a reddish ash, owing to the presence of blood colouring matter in the original sample. In this, the lowest grade of genuine albumin, the ash was less than in the better kinds.

Any admixture of sugar or dextrin would tend to reduce the proportion of ash yielded by a sample of albumin. *Zinc* can be readily detected and determined by dissolving the sulphated ash in dilute hydrochloric acid, adding ammonia, filtering from any precipitate, raising the filtrate to the boiling point, and adding potassium ferrocyanide. A white precipitate will be produced in presence of zinc. If zinc be found, it is best determined by acidulating the solution of the ash with acetic acid, and precipitating the metal by sulphuretted hydrogen.

Serum-albumin, being less costly than egg-albumin, is apt to be substituted for the latter without acknowledgment. The two kinds present the following points of difference :—

1. A solution of serum-albumin containing the ordinary salts is unaffected by agitation with ether, but white of egg gradually coagulates.[1] If the solution be dilute, the precipitate appears at the junction of the ethereal and aqueous layers.

2. Serum-albumin dissolves easily in strong nitric acid, but egg-albumin is only difficultly soluble.

[1] In the absence of salts, the behaviour of egg- and serum-albumin with ether is stated to be exactly reversed, the former being unaffected and the latter precipitated.

3. The specific rotation of serum-albumin for the sodium-ray is between $-55{\cdot}7^\circ$ and -62°; whereas egg-albumin has a rotation ranging from $-35{\cdot}5^\circ$ to -38°. The specific rotation of the albumins is not affected by the presence of neutral salts in any proportion. Hot strong acetic acid raises the rotation of serum-albumin to -71°. The difference between the optical activity of serum- and egg-albumin might be employed for determining their relative proportions in a mixture of the two.

4. According to V. Gauthier, if a solution containing 2 grammes of egg-albumin be treated with 10 c.c. of a reagent made by mixing 250 c.c. of caustic soda (strength not stated), 50 c.c. of a solution of copper sulphate, and 700 c.c. of glacial acetic acid, the liquid will become turbid and a flaky precipitate will separate. In the case of serum-albumin no change occurs.

PROTEIDS OF URINE.[1]

Normal urine is almost, if not entirely, free from any trace of albumin or other proteid, but under particular conditions of fatigue or disease albumin may appear in the urine.[2]

The proportion of albumin present in pathological urine varies greatly. In certain diseases the urine sometimes becomes so highly albuminous that on heating it will undergo coagulation in a manner similar to white of egg. More frequently, comparatively small amounts of albumin are found, and in cases where the urine of convalescent patients is in question it is often of importance to ascertain the presence or absence of mere traces of albumin.[3]

[1] The author is indebted to Dr James Edmunds for perusal and criticism of this article.

[2] Temporary albuminuria is sometimes induced by a cold bath, especially in persons prone to kidney-disease, and it has been observed after excessive muscular exercise, as in the urine of soldiers after a prolonged march.

Any cause which leads to increased blood-pressure in the kidneys tends to induce albuminuria, and many of the cases in which it is the result of disease may be traced to this cause. Albuminuria is a constant accompaniment of the nephritis following scarlet fever, and may occur to a less marked extent in pneumonia, typhoid, and diphtheria. It may also occur in diabetes, and is then a highly unfavourable symptom.

[3] In cases of Bright's disease, the urine rarely contains more than one per cent. of proteids; but taking the volume of urine at 50 oz. daily, this corresponds to a loss of about 220 grains, or 14 grammes per diem. Freund has pointed out that the blood of the average body contains only about 450 grammes of albumin, so that if 7 to 8 grammes of this be lost daily the condition is alarming.

	Serum-Albumin.	Serum-Globulin.	Hetero-Proteose.	Deutero-Proteose.	Peptone.	Acid-Albumin.	Alkali-Albumin.	Mucin.
Dilution with water, .	No change.	Slight opacity.	No change.	No change.	No change.	No change.	No change.	No change.
Saturation with magnesium sulphate (page 40),	No change.	Precipitate.	Precipitate.	No change.	No change.	Precipitate.	Precipitate.	...
Saturation with ammonium sulphate (page 17),	Precipitate.	Precipitate.	Precipitate.	Precipitate.	No change.	Precipitate.	Precipitate.	...
Reaction on boiling after slight acidulation with acetic acid (page 51),	Precipitate.	Precipitate.	No change.	No change.	No change.	No change.	Precipitate.	No change.
Cold concentrated nitric acid (page 52),	Precipitate.	Precipitate.	Precipitate, soluble in excess or on heating, reappearing on cooling.	Precipitated only on adding brine, dissolves on heating, reappears on cooling.	No change.	Precipitate.	Precipitate.	Precipitate.
Metaphosphoric acid (page 57),	Precipitate.	Precipitate.	...	...	...	No change.	Precipitate.	...
Picric acid (page 55),	Precipitate.	Precipitate.	Precipitate.	Precipitate, soluble on heating.	Precipitate, soluble on heating.	Precipitate.	Precipitate.	No precipitate unless acetic acid be added.
Potassium ferrocyanide (page 54),	Precipitate.	Precipitate.	Precipitate.	No change.	No change.	Precipitate.	Precipitate.	...
Potassio-mercuric iodide (page 59),	Precipitate.	Precipitate.	Precipitate.	Precipitate, soluble on heating.	Precipitate, soluble on heating.	Precipitate.	Precipitate.	Precipitate.
Fehling's solution (biuret reaction) (page 12),	Brown-red or violet.	Brown-red or violet.	Rose-pink.	Rose-pink.	Rose-pink.	Brown-red or violet.	Brown-red or violet.	Violet.
Cupric sulphate, . .	Precipitate.	Precipitate.	Precipitate.	No change.	No change.	Precipitate.	Precipitate.	...

Until recently, the proteids liable to occur in urine were classed together under the general name of "albumin," but it is now recognised that several distinct proteids are of common and simultaneous occurrence, and apparently have a varied pathological significance.

The table on page 49 exhibits in a convenient form the chief reactions of solutions of the proteids which are liable to occur in urine.

For clinical use and medical purposes generally, it is necessary to employ simple but fairly delicate tests for the detection of albumin in urine, and many attempts have been made to fulfil the requisite conditions. The following are among those tests which experience has shown to be most generally available and reliable for the purpose, but the recognition of mere traces of albuminous matters in urine is often of great importance, and to effect this with certainty the tests must be applied with care and skill.[1]

Before applying any of the following tests for albumin it is essential that the urine to be examined should be filtered, so as to obtain an absolutely clear liquid, and to ensure its freedom from casual contamination with semen, mucus, epithelial cells, or other débris from the urinary passages.[2]

Previous to filtration, it is important to observe the reaction of the urine. If a slip of blue litmus-paper be promptly reddened when dipped for an instant into the urine, the liquid may at once be filtered. Urine passed during the so-called alkaline tide (after meals) may fail to redden blue litmus, or may even restore the blue colour to reddened litmus-paper. In such case, the urine, before filtration, should be acidulated by adding dilute acetic acid,[3] drop

[1] Urine to be examined for albumin should, by preference, be the mixed excretion of the previous twenty-four hours; but it is easy to lay too much stress on this desideratum. It is better to have a carefully collected sample of the urine passed at one time than a sample of mixed urine collected under conditions open to exception. Thus, in collecting a sample of urine to be examined for albumin, it is important to reject the first ounce or two passed, in order to wash casual discharges out of the urethra. The urine which follows should be passed direct into the sample-bottle, which must be scrupulously clean, and may conveniently contain six ounces.

[2] To ensure perfect filtration, which, when practicable, should be performed on the fresh, warm excretion, a fine close filter-paper should be employed. The importance of operating on carefully filtered urine has been pointed out and insisted on by James Edmunds (*Lancet*, November 9, 1889, page 978).

[3] The use of too strong an acid should be avoided, as an excessive acidity will invalidate the tests to be subsequently applied. *Dilute acetic acid* of the Pharmacopœia contains 4·27 per cent. of real acetic acid, and is a suitable reagent for the purpose. Or *normal acetic acid*, which contains 6 per cent.

by drop, with frequent agitation to ensure perfect homogeneity, until the attainment of a proper degree of acidity is marked by the prompt reddening of a slip of immersed litmus-paper. The urine thus treated will, on filtration, yield a perfectly bright filtrate, which practically is the true urinary excretion, will give no trouble from the presence of mucin, and will yield no precipitate of earthy phosphates or other salts on boiling.

Heat Test.—One of the simplest, and in many cases most satisfactory, tests for the presence of albumin in urine is that of heat. About 10 c.c. (or ½ oz.) of the sample, previously filtered, and, if necessary, acidulated, as above described, is boiled for about one minute in a test-tube. If albumin be present, a cloud is seen to form at the top of the liquid as the boiling point is approached, and, as the urine is boiled, the whole of the albumin separates as a soft, white, opaque precipitate, more or less dense according to the proportion which may be present. On standing for a few minutes this precipitate aggregates into distinct flocculi, and these gradually sink to the bottom of the test-tube, leaving the supernatant liquid clear. After standing for twenty-four hours, the volume occupied by the precipitate will afford a rough indication of the proportion of albumin present. (Compare Esbach's test, page 55.) In highly albuminous urines the precipitate is occasionally so voluminous as to cause the coagulation of the entire liquid.

By the foregoing simple mode of procedure, any proportion of albumin greater than traces will be readily detected. For the detection of smaller quantities, equal measures of the carefully filtered acidulous urine should be placed in two exactly similar test-tubes. The liquid in one tube is then boiled, when, on comparing its appearance with that in the other tube, placed side by side with it, the faintest opalescence will be readily perceived, especially if the tubes be observed in a proper light, with a black background for the line of vision. An alternative plan is to boil the upper part of a column of urine in a somewhat long test-tube by means of a small flame. Any albumin in the upper heated portion of the liquid will thus be coagulated, and will present a marked contrast to the pellucid lower layer.

If not in a distinctly acidulous condition, human urine, on boiling, often yields a precipitate of earthy phosphates, while calcium carbonate is sometimes thrown down from the urine of herbivorous animals. These precipitates are readily and completely re-dissolved on adding a few drops of acetic acid, with

of real acid, may be employed. This can be prepared approximately by diluting 2 fluid ounces of *Acetic Acid*, B.P., with 11 fluid ounces of distilled water.

agitation between each addition, while a precipitate of albumin will remain unchanged under such treatment. A pulverulent precipitate of earthy phosphates, usually $CaHPO_4$, is easily distinguished from the fine flocculi into which an albuminous precipitate soon aggregates. From alkaline urine, albumin is not thrown down by boiling.

In conducting the heat test for albumin, one of the essentials of success is to have the liquid acidulated, as already stated, to a suitable extent. The urine should sharply redden blue litmus-paper, but excess of acid must be avoided. A good plan is to make several tests on portions of the sample to which gradually increasing quantities of acetic acid have been respectively added.[1]

C. W. Purdy (*Practical Uranalysis*, 1894) recommends that the filtered urine should be treated with sufficient of a saturated solution of common salt to raise the gravity to about 1·035. One or two drops of acetic acid should then be added and the perfectly clear liquid boiled, as already described. The addition of the brine is stated to prevent any precipitation of mucin, and hence to avoid the confusion thereby occasioned. But Purdy, apparently, omits to filter the acidulous urine, which treatment would practically remove the mucin.

The quantity of albumin coagulated by boiling may be ascertained by weighing the precipitate (page 29), determining the contained nitrogen (page 21), or observing the diminution of the specific gravity of the urine (page 31).

Nitric Acid Test.—Another delicate and simple test for albumin in urine is due to Heller, and is based on its coagulation by cold nitric acid. The simple addition of some of the urine to strong nitric acid contained in a test-tube, in such a manner as to prevent

[1] According to Tyson, the addition of a few drops of acetic acid may diminish an albuminous precipitate, but on adding more re-precipitation occurs. A large excess, especially if the liquid be boiled, will permanently dissolve the precipitate of albumin. Nitric acid is preferred to acetic acid by some operators, but even more care is necessary to avoid the use of an excess; and the reagent, being corrosive, is unsuited for the study or bedside.

In certain forms of liver-disease the urine gives precipitates which may be mistaken for those of albumin, but which are in reality *bile-pigments*. It has been found, however, that these can be got rid of by previous treatment with acetic or a dilute mineral acid. Grocco, therefore, recommends that all samples of urine likely to contain such matters should be first treated with 2 or 3 per cent. of concentrated acetic acid, set aside in a cool place for two or three hours, and then filtered, before applying the ordinary tests. The precipitate thus formed by the addition of acetic or dilute mineral acids is soluble in alcohol, and does not give the biuret reaction.

the liquids from mixing, suffices for the detection of notable quantities of albumin, but the reaction is much increased in delicacy and reliability by operating in the manner devised by Sir W. Roberts as follows:—A saturated solution of magnesium sulphate is prepared by dissolving 10 parts of the crystallised salt in 13 of hot water and filtering the liquid. To 5 measures of this solution, 1 of nitric acid of 1·42 specific gravity is added. This reagent is so dense that it is easy to avoid admixture with the urine to be tested. Some of the acid mixture is placed in a test-tube, and an equal or larger measure of the filtered urine allowed to flow gently on to the surface, carefully avoiding any mixing of the two layers.[1] In presence of much albumin a more or less opalescent zone is immediately formed at the junction of the two liquids; but when only traces are present a longer time, sometimes extending to a quarter of an hour, is requisite for the development of the band. The turbidity due to albumin occurs at the bottom of the layer of urine, just above the line of demarcation, while any cloudiness due to mucin[2] always appears as a diffused haze well above the plane of contact between the urine and the nitric acid, and therefore quite distinct from the albumin ring. A stratum of uric acid occasionally separates in applying this test, but it almost always disappears on warming. With some urines a crystalline precipitate of nitrate of urea may form, but this cannot be mistaken for albumin.

Ferrocyanide Test.—Another very delicate test for albumin in urine is to treat the suspected liquid with excess of acetic acid, and then add an aqueous solution of potassium ferrocyanide. A white precipitate will form immediately, or after a short interval, if any trace of albumin be present.[3] The reaction is delicate, and

[1] J. E. Saul (*Pharm. Jour.*, [3], xvii. 857) suggests the use of a small glass syringe instead of a test-tube. About an inch of the clear urine is first drawn up into the syringe, and then a sufficiency of the reagent. In this manner the danger of inadvertently mixing the two layers is much lessened.

[2] After taking copaiba balsam or sandal oil, the urine may contain resin acids, precipitable by nitric acid, and therefore liable to be mistaken for albumin. In such cases, Alexander recommends that 10 c.c. of the urine should be treated with two or three drops of hydrochloric acid, which will precipitate the resin acids. If, on adding acetic acid, a precipitate is formed, insoluble in excess of the reagent, this consists of mucin.

[3] A yellow coloration is sometimes produced on adding the ferrocyanide solution to urine. According to J. P. Karplus (*Chem. Centr.*, 1893, ii. 496) this reaction is due to nitrites, which he states are often present in urine which has been kept more than twenty-four hours, but not in the fresh excretion. F. W. Pavy employs tabloids containing citric acid and potassium ferrocyanide, instead of using solutions of the reagents. The test is less

produced only by coagulable proteids, which is important, since peptones may be present in urine without albumin. If true albumin be present, there will be paraglobulin and myosin as well. This occurs in amyloid degeneration of the kidney.[1]

Zouchlos proposes to substitute *potassium thiocyanate* (sulphocyanide) for the ferrocyanide. He prepares the reagent by mixing 10 c.c. of a 10 per cent. solution of the salt in water with 2 c.c. of acetic acid.

A. Ollendorff (*Zeitschr. anal. Chem.*, xxxiii. 120) confirms the value of Zouchlos' test, and states that it is capable of detecting 0·005 per cent. of albumin, while other constituents of urine, with the exception of albumoses, have no disturbing influence.

Fig. 3.—Esbach's Tube.

Picric Acid Test.—A valuable reagent for the detection of albumin in urine is a cold saturated aqueous solution of picric acid, first proposed by Braun, and since strongly advocated by Sir G. Johnson and others. (*Brit. Med. Jour.*, Oct. 11, 1884; *Analyst*, ix. 206.) The reagent may be added either to the original (filtered) urine, or to a portion in which acetic acid has failed to give a precipitate, and either the cold or hot urine may be employed. The reagent may either be allowed to mix with the urine, or the junction of the two layers may be observed, when a turbidity will be produced if any trace of albumin be present. For clinical purposes, a minute quantity of powdered picric acid may be substituted for the solution of the reagent. Peptones, alkaloids, piperazine, and urates (when in large excess) are liable to give precipitates with picric acid; but these precipitates are readily distinguished from that due to albumin by their disappearance on heating the liquid. In the absence of acetic or other added acid, picric acid does not

delicate when citric acid is employed than when acetic acid is used, but Pavy's modification is very convenient for clinical purposes. G. Oliver has proposed to employ strips of filter-paper impregnated with the reagents. By immersing one of the potassium ferrocyanide papers and another of the citric acid papers in a little (5 c.c.) distilled water, the reagent can be prepared in a few minutes.

[1] C. W. Purdy (*Practical Uranalysis*) insists strongly on the importance of adding the acetic acid and ferrocyanide solution simultaneously, or at any rate the latter reagent before the acid. In this way he states that any precipitation of mucin is wholly avoided. To a test-tube half filled with the (filtered) urine he adds a "drachm or so" of a 5 per cent. solution of potassium ferrocyanide, and after agitating adds from 10 to 15 drops of acetic acid. No reaction is produced by mucin, peptones, urates, phosphates, alkaloids, or resin acids. Any precipitate is due to albumin and nothing but albumin.

precipitate mucin, and this fact forms a valuable distinction between that body (said by Sir G. Johnson to be always present in traces even in normal urine) and true albumin.

Esbach's tube (fig. 3) is a contrivance for measuring the volume of the precipitate produced by picric acid, and thus obtaining an approximation to the quantity of albumin contained in urine.[1]

A greatly improved form of Esbach's tube has been devised by C. W. Purdy, of Chicago.[2] The tube is drawn out into a cone at the closed end, so that much smaller quantities of albumin can be measured. Purdy makes the further great improvement of placing the tube in a centrifugal machine (such as the Leffmann-Beam apparatus for estimating fat in milk), whereby complete separation of the albumin can be effected in a few minutes, instead of after standing twenty-four hours.

Trichloracetic acid, $CCl_3.COOH$, in aqueous solution is a reagent for albumin for which special advantages are claimed by Raabe. The reagent does not precipitate peptones nor coagulate mucin. It is said to precipitate a form of albumin not indicated by either of the previously described tests, which form appears to be specially characteristic of the presence of granular, epithelial, or hyaline casts. Trichloracetic acid used in saturated solution, and poured on the cold urine, will detect 1 part of albumin in 100,000 of

[1] Esbach performs the test in a graduated tube, about 6 inches in length and 0·6 inch in diameter. The reagent is prepared by dissolving 10 grammes of picric acid and 20 of citric acid in about 900 c.c. of boiling water, and after cooling making up the volume to 1 litre by adding water. The tube is filled to the mark "U" with the urine to be tested, and the reagent added up to the mark "R." The liquids are mixed by cautiously inverting the closed tube several times, avoiding strong agitation, and then left at rest for twenty-four hours. The volume of the precipitate is then observed, each degree corresponding to 0·1 per cent. of albumin.

Esbach's tubes are obtainable from A. Gallenkamp & Co., Cross Street, Finsbury, London; Southall & Son, Birmingham; Gibbs, Cuxson, & Co., Wednesbury; &c.

The graduation of the tubes is purely empirical. The results are commonly declared to be quite accurate enough for all clinical requirements. In the experience of the author, the results are fairly comparable if the same conditions as to time, &c., are observed, but the absolute quantities of albumin indicated leave much to be desired in point of accuracy, and wide discrepancies occur if the reading be taken much before or after the twenty-four hours prescribed.

Esbach's process is inapplicable to urine containing less albumin than 0·1 per cent., and samples containing more than 0·7 per cent. must be previously diluted with their own or twice their measure of water, as the tubes are not graduated for more highly albuminous urines.

[2] Obtainable from Messrs Eimer & Amend, 205 Third Avenue, New York.

liquid. In presence of quinine or other alkaloids a small addition produces a dense white precipitate, soluble on heating or on adding a large excess of the acid.

Salicyl-sulphonic acid[1] is recommended by M. Roch (*Archiv. des Pharm.*, xxvii. 998) and J. A. MacWilliam (*Brit. Med. Jour.*, i., 1891, page 837) as a precipitant of all varieties of proteids in urine. In applying the test it is simply necessary to add a few crystals of the reagent to a small quantity of the clear urine and agitate, when a turbidity or actual precipitate will be produced in presence of albumin. The precipitate produced by albumins and globulins is not affected by heat, while that due to albumoses and peptones dissolves, reappearing as the liquid cools. No normal or abnormal constituent of urine other than proteids is precipitated by the reagent, but these are very completely separated. One part of egg-albumin in 20,000 of water can be detected.

Metaphosphoric acid, readily obtained by dissolving glacial phosphoric acid in cold water, is recommended by C. Hindenlang (*Chem. Centralb.*, 1881, page 471) for the detection of albumin in urine. The reagent is unstable.[2]

Spiegler's Reagent (*Ber.*, xxv. 375) consists of a solution of 8 grammes of mercuric chloride, 4 of tartaric acid, and 20 of sugar, in 200 c.c. of water. On running on to the surface of this reagent some of the urine (previously acidulated with a little strong acetic acid and filtered if necessary), a distinct white ring is formed at the line of demarcation if albumin be present. Globulin and hemi-albumose behave similarly. Peptones give no reaction. The addition of sugar to the reagent is simply for the purpose of increasing the density, and so avoiding mixture with the stratum of urine.

[1] Salicyl-sulphonic acid or sulpho-salicylic acid is prepared by heating salicylic acid with twice its weight of sulphuric anhydride at 100° C. until dissolved. On cooling and standing, brownish crystals of the compound separate. These are purified by recrystallisation from boiling water.

[2] To obviate the inconvenience caused by the ready conversion of the metaphosphoric into ortho-phosphoric acid, L. Blum (*Chem. Centr.*, 1887, p. 345) recommends the following reagent:—From 0·03 to 0·05 gramme of manganous chloride is dissolved in a little water, acidulated with a few c.c. of dilute hydrochloric acid, and treated with 100 c.c. of a 10 per cent. solution of sodium metaphosphate (best prepared by igniting microcosmic salt in platinum). Lead dioxide is then added, in small quantities at a time, with constant agitation, the liquid is allowed to settle, and filtered. The resulting pink solution of manganic metaphosphate is used as an indicator of albumin. The reagent should be placed in a test-tube and the urine to be examined filtered into it. The solution is said to keep well, and to give no reaction with other constituents of urine.

Potassio-mercuric iodide has been recommended by Tanret as a precipitant of albumin.[1] The reagent is prepared by dissolving 1·35 gramme of mercuric chloride and 3·32 of potassium iodide in 64 c.c. of water, and adding 20 c.c. of acetic acid. Méhu states that this reagent precipitates mucin. Brasse finds peptones and alkaloids to be thrown down, but states that the precipitates due to these bodies dissolve on heating, leaving that due to albumin. The alkaloidal precipitate is soluble in ether, the peptone precipitate is insoluble. Bile-salts give a precipitate not dissolved by heat, but distinguishable from that due to albumin by its solubility in ether. No reaction with Tanret's reagent is produced by creatine, creatinine, xanthine or hypoxanthine.

Various other reagents have been proposed for the detection of albumin in urine, but their behaviour with co-occurring bodies has either been incompletely studied or they present no tangible advantages over the tests already described. It must also be remembered that some of the reagents for albumin in urine are extremely delicate, and this fact should be borne in mind when interpreting their indications, as it may be unsafe or unwise to

[1] Tanret has proposed to determine albumin in urine volumetrically by acidulating the liquid with acetic acid and adding a standard solution of potassio-mercuric iodide until a drop of the liquid yields a precipitate on addition of mercuric chloride. The method is said to give fair clinical results.

F. Venturini suggests a modification of Tanret's process based on the fact that mercuric chloride precipitates albumin from urine acidulated with acetic acid before reacting with potassium iodide. A standard solution of mercuric chloride, containing 10 grammes per litre, is prepared. Each 1 c.c. of this solution precipitates 0·0245 gramme of albumin. To 5 c.c. of the urine, 6 c.c. of a 5 per cent. solution of potassium iodide is added, together with a few drops of acetic acid, and the standard mercuric chloride then added drop by drop, until a permanent yellowish-red coloration is obtained. From the volume used a deduction of 1 c.c. is made for the excess required to show the coloration, and the difference is multiplied by the factor 0·0245 to obtain the amount of albumin.

The method of G. Denigès for the determination of casein described in the article on milk, would probably answer equally well for the determination of albumin in urine.

Georges (*Jour. Pharm.*, [5], xiii. 353) utilises these facts for the detection of peptones in the following manner. The coagulable albumin is first precipitated by heating the urine. The filtrate is precipitated by Tanret's reagent, and the precipitate washed on the filter with cold water charged with acetic acid to the same extent as the urine. It is then washed with the same acidulated water heated to boiling, the washings being kept separate. The clear liquid thus obtained gives a precipitate on cooling if any trace of peptone has been dissolved.

base pathological surmises upon the discovery of minute traces of proteids in urine.

Grainger Stewart (*Edin. Med. Journal*, May 1887) is of opinion that picric acid is the most delicate of all reagents for urinary albumin, Tanret's reagent ranking second. U. Vetlesen, of Christiania, represents the relative delicacy of the various tests by the following figures :—Nitric acid, 85 ; trichloracetic acid, 82 ; potassium ferrocyanide and acetic acid, 82 ; metaphosphoric acid, 72 ; picric acid in solution, 36 ; sodium sulphate and acetic acid, 25. D. Campbell Black (*Urine and Urinary Analysis*), as the result of careful experiments, considers Tanret's reagent the most delicate ; heat, nitric acid, and the aceto-picric solution following in the order named ; while he regards ferrocyanide as one of the least delicate of the tests tried.[1]

Determination of Albumin in Urine.

Esbach's method, as modified by Purdy (page 54), affords quantitative results which are somewhat rough but sufficiently accurate for many purposes. More exact determinations can be obtained by precipitating the albumin, &c., by a suitable reagent, and drying and weighing the washed precipitate, or estimating

[1] Experiments by E. J. Evans (*Pharm. Jour.*, [3], xxv. 913), with a view of comparing the relative delicacy of different reagents, gave the following results with solutions of egg-albumin. Solution No. 1 had a strength of 1 in 40 ; solution No. 2 of 1 in 200 ; while solution No. 3 contained 1 part of albumin in 1000 of water. Half an ounce (8 c.c.) was employed for each experiment. No indication is given in the paper whether dry albumin or fresh egg-albumin was employed.

On boiling, No. 1 showed coagulation and slight opalescence, but only frothing was observed in the case of solutions 2 and 3.

On adding a few drops of acetic acid and heating, No. 1 coagulated ; No. 2 gave a white froth with slight coagulation ; while No. 3 showed a slight froth, without any sign of coagulation or opalescence.

Heated with a few drops of nitric acid, solution 1 gave a cloudy precipitate, which became denser on heating ; No. 2, a white cloud in the line of the drops, and on shaking a white opalescence ; while No. 3 showed only a slight froth without opalescence.

Picric acid gave with No. 1 a bulky yellow precipitate, soluble in excess of ammonia ; with No. 2 a yellow opalescence, and after heating and standing for some time a yellowish precipitate ; and No. 3 behaved somewhat similarly.

With a nitric acid solution of ammonium molybdate No. 1 gave a white precipitate, separating in flocks when heated ; No. 2 a white opalescence ; and No. 3 the same.

Uranium acetate produced with No. 1 a yellowish-white precipitate, curdling on heating ; with No. 2 a yellowish-white opalescence, a precipitate separating on heating ; and much the same with No. 3.

the nitrogen contained in it. Picric acid and potassium ferrocyanide are inapplicable as precipitants, since they contain nitrogen. Tannin, phenol, or trichloracetic acid may be used; or Tanret's solution of potassio-mercuric iodide may be employed, bearing in mind the substances other than albumin which are liable to be thrown down. Of the available precipitants, phenol and tannin appear to be the best.

The following method of determining albumin in urine is strongly recommended by Méhu. 100 c.c. measure of the cold urine is rendered slightly acid with acetic acid, 2 c.c. of concentrated nitric acid added, and the liquid thoroughly agitated. Ten c.c. of a mixture of 1 part by weight of crystallised carbolic acid, 1 of commercial acetic acid, and 2 of rectified spirit is next added, the liquid mixed thoroughly, and filtered after a few minutes. The filtration proceeds rapidly. The precipitate is washed with a cold 4 per cent. solution of carbolic acid in water,[1] when it may either be dried and weighed, or treated (paper and all) by one of the modifications of Kjeldahl's method, and the nitrogen found multiplied by 6·3 to obtain the weight of the proteids precipitated. The presence of sugar or much saline matter in no way affects the accuracy of this process, but in the presence of a large proportion of salts the addition of nitric acid becomes unnecessary.

Van Nuys and Lyons (*Amer. Chem. Jour.*, xii. 336; *Analyst*, xv. 234; xvi. 7) have described a method of determining albumin dependent on its precipitation by a solution of tannin. This is prepared according to the method of A. Almén as described on page 11.[2] Ten c.c. of this solution and an equal measure of the filtered urine are well mixed, and the liquid passed through a dry filter. In 5 c.c. of the filtrate the nitrogen is then determined

[1] According to Méhu's original directions, the precipitate is to be washed with boiling water containing 1 per cent. of carbolic acid. L. Ruisand (*Jour. Pharm.*, [5], xxix. 364) finds that a very appreciable amount of albumin is dissolved by this treatment, but that no appreciable solution occurs when the washing is conducted as described in the text.

[2] Girgensohn mixes the albuminous liquid with half its volume of a 20 per cent. solution of common salt, and then adds a slight excess of tannin. The precipitate is filtered off and washed with water till free from salt, and then with boiling alcohol till tannin can no longer be detected in the filtrate, when the residue is pure albumin. Girgensohn states that the albumin present in the urine of nephritic patients combines with 37 per cent. of tannin, while only 28 per cent. of tannin is contained in the compound formed with the albumin occurring in cases of accidental albuminuria. The albumin of eggs, serum, and pathological secretions in general, is stated to combine with 28 per cent. of tannin. If these statements were verified they might lead to important conclusions.

by Kjeldahl's process, and by the same method the nitrogen is determined in 5 c.c. of the original urine. The difference between the two results represents the nitrogen precipitated by the tannin reagent, and this amount multiplied by 6·3 gives the corresponding weight of albumin and globulin in the precipitate. If the urine contain more than 2 per cent. of proteids, it should be diluted with an equal or double measure of water, and the calculation modified accordingly.

H. O. G. Ellinger (*Jour. prakt. Chem.*, [2], xliv. 256) has described a method of determining albumin in urine by means of an instrument closely allied to Amagat and Jean's refractometer. The sample in its original state is compared with the same urine from which the albumin has been removed by heat and acetic acid. The results agree somewhat roughly with the gravimetric determination.

Distinction and Separation of Urinary Proteids.

Until recently it was not recognised that more than one proteid body was liable to occur in urine, but it is now known that serum-globulin (paraglobulin) frequently co-exists with serum-albumin, and, according to Senator, invariably accompanies the latter substance or even exists alone. Certain albumoses, or proteoses, may also be present, in addition to which peptones sometimes occur either with or without serum-albumin. Traces of mucin are usually present, and fibrinogen and hæmoglobin may occur in certain septic and purpurous conditions.

Paraglobulin may be detected in urine, if present in large amount, by diluting the liquid with two measures of distilled water, rendering it faintly acid, and passing a stream of carbon dioxide. On standing for twenty-four hours or more, the globulin forms a white flocculent precipitate.

Noel Paton (*Brit. Med. Jour.*, 1890, page 197) has described the following method of separating and estimating paraglobulin and serum-albumin when occurring together in urine. The total proteids present are first determined by Esbach's method (page 55). Fifty c.c. measure of the urine is then rendered faintly alkaline, and powdered magnesium sulphate added until the liquid is saturated. It is then allowed to stand in a warm place for twenty-four hours, when the globulin will be completely precipitated. The liquid is then measured, filtered, and a portion again treated by Esbach's method. The result now obtained, after due allowance for the increased volume, represents the albumin of the urine, and the difference between this and the figure previously obtained will be the globulin (*plus* any hemi-albumose) of the urine. The globulin and hemi-albumose ("hetero-proteose") may be separated,

if desired, by redissolving the precipitate produced by ammonium sulphate in a small quantity of water, and treating the solution obtained with ten times its measure of absolute alcohol. The resultant precipitate is collected and digested with cold absolute alcohol for a week or ten days. The liquid is then filtered, when a residue will consist of globulin, and the hemi-albumose will be found in the filtrate.[1]

Instead of employing Esbach's method, A. Ost (*Chem. Centralb.*, 1884, page 500) observes the optical activity of the urine before and after saturation with magnesium sulphate.

An alternative plan is to precipitate the globulin and albumin together by boiling the faintly acidulated urine, and estimate the nitrogen in the washed precipitate by the author's modification of Kjeldahl's method (page 25). In another portion of the urine the globulin is precipitated by saturating the liquid with magnesium sulphate, the precipitate collected and washed with magnesium sulphate solution, and the contained nitrogen estimated by the modified Kjeldahl process. The difference between the two results is the nitrogen corresponding to the albumin of the urine.[2]

Albumoses or Proteoses (page 5) have been proved to exist in certain forms of pathological urine. Thus Gerrard has recently shown (*Pharm. Jour.*, [3], xxiii. 261) that in the milk-

[1] In the urine of a person recovering from a prolonged attack of diarrhœa, Paton found 2 per cent. of total proteids, of which 1·92 was globulin ; and in another case 3·82 of total proteids, of which 3·73 was globulin, which was obtained in elongated rhombic crystals.

[2] According to Senator, paraglobulin occurs in urine in cases of lardaceous disease of the kidneys, and has also been found in excess in the intense hyperæmia resulting from poisoning by cantharides, and in functional albuminuria associated with marked disturbance of the digestive organs. The greater the proportion of paraglobulin present, the more unfavourable appears the diagnosis in Bright's disease. When blood is present in urine, as in nephritis after scarlet fever, there is a large increase in the proportion of paraglobulin. Noel Paton (*Brit. Med. Jour.*, ii., 1890, page 196) finds the ratio of globulin to albumin to vary enormously (from 1 : 0·6 to 1 : 39). It varies much during the day, and in such an erratic manner that no conclusions can be drawn.

According to Daiber, of Zurich, globulin is almost invariably present in larger quantity than albumin in the urine of persons suffering from nephritis and cystitis. To determine the amount, he treats the urine with excess of absolute alcohol, washes the precipitated proteids with warm distilled water, and then dissolves them in dilute acetic acid, added drop by drop. If the solution is coloured it should be filtered through animal charcoal. The solution is then made alkaline by sodium carbonate, and two volumes of a 50 per cent. solution of ammonium sulphate added, when the globulin will be thrown down as a flaky precipitate.

treatment of albuminuria no albumin coagulable by heat exists in the urine, but that nitric acid gives a precipitate soluble in excess or on warming, reappearing on cooling; and saturated brine a flocculent precipitate increased by the addition of acetic acid. These reactions are characteristic of the form of proteose called hetero-albumose, the presence of which in urine is said to be an indication of approaching nephritis. The same substance has been found in cases of osteo-malacia and atrophy of the kidneys, and in the urine of persons who have been rubbed with petroleum.

For the detection of hetero-albumose, Tyson acidifies the urine with a few drops of acetic acid, adds one-sixth of its volume of saturated brine, boils, and filters. Albumin and globulin are precipitated. If the filtrate after cooling gives a precipitate on further addition of brine, which dissolves on heating and reappears on cooling, the presence of albumose is indicated.

For the distinction of the proteoses liable to occur in urine, and their separation from co-occurring proteids, the method described at A in the table on page 18 may be used. It is taken, with slight modifications, from W. D. Halliburton's *Text-book of Chemical Physiology and Pathology.*

Peptones are now known to occur in the urine under a great variety of pathological conditions, especially in acute febrile diseases and nervous complaints, and they probably exist in traces in normal urine. The only reliable method of distinguishing and separating urinary peptones from co-occurring proteids appears to be that of S. H. C. Martin (*Brit. Med. Jour.*, i., 1888, page 842). This consists in saturating the urine, faintly acidulated with acetic acid, with ammonium sulphate. The powdered salt is added gradually to the urine till no more is taken up. The precipitate of proteids rises to the surface of the liquid and can readily be separated by filtration.[1] By this treatment any albumin,

[1] If the precipitate be washed twice with a cold saturated solution of ammonium sulphate, and then redissolved on the filter in distilled water, a solution is obtained in which the proteids are easily differentiated. Thus, on saturating the liquid with magnesium sulphate, the globulin is precipitated, while the albumin can be detected in the filtered liquid by its property of coagulating when heated to 73° C., after slightly acidulating the solution with acetic acid. Hemi-albumose is precipitated at 43° to 50° C., the precipitate being soluble in a few drops of a weak acid, and it is precipitated in the absence of acids, which albumin and globulin are not. It also gives a pink reaction with the biuret test, and with nitric acid a precipitate which dissolves on heating and reappears on cooling. It is likewise precipitated by potassium ferrocyanide from a solution faintly acidulated with acetic acid, and by saturating its solution with magnesium sulphate.

globulin, or proteose is completely precipitated, whatever the reaction of the liquid, while any peptone remains in solution.

Certain precautions necessary when separating albumoses from peptones by ammonium sulphate are pointed out on page 18.

Peptones present the closest similarity in their properties and reactions to the proteoses, and especially to the body called deutero-albumose. Peptones differ from deutero-albumose in not being precipitated on saturating the solution with ammonium sulphate, and in giving no precipitate with nitric acid under any conditions. Deutero-albumose, on the other hand, is precipitated by nitric acid after a considerable quantity of common salt has been added to the liquid. This precipitate dissolves on heating the liquid containing it, but reappears on cooling.

Both peptone and deutero-albumose are precipitated, but not coagulated, by alcohol. They are not precipitated by boiling, nor by cupric sulphate, but are precipitated by phospho-molybdic acid, phospho-tungstic acid, picric acid, tannin, and potassio-mercuric iodide. They resemble other proteids in yielding a yellow coloration on boiling with nitric acid, becoming brownish on adding excess of ammonia (the "xanthoproteic reaction," page 11); and by the pink or rose-red coloration obtained on adding excess of caustic alkali, followed by a few drops of a very dilute solution of cupric sulphate ("the biuret reaction," page 12).

These two reactions can be employed for the detection of peptones in the filtrate from the precipitate produced by saturating the urine with ammonium sulphate, as above described.

Where mucin and albumin are already absent, it is stated that peptones may be detected by treating 50 c.c. of the original urine with 5 c.c. of hydrochloric acid and precipitating the warm liquid with sodium phospho-tungstate. The supernatant liquid is decanted, and the resinous precipitate washed twice with water containing 0·5 c.c. of caustic soda solution of 1·16 specific gravity, which dissolves it. The resultant solution is warmed till a greenish turbidity is produced, allowed to cool, and a 1 per cent. solution of cupric sulphate added drop by drop. In presence of a peptone, a red coloration is produced, which is rendered more evident by filtering the liquid.

Roux (*Jour. Pharm.*, [5], xxv. 544) proposes to determine the peptones in urine volumetrically. The sample is freed from albumin and reducing compounds, and a decinormal Fehling's solution added until the colour changes through light blue, blue-violet, lilac, and rose-purple, to a greyish tint. One c.c. is said to represent 0·004 gramme of peptone.

Mucin is the chief constituent of the mucus derived from the

renal and urinary passages, and is probably the source of the so-called "animal gum" found in the urine by Landwehr. It occurs very commonly (according to Sir G. Johnson, invariably) in traces even in normal urine, and in larger amount in urinary catarrh and other affections of the urinary organs. Mucin is slightly soluble in neutral or alkaline urine, but is precipitated on adding acetic acid, and is insoluble in excess of the precipitant. In many of its reactions it resembles albumin, for which it is apt to be mistaken, but it is not coagulated by heat. It is precipitated by alcohol, alum, dilute mineral acids, and by certain organic acids, including acetic and citric acids. If urine containing mucin be poured on to the surface of acetic acid *saturated* with salt, or on to a strong solution of citric acid, a cloud appears at the junction of the two layers. On the other hand, mucin is soluble in saline solutions of moderate strength, and Purdy actually adds brine in quantity sufficient to raise the density of the urine to 1·035, in order to prevent the precipitation of mucin when acetic acid is subsequently added. In the nitric acid test for albumin the haze due to mucin appears above, and distinct from, that produced by albumin.

Salkowski and Leube test for mucin by treating the urine with two measures of nearly absolute alcohol, separating the precipitate by filtration, and redissolving it in water. The resultant solution gives with acetic acid a cloud which is insoluble in excess, but soluble in hydrochloric or nitric acid. Mucin gives a rose-red biuret reaction with caustic alkali and cupric sulphate, and is completely precipitated by lead acetate.

Mucin may be separated from pus by precipitating the latter with mercuric chloride. On adding acetic acid to the filtrate the mucin is precipitated. Pus is characterised by forming a gelatinous mass with alkalies, whereas these reagents give no reaction with mucin.

There are several varieties of mucin. They all appear to have the constitution of glucosides, being compounds of a proteid (probably variable, but generally a globulin) with animal gum, which latter substance yields a reducing unfermentable sugar by boiling with dilute sulphuric acid.

The ultimate composition of urinary mucin does not appear to have been ascertained, but the following are analyses by Hammarsten of mucin from other sources:—

	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.	Ash.
Mucin from snails,	50·30	6·84	13·62	1·71	27·53	0·33
Mucin from submaxillary gland,	48·84	6·80	12·32	0·84	31·20	0·35

PROTEIDS OF PLANTS.[1]

The proteids existing in plants have the same general properties, and are in many cases probably identical, with those characteristic of the animal kingdom. The amido-compounds, of which asparagine and leucine are types, co-exist in plants with the proteids, and are not improbably intermediate stages in the formation of the latter bodies from simpler compounds.

Crystalloid proteids have been found in a number of plants, and have been frequently described as albumins; but in the majority of cases they appear to belong to the globulin class, or to be a mixture of several distinct proteids. Thus the aleurone grains observed in the peony, castor-oil plant, blue lupine, &c., have been found by S. H. Vines to be partially or entirely composed of proteids, which may be differentiated as (1) vegetable peptone or hemi-albumose, soluble in water; (2) proteids soluble in a 10 per cent. solution of sodium chloride; and (3) proteids partially soluble in the same menstruum. Proteids having characters identical with those of egg-vitellin (page 35), and therefore belonging to the globulin class, have been described by various observers. A proteid forming prisms belonging to the monoclinic system has been obtained by Nägeli by extracting Brazil nuts (*Bertholletia excelsa*) with water at 50° C.; and octahedral crystals were obtained by Grübler, by cooling to 7° C. the liquid resulting from extracting castor-oil seeds with common salt solution at 70°. In the inner rind of the potato, well-formed cubic crystals of proteid nature are easily recognisable.

The true crystalline nature of the foregoing bodies has been questioned, since on addition of a solvent they swell up like starch-corpuscles before dissolving.

Vegetable Albumins have been found in barley, rye, wheat, potatoes, papaw-juice, and in the latex of several caoutchouc-yielding plants.[2] The *myrosin* of mustard (Vol. III. Part iii.) has many of the characters of an albumin, and the *myco-protein* found in yeast and bacteria is stated by Schaffler to be an albumin.

Vegetable Globulins appear to be of very wide occurrence,[3]

[1] The author is indebted to Dr W. J. Sykes for the perusal and criticism of this section.

[2] According to W. J. Sykes, the biuret reaction is not yielded by vegetable albumins until after treatment of their solutions with lead hydroxide.

[3] In those cases in which plant-globulins appear to be soluble in water, as is the case, to some extent, with the varieties existing in barley and malt, the solubility is probably due to the solvent action of the salts naturally present in the grain. W. J. Sykes finds that if malt be thoroughly exhausted with cold water, and then treated with a 10 per cent. solution of

and may be conveniently classified as myosins (myosinogens) and vitellins. The former bodies coagulate at 55° to 60°, and are precipitated by separating the salt from their solutions by dialysis. But the precipitate thus obtained has no longer the characters of a globulin, being imperfectly soluble in saline liquids; it resembles alkaline albuminate by dissolving in weak acid or alkali, with reprecipitation when the liquid is neutralised. A similar change to albuminate occurs if the myosin solution be maintained at a temperature of 35° to 40° for eighteen to twenty-four hours. Plant-myosins are also precipitated by saturating their solutions with common salt. The *vitellins* coagulate at 70° to 75° C., and differ from the myosins in not being precipitated on saturating their solutions with common salt, and in not undergoing conversion into albuminate either by dialysis or by heating their solutions to 35° to 40°. *Plant-vitellin* is crystalline and has all the properties of the vitellin contained in eggs. It is free from admixture with nuclein or lecithin, and is regarded by Halliburton as the purest form of globulin at present known. Other crystalline vegetable globulins have been already mentioned (see above).[1]

Vegetable Albuminates.—This class of proteids is generally stated to be represented by the body called by Braconnot *legumin* or *vegetable casein*, which was prepared by Ritthausen from peas, beans, vetches, and lentils by extracting the powdered substance with dilute alkali, precipitating the strained liquid with acetic acid, washing the precipitate with alcohol, and drying the product over strong sulphuric acid. The legumin thus obtained is soluble both in cold and in hot water, and on treatment with sulphuric acid yields leucine, tyrosine, and glutamic and aspartic acids

sodium chloride, a further quantity of globulin is dissolved out, and will be coagulated on boiling the solution thus obtained.

[1] According to T. B. Osborne (*Amer. Chem. Jour.*, xiv. 662) the crystalloid globulins of the Brazil nut and the oat-kernel are distinct substances, although they agree closely in percentage composition, except as regards nitrogen and sulphur. Oat-kernel globulin is soluble in water at 60°, whereas Brazil nut globulin is insoluble. Saturation of a 10 per cent. sodium chloride solution of the two globulins almost completely precipitates that derived from oat-kernel but not the other. Similar solutions, when saturated with magnesium sulphate, retain nearly all of the Brazil nut but none of the oat-globulin. Finally, when the sodium chloride solutions are heated, the Brazil nut globulin begins to separate at 70° and is largely, but not wholly, precipitated at 100°; whereas oat globulin does not coagulate at all when boiled.

The crystalline globulins of hemp-seed, castor-bean, squash-seed, and flax-seed are almost identical in composition and behave very similarly towards reagents and solvents. They are probably identical.

(compare Vol. III. Part iii. pages 214, 221).[1] A similar body has been prepared from various other sources. That from almonds and lupines is called by Ritthausen *conglutin*.[2] The researches of Hoppe-Seyler, Vines, Green, Martin, and others have shown, however, that legumin is not pre-existent in plants, but is an albuminate produced by the action of the reagents employed in its extraction on the albumins and globulins actually existing in the plant, and that plants contain no ready-formed proteid of the albuminate or casein class.

VEGETABLE PROTEOSES have been found in aleurone grains, latex, cereals, &c. Martin has described two varieties of proteose in papaw-juice, namely, *α-phytalbumose*, with which the ferment papain is associated; and *β-phytalbumose*. A phytalbumose has been described by Martin as existing in wheat-flour, and is regarded by him as the precursor of *gliadin* (see page 70).

VEGETABLE PEPTONES do not appear to exist as such in plants, but are produced, with intermediate products, as the result of digestion. The soluble ferment papain, existing in papaw-juice, is incapable of transforming the accompanying proteids into peptones, the change stopping at phytalbumose; but papain acts on animal proteids in a manner similar to trypsin, in an alkaline medium producing true peptones.

THE GLUTEN GROUP includes several bodies of proteid character, remarkable for being more or less soluble in alcohol. Their characters are described at greater length on page 69 *et seq.*

INSOLUBLE VEGETABLE PROTEIDS have an undoubted existence, in the sense that they cannot be dissolved out of the vegetable tissues by solvents not acting chemically on them; but it is pro-

[1] Osborne and Campbell (*Amer. Chem. Journal*, xviii. 583) find that the chief proteid of peas and vetches—the "legumin" of Braconnot—is a globulin, which is readily precipitated by dialysing its salt solutions. It is freely soluble in a 5 per cent. solution of common salt, but dilution of this solution with water causes precipitation. The legumin from the pea is partly coagulated by heating its strong solutions on a water-bath, and after a time sets to a firm jelly; but the proteid of the vetch is not coagulated by heating nor even rendered turbid by prolonged boiling of its concentrated solutions. These differences are, however, attributed to substances with which the proteid is associated in the two seeds. Another proteid, apparently an albumin or a globulin, exists in both the pea and the vetch, together with traces of a proteose.

In the kidney bean, Osborne (*ibid.*, xvi. 454) finds two distinct proteids. *Phaseolin*, which forms 15 per cent. of the seed, has the characters of a globulin, while *phaselin* exists in smaller proportion and is of a less definite character.

[2] Osborne and Campbell (*Amer. Chem. Jour.*, xviii. 609) find that at least six perfectly distinct proteids have been confounded under the name of vitellin or conglutin.

bable that such insoluble proteids do not pre-exist in the plants, but are produced by the action of ferments similar to those which occasion the coagulation of blood and milk.

E. Fleurent (*Compt. rend.*, cxvii. 790) has described experiments in which plant-proteids were heated with baryta-water, as in Schützenberger's experiments with proteids of animal origin (page 7). The products were qualitatively the same, and the non-volatile products likewise amounted to about 95 per cent. of the original substance, but the ratio of the ammonia to the barium oxalate and carbonate was very different. In the case of proteids of the gluten group it was higher, and in the case of legumin and vegetable albumin lower, than with proteids of animal origin.[1]

The extensive and valuable series of researches on the proteids of plants recently published by T. B. Osborne and his collaborators (see *Amer. Chem. Journal*) have greatly added to the existing knowledge of the subject.[2] An epitome of some of the more important of their results is given in the sequel, but for details the original papers should be consulted.

[1] By the action of baryta, gluten yields glutamic acid (see Vol. III. Part iii. page 227), while legumin and vegetable albumin yield aspartic acid (*ibid.*, page 224). It appears probable that the disproportion between the ammonia and the insoluble barium salts is due to the existence in the molecule of the vegetable proteid of an amide similar to asparagine. Glutamic and aspartic acids are formed, and subsequently decomposed, with production of a further quantity of barium carbonate. This view is supported by the fact that in the case of gluten the proportions of ammonia and oxalic acid remain constant, while the proportion of barium carbonate increases with the duration of the reaction. The same phenomenon is observed in the case of vegetable albumin and legumin. In the fixed residue, the ratio of carbon to hydrogen is $C_n : H_{2n}$, which is identical with that found by Schützenberger in the case of animal compounds ; but it appears from Fleurent's researches that the constitution of vegetable proteids is different from that of animal proteids.

[2] According to Osborne and Campbell (*Amer. Chem. Journal*, 1896, xviii. p. 575), the proteids of the potato consist of a globulin, for which the name *tuberin* is proposed, and a proteose, which latter, however, occurs only in small amounts. The same observers find the proteid of the walnut to be *corylin*, whilst to the proteids of the Brazil nut (*Bertholletia excelsa*) and the oat-kernel the names *excelsin* and *avenalin* respectively are given.

Hemp (*Cannabis sativa*), squash (*Cucurbita maxima*), castor-bean (*Ricinus communis*), cotton-seed and maize contain *edestin*. Maize, in addition, contains *zein*, a proteid allied to gliadin.

Lupin (*Lupinus*) contains, as the principal proteid, *conglutin*.

The sunflower (*Helianthus annuus*) contains *edestin*, contaminated with Ludwig and Kromayer's helianthotannic acid.

The proteid present in the almond and the peach-kernel is stated by Osborne and Campbell to be *amandin*, and not conglutin or vitellin, which are the names usually associated with these seeds.

Proteids of Wheat.

According to the experiments of Osborne and Voorhees (*Amer. Chem. Jour.*, xv. 392; xvi. 524) the seed of wheat contains five distinct proteids, having the composition shown in the following table.

Proteid.	Proportion Present. Per cent.	Elementary Composition; per cent.				
		Carbon.	Hydrogen.	Nitrogen.	Oxygen.	Sulphur.
Globulin[1] ("Edestin"),	0·6 to 0·7	51·03	6·85	18·39	23·04	0·69
Albumin[1] ("Leucosin"),	0·3 to 0·4	53·02	6·84	16·80	22·06	1·28
Proteose,[1] . .	0·3	51·86	6·82	17·32	24·00	
Gliadin (page 72), .	4·25	52·72	6·86	17·66	21·62	1·14
Glutenin (page 71),	4·0 to 4·5	52·34	6·83	17·49	22·26	1·08

It will be observed that the first four of these proteids are present in very small proportion, and the two last are constituents of the "gluten" of wheat.

GLUTEN.

When wheaten flour is kneaded in a stream of water, the starch is gradually washed away, and there remains a sticky cohesive mass which is very rich in nitrogen. This mass, which is generally described as "crude gluten," has a brownish tinge, is almost tasteless, on burning gives an odour resembling that of burnt horn or feathers, and on destructive distillation yields the same products

[1] EDESTIN is a proteid of the globulin class, precipitated from its saline solutions by dilution, and probably more perfectly by removing the salts by dialysis. Edestin solutions are also precipitated by saturation with ammonium or magnesium sulphate, but not by saturation with sodium chloride. Edestin is partially precipitated from its solutions by boiling, but is not coagulated below 100° C. Edestin also exists in barley, rye, and probably in sunflower seeds.

LEUCOSIN is an albumin coagulating at 52° and precipitated from its solutions by sodium chloride or magnesium sulphate, but not precipitated by completely removing salts by dialysis in distilled water.

PROTEOSES.—After dialysing away the salts to precipitate the globulin, and coagulating the albumin by heat, the filtered liquid was saturated with sodium chloride. On concentrating the solution by boiling a coagulum was gradually formed, having the composition shown in the table, and amounting to about 0·3 per cent. of the wheat-kernel. The filtrate contained another body of proteose character which was not obtained in a pure state, but by precipitating the concentrated liquid with alcohol, and determining the nitrogen in the precipitate obtained, was estimated at 0·2 to 0·4 per cent. of the seed. Both these substances and the coagulum are regarded by Osborne and Voorhees as unquestionably derivatives of some other proteid in the seed, presumably the proteose first mentioned.

as animal proteids. It is insoluble in cold water, and in a 10 to 15 per cent. solution of common salt, but dissolves partially in alcohol and in boiling water.

Crude gluten consists of a mixture of proteids (page 73) with small quantities of lecithin, fat, phytosterin, starch, cellulose, fibre, and mineral matter. The proteids of gluten have been the subject of numerous researches, with curiously discordant results. Their nature has been re-investigated in a masterly manner by Osborne and Voorhees (*Amer. Chem. Jour.*, xv. 392), who find that only two proteids of definite character can be detected in gluten, namely:—*glutenin*, which is a body substantially identical with the gluten-casein of earlier investigators, and *gliadin*, remarkable for its solubility in dilute alcohol. They can find no evidence of the existence of mucedin or gluten-fibrin described by Ritthausen as constituents of crude gluten, and they strongly dissent from the views of Weyl and Bischoff and of Sidney Martin (*Brit. Med. Jour.*, 1886, ii. 104) that gluten does not pre-exist in flour, but is a product of the action in presence of water of a soluble ferment or zymaze on other proteids of the grain.[1]

[1] According to Martin, wheat-flour contains two proteids, one of which is a myosin (myosinogen) coagulating between 55° and 60°, and precipitated by sodium chloride and magnesium sulphate; and the other a soluble phytalbumose. Both these bodies are converted into true peptones by the action of pepsin or trypsin. They can be extracted from flour by a 10 to 15 per cent. solution of sodium chloride, and may be considered the precursors of gluten; the myosin forming gluten-fibrin and the phytalbumose an insoluble albumose corresponding with Ritthausen's gliadin and mucedin. Hence flour from which the myosin and phytalbumose have been extracted by a solution of salt no longer yields "gluten" when kneaded with water, or yields it in diminished amount according to the completeness with which the proteids have been extracted. This behaviour of flour when treated with salt solution was first observed by Weyl and Bischoff, and their observation has been confirmed by Osborne and Voorhees, provided that the flour be stirred up with a large quantity of salt solution, extracted repeatedly with fresh quantities of the solvent till no more proteid is dissolved, and the excess of salt solution removed by allowing the residue to drain on a filter as completely as possible. But they further point out that if wheat-flour be first mixed with sufficient salt solution to form a firm dough, this dough may be washed indefinitely with salt solution, and will yield gluten as well and as much as if washed with water alone. They attribute the difference to the fact that, when large quantities of salt solution are applied at once, the flour fails to form a coherent mass, and the particles cannot afterwards be brought together, as is possible when a smaller quantity of salt solution is employed in the first instance.

Osborne and Voorhees also argue that Martin's theory of the formation of

GLUTENIN is prepared by Ritthausen by boiling crude gluten several times with alcohol of 0·890 specific gravity, when the gliadin dissolves and a residue is obtained consisting of glutenin with some impurities. This product should be dissolved in very dilute ($\frac{2}{10}$ per cent.) solution of caustic potash, and the proteid reprecipitated by exactly neutralising the solution with acetic acid. Osborne and Voorhees then direct that the precipitate should be treated in succession with alcohol and ether to remove traces of fat, &c., and then redissolved in very dilute alkali, the solution filtered clear through close paper, and the glutenin reprecipitated by exact neutralisation.[1]

When purified in the above manner, glutenin forms a greyish-white mass which is not sticky. When dried at 100° it forms a slightly brownish, horny substance, which slowly recovers its original condition by contact with water. In a moist state, glutenin readily undergoes decomposition, soluble proteids being first formed, and subsequently products of a very offensive character.[2] Glutenin is practically insoluble in cold water or cold

gluten cannot be correct, since it is founded on the observations that alcohol does not extract proteid matter from the flour when applied directly, and that at least one-half of the proteid matter of the seed is a myosin-like globulin ; whereas neither of these observations is correct.

[1] Unless glutenin be treated in the manner described in the text, the impurities are not removed and the product has a variable composition.

Glutenin was first described by Taddei under the name of *zymom*. Liebig, as well as Dumas and Cahours, named it *plant-fibrin*. Ritthausen, who obtained the substance substantially pure, called it *gluten-casein*. Weyl and Bischoff regarded it as an albuminate form of a myosin-like globulin, which body pre-existed in the grain and was converted into glutenin by the action of a ferment. Sidney Martin held the same view, and he and Halliburton caused confusion by designating the proteid as *gluten-fibrin*. This name had already been employed by Ritthausen for a body soluble in dilute alcohol which he described as existing in gluten.

[2] G. Emmerling (*Berichte*, xxix. 2721) describes the result of experiments on the decomposition of wheat-gluten by *proteus vulgaris*. The gluten was prepared by kneading out the starch from wheat-flour, and treating the crude product with malt-extract, the residue after this treatment being washed with alcohol and ether. The purified substance, suspended in water with calcium carbonate, potassium phosphate, and magnesium sulphate, was sterilised and treated with a pure culture of *proteus*. In four days a copious evolution of gas had occurred. The gas had the composition CO_2, 46 ; H, 38 ; and N, 16 per cent. After six days the strongly alkaline fluid was distilled in a current of steam. The distillate contained phenol and trimethylamine ; dimethylamine and other liquid bases were not found. The residue contained betaïne, acetic acid, and butyric acid, but not propionic acid. Egg albumin was also treated with *staphylococcus pyogenes aureus ;* indole, skatole,

alcohol, but appears to be slightly soluble in these solvents when warmed. After dehydration with absolute alcohol and drying over sulphuric acid, glutenin is soluble in very dilute alkalies (as 0·1 per cent. solution of caustic potash) and in very dilute acids (*e.g.*, 0·2 per cent. hydrochloric acid), with the exception of an insoluble residue, the amount of which depends on the condition of its preparation. Thus, when freshly precipitated and in the hydrated state, glutenin is extremely and completely soluble in the slightest excess of caustic alkali, and in somewhat greater but still very slight excess of acid. In this condition, glutenin is also soluble in the slightest excess of ammonia or sodium carbonate solution. After drying over strong sulphuric acid the substance dissolves only partially in a 0·5 per cent. solution of sodium carbonate.

Glutenin also dissolves with facility in cold dilute organic acids (acetic, citric, tartaric). From its solutions in alkalies and dilute acids it is thrown down by exact neutralisation. Glutenin is precipitated from its solutions by cupric acetate, or by saturating its solution with common salt. In sulphuric acid diluted with an equal measure of water, glutenin dissolves on boiling with brownish colour, which persists on standing. On diluting the solution a clear liquid is obtained. In concentrated hydrochloric acid, glutenin dissolves to a slightly yellowish solution, which becomes of a deep violet colour on standing.

Gliadin[1] is readily dissolved out of wheaten flour or gluten by hot dilute alcohol. In the hydrated condition, gliadin is a soft, sticky substance, which can be readily drawn into threads; but when dehydrated by means of absolute alcohol and subsequent treatment with ether, and dried *in vacuo*, it forms a white, friable mass which can be readily reduced to powder. If moistened with a little water or dilute alcohol and then dried, gliadin forms thin transparent sheets resembling gelatin, but somewhat more brittle.

When treated with a little cold water, gliadin forms a sticky mass, and dissolves somewhat on addition of a larger quantity.

phenol, formic acid, acetic, propionic and higher fatty acids were obtained from this decomposition.

[1] This proteid was first discovered in 1805 by Einhof, and in 1820 was named by Taddei *gliadin* on account of its resemblance to glue. By Liebig it was called *plant-gelatin*, and by Dumas and Cahours *glutin*. The *mucin* of De Saussure and of Berzelius must also be considered as impure gliadin, and the products called by Ritthausen *gluten-fibrin* and *mucedin* were apparently simply impure or altered preparations of his plant-gelatin or gliadin, which, owing to the strength of the alcohol used, were more soluble in the hot than in the cold liquid.

It is much more soluble in boiling water, forming an opalescent solution, but partially separates again on cooling. The aqueous solution of gliadin is coagulated on boiling, and the precipitate formed is insoluble in dilute alcohol or in 0·2 per cent. caustic alkali solution. A solution of gliadin in pure water is instantly precipitated by adding a very minute amount of sodium chloride.

If previously-moistened gliadin be treated with water containing a little common salt, a very viscid product is obtained, which adheres persistently to everything with which it comes in contact, but with a stronger solution of common salt (10 per cent.) a plastic mass is formed which is not adhesive. Gliadin is quite insoluble in absolute alcohol, but up to a certain point becomes increasingly soluble as the alcohol is diluted, after which the solubility again diminishes. Thus, alcohol of 70 per cent. dissolves an almost infinite amount of gliadin, but the proteid is precipitated by adding either much water or strong alcohol to this solution. Gliadin is precipitated from its solutions either in strong or in weak alcohol by adding a few drops of sodium chloride solution, the completeness of the precipitation depending on the strength of the alcohol and the amount of salt added. The precipitation is least complete from alcohol of 70 to 80 per cent.

Gliadin dissolves readily in extremely dilute acids and alkalies, and is precipitated, on neutralisation, in a condition apparently unchanged either in composition or properties. Gliadin gives the general proteid reactions with nitric acid, Millon's reagent, and the biuret test. When dissolved in strong hydrochloric acid, gliadin gradually develops a violet coloration. Warm 50 per cent. sulphuric acid gives a similar reaction, the colour becoming much more intense on boiling. Gliadin is precipitated from its solutions by tannin, basic lead acetate, and mercuric chloride.

Gliadin is entirely distinct in composition and properties from the alcohol-soluble proteids of maize and oats.

On reference to the table on page 69, it will be seen that the analyses of gliadin and glutenin show a very close agreement in the ultimate composition of the two bodies, and Osborne and Voorhees suggest that they may be really two forms of the same proteid, one being soluble in dilute alcohol and the other insoluble (*Amer. Chem. Jour.*, xv. 458).[1]

[1] Osborne and Voorhees instance the case of *zeïn*, a proteid contained in maize, which is wholly insoluble in water and in absolute alcohol; but if water be present solution in alcohol at once takes place, the amount of zeïn dissolved depending, within certain limits, on the quantity of water present. (Compare Chittenden and Osborne, *Amer. Chem. Jour.*, xiii.)

Crude Gluten from wheat-flour consists essentially of glutenin and gliadin, both these proteids being essential for its formation. According to the view of Osborne and Voorhees, the gliadin forms a sticky mixture with water, and the presence of the salts natural to the wheat-flour prevents its ready solution. It tends to bind the particles of flour together, rendering the dough and gluten tough and coherent. The glutenin imparts solidity to the gluten, evidently forming a nucleus to which the gliadin adheres, thus preventing its solution by water. A mixture of one part of gliadin with ten of starch forms a dough, but yields no gluten, the gliadin being washed away with the starch on treatment with water. On the other hand, flour freed from gliadin gives no gluten, there being no binding material to hold the particles together.

Soluble salts are also necessary in forming a gluten-mass, since gliadin is readily soluble in distilled water. In water containing salts it forms a viscid, semi-fluid mass, which acts very powerfully in holding together the particles of flour. The mineral constituents of the seeds are apparently sufficient to accomplish this purpose, for a firm gluten can be obtained by washing a dough with distilled water.

In the opinion of Osborne and Voorhees, "no ferment-action occurs in the formation of gluten, for its constituents are found in the flour having the same properties and composition as in the gluten, even under such conditions as would be supposed to remove completely antecedent proteids or to prevent ferment-action." They consider that "all the phenomena which have been attributed to ferment-action are explained by the properties of the proteids themselves as they exist in the seed and in the gluten."[1]

So far as is known, wheat is the only seed the flour of which yields a tough elastic gluten-mass on treatment with water.[2] It is the gliadin which imparts to wheat-flour the property of

[1] S. H. C. Martin (*Brit. Med. Jour.*, 1886, ii. 104) suggests that the formation of gluten is due to the action of a ferment, and considers this view to be supported by the fact that no gluten-mass is formed when the flour is washed with water cooled to 2° C. If wheat-flour be moistened with alcohol of moderate strength, no dough is formed, the product being a damp, sandy mass, similar to that obtained on moistening oat-flour with water. The same effect is produced by treating the flour with strong brine, or by heating it to about 60° C. for some hours. (Compare page 70).

[2] M. Weybull (*Chem. Zeit.*, xvii. 501) attributes the inferior quality of the rye-bread of 1892 partly to a deficiency, and partly to a changed condition of the gluten. In such case, the defect may be remedied by an admixture of wheat-flour, or by the employment of some substance which precipitates the soluble constituents of the gluten. Alum and copper sulphate have this effect,

forming a stiff, elastic dough, capable of retaining vesicles of gas, and thus producing a light and porous loaf. Rye-meal is stated by Osborne to contain about 4 per cent. of gliadin.[1] The absence of more than traces of gliadin from the glutens of barley and oats is the reason why the flours from these sources do not form a plastic mixture with water, and hence do not make good bread. Gliadin is absent, or nearly so, from leguminous seeds, but is said to be present in the juice of the grape and other fruits, being held in solution by tartaric or other vegetable acid.

An impure gluten is obtained as a waste-product in the manufacture of starch.

Gluten has a high food-value, and bread made from it has been specially recommended as a substitute for ordinary bread in cases of diabetes. This so-called "gluten-bread" is in many cases very unfit for its intended use. Thus, if the starch of flour be reduced by special treatment from 70 to, say, 60 per cent., the product is evidently unfit for use by diabetic patients, who might equally well reduce their consumption of ordinary bread by one-seventh. On the other hand, if the "gluten-bread" be practically free from starch, it fails to satisfy the craving for starch which attends its total prohibition, and may be advantageously replaced by more appetising forms of proteid food.

To ascertain the proportion of crude gluten obtainable from flour, 50 grammes[2] of the sample should be triturated in a mortar

but are inadmissible. Hence Weybull recommends the addition of at least 1 per cent. of common salt, or the employment of skimmed milk instead of water.

[1] As rye-flour yields no gluten-mass when kneaded in a current of water, A. Kleeberg (*Chem. Zeit.*, xvi. 1071) has proposed to detect an admixture of wheaten flour with rye-flour as follows:—Place as much of the flour as will lie on the point of a knife on an object-glass of 7·5 cm. by 2·5 cm., add 5 to 6 drops of lukewarm water (40° to 50° C.), and stir well. The quantity of water has to be so large that the particles of flour still float in the water. The mixture of water and flour is spread over three parts of the object-glass and another object-glass placed on it in such a way that the dry ends protrude on either side. Press the two glasses well, wipe off the liquid, and slide the top glass to and fro several times. During the pressing of the glasses white spots will be observed if wheaten flour be present, which, on being rolled, form "vermicelli"; these are short and thin if the quantity of wheat present is small, and become thicker and longer with increasing amounts of wheaten flour. An admixture of 5 per cent. of wheat-flour is said to be thus recognisable with certainty.

[2] When the gluten is not to be subsequently examined in the aleurometer it is preferable to operate on 10 grammes of the flour, instead of on the larger quantity recommended in the text. Jago recommends the use of 30 grammes of flour and 25 c.c. of water. He ties up the dough in a piece of fine silk, such as is used for dressing flour, about a foot square, and kneads

with 30 c.c. of water. The dough produced should leave the mortar without a trace adhering. After standing at rest for three or four hours, the mass should be placed in a fine linen cloth, which is then tied up tightly and gently kneaded with the fingers, while a fine stream of water is permitted to flow on to it. The kneading and washing are continued until the water which runs away is found to be clear, and hence free from starch. The gluten is then removed from the cloth and dried slowly at 110° to 120° C. Gluten from good flour is elastic and but little coloured; that from damaged or inferior flour adheres to the cloth, is with difficulty united into a single mass, and has less consistency and a higher colour than the product from good flour.[1]

M. Boland has pointed out (*Compt. rend.*, xcvii. 496) that the proportion of gluten obtained from the same flour varies with the mode of operation and the amount of washing. A more hydrated gluten is yielded by flour from soft or old wheat than from hard, and by fresh paste than by paste which has stood several hours before washing. In order to avoid these sources of error, it is recommended that 50 grammes of the flour should be mixed with 25 grammes of water, and the paste allowed to stand for twenty-five minutes. It is then divided into two equal portions, one of which is washed immediately, while the other is allowed to stand for an hour. As soon as the wash-water is clear, the glutens are tightly pressed and weighed, after which they are washed for another five minutes and again weighed. Four numbers are thus obtained, and the mean of these is taken as the true yield of moist gluten. The best flours give a moderately high yield of gluten, but the product is highly elastic, and firm and springy to the touch. Gluten from inferior flour is soft and sticky and possesses but little toughness.

It is sometimes an advantage to ascertain the behaviour on

it in a basin of water instead of under a tap, replacing the water as long as starch continues to wash through.

[1] The following alternative method of determining the yield of gluten is recommended by Wanklyn and Cooper:—10 grammes weight of the sample is mixed on a porcelain plate with 4 c.c. of water, so as to obtain a homogeneous dough. This is placed in a conical measure or other suitable vessel, 50 c.c. of water added, and the dough manipulated with a spatula so as to expel the starch-granules. The water is decanted off, a fresh quantity added, and the kneading repeated till no more starch is extracted from the gluten. The mass is then removed and kneaded in a little ether, after which it is spread out in a thin layer on a platinum dish and dried in the water-oven till the weight is constant. The crude gluten contains ash equal to about ·3 per cent. on the flour, and fat equivalent to 1·00 of the flour. These may, of course, be directly determined in the crude gluten, if desired.

heating of the crude gluten obtained as above. This may be effected by means of the *aleurometer* (fig. 4), an instrument devised by Boland. It consists of a brass cylinder, about five inches in length, furnished with a graduated piston. Adjustable caps are fitted to both ends of the cylinder, the whole length of which represents 50°; but the stem of the piston is graduated from 25° to 50° only, since it is capable of descending only half-way down the cylinder. This contrivance constitutes the aleurometer proper, and is designed for use in a baker's oven. For laboratory purposes, the aleurometer is immersed in a bath of oil, which is maintained by means of a spirit-lamp at a temperature of 150° C. As pointed out by W. Jago, it is important that this temperature should be kept constant during the operation, and hence it is desirable to fix a thermostat to the apparatus.

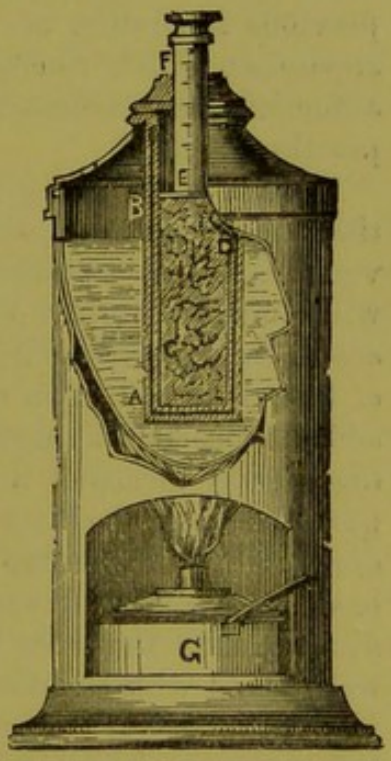


Fig. 4.—Boland's Aleurometer.

In using the aleurometer, from 30 to 50 grammes of the flour should be made into a paste with half its weight of water, and then washed in the manner already described. Seven grammes weight of the freshly prepared gluten is then rolled in a little starch, and placed in the cylinder, the inside of which should be greased to prevent the gluten from adhering. The piston is then pushed down till it registers 25°, and the cylinder is heated in a baker's oven or immersed in the oil-bath maintained at 150° for ten minutes, when the source of heat is withdrawn, and the gluten allowed to remain undisturbed for another ten minutes. On then examining the apparatus, the gluten will be found to have expanded and forced up the piston to an extent dependent on its quality. Good flour yields a gluten which will expand to four times its original volume, but the expansion never exceeds the limit of 50° on Boland's scale. With damaged flour, the gluten does not swell much, but becomes viscous or nearly fluid, adheres to the cylinder, and sometimes exhales a disagreeable odour, whereas good gluten has merely the odour of hot bread. If the gluten does not alter the position of the piston, which therefore will continue to register 25°, the flour may be considered unfit for bread making.[1]

[1] The aleurometer is described in *The Miller* for July 2, 1867. For the

With practice in the use of the aleurometer, very fair results may be obtained, though no fine distinctions are possible. A further knowledge of the character of the gluten is obtainable by drying the swollen product, as taken from the aleurometer, in the water-oven for twenty-four hours. On the average, three parts of moist gluten yield one part of the dry substance.

K. W. Kunis, of Leipzig, has devised an instrument, called by him the *farinometer*, which is intended for use with the dough made from the flour to be tested, instead of necessitating the previous separation of the gluten, as in Boland's process. The apparatus, which resembles the aleurometer, is furnished with an automatic heat-indicator, and is said to yield reliable results in practised hands.[1]

Instead of preparing the gluten, valuable information respecting the bread-making capacity of a sample of flour may be obtained very simply by ascertaining the quantity of water a definite weight will require to form a dough of standard consistency. The test is conducted as follows[2]:—A weight of 25 grammes of the sample of flour is placed in an evaporating basin and 17 c.c. of cold water added to it from a burette, the flour and water being well mixed together by means of a spatula or glass rod. On moulding the paste between the fingers it is easy to determine whether the dough is too stiff or too thin. In the latter case, too much water has been added, and another trial must be made, using a smaller measure. If the paste be too stiff, more water may be added and well mixed with the dough, but it is better to make another test with an increased amount of water. This should always be done before coming to a conclusion as to the strength of the flour, and it is better to leave the dough for one hour before deciding. It is easy to compare the relative strengths of two or more samples of flour by this test, but in the absence of a standard it is difficult without practice to decide exactly when the paste is of a proper consistency. The results are usually expressed in quarts of water required per sack of flour. In London, the sack of flour is taken as weighing 252 lbs., that is, 9 stones; but in some provincial districts a weight of 280 lbs. (=10 stones) is reckoned as one

wood-cut illustrating its construction, the author is indebted to the agents for the apparatus, Messrs Dunham, White & Co., Limited, Harrow Green Works, Leytonstone Road, London, E.

[1] The farinometer is described in *The Miller* for March 5, 1894.

[2] With the exception that decimal weights and measures have been substituted, the test is performed substantially as directed by W. Jago in his *Chemistry and Technology of Bread-making*.

sack. As a quart of water weighs 2·5 lbs., each quart required by a sack of 252 lbs. is practically 1 per cent. Operating on 25 grammes of flour, as directed above, each c.c. of water employed will represent four quarts to the sack of 9 stones; or, with the addition of one-ninth, the gallons of water per sack of 10 stones. Jago regards a flour which requires 68 quarts of water per sack of 252 lbs. as of standard quality. A sack of such flour will make 95 four-lb. loaves or 380 lbs. of bread.

It appears from the facts already set forth that the proteids of wheat and other cereals may be classed broadly as soluble and insoluble, the latter being concerned in the formation of a tough elastic gluten, while the former are rather detrimental than otherwise in the production of bread.

In analysing plant-products, it is very important that the nitrogenised constituents should undergo little or no alteration during the process of extraction. Schulze and Barbieri consider this may be best effected by extracting the substance first with cold water and then with hot water, or else with dilute alcohol.

Chas. Graham has pointed out that a constant ratio exists between the proportion of soluble proteids and the dextrin and sugar found on analysing the flour, and that the longer the flour is digested in cold water the greater the proportion of soluble proteids, and hence of dextrin and sugar, becomes.

Graham has suggested the following simple method of making rough comparative estimates of the soluble proteids of different samples, which, with certain modifications, is as follows:—10 grammes weight of the flour is treated with 40 c.c. of cold water, and the mixture allowed to stand for exactly one hour. The liquid is then passed through a dry filter, the first portions being rejected. 20 c.c. measure of the filtrate (= 5 grammes of flour) is then treated with an equal measure of methylated spirit, when a precipitate of soluble proteids will be produced, the amount of which will depend on the quality of the flour, the best specimens giving the smallest precipitate. A more accurate estimation of the soluble proteids may be made by filtering the liquid, evaporating 20 c.c. of the filtrate to dryness at 100° C., and weighing the residue of sugar, &c. The weight thus obtained is subtracted from that found by evaporating 10 c.c. of the original aqueous solution of the flour, when the difference will be the weight of soluble proteids precipitated by the methylated spirit. It is necessary to adhere strictly to one hour, or other constant time, for the digestion of the flour with water, as higher results are obtained if the treatment be prolonged. Operating in the fore-

going manner, J. W. Downs informs the author that the matter dissolved by cold water from flour ranges from 6·7 in samples of the lowest quality to 3·5 per cent. in the highest quality of flour, the average being about 5 or 5½ per cent.

Cerealin.—The husk of wheat and other cereals contains a soluble nitrogenised ferment or enzyme called cerealin. This body exerts a powerful hydrolytic action on starch, rapidly converting it into dextrin and other soluble bodies.

The presence of cerealin in wheat-bran renders "whole meal" unsuitable for making bread by fermentation with yeast, unless special precautions be taken, though aërated bread can be prepared from it. The cerealin acts like malt-extract, causing a rapid conversion of the starch into dextrin and sugar, and materially modifies the behaviour of the flour in the aleurometer.[1]

Proteids of Barley and Malt.

According to W. J. Sykes (*Trans. Inst. Brewing*, 1891, iv. 173), the proteids in barley and malt are albumin, globulin, albumose, peptone, and glutenin.[2] Other nitrogenous bodies in barley are enzymes, amides, and ammonium salts. The globulin of barley (myosin) is precipitated if its solution be saturated with sodium chloride.

Glutenin is insoluble in cold water, but soluble in slightly acid solutions, such as wort. Its solutions are slowly coagulated on boiling. The presence of glutenin in wort is shown by carefully neutralising, when a precipitate is obtained, most of which dissolves in excess of alkali. On drying the precipitate obtained by saturating wort with ammonium sulphate, a portion always becomes insoluble; this Sykes considers to be the glutenin present. By making use of the above-described properties, a rough separation and estimation of the amounts of proteids present in wort and beer may be made. The only body of the kind present to any extent in beer is an albumose, and this is unable to supply nitrogenous food to yeast.

[1] The use of whole meal is often advocated on account of the high proportion of nitrogenous matter and ash-constituents present in it, but the admixture of oatmeal with wheat-flour might be advocated on precisely similar grounds. The indigestibility of the bran wholly neutralises any advantage supposed to be derivable from its superior nutritive value. (See also the experiments of Rubner, *Jour Chem. Soc.*, xlvi. 622; *Jour. Soc. Chem. Ind.*, 1885, p. 129.)

[2] Sykes adopts Ritthausen's view, according to which wheat-gluten contains the four proteids gluten-casein, gluten-fibrin, mucedin, and gliadin. He regards gliadin as absent from the seed of barley, and if the mucedin and gluten-fibrin are merely modifications of it the only member of the gluten group present in barley is gluten-casein, preferably called glutenin (page 71).

The best known of the amides present is asparagine. No determinations of the amounts of these bodies in wort or beer appear to have been made, but they probably form the chief nitrogenous food of the yeast.

The nitrogenous constituents of a sample of *barley* and of the *malt* made from it were found by Sykes to be:—

	Barley.	Malt.
Total proteids, ...	10·56 per cent.	9·68 per cent.
Total soluble proteids, ...	2·23 ,,	4·05 ,,
Albumin precipitated on boiling, ...	0·70 ,,	0·70 ,,
Albumose, ...	0·55 ,,	1·11 ,,
Amide, ...	0·98 ,,	2·24 ,,

Hilger and van der Becke (*Archiv f. Hygiene*, 1890, x. 477) have published the following figures intended to illustrate the mode of existence of the nitrogen in barley before and during germination, and in malt at different stages of the manufacture. The figures in all cases represent the percentages of nitrogen in the moisture-free substance. The smaller amounts in the steeped barley are due to a portion of the nitrogenised bodies being dissolved by the steep-water.

	Barley.	Steeped Barley.	Green Malt.[1]	Kilned Malt.
Nitrogen as soluble coagulable albumin, .	0·0600	0·0354	0·1671	0·1194
,, ,, peptone, ...	0·0046	0·0009	0·0058	0·0233
,, ,, ammonia, ...	0·0169	...	0·0290	0·0057
,, ,, amido-acids, ...	0·0417	0·0294	0·1417	0·2257
,, ,, amides, ...	...	...	0·0505	0·0029

According to T. B. Osborne (*Amer. Chem. Journal*, 1895, xvii. 539), the seed of *barley* contains the albumin *leucosin*, previously found by him in the kernel of rye (*ibid.*, xvii. 429), together with the globulin *edestin*, and a proteose. Another proteid, called by Osborne *hordein*, also exists in barley-meal. It is soluble in alcohol of 75 per cent., and is apparently identical with the body described by Kreusler. It resembles gliadin in many of its properties, but has a somewhat different ultimate composition. When completely dried at 110°, hordein is nearly insoluble in

[1] The malt was five days old.

water, and the dilute solutions which can be obtained in hot water do not precipitate on cooling or coagulate on boiling, but yield a precipitate on addition of common salt. Hordein is also soluble in very dilute acids and alkalies, but is thrown down again on neutralisation. It dissolves in strong hydrochloric acid with fine crimson coloration, and a warm mixture of equal volumes of water and strong sulphuric acid yields a red colour, whereas gliadin gives a purple-red when similarly treated. Osborne also describes a proteid insoluble in water, saline solutions, and alcohol, but soluble in potash, which exists in barley in the proportion of 4·5 per cent. The hordein amounts to 4·0 per cent., the leucosin to 0·3, and the edestin and proteose together to 1·95 per cent.

Osborne and Campbell (*Jour. Amer. Chem. Soc.*, 1896, xviii. 542) find the proteids of *malted barley* to be not wholly the same as those of the unmalted grain. According to them, the malted grain contains the following proteids:—

1. *Bynedestin*, a globulin far more soluble in very dilute salt solution than edestin, the globulin of barley, and differing moreover from the latter in containing more carbon and less nitrogen. Bynedestin dissolved in 10 per cent. salt solution gives a turbidity at 65° C., and a flocculent coagulum at 84° C., but even after protracted heating at 100° C. the coagulation is incomplete. The proteid is partly precipitated by saturating its solution with magnesium sulphate. 2. *Leucosin*, identical in composition and properties with the albumin obtained from wheat, rye, and barley. 3. A *Proto-proteose*, of the same composition as leucosin, readily precipitated from its aqueous solution by adding an equal weight of alcohol. 4. A *Proto-proteose*, less readily precipitated by alcohol, and differing in composition from the preceding. 5. A *Deutero-proteose*. 6. A *Hetero-proteose*, present in extremely small amount. 7. *Bynin*, a proteid insoluble in water and saline solutions, but readily soluble in dilute alcohol. 8. A proteid insoluble in water, saline solutions, and dilute alcohol. The following proportions of these constituents were found in malt:—

	Per cent.
Proteid, insoluble in salt solutions and in alcohol, . .	3·80
Bynin, soluble in dilute alcohol, ...	1·25
Bynedestin, leucosin, and proteoses soluble in water and salt solutions { coagulable, . .	1·50
{ uncoagulable, .	1·29
Total proteids, . .	7·84

Osborne and Campbell conclude from their researches that "during germination, the proteids of barley undergo extensive changes without acquiring, or before acquiring, the properties of

proteoses; that hordein disappears, and a proteid soluble in alcohol, of entirely different composition, takes its place; and that edestin also disappears and is replaced by a new globulin. The albumin, however, appears to be unchanged in its characters, but its quantity is increased."

The following analyses of two samples of *pale malt* by C. O'Sullivan, in which every constituent was determined directly, are interesting, as showing the action of solvents on the proteids present.

	No. 1.		No. 2.	
Starch,		44·15		45·13
Other carbohydrates (of which 60 to 70 per cent. consist of fermentable sugar), inulin (?), and a small quantity of other bodies soluble in cold water,		21·23		19·39
Cellular matter,		11·57		10·09
Fat,		1·65		1·96
Proteids—				
a. Soluble in alcohol (sp. gr. 0·820) and in cold water,	·63		·46	
b. Soluble in cold water and at 68° C.,	3·23		3·12	
c. Insoluble in cold water, but soluble at 68° to 70° C.,	2·37		1·36	
d. Insoluble at 68° to 70° C., but soluble in cold water (=albumin proper),	·48		0·37	
e. Insoluble in cold water, acid at 70° C.,	6·38		8·49	
		13·09		13·80
Ash,		2·60		1·92
Water,		5·83		7·47
		100·12		99·76

E. Erich (*Der Bierbrauer*, 1895, pp. 145, 161, 177; abst. *Jour. Soc. Chem. Ind.*, 1896, p. 366) has investigated the methods of separating the different proteids of malt- and beer-worts. These, however, he does not differentiate so completely as other observers, but classes as "albumins" such proteids as are precipitable by lead and copper salts, "peptones" (which will include the albumoses), such as are not so precipitated but are thrown down by tannin or phospho-tungstic acid, and "amides," which belong to another of the above groups.[1] His method of analysis was as

[1] From Erich's results, the peptones and amides appear to have the same value as nutrient material for yeast, and to be much superior in this respect to albumin.

Experiments on the influence of germination on the soluble nitrogenous substances of the barley showed that peptonisation of the albumins takes place; and that the longer the period of germination the greater is the amount

follows :—The total nitrogen was determined in the residue from 20 c.c. of a wort. 250 c.c. measure of the same wort was boiled with basic lead acetate, and, after cooling, made up to 500 c.c. When it had stood for seven or eight hours, the precipitate was filtered off, sulphuretted hydrogen passed through the filtrate, and the nitrogen determined in 40 c.c. of the filtered liquid. The numbers obtained represented the nitrogen from peptones and amides, and the difference between this and the total nitrogen of the wort was the nitrogen due to albumin. 20 c.c. of tannin solution was next added to 80 c.c. of the filtrate containing peptones and amides. After standing twelve hours the liquid was filtered, and the nitrogen corresponding to the amides determined in the filtrate.

To effect a separation with copper hydroxide and phospho-tungstic acid, 20 c.c. of the wort was diluted to 100 c.c., warmed, 10 c.c. of copper hydroxide added, and allowed to stand for twenty-four hours. The albuminous precipitate was filtered off, 55 c.c. of the filtrate containing the peptones and amides evaporated, and the nitrogen determined in the residue. To 50 c.c. of the original wort, 10 c.c. of a 10 per cent. solution of sulphuric acid and 50 c.c. of 10 per cent. phospho-tungstic acid was added ; after twenty minutes the precipitate of albumin and peptone was filtered and the nitrogen determined in the residue obtained from 44 c.c. of the filtrate. A comparison of the results revealed the fact that basic lead acetate precipitates more nitrogenous substances than copper hydroxide, and phospho-tungstic acid more than basic lead acetate and tannin together.[1]

The following process has been devised by H. Schjerning (*Zeits. Anal. Chem.*, 1896, xxxv., [3], 285 ; abst. *Jour. Chem. Soc.*, 1896, ii. 631) for determining the nitrogenous compounds in beer-wort. Four precipitants are made use of, and by their means Schjerning professes to be able to determine the quantities of albumin, denucleïn, propeptone (albumose), and peptone.

1. Stannous chloride solution is prepared by dissolving 50 grammes of tin in an excess of concentrated hydrochloric acid, with the addition of a little platinic chloride ; the solution is evaporated to about 130 grammes, diluted to 1 litre, and filtered.

2. An approximately 10 per cent. solution of lead acetate is made by dissolving the normal salt in boiling water, filtering, and adding 10 to 12 drops of acetic acid to the litre.

of nitrogenous bodies rendered soluble. Peptonisation also proceeds during the mashing process, the most favourable temperature being 50° C.

[1] W. J. Sykes finds that treatment with copper hydroxide partially precipitates the albumoses of malt-worts, and that tannin brings down more nitrogenous matter than is precipitated by phospho-tungstic acid.

3. Dilute acetic acid containing 15 c.c. of acid (45 per cent.) in the litre.

4. A saturated (about 10 per cent.) solution of uranium acetate.

Albumin is determined by mixing about 5 c.c. of No. 1 solution with 25 c.c. of wort. The mixture is allowed to stand for four to six hours, the precipitate filtered off, washed with cold water, and the nitrogen determined by Kjeldahl's method.

Denucleïn is precipitated together with the albumin, when about 6 c.c. of No. 2 solution is added to 25 c.c. of wort or beer, and the mixture heated to boiling. Owing to the partial solubility of the precipitate, a correction has to be applied in this case equal to 0·15 c.c. decinormal acid for every 100 c.c. of filtrate and wash water. The denucleïn = Pb − Sn.

Propeptone.—40 c.c. of No. 3 solution is diluted with 100 c.c. of water, and 0·8 gramme of ferric acetate added. The solution is heated to boiling, 20 c.c. of the wort or beer added, the mixture again boiled, and the precipitate at once filtered off and washed. The precipitate (Fe) contains also the albumin and denucleïn, and the propeptone is consequently Fe − Pb.

Peptone.—A mixture of 25 c.c. of wort or beer with 20 to 25 c.c. of No. 4 solution is heated to boiling and then set aside in a dark place until the following day; the precipitate is then filtered off and washed with a cold 1 to 2 per cent. solution of uranium acetate. A correction is applied equal to 0·1 c.c. of decinormal acid for every 100 c.c. measure of filtrate and washings. The peptone = Ur − Fe.

A better method than that given above for the estimation of the propeptone is stated to be the following:—Five drops of acetic acid (45 per cent.) are added to 20 c.c. of wort or beer, which is then heated to about 36°. 18 to 20 grammes weight of pure powdered magnesium sulphate is added, and when this has dissolved the mixture is allowed to stand for half to one hour at the ordinary temperature. The precipitate is finally washed with a cold saturated solution of magnesium sulphate containing 4 to 5 grammes of acetic acid (45 per cent.) to the litre. The precipitate contains the albumin and the propeptone.

Diastase, the soluble ferment of malt, is stated by T. B. Osborne (*Amer. Chem. Jour.*, 1895, xvii. 587) to contain a globulin, an albumin having most of the properties of leucosin, and probably two forms of proteose. Osborne thinks it probable that active diastase is a compound of albumin with proteose, and that it breaks up on heating, yielding coagulated albumin; and, further, that free albumin is also present, which is coagulated at the same time but has no diastasic power.

PROTEIDS OF MILK.[1]

The chief proteid of milk is the substance commonly called casein. It has been contended that all other forms of milk-proteid are merely products of the action of reagents or ferments on the casein, but there is no doubt that a form of albumin, closely allied to, but probably distinct from, serum-albumin, exists normally in milk together with the casein. In addition to these two well-defined proteids, others have been described by different observers under the names of lactoprotein, lactoglobulin, whey-proteid, &c., and some observers regard peptone as a constant constituent of milk.[2]

The determination of the nitrogen of milk affords the means of obtaining a fair approximate determination of the total proteids, but, in some cases at any rate, sensible quantities of bodies of non-proteid character appear to be present. Thus, according to I. Munk (*Jour. Chem. Soc.*, 1894, ii. 106), fresh cows' milk contains from 0·022 to 0·034 per cent. of extractive nitrogen; while human milk contains from 0·014 to 0·026 per cent. The proportion of extractive to total nitrogen is stated to be as 1 : 16 in cows' milk and as 1 : 11 in human milk. If these results be accepted, the proteid nitrogen of cows' milk is 94 per cent., and of human milk 91 per cent., of the whole.

[1] The author is indebted to Mr H. Droop Richmond for perusal and criticism of this section.

[2] H. D. Richmond (*Amer. Chem. Jour.*, October 1893) has summarised the views of different chemists as to the proteids existing in milk as follows:—

Béchamp, . .	Caseinates, albuminates, and galacto-zymase.
Biel, . . .	Syntonin.
Blyth, . . .	Casein, albumin, galactin, lactochrome (nuclein ?).
Danilewsky, . .	Caseo-protalbin and caseo-albumin (nucleo-protalbin and nucleo-albumin), and six other proteids.
Duclaux, . .	Casein only.
Hammersten, .	Casein and albumin only.
Palm, . . .	Hemi-albumose.
Sebelien, . .	Globulin.

Duclaux' view has been definitely disproved, while Hammersten and Chittenden have independently disposed of Danilewsky's view, and with this the alleged existence of lactoprotein (accepted by many), galactin, nuclein, and hemi-albumose becomes untenable. Blyth alleges the presence of lactochrome to the minute extent of 0·01 per cent. only. Richmond considers that the existence of syntonin and galacto-zymase in milk cannot be regarded as fully established, so that casein (caseinogen), lactalbumin, and globulin are the only proteids the existence of which in appreciable amount has been established. The proportion of globulin in milk is very small, so that, except in special cases, it may be ignored.

Munk states that from one-fifteenth to one-thirtieth of the proteids of cows' milk remain in solution after precipitation with alcohol; but that either tannin or cupric hydroxide at the boiling point precipitates the proteids completely, the latter reagent being the more rapid in its action.

Determination of Milk Proteids.

For the determination of the total proteids of milk, Richmond and Boseley (*Analyst*, 1893, p. 172) recommend the following modification of Ritthausen's method (compare page 30):— Dilute 10 grammes of the milk with about 200 c.c. of water, add a few drops of phenol-phthalein solution, and then drop in dilute caustic soda, until a faint pink coloration appears. From 2 to $2\frac{1}{2}$ c.c. of a 6 per cent. solution of copper sulphate is then added, the liquid agitated, allowed to settle, and the precipitate washed five times by decantation, the washings being passed through a tared filter.[1] The precipitate is then transferred to the filter, washed further (spreading the precipitate over the filter), dried slightly in the water-oven, treated with ether to extract the fat, then dried thoroughly at 130° C., weighed, ignited, and the weight deducted from the ash found. The results obtained are about the sum of the casein and albumin determined separately, and a trifle lower than the total proteids as determined by multiplying the nitrogen by Sebelien's factor, 6·37. Instead of drying the filter, J. Muter recommends that the washed precipitate should be treated on the filter with alcohol, and the extraction of the fat by ether at once proceeded with. The author prefers to avoid the tedious drying entirely by treating the moist precipitate, together with the filter, by one of the modifications of Kjeldahl's process.

The *casein* and *albumin* of milk are best separated by Sebelien's method. Ten grammes of milk should be treated with 20 c.c. of a saturated aqueous solution of magnesium sulphate, and crystals of the powdered salt added as long as they dissolve on agitation. An excess of crystals is harmless, but the salt must be free from sodium sulphate. After standing some hours the liquid is filtered, and the precipitate, consisting of casein (caseinogen) mixed with any traces of globulin existing in the milk, is washed with a saturated solution of magnesium sulphate. The precipitate, together with the filter, is then treated with 30 c.c. of strong sulphuric acid, and the nitrogen determined as described on page

[1] In the filtrate the milk-sugar can be directly determined by Fehling's solution or the polarimeter, or deduced from the weight of the residue (less the ash left on ignition) obtained on evaporating an aliquot part of the liquid to dryness.

22. The albumin in the filtrate is determined by treating the diluted liquid with phospho-tungstic acid,[1] and estimating the nitrogen in the resultant precipitate by Kjeldahl's process; or the albumin may be thrown down by Almén's tannin solution (see page 11) and the precipitate treated as above.

Richmond and Boseley state that the foregoing modification of Ritthausen's process answers well with all milk-products except whey, which contains albumoses produced by the action of rennet, called by Richmond chymo-albumoses. Neumeister has shown that the deutero-albumose obtained during digestion-studies by Kühne and Chittenden's method gives a soluble copper salt, while Richmond and Boseley find that in a mixture of whey and milk in about equal parts there exists about 0·3 per cent. of albumoses which are not precipitated by copper sulphate or magnesium sulphate, though they are thrown down with the albumin by either tannin or phospho-tungstic acid. To effect a separation, the diluted filtrate from the magnesium sulphate precipitate should be slightly acidulated with acetic acid and the albumin coagulated by heating. In the filtrate, the albumoses can be thrown down by Almén's tannin reagent.

To ascertain whether a sample of milk has been previously boiled, M. Rubner precipitates the casein by saturating the liquid with common salt and heating it to 30° to 40° C. The clear filtered liquid is heated to the boiling point, when no precipitation will occur if the milk had been previously boiled, but otherwise a coagulum of albumin will be formed.

Casein and Caseinogen.

When milk becomes sour by the formation of lactic acid, owing to the fermentation of the milk-sugar, a point is ultimately reached at which the liquid curdles through the precipitation of a portion of the casein. A similar precipitation may be induced in new milk by adding a small proportion of acetic acid, especially if the milk be warmed, and, preferably, previously diluted. Certain bacterial growths sometimes occur in milk which occasion the so-called spontaneous coagulation.

The casein of milk is also precipitated by contact with rennet, which contains a soluble ferment or enzyme called *chymosin* or *rennin*, possessing in an eminent degree the power of coagulat-

[1] *Phospho-tungstic acid* is prepared by dissolving 50 grammes of sodium tungstate in about 500 c.c. of water, and adding 50 grammes of a solution of phosphoric acid of 1·13 sp. gravity. The liquid is boiled for a short time, allowed to become cold, and then made slightly acid with hydrochloric acid. After standing for twenty-four hours the solution is filtered.

ing casein.[1] A similar property is possessed by the pancreas and portions of other tissues, such as the testes.

For the efficient action of rennet the presence of a salt of calcium, preferably the phosphate, appears to be essential. Rennet will act in a faintly acid, a neutral, or an alkaline solution, and is most energetic at about 40° C. Like other proteolytic ferments, it is rendered inactive by a temperature of about 70° C.

When rennet is added to cows' milk the casein is separated as a firm clot or curd enclosing the fat, while the whey forms a clear yellowish liquid, which contains the lactalbumin, sugar, salts, and albumoses formed by the action of the rennet. If the milk be previously boiled, the casein separates in smaller flocculi, and the same effect is obtained by adding sodium carbonate or lime-water to the milk. Human milk behaves in a similar manner to cows' milk thus treated. These facts have a practical bearing on the feeding of infants, and afford a simple means of preventing the formation of compact curds difficult of digestion.

It has been conclusively proved that the precipitation of casein by acids is a reaction quite distinct from that which occurs when rennet is employed. In the first case there is mere precipitation, and the casein may be again obtained in solution by suitable treatment. In the second case there is true coagulation, the clot formed

[1] Rennet is usually prepared from the fourth stomach of the sucking calf, which is dried and treated with a 5 per cent. solution of common salt. On adding excess of alcohol to this solution the rennin is precipitated, and may be dried and dissolved in water when required. One part of the powdered ferment thus prepared will coagulate 200,000 parts of milk. A less pure form of rennin may be obtained by simply saturating an aqueous extract of rennet with common salt, when the "lab" containing the active principle will rise to the surface.

According to an improved method of preparing rennin devised by C. P. Eyre (*Eng. Patent*, 1893, No. 2675), the cardiac portion of the mucous lining of the stomach of the hog, calf, &c., is washed, chopped, dried, and reduced to powder. The powder is digested for four days with dilute hydrochloric acid (5 per cent.), the mixture being frequently agitated. The solution is then filtered, strong magnesium sulphate solution added until nearly the whole of the pepsin is precipitated, and subsequently the excess of magnesium salts removed by the addition of ammonia. After filtration, the nearly pure solution of rennin is concentrated *in vacuo*, at a temperature not exceeding 105° F., to a thick syrup, mixed with sufficient powdered sugar or starch to yield a stiff paste, and finally dried, preferably in a current of hot air. Prior to drying, the preparation may be granulated by forcing it through a sieve; or it may be converted into any desired form. With ordinary care the dry preparation is stated to remain good indefinitely.

The preparation of a fluid "essence of rennet" has been recently described by J. A. Forret (*Pharm. Jour.*, 1896, Aug. 1, p. 111).

being insoluble in any medium not causing profound chemical change. Under suitable conditions, as good cheese can be obtained by the use of acid-curdled casein as with that curdled by rennet.

The action of rennet does not appear to differ much from that of other proteolytic ferments. Since pepsin is almost inactive in neutral liquids, it does not coagulate casein. Addition of an acid in quantity sufficient to render the pepsin active precipitates the casein. Hence the pepsin cannot act on a solution of casein, but it splits the precipitated substance into soluble caseoses and insoluble dys-casein-peptone or dys-peptocaseose. It appears from recent researches that the insolubility of the latter body is due to its combination with calcium salts (chiefly calcium phosphate). Rennet is active in neutral solutions, but similarly splits casein into soluble chymocaseoses and an insoluble dys-chymocaseose, which latter body forms the curd. This likewise owes its insolubility to combination with calcium salts (preferably phosphate). If calcium salts be completely removed, no insoluble dys-caseose is formed either by pepsin or rennet. Trypsin gives rise to a similar but less insoluble dys-caseose under suitable conditions.[1]

The action of rennin on casein appears to be strictly analogous to that of the blood-curdling ferment on the fibrin of blood, and of the muscle-ferment on the myosin of living muscle. The soluble forms of fibrin and myosin are conveniently called fibrinogen and myosinogen, the names fibrin and myosin being applied to the corresponding coagulated proteids. Similarly, the characteristic proteid of milk may be appropriately called caseinogen, the name casein being applied solely to the coagulated substance. This nomenclature is advocated by Halliburton and other authorities, but it fails to take into account the soluble products formed simultaneously with the coagulated proteid. Further, the term casein has been too long and extensively applied to the proteid in both forms to render it probable that the restricted employment of it proposed will ever become general. Hence, except in the following paragraphs, no attempt will be made to confine the word casein to the coagulated proteid.

For the preparation of caseinogen in a state of purity, W. D. Halliburton recommends that milk should be treated with powdered magnesium sulphate as long as the salt continues to

[1] The presence of both phosphorus and sulphur in the casein (caseinogen) molecule renders it probable that it is a compound proteid of the nucleo-albumin class. In this case, the first action of proteolytic ferments would result in the formation of nuclein as one of the fission-products, and the caseoses would be formed at a later stage.

dissolve. The precipitate of mixed fat and caseinogen which floats on the surface of the saline solution is filtered off and washed with a saturated solution of magnesium sulphate. The precipitate is then treated on the filter with distilled water, which, owing to the presence of the adhering salt, dissolves the caseinogen, leaving a residue of fat. From the filtrate, the caseinogen is precipitated by excess of acetic acid, filtered off, thoroughly washed, dissolved in a dilute alkali such as lime-water, and further purified by repeated precipitation with acid and re-solution in alkali. If the caseinogen so prepared has been washed completely free from calcium phosphate, which is difficult to effect, no coagulation occurs on adding rennet to the solution, but on addition of calcium phosphate or chloride almost immediate coagulation will occur at 40° C.

When prepared by Halliburton's method, caseinogen has a specific rotatory power of $-80°$; in dilute alkali solution of $-76°$; in strong alkaline solutions of $-91°$; and in dilute acid solutions of $-87°$.

Caseinogen is often regarded as ordinary alkali-albumin (page 4), but the latter compound is not coagulated by rennet and is soluble in dilute acids, whereas caseinogen is insoluble. The two bodies are alike in not being coagulated by heat and in being precipitated by neutral salts. A solution of caseinogen in dilute sodium chloride or magnesium sulphate solution becomes somewhat cloudy at 70° C., but there is never any notable flocculent precipitate formed, and the turbidity disappears as the liquid cools, if the heating has not been long continued.

A marked difference between casein and artificial alkali-albuminate is in the proportion of sulphur. Lieberkuhn attributes to the latter compound the formula $M_2C_{72}H_{112}N_{18}O_{23}S$, whereas in casein about one-half of the sulphur is replaced by phosphorus.[1] The mean of the results of Hammersten, Chittenden, Strohmann, Lehmann, and Ritthausen give 0·80 per cent. of sulphur and 0·85 per cent. of phosphorus in casein, which are very nearly atomic proportions. Soldner has also shown that casein unites with lime in proportions very nearly corresponding to CaO : P and 2CaO : P.

As stated on page 90, it is probable that casein (caseinogen) is not a simple proteid, but belongs to the nucleo-albumin class (page 3).

[1] The formula $C_{77}H_{120}N_{20}O_{26}S$ agrees better with the figures of Hammersten and of Sebelien for lactalbumin. It is also in accordance with the results of Chittenden for egg-albumin, and of Chittenden and Bolton for the iron, copper, mercuric, silver, lead, zinc, and uranium compounds of egg-albumin.

Besides containing phosphorus in organic combination, casein is obtainable almost free from metals. The ash of such highly purified casein consists chiefly of *metaphosphoric acid*, HPO_3, and hence is highly acid and attacks platinum at a red heat.

Casein (caseinogen) occurs plentifully in milk, but has not been recognised in the fluids of the textures. It is not present in blood, and exists but rarely in the fluids of cysts.

The determination of casein (caseinogen) in milk is described on page 87. The proportion of casein present in the milk of various mammals is shown on page 100, *et seq.*

Dried casein is a white or yellow, brittle, transparent, hygroscopic body, which swells up in hot water without dissolving, but is soluble in very dilute acid or alkaline solutions.

Casein is unaltered by exposure to a temperature of 100° for some hours. When dried in a vacuum, casein obstinately retains water, which can only be expelled by prolonged exposure to a temperature of 130° to 140° C., after which treatment it is not completely soluble in a solution of sodium carbonate.

Casein reddens litmus, and exercises both an acid and a basic function. Though insoluble in water,[1] it dissolves in ammonia and in dilute solutions of the caustic alkalies and alkaline carbonates, and while still moist and freshly prepared it is soluble in lime-water and baryta-water, and in solutions of the phosphates and borates of alkali-metals. On addition of an acid to such alkaline solutions of casein, precipitates are thrown down containing the precipitating acid in combination, but these compounds are decomposed by water. Casein dissolves in hydrochloric acid containing less than 0·1 per cent. of real HCl, but is precipitated from this solution on addition of hydrochloric acid of medium strength. In hydrochloric acid of 1·0 per cent. strength casein is insoluble, but it dissolves in the strong acid with violet coloration.

The solutions of casein are not coagulated by boiling, but are precipitated by excess of common salt and various other neutral salts, including magnesium sulphate and barium and calcium chlorides. Cupric sulphate, mercuric chloride, lead acetate, and other general reagents for the proteids precipitate casein solutions completely. Precipitated casein is coagulated and rendered insoluble by boiling or treatment with excess of strong alcohol.

The casein of milk is not thrown down on exact neutralisation of the liquid by an acid, the alkaline phosphates apparently pre-

[1] According to Béchamp, pure casein is sparingly soluble (about 1 part per 1000) in pure water, but its solubility is greatly diminished by the presence of minute quantities of salts.

venting the precipitation till a sensible excess of acid is present. The curdling of milk by keeping is due to the conversion of the milk-sugar into lactic acid by the action of the organism *Bacterium lactis*.

If a solution of casein in dilute alkali be boiled, a characteristic scum or pellicle is formed, in the manner familiar in milk.

The acid calcium salt of casein forms a milk-white solution, to which the colour of cows' milk is probably to some extent due. Concentrated solutions of the sodium salt are readily precipitated by acetone, and of the calcium salt by alcohol. If the precipitates be washed first with absolute alcohol, and then with ether, the casein salts are obtained as white powders. (See F. Röhmann, abst. *Jour. Chem. Soc.*, 1896, i. 515.)

The manufacture, in the solid form, of casein salts of the alkalies and alkaline-earths has been protected by Meister, Lucius, & Brüning (*Eng. Patent*, 1894, No. 22,190). One series of compounds are described as neutral to phenol-phthalein, and not coagulated by rennet, while the members of the second series contain about two-thirds of the amount of base present in the former, are acid to phenol-phthalein, and are coagulated by rennet in presence of calcium salts. The compounds are prepared by dissolving casein in the calculated quantity of caustic alkali, alkali-metal carbonate or phosphate, or milk of lime, and evaporating the solution *in vacuo*. The products are dry white powders.

Nutrose (*Pharm. Jour.*, 1896, iii. p. 63) is a food-preparation consisting of a neutral compound of casein with fixed alkali. It is made by mixing dried casein with the calculated proportion of caustic soda, boiling the mixture with 94 per cent. alcohol, and drying. The compound may also be prepared by evaporating an alkaline solution of casein *in vacuo*, or by precipitating an alkaline solution of casein with acetone. Nutrose is a light, finely-divided powder, which dissolves in warm milk, water or broth, without affecting their taste. When placed on the tongue, it has a slight cheesy taste. Nutrose has an alkaline reaction to litmus, but is acid to phenol-phthalein. It is given in quantities of an ounce or two ounces daily, and is readily digestible. The nutritive value of nutrose has been studied by Röhmann, Salkowski, and others, who state that it is able to fully cover the loss of nitrogen, as well as to induce the formation of proteids in general. Nutrose is stated by R. Stüve to contain 13·8 per cent. of nitrogen.

The silver compound of casein has been patented (*Eng. Patent*, 1894, 22,191) under the name of *argenin*.[1] It is prepared by mix-

[1] Argenin must not be confounded with *arginine*, a base of the formula $C_6H_{14}N_4O_2$, which was first obtained by Hedin (*Pharm. Jour.*, No. 1375,

ing 3 kilogrammes of the neutral sodium salt of casein with 300 grammes of silver nitrate, and dissolving the whole in water with the aid of a gentle heat. The solution is then precipitated by alcohol, and the precipitate of casein-silver dried, or the solution may be simply evaporated to dryness *in vacuo*. The substance so obtained differs from a mere mixture of the sodium salt of casein with silver nitrate in the fact that its solution is not precipitated by sulphuretted hydrogen on addition of ammonia, nor is it precipitated by soluble chlorides. Argenin forms a white powder, difficultly soluble in cold water, but dissolving readily on warming. With care, a solution containing 10 per cent. of the solid may be obtained. Argenin and its solutions should be kept in the dark. The substance is neutral. It is stated by A. Liebricht to possess the same bactericidal properties as silver nitrate, while free from the caustic action of that salt. Injected in $7\frac{1}{2}$ per cent. solution, argenin has been found very efficient in the treatment of gonorrhœa.

W. Majert has patented the preparation of an ammonia compound of casein by bringing finely-powdered casein in contact with ammonia gas; or by suspending the casein in alcohol, ether, light petroleum, or benzol, and passing the gas through the liquid. The combination takes place with rise of temperature. The product is a white powder permanent in the air, and readily soluble in water to form a tasteless liquid, which possesses valuable nutritive properties (*Eng. Patent*, 1896, No. 9).

Solutions of casein are employed in commerce as substitutes for albumin in calico-printing.[1] For this purpose the casein is generally dissolved in ammonia, and on evaporation of the solvent becomes fixed and insoluble. A preparation of this kind is known in commerce under the name of *lactarine*. The residue left on volatilisation of the ammonia is soluble in alkaline solutions, but if the ammonia-paste be mixed with milk of lime, and the mixture applied to the cloth, an insoluble lime compound is formed on heating, which resists washing in alkaline liquids.

Skim-milk cheese can be substituted for pure casein for the preparation of lactarine. It is macerated in water, to remove soluble matters, and treated with ammonia. Such products are inferior to albumin for the uses of the calico-printer.

Colour-lakes, which are said by C. Dreher (*Farb. Zeit.*, 1896,

p. 378) as one of the products resulting from the action of hydrochloric acid upon proteid substances, and has since been detected in the blanched sprouts of *Lupinus luteus* and found by Schulze to occur in the tubers and roots of various plants (such as *Brassica rapa*, *Helianthus tuberosus*, *Ptelea trifoliata*, &c.).

[1] See also footnote on page 44.

vii. 164) to be very pure in shade, fast to water, relatively inexpensive, and innocuous, may be produced by precipitating casein from an ammoniacal solution, in the presence of the colouring matter of the shade desired, by means of stannous chloride or acetate, or by aluminium chloride.

A. Dollfus (*Jour. Soc. Chem. Ind.*, 1884, p. 629) treats casein with cold nitric acid, which converts it into a yellow nitro-compound. This is washed with tepid water and dissolved in a minimum of caustic soda solution, and the solution diluted with water in quantity sufficient to reduce it to a suitable consistency for printing. By steaming, it becomes so firmly fixed in the cloth as to withstand the most vigorous soaping and even the action of chlorine. By the use of weaker nitric acid a product of lighter colour is obtained, but it does not fix so well by steaming.

A mixture of casein with slaked lime sets to a hard insoluble mass, which is sometimes employed as a cement for earthenware. Attempts more or less successful have been made to compress casein into a compact form suitable for the manufacture of buttons, knife-handles, billiard-balls, &c. (see a paper by J. Carter Bell, *Jour. Soc. Chem. Ind.*, xii. 14), the product being called *lactite*. A portable food has also been prepared by evaporating mixtures of skim-milk with whey, a specimen of "double lactoserin" obtained from equal measures of the two having, according to J. C. Bell, the following composition :—Fat, 1·34 ; proteids, 22·56 ; carbohydrates, 66·13 ; salts, 6·94 ; and water, 3·32 per cent.

A. Zimmermann (*Eng. Patent*, No. 23,585, 1894) has observed that, while an alkaline solution of casein remains clear when mixed with formic aldehyde (commercial formalin), on evaporation to dryness on a plate of glass or other smooth surface the proteid is left as a thin transparent film, which is quite insoluble in water. A suitable mixture is obtained by dissolving about 100 grammes of casein and 15 grammes of caustic soda in one litre of water, and adding 15 c.c. of commercial formalin of 40 per cent. strength ; or 2 litres of water, containing 100 grammes of casein and 100 c.c. of a 30 per cent. solution of ammonia, are treated with 15 c.c. of formalin. Addition of too large a quantity of formalin must be avoided, or thickening of the liquid and precipitation of the casein will result. Another method of procedure is to evaporate the solution of the casein to dryness, and treat the resultant film with formalin, subsequently allowing the latter to evaporate.

Egg- or blood-albumin, or the product obtained by prolonged boiling of a solution of gelatin containing a little acid or alkali, may be employed in place of casein in the above process.

MILK.[1]

Milk is the fluid secreted by the mammary glands of female mammals for the nourishment of their young,[2] and hence has come to be regarded as a model food.

The formation of milk is not strictly confined to the suckling female. The mammary glands of new-born mammals of both sexes secrete a small quantity of milk for a few days,[3] and in exceptional cases a similar fluid has been secreted by the adult male, both in the case of man and in that of the lower animals.[4] Adult females occasionally secrete milk in cases where there has been neither birth, conception, or connection with the male. With dogs this is not uncommon.

Milk is a more or less opaque fluid, usually of a white or yellowish-white colour, and is somewhat denser than water. The white appearance of milk is principally due to the presence of numerous globules of fat in suspension in the fluid. On allowing the milk to stand at rest for some time a large proportion of the fat-globules will rise to the surface and form a layer of cream, and

[1] The author is indebted to Mr H. Droop Richmond for perusal and criticism of the greater part of this article.

[2] During periods of non-lactation, the acini of the mammary glands are lined by flattened epithelium. During lactation, which begins after the birth of the offspring, the cells are larger, and are constantly undergoing fatty degeneration. The fat-globules liberated by their disintegration float in a clear liquid which is also secreted by the cells from the lymph circulating in the gland.

[3] The following analyses of the lacteal secretion of new-born children ("witch's milk") have been recorded :—

	A.	B.	C.
	Per cent.	Per cent.	Per cent.
Fat,	0·82	1·456	1·40
Casein,	...	0·557	2·80
Albumin,	...	0·490	
Sugar,	...	0·956	6·40
Salts,	0·05	0·826	
Water,	96·30	95·705	89·40
Authority,	Schlossberger and Hoff.	V. Genser.	Gubler and Quevenne.

[4] Schlossberger (*Ann. Chem. Pharm.*, li. 431) has described a sample of milk from a he-goat which had an alkaline reaction and contained 9·6 per cent. of proteids and insoluble salts, 2·65 of butter, and 2·6 per cent. of lactose and soluble salts.

the substratum then appears less white and opaque than the original milk. The separation of cream is effected far more readily and perfectly by the use of a centrifugal separator (page 139). On filtering milk under pressure through a diaphragm of porous earthenware, the filtrate is not only free from fat-corpuscles but also from casein.

It was long supposed that the globules of fat in milk were contained in a solid albuminous envelope which prevented them from coalescing, and this view received support from the difficulty attending the extraction of fat from milk by agitation with ether, unless an alkali were previously added. This difficulty was, however, exaggerated by the earlier observers, who also failed to recognise the fact that the fluid remained cloudy after complete removal of the fat, owing to the presence of particles of casein, &c. The existence of such a membranous envelope is highly improbable on several grounds, and may be regarded as conclusively disproved.

Microscopic Characters of Milk.—Under the microscope milk appears as a clear fluid, conveniently called milk-plasma, having an immense number of minute globules of fat floating in it. These fat-globules vary much in size, the largest being (in the case of cows' milk) fully six times the diameter of the smallest, which latter have a diameter of about 0·00165 millimetre. The smaller globules are far more numerous than the large, and it has been suggested that the number of any particular size is accurately in inverse proportion to their diameter.

A careful microscopic examination of normal milk will detect, besides fat-globules, minute particles of casein and other proteid matters. Colostrum may be recognised by the presence of comparatively numerous cells from the acini of the gland, containing fat-globules which they have not yet liberated by disintegrating.

Blood-corpuscles are occasionally present in milk, and the bacteria characteristic of certain zymotic diseases may be found in milk which has been exposed to infection.

Reaction of Milk.—Human milk always exhibits an alkaline reaction to litmus, and the same is true of the milk of the herbivora when perfectly fresh, but the milk of carnivorous mammals has an acid reaction. Cows' milk often exhibits the so-called *amphoteric reaction*, appearing at once acid and alkaline. This behaviour is commonly attributed to the alkali-metal phosphates of the milk, but the facts require re-investigating with the aid of modern indicators of neutrality. Even when perfectly fresh, cows' milk exhibits an acidity to phenol-phthalein equivalent to 0·1 per cent. or more of sulphuric acid.

The acid reaction of milk gradually increases in intensity, owing to the formation of lactic acid, until a slight rise of temperature, or treatment with carbon dioxide gas, suffices to cause coagulation of the casein. At a later stage, coagulation occurs without any such provocation.

General Composition of Milk.

In chemical composition, milk is qualitatively of tolerably constant character, but the proportions of the leading constituents vary greatly with the kind of animal, and even with individuals of the same species.

The leading constituents of milk are water, milk-fat, and non-fatty solids.

Water in every case forms the most abundant constituent of milk. The total proportion present may be determined with a near approach to accuracy by evaporating a weighed quantity (*e.g.*, 5 grammes) of the milk at 100° C., drying the residual "total solids" till approximately constant in weight, and deducting the amount thus found from the weight of milk employed. Further details respecting this important determination will be found on page 131.

Milk-Fat is well-known as the most valuable constituent of cream and butter. Its colour ranges from nearly white to a bright yellow, the tint varying with the nature of the food eaten by the animal and other circumstances. When kept for some time, and exposed to light and air, the colour of milk-fat is destroyed, and the substance becomes rancid.

The melting point of the fat of cows' milk varies considerably, but is generally between 30° and 40° C.

Milk-fat resembles other animal fats in being a glyceride, and hence yields glycerol and fatty acids on saponification.[1] But it is peculiar in containing a considerable proportion of the glycerides of lower fatty acids of the acetic series, among which that of butyric acid, $C_4H_8O_2$, largely predominates. In fact, the presence of notable quantities of butyrin, the glyceride of butyric acid, distinguishes milk-fat from all other fatty oils, whether of animal or vegetable origin.[2] This peculiarity of composition gives to milk-fat a peculiar interest and value as a food, while affording a means by which the analyst can distinguish true butter from butter-substitutes. The composition

[1] Cholesterin is a constant constituent, or product of the saponification, of milk-fat. It can be extracted in the proportion of about 1 per cent. by agitating the aqueous solution of the saponified fat with ether.

[2] In the fat of the porpoise, the butyric acid of ordinary milk appears to be replaced by valeric acid, $C_5H_{10}O_2$, and the same is not improbably the case with the milk-fat of other marine mammals.

and properties of milk-fat, and the detection of butter-substitutes, were fully described in the article on "Butter" (Vol. ii. p. 145).

THE NON-FATTY SOLIDS of milk consist essentially of milk-sugar or lactose, proteids, and mineral matters.[1] Small quantities of citric acid, and a starch-converting enzyme are also present, with minute traces of cholesterin, urea, lecithin, hypoxanthine, creatine, leucine, tyrosine, &c. The non-fatty solids of cows' milk are far more constant in amount than is the case with the milk-fat, rarely falling below 8·5 per cent., and averaging 8·75 per cent. Their relative proportions are also very constant in normal cows' milk, being stated by P. Vieth at 2 of mineral matter to 9 of proteids and 13 of sugar (*Analyst*, xvi. 203).

Milk-sugar or *lactose* belongs to the saccharose family, containing, when anhydrous, $C_{12}H_{22}O_{11}$, though it commonly crystallises with a molecule of water Lactose is dextro-rotatory ($[a]_D = +55{\cdot}27°$, for the anhydrous substance), reduces alkaline cupric solutions on boiling, undergoes the alcoholic fermentation with difficulty, and is hydrolysed by boiling with dilute mineral acids into the two glucoses dextrose and galactose.

G. Denigès (*Jour. Pharm. et Chem.*, xxvii. 413) has prepared the sugar of human milk in a state of purity and compared it with the sugar from the milk of the ass, mare, cow, goat, ewe, and bitch. The sugars were isolated and purified by repeated crystallisation, and their composition, molecular weights, crystalline form, specific rotation, and cupric oxide reducing-powers carefully determined. As the result of this research, Denigès concludes that the sugar from all the above sources was absolutely identical.[2]

Although lactose is the sugar specially characteristic of milk, its occurrence is not strictly confined to that secretion.[3] Thus milk-sugar has been found in the urine of nursing women by Biot, Kaltenbach, Sinetz, and others (compare page 101).

The characters and manufacture of milk-sugar are described more fully in the sequel.

The *proteids* of milk have already been fully described (page 86). The determination of milk-sugar, fat, and other constituents of milk is fully detailed in the sequel.

The composition of the *mineral matters* contained in milk is considered on page 101.

[1] The milk of the cat was found by Commaille to be acid in reaction and to contain a notable quantity of lactic acid, which in the carnivora appears partly to replace the lactose characteristic of the milk of herbivorous mammals.

[2] Pappel and Richmond state that the sugar of gamoose-milk is distinct from lactose.

[3] According to Bouchardat, lactose occurs in the fruit of Sapotillier (*Achras sapota*).

THE GASES contained in fresh milk are chiefly nitrogen and oxygen, more or less nearly in the proportion in which they exist in the atmosphere, with smaller quantities of carbon dioxide. The free oxygen rapidly undergoes absorption, and the proportion of carbon dioxide enormously increases, probably owing to a fermentative change. The actual figures representing the volumes of gases contained in milk have no interest apart from the circumstances under which the sample was examined.

The following table illustrates the composition of the milk of various mammals. Human milk, cows' milk, and other milks of practical interest are further considered in separate sections.

Animal.	Fat.	Proteids.	Sugar.	Ash.	Water.	Total Solids.	Non-fatty Solids.	Observer.
Woman,	4·13	2·00	6·94	0·20	86·73	13·27	9·14	A. B. Leeds.
Ass,	1·85	3·58	5·04	·52	89·01	10·99	9·14	Vernois & Becquerel.
Mare, mean of 14,	1·05	1·95	6·28	·40	90·32	9·68	8·63	C. A. Cameron.
,, mean of 15,	1·09	1·89	6·65	·31	90·06	9·95	9·86	P. Vieth.
,, average,	·50	1·67	6·74	·41	90·68	9·32	8·82	J. Muter.
,,	1·76	3·58	5·87	·39	88·40	11·60	9·84	J. Bell.
,,	·39	2·45	5·99	·31	91·76	8·24	7·85	H. Gerber.
,, 5 years old, 10 weeks after foaling,	1·27	1·50	5·75	·27	91·15	8·85	7·58	M. Schrodt.
,,	1·16	1·87	7·32	·36	89·29	10·71	9·55	Landowski.
,, mean of 3,	1·31	2·55	5·43	·29	90·42	9·58	8·27	Biel.
Cow (average),	4·02	...	...	...	87·14	12·86	8·84	Vieth & Richmond.
Buffalo, Transylvanian,	9·02	3·99	4·50	·77	81·62	18·38	9·36	F. Strohmer.
,, Egyptian (Gamoose),	4·34	3·39	4·94	·85	85·89	14·11	9·77	Pappel & Richmond.
Sheep, average,	5·72	...	...	·94	82·22	17·78	12·06	S. Macadam.
,, ,,	7·50	...	...	·87	80·57	19·43	11·93	S. Macadam.
,,	11·28	8·83	3·58	1·09	75·22	24·78	13·50	J. Bell.
,, rich,	12·78	6·58	4·66	·98	75·00	25·00	12·22	A. Voelcker.
,, poor,	2·16	5·59	4·93	1·20	86·12	13·88	11·72	A. Voelcker.
,, colostrum,	2·75	17·37	8·85	1·29	69·74	30·26	27·51	A. Voelcker.
Goat, average,	4·41	...	...	·73	86·57	13·43	9·02	S. Macadam.
,, mean of 3,	6·82	3·93	5·06	·86	83·33	16·67	9·85	A. Voelcker.
Llama,	3·15	·90	5·60	·80	89·55	10·45	7·30	Doyére.
Camel,	2·90	3·84	5·66	·66	86·94	13·06	10·16	Chatin.
Sow, mean of 8,	4·55	7·23	3·13	1·05	84·04	15·96	11·41	Various.
,, mean of 2,	5·83	6·18	5·33	·90	81·76	18·24	12·41	C. A. Cameron.
Hippopotamus,	4·51	...	4·40	·11	90·98	9·02	4·51	Gunning.
Elephant, mean of 3,	19·64	3·91	19·64	·63	68·00	32·00	12·36	C. A. Doremus.
Bitch, average,	9·57	9·91	3·19	·73	76·60	23·40	13·83	Various.
,, ,,	8·80	...	1·53	·78	...	...	...	Vernois & Becquerel.
,, mean of 2,	...	9·75	...	1·30	72·40	...	...	G. Bunge.
Cat,	3·33	9·55	4·91	·58	81·62	18·38	15·04	Commaille.
Porpoise,	45·80	11·19	1·33	·57	41·11	58·89	13·09	Purdie.

In addition to the constituents mentioned in the table, Pappel and Richmond found in the milk of the *gamoose* (*Bos bubalus*) 0·30 per cent. of citric acid, and 0·09 per cent. of nitrogenised bases; but as the amount of the latter constituents was calculated from the difference between the total nitrogen and that existing as casein and albumin no great reliance can be placed on the result.

According to Sir C. A. Cameron, no cream can be obtained from *sow's milk*. On drying at a steam-heat, sow's milk exhales the odour of roast pork, and on putrefying that of putrid bacon.

The lacteal fluids secreted by newly-born children and (occasionally) by adult males are described on page 96.

A peculiar milky fluid is secreted by the mucous membrane of the crop of the *pigeon* and certain other birds, for a few days after the chicks emerge from the eggs. The author is informed on excellent authority that the mother-bird feeds her young with this fluid during the first few days of their life. Leconte analysed this "pigeon's milk" and found it to contain 23·23 per cent. of casein and salts, 10·47 of fat, and 66·30 per cent. of water. At other times, the crop secretes a feebly alkaline liquid possessing no nutritive properties or fermentative action.

The Cow-tree, *Brosimum galactodendron*, a native of Central America, yields a juice closely resembling animal milk, and containing, according to an analysis by Boussingault:—fat, 35·2; proteids, 4·0; sugar, &c., 2·8; water, 58; and mineral and undetermined matters, 2·3 per cent. According to L. von Italie, the "milk" of the cocoa-nut has a specific gravity of 1·022, and possesses a faintly acid reaction. No proteids are present, and the sugars are dextrose and sucrose.

Mineral Constituents of Milk.

From the foregoing data it is evident that the proportion of mineral matters in milk varies considerably, being lowest in woman's and mare's milk, higher in the milk of the cow, and still higher in that of the sheep. On the other hand, the constituents of the ash are much the same in all cases.

The following is the average composition of the ash of cows' milk according to Schrodt:—

K_2O.	Na_2O.	CaO.	MgO.	Fe_2O_3.	P_2O_5.	SO_3.	Cl.	O=Cl.
25·42	10·94	21·45	2·54	0·11	24·11	4·11	24·11	3·28 per cent.

Weber and Haidlen have published the following analyses of the ash of four samples of cows' milk:—

	Minimum.	Maximum.	Mean of 4.
Potash,	17·09	33·25	24·67
Soda,	8·60	11·18	9·70
Lime,	17·31	27·55	22·00
Magnesia,	1·90	4·10	3·05
Ferric oxide,	0·33	0·76	0·53
Phosphoric acid (P_2O_5),	27·04	29·13	28·45
Sulphuric acid (SO_3),	...	...	0·30
Chlorine,	9·87	16·96	14·28

The following figures have been published by James Bell (*Food and its Adulterations*, 1883) as representing the composition of the ash of milk from various animals. It will be observed that the proportions of alkalies are considerably lower, and that of phosphoric acid materially lower, than in the analyses previously quoted.

	Mixed Milk from 29 Cows.	Mixed Milk from several Cows.	Woman's Milk.	Goat's Milk.	Mare's Milk.	Ewe's Milk.
Potash, . . .	17·24	19·53	30·80	16·98	16·35	11·42
Soda, . . .	4·29	3·30	3·26	2·67	2·77	1·56
Lime, . . .	24·53	24·48	18·47	25·69	35·19	36·32
Magnesia, . .	2·89	4·76	3·98	4·57	3·40	4·68
Phosphoric acid, P_2O_5,	35·67	32·49	23·93	42·28	32·73	38·99
Sulphuric acid (SO_3),	2·65	0·92	7·97	2·23	3·08	3·32
Chlorine, . .	12·73	14·52	11·59	5·58	6·48	3·71
	100·00	100·00	100·00	100·00	100·00	100·00
Percentage of total ash in milk, .	0·73	0·72	0·29	1·00	0·39	1·09

The following analyses of the ash of milk have been published by Bunge (*Dorpat Dissertation*, 1874). The percentage composition of the ash may be found by multiplying the figures in the table by 100·00 and dividing the products by the total ash per 1000 parts of the milk.

	Human Milk.		Dog's Milk.		Cow's Milk.	Horse's Milk.
	A.	B.	A.	B.		
Potash, ...	0·78	0·71	1·41	1·68	1·76	1·04
Soda, ...	0·23	0·26	0·80	0·69	1·11	0·14
Lime, ...	0·33	0·34	4·53	4·28	1·59	1·23
Magnesia, ...	0·06	0·06	0·19	0·21	0·21	0·12
Ferric oxide, ...	0·003	0·006	0·02	0·01	0·003	0·005
Phosphoric acid, . . .	0·47	0·47	4·93	4·67	1·97	1·31
Chlorine, ...	0·43	0·44	1·62	1·80	1·69	0·31
Total ash per 1000 of milk, .	2·22	2·18	13·15	12·96	7·97	4·17

Phosphoric acid was found to be invariably the chief acid. Of the bases, potash predominated in human milk, but in the other cases lime was in larger amount. In the milk of the dog the proportion of lime was remarkably high.

Bunge found that, while the majority of the constituents of the ash of milk are present in the same proportions as they are contained in the fœtal tissues (of the dog), the proportion of iron in 100 parts of the ash of the milk was only one-sixth of the percentage present in the ash of the fœtus.

Richmond has determined the ratio of the ash to the total solids of cows' milk as deduced from the analysis of 135 genuine samples (*Analyst*, xix. 82). The ash ranged from 7·8 to 9·4 for 100·0 of solids. In a number of instances the ashes were found to have an alkaline reaction to litmus, turmeric, and phenol-phthalein. In cases where the alkalinity was determined it reached a maximum of 0·025 per cent., expressed in terms of sodium carbonate. The following determinations were also made :—

No.	Sol. Ash.	Insol. Ash.	Na_2CO_3.	P_2O_5.	CaO.	Percentages on Insol. Ash.	
						P_2O_5.	CaO.
1	·26	·54	·025	...	...	...	...
2	·24	·53	·010	·228	·166	43·0	31·3
3	·28	·48	·020	·205	·152	42·5	31·7
4	·23	·55	·012	·226	·132	41·1	24·0

The soluble ash consisted of chlorides of alkali-metals, and the carbonates to which the alkalinity was due, with only very faint traces of phosphates. The insoluble ash is regarded by Richmond as a double phosphate of the formula $(Ca,Mg)(K,Na)PO_4$.

The composition of the ash of milk does not correctly represent the salts present in the original milk, since the lactates and other organic salts are decomposed on ignition with formation of carbonates, which are in turn converted into phosphates and sulphates by the phosphoric and sulphuric acids produced by the oxidation of the proteids. The phosphates existing as such in milk are to a certain extent acid phosphates, and to the presence of these compounds the acid reaction exhibited by cows' milk to phenol-phthalein is attributable. Cows' milk, when fresh, and in many cases for some time after, has an alkaline reaction to litmus, a behaviour which is probably due to citrates and to phosphates of the formula M_2HPO_4. The practice of calculating the acidity of milk to phenol-phthalein into lactic acid is therefore incorrect.

According to Duclaux (abst. *Analyst*, xviii. 149), the phosphates existing in milk are present partly in solution and partly in suspension. Those in suspension are the phosphates of iron, magnesium, and calcium, while those dissolved are nearly equal molecular proportions of sodium phosphate and calcium phosphate (held in solution by sodium citrate). From the examination of various kinds of milk, Duclaux concludes that the calcium phosphate in suspension is to that in solution as about 2 to 1, and that the composition of the ash of milk of the most diverse origins is almost identical. Addition of phosphates to the fodder did not affect the proportion of phosphates in the milk produced.

Concretions consisting chiefly of calcium carbonate, with small quantities of phosphates and fat, are occasionally met with in the teats of cattle.

Human Milk.

Human milk is more bluish than cows' milk,[1] and resembles the milk from the mare and ass more closely than the product from ruminants. The specific gravity is usually between 1030 and 1034, the extreme ranges recorded being between 1025·6 and 1046·5. Human milk usually contains from 11 to 14 per cent. of solids, and is remarkable for its high proportion of sugar and low proportion of casein and ash as compared with cows' milk.[2] It is not readily curdled, the casein never separating in a compact clot which settles to the bottom.[3] For this reason, milk from the ass or mare is preferable to that from the cow as a substitute for human milk, for if a child be fed on cows' milk the liquid is apt to curdle on reaching the stomach, a change which is often followed by vomiting.[4] Traces of urea are stated to occur fre-

[1] A. B. Leeds finds that the colour of human milk, whether bluish-white, chalky white, whitish, yellowish-white, or yellow, is no indication of its composition.

[2] Although the percentage of sugar is 2 per cent. higher than in cows' milk human milk is rarely sweet to the taste. Leeds describes it as having a more or less saline, somewhat disagreeable, animal flavour. Woman's milk has a thinner consistence than cows' milk.

[3] Lehmann and Hempel attribute this difference to the fact that the proportion of fat to casein in the curd from cows' milk is much lower (1·16 : 1·0) than that in the precipitate from human milk (3 : 1). They find that, by the addition of fat, cows' milk behaves like human milk on adding acid.

[4] According to MacNaught (*Pharm. Jour.*, [3], xvii. 505), human milk is not curdled by acid so dilute as that which occurs in the human stomach, whilst cows' milk, even when diluted with water, is readily curdled

quently in human milk, and an odorous principle is also present.

According to Lehmann and Hempel (*Arch. Physiol.*, 1894, lvi. 558), the *casein* of human milk is a substance quite distinct from that of cows' milk, differing from it in the proportions of sulphur, phosphorus, and ash. They state the ash of the casein of cows' milk at 7·2 per cent., while that of human milk only averages 3·2 per cent. The casein of human milk is said to contain 1·09 per cent. of sulphur, against 0·72 per cent. in the casein of cows' milk.

W. G. Ruppel (*Zeit. Biol.*, xxxi. 1; abst. *Jour. Chem. Soc.*, 1894, ii. 326) states that the fatty acids resulting from the saponification of the *fat* of human milk are butyric, caproic, capric, myristic, palmitic, stearic, and oleic, all of which occur as glycerides. The presence of formic acid was inferred from the reducing action of the fatty acids. The fat of human milk is very poor in volatile acids.

E. Laves (*Zeit. physiol. Chem.*, xix. 369; abst. *Jour. Chem. Soc.*, 1894, ii. 392) found that the *fat* of human milk yielded on saponification 1·4 per cent. of volatile acids, 1·9 per cent. of acids soluble in water, and 49·4 per cent. of oleic and other unsaturated acids. The volatile acids were composed of caproic, caprylic, and capric acids in about equal quantities, with only the merest trace of butyric acid. The fatty acids present in largest proportion were palmitic, stearic, and oleic; with one or more acids of lower molecular weight, probably myristic acid. The melting point of these mixed acids lies between 37° and 39° C., while the fat itself melts at 30° to 31°.

The *sugar* of human milk is identical with that of cows' milk.

A. B. Leeds (*Chem. News*, l. 263, 280, 281) has published a valuable paper on the composition and methods of analysis of human milk, in which he gives the following figures as represent-

by it. When acted on by rennet-ferment, human milk only gives a few shreds of curd, whilst cows' milk under the same conditions sets to a firm coagulum. MacNaught concluded from his experiments that in infantile dyspepsia there is an increase in the coagulative power of the juices of the stomach, as well as a decrease in their digestive power, and that the curdy masses sometimes vomited are produced by the rennet-ferment with which the milk comes in contact. He therefore recommends the addition to cows' milk, when required for the use of infants, of one-third of its volume of "good" lime-water, which treatment not only prevents coagulation by rennet, but appears to enfeeble or destroy the rennet-ferment. Boiling also lessens the coagulability of cows' milk, and an admixture with arrowroot, after addition of the lime-water, reduces it to a minimum.

ing the average composition of eighty-four samples of human milk:—

	Maximum.	Minimum.	Average.
Specific gravity,	1035·3	1026·8	1031·3
	Per cent.	Per cent.	Per cent.
Fat,	6·89	2·11	4·13
Proteids,	4·86	0·85	2·00
Sugar,	7·92	5·40	6·94
Ash,	0·37	0·13	0·20
Water,	89·08	83·21	86·73
			100·00
Total solids (by evaporation),	16·66	10·91	13·27
Total solids (sum of constituents),	16·79	10·92	13·27
Non-fatty solids,	12·09	6·57	9·14

Leeds states that the milk of women under the age of twenty is richer in each and every constituent than that of older women. The proteids average 2·18 per cent. during the first lustrum, falling to 1·92 for the second and 2·10 for the third. In the first lustrum the sugar is 7·17 per cent., falling to 6·91 in the second, and to 6·77 in the third lustrum.

The following figures show the composition of woman's milk, as deduced by König (*Zusammensetzung d. menschichen Nahrung und Genussmittel*, 3rd edition) from the analysis of 107 samples. The average composition agrees fairly with the results of Leeds.

	Maximum.	Minimum.	Average.
	Per cent.	Per cent.	Per cent.
Fat,	6·83	1·43	3·78
Proteids,	4·70	0·69	2·29
Sugar,	8·34	3·88	6·21
Ash,	1·90 (?)	0·12	0·31
Water,	91·40	81·01	87·41

Lehmann and Hempel (*Arch. Physiol.*, 1894, lvi. 558; abst. *Analyst*, xix. 206) give the following figures as representing the average composition of human milk:—Fat, 3·8 per cent.; casein, 1·2; albumin, 0·5; sugar, 6·0; ash, 0·2; and water, 88·5 per

cent. Care was taken that the women were healthy, and that their glands were fully milked.

The following analyses show the percentage composition of human milk, as recorded by various other observers :—

	Fat.	Proteids.	Sugar.	Ash.	Water.	Authority.
Normal Milks.						
Average, . . .	2·90	3·07[1]	5·87	·16	88·00	A. W. Blyth.
,, . . .	3·68	1·70	7·11	·20	87·31	Marchand.
,, . . .	2·67	3·92	4·37	·14	88·90	Vernois & Becquerel.
,, . . .	3·52	2·01	5·91	...	...	Hammarsten.
Fourteen analyses from same woman, . .	2·53	3·42	4·82	·23	88·36[2]	Simon.
Average, . . .	3·55	1·52	6·50	·45	87·98	Chevalier & Henry.
Mean of 6, aged from 23 to 33, . . .	3·82	2·04	5·93	·42	87·79	H. Gerber.
From woman aged 18, .	3·20	2·39	6·83	·29	87·29	J. Bell.
,, ,, 33 .	2·99	2·51	6·51	·30	87·69	,,
Arab woman, . .	5·31	2·16	6·41	·20	85·92	Richmond.
Four days after delivery,	4·30	3·53	4·11	·21	87·55	Clemm.
Nine ,, ,,	3·53	3·69	4·30	·17	88·31	,,
Twelve ,, ,,	3·34	2·91	3·15	·19	90·41	,,
Abnormal Milks.						
From wet-nurse A., .	6·22	1·38	7·29	·24	84·87	C. Krauch.
,, ,, B., .	1·98	0·75	7·04	·18	90·05	,,
Minimum, . . .	0·67	...	2·52	·05	...	Vernois & Becquerel.
Maximum, . . .	5·64	5·67	5·95	·34	...	,, ,,
Colostrum, . . .	5·00	4·00	7·0	...	82·80[2]	Simon.
,, . . .	5·78	3·23	6·51	·33	84·05	C. M. Tidy.
Immediately after attack of hysteria, . .	·51	5·00	3·49	1·01	89·98	Vogel.
In case of enlargement of the breasts, . .	8·54	8·74	·75	·41	...	Schlossberger.
Four years after birth of last child, . .	2·5	...	...	·2	88·7	H. D. Richmond.

The wet-nurse B., whose milk was analysed by Krauch, was in the eleventh month of lactation, and the child suckled suffered severely from abscesses. According to Marchand, the milk becomes injurious if the fat exceeds about 5 per cent., and the normal proportion of proteids cannot be exceeded with impunity. Yet his casein is lower than is found by any observers except Chevalier and Henry. This may be due to the method of determination employed, for Nencki has shown that the proteids of human milk obtainable by precipitation show a mean of 1·41 per cent., while, if deduced from the nitrogen, the amount found averages 2·53 per cent. Similar discrepancies do not occur with cows' milk. The ash considered normal by these chemists and by König exceeds the maximum found by Vernois and Becquerel, who found 0·18 of ash in the milk from women under twenty, the proportion gradually decreasing to 0·10 in the milk of women between

[1] The proteids in Blyth's analysis are stated to have consisted of casein, 2·40 ; albumin, ·57 ; and galactin, 0·10 per cent.

[2] These figures do not amount to 100·00.

thirty-five and forty. According to the last observers, in nineteen cases of acute disease the average percentage of casein was 5·04, the limits being 3·29 and 5·67; and in twenty-seven cases of chronic disease the range was from 2·52 to 3·99, with an average of 3·26 per cent. of casein. In the milk of a woman suffering from an enlarged breast, Schlossberger found 28·5 per cent. of fat. According to Vernois and Becquerel, the fat increases during the first two months, decreasing again after five or six. In disease, they found the proportion of fat sometimes increased and sometimes diminished. Thus in delirium and fever it was 0·51; in typhus, 0·91; in pleurisy, 2·77; in enteritis, 3·15; and in colitis, 5·41 per cent.

According to L'Heretier, the milk of blondes contains less sugar than that of brunettes, but Vernois and Becquerel found no substantial difference. The latter observers found the proportion of sugar to decrease during the first month after delivery, but that it rises again considerably from the eighth month to the tenth.

J. Szilasi (*Chem. Zeit.*, xiv. 1202; abst. *Jour. Chem. Soc.*, 1892, p. 517) has tabulated the results of the analysis of thirty-six samples of human milk. As showing how the composition of woman's milk varies, Szilasi gives the following examples :—

	Specific Gravity.	Fat.	Proteids.	Sugar.	Ash.	Total Solids.	Remarks.
A.	1·0329	1·00	1·26	7·35	0·20	9·81	Woman aged 30; strong constitution; milk abundant; fourth child, aged 8 months.
B.	1·0318	4·13	1·99	7·14	0·19	13·45	Woman aged 25; weak constitution; milk deficient; child 20 days old.

Referring to the variations in the composition of human milk as recorded by different observers, A. Wynter Blyth remarks that the samples analysed very probably did not represent the average secretion. He considers it impracticable to obtain a complete sample of human milk by any mechanical appliance.

Humanised Milk.—Several brands of condensed milk are now manufactured in such a manner that when diluted they shall have a composition closely resembling that of normal human milk. These preparations are highly recommended, and apparently with justice, as substitutes for woman's milk (see Condensed Milk). The Aylesbury Dairy Company prepare two modifications of unconcentrated milk of the same character, which, according to analyses by H. D. Richmond communicated to the author, have the following

composition. No. 1 is used for a first food and for very delicate infants; No. 2 for children after the first few weeks. An analysis of peptonised milk prepared by the Aylesbury Dairy Company is added for comparison.

	Total Solids.	Fat.	Coagulable Proteids.	Albumoses.	Sugar.	Ash.
						Per cent.
Humanised milk, No. 1,	10·57	4·05	1·33	...	4·70	0·49
,, ,, No. 2,	11·70	3·67	2·23	...	5·22	0·58
Peptonised milk, .	8·80	2·60	0·98	1·45	3·22	0·55

By mixing cows' milk with half its volume of a solution containing 12·3 per cent. of milk-sugar, Soxhlet obtains a product which contains 2·46 per cent. of fat, 2·37 of proteids, 9·40 of milk-sugar, 0·47 of ash, and 85·30 per cent. of water; and which contains, in a given volume, the same quantities of food-constituents as human milk, with the exception that one-third of the fat is replaced by an equivalent (isodynamic) quantity of milk-sugar (*Pharm. Jour.*, [3], xxiii. 787).

According to A. Worcester, the Dresden method of extemporising an imitation human milk consists in slowly and gradually mixing the white of one fresh egg with 50 grammes of milk-sugar, and to the resultant paste gradually adding 1½ pint of water, with constant stirring. The emulsion thus obtained is strained through linen into a pint of milk, and the whole well mixed by agitation.[1]

[1] Coulier prepares an imitation of human milk from 600 grammes cows' milk, 339·5 c.c. of water, 13 grammes of cream, 15 grammes of milk-sugar, and 1·5 gramme of calcium phosphate. Frankland gives the following recipe:—Heat half a pint of skimmed cows' milk to 35° C., and add rennet. After ten to fifteen minutes, break the curd up finely, strain the whey off, and boil it, with the addition of 110 grains of milk-sugar. Strain again, and add the filtrate to a pint of fresh cows' milk, with two teaspoonfuls of cream. This concoction is directed to be made every twelve hours.

Lehmann and Hempel give the following recipe for the manufacture of an artificial human milk:—Dilute cows' milk with water until it contains only as much casein as human milk, and then add cream, milk-sugar, and white of egg until the mixture contains the proportions of fat, sugar, and albumin as in human milk. The white of egg should be added in somewhat larger proportion if the suckling is to be fed from the first, since human colostrum, like that of the cow, is rich in albumin.

Lehmann has produced a "vegetable milk" which is mixed with cows' milk as a substitute for mother's milk. It is prepared from nuts and

Milk of Herbivora.

A. Wynter Blyth (*Foods: their Composition and Analysis*) gives the following figures as representing the average composition of the milk of leading herbivora. The analyses are more detailed than is usual, but in the case of cows' milk, at any rate, the figures are not in close agreement with the results of Vieth and other observers.

	Ass.[1]	Mare.	Sheep.	Goat.	Cow.
Milk-fat,	1·02	2·50	5·30	4·20	3·50
Casein,	1·09	2·19	6·10	3·00	3·98
Albumin,	0·70	0·42	1·00	0·62	0·77
Galactin,	0·10	0·09	0·13	0·08	0·17
Milk-sugar,	5·50	5·50	4·20	4·00	4·00
Ash,	0·42	0·50	1·00	0·56	0·70
Water,	91·17	88·80	82·27	87·54	86·87
	100·00	100·00	100·00	100·00	99·99
Total solids,	8·83	11·20	17·73	12·46	13·13
Solids not fat,	7·81	8·70	12·43	8·26	9·63.
Total proteids,	1·89	2·60	7·23	3·70	4·92

The milks of the ass and mare are remarkable for the large proportion of milk-sugar which they contain. They differ from the

almonds with sugar, and forms a fatty emulsion with a finely flocculent casein like that of human milk. At the National Foundling Hospital at Vienna this mixture is stated to have given very favourable results. Another human milk substitute is Gärtner's *Fettmilch* (Fat-milk, D.R.P., No. 82,510). Its composition, as compared with cows' and women's milk, is given as follows :—

	Fat.	Casein.	Sugar.	Ash.
Women's milk,	3·78	1·03	6·61	·31
Gärtner's fat-milk,	3·20	1·42	5·15	·33
Cows' milk,	3·69	3·02	4·88	·71

[1] In the case of the *asses' milk* the animals were fed on a uniform diet of bran, hay, and oats, the yield of each milking was carefully noted, and the animal in each case milked dry. The yield for commercial purposes did not exceed 2½ to 3 pints daily, the excess being used by the foal. The yield of a single milking ranged from 300 to 400 c.c.

milk of the ruminants in the fact that the casein is not readily curdled by acetic or lactic acid, a fact which renders them specially suitable as substitutes for human milk.

According to C. Besana (abst. *Analyst*, xviii. 248), a sheep ordinarily yields from 45 to 50 litres of milk per annum, but Fleischmann states the range at from 25 to 140 litres, the yield varying greatly with different individuals. The average composition of the milk from three flocks of Italian sheep, the total number of which was 7200, was found by Besana to be as follows :—

Fat,	9·50 per cent.
Proteids,	6·26 „
Sugar,	5·00 „
Ash,	1·01 „
Water,	78·23 „
	100·00
Total solids,	27·77 „
Solids not fat,	18·27 „
Specific gravity,	1·0378

From these figures it appears that the proportion of fat in sheep's milk is about two and a half times as great as that in cows' milk. The proteids are also higher. The large proportion of fat gives sheep's milk a greasy feel, a character which is also possessed by all its products.

Owing to its great density and viscosity, sheep's milk throws up no cream even in forty-eight hours. If diluted with an equal measure of water it yields 14 per cent. of cream in twenty-four and 20 per cent. in forty-eight hours, after which time no more cream rises.

Sheep's milk does not curdle nearly so soon as cows' milk. When curdled with ordinary rennet, the cheese possesses the peculiar odour of the sheep; but if purified rennet (obtained from the ordinary kind by precipitation with common salt) be used, the cheese is indistinguishable from that made from cows' milk.

Besana's paper also contains a description of the butter from sheep's milk, a table for correcting the density of the milk for variations in temperature, and a statement of the effect of various degrees of dilution on the gravity.

A number of complete analyses of *mares' milk* have been recorded by P. Vieth (*Analyst*, x. 218).

The following data relating to the composition of *asses' milk* have been communicated to the author by H. D. Richmond :—

	A.	B.	C.	D.
Milk-fat,	1·22	1·23	1·16	1·11
Proteids,	1·66	...	1·60	1·79
Milk-sugar,[1]	6·37	...	6·91	6·80
Ash,	0·43	0·47	0·46	0·44
Water,	...	...	...	...
	100·00	100·00	100·00	100·00
Total solids,	10·16	10·49	40·13	10·14
Non-fatty solids,	8·96	9·26	8·97	9·03
Specific gravity,	1·0352	...	...	1·03485

The specific gravity of one sample was 1·03840 one hour after milking, and 1·03845 after twenty-four hours.

Asses' milk is white, looks thin and watery, and has a sweet taste. If not quite fresh, boiling causes the coagulation of a portion of the proteids, but the milk remains quite palatable.

Rennet has no immediate action on asses' milk, but eventually causes it to set to a soft, thin curd. Acids throw down the proteids as a very finely-divided precipitate, which is coagulated on boiling.

The fat-globules of asses' milk are about the same average size as those of cows' milk, but somewhat more uniform.

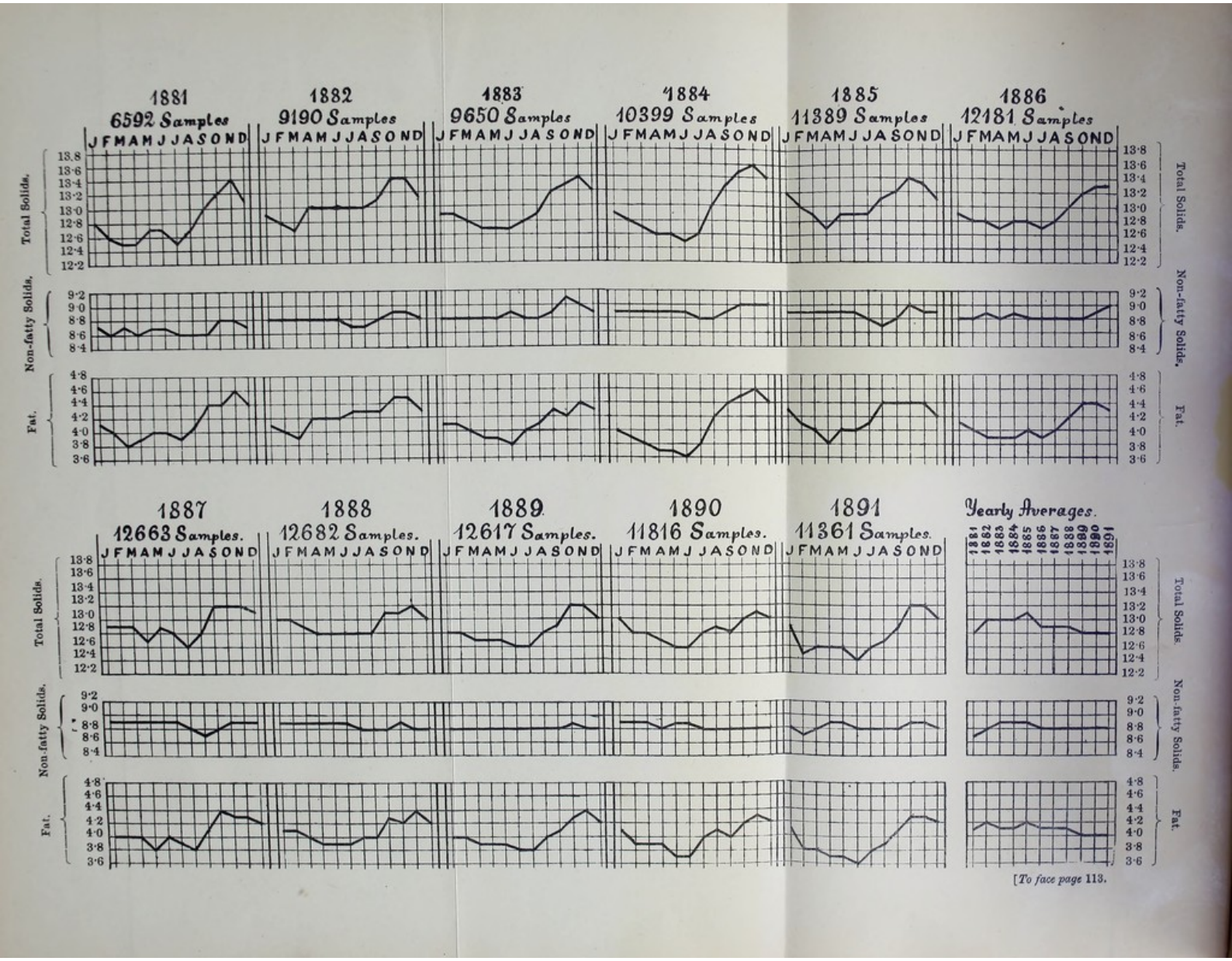
Aubert and Colby (*Chem. News*, lxviii. 168) have published the analyses of two samples of milk yielded by a *mule*, eleven years old, in work, fed on oats and hay, and yielding about two quarts of milk daily.

W. H. Ince has also published (*Pharm. Jour.*, 1896, ii. 176) the following results of the analysis of a sample of milk from a Kentucky *mule* which yielded from five to six quarts a day for several weeks. The milk coagulated slowly with alcohol and hydrochloric acid, and the ash contained a considerable quantity of phosphates but only a trace of chlorides.

Sp. Gravity.	Fat.	Proteids.	Sugar.	Ash.	Total Solids.	Authority.
1·032	1·86	2·94	...	...	10·65	Aubert & Colby.
1·033	1·98	2·31	6·03	0·53	10·86	,, ,,
1·028	1·10	2·19	5·50	0·49	9·28	W. H. Ince.

The milk of the cow is considered in the following section.

[1] The sugar of asses' milk has been identified with lactose. It was prepared by Richmond by precipitating the proteids by acid mercuric nitrate, rendering the filtrate neutral to phenol-phthalein, and precipitating the mercury by sulphuretted hydrogen. On boiling the filtered liquid a precipitate of mixed calcium salts was obtained, and on concentration the milk-sugar crystallised out. By fractional precipitation with alcohol, a small quantity of a dextrinoid body was separated. It was not obtained pure, but appeared to have a low optical activity. The mother-liquors obtained by recrystallising the milk-sugar were inactive or slightly lævo-rotatory.



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Cows' Milk.

The composition of the normal milk of the cow has been established by an enormous number of analyses.[1] Unfortunately, many of these were made some years ago by methods of doubtful accuracy, and the results thereby obtained are consequently not comparable with those of more recent date. In the determination of the fat, especially, the figures given by the earlier observers are apt to be below the truth, owing to imperfect extraction.

It is evident that the mixed milk from a number of cows is less likely to exhibit variations of composition than is the product of individual animals, and it is desirable to bear this carefully in mind in making deductions from the following data.

The division of the milk-solids into fat and "solids not fat" is a practice first suggested by J. A. Wanklyn and now generally adopted. The fat is the most variable constituent of milk, while the sum of the non-fatty solids is comparatively constant.

By far the most extensive series of analyses of new milk ever recorded are those of P. Vieth and H. D. Richmond, the analysts to the Aylesbury Dairy Company. These chemists, up to the end of 1895, examined an aggregate number of 172,093 samples of new milk, representing the produce as received during fourteen years from the farmers supplying the company, and their figures furnish invaluable information of the general composition of dairy milk, and the variations to which it is liable. The results for the eleven years from 1881 to 1891 are expressed graphically in the preceding diagram.[2]

The following table shows the yearly average composition of

[1] In collecting samples of genuine milk for analysis, the milking of the animals should be conducted by, or in the presence of, a responsible person. The previous emptiness of the milk-pails should be ascertained by personal inspection, and care should be taken that each cow is milked *dry*, as otherwise the proportion of fat will be below that corresponding to the entire yield. The quantity of milk yielded by each cow should likewise be noted, as also the length of time since the animal calved, and its age, breed, food, and general condition. Record should also be kept of the time of day at which the milking occurred, and of the number of hours since the last milking. Unfortunately, the whole of the foregoing particulars have rarely been recorded, but the more important of them are usually observed.

[2] This diagram was originally published in connection with a paper read by P. Vieth before the Society of Public Analysts in April 1892, and published in the *Analyst* for May of the same year. The author is indebted to the courtesy of the editor of the *Analyst* for the loan of the block from which the diagram is printed.

the Aylesbury Dairy Company's milk down to the end of 1895.[1] The milk is almost entirely yielded by shorthorns.

[1] The method employed by Vieth for the determination of the total solids remained unchanged throughout. Up to July 1884 the fat was, as a fact, determined by the lacto-butyrometer, but the figures in the table, as also those from that time till the end of 1891, are calculated from the specific gravity and total solids.

The following description of the methods employed in making the above analyses was communicated to the Food Products Adulteration Committee by H. D. Richmond, chemist to the Aylesbury Dairy Company.

Specific Gravity.—The specific gravity is estimated by means of a lactometer; the temperature of the milk is taken at the same time, and all specific gravities are corrected to 60° F. by means of a table drawn up in the Company's laboratory. All lactometers are tested before use in milks of known specific gravity.

Total Solids.—Five grammes of milk are measured into a weighed platinum basin, and the water is evaporated on a steam bath; the skin which forms over the milk is broken with a fine needle, and the drying on the steam bath is continued for about three and a half hours. The basins are then removed to an air bath and dried at a temperature of about 200° F. for four hours; they are then taken out, cooled in the balance case and weighed to the nearest milligramme; the weight of the basin and solids, minus the weight of the basin, multiplied by twenty, gives total solids per cent. The milk is measured by a pipette graduated to deliver 5 grammes of milk (sp. gr. 1·0325) at 60° F.

The percentage of *fat* is ascertained by one of the following methods:—

(1) *Leffmann-Beam Method.*—On 15 cubic centimetres of milk (as described on page 139).

All bottles, before being used, are tested against the Adams' method, using the same acids, &c., employed for general use, and a formula and a table constructed to connect the two.

(2) *The Adams Method.*—The following precautions are taken which are not laid down by the Society of Public Analysts:—(*a*) The coils are well dried; (*b*) The ether is anhydrous; (*c*) The coils are free from any matter soluble in ether; (*d*) The extraction is prolonged to five hours. An exhaustive study has shown that on the one hand all the fat is extracted and weighed as such, and on the other nothing else is weighed as fat.

(3) *The Calculation Method.*—The fat is calculated from the total solids and specific gravity (or *vice versa*) by the formula of Hehner and Richmond. This has been proved by hundreds of experiments to be correct within 0·2 per cent. (See page 155.)

T (total solids) = 254 G. (specific gravity) = 1·164 F. (fat).

The Leffmann-Beam method has only been used wholly since 1st March 1894, and partially during 1893, and January and February 1894. The other results for fat are by the calculation method. The solids not fat are always obtained by subtracting the fat from the total solids.

The results are comparable with the methods adopted by the Society of Public Analysts.

Year.	Number of Samples.	Sp. Gravity at 15° C.	Total Solids.	Fat.	Solids not Fat.	Analyst.
1881, .	6,592	1·0315	12·80	4·12	8·68	P. Vieth.
1882, .	9,190	1·0319	13·03	4·22	8·81	,,
1883, .	9,650	1·0323	12·97	4·10	8·87	,,
1884, .	10,399	1·0323	12·96	4·08	8·88	,,
1885, .	11,389	1·0322	13·06	4·19	8·87	,,
1886, .	12,181	1·0322	12·92	4·07	8·85	,,
1887, .	12,663	1·0322	12·94	4·07	8·87	,,
1888, .	12,682	1·0323	12·94	4·06	8·88	,,
1889, .	12,617	1·0321	12·83	4·01	8·82	,,
1890, .	11,816	1·0322	12·84	4·00	8·84	,,
1891, .	11,361	1·0322	12·76	3·91	8·85	,,
1892, .	13,196	1·0320	12·71	3·91	8·80	H. D. Richmond.
1893, .	14,643	1·0318	12·68	3·91	8·77	,,
1894, .	12,633	1·0322	12·67	3·86	8·81	,,
1895, .	11,081	1·0322	12·66	3·84	8·82	,,
Average,	172,093	1·03215	12·86	4·02	8·84	

The following unusually complete analyses, taken from the *American Experiment Station Record*, v. No. 10, p. 945, show the average composition of the milk of cows of various breeds.

Breed of Cow.	Number of Samples.	Total Solids.	Non-fatty Solids.	Fat.	Casein, &c.	Milk-Sugar.	Ash.	Nitrogen.	Water.	Daily Yield of Milk.
										lbs.
Jersey, . . .	238	15·40	9·80	5·61	3·91	5·15	·743	·618	84·60	14·07
Guernsey, . . .	112	14·60	9·47	5·12	3·61	5·11	·753	·570	85·39	16·00
Devon, . . .	72	13·77	9·60	4·15	3·76	5·07	·760	·595	86·26	12·65
Ayrshire, . . .	252	13·06	9·35	3·57	3·43	5·33	·698	·543	86·95	18·40
American Holderness, .	124	12·63	9·08	3·55	3·39	5·01	·698	·535	87·37	13·40
Holstein-Friesian, . .	132	12·39	9·07	3·46	3·39	4·84	·735	·540	87·62	22·65

The following table, showing the average composition of milk yielded by cows of different breeds, is taken from the records of the New Jersey State Agricultural Station (*Bulletin* 77, 1890).

Breed of Cow.	Specific Gravity.	Percentage.					
		Water.	Total Solids.	Fat.	Casein.	Sugar.	Ash.
Ayrshire, . .	1034·1	87·30	12·70	3·68	3·48	4·84	0·69
Guernsey, . .	1035·0	85·52	14·48	5·02	3·92	4·80	0·75
Holstein-Friesian, .	1032·8	87·88	12·12	3·51	3·28	4·69	0·64
Jersey, . . .	1035·3	85·66	14·34	4·78	3·96	4·85	0·75
Shorthorn, . .	1033·9	87·55	12·45	3·65	3·27	4·80	0·78

The average analysis of the milk of the Aylesbury Dairy Company, for the year 1896, representing 11,633 samples, was as follows :—

	Spec. Gravity.	Total Solids.	Fat.	Solids not Fat.
Morning milk, . .	1·0325	12·60	3·63	8·97
Evening milk, . .	1·0321	12·95	3·99	8·96
Average, . . .	1·0323	12·78	3·81	8·97

An extensive series of analyses of dairy milks has been made by Chas. Estcourt (*Analyst*, 1883, p. 245), who has recorded the number of cows contributing to each sample, their food, and whether the samples were from the morning or evening milkings. The cows were milked in Estcourt's own presence or in the presence of Sanitary Inspectors acting under his instructions.[1]

Dairy Milks.	Food of Cows.	Composition of Milk.		
		Total Solids.	Fat.	Solids not Fat.
Morning Milk.				
Highest of five dairies, .	Grass.	12·87	3·26	10·04
Lowest of five dairies, .	Grass.	11·50[2]	2·10[2]	9·07
Highest of four dairies,	Stall-fed.	12·56	3·06	9·92
Lowest of four dairies, .	Stall-fed.	12·19	2·50	9·50
Evening Milk.				
Highest of nine dairies,	Grass.	13·45	4·27	9·61
Lowest of nine dairies, .	Grass.	12·48	3·20	9·01
Highest of four dairies,	Stall-fed.	13·64	4·46	9·44
Lowest of four dairies, .	Stall-fed.	13·13	3·81	9·10

The mean results of Estcourt's analyses are as follow :—

	Dairies represented.	Cows represented.	Composition of Milk.		
			Total Solids.	Fat.	Solids not Fat.
Morning Milk.					
From grass-fed cows, .	5	83	12·23	2·75	9·47
From stall-fed cows, .	4	50	12·40	2·69	9·71
Average, . .	9	133	12·30	2·74	9·57
Evening Milk.					
From grass-fed cows, .	9	83	12·88	3·68	9·20
From stall-fed cows,[3] .	4	50	13·40	4·11	9·29
Average, . .	13	133	13·04	3·81	9·23
GRASS-FED COWS. Average,	14	166	12·65	3·35	9·30
STALL-FED COWS. Average,	8	100	12·90	3·40	9·50
Grand Average, .	22	266	12·74	3·37	9·37

[1] The analyses were made by subtracting the weight of the fat extracted by gasolene from the weight of the total solids previously observed. The figures for fat are consequently probably somewhat below those which would have been obtained by more modern and perfect methods of fat-extraction.

[2] Cows only half-milked.

[3] The food was grains, linseed cake, chaff and hay.

Estcourt's results show that:—1. The evening milk is richer in total solids than the morning milk, the excess being due to the larger proportion of fat in the latter, whereas the "solids not fat" are somewhat higher in the morning than in the evening produce. 2. The milk from stall-fed cows is fully equal, or rather superior to that of grass-fed animals. 3. Not only is the milk from stall-fed cows better on the average than that from grass-fed animals, but it is also less variable in composition, the range of variation being about twice as great in the latter case as in the former.

The sample which yielded only 11·50 of total solids was the same which contained the minimum of fat. It was taken early in May, and was the produce of grass-fed cows, which were only half-milked. In all the other cases the cows were milked dry. Nearly all the other samples were taken in July 1883.

C. A. Cameron has published the results of the analysis of the milk of forty-two cows kept at the Government Agricultural Institution, Glasnevin, Co. Dublin (*Analyst*, vi. 75). The experiments were made during the winter of 1880. The cows were good animals, and were fed liberally on a daily allowance of 8 to 10 stones of pulped mangolds and turnips, and brewery grains, with from ½ to 1½ stones of hay. From the results of his experiments, Cameron draws the following conclusions:—

1. Cows of mature age (8 or 9 years) give more milk, and milk richer in solids, than cows of 4 or 5 years old. 2. The milk does not appear to become sensibly deteriorated in quality towards the end of the period of lactation, but the quantity yielded is much reduced. This is shown by the following table:—

Period of Lactation.	Daily Yield.	Total Solids, per cent.	
	Quarts.	Morning.	Evening.
Less than one month, . . .	13	12·70	13·21
From one month to two months, .	11¼	13·46	14·12
Four months, ...	10¼	12·20	13·46
From eight to ten months, . .	6¼	13·57	13·96

3. In every instance the quantity of milk yielded in the morning exceeded the amount of the evening yield, in many cases being twice, and in two instances three times, as plentiful. About eight hours elapsed between the two milkings. 4. Thirty of the cows gave milk richer in solids in the evening than in the morning, but in eleven cases the morning milk was the richer, and in one

case the two milkings were alike. The average composition of the morning and evening produce was as follows :—

Time of Milking.	Total Solids.	Fat.	Solids not Fat.
Morning, . .	13·20	3·82	9·38
Evening, . . .	13·74	4·22	9·52

The food of cows also affects the quality of the milk yielded, though to a much less extent than is commonly supposed.

Long periods of cold wet weather, or of heat and drought, occurring during the time cows are kept on pasture, cause a diminution in both the quantity and quality of the milk yielded.

Cows in England are usually milked twice a day, the interval between the first and second milkings being usually about eight hours. In such cases, the morning milk is greater in quantity but poorer in quality than that drawn in the evening. In Holland it is usual to milk the cows three times daily, with the result that 20 per cent. more milk per diem is obtained.[1]

Abnormal Cows' Milk.

In certain comparatively rare cases, milk is met with which exhibits a considerable departure from the usual composition. Such abnormal milks are usually the products of single cows, as the practice of mixing the milk from the various animals in a dairy necessarily tends to disguise the special and peculiar characteristics of the milk yielded by any individual cow. As the milk supplied to the public (at least in towns) is almost invariably the mixed produce from several, and commonly a considerable number, of cows, the variations in the composition of the milk

[1] P. Vieth has described the results of some researches made at Paden on the milk of cows of four different breeds, each represented by four cows. In 124 analyses, the specific gravity only fell once below 1029·0, and rose to 1033·9. The average of the total solids was 11·78, the extreme limits of variation being 10·66 and 13·45 per cent., while the fat ranged from 2·6 to 4·7 per cent., with a mean of 3·23 per cent. The amount of solids not fat varied from 8 to 9 per cent., falling even below 8·0, and rising in very few cases above 9·0.

At the dairy experimental station at Kiel, ten cows gave milk having an average composition over three years of 12·16 of total solids and 3·51 per cent. of fat.

At Proskau a herd of Dutch cows, *milked three times a day*, gave a product containing on the average 11·61 total solids, and 3·19 of fat.

In reference to these and other experiments, Vieth expresses the opinion, based on his own experience, that the milk yielded in England is in general a great deal better than that yielded in North and Central Germany.

from the individual cows has much less practical importance than it would otherwise possess.

All cases in which milk has a highly abnormal composition should be viewed with grave suspicion. Even in cases where the analyst is not the nominee of milk-dealers, or even habitually consulted as to the best and safest methods of systematically sophisticating milk without bringing the adulterator within the meshes of the law, it is easy to submit for analysis a fluid which is far from representing the normal milk of the cow from which it may be the *bona-fide* product. The difference in composition between the fore-milk and the strippings affords a ready means of deceiving the inexperienced inspector or analyst, who may actually see the cow milked, but nevertheless have an abnormal sample palmed off on him as a fair specimen of the milk yielded by the cow in question. Where time elapses sufficient to permit of the milk being affected by an alteration of diet, another efficient means of deception exists, but even when a sample of milk is taken at very short notice, means exist of materially altering its character, where sufficient inducement exists to warrant the adoption of illicit methods.[1]

W. F. Lowe (*Analyst*, 1893, p. 6) has described a sample of milk yielded by a cow which was milked dry in his presence. The cow had calved eight months previously. The milk had an unpleasant saline taste, especially when cold, and on analysis yielded the following results :—Total solids, 8·83 ; fat, 2·73 ; non-fatty solids, 6·10 ; ash, 0·95 ; and chlorine in ash, 0·27 per

[1] B. Dyer (*Analyst*, 1893, p. 2) has described a sample of milk yielded by a cow under four years old which ordinarily yielded milk of normal character and was milked dry by a responsible person. The cow was under exhibition at an agricultural show, and at the time of milking was restless and nervous, and the attendant who milked her observed that she "held up" some of her milk, the total yield being only 16½ lbs., and having the appearance of partially skimmed milk. On the following afternoon the cow yielded milk of normal character and good quality. The following figures were obtained on analysis :—

	Total Solids.	Fat.	Non-fatty Solids.	Ash.
Abnormal milk, . .	10·85	1·85	9·00	0·77
Normal milk, . .	12·75	3·64	9·11	...

The case shows that an "appeal to the cow" may sometimes be misleading. Not only should a cow be milked dry, but she should be milked carefully and without unusual excitement if a fair sample of her yield is desired.

cent. These results render it extremely probable that the cow was diseased, but it was killed some time afterwards and was then said to be healthy. Lowe has also described a milk yielded by a well-fed cow in good condition, which contained: total solids, 8·83; fat, 2·98; and ash, 0·94, containing chlorine, 0·267 per cent. (*Analyst*, 1882, p. 100).

P. Vieth (*Analyst*, 1892, page 89) has published the analysis of a milk having the unusually high specific gravity of 1·036 and containing abnormally high proportions of proteids and ash. He attributes the anomaly to the fact that the cows were highly-fed and in the last stage of the period of lactation. The figures obtained were :—Fat, 3·62; proteids, 4·66; sugar, 4·58; and ash, 0·82 per cent.; the non-fatty solids being 10·06 per cent.

A. Smetham (*Analyst*, 1893, p. 3) has published the following analyses of milk obtained from four shorthorn cows which had been specially fed and kept for competition at the Altrincham Agricultural Society Show for the best cow for dairy purposes.

No.	Weight of Milk.		Total Solids.	Fat.	Ash.	Sp. Gravity.
	lbs.	ozs.				
545[1]	16	10	14·88	5·73	0·68	1·0319
546	8	4	12·79	3·31	0·80	1·0347
547	6	6	19·50	11·06	0·53	1·0266
548[2]	13	6	16·06	7·37	0·72	1·0278

F. J. Lloyd (*Jour. Chem. Soc.*, lvii. 201) has described abnormal milks from two cross-bred shorthorn cows which showed no sign of disease and were receiving food ample in quantity and carefully apportioned in character. All the other cows in the same shed, and receiving the same food, gave rich normal milk. The lowest fat recorded was 2·17 per cent., the non-fatty solids in the same sample being 7·87 per cent. The lowest amount of non-fatty solids was 7·50 per cent. in a sample containing 2·70 per cent. of fat.

J. Pattinson (*Analyst*, 1877, page 47) has described an abnormal milk yielded by a roan-coloured short-horn cow, which gave the following results on analysis :—Fat, 3·00; casein, &c., 2·00; sugar, 3·90; and ash, 0·95 per cent. The total solids were 9·85 per cent. The ash contained 0·27 per cent. of chlorine, equivalent to 0·44 per cent. of sodium chloride.

In the majority of authentic cases of abnormally poor milk it will be found that the milk gives an unusually high ash, owing to

[1] This cow had calved only one week previously.

[2] This cow had had her second calf two months previously.

the presence of an unusually large proportion of chlorides. Richmond has pointed out (*Analyst*, 1893, page 271) that this peculiarity affords a means of distinguishing such abnormal milk from watered milk.

Milk from Half-Starved Cows.—In 1875, J. Campbell Brown (*Chem. News*, xxxi. 226) published the following analyses of milk yielded by half-starved cows :—

	Total Solids.	Fat.	Non-fatty Solids.
Half-starved, middle-aged cow ; yielded 2 quarts, .	10·48	2·31	8·17
Old cow, badly fed ; yielded only 3 pints, . .	11·00	2·14	8·86
Average of several half-starved cows, . . .	11·10	2·33	8·77
,, ,, ,, . . .	10·27	1·29	8·98
,, ,, ,, . . .	10·67	1·09	9·58

The following analyses of milk from half-starved cows have been published by J. Carter Bell (*Analyst*, 1881, p. 63) :—

	Sp. Gravity.	Total Solids.	Fat.	Non-fatty Solids.	Ash.
A. Whole milk from seven half-starved cows, fed on a little hay only :—					
Highest result, . . .	1031	12·34	2·99	9·80	·75
Lowest result, . . .	1028	9·10	1·06 [1]	7·98	·64
Average, . . .	1029·4	11·04	2·39	8·65	·69
B. Whole milk from twenty-two cows on badly-managed farm [2] :—					
Highest result, . . .	1030	13·71	4·34	9·37	...
Lowest result, . . .	1028	10·70	2·78	7·92	...
Average, . . .	...	11·42	2·87	8·55	...

The subject of the composition of milk from half-starved cows would be incomplete without a brief reference to the results of the late Dr Aug. Voelcker made in 1862, and described by him in a lecture delivered before the members of the Farmers' Club in 1874. This lecture was published in full in the *Pharmaceutical Journal* for March 14th of the same year, and detailed criticism of the results will be found in the *Analyst*, 1883, page 256.

[1] The fat fell below 2·2 in one case only.

[2] The poorness of the milk was clearly due to bad feeding, for better food brought the non-fatty solids to about 9 per cent. within a week.

Frozen Milk.—The changes that occur on freezing milk are somewhat remarkable.

H. D. Richmond (*Analyst*, xviii. 53) obtained the following figures :—

	Frozen Portion.	Liquid Portion.
	Per cent.	Per cent.
Water,	96·23	85·62
Fat,	1·23	4·73
Sugar,	1·42	4·95
Proteids,	·91	3·90
Ash,	·21	·80
Specific gravity,	1·0090	1·0345

P. Vieth (*Analyst*, xvi. 65) also found that the frozen portion of the milk contained a larger amount of water than the original milk, and that the part remaining liquid was a concentrated milk.

Colostrum is a term applied to the milk yielded by an animal shortly after giving birth to young. The following description refers mainly to the colostrum of the cow, and is chiefly based on the observations of W. Engling (*Bied. Centr.*, 1879, page 214).

The first three or four litres form a reddish or yellowish viscous fluid, having a peculiar smell and a density of 1·060 to 1·080. When freshly drawn the colostrum has an acid reaction, and on standing a layer of albumin forms on the surface, while the liquid underneath frequently keeps unchanged for many days. The milk coagulates to a cake when heated. The serum exhibits a reddish opalescence, and resembles blood-serum. Colostrum yields cream with difficulty, only a half to three-fourths of the fat separating even on long standing.

The foregoing properties are peculiar to the first milkings, as every subsequent milking produces milk which more nearly approaches the normal character, until after four days the secretion becomes quite normal in the case of mature cows, but the change is more gradual when the milk is derived from young animals.

The *sugar* of colostrum crystallises with difficulty. It contains little or no lactose, and from its reactions appears to be dextrose.

The *fat* of colostrum is distinguished from that of ordinary milk by its peculiar smell, taste, consistency, and melting point (40° to 44° C.). It cannot be churned. The colostrum fat from young cows has an oily consistency.

Lecithin is present in colostrum fat in large quantity and can be readily isolated in a crystalline form. Cholesterin is also present.

Colostrum contains a considerable percentage of *albumin*, apparently identical with blood-albumin. Globulin and nuclein have also been found, as also has urea.

The following analysis of the average composition of the colostrum of twenty-two cows is by Engling:—Fat, 3·37 per cent.; casein, 4·83; albumin, 15·85; sugar, 2·48; ash, 1·78; and water, 71·69 per cent.

The following analyses by Engling represent the percentage composition of the colostrum from a cow eight years old.

Time after Calving.	Sp. Gravity.	Fat.	Casein.	Albumin.	Sugar.	Ash.	Total Solids.
Immediately, .	1·068	3·54	2·65	16·56	3·00	1·18	26·93
After 10 hours, .	1·046	4·66	4·28	9·32	1·42	1·55	21·23
,, 24 ,, .	1·043	4·75	4·50	6·25	2·85	1·02	19·37
,, 48 ,, .	1·042	4·21	3·25	2·31	3·46	0·96	14·19
,, 72 ,, .	1·035	4·08	3·33	1·03	4·10	0·82	13·36

Analyses of milk from different cows shortly after calving have also been published by J. Carter Bell (*Analyst*, ii. 155).

Infected and Diseased Milk.

When a cow is suffering from disease, the milk as it leaves the udder may contain various pathogenic organisms; besides which, milk after being drawn is particularly liable to infection by bacteria from various sources.[1] Many authentic cases are on record in which such diseases as diphtheria, tuberculosis, scarlet fever, typhoid, &c., have been conveyed by milk, and considering the many sources of pollution, and the great rapidity with which bacteria multiply in such an excellent medium as milk, it is surprising that instances of infection are not more numerous.[2]

Besides cases in which milk is infected with the organisms characteristic of virulent diseases, it not unfrequently happens that it affords a medium for the growth of various non-pathogenic

[1] The methods of bacteriological research, as applied to the examination of milk, are outside the scope of this work. They are treated in a thorough but practical manner in *Applied Bacteriology*, by T. H. Pearmain and C. G. Moor.

[2] According to Ernest Hart, fourteen epidemics of scarlet fever and seven of diphtheria have been traced in this country to the use of infected milk, besides a number of epidemics of cholera and typhoid fever.

organisms which render their existence manifest by causing a marked change in the physical characters of the milk. In addition to the lactic and butyric ferments, the effects of which are considered at greater length in the sequel, the phenomena of blue milk, ropy milk, bitter milk, &c., are due to such organisms, and require a brief description.[1]

[1] A fuller account of the micro-organisms of milk will be found in *Milk, its Nature and Composition*, by C. M. Aikman.

In an article in the *Contemporary Review*, G. Clarke Nuttall points out that there are different species of bacteria living in milk, even if it be granted that those causing "souring" are fundamentally one. The various "faults" to which milk is liable are almost all now proved to be the work of different bacteria. The milk bacteria can be divided into two great classes. The aërobic organisms, which can only live in the presence of free oxygen, are not usually harmful; but the anaërobic bacteria have invariably an evil effect. They decompose the proteids of the milk, cause a disagreeable odour, and their presence leads inevitably to bad, unkeepable butter. Hence it is obviously desirable to increase the amount of free oxygen in milk (milk when first drawn from the cow contains relatively very little free oxygen), since by so doing the chances of the presence of these troublesome bacteria are much lessened. This can be done by putting the warm milk into large flat pans, so that a large surface is presented to the air, by cooling it, and by mixing it with air. In the best North American farms, and in Denmark, special apparatus for cooling and aërating the milk is employed. In England, and especially in Germany, the airing is too often neglected. Therefore, if the butter has a soapy, oily, or tallowy taste; a flavour of cheese, or of mangolds (which last peculiarity sometimes appears when there is no possibility of the cows having eaten mangolds); or if the milk is "blue," or "bitter," or "stringy"; or will not churn, or foams in the churning; then it is certain that deleterious bacteria are at work, and the only remedy is to get rid of them by some means. Weigmann claims that by sterilising the milk and then using the pure culture of the right bacteria which he has prepared, any of these faults can soon be eliminated. A single inoculation with the right culture is not sufficient, since the germs are most persistent, but fresh supplies of the culture must be obtained every few days, and the whole process repeated. But, when the faults have been eradicated and the butter or milk is quite satisfactory, or when the dairy is already excellent, it is only necessary to use the pure culture occasionally, say once a month or once in six weeks, for every day a portion of the "fermentation-starter" is left over to begin operations with on the following day. The great excellence of the Danish butter is mainly due to the care exercised in choosing the "fermentation-starter," and at the last dairy exhibition at Oalberg, Jutland, the State Consul Nissen asserted that pasteurised cream and pure cultures for "fermentation-starters" were both generally employed. By bringing bacteriology to bear on the dairy many good results may be looked for. Thus, of the bacteria which have shown themselves suitable for use in butter-making, all do not give precisely the same products in their fermentative action. Hence it may be possible to blend and combine them so that different flavourings may be prepared.

Blue Milk is a phenomenon of somewhat rare occurrence, due to an organism first observed by Ehrenberg, and called by him *Vibrio syncyanus*, but which is more correctly named *Bacillus cyanogenus*. This microbe, which is of a pale blue colour, consists of small motile rods, which are provided with numerous flagellæ. It undergoes fission in the ordinary manner, and forms spores. It does not liquefy gelatin, but stains it bluish-green, and finally a dirty grey tinge. The bacillus of blue milk reduces the acidity of the milk, and sometimes renders it actually alkaline. The fermentation is marked by the production of a blue colour, which is changed to cherry-red by caustic alkalies, but restored by acids. The butter prepared from such milk possesses a greenish colour and disagreeable butyric odour. Reiset states that blue milk may be used for the production of butter, by adding 0·5 gramme of acetic acid per litre. *Bacillus cyanogenus* appears to be harmless. Hueppe fed animals on food mixed with strong cultures of the organism without any ill effects being observed. To prevent the growth of the bacillus it is recommended that the milk-cans, &c., should be washed with boiling water.

Other pigment-forming organisms produce the phenomena of yellow, red, green, and violet milk. *Red milk* is due to contamination with several distinct microbes, among which are *Bacillus prodigiosus* (the spores of which exist in the atmosphere and rapidly develop in any suitable medium), *B. lactis erythrogenes*, *Sarcina rosea*, and *Saccharomyces ruber*.

Ropy Milk is apt to be met with in moist warm weather. When freshly drawn, ropy milk does not exhibit any unusual characters, but after a few hours becomes so viscid as to form a complete string when poured from one vessel to another. Ropiness may be caused by many distinct organisms, among the most prominent of which are:—*Bacillus lactis pituitosi*, described by Löffler as a stout, slightly-curved rodlet, which does not liquefy gelatin; *B. lactis viscosus*, a very short, aërobic rodlet, which does not liquefy gelatin; and *Streptococcus Hollandicus*, which forms chains, and does not liquefy gelatin. It is said to be utilised in Holland in the manufacture of Edam cheese. Ropiness usually develops somewhat suddenly, and generally disappears when the weather becomes cooler.

Bitter Milk.—Liebscher (*Bied. Centralb.*, 1884, 561) has described a case of this bacterial infection of milk which occurred

This would enhance the commercial value of the cultures, for farmers would then be able to choose that flavouring for their butter which best suited their customers, and yet keep it absolutely pure.

on a well-managed farm in Thuringia. The butter became repulsively bitter, and consequently unsalable. On examination, it was found that a number of the cows in the earliest portion of their milking yielded a bitter milk, and that when this was collected separately, the remainder was sweet.[1]

Soapy Milk.—F. J. Herz (abst. *Analyst*, 1892, p. 99) has described a case in which milk was suspected to have been skimmed on account of its high specific gravity (1·0351 to 1·0365 at 15° C.), but was found to contain from 3·7 to 6·1 per cent. of fat, while the specific gravity of the whey of the spontaneously curdled milk was 1·0313 in one instance, the fat in the same sample being 5·03, and the non-fatty solids, calculated by Fleischmann's formula, 10·04 per cent.[2]

On standing for twenty-four hours the milk developed a soapy smell, and gave an extremely persistent froth on shaking. On treating the milk with caustic potash, a fishy odour was produced, recalling that of trimethylamine. The froth or scum on the milk differed from that on normal milk by not consisting of tangible floating particles which can be filtered off. Soapy milk shows but little tendency to curdle. The milk, when kept for twenty-four hours at 40° C., and then exposed to the ordinary temperature of the room, with free access of air and occasional agitation, took from eight to twelve days to curdle. Soapy milk has been observed to be yielded by well-fed cows, and does not appear to depend on the period of lactation. Apart from its disagreeable taste and smell, soapy milk is troublesome to use in butter and cheese-making. Its cause has not been detected.

Tyrotoxicon, a highly poisonous ptomaine observed by Vaughan in stale milk, ice-creams, and cheese, has already been fully described (see Vol. III. Part iii. page 347).

Diseased Milk.—In the majority of cases in which cows are diseased no marked peculiarity can be traced in the chemical composition of the milk yielded. In many instances, no doubt, the diseased milk has a composition different from that of the

[1] From this peculiarity Liebscher suspected that the stalls had in some manner become infected with bacteria, which had commenced their progress into the udders of the cows without having made much advance. The stalls and cattle were thoroughly disinfected, carbolic acid sprinkled frequently, and the cows' udders washed twice daily with lukewarm water, and then with dilute carbolic acid. Under this treatment, the bitterness disappeared in three days, and the milk and butter tasted sweet.

[2] In other cases the values for the non-fatty solids, deduced in the same manner, were 10·05, 10·21, 10·25, and 10·26 per cent. Hence Herz concludes that Fleischmann's formula is inapplicable to these abnormal samples.

secretion of the same individual in a state of health; but the departure from the normal composition of milk of animals of the same species is rarely sufficiently marked to enable the analyst to affirm positively, solely on the result of his analysis, that disease exists. On this account, milk which is found on analysis to have an abnormal composition should be regarded with grave suspicion. In many cases in which a cow has been found to yield milk of a very unusual composition, it is probable that it suffered from some unrecognised complaint, and its use for human consumption should be disallowed.

Following are given, in a tabular form, a number of analyses of milk from diseased cows, published by A. Wynter Blyth (*Foods: their Composition and Analysis*).

	Specific Gravity.	*Fat.*	*Casein.*	*Albumin.*	*Galactin.*	*Sugar.*	*Ash.*	*Total Proteids.*	NaCl *in Ash.*
1. From heifer, suffering from inflammation of the mammæ; two days after calving,	1036·2	2·80	4·02	·56	...	5·54	·92	4·58	·110
2. From heifer with inflamed udder; two days after calving,	1031·3	4·40	...	·62	·27	...	1·16	...	...
3. From cow with pneumonia; fourteen days after calving. Pulse 82; temperature 102·4° F.,	1029·7	2·88	3·75	·41	·09	3·77	·78	4·25	·470
4. From cow with enlargement of rumen and congested liver. Pulse 68; temperature 101° F.,	1032·0	6·06	4·80	1·07	·11	4·48	·67	5·98	·092
5. From phthisical cow, five years old, with extensive tubercular deposit in right lung, December 7, 1878,	1029·7	2·77	3·65	·87	high.	2·82	·87	4·52	·096
6. Same case, February 1879,	1034·0	3·83	5·40	·36	high.	3·34	·77	5·76	·150
7. From cow, two years old, in advanced stage of phthisis, January 29,	1032·9	2·56	3·00	...	...	2·89	·91	...	·10
8. Same case, February 17,	1033·5	3·28	3·98	...	·25	4·10	·78	...	·15
9. From cow with udder infiltrated with tubercular deposit,	1018·0	0·49	1·21	2·39	none.	·47	·76	3·60	·43
10. From cow with parturient apoplexy, third day after calving. Pulse imperceptible; temperature 99·4° F.,	1037·0	3·61	...	3·88	1·09	...	·95	4·97	·100

The following is a summary of the remarks of A. W. Blyth on the milks represented in the foregoing table.

1. It is curious that this milk, taken from a cow suffering from an affection of the udder itself, shows no striking departure

from the chemical composition of normal milks. The high proportion of ash is worthy of notice.

No. 2 was taken from an animal the udder of which was much inflamed in parts. The milk was pink in colour, and contained about 5 per cent. of blood, which was separated by subsidence as much as possible before the analysis was made. The milk was perfectly fresh when examined. It had a feebly acid reaction and rapidly putrefied.

3. From this milk Blyth obtained 0·58 per cent. of cholesterin, being the only instance in which he found an estimable quantity. 0·005 per cent. of urea was found. The microscopic results were negative.

4. This sample presented no marked peculiarities.

5 and 6. The "galactin" was not actually determined, but was present in more than the usual proportion. The sugar in these analyses is abnormally low. Under the microscope the milk presented a normal appearance.

7 and 8. Here again a low proportion of sugar is found in a case of phthisis. Only one gallon of milk was yielded during the whole of January.

9. A milk of this character is never likely to occur in an unmixed state in commerce. It is essentially an albuminous serum, with sufficient casein and milk-sugar to show its origin in a diseased milk-gland. The absence of galactin is noteworthy. The milk contained 0·039 of urea, and 0·018 per cent of nitric acid. The high proportion of chlorides is remarkable. A microscopic examination showed very few fat-globules, and the following abnormal forms :—

a. Clusters of oval or round granular cells, mostly ·0005 inch in diameter, with well-marked oval nuclei.

b. Irregular, granular masses, varying from ·0006 inch to 10 or 12 times that diameter.

c. Granular rounded bodies, taking a brilliant stain on addition of carmine or magenta.

10. The milk had a feebly alkaline reaction and contained much lactochrome but no urea. The high ash is noteworthy. The microscopic examination showed nothing abnormal.

The milk from a herd of cows suffering from a mild form of typhus fever has been analysed by Husson (*Compt. rend.*, 1871, p. 1339), who found the product to contain :—fat, 1·493 per cent. ; albumin, 2·060 ; milk-sugar, 3·140 ; and salts, 1·850 per cent. Husson found the proteids increased from the commencement of the malady, and the milk often contained bloody and purulent admixtures.

According to A. W. Blyth, in the case of cattle suffering from *aphthous fever*, commonly known as "foot-and-mouth disease," the milk suffers a marked change both in chemical constitution and microscopic appearance. The following are the maximum and minimum percentages of each constituent found by Blyth in the milk yielded by eight different cows in various stages of foot-and-mouth disease.

	Fat.	Proteids.	Sugar.	Ash.	Water.	Non-Fatty Solids.
Maximum, .	7·79	14·38		·71	91·24	15·09
Minimum, .	0·39	2·90	4·67	·21	83·85	8·35

These figures show that the variations are enormous. The lowest ash was associated with the highest fat, and was yielded by the milk from a cow in the fourth day of the disease. The highest proportion of non-fatty solids was found with 0·71 of ash, in the product from a cow on the second day of the disease.[1]

In foot-and-mouth disease, unless there is much fever, the milk is secreted during the whole course of the disease. In cases where there are ulcers on the teats, either externally or just inside, the pus exuding from them passes into the milk, which is found to yield a high fatty residue, containing, according to Blyth, sensible traces of cholesterin, lecithin, and nuclein (?). If the local affection is still more severe, blood-cells and even a notable quantity of blood may be found in such milk. Where there are no ulcers on the teats, the milk, in average cases, has a tendency to be deficient in solids, and especially in fat, but returns to its normal condition after seven or eight days.[2] Blyth states that the milk from cows

[1] Blyth states (*Chem. News*, xxxii. 244) that the milk of cows during the first three days of foot-and-mouth disease is occasionally fatal to calves and pigs. A fatal result is by no means invariable, only occurring in a few cases, but the symptoms are always remarkably uniform. In the midst of apparent health the animal is taken suddenly ill, and the illness is as immediately followed by death as if a fatal dose of a violent poison had been administered. On *post-mortem* examination of a calf which had died with extreme suddenness after suckling its mother, the larynx and the base of the tongue were found intensely swollen and congested, the bronchial tubes completely choked with a viscid frothy mucus, the true stomach and the kidneys deeply congested, and the intestines were of a rose colour. There were also a few aphthous patches on the tongue.

[2] A. W. Blyth found in milk, taken from a cow suffering from aphthous fever, elongated, highly refractive and flattened bodies, varying in length from $\frac{1}{5000}$th to $\frac{1}{10000}$th of an inch, and having divisions at intervals. These

suffering from aphthous fever is rendered innocuous by boiling. He quotes a case in which upwards of one hundred adult cattle suffered from foot-and-mouth disease, while the calves, which were fed on boiled milk and water and not allowed to suck their mothers, were not affected.

Klein states that scarlet fever is closely associated with the multiplication and growth of a micro-organism, which consists of spherical micrococci arranged as diplococci, and as straight, wavy or curved chains of streptococci, sometimes of great length. The scarlet fever organism is very similar in appearance and manner of growth to the streptococcus of foot-and-mouth disease, but Klein states that the two may be readily distinguished by their action on milk. Thus the aphthous fever organism causes no apparent change in sterilised milk, whereas the scarlet fever streptococcus, after two days' incubation, converts it into a solid matter. A similar organism has been isolated by Klein from the blood of persons suffering from human scarlatina.

A valuable description of the results of the examination of milk from a cow which appears to have been suffering from *pleuro-pneumonia* has been described by G. W. Wigner (*Analyst*, 1878, p. 251).

The danger attending the use of *tuberculous milk* has received much more attention on the continent than in this country. In Denmark a very thorough system of inspection exists. All cattle found to be tuberculous on examination by experts are at once isolated, and, if necessary, slaughtered and the bodies destroyed.

The milk of cows with tuberculosis of the udders possesses an extraordinary virulence. All animals inoculated show tuberculosis in its most rapid form. The high mortality amongst infants from tubercular intestinal affections is most probably due to the use of milk containing the tubercle bacillus. Cows with apparently sound udders, but affected with tubercle of the lungs, have been known to yield milk containing tubercle bacilli.

did not make their appearance till the third day. On the fourth day they were fewer and larger, and on the fifth day had disappeared.

Blyth's bodies appear to have been identical with those more recently described by Klein (*Fifteenth Annual Report* (1885) *of Local Government Board*), according to whom a special micro-organism is always associated with aphthous fever. This is a micrococcus, which either occurs singly in dumb-bell forms (diplococci) or as streptococci, the chains of which sometimes grow to a considerable length. The individual cells of both the diplococci and the streptococci are spherical, and measure from 0·0006 to 0·0008 mm. The organism is readily cultivated in agar-agar, nutrient gelatin, or broth. Successful cultures can be obtained from milk which has been preserved in tubes for several months.

Determination of the Normal Constituents of Milk.

The analysis of milk for the determination of the constituents normally present is usually limited to a few operations, since the detection of the substances existing in minute traces requires the application of special methods not commonly required or practised. Hence the processes described in this section will be limited to those requisite for the determination of the leading normal constituents of milk. The detection of added water is chiefly inferential, and is dealt with in the following section, as is also the detection of other adulterants, and of preservatives.

One of the simplest and best methods of judging of the quality of milk is the determination of the percentage of milk-solids, and if the result obtained be supplemented by a determination of the milk-fat, so that the proportion of non-fatty solids can also be arrived at, an amount of information is obtained which is sufficient for many purposes. These determinations may be advantageously supplemented by an observation of the specific gravity of the milk, and by ascertaining the proportions of proteids, milk-sugar, and mineral matters present in the non-fatty solids.

Determination of the Total Solids of Milk.

This important determination is best effected by evaporating a known quantity of the sample to dryness at the temperature of boiling water, and weighing the residue obtained. From five to ten grammes of the milk is a suitable quantity to employ for the operation.[1] Either the weight taken must be exactly known, or the specific gravity of the sample must be ascertained and an exact measure taken with a pipette. In this case the results obtained must be divided by the specific gravity of the sample to obtain the true percentage of solids. In technical laboratories, where large numbers of samples are dealt with, it is usual to employ pipettes constructed to deliver five grammes of milk of normal specific gravity (1·031). The difference occasioned by any slight departure of the gravity of the sample from this standard is usually well within the limits of error in working the process.

The evaporation of the sample is ordinarily conducted in a flat-bottomed platinum dish about 2½ inches in diameter, and accurately tared. It is important that the milk should be in the form of a thin layer, so that the evaporation may take place as rapidly as possible. When this condition is fulfilled, the residue obtained

[1] When the solid residue is not to be employed for the determination of the fat or ash, or put to other purpose, a smaller quantity (2 grammes) of the milk may be conveniently employed.

is nearly white, but if the process be prolonged it has a brownish colour. When a number of samples of milk are operated on at once, the dishes containing them may conveniently be heated over a water-bath of copper, furnished with holes of a size to fit the capsules; but a single sample may be placed on a beaker containing water kept constantly boiling. After a time varying with the quantity of milk operated on, but which is about three hours when ten grammes of the sample is employed, the dish is removed to a water-oven and kept there for three hours. As a rule, the above time of heating will dry the residue to a constant weight, but as a precaution it is desirable to return the dish to the water-oven for another hour, and again weigh it. When the loss does not exceed 0·002 gramme the weight may be regarded as constant. Very prolonged heating should be avoided, as liable to occasion decomposition and discoloration of the solids.

Instead of using platinum dishes for determining the total solids of milk, the author employs and prefers flat-bottomed porcelain capsules. Dishes glazed both inside and out are used by A. W. Stokes, but the author finds those which are glazed internally only to be equally satisfactory, while they have the advantage that the weight of the dish and the distinguishing mark of the milk-sample can be written on the outside with a fine lead-pencil, without appreciably affecting the weight.

The residue obtained as above may be employed for the determination of the fat by the Werner-Schmid process (page 135), but is not suited for direct treatment with ether or other solvent of fat. As a rule, however, it is more convenient to employ the residue for the determination of the ash of the sample.[1]

Numerous modifications of the foregoing method of determining milk-solids have been devised, but none have met with such general acceptance as that of simple evaporation in an open dish, and, in the opinion of the author, the modifications present no marked advantage in simplicity, accuracy, or general convenience. The chief of the alternative methods may be epitomised as follows:—

The addition of alcohol or acetic acid to curdle the milk, and thus break the scum formed during evaporation, is advocated by Gerber and by Radenhansen.

Absorption of the milk by sand, cupric oxide (J. Muter), and pumice (Storch) has been recommended.

Ganntner absorbs the milk in wood-pulp contained in an open basin, which is then dried at 100° C. in the usual way.

[1] In the author's laboratory, the determination of the total solids is made in duplicate. One residue is employed for the determination of the ash, while the other is reserved for reference, in case of any dispute arising.

H. D. Richmond employs asbestos in place of wood-pulp, and recommends the following mode of operating (*Analyst*, xvii. 227):—About three grammes of asbestos of the best quality should be ignited in a platinum dish in a muffle-furnace to drive off any combined water. After cooling and weighing, a known weight (about five grammes) of the sample of milk is placed in the dish containing the asbestos, the whole dried on the water-bath, and subsequently kept in the water-oven for about twelve hours, when the weight is again observed. By this method, Richmond obtained absolutely concordant results, and further exposure for twenty-four hours at a temperature of 105° C. did not affect the weight of the residue.

Babcock and Macfarlane have described a method of drying the milk on asbestos contained in a glass tube through which a current of air is passed. Duclaux has used sponge in a similar manner.

Thos. Macfarlane (Canadian Government Laboratories, Ottawa) employs the variety of asbestos called *chrysotile* in the analysis of milk (*Analyst*, xvii. p. 79). The fibre is loosely packed into a short cylindrical glass tube constricted to a funnel at its lower end, which is then weighed. A weight of ten grammes of the sample of milk is carefully run on to the chrysotile, which, if properly packed, absorbs it without any running through. The whole is then dried in a current of air and weighed, the total solids of the milk being deduced from the increase in weight. The fat is then extracted with ether, and the difference regarded as non-fatty solids.

M. A. Adams absorbs the milk in a coil of blotting-paper, which is then dried at 100°.

A useful method of deducing the total solids of milk from the specific gravity and percentage of fat is described on page 155.

Determination of the Mineral Matters of Milk.

The proportion of mineral constituents contained in milk is generally regarded as identical with the ash yielded on igniting the milk-solids, but, as explained on page 103, the two are not strictly identical, owing to the changes resulting from the process of ignition.

The determination of the ash of milk is effected by simply heating the capsule containing the milk-solids to a dull redness, until all the organic matter is consumed. An excessive temperature must be carefully avoided, or loss will occur by volatilisation of the chlorides of the milk.[1]

[1] A plan by which the combustion of the organic matter is greatly facilitated is to char the solid residue, and then, without attempting to burn off

An examination of the character of the ash is always desirable, and is capable in some cases of yielding valuable information not obtainable in any other manner. Thus the ash of cows' milk is very rarely less than 8·0 or more than 8·5 per cent. of the non-fatty solids, and averages 8·3 per cent. In milk containing an abnormally low proportion of non-fatty solids, there is not a corresponding diminution in the ash, which in no case in Richmond's experience has fallen below 0·70 per cent., even in cows' milk containing notably less than 8·5 per cent. of non-fatty solids, while as a rule the ash is considerably in excess of this proportion. On the other hand, dilution of a normal milk with water will reduce the ash almost proportionately to the amount of water added, so that a low ash occurring together with low non-fatty solids affords a strong presumption that the milk has been watered. The ash of genuine cows' milk is free from carbonates and borates, and practically free from sulphates, and the ash soluble in water is about 30 per cent. of the total. Hence, Richmond regards adulteration of a milk as practically certain in a case where the sample contains a low proportion of non-fatty solids, but gives an ash of normal amount, having a marked alkaline reaction, containing borates or a notable quantity of sulphates, or of which much more than 30 per cent. is soluble in water.

Determination of Milk-Fat.

A great number of methods have been proposed and employed for the determination of the fat in milk, but the principle of the survival of the fittest has reduced those now commonly used to a very small number, which may be arranged in four classes.

In the methods of Class A., the fat is extracted from the milk itself by a suitable solvent or mixture of solvents. To this class belong the lacto-butyrometer process of Marchand, the aërometric method of Soxhlet, and the Werner-Schmid process with hydrochloric acid (page 135).

In the methods of Class B. the milk is evaporated to dryness, and the fat extracted from the residue by ether, petroleum-spirit, or other solvent. The processes of J. A. Wanklyn and James Bell belong to this class; but experience has conclusively shown

all the organic matter, to moisten it with a few drops of concentrated sulphuric acid and one drop of nitric acid. On again igniting, the ash will be readily obtained of a pure white colour. This "sulphated ash" contains the alkali-metals as sulphates, and has a weight greater than that of the unsulphated ash from the same milk, in the proportion of 1·45 (to 1·50) : 1·00.

Of course the foregoing treatment of the milk-residue with sulphuric acid prevents the possibility of any further examination of the ash.

that complete extraction of the fat from the residue is very difficult and in some cases practically impossible, and that the amount retained, and thereby ignored, is largely dependent on personal equation. With Bell's method, which was devised and is employed in the Somerset House laboratory, very irregular results were formerly obtained.[1] With care in the manipulation, almost complete extraction may be effected in the case of fresh milk, but with altered milk, in the analysis of which the process finds its chief application, the figures obtained are from 0·2 to 0·3 per cent. below the truth.

In the methods of Class C., the milk is absorbed in some material which at once facilitates the drying and the subsequent extraction of the fat by a solvent. Sand, pumice-stone, asbestos, the variety of asbestos called chrysotile, copper sulphate, plaster of paris, wood-pulp, and various other materials have been employed as absorbents, and their advantages strenuously advocated by their respective proposers. Blotting-paper has been recommended in 1886 by M. A. Adams, and his process was officially adopted and recommended for general use by the Society of Public Analysts.

In the methods of Class D. the milk is treated with certain reagents to facilitate the separation of the fat, and is then subjected to rapid rotation in a centrifugal apparatus, the separated fat being then measured.

The Werner-Schmid Method is capable of yielding very accurate and concordant results. The author has had extensive experience of the process, which he works in the following manner:—Ten grammes weight of the sample of milk to be examined is introduced into a stout glass tube of 50 to 60 c.c. capacity, and having a length of about 8 inches (fig. 5). Ten c.c. of fuming hydrochloric acid is next added, and the tube placed in boiling water until the contents become dark brown, a condition which is generally reached in about ten minutes. Too long heating should be avoided, as it tends to the formation of carameloid bodies, which subsequently contaminate the fat. The tube and its contents are then cooled, ether added to within an inch of the top of the tube, a cork or stopper inserted, and the liquid well agitated. The tube is then allowed to stand for a few minutes, to ensure complete separation of the ethereal layer, which is then

Fig. 5.

[1] This statement is based on the fact, pointed out by O. Hehner, that no relation can be traced between the total solids, fat, and specific gravity of the milks, which relation has been proved by various observers always to exist when the analyses are accurate.

transferred to a tared flask by means of a pipette.[1] Four successive treatments with ether in the foregoing manner are sufficient to ensure the extraction of the whole of the fat.[2] The ether is next distilled off, the residue of fat dried at 100° C. till constant, and weighed.

The Werner-Schmid process can also be conveniently employed for determining the fat in the total milk-solids. For this purpose, the residue should be moistened with concentrated hydrochloric acid (6 or 8 c.c. of acid to 5 c.c. of milk), and heated on the water-bath for about five minutes. The contents of the dish are next transferred to the Werner-Schmid tube, and treated as described above. A. W. Stokes (*Analyst*, xvi. 92) has shown that the results obtained by this modification of the process are in agreement with those yielded when working on the original milk.

The Werner-Schmid process is equally applicable to sour or

[1] Instead of employing a pipette of the ordinary kind, the syringe-pipette illustrated in fig. 6 is very convenient for removing the ethereal layer. It can be readily constructed by drawing out a test-tube so as to form a narrow prolongation, the orifice of which should be turned up so as not to disturb the liquid in which it is immersed. A narrow test-tube, fashioned into a handle at the upper end, serves as a piston, a short length of india-rubber tubing uniting it to the outer tube, while allowing easy movement up and down. Another convenient form of separator, devised by W. Chattaway and shown in fig. 7, is in constant use in the author's laboratory. It is practically a small wash-bottle fitting, which is adjusted to the tube or cylinder containing the layers of liquid it is desired to separate. It is so arranged that the exit-tube can be adjusted in height by sliding it through the india-rubber collar (C), so as to bring the turned-up end just above the junction of the two layers. On then blowing through the side tube (A), the upper stratum is forced up the inner tube, and can be removed, almost to the last drop, without disturbing the lower layer.

Fig. 6.

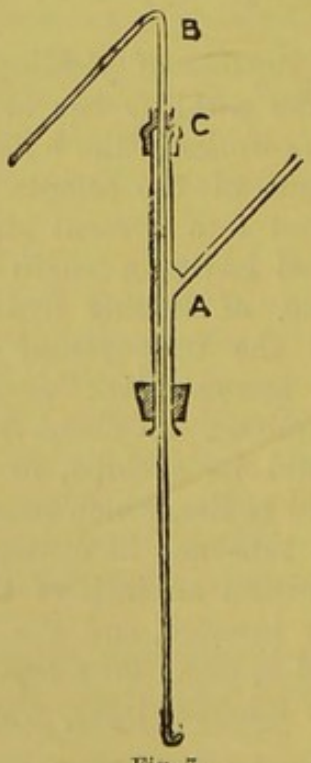


Fig. 7.

[2] Some operators avoid the repeated extraction by ether, by removing an aliquot part of the ethereal layer. The plan is open to several sources of error, and, in the author's opinion, is not to be recommended.

decomposed milk, and with some slight modifications can be used for the determination of the fat in separated or skimmed milk, cream, condensed milk, and cheese.

Bell's Modified Wanklyn Process of Fat Extraction.—The following method of determining the fat and non-fatty solids in milk is that adopted at Somerset House. The description is taken from J. Bell's *Food and its Adulterations*, part ii. (1883).

"When the milk is fresh, a quantity of exactly 10 grammes may be weighed in a platinum capsule containing a glass stirrer. The most suitable size of the capsule for this purpose is one having a diameter of 3 inches and a depth of 1 inch. The capsule is placed on an aperture of a water-bath, and its contents evaporated almost to dryness. It is of advantage to keep the milk well stirred during the process of drying, in order to insure that the solid residue be obtained in a condition favourable for the complete extraction of the fat. The milk residue should neither be too moist nor too dry, as either condition tends to prevent the removal of the last traces of fat. If the evaporation has been carried too far, the residue may be carefully moistened either with a very small quantity of water or of alcohol. When the proper point has been reached, the mass is treated repeatedly with ether, the stirrer being each time used to pulverise the solid matter which, in order to insure that no portion escapes the action of the solvent, should assume a fine state of division. The ether is used warm for the last three treatments. After each washing the ethereal solution of the fat is carefully poured off through a small Swedish filter not exceeding $3\frac{1}{2}$ inches in diameter. To remove the last traces of fat from the filter, the upper part is cut off, divided into small pieces, which are placed in the remaining portion of the filter in the funnel, and washed with a little ether. The filtrates are received into a tared beaker, from which the ether is gently evaporated, and the fatty residue finally dried in a water-oven until the weight is constant.

"The capsule containing the non-fatty residue is placed on the open water-bath for two hours, and subsequently for two or more hours in a closed water-oven kept at 212° F. (100° C.), until a constant weight is arrived at. This result should be obtained in the time stated if the milk solids have been finely pulverised in the process of fat-extraction."

Adams' Coil Process of Fat Extraction.—A very ingenious method of determining fat in milk was described by M. A. Adams in 1885 (*Analyst*, x. 46, 85), according to which a known quantity of the sample is absorbed by a strip of blotting-paper rolled into a coil and freed from fatty and resinous matters. The paper is then dried and exhausted by ether or petroleum spirit. Owing to the enormous surface exposed, the fat can be extracted very completely. The method was adopted in 1886 by

the Society of Public Analysts as their official process, and has been proved by an enormous number of analyses to give very accurate results. If excessive drying of the paper coil be avoided, no appreciable oxidation of the fat occurs; and it has been proved that the ethereal extract consists of pure milk-fat, if the precaution be taken to exhaust the paper with ether before employing it.

As originally described and adopted by the Society of Public Analysts (*Analyst*, x. 217), Adams' process presented several practical difficulties, which are met in the modification described below (Allen and Chattaway, *Analyst*, 1886, p. 71):—A strip, 22 inches long by 2½ wide, is cut from a sheet of "white demy" blotting-paper. This is rolled loosely round a glass-rod, together with a piece of thin string, which serves to prevent contact between the concentric folds of the coil. The string is conveniently passed through holes in the paper, and should, before use, have been boiled in water containing some sodium carbonate, to remove size and resinous matter. A cap of filter-paper is then placed on one end of the coil, and secured by the ends of the string. Thus arranged, the paper forms a coil 2½ inches high by about 1 inch in diameter, and before being used should be deprived of traces of fat, resin, &c., by exhaustion with ether in a Soxhlet-tube, the ether being subsequently driven off.[1] The coil is then suspended by some simple means, the capped end being downwards, and 5 c.c. of the milk to be tested, and the density of which is known, gradually run on to the upper part from a pipette. The milk is rapidly absorbed by the paper, and none filters through the paper cap. The coil is then dried in the water-oven for an hour or two, and then placed in a Soxhlet-tube, in which it is exhausted with ether, at least twelve syphonings being necessary. The ether is then distilled off in the usual way, and the residue of fat dried till constant in weight.

Instead of using a definite measure of the milk, the sample may be weighed in a tared tube or small beaker, poured on the coil, and the vessel rinsed out with a few drops of water, which in their turn are added to the coil. This plan is also available for sour milk, a weighed quantity of which may at once be poured on the centre of the coil. Curdled milk may be previously treated with ammonia and agitated strongly, the resultant emulsion being poured on the coil.

Wm. Thomson has proposed (*Analyst*, 1886, page 73) to replace the thick blotting-paper employed by Adams by a strip of ordinary filter-paper of half the length. One end of the paper is fastened in a clamp, and the other is held by one hand

[1] Richmond extracts the coil with acidulated alcohol.

in a nearly horizontal position, while 5 c.c. measure of the sample is allowed to drop on the strip from a pipette held in the other hand. By this means the milk may be entirely transferred to the strip of paper. The point of the pipette is wiped on a small portion of the strip previously left unmoistened. The paper is next dried by passing it over the flame of a bunsen-burner, or by holding it before a fire. The paper is then coiled on a glass-rod, and extracted with ether in a Soxhlet-tube.

If blotting-paper be substituted for filter-paper in the above modified process, it is apt to break in the act of coiling, besides containing a notable quantity of impurity soluble in ether, which must be previously removed or allowed for. Filter-paper coils easily, yields practically no extract to ether (0·0006 gramme), and allows of the complete extraction of the fat by eight to ten syphonings in the Soxhlet-tube.

In the opinion of the author, Thomson's modification is improved by employing a strip of filter-paper of the dimensions originally recommended (21 to 22 inches by 2½ inches) and increasing the amount of milk from 5 c.c. to 10 c.c.

Centrifugal Separation of Fat from Milk.—The separation of cream from milk by a centrifugal machine is now largely practised on a commercial scale, and has been proposed as a laboratory method. The same principle can be employed for the separation of milk-fat, if suitable means be adopted to liberate it in a pure state. This was first effected by the lactocrite, a centrifugal apparatus which was open to several practical objections and has now been superseded. The Babcock and Thörner instruments have each their advocates, but in the opinion of the author, the two forms of apparatus which are most likely to survive are the Leffmann-Beam and the Gerber instruments. The modes of operating recommended to be employed with these instruments are very similar, though not absolutely identical; but there would be little difficulty in employing either form of separator, and varying the manipulative details as desired.

Leffmann-Beam Process.—This method of fat-separation, which has been extensively used in the author's laboratory, and has given great satisfaction, was devised by H. Leffmann and W. Beam for use with a centrifugal apparatus originally made by Beimling, and shown in figure 8. In using the apparatus, which is constructed to hold four, eight, or twelve bottles, as may be desired, 15 c.c. of the sample of milk should be measured into a specially constructed bottle of a capacity of about 30 c.c. and having a long graduated neck, each division on which represents 0·10 per cent. of milk-fat. Three c.c. of a mixture of

equal parts of amylic alcohol[1] and fuming hydrochloric acid (sp. gr. 1·16) is next added, the liquids well mixed, and 9 c.c. of sulphuric acid (sp. gr. 1·835) slowly added with constant agitation. The liquid becomes hot, and the casein previously separated dissolves completely, forming a dark reddish-brown solution. A hot mixture of two measures of water with one of strong sulphuric acid is then added, until the liquid reaches the zero-mark on the graduated bottle. The bottle is then placed in the centrifugal machine, and whirled for two minutes. In the case of skimmed milk and milk very poor in fat, the rotation should be continued a minute or so longer. On arresting the motion, the fat will be found to have formed a clearly-defined layer in the neck of the

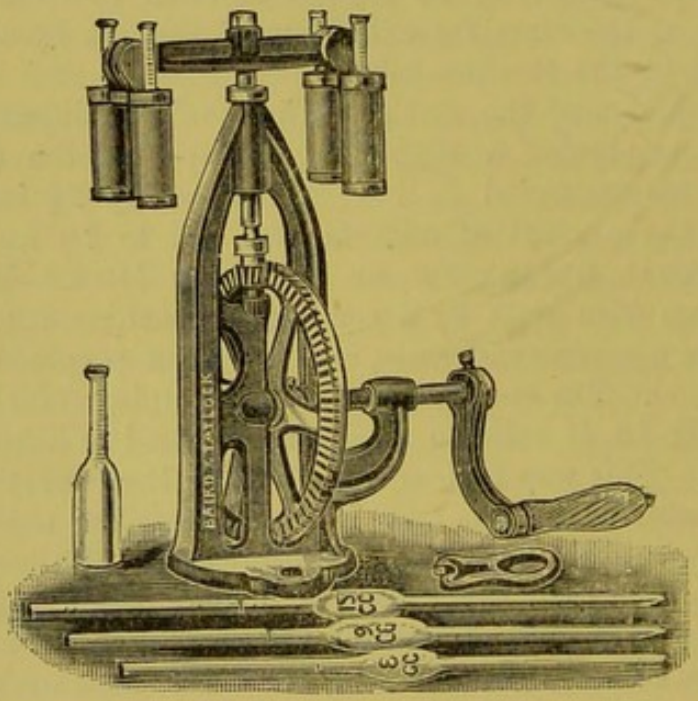


Fig. 8.—Leffmann-Beam (Beimling) centrifugal apparatus.

bottle, the volume of which can be read off. In doing this, it is convenient to use a pair of dividers, the legs of which are adjusted to the upper and lower limits of the fat stratum, due allowance being made for the meniscus. The dividers are then shifted, so that one point is on the zero of the scale. The position of the other will then show the percentage of fat in the sample.

The Leffmann-Beam process is also applicable to the determina-

[1] It is *essential* that the amylic alcohol be anhydrous and practically free from ethylic alcohol. This purification is best effected by agitating it with an equal measure of brine, separating the upper layer, shaking it with excess of recently ignited potassium carbonate, and distilling the decanted alcohol over potassium carbonate.

tion of the fat in *cream*. About 2 c.c. of the sample should be weighed into the bottle, and diluted with water to 15 c.c. The reading is multiplied by 15·45 and divided by the weight (in grammes) of the sample of cream taken.

If but one test is to be made, it is necessary, in order to preserve the balance of the arms of the machine, to place a duplicate test, or a bottle filled with diluted sulphuric acid, in the carrier opposite to that containing the sample to be tested.

Gerber Process.—A very convenient form of centrifugal apparatus has been described by N. Gerber.[1] In the instructions issued with the instrument, it is directed to introduce 10 c.c. of sulphuric acid, of a specific gravity not less than 1·820 nor greater than 1·825, into one of the bottles. One c.c. of chemically pure amylic alcohol is next added, and this is followed by 11 c.c. of the sample of milk to be tested, which should be poured gently down the side of the bottle. The bottle is then closed with an india-rubber stopper, and shaken till the contents are thoroughly mixed. While still hot, the bottle is placed in the rotator, the top of which is screwed on, and the whole made to revolve as rapidly as possible by pulling the cat-gut string. Under ordinary circumstances, the separation of the fat is complete in two or three minutes, when the bottle should be immersed in water at 60° to 70° C. for a few minutes and the volume of fat read off. In the case of skimmed milk, the rotation and immersion in warm water should be repeated several times before observing the volume of the separated fat. The same modified manipulation should be employed in the case of condensed milk, which is previously diluted with nine times its weight of water.

As already stated, there is no difficulty in employing the Leffmann-Beam bottle and proportions of milk and reagents, while taking advantage of the Gerber apparatus.

The results yielded by either of the foregoing methods of centrifugally separating fat from milk are generally in close agreement with those yielded by the Adams and Werner-Schmid processes, the variation, in carefully conducted experiments, rarely exceeding 0·20 per cent., and being often much less.[2] Occasionally, however, erratic results are obtained for some reason which is obscure, and therefore in important cases the indication should always be verified by a gravimetric determination.

A. W. Stokes employs 1½ c.c. of amylic alcohol, 13½ c.c. of sulphuric acid of 1·820 to 1·830 specific gravity, and 15 c.c. of

[1] Gerber has also devised (*Eng. Patent*, 1896, No. 18,282) an improved form of tube for use with his apparatus.

[2] See H. Fresenius (*Zeit. anal. Chem.*, 1897, xxxvi. 31).

the sample of milk to be tested. He has devised (*Eng. Patent*, 1895, No. 12,184) a special form of apparatus calibrated towards one end to allow of the reading of the separated fat, and having constrictions at suitable points, so that the proper quantities of reagents and milk can be added at once without previous measurement.

Stokes finds that fair results are obtainable without the use of a centrifugal apparatus, by thoroughly shaking the tube containing the milk and reagents, and then immersing it for at least an hour in water kept at a temperature from 150° to 180° F.

The following table shows the amounts of milk and reagents prescribed in various forms of the centrifugal method of fat separation.

	Babcock.	Leffmann-Beam.	Gerber.	Stokes.
Milk,	17·5 c.c.	15 c.c.	11 c.c.[1]	15 c.c.
Sulphuric acid, volume,	17·5 ,,	9 ,,	10 ,,[1]	13½ ,,
,, ,, sp. gravity,	1·831 to 1·834	1·835	1·820 to 1·825	1·820 to 1·830
Hydrochloric acid,	None.	1·5 c.c.	None.	None.
Amylic alcohol,	None.	1·5 ,,	1·0 c.c.	1·5 c.c.

Parallel experiments made by the above methods in the author's laboratory gave results decidedly in favour of the method of operating prescribed by Leffmann and Beam. In the case of the other methods, strict adherence to the strength of sulphuric acid prescribed was found to be very important, the use of too strong an acid causing browning of the fat, while with too weak an acid there was a tendency to incomplete separation.

The use of hydrochloric acid is not absolutely necessary, but it tends to effect a sharp separation of the fat. The employment of amylic alcohol gives a marked practical advantage, as it produces a large difference of surface-tension between the two layers of liquid, and thus promotes the ready separation of the fat.

Sebelien and Stören (abst. *Jour. Soc. Chem. Ind.*, 1895, p. 318) have made numerous comparative estimations of the fat in milk by the Babcock-Ahlborn, Thörner, and Gerber forms of centrifugal apparatus; their conclusions are shown in the following table:—

[1] In the process as originally described (*L'Industrie Laitière;* abst. *Analyst*, 1893, p. 137), Gerber directed the use of 10 c.c. of milk, 10 of acid and 1 c.c. of amylic alcohol.

	Accuracy of Results.	Simplicity of Construction.	Remarks on the Centrifugal Apparatus.	Reading-off.	Relative Cost.
Babcock-Ahlborn,	Practically the same in all three methods, the error being usually less than 0·10 to 0·15 per cent., but occasionally reaching 0·25 to 0·30 per cent.	Greatest simplicity.	Easily manipulated; two whirlings necessary for six and one minute respectively.	Difficult.	High.
Thörner,		Less simple.	Less simple; one whirling of three minutes required.	Easy.	Highest.
Gerber,		Unsuitable method of pipetting; rapid deterioration of the caoutchouc stoppers used.	Exceedingly simple circular centrifuge.	Easy.	Low.

Determination of the Proteids of Milk.

In the case of fresh milk, the nitrogen exists almost entirely in the form of proteids. Hence in such cases a fairly accurate measure of the total proteids present can be obtained by determining the nitrogen by Kjeldahl's process (page 21) and multiplying the result by 6·33. In altered milk, ammonia and other decomposition-products are present, which more or less invalidate a determination of the residual proteids by the above means; but the total nitrogen, multiplied by 6·33, is still a measure of the proteids originally present.

For more exact purposes, the method of determining the total proteids described on page 88 should be employed. The separation of the *casein* from the *lactalbumin*, and the determination of *albumoses*, if present, can also be effected as there described.

Tolmatschoff separates the casein from the albumin of human milk by saturating the liquid with magnesium sulphate, and filtering from the precipitate of casein containing entangled fat (compare page 90). From the filtrate the albumin (and globulin) may be thrown down by boiling. Biedert has thrown doubt on the value of this method, but its accuracy has been confirmed by Hoppe-Seyler (abst. *Jour. Chem. Soc.*, 1885, p. 845). H. Faber has applied the same process to cows' milk, and employs it to ascertain whether a sample of milk has been boiled (compare page 88). A more perfect precipitation of albumin and globulin can be obtained by precipitation with tannin or phospho-tungstic acid.

A. Schlossmann (*Zeit. physiol. Chem.*, 1896, xxii. 197; abst. *Jour. Chem. Soc.*, 1897, ii. 62) finds that, by adding to milk

heated to 37° C. a small quantity of a saturated solution of potassium-alum, the casein is precipitated in an insoluble form, the albumin and globulin remaining in solution. On saturating the filtrate with magnesium sulphate, the globulin is precipitated, while the albumin may be coagulated by boiling or determined by difference. The process has been found applicable to the separation of the proteids of human, cows', asses', and pigs' milk. Schlossmann finds, in cows' milk :—Casein (caseinogen), 3·185 ; globulin, 0·154 ; and albumin, 0·374 per cent. He insists on the importance of albumin and globulin in nutrition.

A method of determining the casein of milk, based on its precipitation with potassio-mercuric iodide, has been recently described by G. Denigès (abst. *Analyst*, 1897, p. 11), but experiments made in the author's laboratory to test the value of the process gave very unsatisfactory results.

Determination of Lactose in Milk.

In many of the older analyses of milk the proportion of lactose was determined by evaporating the sample to dryness, extracting the fat with ether, and boiling the residue with strong alcohol, to coagulate the proteids. On then adding some water (milk-sugar being very sparingly soluble in strong alcohol) and filtering, a solution was obtained which, on evaporation, left impure milk-sugar. The residue was weighed and ignited, the loss on ignition being regarded as milk-sugar.

Determinations of lactose made as above are not very accurate, as the residue weighed does not consist strictly of milk-sugar. Traces of citric acid,[1] lactic acid, casein, urea, &c., are also present.

[1] Citric Acid has been proved by Henkel and others to be a normal and constant constituent of cows' milk. A. Scheibe has found that goats' milk also contains citric acid, the proportion with ordinary fodder being from 1·0 to 1·5 gramme per litre, but liable to considerable fluctuations, the proportion, calculated on the total solids, being at times twice as great as at others. The acid does not appear to be derived from citric acid ready-formed in the fodder, as it also occurs, though in smaller proportion, in human milk (0·43 to 0·47 gramme per litre). Nor does the addition of citric acid to the fodder increase the proportion in the milk, or feeding on materials free from citric acid diminish it. The citric acid of milk is apparently not derived from the cellulose undergoing digestion in the intestines of herbivora, since its excretion goes on when food is wholly withheld or meal substituted for ordinary fodder.

In the milk of the gamoose, Pappel and Richmond found 0·30 per cent. of citric acid. For its isolation and determination they precipitated the milk by 4 per cent. of the acid solution of mercuric nitrate, recommended by Wiley (page 145) for the determination of milk-sugar. The filtered liquid was exactly neutralised with caustic soda (phenol-phthalein being employed as an

A more accurate determination of the sugar naturally present in milk may be obtained by observing the optical rotation of the liquid, or by ascertaining its reducing action on an alkaline copper solution.

Richmond and Boseley (*Analyst*, 1892, p. 141) have shown that, when milk is heated to 100° C., the specific rotatory power of the lactose is diminished, but the alteration is not a constant one in different experiments in which the time of heating is the same. Hence no correction can be made. On the other hand, the cupric oxide reducing-power of the milk-sugar is not altered by previous heating. It follows that the polarimetric determination of lactose is inapplicable to milk which has been boiled, but that the copper processes may be used.

For the polarimetric determination of lactose in milk, it is first necessary to obtain a clear solution for observation. This is effected by preparing the "whey" of the milk, which will contain all the milk-sugar. The whey may be obtained by Schmoeger's method, in which precipitation is effected by acetic acid. The resultant liquid is filtered, basic lead acetate added to the filtrate, the solution boiled, cooled, made up to its original bulk, and again filtered; or precipitation may be effected by an acid solution of mercuric nitrate.[1] The filtered solution will be quite clear, and ready for observation in the polarimeter. Mercuric nitrate is not only effective in producing a clear whey from ordinary milks, but is applicable to cream containing upwards of 50 per cent. of fat, if the precaution be taken to dilute the cream with water before precipitation, in order to obtain a sufficient quantity of liquid.

The optical activity of the liquid having been observed, a correction for the volume of precipitated proteids has to be applied. An arbitrary correction was at first made by Wiley, but in a more recent paper (abst. *Analyst*, 1896, p. 186) Wiley and Ewell

indicator), the precipitate filtered off, and decomposed by sulphuretted hydrogen. The filtered liquid was exactly neutralised, calcium chloride added, and the liquid boiled, when calcium citrate was precipitated. The precipitate contained 24·4 per cent. of calcium, as against 24·1 per cent. required by theory (*Jour. Chem. Soc.*, lvii. 754).

Calcium citrate is often deposited on the vessels in which milk is boiled or evaporated.

[1] H. W. Wiley (*Amer. Chem. Journal*, vi. 289), who first recommended the use of acid mercuric nitrate, originally prepared it by dissolving the mercury in twice its weight of nitric acid (sp. gr. 1·42) and diluting the resultant solution with an equal measure of water. More recently he has replaced this by a more dilute solution made by diluting the solution of mercury in nitric acid with five measures of water.

have described a method of double dilution and polarisation, instead of correcting the results of a single observation by a factor.

The idea of double dilution and polarisation first originated with Scheibler, who proposed it for sugar solutions, and it was subsequently suggested by Bigelow and M'Elroy for use in the polarisation of milk-sugar (*Amer. Chem. Journal*, xv. 694). The results of the experiments of Wiley and Ewell show that the factor for correction for the volume of the soluble proteids varies from 2·5 c.c. (when about 60 c.c. of milk were used in a 100 c.c. flask) to 6 c.c., according to the richness of the milk in fat.

The process for the determination of milk-sugar, as modified by Wiley and Ewell, is conducted as follows :—When using a Schmidt and Haensch's triple-field shadow-polariscope, Wiley and Ewell found that 32·91 grammes of pure lactose in 100 c.c. gave a reading of 100 on the scale. Therefore double this quantity, *i.e.*, 65·82 grammes of milk, is placed in a 100 c.c. flask, clarified with about 10 c.c. of the acid mercuric nitrate solution,[1] the volume made up to the 100 c.c. mark, the contents of the flask well shaken, poured upon a filter, and the rotation of the filtrate observed in a 400 mm. tube (which length of tube is used by Wiley and Ewell in order to ensure greater accuracy in the observation). A similar quantity of the milk is placed in a 200 c.c. flask, and subjected to the same treatment. The polarimetric data obtained are employed for calculating the true volume of liquid in the flask, the true percentage of lactose, and the true volume of the precipitate, in accordance with the following rule.

Let x equal the volume of the precipitate ; y, the correct reading of the polarimeter ; a, the reading obtained from the solution in the 100 c.c. flask ; and b, the reading obtained from the solution in the 200 c.c. flask. Then :—

$$\text{(A)}\quad 200-x : 100-x = a : b\,;\ \text{whence}\ x = 100\frac{(a-2b)}{a-b}$$

$$\text{(B)}\quad 100-x : 100 = y : a\,;\ \text{whence}\ y = \frac{ab}{a-b}$$

So that, according to equation B, the true polarisation, as determined by double dilution, is found by dividing the product of the two readings made from the solutions in the 100 c.c. and 200 c.c. flasks by their difference.

Wiley and Ewell's results are very satisfactory, and show that if the method be carefully applied the amount of milk-sugar

[1] In preparing the solution of milk-sugar in the 200 c.c. flask, it may be found necessary to use more than 10 c.c. of the acid mercuric nitrate, in order to obtain a perfectly clear filtrate.

present in a solution may be estimated to within one-tenth per cent.

Richmond and Boseley have criticised the above process (*Analyst*, 1897, p. 98), and give the preference—on the score of smaller experimental error, less time required, and the smaller quantity of milk necessary—to the method described by Vieth (*Analyst*, 1888, p. 63), with some modifications by themselves. They consider that the only objection to Vieth's method is the necessity of making a correction for each sample. In order to avoid this, they make the following compensatory additions to 100 c.c. of the sample of milk :—

a. An addition of 3 c.c. of acid mercuric nitrate, which compensates for the volume of the proteids.

b. The percentage of fat in the sample is multiplied by 1·11 and expressed in c.c. This amount of water is added to compensate for the volume of the fat.

c. The specific gravity of the sample above 1000 is divided by 10, and a volume of water equal to the dividend added.

d. An addition of sufficient water to reduce scale-readings to percentages of milk-sugar. With the Mitscherlich half-shadow polarimeter employed by Richmond and Boseley (identical with that described by Vieth in the *Analyst*, 1886, p. 144), employing a tube of 198·4 mm. length, the value of *d* is 10 c.c. to 100 c.c. of milk.[1] In a sample containing 3·7 per cent. of fat, and having a gravity of 1032·5, the volume of water to be added to 100 c.c. of the milk would be $3+4{\cdot}1+3{\cdot}25+10=20{\cdot}35$; or 10·17 c.c. to 50 c.c. of the sample.

In practice it is better to add to 100 c.c. of the milk 15 c.c. of the weaker mercuric nitrate solution recommended by Wiley and Ewell, making up the required volume with water. If strong mercuric nitrate solution be employed, the proteids are liable to be discoloured by the occurrence of Millon's reaction (page 11).

The test-analyses of Richmond and Boseley show that very good results may be obtained by dilution in the above manner to obtain direct readings. They have also proved that the addition of preservatives in the proportion of 1 gramme per 100 c.c. of milk (and in the case of formalin 2 c.c. for the same volume of milk), the sample being subsequently allowed to stand for a

[1] For other instruments the value of *d* may be calculated by the formula $\left(\frac{55{\cdot}3\,k\times l}{100}-100\right)\times s$; where *k* is the factor necessary to convert angular degrees into scale-readings ; *l* the length of the polarising tube in millimetres ; and *s* the specific gravity of the milk (which may be taken as 1·032 without appreciable error).

week in an incubator at a temperature of 25° C., does not affect the optical activity (except to a limited extent in the case of borax).

P. Thibault (*Jour. Pharm.*, 1896, [6], iv. 5 ; abst. *Jour. Chem. Soc.*, 1897, ii. 80) states that mercuric acetate, lead acetate, and sodium metaphosphate, which give good results with cows' milk, do not yield with human milk a liquid clear enough (after filtration) for optical examination. He recommends a precipitant containing 10 grammes of picric acid and 25 c.c. of glacial acetic acid in one litre. When this reagent is added to an equal measure of human milk, a liquid is obtained which filters perfectly clear.

Milk-sugar may be conveniently determined by observing the cupric oxide reducing-power of the milk, which may be ascertained either gravimetrically or volumetrically.

Among the existent forms of the gravimetric methods of determining lactose in milk, the modification of C. O'Sullivan appears to be the most accurate and convenient. E. W. T. Jones (*Analyst*, xiv. 81) and H. D. Richmond (*Analyst*, xvii. 223) have obtained good results by its use ; the latter chemist, however, finds that he can obtain quite concordant results when the Fehling's solution is not so dilute. O'Sullivan directs that the Fehling's solution be diluted with two measures of water, which is the proportion followed by Jones, but Richmond dilutes the Fehling's solution with an equal measure of water, and thus avoids the filtration of more solution than is necessary. O'Sullivan's method consists essentially in having a large excess of Fehling's solution present, diluted with two volumes of water (about 30 c.c. of Fehling solution for each 0·1 gramme lactose present). The Fehling's solution is brought to the temperature of the water-bath (boiling), the sugar solution added, and the liquid heated for fifteen minutes. The precipitate of cuprous oxide is collected on a filter, ignited gently at first over a Bunsen burner, and then more strongly. Richmond ignites finally in a muffle. He also deducts 2 milligrammes from the weight of the precipitate as a correction for absorption by the filter-paper. The CuO is then calculated into its equivalent of anhydrous lactose by the factor 0·6024 (Jones). Richmond finds 0·6025, whilst by calculation from the mean of Rodewald and Tollens' results he obtained the factor 0·6022.

Milk-sugar may be determined volumetrically by the use of Pavy's ammoniacal cupric solution. This solution may be prepared by mixing 120 c.c. of ordinary Fehling solution with 300 c.c. of ammonia (sp. gr. 0·880), and with 100 c.c. of a ten per cent. solution of caustic soda, or the same volume of a 14 per

cent. solution of caustic potash.[1] The mixture is then made up with distilled water to 1 litre.

The oxidising value of Pavy's solution, prepared as above, is one-tenth that of Fehling's solution, *i.e.*, 50 milligrammes of glucose reduce 10 c.c. of Fehling's or 100 c.c. of Pavy's solution.[2]

When Pavy's solution is heated with a liquid containing milk-sugar the cuprous oxide produced does not separate as a precipitate,

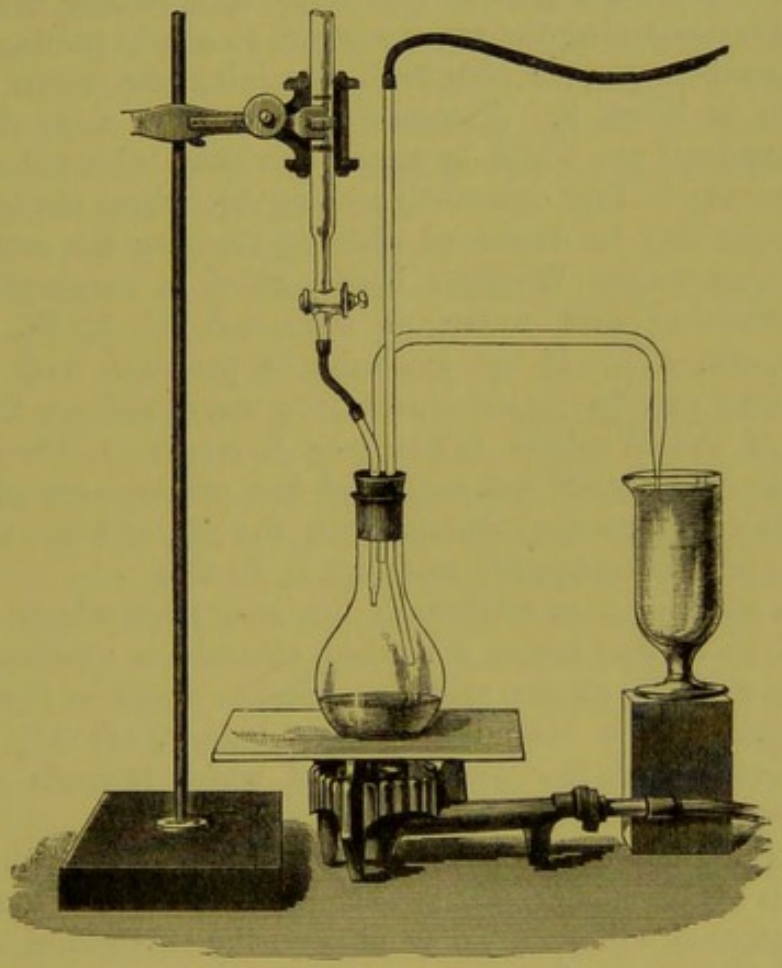


Fig. 9.—Apparatus for Pavy's test.

but remains in colourless solution. The progress of the reaction is therefore indicated by the gradual disappearance of the blue

[1] If Fehling's solution be not at hand, Pavy's solution may be made up directly as follows :—4·157 grammes of pure crystallised copper sulphate is dissolved in about 100 c.c. of hot water and the solution cooled. 21·6 grammes of crystallised Rochelle salt and 18·4 grammes of caustic soda (or 25·8 grammes of caustic potash) are dissolved in hot water, cooled, and added to the copper sulphate solution. To the mixture 300 c.c. ammonia (sp. gr. 0·880) is added, and the whole made up to 1 litre with distilled water.

[2] One molecule of glucose reduces to cuprous oxide 6 CuO existing in Fehling's solution, but only 5 CuO in Pavy's solution prepared as described in the text.

colour of the liquid, and when the reaction is complete—that is, when all the copper is reduced to the cuprous state—the liquid becomes quite colourless. But ammoniacal cuprous solutions are extremely oxidisable, and hence the blue colour indicative of the existence of a cupric compound returns rapidly on exposing the reduced liquid to the air. It is necessary, therefore, to operate in such a manner as will ensure the complete exclusion of air during the process. This is best done by attaching the nose of the burette containing the sugar solution to a tube passing through the india-rubber stopper of a flask containing the copper solution, as shown in figure 9. A second tube conveys away the steam and ammoniacal gas, while, by means of a third tube, a slow stream of coal-gas may be kept constantly passing throughout the operation.[1] The coal-gas may be dispensed with by allowing the exit-tube to pass into an empty Woulffe's bottle, which is connected with a flask containing cold water, as suggested by A. W. Stokes (*Analyst*, xii. 47). If the exit-tube be provided with a valve, the vapours may be passed direct into water without the intervention of the Woulffe's bottle (see J. Steiner, *Chem. News*, xl. 139). The author has suggested the employment of a layer of paraffin oil to prevent contact with the air, and the same proposal has been subsequently made by Z. Peska.

It is a good plan to whitewash the iron plate which supports the flask, as by that means a marked contrast is obtained to the blue colour of the copper solution. Stokes supports the half of an opal-glass globe behind the flask (*Analyst*, xii. 47). H. M. Smith uses a flask the lower third of which is made of white opalescent glass (*Chem. News*, lxxi. 165).

In carrying out Pavy's process, from 40 to 50 c.c. of the copper solution is a convenient quantity to employ. Of course, the amount of solution used will greatly depend upon the strength of the milk-sugar solution. An exact measure of the ammoniacal cupric solution should be placed in the flask and a few fragments of pumice or tobacco-pipe added to prevent bumping. The tubes and burette are then adjusted, a slow current of coal-gas allowed to pass, and the contents of the flask brought to ebullition. The sugar solution is then run in from the burette, the boiling being continued. The process is at an end when the blue colour of the liquid is wholly destroyed. The end-reaction is very sharply marked, but the reduction occurs more slowly than with the ordinary Fehling's

[1] When coal-gas is employed, a brick-red film is formed on the surface of the liquid towards the end of the titration. This is not cuprous oxide, as might be supposed, but cuprous acetylide, Cu_2C_2,H_2O, formed from the acetylene invariably present in coal-gas.

solution, especially in the case of milk-sugar, and hence the process must not be hurried or too low a result will be obtained. According to Stokes and Bodmer (*Analyst*, x. 62), 50 c.c. of Pavy's solution having been used, the measure of sugar solution required to decolorise it contains 0·0481 gramme of milk-sugar.

In sour milk the lactose is more or less converted into an equal weight of lactic acid. This may be determined by titration with standard alkali and phenol-phthalein, and the amount added to the lactose found by Pavy's solution to obtain the quantity of milk-sugar which existed in the fresh milk. Of course, when the decomposition of the milk has proceeded beyond the mere stage of curdling, and alcoholic fermentation has set in, the foregoing method will be quite useless.

Specific Gravity of Milk.

The milk of the cow, about one hour after being drawn, has an average specific gravity of about 1·031.[1]

It was first observed by Recknagel, and the fact has since been fully confirmed by other observers, that after some hours the gravity of cows' milk sensibly increases. Thus P. Vieth (*Analyst*, xiv. 69) found a rise in the gravity in twenty-four hours to vary from 0·001 to 0·002, the average increase being 0·0013. The same phenomenon has been observed by Bourcart in cows' milk, and by Pappel and Richmond (*Jour. Chem. Soc.*, lvii. 754) in the milk of the gamoose. The subject has been further investigated by H. D. Richmond, who in some cases found as great a rise as that observed by Recknagel and Vieth, while in other cases the change was absolutely *nil*. He states that there is some evidence that the rise in specific gravity takes place to a much greater extent in autumn and winter than in the spring and summer.

Recknagel finds the specific gravity of cows' milk to become constant after five hours or less, if the milk is cooled down below 15° C. Above that temperature the gravity increases for twenty-four hours or more. If milk, the gravity of which has become constant, be warmed to 40° C., the density will decrease, and will not become normal again till some time has elapsed.

Recknagel attributed the increase of the gravity to a change in the volume of the casein. Richmond regards enzymic action as the most probable explanation of the phenomenon, and W. J. Sykes has pointed out that hydrolysis due to enzymic action (proteolysis) would produce the effect through the assimilation of water by the molecule.

[1] P. Vieth states (*Analyst*, vii. 53) that of many thousands of milk samples of which he has taken the specific gravity, he never knew it to fall below 1·029 in the case of a normal and well-mixed milk from at least five cows.

The specific gravity of milk is often taken with a hydrometer, but the method is deficient in accuracy, owing to the defective graduation of the instruments commonly sold and on account of the difficulty of correctly reading the position of the spindle in an opaque liquid. For the purpose of calculating the fat or solids by a table or formula a hydrometric determination of the gravity is, in the opinion of the author, unsatisfactory.[1] The density should be taken by a Westphal balance or 10 c.c. Sprengel's tube, and the observation should be made at the standard temperature and not less than six hours after milking. Unless these conditions be observed, determinations of specific gravity to the fourth place of decimals are not to be relied on.

In the opinion of H. D. Richmond, a specific gravity bottle is not a suitable instrument for observing the density of milk, since there is a tendency for cream to separate before the milk has acquired the standard temperature.

Temperature. °F.	Observed Specific Gravity.									
	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035
	Corrected Specific Gravity.									
50	1025·1	1026·1	1027·0	1028·0	1029·0	1029·9	1030·9	1031·8	1032·7	1033·6
51	25·2	26·2	27·1	28·1	29·1	30·1	31·0	31·9	32·9	33·8
52	25·2	26·2	27·2	28·2	29·1	30·1	31·1	32·0	33·0	33·9
53	25·3	26·3	27·3	28·3	29·2	30·2	31·2	32·1	33·1	34·0
54	25·4	26·4	27·4	28·4	29·3	30·3	31·3	32·3	33·2	34·2
55	25·5	26·5	27·5	28·5	29·4	30·4	31·4	32·4	33·3	34·3
56	25·6	26·6	27·6	28·6	29·6	30·5	31·5	32·5	33·5	34·5
57	25·7	26·7	27·7	28·7	29·7	30·6	31·6	32·6	33·6	34·6
58	25·8	26·8	27·8	28·8	29·8	30·8	31·7	32·7	33·7	34·7
59	25·9	26·9	27·9	28·9	29·9	30·9	31·9	32·9	33·9	34·9
60	26·0	27·0	28·0	29·0	30·0	31·0	32·0	33·0	34·0	35·0
61	26·1	27·1	28·1	29·1	30·1	31·2	32·2	33·2	34·2	35·2
62	26·2	27·3	28·3	29·3	30·3	31·3	32·3	33·3	34·3	35·3
63	26·3	27·4	28·4	29·4	30·4	31·4	32·5	33·5	34·5	35·5
64	26·5	27·5	28·5	29·5	30·5	31·5	32·6	33·6	34·6	35·6
65	26·6	27·6	28·6	29·6	30·7	31·7	32·7	33·8	34·8	35·8
66	26·7	27·7	28·7	29·8	30·8	31·8	32·9	33·9	34·9	35·9
67	26·8	27·8	28·8	29·8	30·9	32·0	33·0	34·0	35·0	36·1
68	27·0	28·0	29·0	30·1	31·1	32·2	33·2	34·2	35·2	36·2
69	27·1	28·1	29·1	30·2	31·2	32·2	33·3	34·3	35·3	36·4
70	27·2	28·2	29·2	30·3	31·3	32·4	33·4	34·5	35·5	36·5
71	27·3	28·3	29·4	30·4	31·5	32·5	33·6	34·6	35·6	36·7
72	27·4	28·4	29·5	30·5	31·6	32·6	33·7	34·7	35·8	36·8
73	27·5	28·6	29·7	30·7	31·8	32·8	33·9	34·9	36·0	37·0
74	27·7	28·7	29·8	30·9	31·9	33·0	34·0	35·1	36·1	37·2
75	27·8	28·9	29·9	31·0	32·1	33·1	34·2	35·2	36·3	37·3

[1] From an extensive experience of the use of hydrometers for observing the specific gravity of milk, Richmond considers that, when properly graduated and checked, they are as good as the Westphal balance. The variation from the results obtained on the same samples with Sprengel-tubes did not exceed +0·0002.

Although it is decidedly preferable to observe the specific gravity of milk at the standard temperature (60° F. = 15·5° C.), in cases where this is impossible or highly inconvenient the observation may be corrected by means of the foregoing table, compiled by P. Vieth (*Analyst*, 1885, page 70).

For the determination of the density of curdled milk, M. Weibull (abst. *Analyst*, xix. 24) adds a definite volume of ammonia of known specific gravity, mixes well, and determines the density of the mixture. As no sensible alteration of volume occurs when the liquids are mixed, and the specific gravity of the ammonia is known, the density of the milk can be readily calculated. The results agree well with those obtained on the fresh milk without the use of ammonia.

The specific gravity of milk has often been employed as an indication of its quality, and to a certain extent, and as a preliminary test, the indication is of value. But the gravity, if taken alone, is liable to lead to very erroneous conclusions. Thus the fat, being the lightest constituent of milk, will tend to reduce the density of the liquid in proportion to the amount present, and hence a milk rich in fat will possess a lower specific gravity than a sample containing less fat, and, measured by this test, will be held to be of inferior quality. If a sample of new milk be allowed to stand, and the cream removed by skimming, the skimmed milk will be found to have a density several degrees higher than the original milk. Hence a milk may be first skimmed and then extensively watered, and yet the specific gravity of the sample will afford no indication of the fact. The truth of this proposition is now fully recognised by milk-analysts, and the determination of the specific gravity has been wholly abandoned by them as a means of judging of the quality of a milk of unknown history. For the rapid technical examination of different specimens of genuine milk, the test is capable of useful application, and if employed in conjunction with some method of determining the proportion of fat present, is capable of affording most valuable information.

Milk Formulæ.

The specific gravity of milk is dependent on the absolute and relative proportions of fat, proteids, sugar, and salts present in the liquid. The mineral constituents vary with the proteids, but they are present in small proportion only, and since their solution-density is about the same as that of milk-sugar their effect on the density of the milk is not liable to sensible variation. The proteids and milk-sugar both tend to increase the density of the milk, and not exactly to the same extent, but for practical purposes it is unnecessary to differentiate between them. The fat,

being lighter than water, has a tendency to diminish the specific gravity of the milk, and hence it is necessary to take it into account in drawing any conclusion as to the quality of a sample. The specific gravity of milk-fat (as existing in milk and not in the solid state after separation) has been calculated by H. D. Richmond to be 0·939. This number is distinctly higher than the observations of Blyth, Fleischmann, and others ; but is practically identical with the density calculated from the results of Hehner and Richmond, and with the formula of Fleischmann and Morgen.

In 1879 Behrend and Morgen (*Jour. f. Landw.*, xxvii. 250) pointed out the exact relation between the specific gravity, fat, and total solids of milk, and published a table. Shortly after, Clausnitzer and Mayer (*Forschungen auf dem Gebeite*, ii. 265) published a formula founded on the examination of a single sample of milk, which was analysed, allowed to stand over-night, and then again analysed. From this they found that 1 per cent. of fat lowered the gravity of the milk by 1 degree (water being 1000), while 1 per cent. of non-fatty solids raised it 3·75 degrees. O. Hehner (*Analyst*, vii. 129) subsequently compiled a formula, based on the analysis of twenty-two samples of milk by Wanklyn's method. He took the specific gravity of milk-fat as 0·9275, and assumed that 1 per cent. decreased the gravity of the milk by 0·725 (water being 1000), while each unit per cent. of non-fatty solids raised the gravity by 3·6. Fleischmann and Morgen (*Jour. f. Landw.*, xxx. 293) constructed a formula for use with the plaster process of determining fat. In this they assumed the specific gravity of milk-fat at 15° C. to be ·940, but Fleischmann subsequently found by experiment that the true gravity was 0·930, and altered his formula accordingly.

In 1888 Hehner and Richmond published a formula for use with the Adams' coil process for fat-determination, and this has since been several times modified by H. D. Richmond.

None of these formulæ are absolutely free from objection, as they either contain an assumption which is not strictly correct, or the gravity of the fat is assumed to be the same in milk as it is in the solid state.

It cannot be insisted on too strongly that milk-formulæ necessarily vary with the process of fat-extraction employed. Thus Fleischmann's formula was constructed for use with the plaster process. Similarly, if empirical methods of fat-extraction be employed, which do not always effect complete extraction, the calculation of the unknown must be made by suitably modified formulæ. Where the fat is completely extracted, and is weighed in a state of purity, as is the case with the Adams' coil process in

its various modifications and with the Werner-Schmid method, a constant formula may be used. The formula which has been most generally adopted is that of Hehner and Richmond (*Analyst*, 1888, p. 26), who calculate the fat from the observed total solids and specific gravities of a number of representative samples of milk by the formula:—$F = 0{\cdot}859\,T - 0{\cdot}2186\,G$. In this expression, G is the excess-density or difference between 1000 and the specific gravity of the milk (water = 1000); T the percentage of total solids; and F the percentage of fat in the sample.[1]

A simpler form of what is practically the same formula is $F = \frac{5}{6}(T - \frac{G}{4})$. With average milk either of these expressions will give a close approximation to the truth, but in the case of samples deficient in fat, that is, where $\frac{G}{T}$ exceeds 2·5, the following formula should be used:—

$$F = 0{\cdot}859\,T - 0{\cdot}2186\,G - 0{\cdot}05(\tfrac{G}{T} - 2{\cdot}5).$$

It is evident that the method is equally available for calculating the total solids of milk when the specific gravity and fat are known, the expression for this purpose being:—

$T = \frac{F + 0{\cdot}2186G}{0{\cdot}859} = 1{\cdot}164\,F + 0{\cdot}254\,G$; or, approximately, $T = \frac{24F + 5G}{20}$.

In 1889 (*Analyst*, xiv. 121), as the result of determinations of fat with coils more perfectly freed from ether-soluble impurity than those previously employed, H. D. Richmond proposed the following modified formula:—$T = 1{\cdot}17\,F + 0{\cdot}263\frac{G}{D}$; but admitted that it had little if any practical advantage over that previously proposed by Hehner and himself. More recently (*Analyst*, xvii.

[1] In the analyses on which this formula was founded, 5 c.c. of the milk was pipetted into a weighed dish, and the weight accurately ascertained. The milk was then evaporated and the residue dried till practically constant, the weight obtained being the total solids in 5 c.c. The specific gravities were taken by a Sprengel-tube with very narrow capillaries, or with a Westphal balance of great delicacy.

The samples analysed included normal milk, skimmed milk, skimmed and watered milk, cream, milk enriched with cream, and watered milk. For the determination of the fat, 5 c.c. of the sample was dropped as quickly as possible on a strip of demy blotting paper, which was allowed to dry in the air for about an hour and then coiled up and extracted with ether in a Soxhlet-tube for at least an hour and a half. An allowance of 0·012 gramme was made for ether-soluble impurity contained in each coil, this correction being based on careful blank experiments. In a subsequent paper (*Analyst*, xiv. 125), Richmond states that the error due to matter extracted by ether from paper-coils is by no means constant, varying from 0·04 to 0·55, and being highest where free acid is present. He recommends that the coils should be previously extracted with acidulated alcohol.

Table based on Hehner & Richmond's Milk Formula.

Specific Gravity.	Percentage of Fat.																								
	2·0	2·1	2·2	2·3	2·4	2·5	2·6	2·7	2·8	2·9	3·0	3·1	3·2	3·3	3·4	3·5	3·6	3·7	3·8	3·9	4·0	4·1	4·2	4·3	4·4	4·5
1024·0	8·42	8·54	8·66	8·77	8·89	9·01	9·12	9·24	9·35	9·47	9·59	9·70	9·82	9·94	10·05	10·17	10·29	10·40	10·52	10·63	10·75	10·87	10·98	11·10	11·22	11·33
·5	8·56	8·68	8·79	8·90	9·02	9·14	9·25	9·37	9·48	9·60	9·72	9·83	9·95	10·07	10·18	10·30	10·42	10·53	10·65	10·76	10·88	11·00	11·11	11·23	11·35	11·46
1025·0	8·68	8·80	8·91	9·02	9·14	9·26	9·37	9·49	9·60	9·72	9·84	9·95	10·07	10·19	10·30	10·42	10·54	10·65	10·77	10·88	11·00	11·12	11·23	11·35	11·47	11·58
·5	8·81	8·93	9·04	9·15	9·27	9·39	9·50	9·62	9·73	9·85	9·97	10·08	10·20	10·32	10·43	10·55	10·67	10·78	10·90	11·01	11·13	11·25	11·36	11·48	11·60	11·71
1026·0	8·94	9·06	9·17	9·28	9·40	9·52	9·63	9·75	9·86	9·98	10·10	10·21	10·33	10·45	10·56	10·68	10·80	10·91	11·03	11·14	11·26	11·38	11·49	11·61	11·73	11·84
·5	9·06	9·18	9·29	9·40	9·52	9·64	9·75	9·87	9·98	10·10	10·22	10·33	10·45	10·57	10·68	10·80	10·92	11·03	11·15	11·26	11·38	11·50	11·61	11·73	11·85	11·96
1027·0	9·19	9·31	9·42	9·53	9·65	9·77	9·88	10·00	10·11	10·23	10·35	10·46	10·58	10·70	10·81	10·93	11·05	11·16	11·28	11·39	11·51	11·63	11·74	11·86	11·98	12·09
·5	9·32	9·44	9·55	9·66	9·78	9·90	10·01	10·13	10·24	10·36	10·48	10·59	10·71	10·83	10·94	11·06	11·18	11·29	11·41	11·52	11·64	11·76	11·87	11·99	12·11	12·22
1028·0	9·45	9·57	9·68	9·79	9·91	10·03	10·14	10·26	10·37	10·49	10·61	10·72	10·84	10·96	11·07	11·19	11·31	11·42	11·54	11·65	11·77	11·89	12·00	12·12	12·24	12·35
·5	9·57	9·69	9·80	9·91	10·04	10·16	10·27	10·38	10·49	10·61	10·73	10·84	10·96	11·08	11·19	11·31	11·43	11·54	11·66	11·77	11·89	12·01	12·12	12·24	12·36	12·47
1029·0	9·70	9·82	9·93	10·04	10·16	10·30	10·39	10·51	10·62	10·74	10·86	10·97	11·09	11·21	11·32	11·44	11·56	11·67	11·79	11·90	12·02	12·14	12·25	12·37	12·49	12·60
·5	9·83	9·95	10·06	10·18	10·29	10·42	10·52	10·64	10·75	10·87	10·99	11·10	11·22	11·34	11·45	11·57	11·69	11·80	11·92	12·03	12·15	12·27	12·38	12·50	12·61	12·73
1030·0	9·96	10·09	10·20	10·31	10·42	10·54	10·65	10·77	10·88	11·00	11·12	11·23	11·35	11·47	11·58	11·70	11·82	11·93	12·05	12·16	12·28	12·40	12·51	12·63	12·74	12·86
·5	10·10	10·21	10·33	10·44	10·55	10·67	10·78	10·90	11·01	11·13	11·25	11·36	11·48	11·60	11·71	11·83	11·95	12·06	12·18	12·29	12·41	12·53	12·64	12·76	12·87	12·99
1031·0	10·23	10·34	10·46	10·57	10·68	10·80	10·91	11·03	11·14	11·26	11·38	11·49	11·61	11·73	11·84	11·96	12·08	12·19	12·31	12·42	12·54	12·66	12·77	12·89	13 00	13·12
·5	10·36	10·47	10·58	10·69	10·80	10·92	11·03	11·15	11·26	11·38	11·50	11·61	11·73	11·85	11·96	12·08	12·20	12·31	12·43	12·54	12·66	12·78	12·89	13·01	13·12	13·24
1032·0	10·49	10·60	10·71	10·83	10·93	11·05	11·16	11·28	11·39	11·51	11·63	11·74	11·86	11·98	12·09	12·21	12·33	12·44	12·56	12·67	12·79	12·91	13·02	13·14	13·25	13·37
·5	10·61	10·72	10·84	10·96	11·06	11·18	11·29	11·41	11·52	11·64	11·76	11·87	11·99	12·11	12·22	12·34	12·46	12·57	12·69	12·80	12·92	13·04	13·15	13·27	13·38	13·50
1033·0	10·74	10·85	10·97	11·09	11·19	11·31	11·42	11·54	11·65	11·77	11·89	12·00	12·12	12·24	12·35	12·47	12·59	12·70	12·82	12·93	13·05	13·17	13·28	13·40	13·51	13·63
·5	10·87	10·98	11·09	11·21	11·32	11·44	11·55	11·66	11·77	11·89	12·01	12·12	12·24	12·36	12·47	12·59	12·71	12·82	12·94	13·05	13·17	13·29	13·40	13·52	13·63	13·75
1034·0	11·00	11·11	11·22	11·34	11·44	11·56	11·67	11·79	11·90	12·02	12·14	12·25	12·37	12·49	12·60	12·72	12·84	12 95	13·07	13·18	13·30	13·42	13·53	13·65	13·76	13·88
·5	11·13	11·24	11·35	11·47	11·57	11·69	11·80	11·92	12·03	12·15	12·27	12·38	12·5	12·62	12·73	12·85	12·97	13·08	13·20	13·31	13·43	13·55	13·66	13·78	13·89	14·01
1035·0	11·25	11·37	11·47	11·59	11·69	11·81	11·92	12·04	12·15	12·27	12·39	12·50	12·62	12·74	12·85	12·97	13·09	13·20	13·32	13·43	13·55	13·67	13·78	13·90	14·01	14·13

170), in order to meet the rarely-occurring case of milk with a very high proportion of non-fatty solids, he has proposed to modify the formula as follows :—

$$T = 1{\cdot}164(F + 0{\cdot}2\,[S.\text{ not } F - 8{\cdot}87]) + 0{\cdot}254\,G.$$

The correction has very little practical importance, as it is almost always within the limit of experimental error.

Richmond informs the author that the formula he now uses, and which gives him the best results, is :—$T = 1{\cdot}2\,F + 0{\cdot}14 + 0{\cdot}25\,G$.

An extensive table is appended to the paper of Hehner and Richmond (*Analyst*, 1888, page 32), whereby the fat of milk can be at once deduced from the solids and specific gravity without the use of the foregoing formula. By the preceding table, also based on Hehner and Richmond's formula ($T = 1{\cdot}164\,F + 0{\cdot}254\,G$), the total solids of milk may be ascertained when the fat and specific gravity of the sample are known.

Instead of employing either the formula or table, a convenient plan is to use a milk-scale constructed on the principle of the slide-rule. Such an instrument,[1] shown in fig. 10, has been devised by H. D. Richmond, and by its aid the fat, solids, or specific gravity corresponding to any data observed may be at once read off. The instrument has been used constantly for years in the author's laboratory, with perfect satisfaction.

The establishment of a definite relationship between the specific gravity, fat, and total solids of milk has been of the greatest practical value in the technical examination of milk, and has furnished the analyst with a preliminary method of sorting good milks from bad, the value of which it is impossible to over-estimate.

H. D. Richmond (*Analyst*, xv. 170) has also devised a formula by means of which, the specific gravity, fat, total solids and ash of a sample of

[1] Obtainable from Messrs Baird & Tatlock, Cross Street, Hatton Garden, London, E.C.

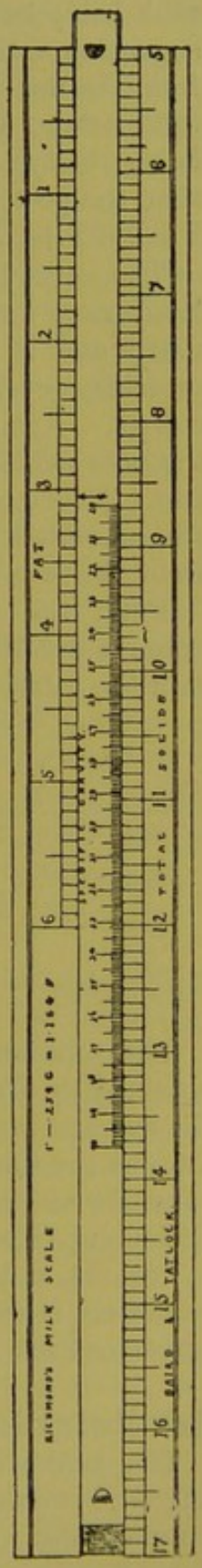


Fig. 10.—Richmond's Milk-scale.

milk being known, the proteids can be calculated, and the milk-sugar then estimated by subtracting the sum of the ash and proteids from the non-fatty solids. Thus if P represent the proteids, T the total solids, A the ash, F the fat, D the density (water at 60° F. being unity), and G the excess-density ($=1000\,D-1000$), then the proteids, P, may be found by the following formula :—
$P=2{\cdot}8\,T+2{\cdot}5\,A-3{\cdot}33\,F-0{\cdot}7\frac{G}{D}$.

The errors between the milk-sugar and proteids found and calculated vary between $+0{\cdot}4$ and $-0{\cdot}4$. These discrepancies are partly due to the assumption that everything which is not ash, proteids, or fat is milk-sugar, which assumption is not strictly correct. Again, the ash left on ignition does not strictly represent the salts existing in the milk, the citrates and other organic salts being decomposed on ignition with formation of carbonates, which are converted into phosphates by the phosphoric acid resulting from the combustion of the proteids.

From the formula may be calculated the influence of 1 gramme per 100 c.c. on the density of the milk, and from these data the solution-densities of the various constituents. Thus :—

	Effect on Density of 1 gramme per 100 c.c.	Solution-Density (calculated).
Fat, ...	0·76	0·929
Sugar, ...	4·00	1·667
Proteids, ...	2·57	1·346
Ash, ...	7·57	4·12

The solution-densities thus deduced agree well with the values obtained by direct determination. Thus, Fleischmann has found the average density of milk-fat to be 0·93 ; various observers give 1·65 as the solution-density of lactose ; Hehner gives 1·31 as the value for casein (not quite pure) ; and, from the figures of Dupré, Richmond calculates the solution-density of albumin and casein to be 1·34, and that of the ash 3·0. As the ash of milk does not accurately represent the salts present in the original milk, no close agreement was to be expected in the last case.

Adulterants and Foreign Matters in Milk.

The methods of analysis described in the previous section had reference merely to the determination of the constituents of genuine milk. The same processes are applicable to the examination of milk which has been sophisticated by the addition of water or the removal of cream, since such treatment necessarily

alters the absolute or relative proportions of the constituents. The detection of added water or the removal of cream is therefore, for the most part, inferential, but valuable confirmation of the fact of sophistication is often afforded by the presence of preservatives, the composition of the ash, the absence of coagulable albumin (as in boiled milk), &c.

Besides the more commonly practised sophistications of milk—such as adulteration with water, removal of cream, and the addition of separated or diluted condensed milk—the adulteration of milk with cane-sugar, glycerin, gelatin, starch, and other foreign matters is occasionally practised. Colouring agents and preservatives are also added.

Milk Standards and Limits. Detection and Determination of Added Water. Abstraction of Fat.

The analysis of milk for the detection of added water and the removal of fat is so closely connected with that of standards and limits that the consideration of these may conveniently precede the description of the means by which confirmation of the deductions therefrom may be obtained.

As already stated, no chemical or physical method has been or is likely to be devised by which the water naturally existent in milk, and which forms about seven-eighths of its entire weight, can be distinguished from water which has been added as an adulterant. Hence the detection of added water is mainly based on the alteration of the composition of the milk caused by its presence.

From the figures recorded in the foregoing sections it is evident that milk varies materially in character according to the animal by which it is yielded, and, within narrower limits, according to the breed of the cow or other animal, the time of year, the nature of the food, and other conditions for which it is not possible to make an exact allowance in all cases. Hence it has become necessary to specify certain limits of composition, which, if not complied with, shall render the milk open to suspicion of having been tampered with, or positively condemned on that account. In practice, cows' milk only is in question, and the frauds which it becomes the duty of the milk-analyst to detect are chiefly those of diluting the milk with water, and removing a portion of the fat. This last fraud can be carried out by allowing the milk to stand, and then removing more or less of the layer of cream by skimming; by the use of a centrifugal separator; or by the addition to new milk of milk from which the cream has already been more or less completely separated.

It is evident that dilution with water will reduce the proportion

of solids in a milk, and that if the original milk be of fair average quality the extent of the sophistication can be accurately judged by their diminution.

The analyses, numbering upwards of 180,000, made by the chemists to the Aylesbury Dairy Company show that new cows' milk contains, on the average, 12·8 per cent. of total solids. If, therefore, a sample of *such* milk were diluted with water in quantity sufficient to reduce the solids to 10·4 per cent., the percentage of the original milk in the mixture would be found by the proportion :—

$$12{\cdot}8 : 10{\cdot}4 = 100 : x.$$

If the percentage of milk found as above be subtracted from 100, the difference will be the percentage of added water in the sample.[1]

Thus, in the case supposed, the adulterated milk would contain 81·25 per cent. of milk and 18·75 per cent. of added water.

On reference to the diagram opposite page 113, it will be observed that the total solids found in cows' milk in different years, and in different months of the same year, are not quite constant, ranging from 13·6 to 12·4 per cent., and that the variation is due chiefly to the varying amounts of fat present in the milk at different periods. The non-fatty solids in the same period ranged only from 9·1 to 8·6 per cent. ; so that the curve showing their proportions presents comparatively little variation, and for months together becomes a perfectly straight line. Hence the fat is the most variable constituent of milk, and this fact is recognised in practice by the popular views as to "poor" and "rich" milk, the difference between these being chiefly due to the fat. In a rich milk, the non-fatty solids are usually somewhat above the average, but not in proportion to the excess of fat.

From these considerations, it is evident that the proportion of non-fatty solids contained in a sample of milk affords a much better criterion of the genuine character of such milk than the percentage of total solids. The percentage of real milk in a watered sample will therefore be better found by substituting 8·84 (the average percentage of non-fatty solids) for the first term of the above proportion, and in the second term placing the percentage of non-fatty solids found by analysis to be present in the sample in question.[2]

[1] The percentage of added water *in* the adulterated sample is not identical with the proportion of water *added to* the milk. Thus a sample made by mixing two parts of milk with one of water would contain 33 per cent. of added water, but the water added would be 50 per cent. of the milk. The latter mode of expression is adopted by Sir Chas. Cameron.

[2] The proposal to calculate the percentage of added water in milk from

Although, as a logical inference from the analytical results, the proportion of added water indicated by the foregoing proportion might be regarded as being present, it would not be proper, in a case in which the composition of the original milk was unknown, to affirm positively the presence of added water in the above proportion. The dairy-chemist is usually well-acquainted with the general character of the milk to be examined, and in many cases his analysis is merely required as a check on the quality of the product. The question in such cases is usually not whether the milk is watered or skimmed, but rather whether it has a particular composition, this required composition practically excluding the possibility of sophistication having been practised. The public analyst, on the other hand, is compelled to take into consideration the natural variations in the composition of milk, and to refrain from condemning a sample which he is morally certain has been watered, unless it falls below the minimum limit which his own experience or acceptance of the authoritative opinion of others leads him to regard as fair and proper.[1]

From the results recorded on page 114 it appears that cows' milk of average quality contains 12·8 per cent. of total solids, of which 4 per cent. consists of fat and 8·8 of non-fatty solids. Such milk may be described as being of "standard" quality. But it would evidently be unjustifiable to condemn all milk which failed to come up to this standard, since the very striking of an average implies that some samples are below and others above the mean. Hence it becomes necessary to lay down a limit of composition, below which, in the absence of positive evidence to the contrary, a sample of milk shall be deemed to have been tampered with. It is clear that the words "standard" and "limit," as here

the proportion of "solids not fat" contained in the sample was first made in a paper by J. A. Wanklyn, "On the Testing of Milk," published in the *Mechanics' Magazine* for October 5th, 1872. The calculations were based on the assumption that genuine milk contained 9·3 per cent. of solids not fat and 3·2 per cent. of fat. Although further experience has shown that the proportion of non-fatty solids in milk is not so constant as was supposed by Wanklyn, their percentage still affords the best available means of judging whether a sample of cows' milk has been diluted with water, though the deduction therefrom may be, and always should be when practicable, supplemented by duly weighing the evidence afforded by other data.

[1] When giving evidence (1896) before the Select Committee on Food Products Adulteration (in his reply to question No. 2074), Mr Richard Bannister made the statement that "The Society of Public Analysts came to the conclusion that a sample of average composition should be the standard, and that anything below that is sold to the prejudice of the purchaser."

applied, have very different significations, and the distinction between them is very clearly understood by analysts. By the magistrates and the public, however, the two terms are very commonly confounded, so that an analyst is frequently asked what "standard" he has adopted in calculating the amount of water added to, or of fat removed from, a sample of milk. The fixing of such limits is necessarily very difficult, in order to avoid injustice on the one hand and to prevent gross adulteration on the other.

The first limit for milk which met with anything like general acceptance was that adopted in 1875 by the then newly-formed Society of Public Analysts, who resolved that milk should contain "not less than 9·0 per cent. by weight of milk solids not fat, and not less than 2·5 per cent. of butter-fat." These figures were based on analyses made by Wanklyn's method, which never effected complete extraction of the fat, the amount remaining varying with the exact method of working pursued, and being largely dependent on personal equation. In 1882, O. Hehner pointed out (*Analyst*, vii. 64) that apparently slight deviations from the (then) commonly adopted process of milk examination lead to widely discrepant results, the modifications all tending to give a lower amount of solids not fat and a larger percentage of fat than the original (Wanklyn's) method.[1] The whole subject was carefully investigated by a committee of the Society of Public Analysts in 1885, and as a result the Society adopted the paper-coil method devised by M. A. Adams as the official method of extracting the milk-fat. As this method was far more perfect than that of Wanklyn, it gave, when employed on the same milk, a materially higher percentage of fat, and, at the same time, a corresponding lower percentage of non-fatty solids. Hence the Society resolved that, concurrently with the adoption of Adams'

[1] "It appears that not a few analysts have forgotten that the results obtained by the analysis of milk are not results laying claim to absolute scientific accuracy, but are only comparative ones, and that the limits adopted by the Society—9 per cent. of solids not fat and 2·5 per cent. of fat—hold good only when each analysis is made in the manner which led to the adoption of those limits; namely, by drying 5 grammes of the milk for two and a half to three hours over an open water-bath, and by exhausting the residue with from three to six successive quantities of boiling ether. Modifications of this plan have gradually crept in, and, concurrently with the adoption of these modifications, instances have multiplied in which undoubtedly genuine milk did fall below the Society's limits. Although it cannot be held that the deficiency in solids not fat was in every case due to the modification in the analytical process, it yet appears certain that in many cases the cause lay less with the milk than with the analyst" (O. Hehner, *Analyst*, vii. 60).

process of fat-extraction, the following should in future be the limits below which cows' milk should not be passed as genuine, namely :—Total solids, 11·5 per cent., consisting of not less than 3 per cent. of fat, thus leaving not less than 8·5 per cent. of non-fatty solids. They further recommended that the non-fatty solids should in all cases be estimated by difference, that is, by subtracting the fat from the total solids as determined by evaporation.[1] This limit remains in force at the time of writing, and has been justified by analyses numbering in the aggregate many scores of thousands.

The limits for many years adopted in the Somerset House laboratory were 8·5 per cent. of non-fatty solids and 2·5 per cent. of fat. The fat was extracted from the total solids by treatment with ether, and the residual non-fatty solids weighed (see page 137). The weight thus obtained, added to that of the ether-residue (fat), was regarded as the "total solids" of the milk. It appeared from the evidence given by Mr R. Bannister in 1894, before the Committee on Food Products Adulteration, that the referees' limit had recently been raised from 2·50 to 2·75 ; but from the evidence given in certain proceedings in which the milk had been submitted to the referees, it is clear that this limit is not adhered to rigidly. The milks submitted to the referees are always in a more or less altered condition, and in this state the method adopted in the Government laboratories does not effect a complete extraction of the fat, from 0·2 to 0·3 remaining with, and consequently increasing the weight of, the non-fatty solids. Hence the referees' present limit of 2·75 per cent. of fat is practically identical with the limit of 3·0 per cent. adopted by the Society of Public Analysts, on the basis of a more perfect method of determination ; but the referees' nominal limit of 8·5 per cent. of solids not fat corresponds to about 8·2 per cent. shown by the Society's method of analysis.

No limit of composition for milk has hitherto been legalised in this country, but definite proportions of fatty and non-fatty solids are required in many States and cities in America.

The following table shows the legal limits for milk-constituents required in various places :—

[1] The above recommendations of the committee were laid before the Society of Public Analysts in November 1885, discussed in detail at a meeting in the following January, and formally adopted on April 10, 1886, the proceedings being fully reported in the *Analyst* (x. 216 ; xi. pp. 1 and 62). It is remarkable that Mr R. Bannister should have remained in ignorance of the limit (of 3·0 per cent.) for fat adopted by the Society, as shown by his evidence given in 1894 before the Committee on Food Products Adulteration (see replies, 1712, 1713, 1715, 1742, 1743, 1744).

State, City, &c.	Total Solids.	Fat.	Non-Fatty Solids.
State:—			
New York, Laws, 1884 and 1893,	12·00	3·00	9·00
Maine, Law, 1893,	12·00	3·00	9·00
Michigan, Law, 1889,	12·50	3·00	9·50
Iowa, Law, 1892,	...	3·00	...
New Hampshire, Law, 1883,	13·00	...	...
Ohio, Law, 1889,	12·50	3·12	9·38
Oregon, Law, 1893,	...	3·00	...
Vermont, Law, 1888,	12·50	3·25	9·25
Wisconsin, Law, 1889,	...	3·00	...
Pennsylvania, Law, 1885,	12·50	3·00	9·50
New Jersey, Law, 1882,	12·00	3·00	9·00
Massachusetts, Law, 1886,	13·00	3·70	9·30
,, ,, May and June,	12·00	...	...
Minnesota, Law, 1889,	13·00	3·50	9·50
City:—			
Columbus, Ohio,	12·50	3·12	9·37
Baltimore, Ind.,	12·00	...	...
Denver, Col.,	12·00	...	...
Lansing, Mich.,	12·50	3·00	...
Madison, Wis.,	...	3·00	...
Burlington, Vt.,	12·50	3·50	...
Des Moines, Iowa,	13·13	3·50	...
Portland, Oregon,	12·00	...	...
Omaha, Nebraska,	12·00	3·00	...
U.S. Treasury Department,	13·00	3·50	9·50
Philadelphia, 1890 Ordinance,	12·00	3·50	8·50
Berne,	12·50	3·50	...

From a return made by the prefect of the Paris police, and handed in by Mr R. Bannister to the Committee on Food Products Adulteration, it appears that the milk sold in Paris contains on the average 130 grammes per litre of total solids, which must contain at least 27 grammes of fat and 45 grammes of milk-sugar. Milk dilution is calculated from a standard 130 grammes of solids per litre, and skimming from 40 grammes of fat per litre. The limit is 115 grammes of total solids per litre, which represents a mixture of 88·5 per cent. of the standard milk with 11·5 per cent. of water. It is considered that dilution can only be detected by a retailer from the external characteristics of the milk when it exceeds 15 per cent., and that below this proportion his *bona fides* cannot be impugned. Hence the Paris police only take proceedings for dilution when this exceeds 19 per cent., calculated from the standard milk. They there pass commercial milks which contain not less than 105 grammes of "extract" per litre (= 10·19 per cent. by weight of total solids) and 27 grammes (= 2·62 per cent.) of fat per litre. But whenever possible the source of fraud is sought by taking samples either from the stocks of itinerant vendors, at the railway stations, or from the dairy companies' depots. Whenever milk on sale supplied from farms in the Department of the Seine is sampled, and found to contain less than 130 grammes of solids and 40 grammes

of fat, samples are taken from the cows, and notes made of their food and breed.

The following limits for milk were suggested by witnesses before the Committee on Food Products Adulteration (1894 to 1896):—

	Total Solids.	Fat.	Non-Fatty Solids.
Aylesbury Dairy Company,	...	3·0 (at least).	8·5
Metropolitan Dairymen's Society,	12·0	3·0	...
Dairy Trade and Cow Protection Society,	...	2·5	8·5
R. Bannister, representing Government laboratories,	...	2·75	8·5
Dairy Products Committee of Central Chamber of Agriculture,	12·0	3·0	...
O. Hehner, representing Society of Public Analysts,	...	3·0	8·5
A. Wynter Blyth (Public Analyst for Marylebone, &c.),	...	2·5	8·5
G. Embrey (Public Analyst for Gloucestershire, &c.),	11·5	3·0	...
F. J. Lloyd (Consulting Chemist to British Dairy Farmers' Association and the Metropolitan Dairymen's Society),	12·0	3·0	...
S. W. Farmer (Wiltshire farmer),	...	3·0	8·5
C. Middleton (Yorkshire dairy farmer),	...	3·0	8·5
James Long, representing Central Chamber of Agriculture and British Dairy Farmers' Association,	12·0	3·2	Would have no figure for non-fatty solids.

The Aylesbury Dairy Company have published the following table showing the proportion of fat contained in the milk of 6462 single cows of various breeds:—

	Number of Samples.	Max.	Min.	Average.
Dairy shorthorns,	2849	10·2	1·1	4·03
Pedigree shorthorns,	1292	7·5	1·9	4·03
Kerrys,	1493	10·5	1·8	4·72
Jerseys,	404	9·8	2·0	5·66
Sussex,	132	7·6	2·9	4·87
Montgomerys,	88	6·5	1·4	3·59
Welsh,	18	8·3	3·0	4·91
Red polled,	186	6·6	2·5	4·34

Out of these 6462 cows, the milk from each of which was collected and analysed separately, in only 241 instances did the milk contain less than 3 per cent. of fat, these cases being as follows :—

Percentage of Fat.	Dairy Shorthorn.	Pedigree Shorthorn.	Kerry.	Jersey.	Sussex.	Montgomery.	Welsh.	Red Polled.
2·9	28	15	6	...	1	3	...	6
2·8	25	15	7	...	...	5	...	1
2·7	21	8	7	...	...	3	...	2
2·6	16	10	2	...	...	1	...	2
2·5	5	5	1	...	...	...	...	1
2·4	8	5	...	1	...	1	...	...
2·3	5	1	...	...	...	2	...	...
2·2	2	...	...	...	...	1	...	...
2·1	5	1	...	...	...	1	...	...
2·0	2	...	...	1	...	...	...	...
1·9	2	1	...	...	...	...	...	...
1·8	...	...	1	...	...	...	...	...
1·7	...	...	...	...	...	1	...	...
1·6	1	...	...	...	...	...	...	...
1·4	...	...	...	...	...	1	...	...
1·3	1	...	...	...	...	...	...	...
1·1	1	...	...	...	...	...	...	...

It follows, from these results, that the milk from individual cows, especially shorthorns, is liable occasionally to contain less than 3 per cent. of fat. But it is evident that this fact does not justify the lowering of the limit, which would have to be fixed at 1·0 per cent. to cover all the abnormal samples in the table. In fact, still lower amounts of fat have been recorded.

On the other hand, these anomalies practically disappear when the mixed milk from a number of cows is in question. This is well shown by the following table, drawn up by H. D. Richmond for the information of the Committee on Food Products Adulteration. It shows the highest and lowest percentages of fat in the milk sent out by the Aylesbury Dairy Company from 1890 to 1893.

	1890.		1891.		1892.		1893.	
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.
January, .	4·5	3·5	4·6	3·3	4·5	3·4	4·3	3·2
February, .	4·4	3·4	4·4	3·1	4·4	3·4	4·3	3·1
March, . .	4·3	3·2	4·6	3·2	4·4	3·4	4·3	3·08
April, . .	4·4	3·0	4·6	3·2	4·2	3·1	4·3	3·1
May, . .	4·3	3·1	4·9	3·1	4·2	3·2	4·2	3·1
June, . .	4·4	3·1	4·3	3·2	4·1	3·0	4·3	3·1
July, . .	4·7	3·4	4·4	3·4	4·1	3·2	4·3	3·2
August, . .	4·5	3·3	4·4	3·5	4·3	3·2	4·4	3·3
September, .	4·6	3·4	4·7	3·3	4·4	3·5	4·6	3·4
October, .	4·7	3·6	5·0	3·5	4·7	3·4	4·8	3·5
November, .	4·7	3·6	4·8	3·7	4·8	3·3	4·7	3·3
December, .	4·7	3·5	4·6	3·4	4·4	3·2	4·5	3·3

From this table it appears that not only was no milk delivered with less than 3·0 per cent. of fat, but that in certain months of the year (autumn and winter) this figure was never approached; indicating that, commercially, not only is there no real justification for a lower standard than 3·0, but that it should be raised in the autumn and winter.[1]

The foregoing data afford abundant material for the adoption of legal limits of composition for milk; but in view of the great difficulty in obtaining any change in a law, however bad or imperfect it may be, it is very undesirable that such limits should be laid down in any Act of Parliament. The Committee on Food Products Adulteration adopted in their report (1896) the recommendation that there should be instituted a Board of Reference, consisting of experts, and that to this Board should be assigned the duty of deciding from time to time the limits of composition which milk and other articles of food should be required to possess. If this were done, and at the same time the method of analysis were prescribed, the grave difficulties under which the duties of public analysts have hitherto been conducted in this country would be materially ameliorated.

In the opinion of the author, based on a very wide experience, the limits of 8·5 for non-fatty solids and 3·0 per cent. for fat (as adopted by the Society of Public Analysts) are as low as is consistent with the interests of the public, and are not liable to occasion injustice to the milk-vendor, provided they are applied with the discretion which a public analyst is presumed to possess. Thus, in the case of a milk very rich in fat, the non-fatty solids may fall somewhat below 8·5 per cent., but the very fact of the

[1] It has been pleaded that a low standard is necessary in order to prevent innocent milk-vendors from being convicted on account of circumstances beyond their control, *i.e.*, the rising of cream by natural force in the milk during delivery, and in hot weather its churning into butter. About 10,000 experiments have convinced the Aylesbury Dairy Company that, under ordinary circumstances, and in ordinary seasons, there is no great difficulty in delivering milk of ordinary average quality, so that every portion to the last drop shall contain the same amount of fat to 0·1 per cent., even in the hottest weather. Moreover, it is not necessary to take this into consideration in fixing a standard, as every such case, should it be proved to occur, would be considered on its merits by the court. In the case of Dyke *v.* Gover, heard before Lord Chief Justice Coleridge and Mr Justice J. Wright (*Law Reports*, [1892], 1 Q.B., 220; 56 J.P., 168), it was held by the court that the onus of preventing the separation of cream from milk in the course of delivery lay on the vendor.

Experiments showing the loss of cream liable to occur in practice during the delivery of milk are described on page 215.

sample containing a full proportion of fat ought to prevent the analyst from condemning the milk unless there is other evidence of adulteration to support the deduction from the low non-fatty solids. In short, the whole of the data should be taken into account in forming an opinion, and this is and always has been done by analysts having a proper sense of their responsibility.

Another limit, which would be very useful in the case of altered milk, where the deduction from the proportion of non-fatty solids is of diminished value, is that of the total nitrogen. This might with advantage be fixed at 0·5 per cent.

In 1883, Chas. Estcourt suggested a valuation of milk solids, instead of a limit or standard (*Analyst*, 1883, page 245). In other words, he proposed to give the sample of milk what may be described as "good marks," in number proportionate to its composition. Thus he suggested that the presence of 8·5 per cent. of non-fatty solids should count as 200, and 3·0 per cent. of fat as 100. From these he deduced the factor 7·85 for non-fatty solids and 11·10 for fat, and proposed that a milk which contained such a percentage of non-fatty solids and of fat as would, when multiplied by their respective factors, together produce 100, should be considered of full value, and therefore not liable to condemnation. Thus a milk containing 8·5 of non-fatty solids and 3·0 per cent. of fat would have a value of 100, for :—

$$8{\cdot}5 \times 7{\cdot}85 = 66{\cdot}7 \text{ ; and,}$$
$$3{\cdot}0 \times 11{\cdot}10 = 33{\cdot}3$$

$$100{\cdot}0$$

It will be seen that the proposed method of valuation covers a very considerable range of composition. Estcourt proposed that the fat should be determined by Wanklyn's method, but it is evident that if the fat were extracted by the more perfect methods since devised some modification of his factors would be necessary. In the opinion of the author, more suitable factors under the existing conditions would be 9·0 for non-fatty solids and 7·8 for fat. This would require 3·36 per cent. of fat in a milk with only 8·2 of non-fatty solids, and 3·6 per cent. of fat if the non-fatty solids were as low as 8·0 per cent.

It is evident that the proportion of water which is regarded as having been added to milk is dependent on the standard adopted. While agreeing that a milk should not be condemned as watered unless the non-fatty solids fall appreciably below 8·5 per cent., some analysts calculate the proportion of added water on the assumption that the original milk was of average composition.

This is a proper course, especially if the percentage of water corresponding to milk of the lowest quality be also stated. But, in practice, such a method of stating the results is apt to cause misunderstanding, and hence it is preferable to calculate the proportion of added water from the limit adopted. Thus with the limit of the Society of Public Analysts the equation for calculating the proportion of milk of lowest quality in the sample will be :—

$$\frac{\text{Percentage of non-fatty solids} \times 100}{8{\cdot}5} = \text{Percentage of genuine milk in sample.}$$

Hence it appears that in the case of a sample of milk containing the average proportion of 8·84 per cent. of non-fatty solids, 3·9 per cent. of water might be added without reducing such milk below the P. A. Society's limit; and it follows that a sample which contains only 8·5 per cent. of non-fatty solids is probably adulterated with 3·9 per cent. of water.[1]

This consideration is, of course, one for the guidance of the analyst, and would never form the basis of a certificate issued with a view to legal proceedings.

In the case of Fortune *v.* Hanson (*Law Reports*, 1896, vol. i., Q.B., Part iii. p. 205; *Analyst*, 1896, p. 53) it was held by Justices Hawkins and Kennedy that the public analyst must state on his certificate the percentage of water present in the sample analysed, and also the percentage of water existing in genuine milk. The court evidently supposed that the difference between these two percentages would be the extent of the adulteration, and others having more excuse have fallen into the same error.[2] As

[1] By a somewhat similar mode of reasoning a well-known chemist (not a public analyst) felt himself justified in stating in evidence that the genuine milk yielded by a certain herd of cows was found by analysis to contain seven per cent. of added water. His meaning was doubtless that the milk in question was of such a quality as would result from the addition of seven per cent. of water to milk of average or superior quality, but this does not appear to have been explained, and the court was left under the impression that genuine milk might naturally contain seven per cent. of added water, thus causing a deplorable failure of justice and throwing undeserved doubt on the value of analysis generally for the detection of adulteration of milk.

[2] The decision in the case of Fortune *v.* Hanson was deplorable as causing much misunderstanding on the part of those called on to adjudicate in cases of milk adulteration, or who are professionally concerned for the defence of persons accused of vending watered milk. Thus a train of false reasoning not unfrequently adopted is to argue that, as the sample in question has been found by the analyst to contain (say) 10·4 per cent. of total solids, and therefore 89·6 per cent. of water, and that, according to the analyst's own showing,

a consequence of the decision in the above case, the Society of Public Analysts recommended that the following wording should be adopted in certifying on samples of watered milk :—

"The said sample contains the parts as under :—

"Milk, ...
"Added Water, ...

100

"This opinion is based upon the fact that the sample contained only ..... per cent. of non-fatty solids, whereas normal milk contains at least 8·5 per cent. of non-fatty solids."

In the subsequent case of Bridge *v.* Howard (*Analyst*, 1896, p. 305), in an appeal heard by Justices Grantham and Kennedy, it was held that the above information was sufficient, and the latter judge modified materially the opinion he had expressed in the case of Fortune *v.* Hanson.

While the proportion of non-fatty solids in milk affords the most valuable and reliable datum for detecting the presence and determining the amount of added water, certain other factors often furnish valuable confirmation of the deduction drawn from the non-fatty solids, and should be duly taken into account in forming an opinion on a sample. The inferences therefrom must not be regarded as conclusive, but the following points may be borne in mind.

H. D. Richmond (*Analyst*, 1893, 271) has pointed out that in the case of a normal milk adulterated with ordinary water both the ash and the non-fatty solids will be low, but the ratio between them will be practically undisturbed; while a naturally abnormal milk will yield a higher ash than corresponds to the non-fatty solids. On the other hand, in a watered milk containing (say)

genuine milk contains on the average 12·8 per cent. of solids and 87·2 per cent. of water, and may contain as much as 88·5 per cent. of water, therefore the adulteration practised is only 1·1 per cent., if any, instead of (say) 18 per cent., as stated by the analyst in his certificate; and that it is well known that the natural variations of milk are far greater than would account for the insignificant proportion of water alleged to have been added. In the face, therefore, of the discrepancy between the analyst's certificate and the conclusion deduced from his figures, the case should be dismissed.

The above example of specious pleading would be ludicrous if precisely similar arguments had not repeatedly resulted in gross failures of justice. It must be remembered that, in the great majority of instances in which proceedings are taken for selling watered milk, the case is conducted by a sanitary inspector or other officer having no scientific or legal training, and in the absence of the public analyst, who rarely has the opportunity of correcting wilful or ignorant misstatements of the above nature.

8·0 per cent. of non-fatty solids, the ash would be about 0·66 per cent., a difference which is small but perfectly perceptible. Of course any mineral additions will invalidate the deductions from the above data, but this will only result in a sample being regarded as abnormal, and not occasion its erroneous condemnation as adulterated. Besides, a further examination of the ash will scarcely fail to afford additional information on this point (compare page 134).

A natural milk which contains an abnormally low proportion of non-fatty solids is generally deficient in milk-sugar, the proteids not decreasing to a marked degree. Hence a sample which shows by Kjeldahl's process less than 0·5 per cent. of total nitrogen may be regarded as very suspicious.

Richmond has further suggested that the presence of nitrates in milk would afford proof of adulteration by added water; but, apart from the fact that a large proportion of natural waters contain no considerable amount of nitrates, it has since been shown by Richmond himself, that a notable quantity of nitrates can be detected in the milk of cows, the food of which had received an addition of potassium nitrate.[1]

The practice, now very common, of adding freshly-separated milk to new milk, is not capable of detection unless the fat in the mixed product fall below the accepted limit. If, however, condensed separated milk be added, its presence may be inferred from the diminished amount of albumin in the sample, since the high temperature to which the article is necessarily subjected in the process of concentration and sterilisation coagulates this constituent (see further, page 189).

Detection of Occasional Adulterants in Milk.

Cane-Sugar has been sometimes added to milk, probably with a view of raising the non-fatty solids and thus masking the addition of water. When present in notable amount, the presence of cane-sugar in milk can be detected by the abnormally sweet taste of the sample. For its determination, a method devised by

[1] A convenient method of testing milk for nitrates is the following:—Place a small quantity of diphenylamine in a porcelain basin, and add to it 1 c.c. of concentrated sulphuric acid free from oxidised compounds of nitrogen. (This must be ascertained by direct experiment, and can be readily ensured by previously heating the acid to its boiling point for a few minutes with a crystal of ammonium sulphate.) Allow a few drops of the serum obtained by warming the sample of milk with a little acetic acid to flow down the side of the basin so as to float on the surface of the acid solution of diphenylamine. If a blue coloration be developed in the course of ten minutes, even if the colour be but faint, the presence of nitrates is certain.

Stokes and Bodmer may be conveniently employed. The milk is coagulated with citric acid, diluted to a definite measure with several times its volume of water, and filtered. The filtrate is then titrated against Pavy's ammoniacal cupric solution in the manner described on page 150. To another portion of the filtrate citric acid is added (in the proportion of about 2 grammes to 100 c.c. liquid), the liquid boiled for at least ten minutes, and the resulting solution cooled, neutralised with ammonia, made up to a known volume, and again titrated with Pavy's solution. The milk-sugar not being affected by the treatment with citric acid, the difference between the reducing powers of the solutions before and after inversion is due to the glucose derived from the cane-sugar originally present. Any addition of cane-sugar will increase the optical activity of the milk without adding to the reducing power. Hence a valuable confirmation of its presence will be found in the want of agreement of the polarimetric determination of the (supposed) lactose with the amount deduced from its reducing power.

A method for the indirect estimation of cane-sugar in milk has been described by J. Muter (*Analyst*, v. 35; xiii. 228). Ten grammes weight of milk is evaporated to dryness upon 4 grammes of hydrated calcium sulphate with frequent stirring, so that nothing adheres to the basin. The powdered dry residue is then extracted in the usual manner in a Soxhlet's tube with ether. The residue in the Soxhlet tube, with the filter containing it, is transferred to a beaker, 20 c.c. of *hot* water added, and the mixture well agitated. 30 c.c. of *rectified spirit* is then added, and the solution allowed to cool, with occasional stirring. The residue is thrown on to a filter, and washed with *proof spirit* until the filtrate measures 120 c.c. (This is usually sufficient unless the amount of cane-sugar be very large, such as is the case in sweetened condensed milks.) The filtrate is next divided into two equal parts, one of which is evaporated, and the residue dried till constant in weight. The ash is deducted from the weight of the total solid matter thus obtained. The weight of the solids *minus* the ash gives, when multiplied by 20, the percentage of total sugars. In the other portion of the filtrate, the milk-sugar is estimated gravimetrically by means of Fehling's solution. The percentage of milk-sugar found, subtracted from the total sugars present, gives the percentage of cane-sugar in the milk. The process is really only applicable to milk containing more than 2 per cent. of cane-sugar. If it should contain less than 1 per cent., a deduction of 0·2 per cent. must be made; if less than 1·5 but more than 1·0 a deduction of 0·1 per cent. will give a fairly accurate result.

Many brands of condensed milk contain an enormous proportion (36 to 40 per cent.) of cane-sugar. It is evident, therefore, that the addition of diluted sweetened condensed milk to fresh milk could be detected by the foregoing methods.

Glucose solution of the same density as milk is stated by Krechel (in a communication to the French Academy of Sciences) to have been employed for the adulteration of milk. A solution containing 8 per cent. of glucose would have a gravity of about 1·032, and if one measure of this were added to seven of milk, the mixture would contain 1 per cent. of glucose. This glucose would exert a reducing action on Pavy's solution equal to 1·924 per cent. of lactose, and hence a milk which originally contained 4·8 per cent. of milk-sugar would, by adulteration with glucose solution in the above proportion, appear to contain 6·12 per cent. An additional indication of the presence of glucose would be found in the want of concordance in the percentage of milk-sugar present, as determined by its cupric oxide reducing power and the optical activity of the sample. The specific rotations of dextrose and milk-sugar are practically identical, but their reducing powers on Pavy's solution are, as already stated, in the proportions of 1 : 1·924. Hence any addition of dextrose would show itself by Pavy's test indicating a higher proportion of sugar than that deduced from the optical activity of the sample.[1]

A further confirmation of the presence of glucose would be found in the disturbance of Vieth's ratio of 2 : 9 : 13 for the ash, proteids, and (apparent) lactose.

J. Muter (*Analyst*, 1878, page 235) has recorded a case of adulteration of milk by *glycerin*. For the detection of this fraud he curdles the milk, evaporates the whey to dryness, and removes the last traces of fat with ether. The glycerin is then dissolved out by a mixture of alcohol and ether, the solution evaporated, and the residual glycerin weighed and identified in the usual manner.

The adulteration of milk by *gelatin* has been observed by A. W. Stokes, who has communicated to the author the following process for its detection :—An acid solution of mercuric nitrate is prepared by dissolving some mercury in twice its weight of nitric acid of 1·42 specific gravity, and diluting this solution to twenty-five times its bulk by the addition of water. Ten c.c. measure of the milk or cream to be examined is mixed with an equal volume of the acid mercuric nitrate solution,[2] the mixture shaken, and

[1] This consideration applies to pure dextrose, but a glucose syrup containing much dextrin might have a high optical activity and low reducing power, which would obscure the indication.

[2] Stokes states that neither copper sulphate nor acetic acid can replace the

then 20 c.c. of water added. The liquid is again shaken, allowed to stand for five minutes, and filtered. In the presence of much gelatin the filtrate will be opalescent, and cannot be obtained quite clear. To a portion of the filtrate, contained in a test-tube, an equal bulk of a saturated aqueous solution of picric acid is added. A yellow precipitate will be produced in the presence of any considerable amount of gelatin; while smaller quantities will be indicated by the cloudiness produced with the picric acid solution. This cloudiness can be best observed on a dark background. In the absence of gelatin the filtrate obtained will be perfectly clear, and will be unaffected by adding picric acid.

Starch, *maize-flour*, and other *farinaceous substances* have been occasionally added to milk to conceal adulteration by water. If the milk has not been boiled or heated, the starch-granules can be detected by allowing the milk to stand in a closed funnel, removing a drop from the bottom with a pipette, and placing it on a microscope slide. On adding a drop of dilute iodine solution, the starch-granules will be coloured blue. A very satisfactory plan of detecting starchy matters is to boil some of the milk in a test-tube, and when cold add sufficient of a solution of iodine in potassium iodide to colour the milk distinctly yellow. The reaction will not be produced if too little iodine be used, and a large excess obscures the blue coloration indicative of the presence of starch. If as little as 0·2 per cent. of starch be added to milk, its presence will be immediately indicated by the above test.[1]

Common Salt is occasionally added to milk, and will be indicated by the large proportion of soluble ash and the abnormal proportion of chlorides therein. In two cases for which convictions ensued in Glasgow (*Analyst*, 1886, p. 238), the milk was found to be adulterated with 22 and 26 per cent. of water, the samples containing respectively 0·21 per cent. (or 147 grains per gallon) and 0·14 per cent. (or 98 grains per gallon) of added salt.

acid mercuric nitrate for throwing down the proteids. Tannic acid may be used instead of picric acid, but its effect must be noted *immediately*, since in a minute or so a dense cloud is formed, even in the absence of gelatin. Picric acid will detect one part of Nelson's gelatin in 10,000 parts of water.

[1] Some analysts prefer to add iodine to the milk and allow the sample to stand over-night, when in presence of starch the layer of cream will be coloured blue. It is claimed that this method of operating is more delicate than that previously described, but in a series of comparative experiments conducted in the author's laboratory it was found that the reaction sometimes failed, and that the immediate test was to be preferred as more reliable and equal to all requirements.

COLOURING MATTERS IN MILK.

Colouring matters are frequently added to milk in some parts of the country; annatto being the colouring agent most frequently employed. Its detection, and that of the coal-tar dyes which may be substituted for it, is described in Vol. III. part i. page 354 *et seq.*

PRESERVATIVES IN MILK.

Until recently, the methods of preserving milk were based solely on the addition of substances having an antiseptic action, but sterilisation by heat is now extensively practised.

Among the antiseptics most commonly added to milk are sodium carbonate and bicarbonate, boric acid and borates, benzoic and salicylic acids, fluosilicates, and, of late years, formaldehyde.

Potassium bichromate is sometimes used as a preservative of milk, but experiments made in the author's laboratory gave very unsatisfactory results unless the antiseptic was used in such proportions as to communicate a distinct colour to the liquid.

Sodium Carbonate and *Bicarbonate* are sometimes added to milk with the object of counteracting acidity, and hence may be classed among preservatives. The addition will be indicated by the marked alkalinity of the ash and the presence of carbonates therein. The determination of the alkalinity does not afford an accurate means of ascertaining the amount of sodium carbonate added, since the salt reacts on ignition with the phosphates of the milk. Hence the alkaline phosphate formed must also be determined. A method for effecting this and estimating the sodium bicarbonate added to milk has been described by L. Pade (abst. *Analyst*, 1896, p. 286).

By the following method, E. Schmidt finds it possible to detect sodium carbonate or bicarbonate when present in milk to the extent of 0·1 per cent.:—10 c.c. measure of the sample is mixed with an equal volume of alcohol, and a few drops of a 1 per cent. solution of rosolic acid added. With pure milk a brownish-yellow coloration is produced, but in presence of sodium carbonate or bicarbonate the colour is rose-red. The difference is best observed by comparing the suspected sample side by side with genuine milk.

Boric acid and *Borates* are extensively employed for the preservation of milk, a mixture of boric acid and borax being very common.

For the detection of boron compounds, the author ignites the solid residue obtained by the evaporation of not less than 10 grammes of the milk, and preferably a larger quantity, and adds

to the ash sufficient dilute hydrochloric acid to render the whole slightly but quite distinctly acid to litmus. A slip of turmeric-paper is then placed in the capsule in such a manner as to be only partly wetted by the liquid, which is then evaporated to dryness at 100° C. If boron compounds be present, the part of the turmeric-paper which was immersed in the liquid will be found to have acquired a well-defined brownish-red colour, owing to the formation of rosocyanin. On moistening the paper with a drop of caustic soda, a variety of colours will be produced—green and purple usually predominating. On acidulating with hydrochloric acid, the red colour is restored, and is again changed to green and blue by treatment with excess of alkali.

Tincture of turmeric may be substituted for the turmeric paper recommended in the foregoing test, but in the author's hands has proved less satisfactory.

The well-known green colour imparted to a flame by boron compounds may be employed for their detection in milk, but unless the test be applied spectroscopically it is less delicate and reliable than the turmeric reaction. The usual way of performing the test is to treat the ash of the milk with a few drops of strong sulphuric acid, then add some strong alcohol, and apply a light. The alcohol will burn with a flame tinged with green at the edges if a boron compound be present. The green colour is most readily perceived if the burning alcoholic mixture be screened from other sources of light. A glass rod should be dipped in the liquid, the drop adhering to it kindled in a small bunsen-flame, and while still burning employed to ignite the liquid in the dish. The green colour is most perceptible at the moment ignition takes place.

The green colour imparted to an alcohol flame by boron compounds is due to the formation of the volatile ethyl borate. If methyl alcohol be substituted for ordinary alcohol, the reaction is improved, since the methyl borate formed burns with a flame of a purer green than that produced by ethyl borate.

M. Kretzschmar (*Chem. Zeit.*, xi. 476; abst. *Analyst*, xii. 159) evaporates from 5 to 6 c.c. of the milk to about one-third of its original bulk, adds five or six drops of fuming hydrochloric acid, and continues the evaporation while directing a non-luminous flame across the dish. In the presence of a boron compound the flame will be tinged green.

According to another plan the ash is macerated with a little glycerin, a drop of the liquid taken up in a loop of platinum wire, and introduced into a bunsen-flame. This is a convenient method if the spectroscope is to be employed.

The determination of boron compounds in milk is troublesome, and, unless the proportion present is considerable, not very accurate. A useful and fairly simple method is based on the observation of R. T. Thomson (*Analyst*, xviii. 184), that free boric acid may be titrated with tolerable accuracy by caustic alkali and phenol-phthalein, provided that the liquid contain at least 30 per cent. of glycerin. The neutral point then corresponds to the formation of $NaBO_2$. Hence each c.c. of decinormal alkali required represents 0·0035 gramme of boric anhydride, B_2O_3; 0·0062 of crystallised boric acid, H_3BO_3; 0·00505 of anhydrous borax, $Na_2B_4O_7$; or 0·00955 gramme of crystallised borax, $Na_2B_4O_7 + 10H_2O$.

Instead of assuming the neutralising power of the standard alkali to be correct, it is desirable to set the solution against a known weight of crystallised boric acid, 0·310 gramme of which should neutralise 50 c.c. of decinormal alkali.

In applying the foregoing method to the determination of boric acid in milk, L. de Koningh recommends that 1 c.c. of a strong solution of caustic soda (1 : 1) should be added to 10 grammes of the milk, the liquid evaporated to dryness, and the residue ignited. The ash is boiled with water, the residue re-ignited and again boiled with water. The two solutions, which will contain all the borates present, are mixed together, a few drops of methyl-orange added, and decinormal sulphuric acid dropped in cautiously until the liquid acquires a faint pink colour after stirring. The liquid is then boiled for a minute or two to expel carbon dioxide, cooled, and half its measure of glycerin added. A few drops of phenol-phthalein solution are now added, and the liquid titrated with decinormal caustic soda till a pink coloration is obtained. For small amounts of boron compounds the method is not very accurate, but with larger quantities the results are reliable.

Another method for the determination of boron compounds in milk is based on their conversion into volatile methyl borate, and the decomposition of this body after distillation by lime or other base. The method, which apparently originated independently with Rosenbladt and Gooch, has been modified by Penfield and Sperry, Gilbert, Cassal, and Hehner, the following being the preferable method of operating (C. E. Cassal, *Analyst*, xv. 230; and O. Hehner, *Analyst*, xvi. 141):—A weight of 50 grammes of cream or 100 grammes of milk is rendered alkaline by caustic soda, evaporated to dryness, and the residue incinerated. The ash, which need not be burnt white, is reduced to powder, and transferred, by means of a little methyl alcohol and a few drops of water, to a conical flask of 200 to 300 c.c. capacity, provided

with a caoutchouc stopper carrying a tapped funnel and delivery-tube. Sufficient acetic acid is then added to render the contents of the flask distinctly acid, and this is followed by five c.c. of methyl alcohol. The flask is attached to a condenser by means of a flexible joint, in such a manner as to permit of the contents being agitated occasionally. The flask is then placed on an oil-bath, and the liquid distilled nearly to dryness. Another addition of 5 c.c. of methyl alcohol is made, and the distillation repeated. Ten such treatments, with subsequent distillation, are ample in all likely cases, and fewer are generally quite sufficient. The residue in the flask should be tested with turmeric-paper, to make sure that the volatilisation of the boric acid is complete. Cassal finds that the addition of a few drops of water prior to distillation greatly facilitates the operation,[1] and that much better results are obtained by repeated distillations of small quantities than by at once adding a large volume of methyl alcohol.

The distillate, containing methyl borate, is usually collected in a vessel containing a known weight of recently-ignited lime, and the boric acid is deduced from the increase of weight when the mixture is evaporated to dryness, and the residue strongly ignited. The manipulative difficulties and sources of error in this operation are such as render the process undesirable. Hehner avoids the difficulty by substituting for the caustic lime a solution of crystallised sodium phosphate. On evaporation with this reagent, the methyl borate undergoes decomposition. In the absence of boric acid the residue left on ignition will consist solely of sodium pyrophosphate, $Na_4P_2O_7$, but in presence of boric acid or methyl borate this will react to form sodium metaphosphate and biborate :—$Na_4P_2O_7 + 2B_2O_3 = 2NaPO_3 + Na_2B_4O_7$.

Hence 0·133 gramme of sodium pyrophosphate, resulting from the ignition of 0·322 gramme of crystallised sodium phosphate, $Na_2HPO_4 + 10H_2O$, will react with and fix 0·070 gramme of B_2O_3, representing 0·124 gramme of crystallised boric acid, or 0·191 gramme of crystallised borax.

In practice, instead of employing a solution of sodium phosphate of exactly known strength, a solution of approximately two per cent. strength may be used. A known volume, *e.g.*, 20 c.c., of this is added to the distillate containing the methyl borate, and

[1] This experience is very remarkable, since methyl borate undergoes hydrolysis with great facility. Experiments made at the author's request by A. R. Tankard showed that when an alcoholic solution of methyl borate was mixed with water and evaporated, the amount of boric anhydride obtained on ignition of the residue was quite as great as when an aqueous solution of an equivalent amount of free boric acid was similarly treated.

an equal quantity, measured with the same pipette, evaporated separately and the residue ignited. The difference between the weights of the two ignited residues will be the B_2O_3 of the sample. The residue left on evaporation to dryness must be heated very cautiously to prevent loss by spitting. The ignition should be conducted in a covered platinum dish, the temperature being ultimately raised until the residue fuses.

O. Hehner now collects the distillate containing methyl borate in a receiver containing caustic soda solution, which he then evaporates, treats with dilute mineral acid till exactly neutral to methyl-orange, then adds glycerin and phenol-phthalein, and titrates with standard caustic soda to determine the boric acid. Experiments in the author's laboratory by A. R. Tankard have proved that the results so obtained agree with those obtained by evaporating the distillate with sodium phosphate and weighing the ignited residue.

Benzoic acid, when present in milk, may be detected, according to E. Meissl (abst. *Chem. News*, 1883, p. 283), by making 250 to 500 c.c. of the milk alkaline with a few drops of lime-water or baryta-water, evaporating the liquid to one-fourth of its original bulk, and mixing it with sufficient plaster of paris to form a pasty mass, the product being then dried on the water-bath. Condensed milk should be treated directly with sufficient plaster of paris and a few drops of baryta-water. The dried mass is finely-powdered, moistened with dilute sulphuric acid, and then exhausted three or four times with about twice its volume of 50 per cent. alcohol, which dissolves benzoic acid freely, but only traces of fat. The alcoholic extract, which contains the benzoic acid, together with some milk-sugar and mineral matter, is thoroughly mixed, neutralised with baryta-water, and evaporated to small bulk. The residue is acidified with dilute sulphuric acid, and agitated with successive portions of ether. On evaporation of the separated ether, the benzoic acid is left almost pure, and may be recognised by its odour, volatility, and crystalline form.

Salicylic acid is sometimes used alone as a preservative of milk, but more often in admixture with boric acid or borax. It is stated that when given with the food, salicylic acid makes its appearance very shortly after in the milk and urine, but disappears after some hours from the milk, and does not again reappear, even though larger quantities of the acid are again administered.

Salicylic acid is not satisfactory as a preservative of milk, as it imparts to it a peculiar sweet taste which becomes increasingly-

noticeable until decomposition ensues. Salicylic acid in milk may be detected by treating about 50 c.c. of the sample with 1 c.c. of an acid solution of mercuric nitrate to remove fat and proteids, as described on page 145. The liquid is then filtered, and shaken with about half its volume of a mixture of equal parts of ether and petroleum-ether. The ethereal solution is evaporated, and a few drops of a neutral solution of ferric chloride added to the residue. In the presence of even very small amounts of salicylic acid the characteristic violet coloration is produced. A more satisfactory plan is to agitate the ethereal solution with dilute ammonia, separate, evaporate the ammoniacal solution at a gentle heat, and test the residue of ammonium salicylate with ferric chloride.

Formic Aldehyde or *Formaldehyde*, H.COH, is now extensively applied to the preservation of milk. It is sold, for this purpose, as an alcoholic solution of about 40 per cent. strength, under the name of "Formalin,"[1] but more dilute solutions are also sold to milk-vendors.

Formaldehyde is probably one of the least objectionable preservatives of milk, since it does not introduce extraneous mineral matters, like boric acid, borax, and fluorine compounds; and the readiness with which it suffers oxidation gives it the preference over antiseptics of the salicylic acid class. But the physiological effects of formaldehyde are at present very imperfectly known, and the practice of adding antiseptics to food is to be deprecated. In this connection, it is noteworthy that several observers have found the casein of milk containing formaldehyde to be dissolved by acid with much less facility than the casein of pure milk. W. N. Yarrow (*Analyst*, xxi. 97) and others have observed that the casein clot produced by hydrochloric acid in pure milk is usually white, but that in presence of formaldehyde it is invariably yellow.

The relative efficiency of various preservatives in milk has been studied by R. T. Thomson (*Glasgow City Anal. Soc. Reports*, 1895, page 5; abst. *Analyst*, xxi. 65), whose results are shown in the following table. Measured quantities of the same milk, to which the preservatives were added, were kept in stoppered bottles under identical conditions, and were examined from time to time.

[1] Formalin is by far the most convenient reagent for preserving milk for the purpose of analysis. One or two drops per fluid ounce is a quantity sufficient to prevent material change for many weeks. An excess should be avoided, or a notable increase in the total solids will result. Before the introduction of formaldehyde, the author habitually preserved milk for analysis by adding to the sample twice its weight of methylated spirit.

Preservative used.	Grains of Preservative per gallon of Milk.	After standing 2 days.	After standing 4 days.	After standing 6 days.	After standing 7 days.	After 8 days.	After 11 days.
						Lactic Acid, per cent.	Lactic Acid, per cent.
(Pure milk), . .	...	Distinctly turned.	Slightly sour.	Sour.	Sour and curdled.	·68	·71
"Formalin," . .	8·75 (= ·0125 p.c.).	Sweet.	Sweet.	Sweet.	Sweet.	Sweet, ·12	Sour and curdled, ·43
,, . .	17·5 (= ·025 p.c.).	Sweet.	Sweet.	Sweet.	Sweet.	Sweet, ·10	Sweet, ·14
,, . .	35 (= ·05 p.c.).	Sweet.	Sweet.	Sweet.	Sweet.	Sweet, ·07	Sweet, ·10
Boric acid, . .	35 (= ·05 p.c.).	Sweet.	Sweet.	Turned.	Sour and curdled.	·42	·52
Boric acid+borax (calculated to boric acid),	35 (17·5 of each).	Sweet.	Sweet.	Sweet.	Sweet.	Sweet, ·10	Sour, ·32
Salicylic acid, . .	17·5 (= ·025 p.c.).	Sweet.	Sweet.	Sweet.	Turned.	Sour, ·26	·42
,, ,, .	35 (= ·05 p.c.).	Sweet.	Sweet.	Sweet.	Sweet.	Sweet, ·10	Sour, ·33
Benzoic acid, . .	17·5 (= ·025 p.c.).	Sweet.	Sweet.	Slightly turned.	Sour.	Sour, ·45	·52

From these results it is evident that formalin is by far the most efficient of the preservatives in question. Boric acid is evidently not so efficient a preservative when employed alone as when used in conjunction with borax, as in the preparation known as "glacialin." Thirty-five grains of boric acid per gallon of the milk to be preserved is the amount generally added.[1]

[1] According to experiments by A. Meyer (*Dingl. polyt. Jour.*, ccxlvii. 376) milk to which boric acid, common salt or salicylic acid had been added and kept at 16° C. began to turn sour and coagulate in the times indicated below :—

	Turned sour after :—	Coagulated after :—
Without addition of preservative, .	25 hours	28 hours
With 0·02 per cent. boric acid, . .	30 ,,	47 ,,
,, 0·04 ,, ,, . .	35 ,,	47 ,,
,, 0·06 ,, ,, . .	56 ,,	60 ,,
,, 0·02 ,, common salt, .	26 ,,	30 ,,
,, 0·04 ,, ,, . .	26 ,,	32 ,,
,, 0·06 ,, ,, . .	26 ,,	34 ,,
,, 0·02 ,, salicylic acid, .	33 ,,	58 ,,
,, 0·04 ,, ,, .	47 ,,	82 ,,
,, 0·06 ,, ,, .	144 ,,	more than eight days.

The presence of boric acid and salicylic acid could not be detected by the taste, but the effect of salicylic acid was perceptible.

On warming a sample of milk preserved with formalin, the characteristic odour of formaldehyde becomes perceptible. Rideal states that one part of formalin in 25,000 parts of milk can thus be detected. When such milk is distilled, the distillate has the odour of formaldehyde, but the preservative is not wholly volatilised. Thus when a solution of formaldehyde is evaporated to dryness at 100°, a very notable quantity of residue is obtained, owing to the conversion of the formaldehyde into non-volatile or difficultly-volatile polymers or condensation-products. This behaviour is still more marked in presence of milk, a portion of the formaldehyde apparently reacting to form non-volatile compounds with certain of the milk-constituents. Thus in employing colour-tests for formaldehyde, a notably weaker reaction is obtained when milk containing formalin is distilled and the distillate tested, than when water containing the same proportion of formalin is similarly treated.[1]

E. J. Bevan (*Analyst*, xx. 152) found that the solid residue obtained on evaporating a sample of milk increased regularly with the amount of formaldehyde added, as is shown by the following figures :—

	Total Solids.
Original milk, without addition,	13·24 per cent.
Five c.c. with two drops of formalin,	13·47 ,,
,, six ,, ,,	13·77 ,,
,, ten ,, ,,	14·10 ,,

O. Hehner (*Analyst*, xxi. 95) has determined the rate of disappearance of formalin when added to milk. He found from a series of experiments that, after one week, no formalin could be detected in a sample which originally contained 1 part of formalin in 100,000 parts of milk ; after two weeks none could be found in the 1 : 50,000 sample ; while after three weeks there was only the faintest trace to be detected in the 1 : 25,000 sample. The experiments were made in cool weather, and the formaldehyde was tested for by Schiff's reagent in the distillate from the milk (see also page 183).

From the foregoing facts it is evident that the determination of formaldehyde in milk is attended with great difficulty, though its

[1] It has been shown by Leonard and Smith (*Analyst*, 1897, page 5) that the separation of formaldehyde from milk is facilitated by acidulation with sulphuric acid previously to distillation (see page 187). From their results it appears that the first 20 c.c. of distillate from 100 c.c. of milk will contain about one-third, and the first 40 c.c. about one-half, of the total formaldehyde present.

mere detection may be effected by several simple tests. The reduction of alkaline solutions of copper and silver,[1] though delicate in their indications, are not sufficiently characteristic of formaldehyde to be accepted as decisive of its presence.

S. Rideal (*Analyst*, xx. 158) has observed that Schiff's reagent is a delicate test for formaldehyde in milk, when employed in a slightly acid solution. Schiff's reagent may be prepared by mixing 40 c.c. of a 0·5 per cent. solution of magenta with 250 c.c. of distilled water, adding 10 c.c. of sodium bisulphite solution of 1·375 specific gravity, and then 10 c.c. of pure strong sulphuric acid. The mixture is allowed to stand for some time, when it will become colourless. It may also be prepared, when required for use, by adding sufficient of a solution of sulphurous acid to decolorise some of the magenta solution. If the sulphurous acid is added in large excess, traces of formaldehyde will not be indicated. As a confirmatory reaction, this test is useful, and may be applied to milk in the manner prescribed by Richmond and Boseley (*Analyst*, xx. 155). The casein is precipitated by the addition of a little sulphuric acid, the liquid filtered, and some of the Schiff's reagent added to the filtrate. The intensity of the red colour produced somewhat roughly indicates the amount of formaldehyde present. In presence of any alkali, combined with a stronger acid than sulphurous acid, a red colour will be produced, even in the absence of formaldehyde. This test has the advantage of requiring no distillation. It has been stated that aqueous solutions of milk-sugar do, under some undefined conditions, produce a red colour on addition of Schiff's reagent; but Richmond and Boseley found the contrary to be the case, milk-sugar solutions giving no coloration, even after boiling with dilute sulphuric acid.[2]

[1] R. T. Thomson recommends that 100 c.c. of milk should be distilled, and the first 20 c.c. of distillate, which is stated to contain the greater part of the formaldehyde, then tested with ammonio-nitrate of silver. In the presence of formaldehyde, the liquid will become strongly blackened on standing for some time in a dark place. When mere traces are present, several hours may be required for the development of the reaction. The distillate from milk containing no preservative gives a negative reaction or at most a light brownish tinge, which should therefore be disregarded.

[2] The value of Schiff's reagent is impaired by the fact that, as has been shown by Richmond and Boseley, the red coloration appears when the solution is warmed, or by blowing air through, or even when it is allowed to stand for a little time in an uncorked bottle. O. Hehner states that, besides the above, he has obtained the reaction when the reagent was allowed to stand for half an hour in a stoppered cylinder with distilled water, or with a distillate quite free from formaldehyde.

O. Hehner (*Analyst*, xxi. 94) has described a test for the detection of traces of formalin in milk, based on the addition to milk of strong sulphuric acid, which in the presence of small amounts of formaldehyde produces a violet-blue coloration.[1] Richmond and Boseley perform the test by diluting the milk with an equal measure of water, and pouring it upon sulphuric acid of 90 to 94 per cent. strength. By operating in this manner, the delicacy of the test is appreciably increased. In the presence of one part of formalin in 200,000 parts of milk, a violet-blue colour is produced at the junction of the two strata, and is permanent for several days. In the absence of formaldehyde, a slight greenish tinge may be produced, and sometimes a brownish-red colour becomes apparent after some hours lower down in acid, but this cannot be confused with the blue colour due to formaldehyde.

Hehner's reaction is simple, delicate, and is not produced by acetaldehyde; but Richmond and Boseley have shown that it fails with larger quantities of formaldehyde. An amount of, say, 0·5 per cent. would give no blue coloration.

Richmond and Boseley state that Hehner's reaction is due to the proteids of milk. They find that egg-albumin and peptone give the reaction, but that gelatin does not. Hehner confirms the last observation, but he could not obtain the reaction with a solution of peptone; and with egg-albumin got only a faint response, which he concludes was due to some impurity rather than to the albumin itself.

N. Leonard (*Analyst*, xxi. 157) has pointed out that, whilst Hehner's reaction is easily obtained when commercial sulphuric acid is used, it fails when the pure redistilled acid is employed. The addition of ferric chloride or platinic chloride to pure sulphuric acid was found to confer on it the power of giving the violet-blue colour with milk containing formaldehyde. The commercial acid was found to contain iron, of which no trace could be detected in the pure acid. Leonard, therefore, concludes that the presence of a feeble oxidising agent is necessary for the production of Hehner's reaction. The test is not improved by the addition of any considerable amount of ferric chloride. Hehner has confirmed the observation of Leonard that the addition of a trace of ferric chloride renders the reaction more distinct.

Another test has been described by O. Hehner (*Analyst*, xxi. 94), which also is only useful when the formaldehyde is present

[1] This reaction was noticed by E. J. Bevan when preparing milks for the Leffmann-Beam method for the determination of the fat.

in small proportion. If to the distillate from a sample of milk or other substance one drop of a dilute aqueous solution of phenol is added, and the mixture poured upon some strong sulphuric acid in a test-tube, a bright crimson colour appears at the point of contact in presence of one part of formaldehyde in 200,000. If a larger proportion be present, a milky-white zone above the crimson ring is seen. Acetaldehyde gives an orange-yellow colour. The reaction only succeeds when carried out exactly as described, and only a trace of phenol must be used.

Plöchl (*Berichte*, xxi. 2117) found that, when a neutral solution of formaldehyde is mixed with ammonium chloride, it becomes acid in reaction. On heating the solution, carbon dioxide is evolved, and trimethylamine is formed. Pulvermacher (*Berichte*, xxvi. 2360) has obtained many condensation-products of formaldehyde with substituted ammonias. In the light of these researches, Richmond and Boseley have found that a reaction takes place between formaldehyde and diphenylamine. A solution of diphenylamine is made by shaking the base with water, and adding just sufficient sulphuric acid to dissolve it. The liquid to be tested (*e.g.*, the distillate from milk) is added to some of the diphenylamine solution thus prepared, and the mixture boiled. In the presence of formaldehyde, a white flocculent precipitate is deposited, which frequently becomes green, especially on continued boiling. It is convenient to distil the milk into the diphenylamine solution, and then boil. This simple test is believed by Richmond and Boseley to be characteristic of formaldehyde. They find, however, that the reaction is not very delicate. Although it gives a reaction with milk to which the amount of formalin prescribed by the vendors had been added, yet it fails with such smaller quantities as give distinct reactions by Hehner's and Schiff's reactions.

Trillat (*Compt. rend.*, cxvi. 891) has proposed the following tests for formaldehyde:—The solution containing the formaldehyde is mixed with dimethylaniline acidified with sulphuric acid, and agitated. The liquid is heated for half an hour on the water-bath, made alkaline, and boiled until the odour of dimethylaniline has disappeared. It is then filtered, and the filter-paper moistened with acetic acid. If some powdered lead dioxide be then sprinkled over the paper, a blue colour will be produced if formaldehyde was present. This blue colour, which is not very stable, is due to the formation of tetramethyl-diamido-diphenylmethane. Another test is based on the fact that, when a solution of formaldehyde is mixed with a 0·3 per cent. solution of aniline, a white precipitate

is produced. This white precipitate, anhydro-formaldehyde-aniline,[1] may be weighed, and the amount of formaldehyde originally present deduced. Acetaldehyde also gives a precipitate. This test must be performed in the cold, as the precipitate is soluble in hot water, reappearing on cooling. The precipitate with acetaldehyde is more soluble than that given by formaldehyde. Trillat states that, owing to the formation of condensation-products, formaldehyde cannot always be detected in preserved foods after some lapse of time. Richmond and Boseley have confirmed this observation, but state that they can always detect formaldehyde by this test when the milk has not curdled.

C. A. Mitchell (*Analyst*, xxi. 97) has described a test for formaldehyde in milk which is dependent upon the action of this substance on Nessler's reagent. A very small amount of formaldehyde is sufficient to produce a yellow colour, which gradually darkens, and produces a precipitate, at first resembling ferric oxide, and finally becoming dark grey. The reaction is quite distinct from that produced by ammonia. By acidulating the sample of milk, and applying the test to the distillate, any interference from the presence of ammonia is wholly prevented. Mitchell states that, when thus applied, the test readily detects the presence of one part of formaldehyde in 250,000, and that acetaldehyde does not interfere.

Richmond and Boseley (*Analyst*, 1896, p. 93) have made a comparison of some of the more important tests for formalin in milk. Hehner's and Schiff's tests they regard as of about equal delicacy, while Trillat's reaction with dimethylaniline is less delicate, and Richmond and Boseley's test with diphenylamine is of about the same sensitiveness. The same observers rely upon Hehner's (modified) test for the detection of formaldehyde in milk, and use Schiff's test (in the whey separated from the clot produced by sulphuric acid) and Trillat's dimethylaniline test to confirm this reaction. If the quantity of formalin is sufficiently large, they prefer to distil the milk into diphenylamine dissolved in a slight excess of sulphuric acid, and boil the liquid.

The determination of formaldehyde, in the small quantities in which it is employed for preserving milk, is attended with great difficulty, and cannot at present be effected with accuracy. The preliminary isolation of the preservative by distilling the milk is

[1] The following equation represents the action :—

$$\underset{\text{Aniline.}}{C_6H_5.NH_2} + \underset{\text{Formaldehyde.}}{H.CHO} = \underset{\text{Anhydro-formaldehyde-aniline.}}{CH_2{:}N.C_6H_5} + H_2O.$$

open to objection, but experiments recorded by Leonard and Smith (*Analyst*, 1897, page 5) show that rough indications of the amount of formaldehyde present can be obtained in this manner, if certain precautions be taken. From their experiments they conclude that—(1) The distillate from fresh milk exerts no appreciable reducing action on alkaline permanganate, but milk three or four days old yields a distillate having marked reducing properties. (2) The separation of formaldehyde from milk is facilitated by acidulating the liquid with sulphuric acid, and blowing open-steam through it. Under these conditions, the first 20 c.c. of distillate from 100 c.c. of milk will contain about one-third, and the first 40 c.c. about one-half, of the total amount of formaldehyde present. (3) The fact that the distillate from milk does not contain the whole of the formaldehyde present is to a great extent explained by the behaviour of solutions of formaldehyde on distillation, and is only partly due to any specific action of the preservative on the constituents of milk.

In a subsequent paper (*Analyst*, 1897, page 92), Leonard, Smith, and Richmond state that the quantity of formaldehyde which passes over on distillation of a dilute aqueous solution may be expressed by the following formula :—

$$100 - y = \frac{(100 - x)^{1.6}}{(100)^{0.65}}$$

in which y is the percentage of formaldehyde contained in the distillate, and x the percentage of the total volume of liquid distilled.[1]

[1] The following experiments on solutions containing known quantities of formalin show that the formula agrees well with the results obtained in practice :—

Volume of Fraction distilled.	Formaldehyde in Fraction.							
	A.		B.		C.		D.	
	Found.	Calculated.	Found.	Calculated.	Found.	Calculated.	Found.	Calculated.
0 to 20 c.c., .	·0024	·00240	·0023	·00234	·0026	·00237	·199	·208
20 to 40 c.c., .	·0021	·00204	·0018	·00196	·0020	·00201	·180	·177
40 to 60 c.c., .	·0017	·00164	·0017	·00160	·0031	·00332	·139	·142
60 to 80 c.c., .	·0011	·00117	·0012	·00114			·105	·1015
80 to 100 c.c., .	·0005	·00055	·0006	·00053			·053	·0475
Total, . .	·0078	·00780	·0076	·00760	·0077	·00770	·676	·676

The amount of formaldehyde contained in the distillate from milk and other very dilute solutions may be ascertained by determining its reducing power on permanganate, ammonio-nitrate of silver, and similar reagents; but in view of the fact that the amount of formaldehyde which is found in the distillate bears no definite relation to that originally added to the milk, the determination has little practical value.[1]

[1] Several good methods exist for determining the proportion of formaldehyde in aqueous solutions, such as commercial formalin (see *Analyst*, xxi. 148; xxii. 4, 5). When present in considerable quantity, formaldehyde may be estimated by titration with a standard solution of ammonia, which converts it into hexamethylene-tetramine, according to the equation:—

$$6CH_2O + 4NH_3 = (CH_2)_6N_4 + 6H_2O.$$

According to this reaction, the accuracy of which has been proved by Trillat and confirmed by Legler, 180 parts of formaldehyde react with 68 parts of ammonia. Lösekan maintains, however, that an equal number of molecules of ammonia and formaldehyde enter into reaction.

In working the above process, S. Rideal (*Analyst*, xx. 157) exactly neutralises any acid contained in the sample by addition of centinormal caustic soda solution, and then agitates a known volume with excess of a standard solution of ammonia. The ammonia in excess is determined by distillation into standard acid.

G. Romijn (abst. *Jour. Chem. Soc.*, 1896, ii. 280) employs the reaction with ammonia for detecting formaldehyde in milk. On distilling the liquid, more or less of the preservative is certain to pass over. A drop of the distillate is mixed on a slip of glass with a drop of ammonia and evaporated to dryness. The crystalline residue, if formaldehyde were present, will be seen under the microscope to consist, "not of rhombohedral, but of regular crystals." On moistening the residue with water, characteristic reactions may be obtained with the general reagents for alkaloids.

O. Hehner (*Analyst*, xxi. 94) suggests that the phenol-sulphuric acid test described by him (see page 185) for the detection of formaldehyde might be used as a means of determining the strength of dilute formalin solutions. The precipitate obtained is very insoluble, and so might easily be washed and weighed if required.

R. Orchard (*Analyst*, xxii. page 4) has applied the reaction of formaldehyde with ammoniacal silver nitrate to its quantitative determination in the following manner:—A convenient measure of the formaldehyde solution is mixed with 25 c.c. of a decinormal solution of silver nitrate and 10 c.c. of dilute ammonia (1 : 50). The mixed solutions are boiled together in a conical flask over a reflux condenser for about four hours. The precipitate, after being washed and dried, is ignited and weighed as metallic silver. The residual silver nitrate in the filtrate may also be determined as a check upon the other result. Since one molecule of the formaldehyde reacts with two molecules of silver oxide, Ag_2O, the weight of the silver precipitated, multiplied by the factor 0·0694, gives the weight of the formaldehyde. Also, 1 c.c.

STERILISATION OF MILK.

Milk can be kept for an indefinite time by heating it to a high temperature, closing the vessel while still hot, and keeping it in a cool place. The exact temperature desirable and the length of heating required have been the subject of considerable controversy, but there is no doubt the success of the treatment depends largely on the perfect destruction and subsequent exclusion of germs.[1]

Sterilised milk is now prepared on a considerable scale, and supplied in glass bottles in its original state of purity without any addition of chemicals, sugar, preservatives, or colouring matter.[2] In appearance the product closely resembles ordinary

of decinormal silver nitrate corresponds to 0·0007495 gramme of formaldehyde. It is therefore possible by this process to estimate extremely small quantities of the aldehyde.

Orchard's analyses of three samples of dilute milk-formalin by this process showed them to be fairly constant preparations of about one per cent. strength.

A method proposed by B. Grützner (*Archiv. de Pharm.*, ccxxxiv. (1896) No. 8) is based on the fact that formaldehyde reduces chloric acid to hydrochloric acid in its salts, and thus, if silver nitrate is added, an amount of silver chloride will be precipitated equivalent to the formaldehyde originally present in the solution under examination. A small measured quantity of the formaldehyde solution is treated with about one gramme of potassium chlorate, dissolved in a little water, in a stoppered flask. Fifty c.c. of decinormal silver nitrate solution is now added, together with about one gramme of nitric acid. The stopper is securely fastened down, and the flask and contents, after being shaken for a time, are warmed gently for half-an-hour. The excess of silver nitrate is determined, on cooling, by thiocyanate solution, using iron-alum as an indicator. The following equation expresses the reaction :—$HClO_3 + 3H.COH + AgNO_3 = 3H.COOH + AgCl + HNO_3$.

Other methods for the determination of formaldehyde have been described by G. Romijn (*Zeit. anal. Chem.*, xxxvi., [8], 18).

[1] Duclaux succeeded in preserving milk in an unchanged condition for five years, by producing a vacuum in the containing vessel and subsequently heating to 120° C. Dietzell found that milk exposed for twenty minutes to a temperature of 105° to 110° C. kept sweet for a few weeks only, but if subsequently heated to 115° for five minutes it could be kept unchanged for three years.

[2] J. A. Forret (*Pharm. Jour.*, 1896, ii. 281) gives the following instructions for sterilising small quantities of milk :—A jar containing a pint of milk is placed in a vessel containing three pints of water, the latter vessel being of such a size that the level of the water and milk are about equal when the jar is supported at about half an inch distance from the bottom of the water-bath. The temperature of the water is now raised to boiling-point, and should require not less than twenty-five minutes, nor more than thirty-five minutes. When the water boils, the milk will be at a temperature of

fresh milk, but the coagulation of the lactalbumin affords a means of differentiating it.

The following figures by C. H. Stewart (*Brit. Med. Jour.*, 1896, p. 626) show the percentage of albumin found in milk raised to various temperatures :—

Time of Heating.	Soluble Albumin in Fresh Milk.	Soluble Albumin in Heated Milk.
10 min. at 60° C.	·423	·418
30 ,, ,,	·435	·427
10 ,, 65° C.	·395	·362
30 ,, ,,	·395	·333
10 ,, 70° C.	·422	·269
30 ,, ,,	·421	·253
10 ,, 75° C.	·380	·07
30 ,, ,,	·38	·05
10 ,, 80° C.	·375	none.
30 ,, ,,	·375	none.

It is evident that no sharp distinction can be drawn between milk which has been raised to a temperature of over 70° C. for a short period, and which is not sterilised in the true sense of the term, and milk which has been heated for sufficient length of time to destroy all microbial life.

75° C.; the water is now allowed to cool, and the milk attains a maximum temperature of 78°–80° C. The milk should be stirred during the whole of the process, so as to prevent the separation of the fat.

P. Cazeneuve (abst. *Jour. Chem. Soc.*, 1896, ii. 120) sterilises milk by heating it in boiling water in screw-stoppered bottles, which are capsuled with tin and completely immersed in the water, the air escaping through capillary orifices in the capsules subsequently closed by compression. As the bottles cool, the capsules and necks are coated with solid paraffin, to prevent the possibility of the entrance of air. Of various samples of milk subjected to this treatment—some fresh, some about to turn sour, and some actually putrescent—Cazeneuve found that none underwent any further change, even when kept for days at 35° C. The lactic ferment seems to be attenuated and, to a large extent, destroyed by the process, for the sterilised samples remained for the most part unchanged after the admission of sterilised air, and did not give rise to colonies when sown in a gelatin medium.

Milk thus treated is stated by Cazeneuve to be more digestible than new milk, and not to have the objectionable colour or taste of milk boiled in an open vessel or sterilised at higher temperatures. The process has been tried on a commercial scale.

The deduction from the albumin test may be confirmed by a method described by Richmond and Boseley (*Analyst*, 1897, page 95), who find that the cream is thrown up from sterilised milk much more slowly and imperfectly than from new milk. Thus they state that practically no cream is observed on the surface of sterilised milk or diluted condensed milk after standing for three hours, and that after six hours the layer is only about one-tenth of that given by new milk. If sterilised milk be allowed to stand for twenty-four hours or more, the bulk of the cream will rise to the surface, but the quantity will still be less than that yielded by new milk. The cream will, however, contain a distinctly larger percentage of fat, namely, about 40 per cent., as against less than 30 per cent. in the cream yielded by new milk. Richmond and Boseley give the following figures in illustration of the foregoing statements:—

Sterilised Milk allowed to stand for Six Hours.

No.	Per cent. of Fat in Milk.	Per cent. Cream Risen.	Per cent. Cream for 1 per cent. Fat.	Per cent. Fat in Cream.	Per cent. Fat in Skim Milk.
1.	4·30	1·3	·30	23·3	4·05
2.	3·80	0·7	·18	22·3	3·67
3.	4·25	1·8	·42	20·6	3·95
4.	4·10	1·9	·46	24·7	3·70
5.	5·35	2·8	·52	31·4	4·60
6.[1]	3·62	0·3	·08	..	..

Sterilised Milk allowed to stand for Twenty-four Hours.

No.	Per cent. Fat in Milk.	Per cent. Cream.	Per cent. Fat in Cream.	Per cent. Fat in Skim Milk.
1.	4·30	7·0	46·8	1·10
2.	3·80	6·0	41·8	1·37
3.	4·25	8·8	39·0	·90
4.	4·10	8·7	41·0	·58
5.	5·35	11·1	41·4	·85
6.[1]	3·62	0·8	..	3·48

[1] Diluted condensed milk.

New Milk allowed to stand for Six Hours.

No.	Per cent. of Fat in Milk.	Per cent. Cream.	Per cent. Cream for 1 per cent. Fat.	Per cent. Fat in Cream.	Per cent. Fat in Skim Milk.
1.	4·05	9·2	2·27	17·4	2·70
2.	4·20	11·2	2·66	16·5	2·65
3.	3·90	9·8	2·51	15·9	2·60
4.	3·70	9·8	2·69	18·9	2·15
5.	4·45	13·5	3·03	16·8	2·30

The samples numbered from 1 to 5 were yielded by the same cows in each case.

Condensed unsweetened milk, which has been diluted to the original volume with water, has all the analytical characteristics of sterilised milk, and no method has hitherto been devised for distinguishing between them.

To distinguish new milk from milk which has been sterilised, Richmond and Boseley propose the following methods :—

(*a*) Place 100 c.c. of milk in a graduated cylinder, and allow it to stand for six hours at 60° F. (15·5° C.); note the percentage of cream. If less than 2·5 per cent. of cream for each 1 per cent. of fat in the milk has risen to the surface, the milk may be considered suspicious; if the quantity of cream falls markedly below 2 per cent. for each 1 per cent. of fat, it is highly probable that sterilised milk is present. The proportion may be calculated by the following formula :—

$$\text{Percentage of sterilised milk} = \frac{2{\cdot}5 - \frac{\text{Cream}}{\text{Fat}}}{2{\cdot}2}$$

(*b*) Estimate the albumin by the method of Hoppe-Seyler, or, better, by that of Sebelein or of Duclaux. If less than 0·35 per cent. is found, sterilised milk may be considered to be present, and the proportion may be deduced by the following formula, which assumes the presence of 0·40 per cent. of albumin in new milk, and its complete absence from sterilised milk :—

$$\text{Percentage of sterilised milk} = \frac{0{\cdot}4 - \text{percentage of soluble albumin}}{0{\cdot}4} \times 100.$$

(*c*) Estimate the milk-sugar by the polarimeter, and also gravimetrically in duplicate. If the difference between the two estimations be more than 0·2 per cent., it will be corroborative evidence

of the presence of sterilised milk. A proportion of sterilised milk much below 30 per cent. could not be detected by this method when mixed with new milk.

The following figures are given by Richmond and Boseley in illustration of the extent to which the foregoing methods are reliable :—

No.	Fat.	Cream.	Cream / Fat.	Albumin.	Sugar.	Sugar; Polarimeter.	Percentage of Sterilised Milk.		
							Actual.	Calc. from Cream.	Calc. from Albumin.
1.	3·86	7·2	1·87	·30	4·85	4·65	33	29	25
2.	4·10	9·6	2·34	·34	4·79	4·68	..	Genuine.	..
3.	3·90	7·5	1·92	·29	4·79	4·64	28	26	27
4.	3·70	4·3	1·16	·16	4·75	4·57	56	61	60
5.	4·10	7·4	1·81	·26	..	..	30	31	35
6.	4·00	7·5	1·88	·27	..	..	30	28	32
7.	3·75	7·9	2·11	·35	..	..	..	Genuine.	..

The analyses numbered from 2 to 7 were made upon mixtures the composition of which was unknown to the operator, those marked 2 to 4 being the work of Richmond, and those from 5 to 7 of Boseley. The results arrived at by analysis are given in the columns marked "calculated," and the agreement between the two methods and with the actual composition is remarkably good.

I. Carcano has proposed to recognise the presence of raw milk in boiled or sterilised milk by warming the sample gently with a few drops of fairly fresh oil of turpentine, and adding tincture of guaiacum. In the presence of raw milk the well-known blue coloration will be produced.

Very discordant opinions have been expressed as to the desirability of employing sterilised milk. Under certain circumstances there can be no doubt that its use is very convenient, but it is very questionable if, in digestibility and consequent food-value, it is equal to new milk.[1]

[1] Flugge (abst. *Analyst*, 1896, p. 102) considers that the sterilised milks of commerce are dangerous preparations, the sale of which should be discontinued. He finds that whilst the pathogenic microbes of tuberculosis, cholera, diphtheria, and typhoid fever are killed by boiling milk for a short time, such is not the case with the microbes of infantile diarrhœa. These latter form spores which resist a temperature of 100° C. for two hours. These organisms form peptones in the milk, which cause diarrhœa. Flugge recommends that milk should be boiled for six minutes, cooled below 18° C., and

Decomposition of Milk.

Milk readily undergoes change at all temperatures above 9° C. The change commences in a few hours, an increase in the specific gravity and an evolution of carbon dioxide being the first indications of alteration. The latter phenomenon is simply a result of fermentation, and may be prevented by subjecting the milk to any efficient antiseptic treatment.

Lactic Acid Fermentation.—The next stage of alteration of milk is marked by the development of an acid reaction, owing to the formation of lactic acid from the milk-sugar. This change is due to the action of certain organised ferments, among which the micro-organism known as *Bacillus acidum lactici* is the best known, and is generally regarded as predominating. As a fact, however, there appear to be a number of organisms which occur in milk, and are capable of converting milk-sugar into lactic acid. Thus, while one ferment may be the chief exciting cause in one district, in another a different bacillus may predominate. In the case of some of these organisms, lactic acid is probably merely a subsidiary product, being incidentally formed together with other bodies. It is very doubtful if any two of these numerous organisms act on milk in an exactly similar manner. In practice, the lactic acid bacillus usually predominates, but the total effect is modified by the action of the co-occurring ferments.[1]

consumed within twelve hours. In this way the growth of peptonising bacteria is retarded. The milk thus sterilised should be kept in closed vessels, hermetic sealing being useless.

Leeds and Conn, in their Report to the Dairy Commission of the State of New Jersey on "The Preservation of Milk" (*Pharm. Jour.*, xxiii. 86), express the opinion that the sterilisation of milk is attended with serious disadvantages. Milk fresh from the cows has a considerable germicidal power, which does not entirely disappear for some hours, whilst the rapid heating in the sterilisation of the milk destroys this property. By the heat employed the lactalbumin is in part coagulated and thus rendered more difficult of digestion. The casein of milk also undergoes a somewhat obscure change, being rendered less easily and less completely precipitable by rennet. Sterilisation also destroys the starch-fermenting property possessed by raw milk. The saliva of adults contains a ferment which converts starch into sugar, but this is absent from the saliva of infants. It is a remarkable fact that fresh milk contains a somewhat similar ferment, which is, however, destroyed on heating the milk to 75° C. The milk-sugar is also affected by the process of heating. The sterilisation of milk has also a marked effect upon the fat, which is originally in a state of emulsion, but by heating is set free and rendered difficult of absorption.

[1] Various bacteria causing lactic fermentation have been isolated and studied. Besides bacilli, they include *micrococci*, *sphærococci*, *streptococci*,

The ordinary lactic acid ferment, *Bacillus acidum lactici*, may be readily cultivated by diluting sour milk with 1000 times its measure of recently boiled sterile water, and adding a single drop of this dilute liquid to a suitable quantity of nutrient gelatin. After a few days the gelatin will be spotted with colonies chiefly composed of the lactic acid bacillus. This assumes the form of short, thick rods, having a length of from 1·0 to 1·7 micro-millimetres and a thickness of 0·3 to 0·4 mmm. When examined by non-immersion lenses the lactic acid bacilli often appear oval, the organisms being united in couples to produce hour-glass forms. The multiplication by fission is marked by a contraction. The characters of the lactic acid ferment are better studied if the bacillus is stained with methylene-blue.

Below 9° to 10° C. the lactic acid bacillus is inactive. Between 10° and 12° there is a slight action, which becomes stronger, but still slow, at 16°. The maximum activity occurs between 35° and 42° C. Above the latter temperature the production of lactic acid lessens, and ceases entirely at about 45°.

W. Thorner (*Chem. Zeit.*, 1891, p. 1108; abst. *Analyst*, xvi. 200) has investigated the degree of acidity requisite to cause coagulation of milk. Using phenol-phthalein as an indicator, and calling the number of c.c. of decinormal caustic alkali required to neutralise the acid in 100 c.c. of milk the "degree of acidity," he finds that, three or four hours after milking, cows' milk has an acidity of 12° to 16°, rising to 14° to 25° when the milk is nine or ten hours old. After twenty-four hours the milk has an acidity of 17° to 60°, and when forty-eight more hours have elapsed the acidity ranges from 30° to 100°. The cooler the milk is kept the more slowly does the acid reaction develop, but exposure to light has little effect. By experiments on samples kept under various conditions, Thorner found that milk coagulates on boiling when the acidity reaches 23°. Adopting 20° as the permissible limit of acidity, he proposes the following test:—10 c.c. measure of the well-mixed sample of milk is diluted with 20 c.c. of water, a few drops of phenol-phthalein solution added, and 2 c.c. of decinormal caustic alkali run in. If a red coloration be produced, even though weak, the milk will not coagulate on boiling.

The formation of lactic acid by the fermentation of milk-sugar is generally represented by the equation:—$C_{12}H_{22}O_{11},H_2O = 4C_3H_6O_3$, but there is good reason to believe that the reaction is of a much less simple character than is thus indicated. Carbon

&c. Lactic bacteria require air for their developement, and do not as a rule produce spores. Hence they have a limited power of resisting heat, a temperature of 70° C. being usually fatal.

dioxide in small quantity appears to be an invariable product, but of the nature of the other subsidiary products no authentic information exists.[1]

In the natural lactic fermentation of milk, the milk-sugar is never entirely converted into lactic acid, since the free acid formed interferes with the growth of the bacillus, but if the acid formed be neutralised, as by the addition of chalk, nearly all the sugar may be transformed.

Under ordinary circumstances, the lactic fermentation of milk is complicated by simultaneous butyric and alcoholic fermentations, but if the lactic acid bacillus be sown in previously sterilised milk a pure lactic fermentation ensues. The formation of lactic acid in decomposing milk very rarely occurs without a very material loss of solids also taking place. In the case of a large number of samples of milk kept from two to forty weeks, F. J. Lloyd found the loss of solids to range from 0·57 to 1·20 per cent., while the free lactic acid formed varied from 1·17 to 2·14 per cent., or approximately one-half the loss of solids. When, however, butyric fermentation occurred actively, the above relationship could no longer be traced (*Analyst*, xii. 234).

Some interesting experiments on the decomposition of milk have been recorded by P. Vieth and C. T. Kingzett (*Analyst*, 1887, p. 2). Kingzett found a formation of alcohol occurred concurrently with that of lactic acid, the amount formed in each case being more than sufficient to account for the deficiency in the amount of

[1] On this point, O. Hehner is reported to have made the following remarks (*Analyst*, 1894, p. 249):—"We did not know with absolute certainty what takes place in the best known fermentations—such as, for example, the alcoholic fermentation. Given a certain quantity of sugar, we did not know exactly how much alcohol and how much carbon dioxide were produced under all possible circumstances. If even this well-known process could not be expressed by a simple formula, what could be said about such products as those of the decomposition of a complex heterogeneous mass of albuminoids and other bodies acted on by an unknown variety of possibly unknown organisms? It was all very well to say that a molecule of milk-sugar was simply converted into two molecules of lactic acid, but no organism works for nothing. In the decomposition which it effects it evidently derives something for its own benefit, and life cannot be carried on without destructive metabolism. There must always be a loss between the stage of sugar and that of lactic acid, and what this is was unknown. Surely the day had gone past for regarding fermentation reactions as having the simplicity of text-book equations. As to the quantity of alcohol produced, we could only approximately calculate the quantity produced back to sugar. . . . It was utterly unjustifiable to pretend to be able to make corrections for decomposition, and to compare the, at best, approximate figures thus obtained with those derived from the analysis of samples when fresh."

lactic acid produced. Kingzett considers that the alcohol formed is not wholly derived from the fermentation of lactose; while, on the other hand, the lactic acid found by titration is in excess of the truth, as the result obtained includes small quantities of butyric and other soluble and fatty acids, which are not derived from lactose.

THE BUTYRIC FERMENTATION OF MILK commonly occurs simultaneously with the lactic fermentation, but at first is far less active than the latter. At a later stage the butyric fermentation becomes more pronounced, and in certain cases completely dominates the other fermentative processes. The butyric acid fermentation can be produced by a number of allied organisms, the best-known of which is a bacillus from ·003 to ·010 millimetre in length and about ·001 millimetre in breadth, and capable of motion. When cultivated in nutrient gelatin, the gelatin liquefies, and a scum forms on the surface. According to H u e p p e, if sterilised milk be inoculated with the butyric acid bacillus and incubated, on the second day a clear, slightly yellowish fluid will be observed below the layer of cream. This increases daily, so that a column of liquid is gradually formed, which is clear above but turbid below. The casein at first forms a firm coagulum, but afterwards diminishes in bulk, and by the end of two or three weeks the greater part has dissolved. The filtered liquid has a bitter taste, contains leucine, tyrosine and ammonia, and gives the biuret reaction (page 13). In the advanced stages of butyric fermentation the liquid has a most offensive odour, and may have an alkaline reaction.

ANALYSIS OF ALTERED MILK.

In examining altered milk, it is often very difficult to obtain a fair representative sample. Occasionally the fat will be found to have partially separated in the form of globules of butter. It is necessary in such cases to weigh the entire sample of milk, separate and weigh the butter-globules, and add their weight to that of any further quantity of fat which may be found by the Werner-Schmid process, which is the method most suitable for the determination of fat in altered milk. Even where no separation of butter has taken place, it is often very difficult, even by vigorous agitation, to convert the curdled milk into a homogeneous mixture. In the experience of the author, the best plan is to transfer the entire sample to a cylindrical beaker, and then agitate it vigorously by several glass rods, kept together at their upper ends by a ring cut from india-rubber tubing. A dipper is constructed from about two inches of test-tube, attached by caoutchouc rings to a glass rod, which serves as a handle. This is plunged into the sample of milk while vigorous agitation is kept up, and then withdrawn. The weight of the portion thus withdrawn having been ascertained,

it should be titrated with standard caustic alkali and phenolphthalein to ascertain the proportion of free acid. If the quantity is too large for further convenient examination, it may be treated with a known quantity of ammonia and agitated briskly. By this means an emulsion is produced which can be readily subdivided. For the determination of the nitrogen, a separate portion should be withdrawn by the dipper, and after weighing, warmed with sulphuric acid until solution of the suspended matters has been effected. The whole or an aliquot part of the resultant liquid can then be subjected to Kjeldahl's process (page 21).

A convenient method of examining milk which has curdled, but has not undergone any other material change, is to ascertain the specific gravity and solids of the *whey*. Many years since the author proved, by the examination of a considerable number of samples, that genuine milk, when curdled by adding a few drops of acetic acid and heating to about 50° C., gave a whey of which the specific gravity was approximately 1·030. The same fact has been observed more recently by Lescœur (abst. *Analyst*, 1895, p. 200), who finds the usual range of gravity to be from 1·029 to 1·031, though occasionally falling to 1·027.[1] He finds the solids of the whey to vary from 6·7 to 7·1 grammes per 100 c.c., the mean being 7·0 grammes and the minimum 6·7 grammes. The serum has the same characters whether the curdling occur naturally or be induced by rennet or acetic acid, and Lescœur regards any milk as watered which yields a whey having a lower gravity than 1·027, or that contains less than 6·7 grammes of solids per 100 c.c. He gives the following table in illustration of the effect of added water on the serum of a sample of genuine milk :—

	Specific Gravity at 15° C.	Solids per 100 c.c.
Pure milk, ...	1·030	7·0 grammes.
,, plus 10 per cent. of water, . .	1·0275	6·4 ,,
,, ,, 20 ,, ,, . .	1·0251	5·9 ,,
,, ,, 30 ,, ,, . .	1·023	5·45 ,,

The *fat* of milk is not liable to material change in any reasonable period of keeping. It may be accurately and conveniently determined in sour milk by the Werner-Schmid process (p. 135).

The *total solids* of altered milk should be determined in a portion

[1] Numerous observations by von Raumer and Späth show the specific gravity of milk-serum to range from 1·026 to 1·033 (*Zeit. angw. Chem.*, 1896, 46, 70).

of the sample which has been previously neutralised by caustic alkali, an allowance being made for the fixed matter thus introduced. In the case of caustic soda, this will be 0·0022 gramme for every c.c. of decinormal alkali employed. If decinormal baryta be used, the correction will be 0·00675, and for strontia 0·0042 gramme. The determination of the total solids of altered milk is neither so easy nor so satisfactory as in the case of fresh milk, material differences in the results being obtained according to the method of drying adopted.

The *non-fatty* solids of altered milk may be deduced in the usual way by subtracting the fat found from the total solids; but as the latter determination is less reliable than in the case of fresh milk, the value of the result is materially reduced. The direct weighing of the non-fatty solids after extraction of the fat is open to grave objections which have been elsewhere stated.

In the judgment of the author, it is undesirable to base an opinion as to the genuine character of a sample of decomposed milk wholly, or even chiefly, on the proportion of non-fatty solids, either with or without correction for change. The *milk-sugar* undergoes changes which are not fully understood, and some of the decomposition-products are volatile. The *proteids* also undergo complex changes, but these do not include the evolution of free nitrogen, and any volatile decomposition-products can be readily and completely fixed by an acid. Further, the nitrogen of these decomposition-products, like that of the original proteids, is readily converted into ammonia by heating with strong sulphuric acid. Hence, in Kjeldahl's process (page 21) there exists a means of determining the nitrogen, and therefore the original proteids, in milk, no matter how far the decomposition may have advanced; and the method is applicable either to the original milk or to the solid residue obtained by its evaporation at any previous time. The lowest percentage of nitrogen contained in any of the milks of which the composition is quoted on page 115, is 0·52 per cent.; and 0·50 per cent. of nitrogen would be a fair and safe minimum limit to adopt.

Additional proof of adulteration having been practised will be afforded by the *ash*, which may be determined in the usual manner, and ought not to fall below 0·70 per cent. The ash should not be strongly alkaline to litmus, should be free from carbonates and borates, and from more than traces of sulphates; and only about 30 per cent. should be soluble in water (compare page 134).

From the foregoing remarks it is evident that the formation of an accurate opinion on the character of a sample of altered milk, from the results of its analysis, is far more difficult than in the case where the fresh milk is available; and where the conclusions

deduced from the analytical results are in conflict, preference should be given to that based on the examination of the fresh milk.

The analysis of altered milk has a very important practical bearing in connection with the examination of samples of milk purchased under the Sale of Food and Drugs Act. In the event of the accuracy of the public analyst's certificate being challenged, the Act provides that, if the court direct,[1] a portion of the sample which has been retained by the inspector shall be referred to the chemists of the Government laboratories at Somerset House for analysis. In no instance can the sample be received by the referees until it is at least a fortnight old, and the usual time varies from four to as much as ten weeks.[2] The chemists of the Government laboratory have, therefore, the problem set them of forming an opinion as to the purity of samples of milk which are always more or less changed, and are sometimes in an advanced stage of decomposition. It appears that, if the referees consider that the milk submitted to them is so changed as to interfere with its analysis, they do not examine it; but this discretion was exercised only four times in the course of nearly twenty years, during which time a total number of 411 samples of milk had been referred to Somerset House, including many which had undergone great alteration[3] (see *Analyst*, i. p. 74).

[1] The Committee on Food Products Adulteration recommend that the present discretion allowed to the court should be withdrawn, and reference be compulsory on demand of either party.

[2] It is difficult to understand that such a notorious fact should have been beyond the cognisance of any of the referees, as it must have been constantly before them in the exercise of their duties; but Mr Richard Bannister, deputy-principal of the Somerset House laboratory, in his evidence before the Committee on Food Products Adulteration in 1896, stated that all samples of milk were necessarily less than twenty-eight days old when received by the referees (reply to question No. 2026).

[3] The facts stated in the text are given on the authority of Mr R. Bannister, deputy-principal of the Somerset House laboratory. The following replies are taken verbatim from the official report of his evidence before the Committee on Food Products Adulteration:—

1673. "We examine whether it is fit for analysis, or whether there is any change in the composition of the sample which prevents an analysis being made."

1674. "We do not state it on our certificate, but if we come across a sample of milk and we find it has been improperly kept, so that it is mouldy or decayed, we do not examine it. We say that it is not in a fit state for examination."

In reply to the Chairman, Mr Bannister said (reply 555):—

"In 311 cases we confirmed the decision of the public analysts, and in 96 we disagreed."

This accounts for 407 out of the 411 milks submitted to the referees, and

It is probable that many failures of justice have been directly attributable to the ambiguous wording of the referees' certificates. Thus for many years it was the referees' practice, continued till recently in spite of repeated protests, to use the expression on their milk-certificates that they were "unable to affirm that water had been added." This expression has been very generally misunderstood, it being frequently held by the court that such a statement was tantamount to a declaration that the milk was genuine. It appears, however, that this interpretation was not intended by the referees.[1]

From the time of the appointment of the Inland Revenue chemists as referees under the Act (1875) till a few years since, samples of milk referred to Somerset House were examined in the ordinary manner (after exact neutralisation of the free acid), but a correction was made in the solids not fat, which took the form of a time-allowance according to the age of the sample, the rate of change being assumed to be approximately constant. Dr James Bell, during that time the principal of the Somerset House laboratory, describes the allowance in the following words [2]:—

"The loss of non-fatty solids is relatively greatest during the first week of keeping, the amount for that period being on the average ·24 per cent.; for the second week the loss averages ·10 per cent. additional; and for each day thereafter ·01 per cent. According to this rate of allowance, the addition to be made to the non-fatty solids would be as follows for the number of days stated:—

7	days,	. .	·24 per cent.
14	„	. .	·34 „
21	„	. .	·41 „
28	„	. .	·48 „
35	„	. .	·55 „

leaves only four samples from 1875 to 1894 on which they declined to express an opinion.

[1] In his evidence before the Committee on Food Products Adulteration, Mr Richard Bannister, in reply to question 1688, asking whether the magistrates, on seeing these words, would not feel inclined to think there was a disagreement between the analyst and Somerset House, said:—"That really would be a misunderstanding, because the expression 'unable to affirm' is only that you cannot tell from an examination of the sample whether water has been added or not."

[2] The quotation in the text is taken from Dr Bell's *Analysis and Adulteration of Foods*, part ii. page 18. Analysts first learned the principle of the referees' allowance from evidence given by Dr Bell and Mr Bannister in a case heard in Manchester in 1883, and fully reported in *The Analyst*, vol. viii. page 185, *et seq.*

"As already mentioned, a slight variation from these figures will be found, according to the conditions under which the milk has been kept; but the difference, whether greater or less, is generally indicated by the acidity of the milk, reckoned as lactic acid. With a carefully-conducted analysis in the manner above described, the error, if any, in making the allowance should not exceed ·10 per cent. of the non-fatty solids, and, in the case of watered milk, the result should come within 1 per cent. of the quantity of water added, as previously estimated from the analysis of the fresh milk."

The referees under the Act can, as a rule, have no knowledge of the circumstances under which a sample of milk has been kept before it is submitted to them, whether on a hot mantelpiece or in a cool cellar; and their time-allowance was necessarily applied with disregard of other variable and unknown conditions, which could not fail to affect the result. It is not surprising, therefore, that the time-allowance, based as it was on the proposition that the rate of change of milk by keeping is approximately constant, was the subject of earnest protest and destructive criticism on the part of public analysts, and has been viewed with the greatest distrust by chemists generally.[1]

In 1887 the author published a table (*Analyst*, xii. p. 231) showing the difference between the actual loss of solids which

[1] Otto Hehner is reported (*Analyst*, 1894, p. 248) to have made the following remarks in a discussion on the subject of the referees' allowances for change in milk:—

"The referees had occasionally declined to examine samples which in their opinion were unfit for analysis, so clearly they were not under legal compulsion, and he thought that it was never intended that such should be the case. The analysis of decomposed milk-samples was a proceeding which the Somerset House chemists had taken entirely on themselves, and it was a pity they did not at the beginning refuse to analyse decomposed samples, or, having analysed them, had not clearly stated in their certificates that they could not come to any definite conclusion. Public analysts would certainly never have ventured to have given opinion on samples five or six weeks old—a course which had been regularly pursued at Somerset House. . . . . The system of allowances had been nothing short of scandalous. . . . . Officials, perhaps, could not be expected, after persisting in making erroneous allowances for seventeen or eighteen years, to confess now that they had been wrong all the time. . . . . The suggestion made by Mr Bannister, in his evidence before the Parliamentary Committee, that such corrections for decomposition did not in any way concern the public analyst, illustrated his inability to appreciate the difficult position of the public analyst, who clearly was deeply concerned in a proceeding which affected his reputation."

had taken place in samples of milk referred to Somerset House, and the loss calculated according to Bell's rule. The figures showed errors in the total solids ranging from +0·79 to −4·07 per cent., and only in a few cases was the agreement moderately close.

The following experiments were recently made in the author's laboratory by F. E. M. Chambers, with the object of determining the influence of time on samples of boiled and unboiled milk, in the original state and after dilution with 20 per cent. by measure of (tap) water. Five c.c. of each sample of milk was measured by a pipette, and placed in a test-tube, the mouth of which was immediately closed with a plug of cotton-wool. The tubes were kept in this condition until the contents were required for analysis. One tube of each series was opened every week, the contents rinsed into a basin, evaporated, and the *total solids* dried and weighed in the usual manner. Except in the case of the fresh milks, the samples were, before evaporation, exactly neutralised by seminormal caustic soda solution, using phenol-phthalein as an indicator. A correction for the amount of soda added was subsequently made. The following were the results obtained :—

Age of Milk.	Original Milk.				Milk + 20 per cent. added water (by volume).			
	A. Unboiled.		B. Boiled.		C. Unboiled.		D. Boiled.	
	Solids.	Acidity.	Solids.	Acidity.	Solids.	Acidity.	Solids.	Acidity.
Fresh, . .	12·68	..	12·68	..	9·93	..	9·93	..
1 week, . .	12·64	1·0	12·66	0·9	9·86	0·9	9·92	0·7
2 weeks, . .	12·32	4·7	12·51	3·7	9·61	3·9	9·91	2·3
3 ,, . .	11·80	4·7	12·43	4·5	9·18	3·9	9·50	3·4
4 ,, . .	11·02	4·1	11·86	5·1	7·88	2·4	9·29	3·0
5 ,, . .	10·70	5·2	11·40	4·1	8·10	3·8	8·87	3·6
6 ,, . .	9·75	3·9	11·29	4·5	7·48	3·6	8·54	2·0
7 ,, . .	9·20	4·1	11·14	3·5	7·40	3·1	8·53	3·6
8 ,, . .	8·39	4·0	10·09	3·7	5·64	2·5	9·25[1]	2·3
9 ,, . .	8·62	4·5	8·96	2·3	6·50	3·7	7·74	2·5
10 ,, . .	8·28	2·6	11·82[1]	7·9	5·61	2·0	7·00	2·9

The numbers under the head of "Acidity" represent the volume

[1] These portions were quite free from odour, and had undergone very little change in appearance.

in c.c. of decinormal alkali required for the neutralisation of the free acid in 5 c.c. of the sample.

It will be observed that even in test-tubes filled and kept side by side, and with every care to secure constancy of conditions, the rate of change is neither equal nor regular.

The following table shows the actual change in the milk-solids by keeping, as compared with Bell's allowance :—

Age of Milk.	Bell's Allowance.	Actual Change in Total Solids.			
		A.	B.	C.	D.
1 week, ...	0·24	0·04	0·02	0·07	0·01
2 weeks, ...	0·34	0·36	0·17	0·32	0·02
3 ,, ...	0·41	0·88	0·25	0·75	0·43
4 ,, ...	0·48	1·06	0·82	2·05	0·64
5 ,, ...	0·55	1·98	1·28	1·83	1·06
6 ,, ...	0·62	2·93	1·39	2·45	1·39
7 ,, ...	0·69	3·48	1·54	2·53	1·40
8 ,, ...	0·76	4·29	2·59	4·29	0·68
9 ,, ...	0·83	4·06	3·70	3·43	2·19
10 ,, ...	0·90	4·40	0·86	4·32	2·92

A similar series of experiments by H. Court showed even greater irregularity in the change of the solids.

It is undesirable to base the proof of the rate of change on the determination of the non-fatty solids, as such a course is open to the contention that the variations observed were due to imperfect and unequal extraction of the fat. But as it is not suggested that any change in the proportion of fat is occasioned by keeping the milk, it is clear that any diminution of the total solids must be caused by change in the non-fatty solids. Hence the determination of the total solids affords at once a measure of the change which has occurred in the milk, and enables the analyst to avoid any but the very simplest manipulation, namely, that involved in drying the total milk-solids to a constant weight. It is, however, not easy to do this, and considerable differences are apt to result in the determination of the total solids of decomposed milk, unless the details of the operation are strictly adhered to.

The value of the referees' time-allowance for change has also been systematically investigated by A. W. Stokes (*Analyst*, xii. 226), who has published the figures yielded by samples of milk

analysed by him when fresh, and again examined by the same method after keeping. The samples were milks received in the ordinary course, and were almost all adulterated. Hence they were of the character which usually form the subject of reference. All the determinations of Stokes were made in duplicate, and some in triplicate. In the case of the stale milk, the quantity taken for analysis (5 grammes) was carefully neutralised by deci-normal caustic soda before being evaporated; and in calculating the results, 0·0022 gramme for each c.c. of standard alkali used was subtracted from the weight of total solids obtained. The times the samples were kept varied from 8 to 117 days, and the season from July 6th to November 3rd, 1887. The following are Stokes' figures:—

Sample.	Decomposed Milk.		Days Kept.	Time-Allowance.	Corrected Solids.	Fresh Milk. Total Solids.	Error of Method.
	Lactic Acid.	Total Solids.					
1	0·52	6·92	8	·25	7·17	6·90	+0·27
2	0·52	11·26	14	·34	11·60	11·26	+0·34
2a	0·99	10·72	36	·56	11·28	11·26	+0·02
3	0·55	10·90	14	·34	11·24	10·92	+0·32
3a	1·78	10·40	36	·56	10·96	10·92	+0·04
4	0·61	10·02	18	·38	10·40	10·54	−0·16
4a	1·09	8·40	40	·60	9·00	10·54	−1·54
5	0·36	10·14	18	·38	10·52	11·13	−0·61
6	1·76	8·54	26	·46	9·00	10·23	−1·23
7	0·97	7·82	28	·48	8·30	10·30	−2·00
8	0·72	8·72	31	·51	9·23	9·72	−0·49
9	0·77	10·32	32	·52	10·84	10·67	+0·17
10	1·18	9·30	35	·55	9·85	10·96	−1·11
11	2·07	7·54	35	·55	8·09	9·63	−1·54
12	1·08	10·12	38	·58	10·70	10·45	+0·25
13	0·82	8·42	38	·58	9·00	9·09	−0·09
14	1·02	9·24	39	·59	9·83	9·93	−0·10
15	1·18	8·26	39	·59	8·85	9·02	−0·17
16	0·82	12·02	40	·60	12·62	12·76	−0·14
17	2·12	11·76	48	·68	12·44	12·02	+0·42
18	2·10	9·50	61	·81	10·31	9·74	+0·57
19	0·10	3·68	63	·83	4·51	8·12	−3·61
20	1·24	12·36	68	·88	13·24	12·74	+0·50
21	1·15	7·52	68	·88	8·40	8·40	0·00
22	0·52	6·22	68	·88	7·10	9·69	−2·59
23	1·35	8·80	69	·89	9·69	10·13	−0·54
24	2·01	7·24	70	·90	8·14	8·25	−0·11
25	2·34	9·40	117	1·37	8·77	10·21	+0·56
26	...	10·54	28	·48	11·02	10·79	+0·23

In the case of twelve samples the time-allowance is too great, in one case it is exactly correct, and in the remaining sixteen cases it is too small. In four cases only did the correction bring the solids within 0·10 per cent. of the truth, which is the limit of error according to Bell.

Sample No. 19, after being kept sixty-three days, developed an extremely disagreeable odour. That the mere time of keeping was

not the cause of this phenomenon is evident by comparing the figures with those given by Nos. 18 and 20, which were kept throughout about the same period and at the same temperature, and actually show a change less than the referees' allowance. In No. 19 the loss of solids, after making the time-allowance, was 3·61, and with this extraordinary deficiency was associated an acidity absolutely the lowest in the whole series.

Sample No. 22 resembled No. 19 in developing the same intolerable odour, accompanied by a very limited production of lactic acid. In the case of each of these samples the analyses were repeated three times with concordant results.

The substantial accuracy of Bell's time-allowance for change has been maintained by Mr R. Bannister, deputy-principal of the Somerset House laboratory, as recently as June 1894, in his evidence before the Committee on Food Products Adulteration; but it came to light in the course of his cross-examination that during the last few years the referees had replaced the old method by one in which an attempt was made to deduce the amount of non-fatty solids present in the fresh milk from the products of their decomposition. The process was described in outline only by Mr Bannister. The following detailed description is taken from a report to the Local Government Board [1]:—

Memorandum on the Analysis of Milk in the Government Laboratories.

The change which takes place in a sample of milk kept out of contact with air, as in a bottle nearly full of the sample, and fitted with a good sound cork sealed with wax, is, as a rule, comparatively slight. The causes and nature of this change have been carefully studied by many observers, and they have been found to be perfectly definite in character. Without going into details concerning the fermentative changes to which milk is liable, it may be stated that the changes which affect the analysis, and, therefore, the inference to be drawn from the results, are concerned with the non-fatty solids only, and more particularly with the milk-sugar. The milk-sugar gives rise, either proximately or remotely, to a variety of products, the most important of which are lactic acid, ethyl alcohol, and acetic acid, but it can be shown that the only quantitative determinations which need be made in order to determine the loss in the non-fatty matter by keeping, are the proportion of alcohol, reckoned as proof-spirit, and the amount of free volatile acid,

[1] The author is indebted to the courtesy of Dr T. E. Thorpe, principal of the Government Laboratories, for an advance-copy of this report, which at the time of writing has not been published. The method was actually applied by the referees to seventy-eight milk samples during the three years 1894, 1895, and 1896, the allowances for change ranging from 0·06 to 0·73 per cent.

together with the ammonia derived from the alteration of the casein, or proteid substance, in the milk. The slight alterations in weight consequent on the hydrolysis and conversion of lactose into lactic acid, and the formation of certain so-called bye-products of alcoholic fermentation, are partly positive and partly negative in direction, but their joint effect is too small to have any appreciable influence on the result.

The entire correction, which, of course, is always additive, in the case of a properly-preserved sample, from three to six weeks old, is fairly constant, and may be said to range from 0·2 to 0·3 per cent. In a few cases, it has been found to be as low as 0·1 per cent., and in very exceptional cases, as in badly-secured samples, or in bottles only partially filled, it has risen to 0·7 or 0·8 per cent.

If the fermentation has passed into the butyric acid stage, the amount of free acid is greatly increased, and, owing to the separated casein, it is sometimes impossible to get the sample into a proper and uniform condition for analysis. In such cases, we decline to proceed with the examination. Such a result, however, practically never happens in the case of samples which have been properly taken and kept by the inspectors pending the appeal to the Government Laboratory.

The analysis of the milk is conducted by what is known as the "maceration" process, and the weight of the non-fatty solids and fat is independently ascertained in duplicate, whilst, as a control, the direct determination of the total solids is made on a third portion of the milk. Before proceeding with the analysis, the total contents of the bottle are transferred to a suitable vessel, and thoroughly mixed with a wire whisk. From 10 to 12 grammes of the samples are weighed out into flat-bottomed platinum capsules, each of which has been tared along with a short glass rod with flattened ends. The weighed portions are next neutralised with decinormal strontia solution, with phenolphthalein as indicator.[1] Decinormal soda answers the purpose perfectly well when the degree of acidity is not greater than 10 c.c. $\frac{N}{10}$ solution for 10 grammes of milk, but above that amount it is of great advantage to use the strontia solution. The milk is then evaporated over the water-bath, until the residue attains the consistency of dry cheese, and, while so warm as to ensure that the fat shall still be in the melted condition, 20 c.c. of dehydrated ether (sp. gr. ·720) are poured over the milk-solids, which are then carefully stirred for some time with the glass rod. The ether containing the dissolved milk-fat is passed through

[1] Respecting the use of standard strontia solution at this point, Professor Thorpe writes to the author as follows :—"We prefer strontia for the reason that the precipitate settles better, as it is less flocculent than that given by baryta. The maceration method can thus be more quickly worked, and less of the precipitate is apt to get transferred to the tared filter. Moreover, of course, strontia has a lower molecular weight than baryta, and therefore the unavoidable error of measurement has less influence on the amount to be deducted as that of the alkaline earth added."

a Schleicher's filter of 10 cm. diameter which has previously been dried and weighed in a weighing-bottle. The maceration of the milk is continued with eight successive additions of 10 c.c. of ether, at which point it has been found that the fat has been wholly separated from the non-fatty solids. The filter-paper, having its edges cut down, is well washed with boiling ether, and the fractions of the filter-paper are replaced in the weighing-bottle, and dried at 100° C. The increase of weight, which with careful work should not exceed a few milligrammes, is added to the weight of the non-fatty solids. At the conclusion of the maceration process, the non-fatty solids should be in a fine state of division, resembling the precipitated chalk of pharmacy. The capsules are next dried over-night in the water-oven, and the first weighing is taken in the morning. Usually, the weight is constant by this time, as shown by the re-weighings taken during the day. As, from the conditions of the analysis, the anhydrous salts of sodium or strontium are present in the dried solids, the correction for the added alkali is in the proportion of 0·0022 gramme per 1 c.c. of $\frac{N}{10}$ soda used, and 0·0042 gramme for each c.c. of the strontia solution required. The ether solution of the milk-fat, contained in tared beakers, is evaporated, and the weight of the dried fat ascertained.

In determining the nature and amount of the loss in non-fatty solids consequent on keeping, the alcohol present is by far the most important item. When the milk-sample under analysis measures 170 c.c., that is, nearly 6 oz. (and it should not be much less than this), 75 grammes of the milk can be spared for distillation, which is carried out in a glass still with glass spiral condenser.

The acidity of the milk having been previously ascertained, the portion taken for distillation is neutralised with soda up to one-half of the total acid present. If nearly neutralised, ammonia may pass into the distillate, and so vitiate the result. The first distillate, which usually contains a little acid, is redistilled with the addition of 0·5 c.c. $\frac{N}{10}$ NaOH, and, after having been made up to the original bulk, the specific gravity at 15·5° C. (60° F.) is taken in a 50 gramme, or in a 1000 grain, weighing-bottle. Supposing that the distillate gave a specific gravity of 999·67, or 0·33 less than 1000, then this difference, multiplied by 1·16,[1] gives the amount of proof-spirit, by volume, present in the milk. The product, multiplied by ·842, equals the actual weight of anhydrous milk-sugar which has been converted into alcohol.

$$(1000 - 999{\cdot}67) \times 1{\cdot}16 \times {\cdot}842 = {\cdot}322 \text{ by weight of milk-sugar lost.}$$

The correction for free volatile acid, reckoned as acetic acid, is ascertained as follows :—

Ten grammes of the milk are neutralised to the extent of one-

[1] This factor has been deduced from Gilpin's tables, as applicable to such small quantities of alcohol as are found in sour milk.

half the total acidity with $\frac{N}{10}$ NaOH, and a little phenol-phthalein added. The mixture is then evaporated to dryness on a water-bath with frequent stirring, and after treatment with 20 c.c. of boiling distilled water, so as to break up and thoroughly detach the milk solids from the capsule, a further addition of $\frac{N}{10}$ NaOH is made, until the neutral point is reached. The difference between the original acidity of the milk and that of the evaporated portion is regarded as acetic acid. The number of c.c. of soda shown, when multiplied by ·0255, or the percentage of acetic acid multiplied by ·425, gives the percentage of loss by weight not recovered in the change of milk-sugar into alcohol, and thence into acetic acid.

Example :—

Acidity of original milk, . .	= 11·6 c.c.	$\frac{N}{10}$ NaOH.
Acidity of evaporated portion, .	= 9·2	„
Difference, . .	2·4	„

$2{\cdot}4 \times {\cdot}0255 = {\cdot}061$ per cent. by weight lost ;

or,

$2{\cdot}4 \times {\cdot}006 \times 10 \times {\cdot}425 = {\cdot}061$ per cent. by weight lost.

The loss arising from the change in the casein is very small. To estimate it, 2 grammes of the milk are made up to 100 c.c. with distilled water, and filtered to a clear solution. Ten c.c. of the filtrate, increased to 50 c.c. by the addition of distilled water, are nesslerised against NH_4Cl solution, equivalent to ·01 milligramme of NH_3 in each c.c. As the Nessler colour produced in presence of milk differs somewhat from that of pure saline ammonia, the blank experiment is carried out with the addition of 10 c.c. of the filtrate from 2 grammes of new milk slightly acidified, and diluted to the same extent as the sour milk. The quantity of test ammonia required varies from 0·5 to 4·0 c.c. In the case of a milk containing ammonia equal to 2·6 c.c. of the test solution, the loss of weight is calculated as follows :—

$$0{\cdot}01 \times 2{\cdot}6 \times 500 \times 5{\cdot}2 = {\cdot}067 \text{ per cent. casein ; or,}$$

$$\frac{2{\cdot}6 \times {\cdot}052}{2} = {\cdot}067 \text{ per cent. casein.}$$

It is evident that any other degree of dilution may be conveniently adopted according to circumstances, or the proportion of ammonia which may be indicated in the milk.

According to the three instances given, the loss of solids, or, in other words, the addition to be made to the non-fatty solids, is as follows :—

Loss from alcohol, . .	= ·322 per cent.	= ·45 per cent.
„ acetic acid, .	= ·061 „	
„ ammonia, .	= ·067 „	

The following table gives the results of analysis of a series of milks analysed in the fresh state, and after a lapse of several weeks. The examination was carried beyond the point of time at which a milk can be referred, under the Amending Act of the Food and Drugs Act, 1875 ; but it will be seen that the changes can be accurately followed and allowed for, and the comparison of the corrected non-fatty solids with the solids of the fresh milk shows that the variations are not greater than may occur in duplicate determinations of the constituents of a fresh milk.

Fresh Milk.		Description of Sample.	Kept Milk.			Total Loss.	
Non-Fatty Solids.	Fat.		Time in Days.	Non-Fatty Solids.	Fat.	Calculated.	Actual.
8·97	4·08	Whole Milks.	21	8·71	4·02	·20	·26
8·97	4·08		27	8·74	3·97	·21	·23
8·97	4·08		50	8·61	4·00	·34	·36
8·97	4·08		85	8·49	4·09	·49	·48
8·99	3·17		27	8·60	3·16	·35	·39
9·12	4·16		42	8·82	4·18	·33	·30
9·12	4·16		102	8·88	4·18	·25	·24
9·02	4·72		41	8·56	4·65	·58	·46
9·02	3·28		52	8·76	3·25	·20	·26
9·02	3·28		79	8·53	3·34	·49	·49
9·02	3·28		93	8·35	3·27	·61	·67
9·13	4·49		57	8·88	4·35	·23	·25
9·52	4·06		74	9·25	3·92	·35	·27
9·27	4·07		77	8·91	4·06	·32	·36
9·11	4·19		91	8·24	4·14	·76	·87
9·34	4·07		91	9·01	4·01	·41	·33
8·42	3·70		91	8·14	3·69	·26	·28
8·83	5·32		91	8·56	5·20	·31	·27
9·20	3·98		91	8·80	3·83	·39	·40
8·14	3·44	Milks to which approximately 10 per cent. of water had been added.	14	7·96	3·41	·27	·18
8·14	3·44		30	7·81	3·40	·42	·33
8·24	2·79		38	7·88	2·78	·33	·36
8·24	2·79		38	7·73	2·76	·50	·51
9·66	·14	Separated Milks.	27	9·25	Not determined.	·22	·41
9·66	·14		36	9·34		·13	·32
9·66	·14		65	9·35		·26	·31
8·91	·09		28	8·62		·28	·29
8·91	·09		37	8·60		·20	·31
8·61	·13		30	8·33		·24	·28
8·61	·13		37	8·29		·28	·32
8·61	·13		91	7·93		·58	·68
8·18	...	Separated milks to which approximately 10 per cent. of water was added.	21	7·86	Not determined.	·24	·32
8·18	...		387	6·71		1·50	1·47
8·18	...		388	6·64		1·56	1·54
8·18	...		401	6·71		1·50	1·47
7·67	...		22	7·22		·35	·45
7·67	...		387	6·24		1·49	1·43
7·76	...		73	7·11		·55	·65
7·80	...		185	6·22		1·59	1·58
7·80	...		188	6·19		1·69	1·61

There has hitherto been no opportunity for the experimental examination of the foregoing process outside the Government

Laboratories. Some points strongly invite criticism, but the method must be welcomed as an ingenious attempt to effect a satisfactory solution of a very difficult problem. A very damaging observation respecting the process has been made by Mr R. Bannister, who, in his evidence before the Committee on Food Products Adulteration in 1894, stated that the method gave results practically the same (replies 1688 and 1689) as the discredited time-allowance which it had superseded.

MILK PRODUCTS.

The products obtained by treating milk in various ways are only second in importance to milk itself. In the following sections the chemistry of the chief of these are considered, including cream and skim milk, butter-milk, condensed milk, koumiss and kephir, cheese, and milk-sugar. Butter was considered fully in Vol. II.

Cream and Skim Milk.

Cream is the thick layer of fat-corpuscles which is formed when milk is allowed to stand, or is otherwise treated with a view of separating the finely-divided suspended matter. Though generally yellowish, the cream produced in winter when the cows are stall-fed is often quite white.

Skim Milk is the lower layer, comparatively poor in fat, which remains when the cream is removed by skimming or similar means. It may be regarded as essentially new milk deprived of the greater part of its fat.[1]

Separated Milk is the secondary product obtained when the cream is removed from milk by a centrifugal apparatus, instead of by hand-skimming after standing in shallow pans, in the manner always practised till recently.[2] By the centrifugal separator a very

[1] If the change which takes place during the rising of the cream consists wholly in the ascent of fat-globules, the relation between the amounts of water and of non-fatty solids will be the same in the cream and in the skimmed milk as it was in the original whole milk. Thus the lower layers may be regarded as an aqueous solution of lactose, casein, &c., containing but little fat in suspension, while the upper layers consist of a precisely similar solution, containing a larger amount of suspended fat. Recent experiments by Leonard and Smith (*Analyst*, 1896, p. 283) have fully established the truth of this proposition.

[2] Some analysts have attempted to draw a sharp distinction between skimmed milk and separated milk, on the ground that the latter product commonly contains a smaller proportion of residual fat than the hand-

perfect separation of the cream can be effected with great rapidity. This is shown by the following analyses by A. Smetham (*Analyst*, 1881, p. 220) of the products from two forms of apparatus, working with the same milk. The results are interesting for comparison, but the machines now in use effect a much more perfect separation than is shown by Smetham's figures.

	By "Laval" Machine running at 29½ gallons per hour.		By "Danish" Machine running at 43½ gallons per hour.	
	Cream.	Separated Milk.	Cream.	Separated Milk.
Fat, . . .	33·44	·29	42·68	·11
Proteids and sugar,	4·56	7·22	4·42	7·32
Ash, . . .	·54	·77	·58	·75
Water, . .	61·46	91·72	52·32	91·36
	100 00	100·00	100·00	100·00

The following analyses by W. Fleischmann are interesting as indicating the composition of cream and skim milk as compared with the original milk.

	New Milk.	Skim Milk.	Cream.
Milk-fat, ...	3·64	·46	67·63
Casein, ...	2·73	2·88	1·17
Albumin, ...	·68	·49	·25
Milk-sugar, ...	4·69	5·34	2·25
Ash, ...	·71	·71	·12
Water, ...	87·55	90·11	28·58
	100·00	100·00	100·00
Total solids, ...	12·45	9·89	71·42
Non-fatty solids, . . .	8·81	9·43	3·79

skimmed milk. The distinction should be borne in mind, but the two products are merely varieties of the same article produced by different processes.

On the other hand, a fine was properly inflicted at Kensington Petty Sessions, in March 1897, for the sale of separated milk under the misleading description of "Pasteurised rich skimmed milk, not deprived of all cream."

The following results were obtained by H. D. Richmond by a similar procedure.

	Milk.	Skim Milk.	Cream.
Specific gravity, ...	1·0308	1·0349	...
Total solids, ...	12·75	9·27	34·61
Fat, ...	4·10	0·28	28·09
Ash, ...	0·71	0·78	0·58
Non-fatty solids, ...	8·65	8·99	6·52
Ratio of non-fatty solids to 100 of water, .	9·91	9·91	9·97
Ratio of ash to 100 of water, . . .	·81	·86	·89

It will be seen, therefore, that by the process of skimming, the percentage of non-fatty solids is rather raised than lowered. By some observers it is contended that the proteids are in part thrown up with the fat, while the sugar mostly remains in the lower layer; but there is strong evidence against this view. The ash of the cream is lower than that of the milk. According to Richmond, the ash of cream has the same composition as that of milk, and is in the same proportion to the water present.

The composition of skim milk, as affected by the various processes for its production, is shown by the following results of Vieth (*Analyst*, ix. 63).

No.	Specific Gravity.	Total Solids.	Fat.	Solids not Fat.	Remarks on System.
1	1·0350	9·75	0·55	9·20	Shallow pans.
2	1·0355	9·90	0·54	9·36	
3	1·0340	10·10	1·00	9·10	
4	1·0355	10·43	0·98	9·45	
5	1·0335	9·68	1·05	8·63	Deep pans.
6	1·0345	9·70	0·60	9·10	
7	1·0355	9·81	0·43	9·38	
8	1·0350	10·26	0·88	9·38	
9	1·0365	9·96	0·46	9·50	Centrifugal system.
10	1·0350	9·28	0·34	8·94	
11	1·0370	9·94	0·34	9·60	
12	1·0370	9·80	0·35	9·45	

The foregoing results do not fairly represent the working of centrifugal separators of more recent construction. The present practice is shown by the following table, in which are incorporated data published by Vieth in 1895 (*Bericht w. Thätigkeit des Milchwirthschaftlichen Institut in Hameln*, 1895, p. 9) and results obtained by the Aylesbury Dairy Company in 1896, communicated to the author by H. D. Richmond. The latter results were obtained with separators arranged for running thick cream, whereas Vieth's data relate to thin cream.

	Separator.	Revolutions per Minute.	Capacity per Hour, in Gallons.	Yield of Cream per cent.	Percentage of Fat in Separated Milk.			
					No. of Samples.	Min.	Max.	Average.
Running thin cream.	Danish (Burmeister & Wain's AA.), . .	2700	247	12·18	274	0·15	0·50	0·24
	Alpha I., . . .	5600	257	12·97	343	0·05	0·25	0·10
	Balance, . . .	7000	169	13·51	267	0·05	0·30	0·15
Running thick cream.	Danish (Burmeister & Wain's B. modified),	4000	80	6·0	118	0·08	0·30	0·15
	Alexandra I., . .	6000	180	6·0	233	0·05	0·30	0·14
	Alpha I., . . .	5600	250	6·0	67	0·08	0·34	0·19
	Standard Russian, .	7800	120	6·0	12	0·15	0·38	0·25

The Balance and Alexandra separators are very similar, and it will be seen that the results are nearly the same. The Alpha machine shows a higher result with thick than with thin cream; but in a few experiments by Richmond with thin cream the separated milk contained on the average 0·10 per cent. of fat, which agrees with Vieth's figure.

L. Chevron (*Le Materiel de Laiterie*, 1889, p. 32) states that the fat contained in the separated milk from the Meloble hand-machine ranges from 0·17 to 0·23, with an average of 0·19 per cent.

The average composition of separated milk produced by the Danish separator A. is stated by Richmond to be:—Fat, 0·25 per cent.; proteids, 3·32; sugar, 4·92; ash, 0·72; and water, 90·74 per cent.[1]

[1] Richmond has published (*Analyst*, 1894, page 86) an analysis of the slime which accumulates in the drums of centrifugal separators. The sample examined contained:—Total solids, 33·76 per cent.; fat, 0·50; milk-sugar, about 0·50; casein, or analogous body soluble in dilute alkalies and precipi-

The change in the composition of milk which may naturally occur owing to the spontaneous rising of the cream has been investigated by J. Carter Bell and J. W. Gatehouse (*Analyst*, 1879, pp. 117, 163). The former chemist requested the Sanitary Inspector to travel with a milk cart, and to take a sample at the beginning of the journey, another at the middle, and a third at the end, when the milk had been nearly all withdrawn from the can. The following were the analytical results :—

	Fat.	Solids not fat.	Ash.	Total Solids.
5.30 p.m., commencement of journey, .	3·55	8·94	·73	12·49
7 p.m., middle of journey, . . .	3·54	9·02	·73	12·56
8·30 p.m., last in can, . . .	3·59	8·98	·70	12·57

J. W. Gatehouse employed an ordinary milk-can, as used for selling milk in the streets. Of eight quarts of milk placed in the can, five were removed during a period of three hours, by dipping at intervals of a few minutes *from the top of the milk*, so as to disturb the cream as little as possible.

The following results were obtained on analysis :—

	Cream.	Fat.	Solids not Fat.	Total Solids.
Original milk at 8.15, . . .	13	3·20	9·40	12·60
Sample taken by dipping at 10.45, .	15	3·40	9·30	12·70
Milk remaining in can at 12.15, . .	9	2·80	9·65	12·45

On the other hand, the milk in a tall tin (about 4 feet high) was found to suffer such change from a rising of the cream as to be greatly altered in richness.

The results of similar experiments have been published by P. Vieth (*Analyst*, viii. 2).

Several patents have been taken out for preventing the rising of cream in delivery-churns.

It is an interesting fact that the first portion of any milking, commonly known as "fore-milk," is less rich in fat than the last portions or "strippings,"[1] which are not unfrequently kept separate

tated by acid, 22·00 ; and ash (of much the same composition as milk-ash, and absolutely neutral to litmus and phenol-phthalein), 3·01 per cent.

[1] This difference has been attributed to a partial separation of the cream in the udder of the cow, but the anatomical structure of that organ renders such

and sold as "cream." The following figures by S. Macadam show the average difference in the composition between the first and last halves of the same milking.

	Sp. Gravity.	Cream.	Fat.	Solids not Fat.	Ash.
First runnings, . .	1033·5	5·4	1·77	9·20	·67
Last runnings, . .	1030·0	11·8	4·06	9·20	·67

Boussingault has published the following figures, yielded by six consecutive fractions into which the product of a single complete milking was divided :—

	1.	2.	3.	4.	5.	6.
Total solids, ...	10·47	10·75	10·85	11·23	11·63	12·67
Fat, ...	1·70	1·76	2·10	2·54	3·14	4·08
Non-fatty solids, ...	8·77	8·99	8·75	8·69	8·49	8·59

It will be observed that the fat increased regularly from the beginning to the end of the milk ; while, on the other hand, the non-fatty solids remained practically constant throughout.

The proportion of cream yielded by milk was formerly ascertained by allowing a known measure of the sample to stand in a wide cylinder graduated at the upper part, and noting the volume which separated after twenty-four hours or other definite time. As a rough guide to the richness of the milk in cream or fat, the observation was serviceable, but the indications were materially affected by the temperature and length of time allowed, the proportion of non-fatty solids, the shape and diameter of the vessel, and other more obscure conditions.

The author found from the examination of several hundreds of samples of milk, in which the cream and fat were determined with great care and under constant conditions, that, although a relation between the volume of cream and the proportion of fat could be traced in many cases, the exceptions were too numerous and obscure in origin to allow of any rule being safely formulated. In the case of diluted and abnormal milk the dis-

an explanation highly improbable. It is more likely due to the greater freedom with which the fat can flow through the secreting vessels and ducts of the udder when the pressure at first existing is removed during the process of milking.

crepancies were still greater.[1] (Compare Allard, abst. *Analyst*, 1892, p. 238.)

Richmond and Boseley have recently called attention to the fact that the cream rises on diluted condensed milk far more slowly than from fresh milk,[2] and have even based on the difference a method of distinguishing between the two (page 191).

Various attempts have been made to deduce the proportion of fat in milk from an observation of its opacity, but none of the contrivances devised for the purpose give uniformly reliable results, probably on account of the variation in the size of the fat-globules in different specimens of milk.

The volume of cream thrown up being no precise indication of the proportion of fat existing in a milk, it follows that the percentage of fat in cream varies between somewhat wide limits. In thirty-six analyses of cream collected by König, the fat ranged from 8·17 to 70·2 per cent. with an average of 25·72, but in the great majority of cases the proportion was between 12 and 60 per cent. The great majority of the samples of cream represented in these analyses were hand-skimmed.

The composition of the cream produced by mechanical separators also varies between wide limits, but is wholly dependent on the adjustment of the apparatus, and therefore under complete control. Hence the average composition of separator-cream has no scientific importance, though it is of interest as an indication of what may be expected in practice. The cream separated by the Aylesbury Dairy Company is now made of a thickness to suit the London taste, and contains nearly 50 per cent. of fat; but in some parts of the

[1] James Bell (*Food and its Adulterations*, Part ii.) has published analyses of various samples of milk believed to be genuine. The proportion of cream, as compared with the fat recorded by him, varies in the most erratic manner; but the evidence therefrom is vitiated by the demonstrably inaccurate determinations of the fat. Still the following figures yielded by ten samples of milk containing a normal proportion of total solids may be quoted.

Specific Gravity.	Total Solids.	Fat.	Cream.
1031·21	12·92	3·76	10
1033·70	12·74	3·45	5
1032·61	12·63	3·55	10
1031·60	12·28	3·70	6½
1032·00	12·41	3·32	8
1029·90	13·02	3·99	5
1031·29	13·04	4·10	5½
1030·00	12·75	3·86	8
1030·50	12·34	3·60	7
1030·30	12·39	3·75	5½

[2] Cazeneuve and Haddon (*Bul. Soc. Chim.*, 1895, [3], xiii. 500) appear to have been the first to call attention to this anomaly.

country a thinner cream is preferred, containing about 35 per cent. of fat. Cream should contain at least 30 per cent. of fat.

The following table shows the composition of the separated cream produced by the Aylesbury Dairy Company during the years 1883 to 1895[1]:—

Year.	Total Solids, per cent.			Fat, per cent.			Solids not Fat, per cent.		
	Average.	Highest.	Lowest.	Average.	Highest.	Lowest.	Average.	Highest.	Lowest.
1883	42·3	47·4	38·9	35·5	41·1	31·8	6·8	7·1	6·3
1884	42·1	45·4	39·6	35·3	39·0	32·6	6·8	7·0	6·4
1885	...	...	...	42·5	51·1	35·9	...	...	...
1886	...	...	...	44·25	46·0	41·5	...	...	...
1887	...	...	...	43·2	46·1	40·6	...	...	...
1888	...	...	...	45·55	48·0	43·5	...	...	...
1889	...	...	...	47·35	49·9	45·5	...	...	...
1890	...	...	...	48·35	50·5	45·3	...	...	...
1891	...	...	...	49·05	51·9	45·7	...	...	...
1892	...	...	...	46·85	49·5	43·9	...	...	...
1893	...	...	...	47·7	50·9	45·0	...	...	...
1894	...	...	...	49·0	51·2	46·5	...	...	...
1895	...	...	...	49·1	50·6	47·4	...	...	...

An unusually thick cream, which was made by H. D. Richmond for experimental purposes (*Analyst*, xxi. 88),[2] gave the following results on analysis:—Total solids, 68·18; fat, 64·88; ash, 0·28; and solids not fat, 3·30 per cent.

In hot weather, the cream sold in London is frequently thickened by means of gelatin, the presence of which may be detected as described on page 173. Preservatives are very frequently present in cream, a favourite preparation being a mixture of boric acid and borax, sweetened with saccharine.

Clotted Cream, an article largely prepared in Devonshire, is produced by heating milk gently, during many hours, in large pans. This treatment causes the fat to coalesce and separate more rapidly than in the ordinary method. Such cream will keep perfectly sweet for many days, even in warm weather, if the superficial layer be preserved intact; but if this be disturbed, and especially if the surface-layer be mixed with the rest of the cream, lactic fermentation rapidly occurs.

[1] The analyses from 1883 to 1890 (inclusive) are by P. Vieth; those from 1891 to 1895 by H. D. Richmond.

[2] This cream was analysed by Richmond to see whether the view put forward in a former paper (*Analyst*, xix. 73), that the ratio of solids not fat to water was the same in cream as in milk, and therefore that the fat did not carry any proteids, was borne out in cream of great thickness. If the fat did carry proteids, the ratio of solids not fat to water should be distinctly higher in very rich cream than in milk. It was, however, found that the ratio was identical with that in the milk used for its preparation.

In a sample of Devonshire clotted cream, A. Wynter Blyth found :—Fat, 65·01 ; proteids, 4·09 ; sugar, 1·72 ; ash, 0·49 ; and water, 28·67 per cent. The ash contained 0·373 of calcium phosphate, and only 0·013 per cent. of chlorine (*Analyst*, iv. 141). These results show that a notable quantity of proteids was thrown up with the fat.

The results of Vieth and Richmond, for an aggregate number of 463 samples of clotted cream analysed during the series of years 1886 to 1895 inclusive, show the following range of composition :—

	Water.	Total Solids.	Fat.	Solids not Fat.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Maximum, . .	45·57	74·84	68·59	11·70	1·17
Minimum, . .	25·16	54·43	45·78	5·26	·42
Average, . . .	34·56	65·44	58·05	7·39	·58

The average figures found by Richmond for clotted cream produced in 1896 were :—Total solids, 67·64 ; fat, 59·16 ; solids not fat, 8·48 ; and ash, 0·68 per cent.

A. Wynter Blyth has met with an artificial clotted cream made of albumin and ordinary cream, and slightly coloured with what was probably carrot-yellow.

Butter-Milk.

Butter-milk is the secondary product of the manufacture of butter by churning milk or cream. Sour milk and either fresh or sour cream are the proper materials for making butter. Attempts to churn sweet milk have given very unsatisfactory results, about one-half of the total fat being left in the butter-milk. When the churning is conducted under proper conditions, the proportion of fat remaining in the butter-milk is generally above 0·5 per cent., but does not exceed 1·0 per cent., unless the operation of churning has been unskilfully performed. Differences in the materials employed influence the composition of the butter-milk to some extent, but other conditions have a much greater effect. Salt is often added to cream in the warm season to preserve it, and this salt appears finally in the butter-milk. When the butter is collected, it is washed with cold water. This water is generally mixed with the butter-milk, thus causing dilution.[1] Vieth (*Anal.*,

[1] In cases heard in Scotland, it has been held that 25 per cent. of extraneous water in butter-milk is not an excessive or fraudulent proportion.

ix. 63) publishes the following analyses of butter-milk as illustrating the above points :—

No.	Total Solids.	Fat.	Non-fatty Solids.	Ash.
	Per cent.	Per cent.	Per cent.	Per cent.
1, . . .	9·77	1·09	8·68	0·69
2, . . .	9·03	0·63	8·40	0·70
3, . . .	10·39	0·78	9·61	..
4, . . .	8·02	0·65	7·37	1·29
5, . . .	9·64	2·51	7·13	0·64
6, . . .	8·13	0·82	7·31	0·64
7, . . .	10·14	0·92	9·22	0·73
8, . . .	8·91	0·50	8·41	0·71
9, . . .	8·98	0·49	8·49	1·32
10, . . .	10·70	0·54	10·16	0·82
11, . . .	9·80	0·76	9·04	0·73
12, . . .	9·72	0·80	8·92	0·73

Condensed Milk.

Commercial condensed milk is prepared by concentrating milk by evaporation. The operation is conducted under diminished pressure. A very sensible decomposition of the proteids and probably of the fat occurs during the operation.

Three distinct varieties of condensed milk are manufactured. Those of Class I. receive no addition, or only a small quantity of preservative, such as salicylic acid or boric acid. The condensed milks of Class II. are treated during or at the completion of the evaporation with a high quality of cane-sugar, the proportion employed being from 4 to 5 lbs. for every gallon of the fully concentrated milk. Starch-sugar and glycerin have also been employed, but the milks thus treated do not appear to be now found in commerce.[1] The milks of Class III. receive special

[1] With the object of preparing a superior kind of condensed milk, Maclaren and Fleming (*Eng. Patent*, 1896, No. 2081) propose to treat 40 gallons of separated milk with 28 lbs. of sugar, and condense the liquid to about 100 lbs. They then add an emulsive mixture consisting of 12 lbs. of refined beef oil (oleo oil), 8 lbs. of lime-water, 1 lb. of corn flour, and 12 lbs. of sugar. The emulsifying is preferably conducted in a steam-jacketed pan provided with an agitator, the lime-water being gradually added during the operation. The "Tiger-nut" is alleged to be largely imported and extensively used in the preparation of some of the inferior kinds of condensed milk.

treatment with a view of giving them a composition approximating to that of woman's milk. The so-called "humanised milk" belongs to this class.

Unsweetened Condensed Milk.—The facility with which the decomposition of unsweetened condensed milk occurs, depends to a great extent on the care and cleanliness with which the various operations connected with the concentration of the milk are conducted. In the manufacture of one of the best known brands of unsweetened condensed milk, milk of a density of not less than 1032 is exclusively used, with a view of avoiding any chance of the introduction of germs or bacteria by dilution with water. After being received, the milk is never handled or touched, and the whole of the operations are conducted in pans thoroughly scoured with sand and hot water and afterwards submitted to the action of high-pressure steam. The only antiseptic agent used is borax, in a proportion not exceeding three parts per million of the unconcentrated milk. The keeping properties of the product are mainly dependent on the extreme cleanliness observed in its manufacture, the heating of the milk to a very considerable temperature after concentration, and the careful packing and soldering in air-tight cans. The product has a density of 1·106 at 26° C., at which temperature it is enclosed in bottles or tins. Three gallons of the original milk produce one gallon of the condensed milk.

Scherff's method of preparing unsweetened condensed milk is employed at Stendorff. The milk is first sterilised by heating it to 100°–113° C. for an hour or two under a pressure of from two to four atmospheres. It is then evaporated to one-half or one-third of its bulk in a vacuum, at a temperature of 65° to 70° C.

According to Nägeli, when the milk has not been heated in closed vessels to a sufficiently high temperature, or for a sufficient length of time, changes gradually take place whereby it acquires an intensely bitter taste, and the casein becomes peptonised. He attributes these changes to fungi which were much attenuated but not actually killed by the heat to which the milk was subjected. According to Biedert, milk can only be permanently preserved by heating to 100° C. for two hours, with exclusion of air, boiling being necessary to destroy any germs which may exist in the milk.[1] These statements apply to milk condensed without the addition of sugar. Sweetened condensed milk may be preserved without change for an indefinite period.

[1] Such prolonged heating to a high temperature is liable to cause clotting and caramelisation. Unsweetened milk may be permanently sterilised by heating it to 80° C for half an hour on three successive days.

The following analyses illustrate the composition of the leading brands of *unsweetened condensed milk* :—

Brand.	Description.	Total Solids.	Fat.	Proteids.	Milk-Sugar.	Ash.	Analyst.
American, .	Mean of 10, .	51·61	15·67	17·81	15·40	2·53	S. Percy.
First Swiss,	,, ,, .	39·41	10·87	11·29	14·26	2·36	R. Fresenius.
,, ,,	,, ,, .	36·10	11·06	12·75	...	...	A. H. Allen.[1]
,, ,,	,, ,, .	36·7	10·5	9·7	14·2	2·1	Pearmain & Moor.[2]
,, ,,	,, ,, .	36·53	12·22	10·30	13·94	2·07	Richmond & Boseley.
,, ,,	,, ,, .	37·03	10·67	9·56	14·55	2·25	H. Faber.
Highland, .	"Evaporated cream," .	31·60	5·90	...	...	..	C. P. Worcester.
Hollandia, .	Best quality, .	43·0	9·8	11·3	18·5	2·5	Pearmain & Moor.
Italian, .	Sold to trade for diluting, .	44·6	9·5	14·7	16·5	3·5	Pearmain & Moor.
St Charles, .	"Evaporated cream," .	31·09	4·35	...	...	...	C. P. Worcester.
Viking, .	Full cream, .	35·1	10·4	9·1	13·8	1·8	A. H. Allen.
,, .	,, ,, .	37·4	11·9	9·6	14·4	2·1	A. H. Allen.

The Italian sample analysed by Pearmain and Moor was a special preparation sold to milk-vendors for diluting, when short of new milk. It contained an unusually high proportion of boric acid.

Sweetened Condensed Milk.—In the manufacture of sweetened condensed milk a high quality of cane-sugar is added to the milk before concentration, and the liquid is then evaporated to the required extent. The concentration now usually practised is such that one part by weight of finished product is obtained from three parts by weight of the original milk. The amount of sugar added is about $1\frac{1}{4}$ lb. to the gallon of milk. Assuming a milk of 1031 specific gravity and containing 12·5 per cent. of solids, the condensed milk would contain 37·2 per cent. of milk-solids, 36·7 of added sugar, and 26·2 per cent. of water.

The following table, rearranged from data published in the *Analyst* for December 1895, shows the composition of a large number of samples of sweetened condensed milk. The analyses by the author were made during the summer of 1894, and are a fairly complete collection of the brands of sweetened condensed milk then in the English market. Some of the brands had dis-

[1] *Analyst*, 1895, page 274.

[2] *Analyst*, 1895, page 268.

appeared from commerce by the latter part of 1895, when the analyses of Pearmain and Moor were made, while a large number of new labels had made their appearance. It will be observed that at this latter date the partially skimmed milks which figure so largely among the author's samples had nearly disappeared from commerce, while the character of some of the surviving brands had undergone material alteration.[1] Another practice, which it is well to bear in mind, is that if, through proceedings in court or other circumstances, a particular brand of condensed milk becomes discredited, certain manufacturers simply rechristen the product, and reissue the old tins with new labels as some other brand.

Composition of Sweetened Condensed Milks.

Brand.	Description on Label.	Total Solids.	Fat.	Proteids.	Milk-sugar.	Ash.	Cane-sugar (by Difference).	Analyst.
Alderney, . .	Guaranteed to contain 60 per cent. of original cream, . .	68·10	11·05	10·95	...	...	...	A. H. A.
Anglo-Swiss, .	Best unskimmed country milk, .	74·4	10·8	8·8	16·0	1·7	37·1	P. & M.
,, .	,, ,,	73·70	9·70	9·87	...	...	...	A. H. A.
Arcadia, .	From best and purest cows' milk,	71·20	8·08	10·25	...	...	...	,,
Beehive, . .	From skimmed milk, . . .	77·7	0·2	...	...	2·6	...	P. & M.
Calf, . . .	,, ,, . . .	58·0	1·0	7·5	16·0	1·6	31·9	,,
,, . . .	Contains skimmed milk, . .	66·30	...	10·19	...	...	...	A. H. A.
Cleeves, . .	From unskimmed milk, . .	71·0	10·8	10·1	17·1	1·7	31·3	P. & M.
Clover-leaf, .	Guaranteed to contain all its original cream, ...	76·0	10·7	8·8	13·6	2·0	40·7	,,
Cow, . . .	From partly-skimmed milk, .	74·9	2·0	11·5	13·0	2·6	45·8	,,
Cowslip, . .	Skimmed; guaranteed to be entirely pure, ...	70·9	1·4	11·4	14·6	1·6	41·9	,,
,, . .	,, ,,	72·10	0·18	10·20	...	...	...	A. H. A.
Cross, . .	From skimmed milk, . . .	75·0	1·2	10·5	16·0	2·6	44·7	P. & M.
Cup, . . .	,, ,, . . .	56·9	1·0	8·5	15·4	1·6	30·4	,,
Daily, . .	,, ,, . . .	68·8	1·3	10·2	13·7	2·5	41·1	,,
,, . .	,, ,, . . .	69·64	0·26	10·58	...	...	...	A. H. A.
Daisy, . .	,, ,, . . .	64·0	0·5	...	...	2·2	...	P. & M.
Darby & Joan, .	Contains nothing but full-cream milk, ...	78·1	9·8	13·3	13·0	2·3	34·7	,,
Devon, . .	From skimmed milk, . . .	70·60	8·50	10·63	...	...	...	A. H. A.
Drummer-boy, .	,, ,, . . .	...	1·0	9·6	...	...	...	P. & M.
Farm, . .	,, ,, . . .	66·60	0·12	10·14	...	...	...	A. H. A.
Farmhouse, .	,, ,, . . .	77·0	0·4	...	...	1·5	...	P. & M.
Favourite, .	,, ,, . . .	70·6	0·3	10·0	...	2·1	...	,,
Fern, . .	Quite genuine, ...	67·7	10·7	10·6	15·0	1·4	30·0	,,

[1] C. P. Worcester, in a report on the condensed milks sold in the State of Massachusetts during the years 1892, 1893, and 1894, gives analyses showing that the Leader, Pine Tree, Interstate, Winner's, Watch, and Star brands contained less than 5 per cent. of fat; the Newport, Superior, Atlantic and Pacific Tea Company, Sovereign, Winner, Fisherman's, Baby, Newport, Full Weight, Highland, Gold Medal, and Red Cross brands, from 5 to 8 per cent. of fat; while only in the Puritan, Twitchel Champlin Company, Dime, and one sample of the J. B. Smith brand, did the fat exceed 10 per cent.

Composition of Sweetened Condensed Milks—(continued).

Brand.	Description on Label.	Total Solids.	Fat.	Proteids.	Milk-sugar.	Ash.	Cane-sugar (by Difference).	Analyst.
Fourpenny,	From unskimmed milk,	76·5	10·4	9·8	13·0	2·0	41·3	P. & M.
,,	From pure fresh milk containing all its cream,	75·36	5·40	13·18	...	...	...	A. H. A.
Full weight,	From unskimmed milk,	76·5	11·0	12·3	13·5	2·5	37·2	P. & M.
,,	Warranted not skimmed,	75·70	11·60	11·27	...	...	...	A. H. A.
Geranium,	Guaranteed perfectly pure,	75·0	9·8	7·5	13·0	1·6	43·1	P. & M.
,,	Full cream,	73·98	10·35	8·56	...	...	...	A. H. A.
Go-ahead,	Guaranteed pure; no part of the cream has been extracted,	76·1	10·0	9·7	14·6	2·1	39·7	P. & M.
Goat,	From skimmed milk,	71·0	1·2	9·9	12·0	2·0	45·9	,,
,,	,,	79·10	4·30	10·44	...	...	...	A. H. A.
Golden Eagle,	Guaranteed pure milk, from which a portion of the cream has been extracted,	...	1·0	6·8	...	...	...	P. & M.
Gowan,	From partly-skimmed milk,	72·0	10·8	10·5	13·4	1·7	35·6	,,
Handy,	From skimmed milk,	75·5	0·3	12·3	17·0	1·6	44·3	,,
,,	,, ,,	67·38	0·17	10·20	...	...	...	A. H. A.
Home,	,, ,,	71·3	1·3	11·1	12·5	2·5	43·9	P. & M.
,,	,, ,,	69·44	0·91	9·13	...	...	...	A. H. A.
Home&Colonial,	No description on label,	72·6	13·5	9·7	17·0	1·9	30·5	P. & M.
Household,	From separated milk,	70·0	0·3	...	...	2·0	...	,,
Imperial Dairy,	From skimmed milk,	70·4	3·7	11·3	12·6	1·6	41·2	,,
Lancer,	,, ,,	67·6	0·3	12·3	16·6	2·6	35·8	,,
Lifeguard,	,, ,,	65·8	0·3	...	...	2·4	...	,,
Lipton's,	Prepared from the richest pure milk,	71·0	9·3	7·9	14·5	2·2	37·1	,,
Lovers,	From skimmed milk,	73·0	0·2	...	...	2·1	...	,,
Lucerne Lion,	Guaranteed finest quality (mountain milk),	71·7	10·8	9·3	15·2	2·0	34·4	,,
Milkmaid,	Swiss milk; genuine,	76·3	11·0	9·7	14·6	2·3	38·7	,,
Milkman,	Warranted to contain all original cream,	73·66	11·80	11·40	...	...	...	A. H. A.
Minstrel,	From skimmed milk,	75·3	0·2	9·7	15·4	1·7	48·3	P. & M.
Mother,	From unskimmed milk,	70·60	5·57	8·32	...	...	...	A. H. A.
,,	Guaranteed to be prepared with the best and richest cows' milk,	72·0	8·8	7·3	13·7	1·7	40·5	P. & M.
Popular,	From partly-skimmed milk,	71·75	2·47	10·14	...	...	...	A. H. A.
Rose,	Warranted not skimmed,	72·40	10·30	9·75	...	...	...	,,
,,	,, ,,	76·6	12·4	8·3	17·6	2·2	36·1	P. & M.
Scandinavian,	Perfectly pure,	74·6	10·8	6·6	14·5	1·5	41·2	,,
Scarboro' Castle,	Containing all original cream,	71·50	8·40	9·11	...	2·1	...	A. H. A.
Shamrock,	From skimmed milk,	71·6	0·5	11·8	18·4	2·0	38·9	P. & M.
,,	,, ,,	71·3	...	10·88	...	...	...	A. H. A.
Springtime,	,, ,,	74·0	0·3	...	...	2·2	...	P. & M.
St Olaf,	From pure unskimmed milk,	71·86	11·30	10·50	...	...	...	A. H. A.
Sunlight,	From unskimmed milk,	71·76	5·60	8·63	...	...	...	,,
Swiss (Nestlè's),	Prepared from pure milk of Swiss cows, and only a small quantity of pure cane-sugar added,	77·2	13·7	9·7	15·0	1·6	37·2	P. & M.
,, ,,	,, ,,	75·00	13·50	10·44	...	...	...	A. H. A.
Threepenny,	From skimmed milk,	66·25	0·30	10·49	...	...	...	,,
Tip-top,	Warranted not skimmed,	74·25	8·12	8·82	...	...	...	,,
,,	,, ,,	74·0	10·7	8·8	15·4	1·7	37·4	P. & M.
Wasp,	From skimmed milk,	73·4	1·0	11·0	12·3	1·9	47·2	,,
World's Tea Co.,	Full cream,	75·0	9·3	...	...	2·0	...	,,

From the figures in the table it will be seen that the proportion of added sugar contained in sweetened condensed milk is fully equal to that of the true milk-solids in the concentrated milk, and

is three times as great as the milk-solids of the milk before concentration. Notwithstanding this fact, the statement is made on some labels that "only a small quantity of pure cane-sugar has been added."

The following recent analyses of sweetened condensed milks have been communicated to the author by C. G. Moor:—

Brand.	Description on Label.	Total Solids.	Fat.	Ash.
Milk Can, . .	Partly skimmed, . .	74·4	0·2	3·0
Dolphin, . .	Partly skimmed, . .	72·0	0·26	2·2
Grass Country, .	Not skimmed, . .	73·1	9·7	2·0
Flamingo, . .	Skimmed milk, . .	71·0	0·29	2·1
Coopers', . .	Unskimmed, . .	74·3	13·9	2·0

In a report by B. Dyer (*Brit. Med. Jour.*, July 27th, 1895) are given the proportions of fat contained in samples of seventeen brands of condensed milk. Some of these are represented in the foregoing tables. Of the rest, the Swiss Dairy, Tea, Wheatsheaf, Marguerite, Clipper, and Gondola brands contained less than 0·8 per cent. of fat; while the As-you-like-it and Nutrient brands contained 4·23 and 2·36 per cent. of fat respectively.

A serious consequence arising from the addition of a large amount of sugar to condensed milk is that the preparation is unsuitable for ordinary purposes, unless mixed with such a proportion of water as to dilute it far beyond the bulk of the milk before concentration. Some labels bear a statement that, if mixed with from three to five volumes of water, the milk may be used as a substitute for cream. As a fact, though the article thus diluted will have the consistence and appearance of cream, it will contain less fat than is present in ordinary uncondensed new milk, instead of 25 to 30 per cent. of fat, which may be taken as the minimum proportion present in true cream.

A highly reprehensible statement, which is made on the labels of many brands of condensed milk, is that, for infants' use, the preparation should be diluted with from six to fourteen parts of water. This direction, if carried out to the extreme limit, would yield a fluid containing only 3 to 4 per cent. of milk-solids (instead of 12 to 13 per cent.), and in some cases less than 1 per cent. of fat (instead of $3\frac{1}{2}$ to 4 per cent.). In some instances the labels bear the statement that nurses are disposed to add too little water.[1]

[1] The Select Committee on Food Products Adulteration, in their report published July 1896, recommended that, in the case of condensed milk made

The following table shows the proportion of fat which would be contained in representative brands of sweetened condensed milk if the contents of the tin were diluted with the amount of water directed on the labels. "Parts" in the directions have been regarded as meaning *parts by measure*, and the calculations have been based on the assumption that the condensed milk before dilution had in each case a specific gravity of 1·28 (compare page 231). This figure is the result of experiment in the author's laboratory on typical preparations of the kind.

Brand.	Number of parts of Water recommended to be added to one part of Condensed Milk for cooking and ordinary use.	Percentage by weight of Fat in Milk diluted for ordinary use.	Number of parts of water recommended to be added to one part of Condensed Milk for Infants' use.	Percentage by weight of Fat in Milk diluted for Infant's use.
Alderney, .	5 to 6	2·32 to 1·94	6 to 8	1·94 to 1·52
Arcadia, .	4 to 5	1·95 to 1·63	7 to 14	1·24 to 0·68
Cowslip, .	4 to 5	0·34 to 0·28	Not stated.	
Devon, . .	4 to 5	2·06 to 1·72	6 to 14	1·50 to 0·71
Farm, . .	4 to 5	0·03 to 0·02	Not stated.	
Fourpenny, .	4 to 5	2·52 to 2·10	7 to 14	1·60 to 0·87
Full Weight, .	4 to 5	2·81 to 2·34	7 to 14	1·79 to 0·97
Goat, . .	5	0·87	Not stated.	
Milkmaid, .	4 to 5	2·66 to 2·22	7 to 14	1·69 to 0·92
Nestlè's Swiss,	4 to 5	3·32 to 2·79	Not stated.	
Rose, . .	4 to 5	3·00 to 2·50	7 to 14	1·91 to 1·04
Threepenny, .	4 to 5	0·07 to 0·06	Not stated.	

From these figures it appears that an attempt to feed an infant in accordance with the directions issued with some brands of condensed milk will either result in the child being half-starved, or will compel it to imbibe such a quantity of fluid as cannot fail to prove a serious strain on its system.

from skimmed milk, the label of the tin should describe the contents in large and legible type, and that a notification should be printed thereon that such milk is not suitable for the purpose of feeding infants and young children. But the Committee do not appear to have realised the grave mischief caused by false statements respecting the dilution which should be practised when the milk is intended for the feeding of infants.

Unfortunately, such flagrant misrepresentations as disgrace the condensed milk trade are not punishable under the Sale of Food and Drugs Act, and the proceedings which are possible under other enactments lack the necessary initiative.

HUMANISED CONDENSED MILK.—Sweetened condensed milk is far from being an ideal food for infants, and even the unsweetened brands possess the disadvantage of readily curdling and of containing less milk-sugar and a larger proportion of proteids than are present in normal human milk. To meet these defects, the so-called "humanised condensed milk" is now manufactured. In these preparations, human milk is imitated by condensing cows' milk to one-third of its bulk, and adding cream and sufficient saturated solution of milk-sugar to bring the mixture to the required composition. The following are analyses of some of these preparations. For comparison, the composition of normal human milk concentrated to one-third is added.

	Solids.	Fat.	Proteids.	Milk-sugar.	Ash.	Authority.
Human milk reduced to one-third, ...	39·8	12·4	6·0	20·8	0·6	A. B. Leeds.
Unknown brand,[1] . .	22·49	6·15	5·35	9·55	1·44	H. Faber.
Cradle brand, . . .	29·9	9·5	3·1	15·5	1·8	Pearmain and Moor.
Edwards' humanised milk, .	44·0	13·5	7·0	21·2	2·0	Pearmain and Moor.

Edwards' humanised milk is prepared according to a patent of C. G. Moor (*Eng. Patent*, 1895, No. 20,219),[2] and is the latest form of the preparation originally introduced as the "Cradle" brand of condensed milk.

The Aylesbury Dairy Company prepare an unconcentrated "humanised milk" (see page 108).

CONDENSED MARES' MILK is prepared by a company at Orenbourg, in South-Eastern Russia, where a stud of mares is kept exclusively for milking purposes. The preparation is recommended as an adjunct to, or substitute for, mother's milk, and favourable

[1] The label on this sample directed that for very young infants the milk should be diluted with seven parts of water. The diluted milk would therefore contain 2·82 per cent. of solids, including 0·77 per cent. of fat (*Analyst*, xiv. 141).

[2] According to Moor's patent, high-class cows' milk is concentrated to one-third, and 33 lbs. of the product mixed with 12 of cream, 10 of milk-sugar, ½ lb. of sodium phosphate, and 44 lbs. of distilled water. Before use, the mixture is diluted with twice its measure of water. The claim is for "concentration and portability over and above all other preparations of humanised milk." By an alteration in the proportion of mineral salts added and reduction in the quantity of water used, a preparation is obtained of the composition of the Edwards' humanised milk.

reports have been made as to its digestibility, nutritious properties, curative power in diarrhœa, and action as an excellent hypnotic.

The following analyses by P. Vieth (*Analyst*, viii. 81 ; ix. 78) show the composition of condensed mares' milk imported to London :—

		Total Solids.	Fat.	Proteids.	Sugar.	Ash.
1	Condensed to one-seventh with 2·33 per cent. added sugar, . . .	82·10	12·07	13·50	54·88	1·65
2	,, ,, ,,	81·20	10·08	15·23	54·09	1·80
3	Condensed to one-eighth with 3 per cent. added sugar, ...	73·27	4·77	13·69	53·07	1·74
4	,, ,, ,,	75·96	6·20	12·17	55·81	1·78

The ash in each case was pink, and contained a notable quantity of iron.

Samples 3 and 4 were of very thick, scarcely fluid consistency, of almost pure white colour, agreeable odour, and had a sweet taste, somewhat resembling that of honey. The condensed milk dissolved readily in water, with the exception of some small flakes, apparently consisting of coagulated albumin.

Vieth concluded that the actual concentration of samples 3 and 4 was somewhat less than that stated on the label.

Analysis of Condensed Milk.—The methods employed for the analysis of fresh milk require certain modifications before applying them to the examination of condensed milk, and especially of such kinds as contain a large proportion of sugar. In the opinion of the author, the following is the best mode of procedure.

The contents of the can should be thoroughly mixed, 10 grammes weighed into a beaker, and made up to 100 c.c. with water. This will give a solution every 10 c.c. of which will contain 1 gramme of the original sample. As the solution is liable to curdle on standing, the various quantities required for the analysis should be measured at once.

1. For the determination of the *total solids*, 20 c.c. of the above solution (= 2 grammes of the sample) should be evaporated in a flat-bottomed capsule (compare page 131), and the residue dried in the water-oven till constant, to effect which heating for at least six hours will be required. The residue is weighed, ignited at a barely visible red heat, and the resultant *ash* weighed.

In the case of sweetened condensed milk, it is very difficult to drive off the last traces of water, and in important cases it is

desirable to absorb the solution in dry asbestos or chrysotile (page 133), and heat in the water-oven till constant.

2. For the determination of the *total proteids*, 10 c.c. may be treated by one of the modifications of Kjeldahl's process (page 21).

3. In cases where it is desirable to determine separately the *casein* and *albumin* of the milk, Sebelien's method may be used (page 87). The amount of soluble albumin (lactalbumin) in condensed milk is considerably lower, in proportion to the casein, than in fresh milk. This fact may be utilised for the detection of diluted condensed milk in new milk (page 190).

4. The *fat* of condensed milk may be determined on 5 c.c. of the solution by Adams' method (page 137), or by evaporating the ethereal extract of the copper-proteid precipitate obtained in Ritthausen's process (page 87). The Werner-Schmid method of fat determination is not applicable without modification to sweetened condensed milks, since the caramel formed by the action of the hydrochloric acid on the cane-sugar dissolves to a certain extent in the moist ether employed for extracting the fat. Dyer and Roberts (*Analyst*, xvii. 81) state the error from this cause may be as high as 1·6 per cent. But the author has found that if the ethereal layer be separated from the acid substratum, and agitated with cold water till no more colouring matter is removed, it will yield the fat in a practically pure state on evaporation. This supplementary treatment is unnecessary if petroleum-ether be substituted for the ether, a perfectly pure fat being, in that case, readily obtained.

Pearmain and Moor (*Analyst*, 1895, p. 270) have succeeded in applying the Leffmann-Beam process to the determination of the fat in sweetened condensed milk, and state that fairly accurate results are obtainable, if the following details, especially the prescribed strength of sulphuric acid, are adhered to in every respect. The chief objection to the method appears to be the large factor which has to be employed (15·5), since it is necessary to work on a quantity of solution representing only 1 gramme of the original sample. Pearmain and Moor recommend that 10 c.c. of a 10 per cent. solution of the sample should be run into the bottle, and 3 c.c. of the hydrochloric acid fusel-oil mixture added, the bottle well shaken, and then 15 c.c. of sulphuric acid of 85·0 per cent. added with agitation. Sufficient of a hot mixture of sulphuric acid and water (1 : 2) is then run into the bottle to bring the top of the liquid nearly up to the zero-mark. The bottle is then whirled in the machine for three minutes. The fat will not all come up at once, but after placing the bottle in the water-oven for two or

three minutes, and again whirling, the entire amount of fat will be obtained, and may then be carefully measured off with a pair of dividers.

5. The determination of the *milk-sugar* of unsweetened condensed milk presents no great difficulty. In ordinary cases, it may be effected with sufficient accuracy by subtracting the sum of the other constituents from 100·00. The polarimeter is not suitable for the determination of the milk-sugar in condensed milk, since the heat to which the milk has been subjected changes the optical activity to an indefinite extent (Richmond and Boseley, *Analyst*, 1893, page 141).

A satisfactory determination of the milk-sugar can be made in the filtrate from the copper-proteid precipitate obtained in the Ritthausen process (page 87), by either Fehling's or Pavy's copper solution. J. C. Shenstone (*Analyst*, xiii. 222) dilutes 30 grammes of condensed milk with about 50 c.c. of water, boils, and makes the cooled solution up to 97 c.c. To this he adds 3 c.c. of the acid solution of mercuric nitrate described on page 145. On pouring the liquid several times from one beaker to another, the proteids coagulate immediately and very uniformly, so that a perfectly bright whey is obtained on filtration. To prevent the possibility of an error due to bi-rotation, the filtered solution should be heated to the boiling-point. Immediately sufficient whey has been separated, 10 c.c. should be diluted to 100 c.c. and a portion examined in the polarimeter without delay, since cane-sugar gradually undergoes inversion in presence of the mercuric reagent. Another portion of the diluted liquid is titrated by Pavy's ammoniacal cupric solution, which will give the means of calculating the amount of milk-sugar present (compare page 148).

In the case of unsweetened condensed milk, the extent to which the concentration has been carried may be judged from the proportion of total solids. The percentage of ash affords an independent criterion, but this is liable to be vitiated if mineral preservatives have been added. Further, a deposition of certain salts is liable to occur during the evaporation of the milk, and this circumstance tends to diminish the proportion of ash in the finished product.

As the fat of whole cows' milk is always sensibly in excess of the proteids, it will be at least as high as the proteids in the condensed preparation, provided that none of it has been removed. There is considerable inducement to remove part of the milk-fat prior to concentration, as a portion is liable to separate from very rich milk, and this difficulty has only been overcome of late years.

In the case of sweetened condensed milk, the proportion of total solids is not available as a criterion of the extent to which concentration has been effected. The amount of ash will serve as a rough guide, but the proportion of proteids affords the most reliable datum. As the proteids of genuine uncondensed milk average 3·5 per cent., the condensation is readily calculated, making allowance for the increase in the weight of the product due to the addition of a large proportion of cane-sugar. Thus, the "Milkmaid" brand, which is a typical make of sweetened condensed milk, contains, according to the analysis of Pearmain and Moor, 37·6 per cent. of milk-solids, 38·7 per cent. of cane-sugar, and 23·7 per cent. of water. The milk-solids are three times as high as in uncondensed milk of fair quality, which may be taken as containing 12·5 per cent. of solids and 87·5 per cent. of water. The water originally associated with 37·5 per cent. of milk-solids would be $87{\cdot}5 \times 3 = 262{\cdot}5$, whereas the condensed milk contains only 23·7 parts of water for the same amount of milk-solids. Therefore, 238·8 parts of water out of 262·5 originally present, or about ten-elevenths of the whole, must have been evaporated. But the addition of cane-sugar dilutes the milk thus concentrated, so as to produce a finished product containing only three times the proportion of milk-solids present in unconcentrated milk. Hence the addition of twice its weight of water to the condensed milk will reduce the milk-solids to their original proportion. But, as the dilution is in practice effected by measure rather than by weight, the number of measures of water (W) required to reduce one measure of condensed milk to its original concentration may be found by the following equation :—

$$\text{Measures of water required for dilution } (W) = \frac{\text{Milk-solids in condensed milk (C)} \times \text{sp. gr. (G)}}{\text{Milk-solids in diluted milk (D)}} - 1.$$

Sweetened condensed milk of the character of the "Milkmaid" brand has a specific gravity of about 1·28. If this figure be accepted, and the milk-solids of the original milk be taken at 12·5 per cent., the above equation becomes :—

$$W = \frac{C \times 1{\cdot}28}{12{\cdot}5} - 1 \text{ ; or } W = 0{\cdot}1024C - 1.$$

Taking as an example the "Milkmaid" brand with 37·6 per cent. of milk-solids, somewhat less than three measures of water will reduce it to the primary concentration :—

$$W = 0{\cdot}1024 \times 37{\cdot}6 - 1 = 2{\cdot}85.$$

Similarly, the percentage of milk-solids which will be contained in a diluted condensed milk may be found by the equation :—

$$\text{Milk-solids in diluted milk (D)} = \frac{1{\cdot}28C}{W+1}.$$

Thus, taking the "Milkmaid" brand, if the milk be diluted with the maximum quantity of water directed on the label, namely, 14 parts, the diluted milk will contain 3·21 per cent. of milk-solids :—

$$\frac{1{\cdot}28 \times 37{\cdot}6}{14+1} = 3{\cdot}21.$$

If for the value of C in the above equation the percentage of fat (F) in the condensed milk be substituted, the proportion of fat in the diluted milk will be found. In the case of the "Milkmaid" brand, diluted to the maximum as before, this will be :—

$$\frac{1{\cdot}28 \times 11{\cdot}0}{14+1} = \frac{14{\cdot}08}{15} = 0{\cdot}94 \text{ per cent. of fat in the diluted milk.}$$

The foregoing equations give the dilution necessary to bring the parts of milk-solids per 100 measures of the diluted condensed milk to that contained in the original milk before concentration. If unity, when it appears in the above equations, be multiplied in each case by the specific gravity of the condensed milk (=1·28), the equations will give the dilution necessary to yield a product containing 12·5 per cent. by weight of milk-solids.

Products of the Alcoholic Fermentation of Milk.

It has been already stated (page 196) that the production of more or less alcohol almost constantly attends the lactic fermentation of milk. In cases where the fermentation is effected by pure cultivations of *Bacillus acidum lactici*, no alcohol appears to be formed ; but in practice the above-named organism is associated with, or possibly actually replaced by, others which produce alcohol. The number of lactic acid ferments are very numerous and of a varied nature, and it is probable that alcohol is a normal product of the action of some of them, altogether apart from its formation by organisms of the yeast type. The possibility of fermenting milk-sugar by means of yeast has long been a vexed question, but appears to be settled in the negative so far as the ordinary yeasts are concerned. But recent experiments by Duclaux, Adametz and Kayser have shown that one or more forms of yeast exist which have the power of setting up an alcoholic fermentation of milk-sugar. These milk-sugar yeasts

were obtained from a dairy in which much trouble had arisen from the fermentation of the milk. It has been found that these yeasts not only possess the unique property of fermenting milk-sugar, but that they are also far more effective than brewers' yeast in fermenting galactose, while, on the other hand, they are much inferior to brewers' yeast in bringing about the fermentation of maltose.

In consequence of these new yeasts giving rise to a pure alcoholic fermentation of milk-sugar, it has been suggested that they might be used for manufacturing a sparkling and highly nutritious beverage from the whey which is produced on so large a scale in the manufacture of cheese, for the production of which some 224 millions of gallons of milk are annually employed in the United Kingdom alone. The *whey wine* thus obtained is stated to have a flavour resembling that of cider. The following figures show the composition of the product. In the first two cases cane-sugar was added before fermentation, to increase the alcoholic strength. In the third case, the whey was concentrated with the same object, but this plan was found to yield a product of too saline a flavour.

	A. Whey and Sugar.	B. Whey and Sugar.	C. Concentrated Whey.
	Per cent.	Per cent.	Per cent.
Residual sugar, . .	2·92	1·17	2·37
Alcohol (by weight), .	2·88	3·68	3·00
Acidity, ...	0·14	0·15	0·60

This whey wine must not be confounded with koumiss, which is a complex product resulting from the simultaneous action of several ferments.

Koumiss is a whitish, effervescent, alcoholic beverage obtained by the fermentation of milk. True koumiss is prepared from mares' milk, but an excellent imitation can be obtained from skimmed cows' milk to which a little cane-sugar has been added by fermenting it with brewers' yeast.[1] Camels' milk is also employed for the production of koumiss.

Koumiss is prepared by the Tartars by adding a little sugar to ten parts of fresh warm milk, and treating the mixture with one part of sour milk (therefore undergoing the lactic fermentation).

[1] When cows' milk is employed the greater part of the cream should be previously removed, and it is sometimes advisable to add some milk-sugar.

The whole is allowed to stand for a few hours, during which it is repeatedly stirred.

The following analyses of koumiss suffice to show its general composition :—

	Mean of 10.	From Mares' Milk.	From Cows' Milk.	From Mares' Milk. Mean of 8.[1]
Authority, . .	König.	Fleischmann.	Fleischmann.	H. W. Wiley.
Carbonic acid, .	0·88	0·88	1·03	0·83
Alcohol, . .	1·59	1·85	2·65	0·76
Fat, . . .	0·94	1·27	0·85	2·08
Proteids, . .	2·83	1·91	2·03	2·53
Lactic acid, . .	1·06	1·01	0·79	0·47
Milk-sugar, . .	3·76	1·25	3·11	4·38
Ash, . . .	1·07	0·29	0·44	...
Water, . .	87·88	91·53	88·93	89·32

H. Suter-Naef (*Ber.*, v. 286) gives the following as the percentage composition of Swiss koumiss of 1·1286 specific gravity, manufactured at Davos :—Carbonic acid, 0·177 ; alcohol, 3·21 ; fat, 1·78 ; albuminates, 1·86 ; lactic acid, 0·19 ; milk-sugar, 2·10 ; salts, 0·51 ; and water, 90·35.

The foregoing analyses afford no information as to the age of the sample when analysed, a point which is of much importance in view of the rapid change which koumiss undergoes by keeping.[2] P. Vieth (*Analyst*, x. 221 ; xi. 69 ; xii. 43) has supplied this deficiency in the following series of analyses of koumiss of different descriptions. Each sample was analysed when one day, eight days, and three weeks old. The carbonic acid present was not taken into account.

[1] These specimens of koumiss were prepared in Indianapolis and contain less alcohol and more fat than is shown in analyses by other chemists.

[2] "A mixture of fresh and fermented mares' milk, when twenty-four hours old, is called 'fresh koumiss.' It is put up in champagne-bottles, corked, and wired. In the bottles the alcoholic fermentation advances, and the koumiss, in the course of from twenty-four to forty-eight hours, enters into its second stage. So much of the sugar is then decomposed that the whole contents of the bottle may be drawn off by means of a champagne-tap, presenting a highly effervescent liquid. After a fortnight has elapsed, little or no sugar remains undecomposed, and we have now 'old koumiss'" (P. Vieth, *Analyst*, x. 220).

	Full Koumiss.			Medium Koumiss.			Whey Koumiss.			Diabetic Koumiss.			Russian Koumiss.			Cows' Milk Koumiss.			
	Days Old.			Days Old.			Days Old.			Days Old.			Days Old.			Days Old.			
	1	8	22	1	8	22	1	8	22	1	8	22	1	8	22	1	8	22	90
Water,	88·90	90·35	90·57	87·55	88·39	88·62	89·74	90·63	91·07	92·24	92·38	92·55	91·87	92·26	92·52	88·50	90·15	90·31	90·36
Alcohol,	·15	·94	1·04	·29	·97	1·05	·30	1·03	1·38	·28	·35	·57	·22	·45	·57	·17	·92	1·03	1·12
Fat,	1·35	1·36	1·38	1·54	1·56	1·58	·11	·13	·15	·51	·52	·51	·34	·33	·33	1·65	1·48	1·58	1·57
Casein,	2·01	1·96	1·88	1·46	1·40	1·30	·15	·14	·11	2·19	2·13	2·05	2·32	2·17	2·03	2·06	2·00	1·93	1·70
Albumin,	·30	·23	·20	·43	·25	·14	·39	·36	·32	·30	·25	·18	·08	·07	·07	·32	·22	·21	·09
Lactoprotein and Peptones,	·34	·53	·77	·48	·76	·97	·44	·49	·58	·36	·48	·65	·32	·48	·63	·32	·56	·74	·91
Lactic acid,	·34	·96	1·40	·68	1·20	1·67	·60	·91	1·26	·75	·86	1·22	·06	·31	·56	·26	·97	1·39	1·94
Sugar,	6·03	3·10	2·18	6·80	4·70	3·90	7·48	5·52	4·34	2·78	2·42	1·64	3·95	3·08	2·45	6·16	3·14	2·23	1·73
Ash, Insoluble,	·41	·34	·35	·49	·45	·44	·42	·42	·42	·37	·37	·37	·38	·36	·35	·42	·34	·35	·33
Ash, Soluble,	·17	·23	·23	·28	·32	·33	·37	·37	·37	·22	·24	·26	·46	·49	·49	·16	·22	·23	·25

The following hitherto unpublished analyses of mares'-milk koumiss are also by Vieth. Three samples of each age were analysed. The proportions of alcohol are much higher than in the analyses in the previous table.

	Samples 1 Day Old.			Samples 8 Days Old.			Samples 22 Days Old.		
Water, . . .	90·99	91·87	91·42	91·95	92·38	92·04	91·79	92·42	91·99
Alcohol, . . .	2·47	3·29	2·25	2·70	3·26	2·84	2·84	3·29	2·81
Sugar, . . .	2·21	·39	2·30	·69	·09	·73	·51	...	·19
Lactic acid, . . .	·64	·96	·70	1·16	1·03	1·06	1·26	1·00	1·54
Casein, . . .	·83	·80	·67	·81	·85	·88	1·01	·79	·69
Albumin, . . .	·37	·15	·23	·23	·32	·27	·29	·32	·11
Lactoprotein and Peptone,	1·05	1·04	·85	·96	·59	·74	·67	·76	·89
Fat, ...	1·08	1·17	1·22	1·13	1·14	1·10	1·27	1·20	1·44
Ash, ...	·36	·33	·36	·37	·34	·34	·36	·35	·37

The foregoing tables clearly show the change in the composition of koumiss by keeping. The progress of the lactic and alcoholic fermentations may be traced by the diminution of the sugar and the increase in the proportions of lactic acid and alcohol; while the process of peptonisation is indicated by the decrease in the coagulable proteids, and the notable increase in the peptonoid bodies. This change is of great importance, since the value of koumiss as a food for invalids (apart from the stimulating effect of the contained alcohol) appears to be largely dependent on the peptonised condition of the casein, which is thus easily assimilated, and the fact that the unaltered casein does not readily form clots. Another point of interest is the increase in the soluble ash and decrease in the proportion of insoluble ash. This change is considered by Vieth to be due to two causes:—the increasing amount of lactic acid, and the transformation of the casein, with which the phosphates in milk are chemically combined.

Kephir is a Caucasian product somewhat similar to koumiss, but prepared from cows' milk in leather bottles by the aid of a peculiar ferment known as "kephir grains."[1] The following

[1] According to E. Kern, the kephir ferment is an elastic, cauliflower-like mass found below the snow-line on certain bushes. Kern states that the fungus consists of bacilli and yeast-cells, each cell containing two round spores, whence the name *Dispora Caucasina*. When dried, the kephir fungus forms hard, yellowish grains about the size of peas. By soaking these in water and adding them to milk, alcoholic fermentation ensues, and the kephir is

figures show the comparative percentage composition of fresh milk, kephir, and koumiss[1] :—

	Fresh Milk.	Kephir.	Koumiss.
Fat, ...	3·8	2·0	2·05
Proteids, . . .	4·8	3·8	1·12
Sugar, . . .	4·1	2·0	2·20
Lactic acid, . .	trace.	0·9	1·15
Alcohol, . . .	none.	0·8	1·65
Water and salts, . .	87·3	90·49	91·83

The most complete analyses of kephir are those of König and Hammersten, who have published the following data :—

	König.	Hammersten.
	Per cent.	Per cent.
Fat, ...	1·44	3·09
Casein, ...	2·83	2·90
Lactalbumin, ...	0·36	0·19
Hemi-albumose, ...	0·26	} 0·07
Peptone, ...	0·04	
Sugar, ...	2·41	2·68
Lactic acid, ...	1·02	0·73
Ash, ...	0·68	0·71
Alcohol, ...	0·75	0·72
Water, ...	90·21	88·91
	100·00	100·00

mature in a few days. During the fermentation a considerable quantity of the ferment is formed. This is removed, dried in the sun, and preserved for future use, to effect fresh fermentations. H. Struve (*Ber.*, xvii. 1364) found kephir grains to have the following composition :—water, 11·21 ; fat, 3·99 ; soluble peptone-like substances, 10·98 ; proteids soluble in ammonia, 10·32 ; proteids soluble in potash, 30·39 ; and insoluble residue, 33·11 per cent. The insoluble residue was found on microscopic examination to consist of a mixture of yeast-cells with Kern's *Bacterium dispora caucasina*. In a few cases, *Lepthothrix* and *Oidium lactis* were also observed. The whole of the active matter of the ferment is stated by Struve to be contained in the insoluble residue. The fermentation produced by kephir grains is of a very complex character. Struve regards the bacterium as inactive, the fermentation being entirely due to the yeast-cells, which have been modified by having grown in contact with the leather of the bottles.

[1] An article in the London *Medical Record* for February 15th, 1886, from which the figures in the text are taken, describes kephir as more agreeable and cheaper than koumiss, and not liable to disturb the digestion. It is stated to have been used with advantage in all kinds of dyspepsia, anæmia, catarrh of the stomach, &c., &c.

The following analyses illustrate the change undergone by kephir during maturation [1]:—

	First Day.	Second Day.	Third Day.
	Per cent.	Per cent.	Per cent.
Fat, ...	...	1·75	1·70
Casein, ...	3·34	2·87	2·99
Lactalbumin, . . .	0·11	0·03	0·00
Acid albumin, . . .	0·09	0·10	0·25
Hemi-albumose, . .	0·09	0·28	0·40
Peptone, ...	0·03	0·04	0·08
Lactose, ...	3·75	3·22	3·09
Lactic acid, . . .	0·54	0·56	0·65
Alcohol, ...	...	0·80	1·00

Kephir may be prepared by adding one part of the three-days-old product to four or five of new milk, and allowing it to ferment for two days at the ordinary temperature, with occasional agitation. When well-prepared, kephir effervesces when the containing bottle is opened, has the consistence of cream, with a pleasant sour-sweetish taste, and an odour of sour cream. Strong kephir is thinner, more sour and less palatable, and is richer in alcohol and carbonic acid. The curdled casein should form a perfect emulsion, which should not feel lumpy to the tongue. When left at rest for some time, kephir separates into two layers, but should become homogeneous on being shaken.

Cheese.

Cheese consists essentially of a compressed mass of milk-curd. The coagulation of the milk would ultimately occur spontaneously by the action of the micro-organisms present, but in practice the process is hastened by the addition of rennet, the nature and action of which have already been explained (page 88). The acidity of the milk should not vary much above or below 0·2 per cent. The temperature employed varies with the weather and other circumstances, the conditions being regulated in practice so as to effect complete coagulation in a time varying from forty to sixty minutes. The

[1] The figures are taken from an article by C. D. Spivak ("Food and Sanitation" May 22, 1897; from *The Dietetic and Hygienic Gazette*) containing interesting particulars respecting the manufacture and employment of kephir. See also Thorpe's *Dictionary of Applied Chemistry*, ii. 616.

curd is then carefully broken and drained from the fluid, after which it is allowed to stand for a time to harden it and promote separation of the whey.[1]

A certain proportion of annatto or other colouring matter is usually added with the rennet, and after the curd is separated from the whey an addition of common salt is made. The curd is then pressed for several days, and is subsequently subjected to a process of curing in a room kept at a constant temperature.

In Britain, cheese is made exclusively from cows' milk, but on the continent of Europe certain kinds are manufactured from the milk of sheep and goats.

The main constituents of cheese are casein, milk-fat, and water. Among the minor constituents, so far as quantity is concerned, are milk-sugar, lactic acid, mineral matter, including the salt added as a preservative, and various compounds resulting from the bacterial fermentation of the casein. These last include ammoniacal salts, leucine, tyrosine, &c. The milk-sugar which is present in new cheese becomes converted into lactic acid, alcohol, and other decomposition-products during the process of ripening.

The chemical changes which occur during the ripening of cheese are very imperfectly understood. A loss of water naturally occurs, and the formation of ammonia and other basic compounds causes the acid reaction of the fresh cheese to be replaced by an alkaline reaction in the matured product.[2] The vexed question whether the casein undergoes partial conversion into fat during the process of ripening, cannot be regarded as definitely settled, though many of the results which have been supposed to prove

[1] THE WHEY obtained as a secondary product in the manufacture of cheese is used to a limited extent for the preparation of milk-sugar, but by far the larger proportion is employed for feeding pigs. According to König, cheese-whey has the following average composition :—Water, 93·31 per cent.; fat, 0·24 ; lactose, 4·65 ; lactic acid, 0·33 ; nitrogenous matters (*e.g.*, albumin and peptones), 0·82 ; and salts, 0·65 per cent.

Experiments made by the New York State Dairy Commission showed the average loss of fat in the whey to be 0·29 per cent., or about 7½ per cent. of the total fat. The proteids lost in the whey averaged 0·74 per cent. (or about 24 per cent. of the total amount), of which 0·15 consisted of casein and 0·59 per cent. of albumin.

The average proportions of casein and albumin present in the original milk are stated at 2·4 and 0·72 (*sic.*) per cent. respectively. Hence, about 6 per cent. of the total casein and 82 per cent. of the total albumin were lost in the whey.

[2] The formation of free organic acids in cheese results in the removal of a portion of the lime from its casein-compound, and this reaction explains the considerable quantity of calcium salts present in the aqueous extract of all kinds of ripe cheese.

the occurrence of such a change are clearly due simply to the concentration of the previously-existing fat by the loss of water undergone by the cheese by keeping. The whole subject requires re-investigation with the aid of improved methods of analysis.

The characteristic flavours possessed by various descriptions of cheese are due primarily to the details of the process of manufacture. The art of cheese-making may be said to consist in establishing conditions which will favour the predominant development of those kinds of bacteria specially implicated in the ripening of the particular kind of cheese required.[1] By carrying this principle into practice, cheeses indistinguishable from numerous foreign varieties have been successfully produced in England (page 242).[2]

The coloured moulds which are often seen in cheese are fungi, the red being commonly *Sporondonema casei* and the blue *Aspergillus glaucus*.[3] The cheese-mite, *Acarus domesticus*, belongs to the articulata. The "jumpers" often found in cheese are the larvæ of the fly, *Piophila casei*.

[1] See H. L. Russell (*Food and Sanitation*, June 26, 1897).

[2] E. Duclaux (abst. *Jour. Chem. Soc.*, xlii. 436) states that in manufacturing fine cheeses only a small quantity of rennet is added, and the coagulation takes a long time, the curd remaining soft and containing much whey. It is drained slowly, and as perfectly as possible, in order to eliminate the milk-sugar, part of which is oxidised and part converted into lactic acid, thus rendering the curd acid. The casein-ferments then develop, and give rise to ammonium carbonate, which neutralises the lactic acid, and thus the curd becomes alkaline. At the same time, "diastases" are formed, which penetrate the mass of curd, producing a yellow translucent layer which gradually extends to the centre of the mass. This takes the place of the white opaque casein. It is from these diastases that the different varieties of cheeses are matured, and the skill of the manufacturer consists in making repeated use of the same ferments, and preventing the access of others.

In the manufacture of Gruyère cheese, the difficulty is in heating the curd to 50° C., which is done to rapidly eliminate the serum, in order that the curd may be quickly pressed into a mould. Slow heating and constant stirring are necessary to ensure the production of fine granules, without which an impermeable coating would be formed in the press, and so the serum would be retained. The residual milk-sugar is got rid of by fermentation.

For the making of Cantal cheese the milk is rapidly curdled, and the curd is then so treated (without heating) that about half its weight of serum remains with it. It is not immediately pressed, but subjected to fermentation, which causes the entire disappearance of the milk-sugar, and the casein undergoes a curious molecular change.

[3] Roquefort and many other varieties of cheese are ripened by means of *Penicillium glaucum*, which is cultivated at a temperature as near 0° C. as possible, in order to prevent the invasion of other undesirable organisms. The spores are often introduced in the form of mouldy bread-crust.

Cheese is usually made from whole milk, but some varieties, such as Dutch cheese, are made from partially skimmed milk. Other kinds are made from milk to which cream has been added. "Filled cheese" is made from milk from which the milk-fat has been wholly or partially removed, and replaced by margarine or lard. In cheese made from unskimmed milk, the fat is always sensibly in excess of the proteids (*i.e.*, the nitrogen × 6·3).[1]

The various descriptions of cheese may be conveniently classified as "hard" and "soft." Cheddar, Cheshire, American, Dutch, Stilton, and Gruyère belong to the first class; while Camembert, Roquefort (a cheese made from ewe's milk), Neufchatel, and ordinary cream cheese are examples of the second class.

The following analyses by J. Muter (*Analyst*, x. 3) illustrate the composition of different kinds of cheese sold in London in 1885. The fat was prepared by exactly neutralising the free acid of the sample, mixing it with sand, drying at 100° C., and extracting with redistilled petroleum-spirit. The figures obtained by the analysis of the fat are interesting as showing the change in its character resulting from the ripening of the cheese.

	American Cheddar.	Bondon.	Camembert.	Cheddar.	Double Gloucester.	Dutch.	Gruyère.	Roquefort.	Stilton.
Water,	29·70	55·20	48·78	33·40	37·20	42·72	33·20	21·56	28·60
Fat,	30·70	20·80	21·35	26·60	22·80	16·30	27·26	35·96	30·70
Lactic acid,	0·90	0·90	0·36	1·53	1·80	1·35	1·35	0·72	1·08
Lactose,	trace	0·74	trace	...	...	...	...	...	...
Ash, insoluble,	2·16	0·52	0·16	2·30	2·56	2·26	3·12	1·70	1·80
Ash, soluble,	1·54	6·46	8·64	2·00	2·00	9·10	1·58	8·54	2·22
Containing NaCl,	1·20	3·16	3·46	1·52	1·64	4·02	1·05	3·42	0·75
Data of Fat Analysis.									
Insoluble fatty acids,	89·98	87·34	87·15	87·66	87·00	87·20	87·32	87·00	86·20
Soluble fatty acids,	3·30	5·95	6·09	5·60	6·28	6·09	5·98	6·27	7·02
Mgrms. of KHO required by 1 gramme,	220·2	228·0	229·0	227·5	229·3	228·7	228·0	229·3	231·7

[1] Vieth states that cheeses made from whole milk in various parts of the world show that the casein and fat are in the average proportion of 100 : 122, never exceeding the ratio 100 : 150, or falling below 100 : 100. Experiments by the New York State Dairy Commission gave the ratio of 140 to 150 of fat to 100 of proteids. Partial skimming reduced this ratio to 122 : 100, while addition of cream raised it to over 200 : 100.

The following analyses of various descriptions of cheese are selected from a larger number published by Chattaway, Pearmain & Moor (*Analyst*, xix. 145).

No. of Sample.	Kind of Cheese.	Water.	Fat.	Ash.	Nitrogen.	Proteids = N×6·3.	Reichert figure for Fat.	Valenta Test.
1	Cheddar (English), .	33·8	30·5	4·1	4·20	26·7	26·4	31·0° C.
2	Cheddar (Canadian), .	33·3	30·6	3·6	4·34	27·6	24·0	41·5
3	American, . . .	29·8	33·9	3·7	4·76	30·3	26·2	47·5
4	American, . . .	30·6	27·7	3·6	4·84	30·8	3·0	82·0
5	Gorgonzola, . .	40·3	26·1	5·3	4·36	27·7	22·1	26·5
6	Dutch, . . .	41·8	10·6	6·3	5·11	32·5	27·0	40·0
7	Gruyère, . . .	35·7	31·8	3·7	4·49	28·7	31·1	41·0
8	Stilton, . . .	21·2	45·8	2·9	4·14	26·3	32·0	45·5
9	Cheshire, . . .	37·8	31·3	4·2	4·03	25·7	31·6	43·0
10	Double Gloucester,[1] .	37·4	28·1	4·6	4·45	28·3	32·3	41·0
11	Camembert, . .	47·9	41·9	4·7	3·43	21·8	31·0	32·0
12	Camembert, . .	43·4	22·6	3·8	3·83	24·4	35·0	33·0
13	Parmesan, . . .	32·5	17·1	6·2	6·86	43·6	28·0	28·0
14	Roquefort, . . .	29·6	30·3	6·7	4·45	28·3	36·8	19·0
15	Double cream, . .	57·6	39·3	3·4	3·14	19·0	31·2	40·0

No. 4 is evidently a "filled" or margarine cheese. The figures for No. 6 confirm the fact that Dutch cheese is made from partially-skimmed milk.

The following analyses of cheeses of English manufacture, made in imitation of different well-known varieties, have been published by Chattaway, Pearmain & Moor (*Analyst*, xx. 132):—

No.	Kind of Cheese.	Water.	Fat.	Ash.	Nitrogen.	Proteids = N×6·3.
1	Port de Salut, .	31·3	36·2	4·6	4·2	26·5
2	Caerphilly, .	24·8	30·4	3·4	5·9	37·2
3	Culommier, .	37·8	24·1	4·1	3·9	24·6
4	Cleveland, . .	38·0	35·0	3·4	4·4	27·7
5	Cambridge, .	32·1	47·1	4·4	3·9	24·6
6	Gorgonzola, .	33·5	33·2	3·5	6·0	37·8
7	Double cream, .	14·0	68·1	1·2	3·2	20·1
8	Camembert, .	35·0	33·2	2·0	5·5	34·6
9	Gervais, . .	15·8	69·3	0·6	3·0	18·9
10	Wensleydale, .	28·3	33·3	3·7	4·3	27·2
11	Cheddar, . .	37·7	30·5	3·9	4·6	29·0
12	Stilton, . .	25·0	34·6	4·1	4·5	28·4

[1] Pearmain and Moor state that, contrary to the popular belief, the terms "Single Gloucester" and "Double Gloucester" refer to the size of the cheese only, and have no relation to the quality.

The following analyses by A. Smetham show the composition of the milk, whey, and curd, as compared with the finished (Cheshire) cheese :—

	Water.	Fat.	Ash.	Proteids (=N×6·33).	Milk-Sugar, &c. (by difference).
Milk, June 28, 1892, . .	88·17	3·23	0·70	3·30	4·60
Whey, June 28, 1892, . .	93·33	0·24	0·49	0·88	5·06
Curd, June 28, 1892, . .	47·90	26·00	2·26	20·37	3·47
Cheese, Sept. 16, 1892, . .	39·55	29·56	3·95	24·83	2·11

A large number of samples of commercial cheese (exclusive of cream-cheese), purchased under the Sale of Food and Drugs Act during the years 1892–97, were found, on analysis in the author's laboratory, to have the following range of composition. The fat varied from 47·4 to 16·8 per cent., the latter being found in a Dutch cheese. The next lowest percentage of fat (18·2) was likewise met with in a sample of Dutch cheese, which also contained the highest ash, 8·7 per cent., of which 4·8 was common salt.[1] The lowest ash met with was 2·45 per cent. The Reichert-Wollny determinations on the extracted fat, exclusive of samples which were evidently "filled" cheese, ranged from 22·4 c.c. (for 5 grammes of fat) to 38·6 c.c. As a rule, the volume of alkali neutralised by the distillate from cheese-fat is sensibly higher than that required by the fat of fresh milk or butter.

The following analyses of four samples of *cream-cheese*, manufactured by the Aylesbury Dairy Company, have been recorded by P. Vieth (*Analyst*, 1886, p. 162). The samples were analysed when fresh, and then one half of each cheese was kept loosely covered at the ordinary temperature, and the fat analysed after the lapse of one, two, three, and four months respectively, to ascertain any change in its constitution.

	I.	II.	III.	IV.
Water, ...	30·24	32·40	27·69	32·30
Fat,[1]	62·80	60·48	66·80	61·88
Casein, &c. (by difference), ...	5·17	5·90	4·20	4·51
Lactic acid, ...	0·31	0·14	0·30	0·28
Ash,[2]	1·48	1·08	1·01	1·03
	100·00	100·00	100·00	100·00
[1] Insoluble fatty acids of fat, fresh, . . .	87·22	87·30	87·27	87·46
,, ,, ,, after 1 month, . .	87·12	...	...	...
,, ,, ,, ,, 2 months, . .	...	88·02	...	...
,, ,, ,, ,, 3 ,, . .	...	...	87·96	...
,, ,, ,, ,, 4 ,, . .	...	...	...	87·58
[2] Containing Cl equal to sodium chloride, . .	1·15	0·70	0·70	0·87

[1] C. G. Moor found from 10·7 to 28·4 (average of nine, 18·1) per cent. of fat in Dutch cheese.

The following figures are the average of those obtained by Vieth by the analysis of four samples of a cream-cheese, "*Fromage Gervais*," made according to a French recipe :—Water, 42·32 ; fat, 49·18 ; lactic acid, 0·27 ; casein, &c., 7·50 ; and ash, 0·48 per cent.

The following are examples of what must be regarded as spurious cream-cheeses :—

	Water.	Fat.	Lactose.	Lactic Acid.	Casein, &c.	Ash.	Remarks.
English cream (J. Muter),	63·64	15·14	·90	·90	18·5	·92	Insol. acids in fat, 90·01. Casein by difference.
York cream (P. Vieth), .	72·04	9·89	...	1·79	15·31	·97	Insol. acids in fat, 86·44. Casein by difference.
York cream (Chattaway, Pearmain, & Moor), .	63·1	6·5	...	...	17·9	1·4	Nitrogen, 2·76 × 6·3 = casein.

Sheep's-milk Cheese usually possesses a peculiar taste, but according to C. Besana (abst. *Analyst*, xviii. 248) this may be avoided if the rennet used be previously purified by precipitating the crude article with brine. Twice the amount of rennet requisite for curdling cows' milk was found to be necessary. By this means, Besana obtained a product very similar to Parmesan cheese (made from partly-skimmed goats' milk), and which could not be distinguished from cheese made from cows' milk.

G. Sartori (abst. *Jour. Chem. Soc.*, 1891, p. 951) gives the following analyses of cheese from sheep's milk. Nos. I. and II. were prepared by Besana's method; the others in the ordinary manner :—

	I.	II.	III.	IV.	V.
Water,	27·47	29·70	28·50	29·13	32·90
Fat,	30·50	31·30	30·93	30·30	29·96
Nitrogenous matter,	35·59	33·69	34·19	34·00	30·74
KCl,	5·39	4·34	5·03	5·51	4·58
Ash (less NaCl),	1·05	0·97	1·35	1·33	1·82
	100·00	100·00	100·00	100·27	100·00
Proteids,	31·57	28·12	27·95	28·93	24·63
Proteid decomposition-products,	4·00	5·27	5·94	4·86	6·08
Nuclein,	0·183	0·162	0·261	0·256	0·201
Ammonia,	0·162	0·169	0·491	0·152	0·143
Total nitrogen,	5·26	4·72	4·83	4·70	4·30
Proteid nitrogen,	4·84	4·28	4·27	4·40	3·76
Nitrogen of decomposition-products,	0·42	0·41	0·54	0·25	0·54
Nitrogen of ammonia,	0·150	0·138	0·157	0·125	0·117
Free fatty acids,	1·00	0·85	0·95	0·73	0·84

Sartori states that "*Ricotta*," prepared from sheep's milk, contains more fat and less proteids than that prepared from cows' milk. He gives the following analyses of ricotta obtained from sheep's milk and cows' milk respectively :—

	Sheep's Milk Ricotta (mean of 3).		Cows' Milk Ricotta.	
	Fresh.	Dry.	Fresh.	Dry.
Water, ...	43·27	...	68·47	...
Fat, ...	33·31	58·76	5·22	16·56
Proteids, ...	11·73	20·66	18·72	59·37
Milk-sugar, ...	10·42	18·37	3·97	12·59
Lactic acid, ...	0·43	0·76	...	...
Ash, ...	0·81	1·43	3·62	11·48

The following preliminary analyses by Sartori (*Analyst*, xviii. 17) show the composition of "*Cacio-Cavallo*," which is the typical cheese of Southern Italy. No. 1 was made from whole sheep's milk, and No. 2 from a mixture of separated milk and milk containing 8 per cent. of fat. The first was pale yellow, and had a normal odour. The second was strongly coloured, and had a slight smell of the sheep :—

	No. 1.	No. 2.
Water, ...	19·76	22·09
Fat, ...	36·70	35·90
Total proteids, ...	37·82	36·06
Ash (without NaCl), ...	2·34	2·64
Sodium chloride, ...	3·26	3·16
Total, ...	99·88	99·85
Pure proteids,[1]	34·12	35·57
Nitrogen as ammonia, ...	·06	·05
Amidic nitrogen, ...	·66	·61
Reichert-Wollny figure of the fat, . . .	25·30	28·71

A. Kalantaroff (abst. *Jour. Chem. Soc.*, 1884, p. 700) has

[1] The pure proteids were separated by Stützer's method (page 30), and the nitrogen determined in the copper precipitate.

published figures showing the composition of cheese of Russian manufacture.

Filled Cheese, also known as *lard cheese* and *oleomargarine cheese*, is now manufactured extensively in America, and has found its way into many parts of Britain.[1]

The following analyses of filled cheese were recorded in 1882 by P. Vieth (*Analyst*, vii. 137).

	Cheese containing Lard.	Cheese containing Oleomargarine.
	Per cent.	Per cent.
Water, ...	38·26	37·99
Fatty matters, ...	21·07	23·70
Casein, &c., ...	35·55	34·65
Mineral matter, ...	5·12	3·66
	100·00	100·00
Insoluble fatty acids in fat, ...	90·46	91·82

[1] In Bulletin No. 13 (1887) of the United States Department of Agriculture, H. W. Wiley gives the following description of the manufacture of filled cheese :—"An emulsion of lard is made by bringing together in a disintegrator lard and skimmed milk, both previously heated to 140° F. in steam-jacketed tanks ; the disintegrator consists of a cylinder revolving within a cylindrical shell ; the surface of the cylinder is covered with fine serrated projections, each one of which is a tooth with a sharp point ; as this cylinder revolves rapidly within its shell the mixture of melted lard and hot skimmed milk is forced up into the narrow interspace and the lard becomes very finely divided and most intimately mixed or emulsionised with the milk. This emulsion consists of from two to three parts of milk to one of lard ; it can be made at one factory and taken to another to be used for cheese, but it is usually run at once into the cheese vat.

"In making the cheese a quantity of this emulsion, containing about 80 lbs. of lard, is added to 6000 lbs. of skimmed milk and about 600 lbs. of butter milk in the cheese vat, and the lard that does not remain incorporated with the milk or curd (usually about 10 lbs.) is carefully skimmed off. These quantities of materials yield from 500 to 600 lbs. of cheese, containing about 70 lbs. of lard, or about 14 per cent. About half of the fat removed in the skimming of milk is replaced by lard."

An Act of Congress, passed to prevent the sophistication of cheese, describes filled cheese as "all substances made from milk or skim milk, with the admixture of butter, animal oils or fats, vegetable or other oils, or compounds, foreign to such milk, and made in imitation or semblance of cheese." A tax is levied on the weight of filled cheese made, besides which the manufacturer has to pay a tax on the factory, and wholesale and retail dealers in the article are also taxed.

Both samples had the appearance of Cheddar cheese, one being very highly coloured. They were well-prepared, and tasted fairly palatable, the lard cheese, however, having a peculiar flavour.

From the proportion of insoluble fatty acids yielded by the fat of the samples on saponification, Vieth concluded that the fat of the lard cheese contained 63 per cent. and that of the oleomargarine 46 per cent. of milk-fat. In another sample (*Analyst*, x. 9) Vieth found 18 per cent. of fat, yielding 90·78 per cent. of insoluble acids. J. Muter finds the fat from American filled cheese to yield from 90·5 to 92·0 per cent. of insoluble acids.

The author has met with a cheese-fat yielding a Reichert-Wollny figure (on 5 grammes of fat) of 5·74 c.c., representing only about 17 per cent. of milk-fat. R. Bodmer (*Analyst*, 1895, p. 268) found the fat of two samples of filled cheese to contain practically no milk-fat.

R. R. Tatlock found a cheese sold in Glasgow to contain 12·56 per cent. of milk-fat and 14·12 per cent. of foreign fat.

The mineral matter of cheese, apart from added salt, &c., consists chiefly of calcium phosphate. According to experiments by Mariani and Tasselli (abst. *Analyst*, xx. 168), the proportion of phosphoric acid in cheese-ash is always greater than is necessary to convert the lime into tricalcic orthophosphate, the excess of P_2O_5 being greatest in Edam and skim-milk cheese, and least in cheese made from sour milk. The excess of phosphoric acid is supposed by Mariani and Tasselli to exist as acid calcium phosphate. The largest amounts of lime and phosphoric acid were found in sheep's-milk cheese and cheese made from sour milk. Hence, the acidity of milk does not appear to prevent the precipitation of calcium phosphate with the curds.

The proportion of *sodium chloride* present in cheese varies necessarily with the kind of cheese and mode of salting adopted, poor cheese requiring for its preservation a larger amount of salt than cheese rich in fat. The highest proportion of salt met with in the author's experience was 4·8 per cent., which occurred in a Dutch cheese. The proportions found by Muter in various kinds of cheese (other than "English cream-cheese") ranged from 0·75 per cent. in Stilton to 4·02 per cent. in Dutch cheese.

Heavy metals are occasionally met with in cheese, owing to the improper employment of preparations containing them. Thus *lead chromate* has been found in cheese-rind, owing to the cheese having been wrapped in a cloth covered with that pigment. It is not improbable that lead chromate has been occasionally used as a substitute for the annatto or other harmless vegetable colouring matter almost universally employed for colouring ordinary cheese. According to James Bell, it is the practice in some

cases to paint the outside of cheese with *venetian red*, and it is said that solutions of poisonous metals, such as salts of *copper* and *lead*, have sometimes been brushed over the outside of the cheese to preserve it from parasitic attacks.

F. W. Stoddart (*Analyst*, 1896, page 208; 1897, page 2) has recorded a case in which cheese of Canadian manufacture was found to be darkened in parts. The discoloration, which to the eye exactly resembled moulding, was due to an amorphous powder, apparently consisting of lead-dust. The specimen of cheese examined contained on the average 1¼ grain of lead per lb., the metal being distributed in veins, which gave the cheese a mottled appearance.[1] Out of a consignment of 200 cheeses, about 150 were affected in various degrees. The absence of sulphides from the cheese negatived the view that the colouring matter consisted of lead sulphide produced from some other lead compound, while the fact that the blackened portions reduced copper and silver from their salts (parallel experiments with sound cheese giving a negative reaction in the former case and only slight reduction in the latter), render it probable that the pigment consisted of finely-divided metallic lead. Stoddart considers it probable that the impurity was introduced accidentally by friction upon some leaden surface, but in the opinion of the author it is more likely to have had its origin in some compound of lead (*e.g.*, chromate, carbonate, acetate) purposely added to the cheese.

F. Hudson-Cox has called attention to the fact that in some places in Wiltshire *zinc sulphate* is habitually used "to prevent the heading and cracking of cheese." This objectionable practice is less common than was formerly the case, but zinc sulphate is still openly supplied by qualified druggists, under the description of "cheese-spice."[2] One preparation purchased by the author in 1897 consisted simply of crystallised zinc sulphate, while another was an aqueous solution containing zinc sulphate in quantity corresponding to 38 grammes of the crystallised salt per 100 c.c. R. Bodmer recently found zinc in two specimens of cheese sold in Southwark. One of these, a pale cheese resembling Cheddar, contained *zinc* in a proportion corresponding to 3·7 grains of crystallised sulphate per pound; while

[1] For many months, mice refused to eat this cheese, though they consumed the greasy paper in which it was wrapped. On the other hand, the cheese ultimately became infested with "jumpers" to such an extent as to necessitate its destruction.

[2] In the case of cheese prepared on a small scale by Hudson-Cox and the author, with the addition of a solution of one gramme of crystallised zinc sulphate per gallon of the milk, it was found that upwards of 99 per cent. of the total zinc combined with the curd, only traces being contained in the whey.

the other sample, a reddish cheese resembling Gloucester, contained zinc equivalent to 2·5 grains of the crystallised sulphate per pound. There is little doubt that the presence of zinc in these cheeses was due to the employment of the sulphate as a "cheese-spice." (See *Analyst*, 1897, page 187.)

In 1841, several persons died at Chantillon from eating cheese to which a preparation of *arsenic* had been added as a preservative, and in 1854 a family in Paris was poisoned, though not fatally, from the same cause.

A cheese which caused the illness of about 300 persons in Michigan, in 1886, was found by V. C. Vaughan to contain the poisonous ptomaïne *tyrotoxicon* (Vol. III. Part iii. page 347. See also H. A. Weber, *ibid.*, page 350).

Cheese which produces symptoms of irritant poisoning in human beings is not infrequently fatal to mice and guinea-pigs.

ANALYSIS OF CHEESE.

The analytical examination of cheese presents no great difficulties, so far as the main constituents are concerned.

Moisture.—For the determination of the water in cheese, about 5 grammes should be removed from the cheese by means of a cork-borer or cheese-sampler, and the exact weight ascertained. It is then mixed in a mortar with an exactly-known weight (about 20 grammes) of recently-ignited sand or pumice-stone, transferred to a flat-bottomed capsule by means of a spatula, and the last portions of the mixture removed from the mortar and spatula with a small piece of filter-paper of known weight. The whole is then exposed in the water-oven till practically constant in weight, the loss being regarded as the water in the portion of cheese taken for analysis.

A simpler method, which answers fairly well when the cheese is not very rich in fat, is to reduce the weighed portion to thin slices or shavings, and dry these direct in the water-oven.

Fat.—The portion of cheese already used for the determination of water may be employed for ascertaining the amount of fat. If not already mixed with sand, it is incorporated with sufficient of this or similar substance to allow of its being reduced to powder, and is then exhausted with ether, petroleum-spirit, or other solvent of fat. The process may be conducted in a Soxhlet's tube, but it is preferable, in the author's opinion, to boil the mixed cheese and sand direct with the solvent, which is then poured off into a suitable flask, and the treatment repeated three or four times. The solvent is then distilled off and the residual fat weighed.

Petroleum-spirit, which has been redistilled below 100° C., is preferable to ether for the extraction of cheese-fat, as the latter solvent is liable to dissolve sensible quantities of lactic acid.

It not infrequently happens that a determination of fat is required where the proportion of water is not of interest. In such cases, about 2 grammes of the cheese should be mixed with about five times its weight of copper sulphate, previously rendered anhydrous by gentle ignition. The salt rapidly combines with all the water of the cheese, and the mixture may be at once extracted in the Soxhlet-tube.

In cases where an approximate determination of the fat in cheese will suffice, a convenient plan is to reduce 10 grammes of the sample to the condition of shavings or thin slices, and treat it in a graduated tube with 30 c.c. of fuming hydrochloric acid. The tube is immersed in boiling water until the melted fat forms a well-defined layer on the surface. The volume of fat is read off without allowing cooling to take place. Each 0·1 c.c. of cheese-fat measured at the boiling point of water weighs 0·0865 gramme.

Instead of measuring the volume of the separated fat, the contents of the tube may be allowed to cool, and then agitated with ether or petroleum-spirit, in the manner described on page 135.

Pearmain and Moor determine the fat on 2 grammes of cheese by an adaptation of Leffmann and Beam's method (page 139).

It is often requisite not merely to determine the fat in cheese, but to extract sufficient of it to allow of its analytical examination for the detection of foreign fats, as in filled cheese. For this purpose, at least 50 grammes of the sample should be reduced to shavings, and placed in a beaker. This is heated in the water-oven, the contents being stirred at intervals, until the greater part of the fat has separated. This is then poured off, and submitted to further examination. If requisite, the fat may be filtered through paper, in the water-oven.

O. Henzold prepares the fat of cheese by crushing 300 grammes in a mortar, and shaking the crushed sample with 700 c.c. of a 5 per cent. aqueous solution of caustic potash. The casein dissolves in a few minutes, and the fat rises to the surface in small lumps, which can be made to coalesce by gently moving the flask. The flask is then filled with cold water, when the solidified globules of butter can be collected, washed in cold water (to remove traces of alkali, and assist the solidification of the fat), pressed to remove as much of the water as possible, melted, and filtered. The fat is said to be unchanged by the foregoing treatment.

Lactose.—The unchanged sugar contained in cheese may be determined by macerating the sample with hot water, filtering, and ascertaining the reducing action of the filtrate on Fehling's or Pavy's solution.

Lactic acid.—The free acid of cheese is chiefly lactic acid, and

is generally expressed in terms of that body. But in matured cheese the acidity is by no means a measure of the total lactic acid present, since the ammonia resulting from the fermentation of the casein neutralises any free acid previously present. (The determination of the total lactic acid of cheese may be effected in the manner described in Vol. III. Part iii. page 411, *et seq.*)

Proteids.—For ordinary purposes, a sufficient approximation to the amount of total proteids present in cheese is obtained by determining the nitrogen by Kjeldahl's method (page 21) and multiplying the result by 6·33. The product represents, with a fair approach to accuracy, the casein or other proteids originally present. But during the process of maturing, which is essentially one of bacterial fermentation, the original proteids undergo material changes, so that the ripened cheese contains, besides unchanged casein, &c.:—more or less albumoses and peptones; amido-compounds, such as leucine; ammonia; and certain bodies of unknown constitution.

Van Ketel and Antusch (*Nederl. Tijdschr. Pharm.*, 1897, 82) conclude, from the analysis of a number of cheeses, that only about 80 per cent. of the nitrogen exists in the form of proteids, the remainder being present chiefly as ammonia and amido-bodies. The nitrogen existing as ammonia is determined by distilling the sample, previously powdered with the addition of sand, with water holding barium carbonate in suspension. The distillate is received into a measured quantity of standard sulphuric acid, and, after boiling, the excess of acid is neutralised with standard soda, using rosolic acid as indicator. The nitrogen existing as amido-compounds is estimated by macerating the powdered cheese with water for fifteen hours at the ordinary temperature. After adding a little dilute sulphuric acid (1 : 4), the proteids and peptones are precipitated by phospho-tungstic acid. The precipitate is filtered off, and then washed with water containing a little sulphuric acid. The filtrate is made up to a definite bulk, and the nitrogen is determined in an aliquot part of the liquid by Kjeldahl's process, allowance being of course made for the nitrogen existing as ammonia. The peptones and albumoses are determined jointly by boiling the powdered cheese (mixed with sand, as before) with water and filtering from the undissolved casein and albumin. In an aliquot part of the filtrate, the peptones and albumoses are precipitated by adding dilute sulphuric acid and phospho-tungstic acid. After washing with acidulated water, the precipitate is submitted to Kjeldahl's process. The total nitrogen of the cheese is also determined by Kjeldahl's process, and, after allowing for the nitrogen existing in other forms, the balance is

calculated to casein. The amount of indigestible casein was found to be very trifling.

An elaborate method for differentiating between the various classes of nitrogenised compounds existing in matured cheese has been described by A. Stütz er (*Zeit. Anal. Chem.*, 1896, xxxv. 493; *Analyst*, xxii. 14). The following figures show the results obtained by Stützer in three cases :—

	Camembert.	Swiss.	Gervais.
	Per cent.	Per cent.	Per cent.
Water,	50·90	33·01	44·84
Fat,	27·30	30·28	36·73
Fat-free organic matter,	18·66	31·41	15·48
Ash,	3·14	5·30	2·95
The ash contained—			
Calcium,	0·03	1·56	0·14
Phosphoric acid,	0·76	0·82	0·23
Sodium chloride,	2·21	1·56	0·76
Total nitrogen,	2·900	5·072	1·923
Nitrogen as ammonia,	0·386	0·188	0·031
,, amides,	1·117	0·459	0·069
,, albumoses and peptones,	0·885	0·435	0·298
,, indigestible matter,	0·115	0·119	0·166
,, casein and albumin,	0·397	3·871	1·329
In 100 parts of nitrogen there existed :—			
As ammonia,	13·0	3·7	1·6
,, amides,	38·5	9·0	5·2
,, albumoses and peptones,	30·5	8·6	15·5
,, indigestible matter,	4·0	2·4	8·6
,, casein and albumin,	14·0	76·3	69·1
Percentage of casein and albumin dissolved by pepsin solution :—			
In 30 minutes,	100	68	52
,, 60 ,,	100	91	75

The water and ash were determined in the usual way. The fat was determined by extracting the dry residue from the water determination with dry ether.

Filled cheese may be recognised by the results yielded by the analysis of the fat after isolation in the manner directed on page 249. The Koettstorfer and Reichert-Wollny methods are to be preferred, and have the advantage of requiring only a limited quantity of the fat for their application. Owing to the changes undergone during the ripening of the cheese, the figures yielded by cheese-fat are somewhat different from those ordinarily obtained from butter. Thus the volume of alkali required when the Reichert-Wollny process is applied to cheese is sensibly higher

than in the case of butter, frequently exceeding 30 c.c. of decinormal alkali for neutralisation of the distillate from 5 grammes of fat, and on one occasion in the author's experience reaching the outside figure of 38·6 c.c. The amounts of alkali neutralised by the fat when saponified (as in Koettstorfer's process) are similarly abnormal, when fully-matured cheese is under examination.

E. Solberg (*Bied. Centralb.*, 1896, xxv. 15; abst. *Jour. Chem. Soc.*, 1896, ii. 378) has recorded the following figures obtained by the analysis of the fat obtained from samples of cheese manufactured respectively from goat's milk and reindeer's milk:—

	Goat-milk Fat.	Reindeer-milk Fat.
Melting point, ° C.,	27 to 38·5	37 to 42
Solidifying point, ° C.,	24 to 31	34 to 39
Specific gravity (average) at 15° C.,	0·9312	0·9428
Specific gravity (average) at 100° C.,	0·8669	0·8640
Refraction-coefficient,	1·4596	1·4647
Acid number (=per cent. of KHO required for neutralisation),	0·395 to 1·388	2·760
Saponification-number (= per cent. KHO required for saponification),	22·16	21·92
Insoluble fatty acids (Hehner's number),	86·46 to 87·34	86·89
Reichert-number,	23·1 to 25·4	31·4
Iodine absorption,	30·4 to 34·6	25·1
Lecithin, per cent.,	0·1 to 0·2	0·21

Goat's milk fat presents many resemblances to the fat of cow's milk, but contains a larger proportion of insoluble volatile acids. In reindeer-milk fat the amount of insoluble volatile acids is much less.

Solberg's results must be received with caution, as the original fat is likely to have undergone change during the ripening of the cheese.

Milk-Sugar. Lactose.

Milk contains a peculiar kind of sugar called lactose, which differs in certain particulars from the sugars obtained from other sources.

The occurrence of lactose is, however, not strictly confined to milk. It has been found in the urine of nursing women,[1] and galactose has been alleged to occur in certain plants.

[1] Lactose was isolated from urine by Hofmeister (*Zeit. physiol. Chem.*, i. 101), by precipitating the liquid with neutral lead acetate, treating the

The question of the identity of the sugar contained in the milk of various mammals has been studied with discordant results. Denigès concludes that they are strictly identical (see page 99). H. D. Richmond states that the sugar of asses' milk is identical with that of cows' milk. From a specimen of the milk of the gamoose or Egyptian buffalo, Pappel and Richmond isolated a distinct species of sugar, to which they gave the name "tewfikose" (*Jour. Chem. Soc.*, lvii. 754); but a second preparation of sugar from the milk of the gamoose agreed in all respects with the lactose of cows' milk.[1]

Milk-sugar belongs to the saccharose family, containing, when anhydrous, $C_{12}H_{22}O_{11}$, though it commonly crystallises with a molecule of water. The hydrated crystals are hard, white, semi-transparent, slightly hygroscopic, hemihedral, rhombic prisms or saccharoid masses, containing $C_{12}H_{22}O_{11}+H_2O$. Hydrated lactose is unaltered at 100° C., but the water is driven off with some difficulty by heating to 130°, with production of melted anhydrous lactose, which solidifies on cooling to a crystalline, very hygroscopic

filtrate with ammonia, and precipitating the refiltered liquid once again with lead acetate and ammonia. These two last precipitates were decomposed by sulphuretted hydrogen, the filtrate shaken with oxide of silver, and the filtered liquid freed from silver by sulphuretted hydrogen. Barium carbonate was next added, and the liquid evaporated. On treating the residue with alcohol, the milk-sugar was dissolved, and obtained in a crystalline form by evaporating the solution *in vacuo* over sulphuric acid. Kaltenbach obtained mucic acid and galactose from the body thus isolated, thereby conclusively identifying it with milk-sugar.

F. A. Lemaire has more recently (*Zeit. physiol. Chem.*, 1896, xxi. 442; abst. *Jour. Chem. Soc.*, 1896, ii. 490) confirmed the presence of lactose in the urine of women after child-birth, by employing Baumann's benzoic chloride method of separating carbohydrates, and then preparing the osazones. He confirms Baisch's observation that normal urine contains glucose and a dextrinoid body, and isomaltose was also found. In fifteen cases lactose was found after child-birth, but not before delivery. Lemaire gives the following figures :—

	Ante Partum.	*Post Partum.*
Glucose, . . .	0·004 to 0·008 per cent.	0·007 to 0·014 per cent.
Isomaltose, . . .	0·001 to 0·002 ,,	0·002 to 0·0035 ,,
Lactose, . . .	None.	0·01 to 0·04 ,,

[1] From a private communication of H. D. Richmond to the author, it appears that the sugar of human milk crystallises in a different form from, and more easily than, ordinary lactose. The value of $[a]_D$ is only +48·7°, but the cupric oxide reducing-power differs only slightly from that of the latter sugar. The sugar of human milk gives much mucic acid on oxidation with nitric acid, and by reaction with phenyl-hydrazine yields an osazone resembling phenyl-lactosazone.

mass. The product is not further altered at 150° C., but at 170° to 180° it turns brown and yields lacto-caramel, $C_6H_{10}O_5$, with the loss of the elements of water.

When an aqueous solution of milk-sugar is evaporated rapidly to dryness, as occurs in the process for determining the total solids in milk, the sugar is obtained in the anhydrous state, but the product is not hygroscopic.

According to Hesse, a freshly-prepared saturated aqueous solution of milk-sugar contains 14·55 per cent. of the hydrated substance, but after standing over the crystals (or immediately on boiling the liquid) the solution is found to contain 21·64 per cent., or about half as much again.[1] This change in solubility is regarded by Hesse as related to the size of the molecules, for the specific rotatory power of the two modifications of milk-sugar which may be assumed to exist in the solutions is in inverse proportion to their solubility in water. Thus a freshly-prepared solution of lactose is supposed by Hesse to contain the α-modification, and a solution which has been kept the β variety. The value of $[a]$ for the α variety is $+80°$, and for the β kind $+52·7°$ for a concentration of 12 per cent., and $+53·2°$ for solutions of 3 per cent. As the latter modification is more soluble than the former, in the proportion of about two to three, it follows that saturated solutions of either modification will exert the same angular rotation on a ray of polarised light.

This peculiar behaviour to polarised light must be borne in mind when it is desired to effect the determination of milk-sugar by the polarimeter.

As it occurs in solution in milk, milk-sugar exhibits a dextrorotation which, for solutions not exceeding five per cent. in concentration, is, according to the most reliable observations, $+52·5°$ for the sodium-ray.[2] This value, which happens to be almost

[1] On the other hand, Richmond finds the initial solubility of hydrated milk-sugar at 15° C. to be about 7·5 grammes per 100 c.c., the solution being attended with a fall of temperature of about 0·5° C. No thermal change (or less than 0·01° C.) occurred in a solution the rotation of which was rapidly falling.

[2] L. Grimbert (abst. *Jour. Chem. Soc.*, 1888, p. 329) gives the rotation of crystallised milk-sugar as $+52·37°$, and states that this figure is independent of the concentration of the solution. He finds that the specific rotation of the substance when freshly dissolved to that after it has become stationary is in the ratio of 8 : 5. Schmöger has published results obtained with solutions of milk-sugar ranging in strength from 4 to 36 per cent., and finds the value of $[a]^D$ for hydrated lactose to be $+52·53° + (22 - t) \times 0·055$. This gives a value of $+55·30°$ for anhydrous lactose at 20° C. The results of

identical with the specific rotation of dextrose under similar conditions, corresponds to $[a]_D = +55{\cdot}27°$ for the anhydrous sugar.

When crystallised milk-sugar is dissolved in cold water the solution at first exhibits strongly the phenomenon of bi-rotation, that is, the optical activity of the newly-made solution approaches double of the activity of milk-sugar as it exists in milk.[1] On keeping the solution at the ordinary temperature for twenty-four hours, or immediately on heating it to the boiling point, the optical activity falls to the normal value of $[a]_D = +52{\cdot}5°$ for the hydrated sugar.

If crystallised milk-sugar be exposed to a temperature of about 130° C., the anhydrous substance so obtained exhibits strong bi-rotation when dissolved in cold water, but on keeping the solution at the ordinary temperature, or immediately on boiling it, the activity falls to the normal value.

On the other hand, if a solution of pure milk-sugar be rapidly evaporated, the anhydrous substance so obtained has an optical activity far below the normal, but this "semi-rotation" disappears on keeping or boiling the solution.

A modification of anhydrous milk-sugar exhibiting only slight bi-rotation is obtained by quickly evaporating a solution of milk-sugar in presence of some indifferent material which ensures distribution over a large surface. This modification, which is not impossibly a mixture of two of those previously mentioned, exists in the residue obtained by evaporating milk for the determination of total solids.

The specific gravity of ordinary crystallised milk-sugar is about 1·545, while that of the anhydrous substance is only about 1·53 (Richmond).

According to O. Hehner, an aqueous solution containing 10 grammes of anhydrous lactose per 100 c.c. has a specific gravity of

Denigès and Bonnans (abst. *Jour. Chem. Soc.*, 1888, p. 933) agree closely with the above.

Parens and Tollens find the value of $[a]_D$ to be +52·53° for hydrated milk-sugar in 10 per cent. solution.

E. W. T. Jones (*Analyst*, xiv. 82) finds a somewhat lower value. Using a Soleil-Ventzke-Scheibler polarimeter, he finds the specific rotatory power of hydrated milk-sugar in 5 per cent. solution to be +51·9° for the D ray, which corresponds to +54·6° for anhydrous lactose.

[1] Richmond finds that when the α-modification of lactose is dissolved in cold water the specific rotation remains constant for a short time and then gradually diminishes, the ratio of the initial to that of the constant optical activity averaging 1·601 : 1·000. Mixtures of the α- and β-modifications of lactose, as obtained by precipitating an aqueous solution with alcohol, also exhibit a stable initial rotation for a short period.

1039·1 at 15·5° C. (= 60° F.), and E. W. T. Jones finds a solution of 5 grammes of crystallised lactose per 100 c.c. to have a gravity of 1018·6. These results are in absolute concord, and show that the number of grammes of anhydrous milk-sugar contained in 100 c.c. of an aqueous solution may be found by dividing the excess-gravity (= sp. gr. − 1000) by 3·91. For hydrated milk-sugar the divisor is 3·72.

Lactose reacts with caustic alkalies and alkaline earths to form compounds of an unstable character. By the prolonged action of alkalies on milk-sugar, lactic acid and catechol are formed, while by the action of fused potash oxalic acid results.

When boiled with dilute sulphuric or hydrochloric acid, milk-sugar undergoes hydrolysis, with conversion into two glucoses, dextrose and galactose, both of which are dextro-rotatory. The latter product is said to occur naturally in certain plants, and appears to be identical with the "cerebrose" obtained by Thudichum by the action of dilute sulphuric acid on certain constituents of the brain.

It has been observed by Stokes and Bodmer (*Analyst*, x. 62) that milk-sugar does not undergo hydrolysis when its solution is boiled with citric acid, whereas cane-sugar suffers rapid and complete inversion. Upon this fact is based their method of determining cane-sugar in presence of milk-sugar, as required in the analysis of sweetened condensed milk (see page 172). Stokes and Bodmer add 2 grammes of citric acid to 100 c.c. of the milk-sugar solution and boil the liquid for not less than ten minutes. E. W. T. Jones (*Analyst*, xiv. 82), who confirms the value of their process, adds 1·6 grammes of citric acid per 100 c.c., and heats the flask in boiling water for half an hour.

On treatment with nitric acid, lactose first suffers hydrolysis, the dextrose subsequently undergoing conversion into saccharic acid and the galactose into the isomeric body mucic acid, $C_6H_{10}O_8$. Smaller quantities of tartaric, racemic, and oxalic acids are also formed, and if concentrated nitric acid be employed for the oxidation, the last product predominates. The formation of mucic acid affords a means of detecting and identifying milk-sugar, but mucic acid is also produced by the oxidation of dulcite and melitose.[1]

[1] For the preparation of mucic acid, milk-sugar should be heated on the water-bath with about four times its weight of nitric acid of 1·27 specific gravity until gas is copiously evolved, when the mixture is maintained at about 60° C. until it begins to acquire a brown colour or the evolution of gas ceases. The liquid is then diluted with half its measure of water and allowed to stand. On cooling, mucic and oxalic acids crystallise out, saccharic acid

On gradually adding milk-sugar to a well-cooled mixture of concentrated sulphuric and nitric acids, nitro-lactoses are formed, and are precipitated on diluting the solution with water. If the temperature be allowed to rise the lactose is oxidised with violence. By heating with acetic anhydride, lactose yields several acetates, at least one of which, the hexacetate, containing $C_{12}H_{16}O_5(C_2H_2O_2)_6$, is obtainable in a crystalline form.

Milk-sugar reduces Fehling's and Pavy's alkaline copper solutions on boiling, and precipitates metallic silver from the ammonio-nitrate even in the cold. The reducing action of lactose on cupric solutions is employed for its determination (see page 148).

Deon has observed (*Bul. Soc. Chim. Paris*, xxxii. 123) that if a solution of milk-sugar be boiled with lead acetate, and a little ammonia added, a yellow coloration is produced, changing to a cinnabar-red precipitate.

With phenyl-hydrazine, milk-sugar reacts to form a crystalline phenyl-lactosazone, $C_{24}H_{32}N_4O_9$, which somewhat resembles the analogous body yielded by dextrose. The compound is best prepared by heating a solution of 1 part milk-sugar in 30 parts of water, with $1\frac{1}{2}$ parts of phenyl-hydrazine hydrochloride and 2 parts of sodium acetate on the water-bath, for an hour or two. On allowing the liquid to cool, the phenyl-lactosazone crystallises out in yellow needles. The crystals are much broader than those of phenyl-dextrosazone, melt at 200° instead of at 204°, and dissolve more readily in alcohol and in hot water; but separate out again on cooling. Phenyl-lactosazone is converted with great facility (as by heating with very dilute sulphuric acid) into an anhydride which crystallises in long, yellow, silky needles, which are almost insoluble in hot water and melt at 223° with evolution of gas. This anhydride is often obtained instead of the parent lactosazone on applying the phenyl-hydrazine test to milk-serum.

For the detection of milk-sugar, the physical characters of the isolated sugar and its reactions with nitric acid and phenyl-hydrazine are the most serviceable. For the determination of lactose, the sugar may be isolated in a state of purity, or the amount present deduced from the specific gravity, optical activity, or cupric oxide reducing-power of the solution.

remaining in solution. The mucic acid may be purified by treatment with warm alcohol, in which only the oxalic acid dissolves.

Mucic acid forms a sandy crystalline powder or crystalline plates. It is nearly insoluble in cold water or alcohol, and requires sixty-six parts of boiling water for solution. The mucates are mostly insoluble. Neutral potassium mucate is only sparingly dissolved by water, but the acid salt is somewhat more soluble.

Milk-sugar is not directly fermentable by yeast—that is, lactose is not a food for yeast; but when, by the action of acids, it has suffered hydrolysis with formation of dextrose and galactose, both these glucoses are susceptible of the ordinary alcoholic fermentation.

Milk-sugar is directly converted into alcohol by another organism which requires further investigation. This organism is developed when milk is allowed to stand in wooden vessels, and is predominant in the production of koumiss (page 233). An alcohol-producing organism is concerned in the production of kephir (page 236).

The conditions under which milk-sugar, in the pure state and as present in milk, undergoes the alcoholic fermentation have been studied by P. Vieth (*Analyst*, xii. 3).

In presence of cheese or gluten, milk-sugar readily undergoes the lactic fermentation (page 194). In practice, a small amount of alcohol is always produced simultaneously, especially if the lactic acid is not neutralised as it is formed (*e.g.*, by addition of chalk). The formation of alcohol occurs with greater facility in dilute solutions.

Milk-sugar is not affected by pepsin, rennet, or pancreatic extract.

PREPARATION OF MILK-SUGAR.

On the small scale, milk-sugar is commonly prepared by curdling milk with rennet or sour whey, removing the coagulum, and evaporating the whey to a thin syrup. The crystals which gradually separate on standing in the cold are purified by crystallisation from hot water, re-solution, filtration through animal charcoal, and re-crystallisation on strings or pieces of wood placed in the solution. The product may be further purified by re-solution in water and precipitation by alcohol, in which menstruum milk-sugar is but sparingly soluble.

A very similar process is employed for the preparation of milk-sugar on the large scale. J. Kunz, writing in 1884 (*Pharm. Jour.*, [3], xv. 443), states that in Switzerland[1] the manufacture of milk-sugar is carried on almost exclusively in two stages. The first of these is the production of the crude milk-sugar or "sugar-sand," and

[1] Kunz points out that as the previous removal of the proteids and fat is essential, the manufacture of milk-sugar is almost exclusively combined with that of "Swiss" cheese, particularly in the Alps, where the uniform method of feeding, the low temperature, and the fact that the milk is usually worked up for cheese at the place of its production, combine in facilitating an almost complete separation of the various constituents of the milk. Though insignificant in its proportions compared with the production of cheese and butter, the manufacture of milk-sugar is of considerable magnitude. It is carried on chiefly in the cantons of Berne and Lucerne.

the second is the purification of this crude material by recrystallisation. The two processes are usually conducted in different establishments and by different firms. From 300 to 1000 litres of whey is boiled and sufficient acid whey or whey-vinegar added to cause the separation of a cream-like scum on the surface. This is skimmed off, and more of the whey-vinegar added in the proportion of about 3 per cent. This effects the breaking of the whey, the proteids still present rising to the surface in large lumps. The coagulum is skimmed off, and the resultant limpid, greenish liquid (amounting to about 70 per cent. of the original milk) boiled down in flat-bottomed copper "kettles," so set as to be heated at the bottom only. When reduced to about one-fifteenth of its original volume, the solution is baled out into a wooden vessel and allowed to cool. The crystals which form during twenty-four to forty-eight hours have a saline taste, to remove which they are treated with very cold water. After separation of the water, the sugar, which now varies in colour from bright yellow to nearly white, is placed in sacks, from which the excess of water gradually drains away.[1] The strongly saline residuary liquors contain much potash, phosphates, &c., and find an application as fertilisers.

The sugar-sand obtained in the foregoing manner is sent to the refiner, by whom it is again washed with cold water, and then dissolved in hot water, more of the sand being added until a solution of the proper concentration is obtained. The dark scum which collects on the surface is removed from time to time, until only a faintly-coloured skin is formed. Various clarifying agents are sometimes added at this stage, to effect a more perfect removal of the impurities. The liquid is removed while still boiling-hot to copper or copper-lined vessels, in which the sugar is allowed to crystallise. To promote the formation of large crystals, sticks of wood of the diameter of a quill are hung in the liquid. When the crystallisation is complete, which occurs in about ten days, the mother-liquor is run off rapidly, since while in motion it deposits sugar in powdery crystals which spoil the appearance of the main product. The crystals are next washed and dried at a moderate temperature. The drying must be thorough, or the sugar will eventually become mouldy.

In the manufacture of milk-sugar, the quality of the clarified

[1] When properly boiled, 100 litres of clarified whey yield on the average about 3 kilogrammes of washed, drained, and marketable "sand." This product often contains from 15 to 20 per cent. of water, a portion of which drains away during transit. In the wet state, the sand readily becomes mouldy, and hence it is important that it should reach the refiner as soon as possible.

whey, which should be clear and sweet, the manner of setting the copper kettles, and the manipulation of the boiling, all have an important influence on the success of the process.[1] All attempts to manufacture milk-sugar direct from the whey, on the principle on which loaf-sugar is made from beet-juice, have failed to yield good results in practice, the removal of the proteids, fat, and salts being essential to the production of a good crystalline product.[2]

According to an invention by J. Y. Johnson (*English Patent*, No. 13,444, 1890), the whey is neutralised by slaked lime or chalk, boiled, and filtered from the partially-precipitated proteids. The greenish turbid liquid is evaporated to half its bulk, and is treated whilst still hot with a sufficient amount of a hot concentrated solution of equal parts of washing soda and alum. Any acid (except nitric acid), or any one of the known precipitants of proteids, together with a solution of rennet, may be used instead of the washing soda and alum. The filtrate is neutralised as before, passed through filters of animal charcoal, and evaporated *in vacuo* to the crystallising point. The crystals are washed free from salts, dried, and powdered, whereby a marketable article is obtained.

C. L. Penny, of the Delaware Experiment Station, has recommended the following method as being specially suited for use in creameries. The heated whey is clarified by the addition of a hot solution of aluminium sulphate, and after filtration through wire-gauze, powdered chalk is added. The excess of alumina added is precipitated, together with any proteids not already got rid of. The prevention of foaming of the solution is secured by the addition of ground oak-bark, three or four lbs. of oak-bark to every 100 lbs. of skim-milk being sufficient. A more convenient form of the oak-bark—the commercial tanner's extract of oak-bark—may be used, about half-a-pound being sufficient for the 100 lbs. of skim-milk. The foaming of the solution is the cause of much

[1] Kunz states that, when the boiling is badly managed, the syrup in the kettle foams strongly, its volume, in spite of the continued boiling, becomes greater, and it acquires a darker colour. On cooling such a syrup, it yields a jelly-like mass, intermingled with but a small quantity of sugar crystallised in the form of grains of the size of small shot.

[2] The milk-salts alone amount to 0·5 per cent. of the whey, or about 10 per cent. of the contained sugar. The specific gravity of the whey ranges from 1·026 to 1·028, and is stated by Kunz to contain :—

Milk-sugar,	4·5 to 5·0 per cent.
Lactic acid,	0·2 to 0·5 ,,
Nitrogenised substances and fat,	0·5 to 1·0 ,,
Salts,	0·5 to 0·7 ,,
Water,	94·2 to 92·8 ,,

loss of sugar, besides retarding the process. The purified whey is then evaporated as usual.

COMMERCIAL MILK-SUGAR.

Milk-sugar is now largely employed in both allopathic and homœopathic pharmacy, and enters extensively into the composition of certain infants' foods. It also finds applications in the preparation of lactic acid, in silvering glass, &c.

When kept for some time in dry, warm rooms, crystallised milk-sugar loses any water mechanically enclosed in the crystals. Hence old stock is always preferred to new. Unseasoned milk-sugar is apt to become sticky in the process of grinding, and often forms a mass resembling caoutchouc. With well-dried milk-sugar no such difficulty occurs.

The British Pharmacopœia (1885) describes the characters of milk-sugar as follows :—"Usually in cylindrical masses, two inches in diameter, with a cord or stick through the axis, or in fragments of cakes ; greyish-white, crystalline on the surface and its texture translucent, hard, scentless, faintly sweet, gritty when chewed. Soluble in seven parts of water at common temperatures and in about one part of boiling water." The United States Pharmacopœia (1890) describes milk-sugar as soluble in about six parts of cold water, forming a solution neutral to litmus. On treating a hot saturated solution of milk-sugar with an equal measure of dilute caustic soda solution and gently warming, the liquid turns yellow and brownish-red. The absence of cane-sugar is proved by sprinkling one gramme of the powdered sample upon about 5 c.c. of cold sulphuric acid contained in a flat-bottomed capsule, when the acid may acquire a greenish or reddish, but no brown or brownish-black, colour within half an hour.

Lorin states that a mixture of equal parts of milk-sugar and oxalic acid melts when warmed upon the water-bath, and becomes only very faintly darker in tint. An addition of one per cent. of cane-sugar causes the rapid development of a dark tint on heating, and with several units per cent. the mass acquires a greenish-brown or black colour. Lorin's observation is confirmed by Geissler.

J. O. Braithwaite (*Pharm. Jour.*, 1894, xxiv. p. 853) has called attention to the fact that samples of commercial milk-sugar, which answered all the requirements of the British, German and United States Pharmacopœias, coagulated fresh milk when heated with it nearly to the boiling point. This he found to be due to the presence of magnesium lactate. It was noticed that the coagulation occurred only with those samples which left a high percentage of ash on ignition, and the ash of four samples out of twelve was found to contain magnesia, while in one case calcium

was present in addition. It appeared probable, therefore, that the manufacturer had added magnesia or the carbonate to neutralise the acidity of the whey during crystallisation, with the result that magnesium lactate crystallised out with the milk-sugar. Neutral magnesium lactate was found to coagulate milk, and when 5 per cent. of this salt was added to a milk-sugar which had previously had no action on boiling milk, the mixture caused immediate and complete coagulation.

The following figures by Braithwaite show that the amount of ash should not be allowed to exceed 0·25 per cent., and he suggests that this would be a desirable additional test to those already laid down in the pharmacopœias.

No.	One gramme boiled with ten c.c. of Fresh Milk.	Ash; per cent.	Composition of Ash per cent.	Corresponding to Lactate (Mg or Ca) in Sample.	Acidity in Terms of Lactic Acid per cent.
1	Coagulates.	1·53	1·343 MgO.	6·78 Mg.	0·018
2	No effect.	·21	Mg and Ca.[1]	...	0·018
3	,,	·32	Fe, Mg, and Ca.[1]	...	0·072
4	,,	·10	Mg.[1]	...	0·054
5	,,	·09	Mg.[1]	...	0·036
6	,,	·08	Mg.[1]	...	0·018
7	,,	·03	Mg.[1]	...	...
8	Coagulates.	1·6	1·35 MgO.	6·8 Mg.	...
9	No effect.	·03	Mg.[1]	...	0·072
10	Coagulates.	1·16	0·76 MgO and 0·22 $CaCO_3$	3·83 Mg and 0·47 Ca	0·036
11	No effect.	·02	Mg.[1]	...	...
12	Coagulates.	1·48	1·36 MgO.	6·9 Mg.	0·018

MEAT AND MEAT PRODUCTS.

The lean of meat, or muscle, consists anatomically of the supporting *connecting tissue* and the *muscular fibres*, the latter being themselves composed of the *sarcolemma* (an albuminoid substance closely resembling elastin) and the *contractile substance* enclosed by it.

The *gelatin* and *fat* shown in proximate analyses of muscle are constituents of the connective tissue.

[1] Traces only.

Proteids of Muscle.—During the life of an animal the contractile substance of the muscles has a semi-fluid consistency, and contains a large percentage of proteids together with smaller quantities of extractive matters and salts. By pressing the ice-cold purified muscle of the frog immediately after death under suitable conditions, Kühne obtained the muscle-plasma as a syrupy liquid of faintly alkaline reaction, which at the ordinary temperature soon clotted after the manner of blood-plasma (page 37). The clot consists of *myosin* or *myofibrin*, which bears the same relation to the myosinogen of the live muscle that blood-fibrin does to fibrinogen (compare page 41).

W. D. Halliburton distinguishes five proteids in muscle-plasma, in addition to the hæmoglobin present in red muscle, namely:—*myosinogen* and *paramyosinogen*, which pass into the clot of myosin; *myoglobulin*, which coagulates at 63° C. but otherwise closely resembles the globulin of blood-serum; *muscle-albumin*, apparently identical with blood-albumin; and *myo-albumose* or *myoproteose*, which gives the reactions of Kühne and Chittenden's deutero-proteose, and is closely allied to or actually identical with the muscle-ferment which causes the coagulation of the myosinogen.[1]

Extractive Matters of Flesh.—The nitrogenous non-proteid extractive matters of muscle include creatine (from 0·07 to 0·32 per cent.), creatinine, xanthine and allied bases, urea, taurine, and inosinic acid. Among the non-nitrogenous extractive matters are

[1] O. von Fürth (abst. *Jour. Chem. Soc.*, 1896, ii. 48) agrees with Halliburton with respect to the presence of two proteids in the muscle-clot of myofibrin. Paramyosinogen passes into the condition of myofibrin directly, while in the passage of myosinogen into the same state there is an intermediate soluble stage coagulated by heat at the remarkably low temperature of 40° C. Paramyosinogen forms from 17 to 22 per cent. of the total proteid of muscle, and is regarded by von Fürth as a typical globulin. Myosinogen constitutes from 77 to 83 per cent. of the total proteid, and differs from a globulin in many particulars. The proteid of muscle-serum, called by Halliburton myoglobulin, is not regarded by von Fürth as a definite substance, but merely as a part of the myosinogen which has escaped coagulation. Traces of serum-albumin were detected, but no peptones, albumoses, or nucleo-proteids were found by von Fürth. Whitfield has also failed to find albumoses, peptones, or nucleo-proteids in muscle (abst. *Jour. Chem. Soc.*, 1894, ii. 358), but C. A. Pekelharing (abst. *Jour. Chem. Soc.*, 1897, ii. 61) attributes this last result to Whitfield having extracted the muscle with water, which rapidly becomes acid, and nucleo-proteids are insoluble in dilute acids. By extracting with water containing 0·25 per cent. of sodium carbonate, Pekelharing obtained 2 grammes of a nucleo-proteid precipitable by acetic acid from 543 grammes of flesh.

lactic acid, glycogen,[1] inosite, and a fermentable sugar; besides traces of formic, acetic, and butyric acids.

Mineral Constituents of Flesh. — The ash of muscle ranges from 0·8 to 1·8 per cent. in the flesh in its natural condition, or from 3·2 to 7·5 per cent. in the water-free flesh. The inorganic salts consist chiefly of calcium and potassium phosphates and sodium chloride.

J. König gives the following figures as representing the percentage composition of the *ash* (free from carbon dioxide) of the flesh of terrestrial animals:—

	Minimum.	Maximum.	Mean.
	Per cent.	Per cent.	Per cent.
K_2O,	25·0	48·9	37·04
Na_2O,	0·0	25·6	10·14
CaO,	0·9	7·5	2·42
MgO,	1·4	4·6	3·23
Fe_2O_3,	0·3	1·1	0·44
P_2O_5,	36·1	48·1	41·20
SO_3,	0·3	3·8	0·98
Cl,	9·6	8·4	4·66
SiO_2,	0·0	2·5	0·69

From the recent analysis of the flesh of a large number of animals, J. Katz (*Pflüger's Archiv.*, 1896, lxiii. 1) finds the ash-constituents to vary between the following limits. The figures are parts per 1000 of the fresh flesh:—Potassium, 2·4 to 2·6; sodium, 0·3 to 1·5; calcium, 0·02 to 0·39; magnesium, 0·18 to 0·37; iron, 0·04 to 0·25; and chlorine, 0·32 to 0·8. The phosphorus from phosphates ranged from 1·22 to 2·04; from lecithin, 0·13 to 0·48; and from nuclein from 0·09 to 0·32 parts per 1000.

R. Stockman (*Jour. Physiol.*, 1895, xviii. 484) states that the *iron* contained in the daily diet of an average adult is from 9 to 10 milligrammes; but in the case of chlorotic persons, who take but little food, it is as little as 3 milligrammes. Stockman gives the following as the amounts of iron contained in leading articles of food.

Milk, from 0·002 to 0·0043 gramme per litre.
Oatmeal (dried), 0·0035 per cent.

[1] Glycogen is present in relatively large proportion in the flesh of the horse, and this fact has been utilised as a means of distinguishing it from the flesh of other animals (page 284).

Bread (dried), from 0·0061 to 0·0085 per cent.
Yellow ox-marrow (dried), from 0·0025 to 0·004 per cent.
Red calf-marrow (dried), from 0·0076 to 0·0087 per cent.
Beef-steak (dried), 0·0039 per cent.

Human muscle is stated by W. D. Halliburton to contain 73·5 per cent. of water and 26·5 per cent. of solids, these latter consisting of :—

	Per cent.
Proteids ; including sarcolemma, proteids of connective tissue, vessels and pigments, . . .	18·02
Gelatin, Fat } from the connective tissue of muscle, . .	1·99 3·27
Extractives ; creatine, lactic acid, glycogen, &c., .	0·22
Inorganic salts, ...	3·12
	26·62

According to J. König, the following represents the general composition of *pure muscle* freed from adherent fat, &c.

Water, ...		75·0 to 77·0
Nitrogenised compounds,	Sarcolemma (muscle fibre), . . .	13·0 to 18·0
		2·0 to 5·0
	Albumin, ...	0·6 to 4·0
	Creatine, ...	0·07 to 0·34
	Hypoxanthine, ...	0·01 to 0·03
	Creatinine, Xanthine, Inosinic acid, Uric acid, ...	Undetermined
	Urea, ...	0·01 to 0·03
Fat, ...		0·5 to 3·5
Other Nitrogen-free compounds,	Lactic acid, ...	0·05 to 0·07
	Butyric acid, Acetic acid, Formic acid, Inosite, ...	Undetermined
	Glycogen, ...	(0·3 to 0·5)
Salts, ...		0·8 to 1·8
	Composed of :—	
	Potash, ...	0·40 to 0·50
	Soda, ...	0·02 to 0·08
	Lime, ...	0·01 to 0·07
	Magnesia, ...	0·02 to 0·05
	Oxide of iron, ...	0·003 to 0·01
	Phosphoric acid, ...	0·40 to 0·50
	Sulphuric acid, ...	0·003 to 0·04
	Chlorine, ...	0·01 to 0·07

The following table by Hofmann (*Lehrbuch der Zoochemie*) shows the average composition, in parts per 1000, of the *muscles of vertebrate animals* :—

		Mammals.	Birds.	Cold-blooded Vertebrates.
	Water,	745 to 783	717 to 773	800
Organic matters.	Coagulated albumin, sarcolemma, nuclei, vessels,	145 to 167	150 to 177	?
	Alkaline albuminate,	28·5 to 30·1	...	...
	Creatine,	2·0	3·4	2·3
	Xanthine and hypoxanthine,	0·2	...	...
	Taurine,	0·7 (horse)	0·0	1·1
	Inosite,	0·03	...	...
	Glycogen,	4·1 to 5·0	...	30 to 50
	Lactic acid,	0·4 to 0·7	...	...
Salts.	Potash,	3·0 to 3·9	...	...
	Soda,	0·40 to 0·43	...	...
	Lime,	0·16 to 0·18	...	...
	Magnesia,	0·40 to 0·41	...	...
	Oxide of iron,	0·03 to 0·10	...	...
	Phosphoric acid,	3·4 to 4·8	...	...
	Sodium chloride,	0·04 to 0·10	...	...

Composition of Meat.

The following is the percentage composition of the lean of some of the principal kinds of flesh used for food (Munk's *Physiologie*):—

	Ox.	Calf.	Pig.	Horse.	Fowl.
Water,	76·7	75·6	72·6	74·3	70·8
Proteids and gelatin,	20·0	19·4	19·9	21·6	22·7
Fat,	1·5	2·9	6·2	2·5	4·1
Carbohydrates,	0·6	0·8	0·6	0·6	1·3
Salts,	1·2	1·3	1·1	1·0	1·1

Hence the lean of meat contains four times the proportion of proteids present in milk, and about the same proportion as is contained in the white of egg (page 32).

The following analyses, showing the average composition of fresh meat, are by König:—

Description of Meat.	No. of Samples contributing to average.	Water.	Nitrogenous Matters.	Fat.	Ash.
Very fat ox-flesh,	7	55·42	17·19	26·38	1·08
Moderately fat ox-flesh,	21	73·25	20·78	5·33	1·33
Lean ox-flesh,	9	76·71	20·78	1·50	1·18
Fat cow-flesh,	9	70·98	19·86	7·70	1·07
Lean cow-flesh,	6	76·35	20·54	1·78	1·32
Very fat mutton,	3	47·91	14·80	36·39	0·85
Moderately fat mutton,	8	75·99	17·11	5·77	1·33
Horse-flesh,	12	74·27	21·71	2·55	1·01

A. H. Church[1] gives the following as the composition of a *mutton-chop*, exclusive of the bone, when quite fresh :—Water, 44·1 ; albumin, 1·7 ; fibrin (true muscle), 5·9 ; ossein-like substances, 1·2 ; fat, 42·0 ; organic extractives, 1·8 ; mineral matters, 1·0 ; and other substances, 2·3 per cent.

The following analyses of *animal foods* are also by Church :—

	Water.	Nitrogenous Matters.	Fat.	Mineral Matter.	Remarks.
Tripe, . . .	79·5	10·0	10·0	0·5	The sample was cleansed, boiled, and freed from excess of fat.
Fowl, . . .	...	...	...	...	The nitrogenous matters included ossein-like substances.
Streaky bacon, .	22·3	8·1	65·2	4·4	The ash includes 3·8 per cent. of common salt.
Mackerel, . .	68·7	13·5	12·5	5·3	The ash includes 2·2 per cent. of common salt.

J. König gives the following analyses of the flesh of *wild animals* and of *birds* :—

	Water.	Nitrogenous Matters.	Fat.	Other Nitrogen-free Substances.	Ash.
Hare, ...	74·16	23·34	1·13	0·19	1·18
Rabbit, ...	66·85	21·47	9·76	0·75	1·17
Deer, ...	75·76	19·77	1·92	1·42	1·13
Domestic hen, . . .	76·22	19·72	1·42	1·27	1·37
Wild duck, ...	70·82	22·65	3·11	2·33	1·09
Partridge, ...	71·96	25·26	1·43	...	1·39
Pigeon, ...	75·10	22·14	1·00	0·76	1·00

The following analyses of *animal foods* are due to Payen :—

Food.	Water.	Nitrogenous Matters.	Carbo-hydrates, &c.	Fat.	Ash.
Calves' liver, . . .	72·33	20·10	0·45	5·58	1·54
Sheep's kidneys, . .	78·20	17·25	1·32	2·12	1·10
Foie gras, ...	22·70	13·75	6·40	54·57	2·58
Lobster (fresh), . . .	76·62	19·17	1·22	1·17	1·17
Oysters, ...	80·38	14·01	1·40	1·51	2·69
Mussels, ...	75·74	11·72	7·39	2·42	2·73

[1] "Food ; *Some account of its Sources, Constituents and Uses.*"

Lawes and Gilbert, in their elaborate essay, "On the Composition of some of the Animals Fed and Slaughtered as Human Food" (*Phil. Trans.*, 1859, ii. pp. 493 to 680), give a large number of analyses showing the composition of the entire animals and of various parts thereof. Their results show that the total edible parts of the ten animals analysed contained 3·5 parts of fat for 1 of dry nitrogenous matter. Bread contains 6·8 parts of starch or starch-equivalent to 1 of nitrogenous matter. But 1 part of fat has the same value as a heat-producer as 2·5 parts of starch or other carbohydrate, and hence 3·5 parts of fat are equivalent to 8·75 parts of starch. From these and similar facts, Lawes and Gilbert draw the startling deduction that the employment of animal food to supplement a farinaceous diet results in reducing (and not in increasing) the ratio of flesh-forming material to the peculiarly respiratory and fat-forming capacity of the food consumed. The fallacy of this reasoning lies in the assumption that all the fat of the edible portions of the animals is consumed with the lean, whereas much is removed by the butcher, a further quantity by the cook, and an additional loss occurs in the form of dripping. Hence, instead of the ratio of fat to proteids being 3·5 : 1 the fat actually consumed will, for all kinds of meat except bacon, be less, and often considerably less than the proteids.

Although statements of the composition of various kinds of animal food in the raw state are very numerous, analyses showing the composition of cooked meat are comparatively rare. The following figures may be quoted.

A. H. Church cites the following figures illustrating the composition of cooked *mutton chops*. The two analyses are evidently quite independent, and do *not* represent the composition of the same chop, with and without the gravy and dripping.

	Water.	Nitrogenous Matters.	Fat.	Mineral Matter.	Other Substances.
Cooked chop, including gravy and dripping, ...	54·0	27·6	15·4	3·0[1]	...
Cooked chop, exclusive of gravy and dripping, . .	51·6	36·6	9·4	1·2	1·2

The following results were obtained in the author's laboratory by A. R. Tankard. They represent the composition of various kinds of meat cut from the cold roast joint, and wholly edible. They include such a proportion of fat as would be com-

[1] This figure is evidently a determination by difference, and is in excess of the truth.

monly helped and eaten with the lean, but are exclusive of skin, gravy, and dripping.

	Mutton.	Lamb.	Beef.	Veal.	Pork.	Duck.	Fowl.
Water (loss at 100°), .	39·76	59·89	45·63	51·88	44·90	64·13	67·40
Fat (ether extract), .	26·80	11·95	24·21	11·39	19·67	6·06	6·68
Proteids (N×6·3), . .	29·04	24·69	26·50	32·19	32·63	27·12	24·26
Ash (sulphated), . .	1·93	1·63	1·21	1·57	1·86	2·04	1·37
	97·53	98·16	97·55	97·03	99·06	99·35	99·71
Cold water extract, .	3·74	2·81	3·60	6·55	...	3·70	4·00
containing ash, . .	0·97	0·92	1·10	1·30	...	1·20	0·60

Composition of the Flesh of Fish.

W. O. Atwater (*Amer. Chem. Jour.*, 1887, ix. 421) has published the results of his analyses of a large number of fish caught in American waters. Of these, the following may be quoted :—

Fish.	No. of Samples contributing to average.	Water.	Nitrogenous Matters.	Fat.	Ash.
Herring, ...	2	74·6	14·5	9·0	1·78
Mackerel, ...	8	71·2	19·4	8·0	1·36
Halibut, ...	3	75·2	18·5	5·2	1·06
Conger-eel, ...	2	71·4	18·5	9·1	1·00
Salmon, ...	8	64·3	21·6	12·7	1·39
Cod, ...	6	82·2	16·2	0·33	1·36
Plaice, ...	2	78·3	18·7	1·9	1·01
Sole, ...	1	86·1	11·9	0·25	1·22
Carp, ...	1	76·9	21·86	1·1	1·33

In a subsequent paper (*ibid.*, x. 1) Atwater gives the following data respecting the nature and proportions of nitrogenised matters, &c., in the water-free *flesh of various fishes* :—

Fish.	Coagulable Albumin in Cold-Water Extract.	Non-coagulable Matter in Cold-Water Extract.	Gelatinoïds extracted by Hot Water.	Insoluble Proteids.	Phosphoric Acid.
Herring, .	5·23	4·51	9·46	...	1·77
Mackerel, .	7·27	8·61	5·74	47·37	2·11
Halibut, .	0·42	7·04	12·89	28·14	1·81
Pike, . .	6·95	9·55	10·20	56·71	2·21
Haddock, .	7·89	6·18	16·36	65·06	2·49

Atwater has also compared the composition of the *ash* yielded by the ignition of the flesh of the haddock and the pike, as typical of salt-water and fresh-water fishes respectively :—

Fish.	Ash in Dry Flesh.	Percentage Composition of Ash.						
		K_2O.	Na_2O.	CaO.	MgO.	P_2O_5.	SO_3.	Cl.
Haddock, .	11·26	13·84	36·51	3·39	1·90	13·70	0·31	38 11
Pike, . .	6·13	23·92	20·45	7·38	3·81	38·16	2·50	4·74

From these figures the presence is apparent of a considerable proportion of sodium chloride in the flesh of salt-water fish ; but the flesh of the pike also shows a much larger proportion of soda than is found in the flesh of ruminants.

The following recent analyses of *cooked fish* are selected from a number published by Miss K. I. Williams (*Jour. Chem. Soc.*, lxxi. 649).[1]

Name of Fish.	Date.	Portion analysed.	As served at table.			
			Waste Bones, &c.	Gelatin.	Water.	Nutrients
Herrings, .	February	Whole	11·74	0·63	52·99	34·54
Salt herrings,	January	Flesh	...	...	46·03	53·97
Sprats, .	November	Whole	17·90	0·90	61·50	19·70
Sardines, .	March	Whole	4·91	...	42·17	52·92
Salmon, .	July	Section	5·99	0·53	61·06	32·02
Trout, .	May	Whole	8·23	0·55	67·12	24·10
Eels, . .	October	Heads removed	11·66	1·09	53·29	33·96
Mackerel, .	April	Whole	10·51	0·25	65·21	24·03
Cod, . .	January	Section	15·99	0·43	63·78	19·79
Salt cod, .	February	Section	6·13	0·33	67·68	25·86
Haddock, .	January	Whole	35·10	0·80	46·46	17·64
Whiting, .	January	Whole	25·10	0·86	61·29	16·35
Turbot, .	February	Anterior and head	31·20	0·59	53·09	15·12
Halibut, .	May	Section	6·84	0·03	69·35	23·78
Plaice, .	December	Flesh	...	...	79·86	20·14
Soles, . .	March	Whole	22·02	0·74	61·18	16·06
Lemon soles,	January	Whole	26·17	1·42	56·56	15·85
Oysters, .	March	Shell contents	...	...	77·71	22·29

[1] The fish was prepared just as it would be served at table, being first cleaned and then boiled in water of 26 degrees of hardness (chiefly due to calcium carbonate). The salt cod and herrings were previously soaked in cold water for several hours, while the sardines were well washed in both boiling and cold distilled water, to remove as much of the surface-oil as possible. When cold, all the bones, head, and such portion of the skin as would not be eaten were removed and carefully weighed, crushed in a mortar, boiled in distilled water, and the liquid siphoned off and evaporated over a water-bath. The residue, when constant in weight, was taken as gelatin.

The following additional data were obtained by the analysis of the same samples of *cooked fish* :—

Name of Fish.	Water in Flesh of Fish.	Analysis of the Dried Substance.						
		Ash.	Nitrogen.	Phosphorus.	Fat ; or Ether Extract.	Proteids.	Reducing substances reckoned as Glucose.	Nitrogen Pentoxide.
Herrings, . .	60·54	5·56	11·11	0·91	25·25	67·07	...	0·66
Salt herrings, .	46·03	19·69	7·12	0·89	21·90	38·88	17·59	1·64
Sprats, . .	75·77	6·42	9·26	1·17	27·37	57·94	9·88	...
Sardines, . .	44·35	12·03	8·54	0·97	33·49	55·44	...	...
Salmon, . .	65·32	4·94	10·70	0·51	29·43	56·65	14·89	0·46
Trout, . .	73·58	6·60	11·96	1·13	8·84	80·00	4·68	...
Eels, . .	61·08	2·11	7·36	0·42	44·68	42·88	8·91	...
Mackerel, .	73·13	4·07	10·46	0·85	25·73	62·32	13·93	0·33
Cod, . . .	76·32	3·31	15·30	0·62	1·15	91·55	6·67	0·63
Salt cod, . .	72·35	14·26	12·41	0·29	0·94	76·06	7·14	0·31
Haddock, .	72·37	3·28	13·11	0·53	1·29	79·57	13·15	0·43
Whiting, . .	78·78	1·92	13·28	0·73	1·86	79·55	17·54	...
Turbot, . .	77·84	2·41	13·76	0·57	4·75	84·71	11·81	...
Halibut, . .	74·46	4·11	13·32	0·67	15·81	79·67	...	...
Plaice, . .	76·86	4·06	13·02	0·71	9·84	75·16	11·56	2·78
Soles, . .	79·20	3·47	14·00	0·52	1·71	86·71	11·87	...
Lemon soles, .	78·11	4·42	11·04	0·54	12·96	69·88	14·80	...
Oysters, . .	77·71	12·16	11·85	0·49	7·77	65·42	18·32	...

The proteids in these analyses were estimated by multiplying the nitrogen determined by the soda-lime method by 6·25.

For the determination of the reducing substances, the fat was first removed by benzene, and the residue digested with 100 c.c. of water and 10 c.c. of hydrochloric acid (sp. gr. 1·125) in a flask connected with a reflux-condenser. The whole was heated as strongly as possible over a water-bath for three hours, the liquid filtered, treated with basic lead acetate, and sulphur dioxide passed through the filtered liquid. The solution was again filtered, concentrated at 100°, and a little washed alumina added until it was no longer dissolved. The filtered liquid was then evaporated to dryness at 100°, the residue treated with boiling alcohol, the liquid filtered, and the alcohol distilled off. The residue was dissolved in water, the solution boiled with animal charcoal and a few drops of milk of lime, filtered, and the filtrate titrated with Fehling's solution.

The proportion of reducing substances shown in the foregoing analyses by Miss Williams of the flesh of fish is, in most cases, remarkably large. As the method of determination involved treatment with hydrochloric acid for some hours at the boiling point of water, it seems probable that the reducing substances did not pre-exist as such, but were the products of the hydrolysis of bodies of the gluco-protein class. These compounds have been

observed by Hammarsten (*Zeit. physiol. Chem.*, xix. 19; abst. *Jour. Chem. Soc.*, 1894, i. 310), Pavy (*Physiology of the Carbohydrates*, 1894), and others to result from action of hot dilute acids on proteid matters. The conjecture receives support from the fact that the sum of the ash, fat, proteids, reducing substances, and nitrogen pentoxide is in some cases materially in excess of 100·00.

The presence of notable quantities of nitrates in the flesh of fish is remarkable. The analyses are not in accordance with the popular belief that the proportion of phosphorus is materially in excess of that present in the (dry) flesh of terrestrial animals.

Fish-roe and Caviare.—J. König gives the following analyses illustrating the percentage composition of caviare :—

	No. of Samples.	Water.	Nitrogenous Substances.	Fat.	Nitrogen-free Extract.	Ash.	Common Salt.
Caviare, .	5	43·89	30·79	15·66	1·67	8·09	6·02
Paionsnàja,[1] .	1	30·89	40·33	18·90	...	9·88	...
Fish - roe cheese,[2] .	1	19·38	34·81	28·87	(6·33)	10·61	...

Gobley gives the following as the composition of the *eggs* of the *carp* (compare hens' eggs, page 34):—water, 64·08; paravitellin, 14·06; fat, 2·57; cholesterin, 0·27; lecithin, 3·04; cerebrin, 0·21; membranous substance, 14·53; extractive matters, 0·39; colouring matters, 0·03; and salts, 0·82 per cent.

General Characters of Meat.

It will be seen, from the foregoing analyses, that the composition of meat is largely affected by the proportion of fat, and the higher the proportion of this constituent the lower will be the percentage of water. If the adipose tissue be excluded, flesh is found to be remarkably uniform in composition, even when derived from animals of the most diverse natures. On the average, the composition of the muscles of terrestrial animals may be stated thus :—

Water, ...	75 per cent.
Proteids and albuminoids, . .	21 „
Fat, extractives, and salts, . .	4 „
	100

[1] Paionsnàja consists of salted and pressed caviare.

[2] Fish-roe cheese is prepared in the Dardanelles by pressing and drying fish-roe in the air.

The flesh of fishes, if free from fat, contains as much as 80 per cent. of water, the solid matters being proportionately less.

The flesh of young animals is richer in gelatinoïd bodies than that of the corresponding adult animals. Castration greatly improves the flavour of the flesh of male animals, as instanced by the cases of the bullock and capon. The flesh of large animals is generally coarser than that of small, and this rule applies, not only to animals of different kinds, but also to the flesh of large individuals of the same species.

According to H. Letheby (*Lectures on Food*, 1870) good meat has the following characters:—"(1) It is neither of a pale pink colour nor of a deep purple tint, for the former is a sign of disease, and the latter indicates that the animal has not been slaughtered, but has died with the blood in it, or has suffered from acute fever. (2) It has a marbled appearance, from the ramifications of little veins of fat among the muscles. (3) It should be firm and elastic to the touch, and should scarcely moisten the fingers; bad meat being wet, and sodden, and flabby, with the fat looking like jelly or wet parchment. (4) It should have little or no odour, and the odour should not be disagreeable, for diseased meat has a sickly cadaverous smell, and sometimes a smell of physic. This is very discoverable when the meat is chopped up and drenched with warm water. (5) It should not shrink or waste much in cooking. (6) It should not run to water or become very wet on standing for a day or so, but should, on the contrary, be dry on the surface. (7) When dried at a temperature of 212° F. or thereabouts, it should not lose more than 70 to 74 per cent. of its weight, whereas bad meat will often lose as much as 80 per cent."

In addition to possessing the foregoing characters, meat should, of course, be free from any indication of the presence of parasites.

Parasites in Meat.—Meat, and especially pork, is liable to contain *Trichina spiralis* and *Cysticercus cellulosæ*, the latter of which gives rise to the so-called "measly" pork. If such meat be eaten without the parasites being killed by thorough cooking, the cysticerci develop into tape-worms on reaching the alimentary canal. The cysticercus occurs in the meat in cysts, and in the pig is of notable size and thus readily perceived; but the cysticerci of veal and beef are much smaller.

For the detection of cysticerci and trichinæ the fat should first be removed by ether-alcohol. Schmidt recommends that ten grammes of finely-divided meat or sausage should be treated with 100 c.c. of water containing 0·5 per cent. of HCl, and a suitable quantity of pepsin added. The mixture is allowed to stand for

about six hours at 40° C. The flesh is thereby dissolved, the fat forms a layer on the surface of the liquid, and the parasites sink to the bottom and can be readily recognised by the microscope. Cysticerci have tape-worm-like heads and bladder-like tails. Trichinæ, when encysted in the muscular tissue, occur as minute thread-like worms, coiled into flat spirals. Neither salting, smoking, nor moderate heating will suffice to destroy these formidable parasites, but they are killed by exposure to the temperature of boiling water.

The change undergone by meat under certain obscure conditions, whereby it produces symptoms of irritant poisoning in those who eat it, is apparently due in most cases to the formation of decomposition-products of the ptomaïne class (Vol. III. Part iii. page 321). Pork and veal appear to be specially liable to such putrefactive change. The poisonous effects are usually exhibited in the very first period of change, before the meat has acquired any marked peculiarity of appearance, taste, or odour which would serve to warn the consumers of the danger incurred by eating it. The change in question is quite distinct from that undergone by game when it becomes "high." High game, however, is distinctly unwholesome to some persons.

Mutton and *beef*, when of good quality, possess a rich, bright, uniform colour, and a firm texture, quite free from flabbiness, though moderately soft and elastic. Damp and clammy meat, from which moisture has a tendency to exude, is generally unwholesome. Very young meat, from animals of forced growth, is inferior in flavour and probably in digestibility. The flesh, or true muscular fibre, is not properly developed, while, on the other hand, the connective and other gelatinous tissues are present in undue proportion. Thus, according to Liebig, 1000 parts of beef contain, on the average, 6 parts of "gelatin," against an average of 50 parts contained in *veal*.

Horseflesh is extensively used for human food in some parts of Europe, though its employment for this purpose has met with but little favour in the United Kingdom. M. Humbert states that, in the year 1892, upwards of 20,000 horses were slaughtered in Paris for use as food, and that much of their flesh was made into sausages, the vendors of which are required to indicate the nature of the articles sold by them (see page 282).[1]

[1] James Bell, as the result of inquiries on the subject in 1886 (*Chem. News*, lv. 15), states that in Paris the Inspector-General of Slaughter-houses relies on the following characters for recognising horseflesh :—

"1. Horseflesh is reddish-brown, more or less dark, according to the quality, and gradually becomes darker when exposed to the air.

The flesh of *birds*, especially when wild, is remarkable for the almost entire absence of intra-muscular fat.

Frozen Meat is now imported to Britain in enormous quantities.[1] For its recognition, Maljean (*Jour. Pharm. et*

"2. It has an odour peculiar to itself.

"3. It is soft and but slightly tenacious, and the finger sinks easily into it. On working up the fibres a little, they break up and become pulpy.

"4. The muscular fibres are long and fine, and united by very compact muscular tissue.

"5. In cooking, horseflesh hardens and becomes more dense and compact than beef.

"6. Under the microscope, the fibres and striations of the muscular tissue are finer than in the flesh of the ox."

Bell quotes the following table taken from Baillet's *Treatise on the Inspection of Butcher's Meat*, comparing the characters of the flesh of the ox, cow, and horse.

	Ox-Flesh.	Cow-Flesh.	Horse-Flesh.
Colour, . . .	Bright red.	Bright red.	Dark red.
Consistence, . .	Firm, hard, often even tough.	Firm, but soon becomes tender and unctuous.	Firm, hard, tough.
Cut, . . .	Resistant and large grain. Not mottled.	Easy and fine grain. Mottled.	Resistant, large grain. Not mottled
Odour, . . .	Fresh, only that of beef.	Fresh, aromatic.	Musk-like.
Fat, . . .	No covering fat. Interior fat white.	Covering fat white or yellowish.	No covering fat. Interior fat white and firm.
Articular surface,	Rosy white.	Rosy white.	Deep rose.
Anatomical constitution, . .	Muscular bundles with fibres smooth, long, and united by conjunctive tissue, loose, easily penetrated by the fat.	Muscular bundles more compact, more resistant. Conjunctive tissue loose or compact, according to the quality.	Muscular fibres short, crowded in long and thin bundles.

It will be observed that whereas, according to the first description, the muscular fibres of horse-flesh are stated to be "long and fine," Baillet describes them as "short," but "crowded in long and thin bundles."

Bell found the foregoing characters insufficiently defined to enable him to positively identify horseflesh and distinguish it from beef. He considered the characters of the accompanying fat more reliable (see page 287).

[1] According to a pamphlet published by The Colonial Consignment and Distributing Company, Limited, Nelson's Wharf, Lambeth:—"During the year 1895 no fewer than 3,423,347 carcases of frozen sheep and lambs, and 323,698 quarters and pieces of beef arrived here from New Zealand and Australia alone (without counting 1,652,625 sheep and 12,418 quarters beef

Chim., 1892, xxv. 348) expresses a little blood or juice from the sample, and examines it without delay under the microscope. The juice of fresh meat is seen to contain numerous red corpuscles, which are normal in colour and float in a clear serum. In the case of blood from frozen meat, on the other hand, the corpuscles have dissolved in the serum under the influence of the low temperature, and not a single normal red corpuscle can be observed. The hæmoglobin escapes into the serum and appears as irregular yellow-brown crystals, which are not always visible to the naked eye but can always be readily detected under the microscope.

By salting, meat loses a small proportion of its soluble constituents, which are dissolved out by the brine. *By smoking*, the outer surface of the meat is coagulated and hardened, the creasote and other constituents of the smoke acting as antiseptics.

Cooking of Meat.—If properly cooked, meat is tender before the occurrence of *rigor mortis*, but when once that condition has set in it requires to be kept some days before it again acquires this character.

The tangible change in composition undergone by meat in the process of roasting consists chiefly in the loss of water by evaporation and formation of gravy, with the loss of fat in the form of dripping. The proteids become concentrated in the cooked meat, while at the same time certain dark-coloured substances of carameloid nature are formed, which greatly modify and improve the flavour and odour of the meat.

When meat is boiled in water, a considerable quantity of organic and inorganic matters pass into solution, and when the liquid is not intended to be consumed the loss should be reduced to a minimum by immersing the meat in boiling water for a few minutes, and then adding more water, in quantity sufficient to reduce the temperature of the liquid to about 77° C., which temperature should not be greatly exceeded during the remainder of the process of cooking. By operating in this manner an insoluble coating of coagulated proteids is formed on the meat, and loss by solution reduced to a minimum. On the contrary, when it is desired to extract the meat as thoroughly as possible, as in pre-

from the River Plate, and 19,432 sheep from the Falkland Islands), a total of 5,095,404 frozen sheep and lambs, and 336,116 quarters beef in the year, and for the first six months of this year a total of 3,214,660 frozen sheep and lambs, and 155,195 quarters of beef have been received from the same sources of supply." "It is no unusual thing for 12,000 sheep to be received here in the course of a day from the eight barges which are kept constantly working, though 10,000 is regarded as an average day's work."

paring soup, beef-tea, or mutton-broth, the meat should be placed in cold water, and the temperature gradually raised.

Soup contains the extractives of the meat from which it is prepared, a portion of the proteids, and most of the gelatinoïds.

Beef-tea contains only insignificant quantities of proteids, gelatinoïds or fat, and hence possesses true nutritive properties to but a very limited extent. Its value appears to be due to the stimulating action of the extractives, especially creatine, creatinine, xanthine, lactic acid, and salts.

Liebig's extract of meat is practically concentrated beef-tea, and owes its value to the same constituents. The nature of commercial meat-extracts is discussed fully in the sequel.

Sausages.

Sausages are well-known to be manufactured from meat of various sorts, with condiments and (frequently) amylaceous additions. Gristle and the unsalted scraps and trimmings from bacon factories are constituents of the inferior kinds.

German Sausages are extensively made from blood, liver, heart, brain, &c., and from fresh, dried, smoked, and salted meat. An addition of farinaceous material, usually in the form of flour or pea-meal, is very common.

The following description of the chief varieties of German sausage is an abstract of that of J. König:—

Blutwurst or *Rothwurst* is made with hogs' blood, bacon, and pork, sometimes with the addition of heart or kidney, and either with or without flour. These sausages are similar to those known in England as "black pudding." They soon undergo decomposition.

Mettwurst (Bologna or thick sausage) is made from pork and lard, with an addition (*inter alia*) of beef or horseflesh. It is often coloured with rosaniline or other coal-tar dyes.

Cervelatwurst or brain-sausage is somewhat similar to the last. The Italian sausage *Salamiwurst* receives an addition of red wine.

Knackwurst is a hard sausage, of a similar composition to cervelat, but the meat is previously cooked.

Leberwurst or liver-sausage is composed of liver, lung, kidney, skin, and lard or suet, with or without flour. Liver-sausages readily undergo change, and are then liable to occasion symptoms of irritant poisoning. *Trüffelwurst* is made from meat, fat, and flour, with an admixture of truffles.

Schwartenwurst, *Sülzenwurst*, and *Magenwurst* are German sausages made from skin, stomach, &c., boiled soft, and mixed with unsalted bacon and a little blood.

Bratwurst is made from fresh raw pork and bacon, flavoured with salt, pepper, and lemon-peel or cumin.

Frankfort and *Vienna sausages* are small, filled into sheep's gut, and composed of raw, slightly-smoked pork, flavoured with salt, nitre, and pepper.

Reiswurst and *Grützwurst* are sausages commonly manufactured in North Germany from oat- or buckwheat-meal, blood, soft-boiled skin, bacon, spices, &c.

Erbswurst is composed of beef-suet, bacon, peas, onions, spices, &c. Sausages of this kind, when of French manufacture, often contain much coarse meal and husk. Hence the woody fibre is high (4·3 per cent.) as compared with that in German pease-sausages (0·88 to 1·08 per cent.). G. Heppe examined three samples of *Erbswurst* which contained 7·32 per cent. of flesh constituents. F. Hofmann found in one sample a mere trace of animal proteids, while another contained 16·45 of total proteids, of which from 2 to 3 per cent. was of animal origin.

The following analyses also show the variation in the composition of pease-sausages :—

Authority.	Date.	Water.	Nitrogenous Matters.	Fat.	Starch, &c.	Salts.
Ritter, . . .	1870	29·25	16·02	29·70	11·94	13·19
König, . . .	1884	11·00	19·65	15·52	41·05	11·88

The so-called blood- and liver-sausages often contain more flour than blood, liver, or flesh.

A specimen of cooked Lorraine sausage, recently examined by the author, contained smoked meat, gristle, pea-meal, and onions, and gave the following figures on analysis :—Water, 46·04 ; fat, 25·67 ; proteids, 15·49 ; gristle, 3·65 ; starch, 4·00 ; and ash (sulphated), 6·36 per cent.

Sausages which are intended to be kept should not contain milk, flour, bread, onions, or brains. O. Dietch found in two cases in which sausages became phosphorescent by keeping that good meat had been used, but that the lard was bad.

In a certain stage of decomposition, sausages are very apt to occasion symptoms of irritant poisoning. According to Chodounsky (*Chem. Centralb.*, 1887, page 119) the toxic principle is not of a basic character.

The occurrence and detection of *cysticerci* and *trichinæ*, parasites which are specially liable to be present in German sausages, are described on page 274.

The following analyses of different varieties of German sausages are by J. König.

	Water.	Nitrogenous Matters.	Fat.	Carbo-hydrates.	Ash.	Sum of Constit-uents.
Cervelatwurst (brain-sausage),	37·37	17·64	39·76	...	5·44	100·21
Mettwurst (Bologna or thick sausage),	20·76	27·31	39·77	5·10	6·95	99·89
Frankfurter Würstchen (Frankfort small sausages),	42·79	11·69	39·61	2·25	3·66	100·00
Blutwurst (black pudding), best quality,	49·93	11·81	11·48	25·09	1·69	100·00
Blutwurst (black pudding), ordinary quality,	63·61	9·93	8·87	15·83	1·76	100·00
Leberwurst (liver-sausage), best quality,	48·70	15·93	26·33	6·38	2·66	100·00
Leberwurst (liver-sausage), third quality,	47·58	10·87	14·43	20·71	2·87	96·46
Leberwurst, without flour,	35·89	16·13	45·51	...	3·72	101·25
Sülzenwurst,	41·50	23·10	22·80	...	12·60	100·00
Knackwurst,	58·60	22·80	11·40	...	7·20	100·00
Erbswurst (German pease-sausage),	6·53	15·46	37·94	31·38	8·69	100·00
Trüffelwurst, best quality,	43·29	13·06	41·27	...	2·41	100·03
Schinkenwurst (ham-sausage),	46·87	12·87	24·43	12·52	3·31	100·00

English Sausages are generally very different from those of German manufacture. As sold (with the exception of "polonies"), they are made of uncooked and unsmoked meat, and are intended to be cooked and eaten while quite fresh. The addition of dry bread or biscuit is very common, but by no means invariable.

The following analyses of sausages obtained from respectable dealers in Sheffield were recently made in the author's laboratory :—

Description of Sausage.	Price per lb.	Water.	Fat.	Proteids.	Gristle, &c.	Starch.	Ash.
Pork,	9d.	54·99	21·04	12·28	0·67	1·05	3·52
"Cambridge" pork,	9d.	51·54	29·72	9·45	0·72	2·20	3·47
Mutton,	1s.	55·58	30·51	1·89	3·11	3·90	2·50
German,	8d.	46·54	17·87	16·38	1·13	15·00	4·47
Polony,	10d.	45·57	32·66	17·26	0·54	2·30	2·80

In these analyses, a weight of 10 grammes was dried at 105° C. for the determination of the *water*. The dried substance was then extracted with ether in a Soxhlet tube, the solution evaporated, and the residual *fat* weighed. The residue insoluble in ether was moistened with sulphuric and nitric acids, ignited,

again moistened with sulphuric acid, re-ignited, and the *sulphated ash* weighed.

For the estimation of the *gelatinoids*, a weight of 20 grammes of the sausage was disintegrated by stirring it in a basin with cold water, the excess of water drained off, and the fragments of gristle picked out with a pair of forceps with the aid of a lens. They were then washed in succession with methylated spirit and with ether, dried at 100° C., and weighed.[1] The nitrogen contained in the *gristle*, &c., thus found, was then determined by Kjeldahl's process, and the amount deducted from the total nitrogen found by the same process in the original sausage. The difference was regarded as *proteid* nitrogen, and multiplied by 6·3 to find the proportion of these compounds present.

The *starch* was determined by Mahrhofer's process (see below). No allowance was made for that derived from the pepper, or other spices. No wheat-starch could be observed by the microscope in the case of the first two samples. The dry bread used in the manufacture of sausages may be taken as containing 60 per cent. of starch.

A. W. Stokes, in a communication to the author, states that in 1894 he found that sausages which were being extensively sold on street-stalls in the east of London contained 10 per cent. of flesh, 20 of fat, and 70 per cent. of bread. On being placed in water they disintegrated, the meat sinking rapidly, so that a fairly good separation of the constituents could be effected by elutriation. No proceedings were taken against the vendors, owing to the absence of any legal or authoritative definition of a sausage.

Starch in sausages, when present in more than the trifling proportion due to the pepper, indicates an admixture of flour, bread, or other farinaceous material. Broken and waste biscuits are extensively used for the purpose. The presence of such amylaceous substances may be detected by moistening the sausage with a dilute solution of iodine, and examining the mixture under the microscope. The starch-granules, more or less altered by the action of heat, will be readily recognised by their blue colour.

For the determination of the amount of starch in sausages, Medicus and Schwab (*Berichte*, xii. 1285) recommend that a weighed quantity of the material should be digested for two hours at 30° to 40° C. with a known measure of an infusion of malt, and then allowed to stand for about eighteen hours at the ordinary

[1] This method has, of course, no pretensions to great accuracy, but is useful as indicating the character of the materials used in manufacturing the sausage.

temperature. The mixture is then filtered and well washed, the filtrate boiled, and the coagulated albumin filtered off. The filtrate is then boiled with hydrochloric acid to convert the dextrin and maltose into dextrose, which is determined in the usual way by Fehling's solution. The dextrose yielded by the malt extract employed is also determined and deducted from the gross amount. Ten parts of dextrose thus found represent nine parts of anhydrous starch originally present. An allowance of 1 per cent. should be made for starch existing in the form of pepper.

In the place of the above or similar methods, based on the conversion of the starch and determination of the resultant dextrose, J. Mahrhofer (abst. *Analyst*, 1897, page 2) recommends the following process as simpler and more satisfactory. From 60 to 80 grammes of the sample should be heated on the water-bath with alcoholic potash (about 8 per cent.), which in the case of pure sausages dissolves almost everything except a little cellulose. The solution is diluted with warm alcohol, to prevent gelatinisation, and filtered through a paper or asbestos filter. The insoluble residue, containing any starch which may be present, is washed with alcohol until the washings are no longer alkaline, then treated with *aqueous* potash, and the starch solution made up to a definite volume. On adding alcohol to an aliquot portion, the starch falls as a flocculent precipitate, which rapidly subsides. This is collected on a weighed filter, washed with alcohol and ether, dried and weighed.

In order to avoid a determination of the ash in this precipitate, it is advisable to operate in a weak acetic acid solution instead of in an alkaline solution, since the potassium acetate then formed is readily soluble in alcohol. By this means the starch is obtained quite free from ash.[1]

As a test of the accuracy of the method, mixtures were made of about 60 grammes of sausage with varying amounts of pure potato starch—dried at 100° C., and analysed as described above, with the following results :—

Starch *added*, in milligrammes,	58	133	97	98	341	260
Starch *found*, in milligrammes,	56	132	97	98	340	255

Horseflesh Sausages.—On the continent of Europe, and especially in France, horseflesh is extensively used for the manufacture of sausages, the vendors of which are required to indicate

[1] Instead of weighing the starch precipitated by alcohol, the author has found it convenient to convert it into dextrose by prolonged boiling with dilute acid, and determine the cupric oxide reducing power of the resultant liquid.

the nature of the articles they sell. Hence a means of recognising horseflesh, and detecting it when mixed with the flesh of other animals, is of practical importance.

The physical characters of horseflesh have been employed for its recognition (see page 275) but are not very conclusive under any circumstances, and are useless when the flesh is minced and mixed with other kinds of meat, as in sausages.

Horseflesh is remarkable for the comparatively large proportion of *glycogen*[1] usually contained in it, and this fact has been utilised

[1] GLYCOGEN $(C_6H_{10}O_5)_n$, was first found in the liver, but has been more recently met with in many other parts of the body. It has been termed "Animal Starch" from its close analogy to soluble starch. The physiological significance of glycogen has been the subject of much controversy, and the question is not yet settled.

Glycogen may be prepared by rapidly cutting up the liver of an animal killed immediately previously, and throwing the fragments into five times their weight of boiling water. After boiling for a short time, the fragments of liver are mixed with sand and reduced to powder in a mortar, and then returned to the water, which is again boiled. The liquid is strained, and faintly acidified with acetic acid while still hot. The filtrate from the coagulated proteids is rapidly cooled, and the remaining proteids precipitated by the alternate addition of hydrochloric acid and potassio-mercuric iodide. The filtered liquid is mixed with such a volume of strong spirit as to make it contain 60 per cent. of absolute alcohol, when the precipitated glycogen is filtered off, washed first with 60 per cent. spirit, and then with absolute alcohol and ether.

Kistiakoffsky (*Jour. Russ. Chem. Soc.*, xxv. 60; abst. *Jour. Chem. Soc.*, 1893, i. page 618) prepares glycogen from the liver and muscles, taken as before immediately after the death of the animal, by cold extraction. The material is rubbed up in an iron mortar, cooled to a very low temperature to prevent fermentation, and the homogeneous mass then extracted with ice-cold water containing 1 to 2 per cent. hydrochloric acid. This operation is repeated until the last extract ceases to give the glycogen reaction with iodine. If it is not essential that the whole of the glycogen should be extracted, water containing from 0·2 to 0·7 per cent. of acid may be used. The solution obtained is coloured by hæmoglobin, and contains albuminous matters, which are precipitated by means of mercuric iodide. This precipitate is filtered off and washed with dilute mercuric iodide solution until free from glycogen. The glycogen is precipitated from the filtrate and washings by the addition of about one and a half volumes of alcohol. It is collected on a filter, and washed first with 75 per cent. alcohol, and then with ether-alcohol. After being dried over sulphuric acid, the product forms a white, amorphous powder, containing no nitrogenous compounds, and leaving only traces of ash on ignition. If dried in the air, a resinous mass difficult to powder is obtained. This method may be used for the estimation of glycogen in animal tissues, the results obtained being somewhat under those found by Brücke's method. (See further, Kistiakoffsky, abst. *Jour. Chem. Soc.*, 1896, ii. 80.)

Brücke advocates the use of 0·1 to 0·3 per cent. solutions of alkali for the

by Brautigam and Edelmann (*Chem. Centralb.*, 1894, i. 485; abst. *Analyst*, xix. 24) for its detection in sausages. They state that 10 per cent. of horseflesh or horse-liver can be detected by

extraction of glycogen, instead of the 2 per cent. alkali solutions as sometimes used. If the extraction of the glycogen be effected with boiling water, the aqueous liquid contains, besides glycogen, alkali albuminates, glutin, and traces of peptone (Kistiakoffsky, abst. *Jour. Chem. Soc.*, 1896, ii. 80). These are all precipitated by hydrochloric acid and potassio-mercuric iodide as already described.

Of the substances proposed for the extraction of glycogen, boiling water, trichloracetic acid, sulpho-salicylic acid, and formaldehyde all extract albumoses, in some cases in considerable quantities, from the animal substances used. The separation of the albumoses is attended with some difficulty. Their presence may be conveniently recognised by treating the glycogen solution with a reagent containing 100 parts of sodium tungstate, 50 parts of phosphoric acid, 10 parts of concentrated hydrochloric acid, and 500 parts of water. On treatment with Millon's reagent, the dried precipitate will show the presence of 0·02 per cent. of albumose. The presence of glycogen does not interfere. This test is due to D. Huizinga (*Pflüger's Archiv.*, lxi. 32; abst. *Jour. Chem. Soc.*, 1896, i. 6), who finds that the best results are obtained when the liver or other animal substance is treated with a mixture of equal parts of a saturated solution of mercuric chloride and Esbach's reagent, made by dissolving 10 grammes of picric acid and 20 grammes of citric acid in water, and making the solution up to one litre. This treatment does not extract the whole of the glycogen from the animal substance.

Pure glycogen is a snow-white, amorphous powder, readily soluble in water to form a solution which is usually, but not invariably, opalescent, and which becomes more limpid on adding acetic acid or an alkali. Glycogen is precipitated from its aqueous solution by alcohol whenever the alcohol amounts to 60 per cent. of the liquid. If the solution be quite free from salts, the separation is sometimes very difficult, but takes place instantly on adding a minute quantity of common salt. The precipitation of glycogen in liquids containing 60 per cent. of alcohol distinguishes it from the different varieties of dextrin, none of which are precipitated by alcohol of less than 85 per cent. strength. On the other hand, glycogen exactly simulates erythro-dextrin in its behaviour with a solution of iodine, which produces a port-wine colour, disappearing on heating, and returning as the liquid cools.

Glycogen is strongly dextro-rotatory, the value of $[\alpha]_D$ varying from $+203°$ to $+234°$, according to the concentration of the solution. (Huppert found the specific rotation to be $+196·6$.)

Glycogen does not reduce Fehling's solution. It is precipitated by baryta-water as $BaO(C_6H_{10}O_6)_3$, and by basic lead acetate as $PbO(C_6H_{10}O_6)_2$.

When boiled with dilute nitric acid, glycogen yields oxalic acid. Boiled with dilute sulphuric or hydrochloric acid, it is converted into dextrose. It does not ferment with yeast, but diastase and saliva convert it into maltose and achroo-dextrin, a little dextrose being also formed. On the other hand, in the hydrolysis of glycogen in the liver, dextrose and not maltose is the chief product.

this means, the proportion of glycogen therein ranging from 0·37 to 1·07 per cent., while the flesh of other animals used for food contains little or none,—ox-flesh coming next with 0·20 per cent. On the other hand, the flesh of the fœtus, both of man and of the lower animals, is rich in glycogen. M. Humbert (*Jour. Pharm. et Chim.*, 1895, 195; abst. *Analyst*, 1895, p. 95) confirms the value of the observation of Brautigam and Edelmann and recommends the following method of procedure:—About 50 grammes weight of the muscular tissue should be cut into small pieces and boiled for an hour with 200 c.c. of water. After cooling, nitric acid is added in the proportion of 5 c.c. to 100 c.c. of broth, and the liquid filtered. To a portion of the filtrate contained in a test-tube iodine-water [1] is added drop by drop, so as not to mix the liquids, when the formation of a reddish-violet zone at the junction of the two strata will indicate the presence of glycogen in the original sample. The reaction is said to be quite characteristic of horseflesh. Humbert states that of ten samples of horseflesh obtained from different dealers in Paris, seven showed the colour very plainly, in two it was less pronounced but distinct, while in one case it was doubtful. In no instance was there any coloration with beef, veal, mutton, or pork. Beef-broth left in contact with the iodine for ten days gave no reaction. The flesh of the ass gave a negative result, but with that of the mule the reaction was the same as with horse-flesh. A mixture of equal parts of horse-flesh, beef, mutton, veal, and pork showed the coloration, but it was less pronounced than with horseflesh alone.

Courlay and Coremons (abst. *Analyst*, 1896, p. 231) recommend a slight modification of the foregoing test. About 50 grammes of the substance, in as fresh a state as possible, should be finely-divided and boiled for fifteen to thirty minutes with 200 c.c. of water. After cooling (*no* addition of nitric acid being made) the broth is filtered and then tested with a few drops of iodine solution, prepared by dissolving 2 parts of iodine and 4 of potassium iodide in 100 parts of water. A brown coloration, disappearing on warming to 80° C. and reappearing on cooling, shows the presence of horseflesh.[2] In the presence of starch (*e.g.*, in sausages) the blue reaction with iodine may entirely mask the

[1] The reagent should be recently prepared by saturating hot water with iodine. In the original form of the test, Brautigam and Edelmann employed hydriodic acid instead of the hot iodine water recommended by Humbert.

[2] Courlay and Coremons state that, by their modification of the iodine test, no glycogen reaction was obtained from the flesh of calves, pigs, dogs, or cats; but that this observation does not apply to the fœtus of any of these animals.

brown coloration due to glycogen; but this is obviated by treating the broth with two or three times its measure of strong acetic acid, filtering, and applying the iodine test to the filtrate.

W. Niebel (*Chem. Centralb.*, 1893, p. 323; abst. *Analyst*, xx. 252) criticises the foregoing methods on the ground that the reaction with iodine is uncertain, since glycogen is also found in the flesh of dogs, cats, and very young calves,[1] in the livers of cattle, and in meat-extract to the amount of 1·5 per cent. With old sausages from horseflesh Brautigam and Edelmann always obtained the glycogen reaction, although that substance would usually be completely decomposed under these circumstances. There is also an uncertainty in the reaction caused by the fact that the erythro-dextrin formed from the starch gives a similar coloration with iodine, and no means of removing it is known. Niebel considers that the red colour with iodine is not sufficient proof of the presence of glycogen, which should be isolated in a pure condition. Nevertheless, the iodine coloration, and the occurrence of more than 1 per cent. of glucose in the fat-free substance, points to the presence of horseflesh in a sample, even when all the glycogen has been decomposed. The red colour only fails in the case of the flesh of young foals.

A. Bujard (*Forsch. Ber.*, 1897, iv. 47; abst. *Analyst*, 1897, p. 160) has published the following determinations of glycogen made by the method of Niebel and Salkowsky (*Zeit. Fleish und Milkhyg.*, 1891, 185).

	Per cent.		
	Water.	Glycogen; Direct.	Glycogen on Dried Substance.
Horseflesh,	61·83	0·846	2·24
,,	72·90	0·174	0·64
,,	70·47	1·366	4·62
,,	71·84	0·59	2·09
Horseflesh smoked,	43·00	0·108	0·19
Beef (ox),	73·62	0·206	0·74
Beef,	75·55	0·018	0·073
Veal,	76·12	0·346	1·44
,,	74·47	0·066	0·25
Pork,	54·05	trace	trace
,,	66·29	...	...
Horse Sausages:—			
Red sausage,	70·04	0·504	1·68
Liver sausage,	67·00	1·762	5·34
Salami,	33·60	0·034	0·05
Sausages:—			
Salami,	20·00	trace	trace
Thuringian,	12·93	,,	,,
,,	29·16	,,	,,

[1] See footnote 2 on page 285.

The following figures were obtained by Bujard more recently. Some of the samples were analysed both by the method of Niebel and Salkowsky and by that of Mayrhofer (*Forsch. Ber.*, 1896, 141), to which he gives the preference on the ground of simplicity. In the latter process, the finely-divided flesh is dissolved in aqueous potash, the proteids precipitated by hydrochloric acid and Nessler's solution, and the clear filtrate treated with alcohol. This throws down the glycogen, which is filtered off, washed with dilute alcohol and with ether, and dried at 110° C.

	Water per cent.	Per cent. Glycogen Direct.		Per cent. Glycogen calculated on Dried Substance.	
		Niebel.	Mayrhofer.	Niebel.	Mayrhofer.
Horseflesh, ...	74·44	0·440	0·445	1·721	1·741
,, ...	74·87	0·600	0·520	2·388	2·069
,, ...	76·17	1·827	1·727	7·667	7·247
,, ...	76·00	0·592	0·610	2·466	2·542
Red sausage (Knackwurst),	69·26	...	0·038[1]	...	0·124
Pork sausage, . . .	67·25	...	0·24[1]	...	0·733
Veal, ...	74·6	...	0·086	...	0·342
Pork, ...	75·0	...	0·186	...	0·744

In Bujard's opinion, his figures show that only in exceptional cases (where the amount is large) can the glycogen be taken as conclusive of the presence of horseflesh, especially when the latter is mixed with other kinds of flesh.

James Bell has pointed out (*Chem. News*, lv. 15) that the fat of the horse differs materially in its characters from that of the ox. Thus the fat isolated from different parts of the horse—such as the round, flank, ribs, kidneys, and heart—was found to have a specific gravity at 100° F. ranging from 0·9086 to 0·9088. The intra-muscular fat had a gravity of 0·9084 at the same temperature, and hence was not greatly different in character from that obtained directly from the adipose tissue. The horse-fat melted to a clear oil at 70° C. and the amount of solid fat deposited at a lower temperature was comparatively small. A series of similar experiments made with beef-fat showed a melting-

[1] In these samples pepper-starch could be detected microscopically, and on testing with iodine only the blue starch reaction could be obtained, whilst in all the other cases the brown glycogen reaction was marked.

point of 110° to 116° F. The specific gravity was taken at 120° F., and when the results were corrected to 100°, to render them comparable with those obtained with horse-fat, the specific gravity was found to range from 0·9036 to 0·9040. These figures show a substantial difference between beef-fat and horse-fat, and the distinction is still more marked in the case of mutton-fat. The low melting-point of horse-fat is an important characteristic, and in cases where the flesh is not of mixed origin ought usually to be conclusive as to its nature.

R. Frühling (*Zeit. ang. Chem.*, 1896, p. 352; abst. *Analyst*, xxi. 231) was led to study the fat of horseflesh, being unable to obtain conclusive results by the reaction for glycogen. He found the fat extracted from sausages made wholly from horseflesh to have an iodine-absorption of 72·5 per cent., while that from sausage composed of horseflesh with 15 per cent. of pork gave 62·3; and from sausage composed of equal parts of horseflesh and pork 57·2 per cent. As pure pork-fat (lard) shows an iodine-absorption averaging about 60, it is evident that the method is inconclusive.

H. Bremer (*Forsch. Ber.*, 1897, iv. 1; abst. *Analyst*, xxii. 104) has reviewed the work of other chemists, and proposed the following method of detecting horseflesh, based on the characters of the intra-muscular fat.

The meat preparation, from which all visible fat has been removed, is minced in a sausage-machine, and heated for about an hour on the water-bath with water. The fat rising to the surface is poured away with the water, and the flesh, after having been washed several times with hot water, is dried at 100° C. for twelve hours, and extracted for several hours with petroleum-spirit of low boiling-point. Part of the intra-muscular fat thus obtained is taken for the determination of the iodine-absorption, refractive index, and Reichert number. The remainder is saponified, the excess of alkali neutralised with acetic acid, and the alcohol evaporated on the water-bath. The soap is dissolved in hot water, a hot solution of zinc acetate (1 part to 2 parts of fat) added, and the zinc soap washed with hot water and alcohol, pressed between filter-paper, and extracted with about ten times its volume of water-free ether for fifteen to thirty minutes under a reflux-condenser. After cooling, the solution is filtered into a weighed flask, the ether evaporated, and the iodine-absorption of the residue determined.[1] Every precaution must be taken to prevent access of air during filtration and drying.

[1] There appears to be some direction omitted here. As prescribed, the iodine-absorption of the zinc soap soluble in ether would be ascertained. If

The subjoined table gives the results obtained by this method :—

	Iodine No. of Intra-muscular Fat	Iodine No. of Liquid Fatty Acids.
I. Horseflesh sausage without bacon, ...	75·8	108·1
II. Horseflesh sausage with about 6 per cent. of bacon,	74·0	104·1
III. Horseflesh cervelat (brain) sausage with about 22 per cent. of well-smoked bacon, ...	53·7	92·4
IV. Horseflesh cervelat sausage with about 25 per cent. of bacon, ...	74·1	102·1
V. Ordinary sausage with some bacon, ...	57·6	94·2
VI. Thuringian cervelat sausage with about 65 per cent. of pig's fat, ...	64·3	95·8
VII. Mixture of I. and V. in equal parts, ...	66·4	103·1
VIII. Mixture of IV. and VI. in equal parts, . . .	65·2	99·5

It is stated that whenever horseflesh is present the petroleum-ether extract has a red to reddish-brown colour, and that even the liquid fatty acids have a more or less reddish-yellow shade. On the other hand, bull's flesh gives a similar colour, so that too much reliance must not be placed on this fact, except as a confirmatory test. When, however, this is found to be the case, when at the same time glycogen is detected, and when the iodine number of the intra-muscular fat exceeds 65, and that of the liquid fatty acid is considerably over 95, there can be but little doubt as to the presence of horseflesh.

COLOURING MATTERS are often added to sausages, and are occasionally of an objectionable character.

Red ochre is said to have been used, but would make its presence evident in the ash of the material. *Aniline-red* has been detected, but certain varieties of *benzopurpurin* (Vol. III. Part i. page 207) are now most commonly employed. This colouring matter is allied to congo-red, and has the property of dyeing vegetable fibres without a mordant. It is added to the meat together with the dry bread or biscuit, which it effectually colours.

Aniline-red, if present, can be detected by extracting the finely-divided sausage with methylated spirit, evaporating the solution after straining or filtering, taking up the residue with water, and

the ethereal extract of the zinc soap were shaken with dilute sulphuric acid, the zinc would pass into the aqueous liquid as sulphate, and the ether would yield on evaporation the "liquid fatty acids," or acids corresponding to the portion of the zinc soap soluble in ether.

immersing white wool in the boiling liquid, when the fibre will be dyed red if rosaniline is present.[1]

For the detection of *cochineal-carmine* in sausages, Klinger and Bujard (*Zeit. angw. Chem.*, 1891, p. 515; abst. *Jour. Chem. Soc.*, 1893, ii. 56) recommend that about 20 grammes of the cut-up sausage should be heated in a water-bath with a mixture of equal parts of water and glycerin. In the absence of this colouring matter only a slight yellow colour is produced, but in its presence the liquid becomes decidedly reddish in colour. The filtered solution is heated, if necessary, with another 20 grammes of the sausage. The clear liquid is then examined in the spectroscope, when cochineal-carmine can be readily recognised by its characteristic absorption-bands. A preferable plan is to precipitate the colouring matter as a lake, and, after washing, to dissolve it in a little tartaric acid. A more concentrated solution is thus obtained, and the spectroscopic test is consequently more satisfactory.

H. Bremer (abst. *Analyst*, 1897, p. 216) confirms the value of the foregoing method for the detection of cochineal-carmine. He heats the finely-divided sausage for several hours on the water-bath with two measures of the slightly acidulated glycerin-water mixture. The yellow solution is freed from fat and filtered, and the colouring matter precipitated as a lake by the addition of alum and ammonia. The absorption-bands lying between *b* and D, which are characteristic of carmine-lake, may be readily observed in the spectroscope. In one instance Bremer found a cervelat sausage coloured with cochineal-carmine to have all the appearance of good meat when cut, but further examination showed it to be quite unfit for food, the "acidity number" of the fat being 76·0 (*e.g.*, the separated fat required 7·6 per cent. of KHO for its neutralisation).

Weller and Riegel (*Forsch. Ber.*, 1897, iv. 204; abst. *Analyst*, xxii. 324) state that the hæmoglobin of pig's blood is converted by nitre into a modified form which dissolves with red colour in diluted glycerin, alcohol, amylic alcohol, and ether, but can be distinguished from cochineal-carmine by its absorption-spectrum after reduction by ammonium sulphide. They consider the method of extraction employed by Klinger and Bujard (which is that adopted by the Berlin Police Council) to be useless.

Preservatives in sausages may be sought for as in milk (page 175). *Boric acid* may also be determined by the method of C. Fresenius and Popp (page 331).

[1] O. Schweissinger (abst. *Analyst*, xii. 53) has described a red colouring matter in sausages which gave a negative reaction by the above process. A freshly-cut surface of the sausage did not become paler on drying, and on examination under the microscope portions of the material, and

Potted Meat, &c.

The following analyses by J. König show the composition of commercial potted foods. The foie-gras paste was obtained from Strassburg, and the remaining samples from Crosse & Blackwell, London.

	Water.	Nitrogenous Matters.	Nitrogen-free Extract.	Fat.	Ash.	Common Salt.
Foie-gras paste, .	46·04	14·59	2·67	33·59	3·11	·22
Potted beef, . .	32·81	17·17	3·36	44·63	2·03	...
Potted ham, . .	25·57	16·88	...	50·88	6·78	5·72
Potted tongue, .	41·52	18·46	0·46	32·85	6·71	5·98
Potted salmon, .	37·64	18·48	0·70	36·51	6·67	5·65
Potted lobster, .	51·33	14·87	4·04	24·86	4·90	·38
Anchovy paste .	36·81	12·33	5·18	1·59	44·09	40·10

A. H. Hassall, writing in 1860, stated that he had examined twenty-eight samples of potted meat and fish, out of which twenty-three were coloured with a red ferruginous earth. All the samples of anchovy-paste examined were coloured with this material, and two contained wheat-flour in addition.

Canned Meat.

The preservation of meat and other kinds of food by confining it in hermetically sealed vessels is now practised on an enormous scale. In the case of meat, the material, freed from bone, is packed in the tins, and an addition of jelly or gravy, salted and flavoured, is often made. The tins are then closed, with the exception of a small orifice, and immersed in a bath of boiling calcium chloride solution, or other bath of sufficiently high temperature. The current of steam issuing from the tin through the aperture left for the purpose carries the air with it, and the high temperature effectually destroys any lower organisms. When the air is judged to be thoroughly expelled, the orifice is closed by solder. The destruction of organisms and the absorption of the last traces of oxygen are in some cases further ensured by the introduction of a small quantity of calcium sulphite. When the operation is carefully performed, the contents of the tin will keep in a good condition for an indefinite period.

especially the connective tissue, were observed to have a bright red colour, quite different from the yellow tint due to hæmoglobin. The colouring matter could not be extracted either by amylic alcohol or ordinary alcohol. On treatment with sulphuric acid it was gradually turned orange, and was completely decolorised by caustic soda.

Tinned meat, preserved in the foregoing manner, is frequently over-cooked, though this fault has been less evident of late years. Occasionally, through imperfect sealing of the tins, the contents undergo change, and when there is any evidence of this they should on no account be eaten. Incomplete sterilisation will result in gradual bacterial fermentation of the meat, with production of gas, and sometimes with formation of poisonous ptomaïnes. Hence any can which is bulged by internal pressure, or from which gas issues on opening, should be absolutely rejected. Occasionally severe and even fatal ptomaïne-poisoning has occurred by the use of decomposed canned foods, but such cases bear such a small proportion to the enormous number in which the meat is found good and wholesome, that, with care in the directions above-named, danger from this cause is very remote.

Tin is dissolved from the containing can very readily in the case of acid fruits, and sometimes to such an extent as to communicate a distinct metallic taste to the food. Canned meat, soup, and fish are less liable than fruits and vegetables to contamination from this cause, but the evidence on the subject is very conflicting. Thus, J. Attfield (*Pharm. Jour.*, [3], xiv. 719) failed to find more than very minute traces of tin compounds in various canned foods, but states that he not unfrequently detected minute particles of metallic tin by carefully washing the external surfaces of a mass of meat just removed from a can. Out of fifty cans of preserved animal food, G. W. Wigner (*Analyst*, 1880, p. 219) found only one to contain tin in appreciable quantities.

On the other hand, A. E. Menke (*Chem. News*, 1878, xxxviii. 971) detected and determined tin in canned pine-apple, apple, and lobster. In 1880, G. J. Wishart (*Chem. News*, xlii. 47) found tin in canned pine-apples, apples, and greengages, in quantities ranging from 0·21 grain to 0·36 grain of SnO_2 per two-pound can, together with much larger proportions of iron. The taste was distinctly metallic and the fruits were uneatable. In 1883, A. Wynter Blyth found tin in every one of nineteen samples of canned fruit (apricots, pine-apples, tomatoes), the proportions ranging from $1\frac{1}{2}$ to 11 grains per lb.

In 1889, Sedgwick showed that poisonous effects were produced by pears which had been cooked in a tinned saucepan. Beckurts, in the same year, called attention to the formation of tin sulphide by the action of albuminous matters on tin, and Nehring recorded the presence of tin in preserved asparagus in quantities ranging from 0·19 to 0·31 per cent. Bettink, in 1890, found from 19 to 72 milligrammes of tin per kilogramme of tinned lettuce and meat which had occasioned the illness of a number

of soldiers. Kayser found 0·19 per cent. of tin in preserved eels which had proved injurious to several persons who had partaken of them. The author recently found 0·21 per cent. of tin in tomato-sardines suspected to have occasioned colic and diarrhœa.

The amount of tin dissolved necessarily depends on the length of time the article of food has been in contact with the metal, but van Hamel Roos, in an extensive research on the subject, found all tinned foods, whether of animal or vegetable nature, to contain more or less tin (abst. *Analyst*, 1891, p. 195).

O. Hehner (*Analyst*, 1880, p. 219) found canned animal foods of almost every variety to contain more or less tin. The weight of tin found in one of the soups was 0·035 gramme in a one-pound can; in a can of preserved milk, 0·008 gramme; and in a one-pound can of preserved oysters 0·045 gramme, in addition to a considerable quantity of copper.[1] The tin was found throughout the mass of the soups and pasty foods, but in the cases of the hard meats existed chiefly on the surface. In many cases the cans were much discoloured and blackened on the inner surface, but in others the surface of the metal was perfectly bright, although there was an abundance of tin in solution.[2]

For the detection of the tin in the above analyses, Hehner incinerated about 30 grammes of the material in a platinum basin, and heated the ash with strong hydrochloric acid. The greater part of the acid was then boiled off, about 30 to 40 c.c. of water added, and the liquid filtered. The alternate treatment of the residue with acid and water was repeated until no more tin could be extracted. The clear (and, as a rule, colourless) solutions thus obtained were then treated with sulphuretted hydrogen, and any yellow precipitate of stannic sulphide further treated, if necessary, in the usual manner.

In the foregoing process it is assumed that boiling hydrochloric

[1] Copper has been found to occur naturally in oysters.

[2] Tin is not usually regarded as a very active poison, but much evidently depends on the condition, as to solubility or otherwise, in which the metal is exhibited, and whether it be in the stannous or the stannic condition. Hehner has found that freshly-precipitated and moist stannous hydroxide, when given to a guinea-pig, acted as a powerful irritant poison, whereas freshly-precipitated and moist stannic hydroxide was comparatively inert.

The action of tin on the animal organism has been systematically investigated by T. P. White (*Pharm. Jour.*, [3], xvii. 166), who concludes "that tin, though possessing decided toxic properties when introduced into the blood, is entirely devoid of danger when taken internally in any form that could arise from being in contact with fruit or vegetables."

In a case recorded by Luff (*Brit. Med. Jour.*, April 12, 1890), preserved cherries contaminated with tin appeared to act as an irritant and cardiac poison.

acid can be relied on to dissolve tin from the ash of food, but in the experience of the author its complete solution is always difficult and sometimes impossible to effect. It is highly probable that the negative results obtained by some chemists when examining canned foods for tin (*e.g.*, J. Attfield, *Pharm. Jour.*, [3], xiv. 719) have been due to the use of inefficient methods of analysis.

The author has devised the following process for the detection of tin and certain other heavy metals in organic products. With obvious modifications, the method can readily be applied quantitatively.[1] The substance to be examined for heavy metals is placed in a porcelain capsule,[2] and concentrated pure sulphuric acid dropped on it and incorporated with the aid of a glass rod. The acid should be in sufficient quantity to moisten the substance, but an excess should be avoided.[3] The dish is then heated on a water-bath for a short time, after which the temperature is gradually raised to complete the decomposition of the chlorides. About 1 c.c. of strong nitric acid should now be added, and the heating continued till red fumes are evolved. Ignited magnesia in the proportion of 0·5 gramme for each c.c. of sulphuric and nitric acid previously used is now gradually added and incorporated with the material. The dish containing the mixture is then ignited at a *dull red* heat, preferably in a gas-muffle. After cooling, the ash is moistened with nitric acid and then gently reignited, this treatment being repeated till the carbon is wholly consumed. The residue is then treated with eight to ten drops of strong sulphuric acid, heated till fumes are evolved, cooled, boiled with water, diluted, *without filtration*, to about 100 c.c., and sulphuretted hydrogen passed through the liquid to saturation. The solution is then filtered, and examined according to the following scheme of analysis[4]:—

[1] Numerous experiments made in the author's laboratory by F. Hudson-Cox have shown the substantial accuracy of the process, but various modifications were tried before this result was attained.

[2] When it is desired to examine a liquid, it should be evaporated to dryness or concentrated to a syrup before adding the acid. The subsequent steps are the same as when a solid substance is under examination.

[3] Two c.c. of strong sulphuric acid to 25 grammes of material will generally be found a suitable proportion.

[4] By the action of the acids, a large part of the organic matter undergoes oxidation before the stage of ignition is reached. All chlorides and organic salts of the light metals are converted into sulphates, which, not being readily fusible, do not impede the combustion of the remaining carbon. The use of magnesia is advantageous in preventing the formation of metaphosphates, or the conversion of lead sulphate into phosphate. The magnesium sulphate and nitrate formed act as oxidising agents at a red heat, and greatly facilitate the combustion of the carbon. In the end, all tin is left insoluble as oxide

<table>
<tr><td colspan="3">AQUEOUS SOLUTION may contain zinc, iron, earthy phosphates, &c. Add bromine-water to destroy sulphuretted hydrogen and ensure the existence of any iron in the ferric state, boil, then add excess of ammonia, boil again, and filter.</td><td colspan="3">PRECIPITATE AND RESIDUE may contain PbS, SnO_2, SnS_2, CuS, $CaSO_4$, &c. Fuse in porcelain for ten minutes with two grammes of mixed potassium and sodium carbonates and one gramme of sulphur. When cool, boil with water and filter.</td></tr>
<tr><td>PRECIPITATE may contain iron, phosphates, &c.</td><td colspan="2">FILTRATE, if blue, contains nickel. Divide into two portions :—</td><td colspan="2">RESIDUE. Boil with strong hydrochloric acid as long as sulphuretted hydrogen is evolved, add a few drops of bromine-water to complete the oxidation of the copper sulphide, and filter if necessary. To the filtrate add excess of ammonia, when a blue coloration will be indicative of copper. Acidulate the liquid with acetic acid and divide into two portions.</td><td>FILTRATE. Acidulate with acetic acid. A yellow precipitate of SnS_2 indicates Tin.[2]</td></tr>
<tr><td></td><td>I. Heat to boiling and add potassium ferrocyanide. White precipitate or turbidity indicates Zinc.</td><td>II. If zinc be found in I., for its determination acidulate the ammoniacal solution strongly with acetic acid, filter if necessary, and precipitate the zinc from the filtrate by sulphuretted hydrogen. Any nickel present will be co-precipitated.</td><td>I. Add potassium bichromate. A yellow precipitate of $PbCrO_4$ indicates Lead.</td><td>II. Add potassium ferrocyanide. A brownish precipitate or coloration indicates Copper.[1]</td><td></td></tr>
</table>

or phosphate, the lead as insoluble sulphate or phosphate, while copper and zinc are converted into soluble sulphates. The presence of phosphates in the aqueous extract of the ash must be borne in mind, as certain tests and methods are thereby rendered inapplicable ; in fact, it is chiefly to avoid the difficulty in effecting the determination of tin and lead in the presence of phosphates and sulphates that the particular method of analysis is prescribed.

[1] Exceedingly minute traces of copper are perhaps best detected by introducing a knitting needle into the slightly acidulated and tolerably concentrated extract of the ash, removing it after some hours, cautiously rinsing it in water, and then immersing it in dilute ammonia, with free contact of air. The copper precipitated on the iron will pass into solution, and may be detected by acidulating the ammoniacal liquid with acetic acid and adding potassium ferrocyanide, when a chocolate or brownish coloration will be produced, if a trace of copper be present.

[2] When tin is known to be present, the amount may be found by treating the precipitate of stannic sulphide with strong nitric acid, igniting the metastannic acid formed, and weighing the resultant SnO_2. For the detection of tin, the stannic sulphide should be treated with hydrochloric acid and bromine-water, and the filtered liquid boiled with metallic iron to reduce the tin to the stannous condition. The liquid is diluted and decanted from the undissolved iron (and any precipitated *antimony*, &c.). The tin can then

G. Denigès recommends the violet coloration produced with kakotelin as a delicate test for stannous compounds in solution. The reagent is prepared by dissolving 0·5 gramme of brucine in 5 c.c. of cold nitric acid, adding 250 c.c. of water, boiling for ten to fifteen minutes, and making up the cooled liquid to 250 c.c. A few drops of this reagent should be added to the liquid supposed to contain tin, when an amethyst coloration will be produced, which becomes blue with alkali in the absence of air, and green in the presence of air. The coloration is destroyed by excess of stannous salt. Ferrous and cuprous salts do not yield this reaction, but thiosulphates and some other reducing agents produce the coloration.

Warden and Bose have published (*Chem. News*, 1890, lxi. 304) some unusually complete *analyses of typical samples* of canned beef and mutton. They found the moisture to range from 49 to 57 per cent.; the fat from 10 to 22; the proteids (*i.e.*, N × 6·25) from 24·5 to 29; the ash from 0·62 to 4·36; the chlorine from 0·11 to 2·65; the phosphoric acid from 0·31 to 0·40; the hot-water extract from 5·35 to 10·14 per cent., with a content of nitrogen ranging from 0·88 to 1·10 per cent.

In making these analyses, the entire contents of a can were thoroughly pulped in a large mortar, great care being taken to scrape the interior of the can free from fat and jelly. The plan of taking a slice of the contents and regarding that as a fair sample Warden regards as fallacious.

For the determination of *moisture*, a weight of from 5 to 6 grammes of the sample was teased with forceps in a flat platinum dish, and dried first at 100° and subsequently at 120°. The samples were then moistened with absolute alcohol, and re-dried. The whole time of heating occupied from eight to nine hours. In another large platinum dish from 30 to 40 grammes weight of pulp was similarly heated, reduced to fine powder, and again heated. This dried pulp was preserved in a closely-stoppered bottle and employed for the determination of fat, nitrogen, and aqueous extract. The ash and ash-constituents were conveniently determined on the undried pulp.

In determining the *ash*, the portion of the pulp used for ascertaining the moisture was charred at a temperature below redness, crushed with a glass rod, exhausted with boiling water, and again ignited. The residue was again treated with boiling water, and the *insoluble ash* ignited and weighed. The aqueous extract

be detected by adding a drop of mercuric chloride solution, which will produce a white or a grey turbidity, according to the amount of tin present; or the tin may be determined volumetrically by titration with a very dilute standard iodine solution :—$SnCl_2 + 2HCl + I_2 = SnCl_4 + 2HI$.

was evaporated to dryness, the residue heated nearly to redness, and weighed to find the *soluble ash*. The *total ash* was regarded as the sum of the soluble and insoluble ashes determined as just described, and it was found that the figures thus obtained agreed well with determinations of the total ash by direct ignition, while avoiding the difficulty experienced in the latter case in effecting complete combustion of the carbon without losing a portion of the alkali-metal salts by volatilisation.

The soluble ash was used for the determination of *potassium* and *sodium*, by dissolving it in water and adding in succession barium chloride, ferric chloride, and ammonia to the warm solution, the last reagent being employed in quantity sufficient to render the liquid just alkaline. The precipitate (consisting of $BaSO_4$, $FePO_4$, and FeH_3O_3) was filtered off, the filtrate treated with ammonium carbonate and ammonium oxalate, and warmed for some time on the water-bath. The precipitate (consisting of $BaCO_3$ and CaC_2O_4) was removed by filtration, the filtrate evaporated to dryness in platinum and the residue gently ignited. The residue was redissolved in water, the solution filtered from a little barium carbonate, the filtered liquid treated with a drop of hydrochloric acid, and evaporated with platinic chloride to effect a separation of the potassium and sodium.[1]

For the determination of the *chlorine* and *phosphoric acid*, Warden and Bose mix 20 grammes of the freshly pulped meat with about 2 grammes of pure sodium carbonate, dissolved in sufficient water to cover the pulp. The resulting magma is evaporated to dryness, carbonised, extracted successively with water and with nitric acid, the residue again ignited and dissolved in nitric acid, and the chlorine and phosphates determined in the mixed solution by the usual methods.

The *total nitrogen* was determined in the dried pulp by Kjeldahl's process, and multiplied by the factor 6·25 to find the *proteids*.

The *extractive matter* was determined by boiling 1 gramme of the dry pulp with distilled water in a 100 c.c. flask, and when cold diluting to 100 c.c. The liquid was passed through a dry filter, and an aliquot portion of the very faintly opalescent filtrate evaporated to dryness in a platinum dish and the *residue* weighed. The greater part of the filtrate was used for the determination of *extractive nitrogen* by Kjeldahl's method.

The *fat* was determined by treating about 0·5 gramme of the

[1] Warden and Bose (*Chem. News*, lxi. 292) describe an indirect method of determining potassium and sodium in the mixed chlorides isolated as above. This they find to yield fairly accurate results, and to be specially applicable to cases in which small amounts of sodium and potassium have to be determined.

dried pulp in a small accurately-stoppered weighing bottle, and adding a measured volume of light petroleum-ether from a burette. The mixture was allowed to stand for two days, with occasional agitation, when a portion of the perfectly clear supernatant liquid was withdrawn by a small burette, and a carefully-measured volume discharged into a small beaker. The petroleum-ether was then distilled off, and the residual fat dried at 100° and weighed. This method, which is due to Dragendorff, was found by Warden and Bose to give results which agreed closely with those obtained by exhausting the substance with a solvent of fat in the usual way.

Warden and Bose have compared their analyses of canned meats examined by the foregoing process with the figures obtained by König by the analysis of fresh beef and mutton (page 267). They find that, while the percentage of moisture in the canned meat is usually lower than in fresh meat, the fat in canned meat as a rule exceeds the proportion in fresh. Taking the nitrogenous matters as representing the nutritive value of the meat, and ascertaining their amount by multiplying the total nitrogen by 6·25, they obtain the following amounts of albuminous matters in the anhydrous and fat-free samples of meat examined :—

	Albuminous matters in anhydrous, fat-free meat.
Average of canned beef samples . .	87·06 per cent.
Average of canned mutton samples, .	87·19 ,,
Average of all fresh cow- and ox-flesh, .	93·94 ,,
Average of all fresh mutton, . .	93·81 ,,
Average of all canned meat samples, .	87·12 ,,
Average of all fresh meat samples, .	93·87 ,,

Analyses by König of seven specimens of canned meat showed them to have the following average composition :—Proteids, &c., 28·97 ; fat, 12·63 ; ash, 3·71 ; and water, 54·69 per cent. These figures correspond to 10·33 per cent. of nitrogen and 27·27 per cent. of fat in the anhydrous samples, and to 88·63 per cent. of albuminous matters in the anhydrous and fat-free samples.

From the foregoing figures Warden and Bose conclude that canned meat has a sensibly lower nutritive value than fresh meat, and that this inference from purely chemical data is quite in accordance with arguments based on physiological grounds. The apparent difference is, however, increased by the salt which has evidently been added to some of the canned meat samples.

Preservatives in canned foods can be sought for and determined by the methods employed for the examination of milk (page 175. For boric acid, see also page 331).

The gases of canned meat may be conveniently collected for analysis as described by C. A. Doremus (*Amer. Chem. Jour.*, 1897, xix. 733).

Extracts of Meat.

A variety of preparations occur in commerce, under the titles of meat-extract, fluid beef, beef-juice, &c. These articles, while useful and valuable in their way, do not justify the extravagant claims made respecting certain of them.

Liebig's Extractum Carnis.

The oldest preparation of the nature of a meat-extract was that of Justus von Liebig.[1] According to the original directions, the extract was to be prepared by treating lean beef (chopped fine) with eight times its weight of cold water, straining from the insoluble fibrous matter, heating the liquid to a temperature sufficient to coagulate the dissolved albumin, filtering, and evaporating the filtrate to a syrupy condition. It is evident that both proteid and gelatinoid bodies are excluded from an extract prepared in the cold in the above manner. But this method of preparation was admitted by Liebig to be impracticable on a manufacturing scale, and in 1865 he stated that the only available plan was to mix the chopped flesh with water free from gypsum, and to raise the temperature of the mixture to 180° F. (*Pharm. Jour.*, [3], xiii. 414).

In the following passage, actual boiling with water is recommended by Liebig:—"Those who may feel inclined to prepare extract of meat as an article of commerce, will entirely miss their aim, unless they most carefully and conscientiously seek to avoid the errors of those who have hitherto attempted it. Half an hour's boiling of the chopped meat with eight or ten times its weight of water suffices to dissolve all the active ingredients. The decoction must, before it is evaporated, be most carefully cleansed from all fat (which would become rancid), and the evaporation must be conducted in the water-bath. True extract of meat is never hard and brittle, but soft, and it strongly attracts moisture from the atmosphere" (Liebig's *Letters on Chemistry*).

Liebig himself has stated that 34 lbs. of meat are required to produce 1 lb. of extract, a fact which shows how completely the real nutritive portion of the meat must be excluded. In short, an extract of meat prepared according to Liebig's original directions is

[1] Extract of meat was first described by Proust, in 1801, but the method of manufacturing it on a commercial scale is due to Liebig, and was described by him as early as 1847. The Liebig's Extract of Meat Company was established in 1865, but the article itself has been extensively made and sold under the designation of Liebig's Extract since the year 1856.

practically free from albumin, gelatin, and fat, and may be said to comprise the saline and extractive matters of the meat.[1] Among these constituents, creatinine, lactic acid, phosphates, and potassium salts occupy a prominent position. The true nature and value of Liebig's extract is now becoming generally recognised. Though not strictly of alimentary value, it possesses marked stimulant and restorative properties, which render it useful in exhausted states of the system. Like tea and coffee, it is a food-adjunct rather than a true food.[2] Being rich in the flavouring matter of cooked meat ("osmazome"), Liebig's extract is often used for flavouring soups.[3]

Druitt (*Trans. Obstetr. Soc.*, 1861, p. 143), in describing the characters of a liquid essence of beef which had been prepared according to his instructions, states that it exerted a rapid and remarkable stimulating action on the brain, and proposed it as an auxiliary to, and partial substitute for, brandy in all cases of great exhaustion or weakness attended with cerebral depression or

[1] Various recent analyses of the extract of meat manufactured by the Liebig Company show that the commercial preparation contains material quantities of gelatinoid bodies and soluble, non-coagulable proteids.

[2] In a letter to *The Times* (October 1, 1872) Liebig wrote:—"Neither tea nor extract of meat is nutriment in the ordinary sense; they possess a far higher importance by certain medicinal properties of a peculiar kind. The physician does not employ them as specific remedies. They serve the healthy man for the preservation of his health. Taken in proper proportions they strengthen the internal resistance of the body to the most various external injurious influences, which combine to disturb the general vital processes, and adjust these latter. . . . It is surely a grave offence against all the laws of physiology to compare tea, coffee, and extract of meat with the more common articles of food, and, because they are not that, to draw the inference, as Dr Edward Smith has done, that they are nothing at all. . . . Extract of meat is beef-tea made from fresh meat—not roasted—in the purest state, condensed to the consistency of a thick honey, to which nothing whatever is added by the manufacturer. The assertion that common salt is added to the extract is an unjustifiable invention. . . . The necessity for the consumption of meat is considerably lessened when extract of meat is added to the vegetable food; in addition to the nutritive value which vegetables possess in themselves, they acquire in the soluble component parts of meat those substances which give a meat-diet its peculiar effect."

[3] Kemmerich failed to keep animals alive on a diet of meat-extract, and the urine contained an abnormal proportion of carbon. It is not clear, however, that sufficient extract was ingested to correspond to ordinary food in the carbon and nitrogen content. M. Rubner found that the urine of dogs fed on Liebig's preparation acquired on concentration the peculiar odour of meat-extract. He concludes that the meat-extract does not contribute to the bodily heat, that the waste of tissue is neither hastened nor retarded by it, and that it passes away unaltered in composition (abst. *Jour. Chem. Soc.*, 1885, page 409).

despondency. Similar stimulating effects have been observed as resulting from a copious employment of Liebig's extract. The effect of a feast of animal food on savages whose customary diet was almost exclusively vegetable has been observed to be similar to the administration of an intoxicating spirit or drug.

COMMERCIAL MEAT-EXTRACTS.

A great number of preparations having the general nature of Liebig's extract of meat are now articles of commerce.[1] Some of these have received additions of gelatin, blood-albumin, meat-fibre, &c., while in certain cases the albumin has been more or less peptonised.[2] It is claimed on behalf of these preparations that the various additions and methods of treatment give them value as real foods, but this is true in but a very limited sense, since the amount of such preparations which would require to be taken to furnish the carbon and nitrogen requisite to support life is enormously beyond the quantity of any of the preparations which could be consumed without upsetting the system, to say nothing of the extravagant cost of all such preparations if used in quantity necessary to sustain life.[3]

[1] The term "Liebig's extract" has now a wide significance and has been decided by High Court of Justice to be public property. Hence it does not always imply an article manufactured by the Liebig's Extract of Meat Company.

[2] An interesting light on the methods of manufacturing meat-extracts and pseudo-peptones is afforded by the following process, which forms the subject of a patent by Etienne and Delhaye (1890, No. 10,961). After removing the tendons and grease, the meat is chopped and mashed, mixed with about half its weight of water, and heated by steam under pressure for one hour to a temperature ranging between 150° and 175° C. A portion of the albuminoid matter is rendered soluble, and goes into solution with the extractives. The residue, when pressed, forms a friable mass amounting to about one-third of the fresh meat used. This residue is treated on the water-bath with an equal weight of concentrated hydrochloric acid, until the fibro-muscular tissue is quite disintegrated and decomposed, when the liquid is filtered. The insoluble residue is sold as manure. The liquors are neutralised with sodium carbonate, and then contain "peptone" and sodium chloride in solution. If pure "peptone" is required, the liquid is decolorised with animal charcoal, and dialysed to remove the salts; but if only a meat-extract is required, the liquors from the steam treatment of the meat and the neutralised liquors from the acid treatment are mixed and evaporated in a vacuum until sufficiently concentrated.

[3] In judging of the amount of credence to be attached to statements of the nutritive value and concentration of meat-extracts and similar preparations, it should be borne in mind that fresh lean meat contains about 20 per cent. of nutritive matter and 75 per cent. of water. Hence by the desiccation of 4 lbs. weight there will be obtained 1 lb. of dry substance, of which 80 per cent. is nutritive proteid matter, the remaining 20 per cent. consisting of fat, meat-

It appears, therefore, that meat-extracts have a true value as stimulants and restoratives, the proportions of meat-bases, extractives, and salts present being an index of their value in this respect. On the other hand, all attempts to give them the characters of true nutritive concentrated foods can meet with but a very limited success.

A failure to appreciate these facts has caused very delusive values to be placed on such preparations, and the errors have been further magnified by the discordant methods of judging of the value of such articles. Thus Stutzer has expressed the opinion that the albumoses and peptones are the only constituents of value in a meat-extract, and he ignores any meat-fibre, gelatin, or coagulable albumin which may be present. Another well-known analyst regards the matters precipitated by alcohol as being the only constituents of value; but such a contention is clearly untenable, since the precipitate formed by alcohol contains a variable but very considerable percentage of non-nitrogenous extractives and salts.[1]

In the opinion of the author, the following are the chief considerations on which a judgment should be formed of the value of a meat-extract :—

The percentage of *water* should first be taken into account. Thus a preparation which contains only 10 per cent. of solid matters must evidently have less than half the food-value of the meat from which it is derived; and it might happen that, exclusive of the meat-bases (valuable merely as stimulants) and the gelatin (of questionable nutritive value), such a preparation contained a smaller proportion of nitrogenised organic matter than is present in ordinary beer.

It is usual in analyses of meat-extracts to state the whole of the *chlorine* in terms of sodium chloride. This convention is not scientifically accurate, since the chlorine derived from the meat

bases, salts, &c. By no possible means can further material concentration of the nutritive matter be effected. Statements that meat-extracts, meat-essences, fluid meats, &c., contain the nutritive matter of thirty, forty, or fifty times their weight of fresh meat are unjustifiable. Preparations still containing nearly half their weight of water, but of which a table-spoonful is said to be equal in nutritive value to a full meal of fresh lean meat, and meat-lozenges and tablets weighing less than a gramme, one or two of which are alleged to suffice for a meal, are evidently quite inefficient for their pretended purpose as concentrated forms of food.

[1] On treating an aqueous solution of Liebig's extract of meat with excess of strong alcohol, the author obtained a precipitate weighing 31·8 per cent. of the original extract, and containing 11·7 per cent. of ash. The nitrogen in the precipitate corresponded to 10·6 per cent. of proteids, leaving 9·5 for non-nitrogenous extractive matters.

exists chiefly, if not entirely, in the form of potassium chloride, the balance being as sodium chloride, added in the form of common salt. Making an allowance of, say, 0·06 per cent. of sodium chloride for every unit per cent. of dry solid matter present in the preparation, any excess may be fairly regarded as having been added in the form of *common salt*. Thus, if a meat-extract contain 25 per cent. of water (=75 per cent. of solids) and 10 per cent. of chlorine in terms of sodium chloride, the allowance for natural chlorides would be 4·50 per cent. (=75 × ·06); and 5·50, that is, the difference between this figure and 10·00, will represent the added common salt of the sample. Added salt should, of course, be deducted in estimating the effective concentration of the preparation.

The *bases* are among the most important of the natural constituents of meat-extracts, but unfortunately the existing methods for their determination are far from satisfactory. The amount of meat-bases in a preparation is often deduced from the percentage of nitrogen over and above that found to exist in other forms. Apart from the errors attendant on this indirect method of determination, it is difficult to fix on a suitable factor for calculating the actual amount of meat-bases from the nitrogen ascribed to that form of combination. Stutzer adopts the factor 3·12, which would be correct if the bases were wholly creatine. Hehner prefers to use the albumin-factor (6·25) for all nitrogenous constituents of meat-extracts for convenience of comparison, with the knowledge that it is too high in the case of the meat-bases, but he points out that by adopting it the figure obtained (by difference) for the non-nitrogenised extractive matters is much lower and probably a better approximation to the truth than when Stutzer's factor is employed. Still, with the exception of leucine, tyrosine, and carnine, the factors for calculating the nitrogen to the bases are all lower than that for creatine.[1]

[1] The following are the factors corresponding to the chief bases, &c., of muscle:—

Substance.	Formula.	Proportion of Nitrogen.	Factor.
Creatine,	$C_4H_9N_3O_2$.	42 in 131	3·12
Creatinine,	$C_4H_7N_3O$.	42 in 113	2·69
Xanthine,	$C_5H_4N_4O_2$.	56 in 152	2·71
Xanthocreatinine,	$C_5H_{10}N_4O$.	56 in 142	2·54
Hypoxanthine,	$C_5H_4N_4O$.	56 in 137	2·44
Carnine,	$C_7H_8N_4O_3$.	56 in 196	3·50
Leucine,	$C_6H_{13}NO_2$.	14 in 131	9·36
Tyrosine,	$C_9H_{11}NO_3$.	14 in 181	12·93
Urea,	CH_4N_2O.	28 in 60	2·14
Uric acid,	$C_5H_4N_4O_3$.	56 in 168	3·00

The added *albumin* and *meat-fibre* present in some commercial meat-extracts have, of course, a true food-value, but the amount of these constituents present in such a quantity of a meat-extract as is usually, or could be, taken at a time is too insignificant to give it any appreciable value as nutriment.[1]

The same remark applies to *gelatin*, which is present in some preparations as a product of the hot water or superheated steam employed for the extraction, and in other cases has been added as such. In fact, that gelatin has any value as a food is open to question, and has been the subject of much dispute.

The *albumoses* and *peptones* present in some meat-extracts have the advantage of being readily assimilated, and so far as they go are desirable constituents of such preparations. It is, however, almost certainly the fact that the proportion of the peptones has been greatly over-estimated, and that some preparations in which certain analyses show a material proportion of peptones are in reality almost, if not entirely, devoid of such constituents.[2]

Composition of Commercial Meat-Extracts.[3]

Numerous analyses of meat-extracts and allied preparations have been published, but they have little value unless the exact method of analysis has been specified, and are useless for comparison, except where the various analyses of a series have been made by the same method. It must be borne in mind that preparations bearing the same names, and produced by the same firms at different dates, are liable to considerable variations in their character.

The following valuable table shows the composition of the chief commercial meat-extracts, according to results obtained in the years 1896 and 1897, by Otto Hehner. The analyses were made by Stutzer's method (page 320) except that in some of the later analyses the albumoses were precipitated by zinc sulphate instead of by ammonium sulphate. The peptones were precipitated by phospho-tungstic acid. The various nitrogenised matters were in all cases calculated from the nitrogen found by the factor 6·25 (compare page 303). As all the analyses and

[1] In the lean of meat, the proteids bear to the sum of the extractives, meat-bases, and salts the proportion of about 4 to 1. Hence, if all the meat-fibre, &c., were again added to the extract the product would contain the solids of meat in the same relative proportions. Assuming in a preparation the presence of 70 per cent. of extractives, &c., and 10 per cent. of meat-fibre, it follows that only about one part out of twenty-eight of fibre separated has been returned to the extract.

[2] Analyses of various commercial "peptones" are given on page 383 *et seq.*

[3] The following list of papers, treating of meat-extracts and commercial peptones, has been compiled at the author's request by A. R. Tankard. Many

calculations were made by the same method, the results are comparable.

of the references are to abstracts of foreign papers in English journals, since these are more readily accessible than the originals.

Year.	Author.	Reference.	Remarks.
1880	A. Stutzer, . . .	*Jour. Chem. Soc.*, xxxviii. 676.	Estimation of "protein compounds" by cupric hydroxide.
1880	M. Rubner, . . .	*J. C. S.*, xxxviii. 904; xl. 451.	Nutritive value of fluid meat.
1881	C. Estcourt, . .	*Analyst*, vi. 201.	Composition of meat-extracts.
1881	S. Darby, . . .	*J. C. S.*, xl. 450.	Fluid meat.
1881	T. Defresne, . .	*Pharm. Jour.*, [3], xii. 8	Determination of peptones.
1881	C. Tanret, . . .	*J. C. S.*, xl. 832.	Character of peptones.
1882	A. Stutzer, . . .	*J. C. S.*, xlii. 1239.	Precipitation of proteids by cupric hydroxide.
1882	A. H. Chester, . .	*Analyst*, vii. 124.	Composition of various meat-extracts.
1882	Justice Field (Judgment),	*Pharm. Jour.*, [3], xiii. 412.	Liebig Company *v.* Anderson.
1885	O. Hehner, . . .	*Analyst*, x. 221.	Analyses of beaf-tea.
1885	F. Szymanski, . .	*J. C. S.*, xlviii. 822.	Characters of peptone.
1885-6	A. Stutzer, . . .	*Analyst*, x. 57, 73; *J. S. C. I.*, v. 37.	Analysis and composition of various meat-extracts.
1886	H. Weiske, . . .	*J. C. S.*, l. 1087.	Peptones are not precipitated by cupric hydroxide.
1886	Kühne & Chittenden,	*J. C. S.*, l. 818.	Determination of albumose and peptone.
1886	S. H. C. Martin, . .	*J. C. S.*, l. 636.	Separation of peptones from other proteids by ammonium sulphate.
1888	E. Schumacher-Kopp,	*J. S. C. I.*, vii. 130.	Analyses of Maggi's meat preparations.
1888	J. König, . . .	*J. S. C. I.*, vii. 449.	Valuation of peptones.
1889	König & Kisch, . .	*J. C. S.*, lvi. 803.	Determination of albumose and peptone.
1890	G. Bruylants, . .	*J. C. S.*, lviii. 1351.	Analysis of peptones.
1890	A. Denaeyer, . .	*J. C. S.*, lviii. 1351; *Analyst*, xv. 101.	Analysis of peptones.
1891	A. Denaeyer, . .	*Analyst*, xvi. 98, 234.	Analysis of peptones.
1891	Etienne & Delhaye, .	English Patent, 10,961, 1890.	Improvements in preparation of peptonised soluble meat and peptone.
1892	Heaton & Vasey, .	*Analyst*, xvii. 28; *J.C. S.*, lxii. ii. 1535.	Analysis of peptones and review of literature.
1892-3	S. Riva-Rocci, . .	*J. C. S.*, lxii ii. 1136; *Ch. News*, lxvii. 254.	Determination of albumose and peptone in stomach contents.
1892-3	L. A. Hallopeau, .	*Ph. J.*, [3], xxiii. 181; *J. C. S.*, lxiv. ii. 104.	Determination of peptones by precipitation with mercuric nitrate.
1893	A. Stutzer, . . .	*J. C. S.*, lxiv. ii. 146.	Determination of nitrogenous constituents of commercial peptones.
1893	W. Kühne, . . .	*J. C. S.*, lxiv. i. 233.	Characters of albumoses and peptones.
1894	E. Kemmerich, . .	*J. C. S.*, lxvi. ii. 150.	Composition of South American meat-extract and meat-peptone.
1895	E. O. Beckmann, .	*Analyst*, xx. 44; *J. C. S.*, lxviii. ii. 375.	Determination of gelatin and albumin in peptone.
1895	L. Hugounenq, . .	*Analyst*, xx. 94.	Analyses of adulterated peptones.
1895	A. Stutzer, . . .	*Analyst*, xx. 182; *J. C. S.*, lxviii. ii. 543.	Composition of various meat-extracts.
1895	A. Stutzer, . . .	*Analyst*, xx. 246; *J. S. C. I.*, xiv. 897.	Nitrogenous constituents of meat-extracts and commercial peptones.
1895	— Dutto, . . .	*J. C. S.*, lxviii. ii. 468.	Assay of peptones by precipitation with potassio-bismuth iodide.
1896	König & Bömer, . .	*Analyst*, xxi. 17; *J. C. S.*, lxx. ii. 82.	Composition of various meat-extracts.
1896	A. Bömer, . . .	*Analyst*, xxi. 16; *J. C. S.*, lxx. ii. 83.	Precipitation of albumoses by zinc sulphate.
1896	A. Stutzer, . . .	*Analyst*, xxi. 19; *J. C. S.*, lxx. ii. 84.	Determination of gelatin in meat-extracts and peptones.
1896	L. de Koningh, . .	*J. C. S.*, lxx. ii. 552.	Determination of solids in beef-tea.
1897	G. Bruylants, . .	*J. S. C. I.*, xvi. 640.	Analysis of meat-extracts.
1897	A. Denaeyer, . .	*Ph. J.*, [4], 1897, ii. 3.	Value of peptones.
1897	Rideal & Stewart, .	*Analyst*, xxii. 231.	Precipitation of proteids by chlorine.
1897	Allen & Searle, . .	*Analyst*, xxii. 258.	Precipitation of proteids by bromine.
1897	H. Schjerning, . .	*Zeits. anal. Chem.*, 1897, p. 643.	Precipitants of proteids.

Number.	Description.	Water.	Fat (Petroleum-Ether Extract).	Gelatin.	Albumin.	Meat-fibre and Coagulated Albumin.	Albumoses.	Peptones.	Meat-bases.	Ash.	Difference.	Sodium Chloride.	Phosphoric Acid.	Total Nitrogen.
1	Liebig Company's *Extractum Carnis*, ...	15·26	0·34	5·18	..	2·12	2·01	8·06	39·32	23·51	4·20	5·81	6·97	9·07
2	Armour's Extract of Meat, .	15·97	0·21	3·31	..	..	1·75	5·13	41·12	29·36	3·15	9·74	6·76	8·21
3	Brand & Co.'s *Extractum Carnis*, ...	17·85	0·38	4·56	..	1·81	4·19	10·16	38·90	18·80	2·87	3·31	5·16	9·80
4	Liebig's Extract (Bovril & Co.'s make), ...	22·24	0·29	5·50	..	1·30	3·62	8·44	38·58	20·45	−0·42	5·14	5·50	9·19
5	Brand & Co.'s Meat-Juice, .	55·48	0·10	0·69	1·00	..	1·06	2·50	12·50	11·06	15·61 Glycerin ?	4·43	1·52	2·84
6	Valentine's Meat-Juice, . .	55·53	0·10	0·75	0·25	..	2·00	2·87	12·48	12·01	14·01	2·35	2·85	2·92
7	Wyeth's Meat-Juice, . .	61·61	0·08	1·12	5·62	..	1·08	1·86	9·44	14·78	4·41	6·96	3·01	3·06
8	Borthwick's Bouillon, . .	36·19	0·25	1·37	..	4·00	1·16	11·09	24·25	17·93	3·76	6·09	3·58	6·70
9	Vitalia Meat-Juice, . .	70·19	0·32	0·45	16·44	0·37	0·05	0·37	2·82	6·65	2·34	5·11	0·37	3·28
10	Brand & Co.'s Essence of Beef,	89·68	0·06	5·12	..	..	0·19	0·57	3·43	1·00	−0·05	0·33	0·40	1·49
11	Bovril Company's Fluid Beef,	28·34	1·02	3·81	..	5·37	8·38	13·18	19·38	17·67	2·85	9·07	4·05	8·02
12	Bovril Fluid Beef (unseasoned),	44·75	0·62	1·06	..	7·31	2·38	6·25	17·12	19·90	0·61	11·42	3·34	5·46
13	Bovril for Invalids, . .	24·34	1·07	4·56	..	5·87	5·56	6·44	34·07	16·50	1·59	5·23	3·35	9·20
14	Bovril for Invalids, . .	17·47	0·51	2·56	4·43	15·25	1·06	8·82	31·89	16·30	1·91	2·46	1·43	10·21
15	Caffyn's *Liquor Carnis*, . .	48·46	0·11	0·25	2·19	0·94	3·65	0·98	11·30	9·05	22·17 Glycerin.	4·43	0·62	3·09
16	Extract of Meat with Vegetable Extract, . . .	30·03	0·10	1·69	6·12	..	1·74	4·85	16·97	23·47	15·05 Carbohydrate.	11·56	3·02	5·02

The following table shows the amounts of nitrogen existing in different forms in the foregoing preparations :—

Number.	Description.	Nitrogen existing as						
		Gelatin.	Albumin.	Meat-fibre and Coagulated Albumin.	Albumoses.	Peptones.	Meat-bases.	Total Nitrogen.
1	Liebig's *Extractum Carnis*,	0·83	..	0·34	0·32	1·29	6·29	9·07
2	Armour's Extract of Meat,	0·53	..	..	0·28	0·82	6·58	8·21
3	Brand & Co.'s *Extractum Carnis*,	0·73	..	0·29	0·67	1·69	6·42	9·80
4	Liebig's Extract (Bovril Co.'s make),	0·88	..	0·21	0·58	1·35	6·17	9·19
5	Brand & Co.'s Meat-Juice,	0·11	0·16	..	0·17	0·40	2·00	2·84
6	Valentine's Meat-Juice,	0·12	0·04	..	0·32	0·46	1·98	2·92
7	Wyeth's Meat-Juice,	0·18	0·90	..	0·17	0·30	1·51	3·06
8	Borthwick's Bouillon,	0·22	..	0·64	0·19	1·77	3·88	6·70
9	Vitalia Meat-Juice,	0·07	2·63	0·06	0·01	0·06	0·43	3·28
10	Brand & Co.'s Essence of Beef,	0·82	..	..	0·03	0·09	0·55	1·49
11	Bovril Fluid Beef,	0·61	..	0·86	1·34	2·11	3·10	8·02
12	Bovril Fluid Beef (unseasoned),	0·17	..	1·17	0·38	1·00	2·74	5·46
13	Bovril for Invalids,	0·73	..	0·94	0·89	1·03	5·61	9·20
14	Bovril for Invalids,	0·41	0·71	2·44	0·17	1·41	5·07	10·21
15	Caffyn's *Liquor Carnis*,	0·04	0·35	0·15	0·58	0·16	1·81	3·09
16	Extract of Meat and Vegetables,	0·27	0·98	..	0·28	0·77	2·72	5·02

The foregoing preparations may be roughly classified as :—concentrated meat-extracts, represented by analyses 1 to 4 on the table ; articles of the bouillon and "meat-juice" class, represented by Nos. 5 to 10 ; and preparations which have received an addition in material quantity of a substance not naturally a constituent of a meat-extract. Thus, the finished preparations of the Bovril Company contain a variable percentage of finely-divided meat-fibre and sometimes added albumin. "Bovril" is stated to contain "the entire nourishment of prime ox-beef." "Invalid Bovril" "differs from ordinary bovril in being more concentrated and quite devoid of seasoning," and is described as "the most perfect form of concentrated nourishment at present known."

The following analyses of Bovril preparations are by A. Stutzer (abst. *Analyst*, 1895, p. 182).

	Bovril Fluid Beef.	Bovril Fluid Beef (seasoned).	Bovril for Invalids.	Bovril Beef-Jelly.	Bovril Lozenges.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Water,	29·14	44·42	28·13	89·15	9·47
Sodium chloride,	14·12	10·72	4·57	0·26	1·63
Other salts,	3·38	7·60	11·50	1·04	5·71
Organic matter,	53·36	37·26	55·80	9·55	83·19
Total nitrogen,	8·25	5·12	8·69	1·46	11·94
Meat-fibre nitrogen,	0·73	0·90	0·70	..	0·57
Gelatin nitrogen,	0·09	0·09	0·15	0·29	0·70

Samples 15 and 16 are other examples of meat-extracts to which additions have been made.

The following are additional published analyses of preparations which appear in the table on page 306. The analyses are somewhat wanting in detail, but are of interest as confirmations of the general character of the articles in question, and as illustrating their variation from time to time.

	Valentine's Meat-Juice.			Wyeth's Beef-Juice.		Brand & Co.'s Essence of Beef.			Cibil's Fluid Extract of Beef.	
	R. R. Tatlock.	R. H. Chittenden, 1891.	O. Hehner, 1893.	R. R. Tatlock.	R. H. Chittenden, 1891.	A. Dupré, 1886.	R. R. Tatlock.	O. Hehner, 1893.	A. Dupré, 1886.	R. R. Tatlock.
Water,	51·40	60·31	55·24	56·13	57·88	89·45	90·48	91·23	62·50	63·85
Ether extract,	0·04	0·78	4·80	Trace.	0·85	Trace.	Trace.	0·18	Trace.	0·04
Gelatin and albumin,	7·66	0·55 (Albumin.) None.	0·93	11·16	0·47 (Albumin.) None.	5·88	4·83	3·79	8·00	10·13
Peptone,			1·55							
Creatine and meat-extractives,	18·56	29·15	18·27	10·04	35·08	4·49	2·98	3·96	..	7·78
Non-nitrogenous extractives,	11·96		8·08	7·23			0·47	None.	..	2·06
Sodium chloride,	10·38	11·30	2·62	15·44	17·52	1·25	1·24	0·45	19·15	16·14
Other mineral matters,			8·51					0·39		
Containing P_2O_5,	..	4·00	..	..	3·94	0·59	..	..	1·74	..

Tatlock's and Dupré's analyses were probably made by some modification of the alcohol process (page 311). The figures of R. H. Chittenden are from his address to the Philadelphia County Medical Association (May 1891). The results show generally that the preparations contain a large percentage of water, and, though of value as stimulants or food-adjuncts, that they cannot be regarded as concentrated forms of nutrient food.

"Cibil's Fluid Extract of Beef" is said to be manufactured with the aid of papain, a vegetable ferment which possesses no true peptonising power.

The "Perfected Wyeth Beef-Juice" is stated by the manufacturers to contain "not only the hæmoglobin but also the valuable nutritive albuminous elements of beef active and unchanged. It is carried to a very high degree of concentration, each tablespoonful containing the nutrient and stimulating principles of three-quarters of a pound of fresh lean beef. It contains many times more pure serum-albumen than any of the

ordinary preparations of this class, and it does not owe any of its nitrogenous material to added egg-albumen." An analysis quoted by the manufacturers states the preparation to contain :—Moisture, 44·87 per cent.; organic matter (including 4·57 of nitrogen), 38·01 per cent.; and mineral matter, 17·12 per cent.

A valuable table by A. D. Chester, showing the composition of various meat-extracts sold in New York State in 1882, will be found in the *Analyst*, vol. vii. p. 124.

The following are published analyses of certain varieties of meat-extracts, &c., which do not appear in the table on page 306.

	Bouillon Fleet.	Bovinine.		Pure Beef Company's Beef Tea.
	R. R. Tatlock.	O. Hehner, 1893.		J. Hughes.
Water,	61·95	80·70[1]	78·42[1]	25·0
Fat (ether extract),	0·06	1·21	0·09	0·90
Gelatin, albumin, and peptone,	11·81	13·54	13·32	45·60[2]
Creatine and meat-extractives,	9·87	0·21	0·55	21·10
Non-nitrogenous extractives,	3·81	3·48	6·01	
Sodium chloride,	12·50	0·78	1·04	7·35
Other mineral matters,		0·08	0·57	
Containing P_2O_5,	..	..	..	2·55

"Bovinine" is described by the manufacturers as a beef-juice made by a cold process, unique as a nutrient, free from cooked taste and added flavour.

"Vimbos" is a preparation stated by the manufacturers to contain "not only the 'Vital Principle,' but the entire nutritive constituents of prime ox-beef." The following analysis of "Vimbos" is by Stevenson Macadam:—"Nitrogenous organic matter (albuminous and flesh-forming), 50·86 per cent.; fatty bodies, 1·51; saline matters, including phosphates (bone-forming), 23·51; moisture, 24·12 per cent."[3]

"Zebril" is a concentrated animal food for dogs and poultry. It is described as a pure extract of meat, and each penny tablet

[1] Including the alcohol used for the preservation of the liquid.

[2] Including 5·78 of meat-fibrin and insoluble albumin. The Pure Beef Company make a point of the fact that their preparations "include the gelatines and all-important albumens, which are purposely excluded from all forms of the so-called Extractum Carnis."

[3] The manufacturers of "Vimbos" add that "the facts of the above analysis have only to be understood, and compared with the published analysis of any other fluid beef preparation, to secure the unrivalled favour of the public."

(about 2½ oz.) is said to be equal to at least 5 lbs. of lean beef in stimulating and nutritive properties.[1]

The following are recent analyses made by A. R. Tankard, in the author's laboratory, by the method described on page 315.

	Liebig Company's Extract.	Bovril Fluid Beef. Seasoned.	Bovril for Invalids.	Brand & Co.'s Beef Bouillon.	"Esco" Beef Juice.	Vimbos.	"Viking" Beef Essence.
Water,	18·35	38·10	21·16	36·27	52·43	34·93	90·68
Insoluble proteids, meat-fibre, &c.,	traces	8·40	8·47	3·84	0·56	7·23	none
Soluble proteids and gelatin,	9·45	3·84	8·19	5·73	7·10	4·28	3·63
Meat-bases (N×3·12),	29·67	12·04	16·13	19·34	5·90	12·60	1·85
Non-nitrogenous extractive matters,	18·85	19·75	29·23	19·75	13·62	23·61	2·41
Mineral matters,	23·68	17·87	16·82	15·07	20·39	17·35	1·43
	100·00	100·00	100·90	100·00	100·00	100·00	100·00
Chlorine, in terms of sodium chloride,	3·79	10·84	5·85	6·00	11·93	7·02	..
Total nitrogen,	11·01	5·80	7·81	7·11	3·02	5·86	1·17
N in insoluble proteids, &c.,	trace	1·33	1·34	0·61	0·09	1·15	none
N in precipitate by bromine in filtrate from insoluble proteids, &c.,	1·50	0·61	1·30	0·91	1·13	0·68	0·58
N not precipitated by bromine,	9·51	3·86	5·17	6·20	1·89	4·04	0·59

Analysis of Meat Extracts, Commercial Peptones, &c.

The complete analysis of extracts of meat and allied preparations is both difficult and tedious, and in some respects cannot be effected satisfactorily by existing methods. The following processes are those which, in the experience of the author, are the most satisfactory for their intended purpose.

Water and *Total Solid Matters* in meat-extracts may be determined by evaporating a known weight of the sample to dryness at 100° C. and drying the residue till constant. From 3 to 25 grammes should be employed, according to the nature of the preparation. Stutzer weighs the quantity intended for the determination of water into a thin basin of tin-foil (about 20 mm. high and 55 mm. in diameter), dissolves it in a little hot water, and adds sand (previously ignited and freed from fine dust by a sieve) in sufficient quantity to absorb the liquid almost completely. The basin is then heated in the water-oven until the weight is constant.

[1] A. R. Tankard found "Zebril" to contain :—Water, 21·06 ; sulphated ash (including Fe_2O_3), 5·30 ; blood-fibrin and altered hæmoglobin, 42·75 ; soluble proteids, 9·88 ; total nitrogen, 11·69 per cent.

The weight of the tin-foil and sand being deducted, the *solid matter* of the extract is obtained. The tin basin and its contents are then used by Stutzer for the determination of the gelatin (p. 326).

In the case of samples containing gelatin, or which from other circumstances cannot be readily dried, L. de Koningh treats the preparation with a weighed quantity of tannin, containing a known amount of dry matter. The mixture is evaporated and dried in the water-oven till constant, when the weight of dry tannin is deducted from the residue obtained.

The Ash of meat-extracts may be determined by igniting the residue obtained in the determination of the solids. A further examination of the ash is rarely requisite, except for the determination of the *chlorine*, which is interesting as affording a rough measure of the quantity of *common salt* present in the preparation.

Fat is rarely present in material quantity in meat-extracts. Ether extracts certain organic matters besides fat, and hence the results obtained are above the truth. Petroleum-ether extracts the fat only and therefore is to be preferred to ether.

The Total Nitrogen of meat-extracts, &c., may be determined by treating from 1 to 5 grammes, according to the nature of the preparation, by Kjeldahl's process.

No analysis of a meat-extract or similar preparation can be regarded as affording reliable information as to the quality of the sample which does not make some distinction between the different forms in which the nitrogen exists. Thus while the extractive matters and meat-bases have a special value of their own, they are not nutritive. Albumin, albumose and peptone, on the other hand, are true nutritive compounds, and are superior in value to gelatin.

Assay of Meat-Extracts by Alcohol Precipitation.

A simple means of roughly differentiating between the soluble forms of nitrogenised matters in meat-extracts has been in use for many years in the laboratories of manufacturers of such preparations. It consists in treating the sample with alcohol of such strength as to precipitate as much as possible of the proteid and gelatinoid constituents of the extract, and as little as possible of the meat-extractives and salts. For this purpose, O. Hehner recommends (*Analyst*, x. 221) that 2 grammes of the sample should be dissolved in 25 c.c. of water, and 50 c.c. of strong methylated spirit added to the solution. The precipitate is allowed to settle over-night, and the clear liquid then decanted as completely as possible. The (unwashed) precipitate is dissolved in a little hot water, the solution evaporated in a weighed basin, and the residue dried at 100° and weighed.

O. Hehner (*Analyst*, x. 221) gives the following results of

analyses of some well-known preparations which he examined by the above method. The results yielded by two samples of "Essence of beef" of South African manufacture, analysed in the author's laboratory by the same process, are added for the purpose of comparison :—

Description.	Water.	Total Solids.	Alcohol Precipitate.	Ash.	Phosphoric Acid.	Nitrogen.	Authority.
Liebig's Extract, .	18·70	81·30	5·16	23·38	6·07	7·94	O. Hehner.
Nelson's Gelatin, .	..	..	93·19	3·25[1]	None.	..	,,
Concentrated Beef Tea:—							
English, . . .	36·96	63·04	27·40	4·36	1·16	8·25	,,
English, . . .	31·00	69·00	30·30	4·13	1·00	8·36	,,
English, . . .	41·93	58·07	25·50	4·92	1·10	7·52	,,
Russian, . .	24·56	75·44	35·40	6·72[2]	0·95	9·89	,,
X, ...	54·31	45·69	32·30	7·57	2·11	6·79	,,
Commercial Essence of Beef:—							
English, . . .	89·25	10·75	3·07	1·17	0·34	1·36	,,
English, . . .	89·61	10·39	3·74	1·00	0·26	1·36	,,
English, . . .	92·32	7·68	1·99	1·30	0·38	0·79	,,
South African, .	90·50	9·50	2·98	1·74	0·50	1·22	A. H. Allen.
South African, .	87·55	12·45	2·88	2·36	0·17	1·41	,,

Hehner considers, from the above analyses, that the ash of these products should be high, containing about one quarter its weight of phosphoric acid, and that alcohol should not precipitate much more than 25 per cent. of the total dry matter of meat-essence, nor more than 44 per cent. of that of beef-tea.

J. Bruylants (*Jour. Pharm. et Chim.*, 1897, v. 515) has described a method of analysing meat-extracts, based on fractional precipitation by alcohol of different strengths.[3] Thus, gelatin is thrown down by alcohol of 40 per cent., albumoses by 80 per cent., and peptones by 93 to 94 per cent. alcohol. The following results were obtained by the analysis of typical preparations :—

[1] This ash was insoluble in water, and consisted chiefly of calcium carbonate.

[2] This ash was only partly soluble in water, and was almost entirely composed of calcium carbonate. The ashes of the other samples, with the exception of Nelson's gelatin, were completely soluble in water, and practically devoid of lime.

The phosphoric acid was determined in the samples by precipitation with molybdate solution, re-solution of the precipitate in ammonia, the solution evaporated at 100° C., and calculated to P_2O_5.

[3] K. Micko has found Bruylants' method to work well (*Zeits. des allgem. österreich. Apotheker-Vereins* xxx. 1).

	Liebig's Extract.	Solid Bovril.	Bovril for Invalids.	Liquid Bovril.
Water,	16·75	19·20	2·35	43·25
Sodium chloride,	2·95	4·50	4·00	9·75
Other mineral salts,	18·24	16·20	17·05	6·25
Insoluble in water (meat fibre),	..	..[1]	7·10	8·19
Organic matters,	62·06	60·10	54·50	32·06
Total nitrogen,	9·30	8·85	9·12	4·85
Nitrogen in part insoluble in water,	..	..[1]	1·09	1·19
Nitrogen, ammoniacal, uric acid, &c.,	0·60	0·50	0·45	0·30
Nitrogen, from lead precipitate (non-proteid matters),	0·65	0·57	0·45	0·27
	} 5·09	} 4·56	} 4·48	} 2·67
Nitrogen, non-proteid, from 80 per cent. alcohol,	0·15	0·20	0·18	0·05
Nitrogen, soluble in strong alcohol,	3·69	3·29	3·40	1·05
Nitrogen, from gelatin,	0·19	0·25	0·12	0·05
,, from albumoses,	0·80 } 3·93	0·95 } 3·78	0·75 } 3·57	0·45 } 1·83
,, from peptones,	2·94	2·58	2·70	1·33
Total soluble proteids,	24·56	23·62	22·40	11·43
Insoluble albumin (meat fibrin),	..	..[1]	6·81	7·43

All methods of examining meat-extracts based on precipitation of various proteids by alcohol of certain strengths, though useful in practice, are open to objection as deficient in accuracy. The method has been subjected to criticism by König and Bömer (*Zeit. anal. Chem.*, 1895, v. 548; abst. *Analyst*, xxi. 17), who point out that in meat-extracts prepared at low temperatures, and which are only concentrated to the required consistency after filtration, the amount of gelatin present must be excessively small. Thus E. Beckmann (Hilger's *Forsch. im Lebensmittel*, 1894, p. 423) could only find 0·5 per cent. of albumin and gelatin in Liebig's extract by precipitation with formalin. On the other hand, C. Karmrodt found 10·4 per cent. of gelatin in Liebig's extract, and Stutzer from 20·5 to 22·6 per cent. of peptone. Kemmerich (*Zeit. f. Physiol. Chem.*, 1894, xviii. p. 409; abst. *Jour. Chem. Soc.*, 1894, ii. 150) found in South American meat-extract about 6 per cent. of gelatin and 30 per cent. of proteids, in the form of albumoses, peptones, and other soluble compounds. Kemmerich employed fractional precipitation with alcohol of different strengths, as well as precipitation with ammonium sulphate and sodium phospho-tungstate. By these means he found, in addition to

[1] The meat-fibre in this sample does not appear to have been separately determined.

flesh-bases, the following amounts of proteids in this meat-extract :—

		Per cent.
Gelatin, &c., precipitated by 50 to 60 per cent. alcohol,		6·19
Albumoses, &c., precipitated by 80 per cent. alcohol,		14·76
Of these, there were precipitated by $(NH_4)_2SO_4$,	9·89	
Other soluble proteids not precipitated,	4·87	
Peptones, &c., soluble in 80 per cent. alcohol, but precipitated by sodium phospho-tungstate,		12·31
Other organic matters (including 4·33 of creatinine),		32·56
Mineral matters (chiefly phosphates),		22·29
Water (by difference),		11·89

The meat-extract in question contained 8·13 per cent. of total nitrogen, and these figures give 6·5 per cent. of this to the proteids, which König and Bömer consider extremely improbable. By precipitation of the meat-extract with alcohol of different strengths and determination of the nitrogen in the precipitates they obtained results considerably lower than those of Kemmerich. Thus in South American meat-extract the following results were obtained :—

	Kemmerich.	König and Bömer.
Gelatin (?) precipitated by 50 to 60 per cent. alcohol,	6·19 per cent.	1·83 per cent.
Albumoses precipitated by 80 per cent. alcohol,	14·16 ,,	4·50 ,,

These differences were considered too great to be accounted for by variation in the meat-extracts, and are regarded by König and Bömer to have been due to difference in procedure, Kemmerich having determined the amount of his precipitates gravimetrically, and not by direct estimation of the nitrogen.

König and Bömer then made comparative determinations by precipitation with 80 per cent. alcohol and with ammonium sulphate, with the following results :—

	Liebig's Extract.	Kemmerich's Extract.	Kemmerich's Peptone.	Cibil's Extract.
	Per cent.	Per cent.	Per cent.	Per cent.
Total nitrogen,	9·32	8·94	9·88	2·77
Nitrogen in precipitate by 80 per cent. alcohol,	0·69	1·05	4·05	0·61
Corresponding to albumoses,	4·31	6·56	25·31	3·81
Albumoses obtained by saturation with ammonium sulphate,	7·32	9·71	34·44[1]	5·97

[1] This figure includes the syntonin present in Kemmerich's "peptone."

These results, and the fact that in the filtrates from the 80 per cent. alcohol precipitation the biuret reaction was always obtained, showed that proteids were still present, but it was considered extremely doubtful whether these were to any extent peptones.

Assay of Meat-extract by Bromine Precipitation.

The fact that aqueous solutions of the proteids and of gelatin are precipitated by chlorine has been recently utilised by Rideal and Stewart (*Analyst*, 1897, p. 228) as the basis of a process of assaying meat-extracts, &c. As employed by these chemists, the method consists in passing a current of chlorine-gas through the solution to be tested, filtering off and washing the resultant precipitate, and weighing it after drying at a temperature not exceeding 70° C. or preferably *in vacuo* over sulphuric acid. The precipitate is stated to be remarkably stable at ordinary temperatures but to be readily decomposed on heating, becoming nearly black and rotting the filter-paper.

In order to avoid the inconveniences attaching to the use of chlorine-gas, and the drying and weighing of an unstable precipitate, the author has devised a modified process (*Analyst*, 1897, p. 258), which is rapid, easily worked, and gives concordant results. In this method, bromine-water is employed as the precipitant, in place of chlorine. The precipitate is filtered through asbestos, treated with strong sulphuric acid while still moist, and the contained nitrogen determined by the Kjeldahl-Gunning process (p. 21). The following are the details of the operation :—

A quantity of the solution containing about 1 gramme of the albuminoid matter is diluted with cold water to a volume of about 100 c.c., and treated in a conical beaker with sufficient hydrochloric acid to render the liquid distinctly acid to litmus. Bromine-water is then added in considerable excess, and the liquid stirred vigorously for some time. The yellowish precipitate which separates is at first flocculent, but becomes more viscous on stirring, and finally adheres in great part to the sides of the beaker. When this occurs the liquid is allowed to stand at rest for about half an hour, or until the precipitate has settled. It is then decanted through an asbestos filter.[1]

The precipitate adhering to the sides of the beaker is washed several times with cold distilled water, the washings being poured through the filter. Occasionally, when the greater part of the bromine has been washed out of the precipitate, the liquid does

[1] The filter is made by placing a plug of glass-wool in a cylindrical funnel (constructed of a vertical glass tube drawn out at the lower end), and covering it with a pad of pulped asbestos. If the filter be properly constructed, no water-pump will be required, and a perfectly clear filtrate will be obtained.

not filter clear. It is therefore advisable to keep the washings separate from the filtrate, and, if necessary, to add bromine or sodium sulphate to the wash-water.

The contents of the filter-tube (including the asbestos, and, if necessary, the glass-wool) are returned to the beaker used for the precipitation, 20 c.c. of strong sulphuric acid added, and the beaker covered with a watch-glass and heated over wire-gauze. The substance chars and bromine-vapour is evolved. When frothing has ceased, about 10 grammes of powdered potassium sulphate should be added, and the liquid boiled vigorously until colourless. It is then allowed to cool, diluted with water, an excess of caustic soda added, the ammonia distilled off, and the distillate titrated with standard acid. From the nitrogen found the amount of proteid or gelatinoid body present is deduced by a suitable factor.

As the results of experiments by the foregoing process, it was found (Allen and Searle, *Analyst*, 1897, p. 259) that practically the whole of the nitrogen of gelatin, gelatin-peptone, egg-albumin, syntonin, and of the mixed products of the acid-pepsin digestion of egg-albumin was thrown down in the precipitate produced by bromine.[1] In the case of syntonin, fairly good results were obtained whether the acid used for conversion of the egg-albumin was left unneutralised or was exactly saturated by caustic soda; but if the solution was made alkaline and bromine then added, the proteid could not be completely precipitated by subsequent free acidulation of the liquid.[2] The mixed peptones formed

[1] The following is a tabular statement of the chief results obtained:—

Substance.	Nitrogen per cent.		Nitrogen multiplied by factor.		Factor employed.
	Total in Original Substance.	Precipitated by Bromine.	Total in Original Substance.	Precipitated by Bromine.	
Commercial gelatin,	14·10	14·00	76·42	76·14	5·42
Gelatin-peptone,	14·10	13·90	76·42	75·44	
Commercial scale-albumin,	8·80	8·72	55·8	55·2	6·33
Syntonin from scale-albumin,	9·86	9·76	62·41	61·78	
Digested scale-albumin,	8·89	8·81	56·3	55·8	
Fresh white of egg,	1·89	1·88	11·96	11·90	
Syntonin from white of egg,	1·89	1·89	11·96	11·96	
Peptone from white of egg,	0·70	0·69	4·43	4·37	
Beef-extractives,	0·33	0·004	2·11	0·03	

[2] A sample of commercial scale-albumin was converted into acid-albumin (syntonin) by heating it in 1 per cent. solution with hydrochloric acid for six hours. The nitrogen was then determined directly in the resultant liquid and in the bromine precipitate produced in different ways, as shown below. The

by the acid-pepsin digestion of the white of hard-boiled eggs were also completely precipitated by bromine.

On the other hand, bromine produced no precipitate in acidulated solutions of creatine, creatinine, asparagine, or aspartic acid. A meat-extract prepared by soaking raw beef in ten parts of cold water, straining, boiling, and filtering from the coagulated albumin, gave only a trifling precipitate on addition of bromine-water. In the liquid concentrated to one-tenth a larger precipitate was obtained, the greater part of which dissolved on diluting the liquid with an equal measure of water, and almost the whole on addition of a few drops of hydrochloric acid. On the other hand, the complete precipitation of albumin and gelatin by bromine seemed to be quite unaffected by dilution or the presence of free hydrochloric acid.

On applying the bromine-method to commercial meat-extracts the following results were obtained. The solutions were not previously filtered, and therefore the figures include the nitrogen of any meat-fibre present in the preparations :—

	Nitrogen in precipitate by Bromine.	×6·3 = Proteids.
Liebig Company's Extract, . .	1·41 per cent.	8·88 per cent.
Seasoned Bovril, ...	1·94 ,,	12·22 ,,
Bovril for Invalids, . . .	2·64 ,,	16·63 ,,
Brand's Beef Bouillon, . . .	1·52 ,,	9·58 ,,
Vimbos, ...	1·83 ,,	11·53 ,,

Another sample of Liebig Company's extract recently analysed in the author's laboratory by entirely different methods gave 9·37 per cent. of total proteids.[1]

figures are calculated to 100 parts of the original albumin, which was found to contain 9·38 per cent. of nitrogen and to yield 8·32 per cent. of ash on ignition. Hence the sample was far from pure.

	Nitrogen.
A. By direct Gunning-Kjeldahl process on syntonin solution,	9·86 per cent.
B. By precipitate from unneutralised syntonin solution, .	9·76 ,,
C. By precipitate from nearly neutralised syntonin solution,	9·69 ,,
D. By precipitate from syntonin solution, rendered strongly alkaline and then re-acidified, ...	9·60 ,,
E. Precipitate from syntonin solution, made strongly alkaline by soda, bromine added, and the liquid acidulated after half an hour, ...	6·69 ,,
F. Precipitate from syntonin solution, made strongly alkaline by soda, bromine added, and the liquid acidulated after twenty-four hours, ...	3·52 ,,

[1] König and Bömer have recently shown that the proteid nitrogen in meat-extracts has generally been much over-estimated. They found a total of 1·17 per cent. of proteid nitrogen in Liebig Company's extract, which is equivalent to 7·41 of total proteids (mostly albumose).

In another experiment, 5 grammes of the Liebig Company's extract was dissolved in 100 c.c. of water, and the solution saturated with zinc sulphate. On adding bromine to the filtered liquid, a precipitate was produced which redissolved on diluting with water and adding hydrochloric acid. When 50 c.c. of the filtrate from the zinc sulphate was diluted with water to 250 c.c., and freely acidulated with hydrochloric acid, no precipitate was produced on subsequently adding bromine. This result appears to negative the presence of considerable quantities of real peptones in Liebig's extract, and confirms the conclusion of König and Bömer on this point.

A parallel experiment with Bovril gave a precisely similar result.

If the novel and unexpected observation that bromine-water in presence of hydrochloric acid completely precipitates all proteid and gelatinoid matters tried, without throwing down meat-bases, is fully confirmed on wider experience, and found to hold good under varied conditions,[1] it will afford a means of solving one of the most difficult problems connected with the analysis of meat-extracts and products of digestion. Thus by first saturating the liquid with zinc sulphate, as directed by Bömer (page 322), the whole of the proteid and gelatinoid bodies may be thrown down, with the exception of the peptones. If the filtrate be then diluted with about five measures of cold water, strongly acidulated with hydrochloric acid, and treated with excess of bromine-water, the peptones will be precipitated while the meat-bases remain in solution. The amount of peptone can then be directly deduced from the nitrogen contained in the precipitate produced by bromine.

Proximate Analysis and Determination of the Nitrogenised Constituents of Meat-Extracts, &c.

In the fullest possible analysis of a meat-extract, an attempt will be made to discriminate between and determine the amount of nitrogen existing in the various forms of meat-fibre and insoluble albumin, coagulable albumin, acid-albumin, albumoses, peptones, coagulable gelatin, gelatin-peptone, meat-bases, amido-compounds, and ammonia. Such an analysis is necessarily tedious and rarely necessary, but some of the more important of the above determinations can be effected with reasonable ease and accuracy, and are not uncommonly required of the analyst.

In consequence of the uncertainty attaching to the composition

[1] The precipitation of peptones in the filtrate from the precipitate formed by zinc sulphate, on addition of excess of bromine-water, has been found to be as complete in dilute as in concentrated solutions, and to be independent of the presence of zinc sulphate.

of certain of the nitrogenised constituents of meat-extracts, it is often convenient to state simply the amounts of nitrogen found to exist in the various forms, and in cases where it is preferred to state the actual amounts of the nitrogenised bodies present the corresponding amounts of nitrogen should always be given in addition.

Ammoniacal nitrogen should be determined by distilling the aqueous solution of a known weight of the preparation with barium carbonate, which is preferable to magnesia.

Unaltered Proteids and Meat-Fibre.—Bovril and certain allied preparations contain finely-powdered meat-fibre. This may be detected by treating the meat-extract with cold water, and examining the insoluble portion under the microscope. If meat-fibre be found, 5 grammes of a dry preparation, 8 to 10 grammes of an extract, or 20 to 25 grammes of a fluid preparation should be treated with cold water, the insoluble matter collected on a filter, washed with cold water, dried at 100° C., and weighed. The weight obtained represents the *meat-fibre* and *insoluble matter* of the preparation. An alternative and in some respects preferable plan is to treat the moist residue by Kjeldahl's process. The nitrogen found, multiplied by the usual factor, will give the *meat-fibrin*, as distinguished from the crude meat-fibre, &c., obtained by weighing the insoluble matter.

Coagulable Albumin can be determined in the filtrate from the insoluble matter, by rendering the liquid distinctly acid with acetic acid (see page 29), boiling for five minutes, filtering, and determining the nitrogen in the coagulum.[1] Only insignificant amounts of albumin are usually present in meat-extracts, but in certain preparations which have received an addition of scale-albumin the amount may be considerable.

Syntonin.—An aliquot portion of the liquid filtered from the coagulable albumin should be further acidulated with acetic acid and tested with potassium ferrocyanide. If any precipitate be formed the liquid should be heated, and if re-solution does not ensue the presence of *acid-albumin* is certain. If found, the remainder of the liquid should be rendered exactly neutral to litmus, the precipitate filtered off, and the contained nitrogen determined. Syntonin is stated by Denaeyer to be present in considerable proportion in "Kemmerich's meat peptone," while "Somatose" consists largely of alkali-albumin, which will be determined as syntonin by the above process.

[1] The filter-paper used for the separation of these albuminous precipitates must be as free as possible from nitrogen, or a correction must be made for the amount present. Stutzer recommends the filters of Schleicher and Schüll.

Albumoses and Peptones.—The filtrate from the precipitate of syntonin, or, in the absence of syntonin, the liquid filtered from the coagulable albumin, is saturated with zinc sulphate, as described on page 322. The precipitate produced contains all the albumose of the extract, together with any gelatin which may be present and any coagulable or insoluble proteids not previously removed. Peptones, meat-bases, amido-compounds, and ammoniacal salts are not precipitated.

In aliquot parts of the filtrate, peptones may be determined by precipitation with bromine (as on page 318); ammonia by distillation with barium carbonate; and total nitrogen by Kjeldahl's process. These two latter determinations are, however, preferably made on a filtered aqueous solution of the original sample. The difference between the total nitrogen and that found in other forms is regarded as existing as *meat-bases, &c.*, the actual weight of which is usually calculated by multiplying the nitrogen by the factor 3·12 (compare page 303).

For the determination of the nitrogen existing as albumoses and peptones, Stutzer (*Zeit. anal. Chem.*, 1895, iii. 372; abst. *Analyst*, xx. 248) recommends the following method:—Of a dry preparation, 5 grammes should be warmed in a beaker with 25 c.c. of water; of extracts, 10 grammes should be treated with 10 c.c. of water; of fluid preparations, 20 to 25 c.c. should be taken, and no water added; and in the case of very fluid preparations, 50 c.c. should be concentrated on the water-bath to about 25 c.c. To the solution thus prepared, 250 grammes of absolute alcohol should be added with constant stirring, which is continued for some minutes after the whole of the alcohol has been added. The liquid is allowed to stand for twelve hours, filtered, and the precipitate repeatedly washed with alcohol. The precipitate is then washed into a beaker with water, the alcohol completely evaporated on the water-bath, and the liquid filtered. A small portion of albumose is liable to be rendered insoluble by the action of the alcohol, and the nitrogen in this residue should be determined and added to the albumose-nitrogen subsequently found.

Stutzer directs that the filtrate be made up to 500 c.c. and aliquot portions taken for the following determinations:—

a. Fifty c.c. is employed for the determination of the *total nitrogen* by Kjeldahl's process.

b. Fifty c.c. is mixed in the cold with an equal volume of dilute sulphuric acid (1 : 3), and phospho-tungstic acid added until no further precipitation occurs. The precipitate is washed with dilute sulphuric acid, and the contained nitrogen determined. The

amount found represents the nitrogen existing in the three forms of gelatin, albumose, and peptone.

c. If peptone be found by a qualitative application of the biuret test,[1] Stutzer concentrates 100 c.c. to about 10 c.c., and when cool adds at least 100 c.c. of a saturated solution of ammonium sulphate. The precipitate is washed with saturated ammonium sulphate solution, dissolved in boiling water, and the solution evaporated to dryness with sufficient barium carbonate to expel all the ammonia. The residue is treated with hot water, the barium sulphate and carbonate filtered off, and the nitrogen in the filtrate determined as usual. The amount found represents the nitrogen present in the forms of albumose and gelatin. This amount deducted from that found in *b* gives the nitrogen existing in the form of *peptone*. The gelatin having been determined independently, the nitrogen corresponding to it is deducted from the amount contained in the ammonium sulphate precipitate. The difference is the nitrogen existing as *albumose*, which is corrected by adding the small quantities of albumose-nitrogen previously found.

The foregoing indirect method of determining peptones, although recommended by as high an authority as Stutzer and widely employed, is very unsatisfactory. It assumes that meat-bases are wholly unprecipitated from a freely acidulated solution by excess of phospho-tungstic acid. This experience is not in accordance with the experience of other chemists, and, according to König and Bömer, all flesh-bases, together with the rest of the constituents of meat-extracts, are precipitated by phospho-tungstic acid if sufficient time be allowed. As the nitrogen of any bases so precipitated will be calculated as existing as peptone, the amount of this constituent is liable to be seriously over-estimated. Thus König and Bömer (*Analyst*, xxi. 18) obtained the following figures :—

	Liebig's Extract.	Kemmerich's Extract.	Kemmerich's Peptone.	Cibil's Extract.
	Per cent.	Per cent.	Per cent.	Per cent.
Nitrogen in phospho-tungstate precipitate, . .	6·27	5·59	8·29	2·00
Nitrogen in ammonium sulphate precipitate, .	1·17	1·55	5·51	0·96
Peptone (?) nitrogen, . .	5·10	4·04	2·78	1·04

[1] For this purpose Stutzer directs that a considerable quantity (300 c.c.) of the solution should be concentrated by evaporation to a small bulk, and the gelatin and albumose precipitated by adding solid ammonium sulphate until a little of the salt remains undissolved. A few drops of a very dilute solution of copper sulphate are added to the filtered liquid, and this is followed by a

From these figures König and Bömer consider it obvious that so large a quantity of peptone-nitrogen cannot be present—at any rate in the meat-extracts—and that the flesh-bases must claim a considerable amount of it.[1] Basing their conclusions largely on the absence of the biuret reaction in the filtrate of a meat-extract, König and Bömer believe that the extracts examined contained either no peptone at all, or, at most, very slight quantities (2 to 3 per cent.).[2]

An important improvement on the ordinary method of separating albumoses from peptones has been devised by A. Bömer (*Zeit. anal. Chem.*, 1895, v. 562 ; abst. *Analyst*, xxi. 16), who substitutes zinc sulphate for the ammonium salt. 50 c.c. of the solution, containing from 1 to 2 grammes of solid matter, is freed from insoluble and coagulable matters, and treated with 1 c.c. of dilute sulphuric acid (1 : 4), to prevent the precipitation of zinc phosphate. It is then completely saturated with zinc sulphate at the ordinary temperature, by adding the powdered salt as long as it continues to dissolve on stirring. The precipitate, which will contain any gelatin and all proteids other than peptones, is filtered off and washed with a cold saturated solution of zinc sulphate. The filter and its contents are then transferred to a flask and treated by Kjeldahl's process.[3]

In the filtrate from the precipitate produced by zinc sulphate, the peptone, if present, may be detected by the biuret test.[4]

Bömer gives the following figures in illustration of the process :—

	Percentage of Nitrogen precipitated by		
	Ammonium Sulphate.	Zinc Sulphate.	Phospho-tungstic Acid.
Liebig's meat-extract, ...	1·17	1·19	5·31
Kemmerich's meat-extract, . . .	1·55	1·52	4·05
Kemmerich's meat-peptone, . . .	5·51	5·44	3·16
Cibil's meat-extract, ...	0·96	0·92	1·11

considerable quantity of strong caustic soda, when the characteristic rose-red coloration will be produced if peptone be present. An excess of copper solution must be carefully avoided, or the rose coloration will be obscured.

[1] This conclusion is supported by the negative reaction of Liebig's extract with bromine-water (Allen and Searle, page 318).

[2] Hehner considers the error from this cause to be exaggerated.

[3] Meat-extracts often contain ammonia, which might possibly be carried into the precipitate in the form of the sparingly soluble ammonio-sulphate of zinc. Bömer found that this never occurred in practice ; but the whole of the ammonia originally present could be obtained by distilling the filtrate with magnesia in sufficient quantity to precipitate all the zinc and leave the liquid strongly alkaline.

[4] This may be applied directly by adding caustic soda in large excess and

No biuret reaction was in any case obtained in the filtrate from the albumose precipitate, whether zinc sulphate or ammonium sulphate was employed. This result confirms the statement of Denaeyer (page 383), that Kemmerich's "meat-peptone" contains no true peptone. The acid-albumin contained in it would behave like albumose with ammonium or zinc sulphate. As peptone was absent in each case, the nitrogen contained in the precipitate produced by phospho-tungstic acid must have existed in the form of meat-bases and decomposition-products insoluble in alcohol.

As the result of the foregoing and similar experiments, König and Bömer hold the following views with respect to the chemical examination of meat-extracts and commercial peptones :—

1. Precipitation with 80 per cent. alcohol is of no value in determining the form of combination in which the nitrogen exists. 2. Albumoses should be determined by salting out with ammonium sulphate or zinc sulphate. 3. The filtrate from the ammonium or zinc sulphate precipitate should be decolorised with animal charcoal, and tested for peptones by the biuret reaction. 4. A determination of the ammonia by distilling an aqueous solution of the extract with ignited magnesia is valuable. 5. When peptone has been proved to be absent, the nitrogen in the phospho-tungstate precipitate, after deducting the nitrogen derived from gelatin, albumoses, and ammonia, may be ascribed to the flesh-bases. The phospho-tungstate precipitate should stand at least one day before filtration. 6. The difference between the total nitrogen and the sum of the nitrogen in the forms of gelatin, albumoses, flesh-bases, and ammonia gives the amount of nitrogen present in compounds not precipitated by phospho-tungstic acid. No evidence was obtained of the presence of amido- or acid amido-compounds.

By the application of these principles to the analysis of typical preparations, König and Bömer obtained the following results :—

then a drop or two of very dilute solution of copper sulphate. Bömer considers it better, however, to destroy any colour as far as possible, by shaking the liquid with animal charcoal, and then to remove the zinc. This may be effected by precipitation with sodium carbonate, with subsequent concentration of the filtrate ; or both zinc and sulphate may be precipitated by cautious addition of baryta-water. An aliquot part of the filtrate may be treated by Kjeldahl's process, and in another portion the peptones and flesh-bases precipitated together by phospho-tungstic acid, after adding an equal measure of dilute sulphuric acid (1 : 4).

	Liebig's Extract.		Kemmerich's Extract.		Kemmerich's Peptone.		Cibil's Extract.[1]	
	Per cent. of Substance.	Per cent. of Total Nitrogen.	Per cent. of Substance.	Per cent. of Total Nitrogen.	Per cent. of Substance.	Per cent. of Total Nitrogen.	Per cent. of Substance.	Per cent. of Total Nitrogen.
NITROGEN in the form of—								
1 Soluble albumin, . . .	trace	trace	0·08	0·87	0·06	0·59	trace	trace
2. Nitrogenous compounds insoluble in 60–64 per cent. alcohol, . . .	0·21	2·26	0·33	3·61	1·36	13·49	0·25	9·02
3. Albumoses, ...	0·96	10·34	1·21	13·24	4·15	41·17	0·70	25·27
4. Peptones, ...	0 to trace	0 to trace	0	0	0	0	0	0
5. Flesh-bases, ...	6·81	73·38	5·97	65·32	3·97	39·38	1·56	56·31
6. Ammonia, ...	0·47	5·06	0·41	4·49	0·29	2·88	0·09	3·25
7. Other nitrogenous compounds, ...	0·83	8·96	1·14	12·47	0·25	2·49	0·17	6·15
TOTAL, . . .	9·28	..	9·14	..	10·08	..	2·77	..

[1] Cibil's Extract is a preparation stated to be peptonised by an extract of Papaya. This vegetable ferment has been proved to form little or no true peptone.

These amounts of nitrogen represent the following percentages of nitrogenous compounds :—

	Liebig's Extract.	Kemmerich's Extract.	Kemmerich's Peptone.	Cibil's Extract.
1. Soluble albumin, . . .	trace	0·50	0·38	trace
2. Gelatin and proteids insoluble in 60–64 per cent. alcohol, ...	1·14	1·80	7·40	1·36
3. Albumoses, ...	6·05	7·62	26·15	4·41
4. Peptones, ...	0 to trace	none	none	none
5. Flesh-bases, ...	21·25	18·63	12·39	4·87
6. Ammonia, ...	0·57	0·51	0·34	0·11
7. Other nitrogenous matters, .	5·23	7·18	1·58	1·07
TOTAL, ...	34·24	36·24	48·24	11·82

E. Beckmann has based a process of analysing meat-extracts, &c., on the fact that albumin, casein, hemialbumose and gelatin, are rendered completely insoluble, by evaporating their solutions to dryness with formaldehyde, whereas peptone and gelatin-peptone are unaffected by such treatment. A solution of the sample, containing about one gramme of proteids, is rendered faintly alkaline by sodium carbonate, and treated with five or six drops of commercial formalin (containing about 40 per cent. of formic aldehyde). The liquid is then evaporated to dryness on the water-bath, the residue moistened with a drop or two of formalin, and heated for half an hour in the water-oven to 100°. The residue is next digested with water at 70° C. to dissolve the trioxymethylene formed, the liquid filtered, and the treatment with water repeated several times. The residue is then dried at 100° till constant in weight, ignited, and the resultant ash deducted to find the true weight of the proteid rendered insoluble. From the solution the peptone and gelatin-peptone can be thrown down by tannin or phospho-tungstic acid, or preferably by bromine.

Beckmann states that true meat-extract yields by the above process but very little residue, the amount ranging from 0·22 to a maximum of 3·25 per cent.

Beckmann quotes the following results obtained by the application of the foregoing process to commercial "peptones" :—

	Formalin Residue.	Containing Albumin.
	Per cent.	Per cent.
Merck's hydropeptone, ...	1·45	0·76
Kemmerich's meat-peptone, ...	14·15	1·92
Denaeyer's peptone, ...	13·87	0·48
Bovril lozenges (peptonised), ...	46·49	5·96

Gelatin is present in notable quantity in some meat-extracts, and its determination presents considerable difficulty. Gelatin is precipitated more or less completely by most of the reagents for proteids, including tannin, phospho-tungstic acid, bromine-water, and ammonium and zinc sulphates. Unchanged gelatin is said not to be precipitated by Stützer's copper reagent (page 30), but the commercial article is largely thrown down. The same is the case when a solution of commercial gelatin (Nelson's) is treated with mercuric chloride or with potassio-mercuric iodide, although these reagents are stated by Denaeyer not to precipitate gelatin; in fact the first reagent is employed by him to separate gelatin from peptone, and the second to differentiate gelatin from albumose.

Unchanged gelatin is precipitated by alcohol of very moderate strength (50 to 60 per cent.), but the non-jellifiable modification produced by the prolonged action of hot water or of weak acids on gelatin is only precipitated by very strong alcohol (95 per cent.). This modified gelatin, often called *gelatin-peptone* or *gelatone*, has been but imperfectly examined, but differs materially from colloidal gelatin in its chemical reactions.[1]

On the whole the gelatin of meat-extracts is best determined by the following modification by Stutzer of a method devised by Denaeyer, but the process cannot be regarded as wholly satisfactory :—

The tin-foil capsule containing the dry residue resulting from the determination of the total solids of the sample (page 310), together with the sand, is cut into small strips and exhausted in a beaker four times with absolute alcohol. The alcohol is passed through an asbestos filter, taking care to leave the insoluble matter

[1] Various experiments have been conducted in the author's laboratory by A. B. Searle with a view of finding a reliable chemical method of separating gelatin from proteids. So far, none of the reagents generally credited with effecting a separation of gelatin from albumoses have been found to behave in accordance with published statements, and no method of distinguishing sharply between gelatin and gelatin-peptone has been devised up to the time of writing.

as far as possible in the beaker. After removal of the adherent alcohol, the mixture of tin-foil, sand, and gelatin is treated with ice-water, to which 10 per cent. of alcohol has been added, the temperature being kept below +5° C. by the gradual addition of small pieces of ice. After being shaken for two minutes in a suitable apparatus, the extraction with ice-water is repeated.[1] The insoluble portion (together with the tin-foil and sand) is washed with alcoholised ice-water until the filtrate is colourless. The residue is then boiled with water, the solution of gelatin filtered, and the contained nitrogen determined by Kjeldahl's method. This may be applied to the liquid itself, or the gelatin may be thrown down by bromine-water, and the resultant precipitate treated. The nitrogen found, multiplied by 5·44, gives the gelatin of the sample.

A method of determining gelatin in meat-extracts, &c., based on its coagulation by formaldehyde is described on page 325.[2]

[1] The following is Stutzer's most recent method of operating (*Zeit. Anal. Chem.*, 1895, xxxiv. 568) :—The beaker (marked *a*) together with four others (marked *b*, *c*, *d*, *e*) and a flask containing a mixture of 100 c.c. of alcohol, 300 grammes of ice, and cold water to 1 kilogramme, are then immersed in a bath containing crushed ice. About 100 c.c. of the mixture in the flask, the temperature of which must not exceed 5° C., is poured on the sand, stirred with a glass rod for two minutes, and decanted into beaker *b*, a piece of ice being added to keep down the temperature. The extraction in the beaker is repeated with a second quantity of alcoholised water which is poured into *c*, and the treatment repeated until the last washing is colourless, a fragment of ice being added to each quantity of extraction liquid as soon as it is poured off. Three funnels, of about 7 centimetres diameter, are then arranged with filter-beds of long-fibred asbestos supported by perforated porcelain plates about 4 centimetres in diameter, and connected with a pump by which gentle and gradually-increasing suction can be applied. The contents of beaker *a* are filtered into the first, *b* is poured into the second, and *c*, *d*, and *e* into the third. The filters, as well as that through which the absolute alcohol extract has been filtered, are then thoroughly washed with the ice-cold alcoholised water, transferred to a porcelain basin, and repeatedly extracted by boiling with water. The aqueous extract is filtered, concentrated, and treated by Kjeldahl's process.

Stutzer finds that when the process is conducted exactly in the manner prescribed above, that from 95 to 98 per cent. of the total gelatin present is obtained, and that the small quantities of gelatin-peptone present in meat-extracts are precipitated by alcohol together with the gelatin proper, and may be suitably determined therewith.

[2] An experiment made in the author's laboratory by A. B. Searle, with a sample of Nelson's gelatin, gave a precipitate with formaldehyde which contained only 10·03 out of the 14·10 per cent. of nitrogen actually present. It is possible that the deficiency may have been due to the presence of gelatin-

A. Denaeyer (abst. *Analyst*, xv. 102) has described a method of determining gelatin in meat-extracts based on separation of coagulable and acid albumin, and precipitation of the albumoses by Mayer's solution.[1] The filtrate is concentrated to a small bulk, and treated with excess of a saturated aqueous solution of ammonium sulphate, which precipitates the gelatin. On boiling the liquid and giving it a centrifugal motion, the gelatin adheres to the sides of the beaker, and after cooling may be washed with saturated ammonium sulphate, then rapidly with a little cold water, and finally treated with boiling alcohol, to remove traces of potassio-mercuric iodide. The beaker, with the adherent gelatin, is then dried at 100° C. and weighed. The result is corrected by estimating and deducting the weight of ammonium sulphate adhering to the gelatin.[2]

L. Crismer recommends an acid solution of chromic acid as a reagent for gelatin. Gelatin-peptone is stated to be unaffected. Any syntonin or other proteid precipitable by potassium ferrocyanide in acetic acid solution must be previously removed.

Nitrogen of Flesh-Bases and Decomposition-Products. — The nitrogen of the meat-bases, &c., insoluble in alcohol, is found by Stutzer by subtracting the nitrogen contained in the precipitate produced in the solution by phospho-tungstic acid (*b*) from the total nitrogen present in the liquid (*a*). The result is generally much below the truth, for the same reason that the peptones are overestimated.

The liquid filtered from the precipitate produced by alcohol

peptone in the sample, since a specimen of the latter body, prepared by boiling gelatin with water containing one per cent. of HCl for twenty-four hours under a reflux condenser, gave only a very slight precipitate with formalin.

[1] Most discordant statements are made respecting the behaviour of the constituents of meat-extracts with Mayer's solution (potassio-mercuric iodide). According to L. Crismer (*Bul. de l'Assoc. belge des Chimistes*, iv. 135, 233) the reagent precipitates neither gelatin, syntonin, albumose, nor peptone from neutral solutions; but in presence of dilute acids or neutral salts precipitates are formed which are soluble in excess either of peptone or the precipitant, so that the reagent has little practical value. Crismer finds an alkaline solution of potassio-mercuric iodide to give no reaction with albumin, syntonin, peptone, or gelatin. The author has confirmed this statement for gelatin and gelatin-peptone, but in neutral solutions commercial gelatin (Nelson's) is in part precipitated by Mayer's reagent.

[2] In an experiment made in the author's laboratory in which zinc sulphate was substituted for the ammonium salt, 13·3 out of a total of 14·1 per cent. of nitrogen was precipitated. In the case of gelatin-peptone, only 8·4 per cent. out of the same total was thrown down by the zinc sulphate.

will contain any gelatin-peptone,[1] leucine, tyrosine, and indefinite decomposition-products of advanced digestion, together with a portion of the flesh-bases. The alcohol is completely removed by distillation, the residue dissolved in water, and any insoluble matter filtered off. The nitrogen contained in it is estimated, corrected for any coagulable albumin present in the sample, and the amount found added to the albumose-nitrogen precipitated by ammonium sulphate (page 321).

The clear solution is diluted by Stutzer to 500 c.c., of which 100 c.c. is taken for the total nitrogen present, 100 c.c. for the separation of decomposition-products, and a similar quantity for the determination of ammoniacal nitrogen. The amount of ammoniacal nitrogen found is deducted from the total, the difference being the nitrogen present in the form of flesh-bases and decomposition-products.

For the partial separation of the decomposition-products, Stutzer directs that 100 c.c. of the liquid from which the alcohol has been driven off should be warmed to about 40° C., and treated with 10 to 15 c.c. of a paste containing about 15 per cent. of mercuric oxide. This is prepared by pouring mercuric chloride solution into dilute caustic soda in excess, washing thoroughly, and preserving the precipitate in the dark. The mixture is stirred for a few minutes, filtered, and the nitrogen determined in the precipitate and filtrate. The former contains the gelatin-peptone,[1] with unknown decomposition-products of albumose and peptone. The filtrate contains the leucine, tyrosine, and other products of a pancreatic digestion which has been carried to excess, together with part of the flesh-bases (creatinine, &c.) which are very sparingly soluble in 95 per cent. alcohol. In place of the paste of mercuric oxide, phospho-tungstic acid may be employed. According to Stutzer, when used in excess it precipitates none of the flesh-bases except xanthine and hypoxanthine, of which, from their sparing solubility, only traces can be present in the alcoholic solution of the substance.

This statement is certainly incorrect, for the author has proved that creatinine yields an abundant white granular precipitate with phospho-tungstic acid in solutions strongly acidulated with sulphuric acid. On the other hand, creatine is not precipitated under these conditions. Kemmerich found the phospho-tungstic acid precipitate produced in the portion of a South American meat-extract soluble in 80 per cent. alcohol to amount to 16·74 per cent.

[1] In a later paper (*Zeit. anal. Chem*, 1895, xxxiv. 372) Stutzer states that gelatin-peptone is practically insoluble in 95 per cent. alcohol, and hence is not likely to be found at this stage.

From this precipitate by treatment in neutral concentrated solution with alcoholic zinc chloride (see Vol. III. Part iii. p. 292) he isolated 4·33 per cent. of creatinine.[1] Assuming no other flesh-base to have been co-precipitated with the peptone by phospho-tungstic acid, the nitrogen in this amount of creatinine would be 1·61 per cent., and this when multiplied by the factor 6·3 would give apparent peptone to the extent of 10·14 per cent., which peptone had no actual existence in the extract.

The question is further complicated by the observation of Kemmerich (*Zeit. physiol. Chem.*, xviii. 409) that fresh good meat-extract, contrary to the general statement, contains hardly any creatine, but a large proportion of creatinine. At any rate, when fresh meat-extract is diluted with a little water or glycerin, and examined under the microscope without delay, it shows the characteristic whetstone-form crystals of creatinine (Vol. III. Part iii. p. 291.) The creatinine gradually takes up water with formation of creatine, so that on long standing columnar crystals of this base are deposited (*ibid.*, p. 286). This confirms the observation of G. S. Johnson that perfectly fresh meat contains creatinine but no creatine, whereas in about thirty-six hours the latter base predominates.

Non-Nitrogenous Extractive Matters.—Of these constituents of meat-extracts very little is known quantitatively. *Lactic acid* and *lactates* probably predominate, but their actual amount does not appear to have been ascertained.[2] *Glycogen* is present in sensible quantity, and is determined by E. Kemmerich by dissolving the sample in a little water and precipitating the solution with alcohol of 60 per cent. The resultant precipitate is treated with a dilute solution of caustic potash, and the solution obtained treated by Brücke's method (page 283).

Kemmerich states that meat-extract is free from dextrin, sugar, and similar bodies, and contains no substance which is converted into glucose by boiling with dilute sulphuric acid.

The Salts of meat-extracts have already been considered. They consist chiefly of *earthy phosphates* and *potassium chloride* and *acid phosphate.* Lactates and other organic salts of potassium are also present, and on ignition of the residue obtained by evaporating

[1] The same meat-extract was found by Kemmerich to contain from 0·25 to 1·00 per cent. of carnine, 0·9 per cent. of ammonia, 1·22 per cent. of glycogen, and 18 to 22 per cent. of other extractives.

[2] Taking the proportion of lactic acid in fresh meat at 0·06 per cent., and assuming that thirty-four parts of meat are required for the production of one part of extract, the proportion of lactic acid in the latter would be 2·04 per cent.

the extract are of course converted into carbonates. The acid potassium ortho-phosphate is also decomposed with formation of metaphosphate ($KH_2PO_4 = KPO_3 + H_2O$). These reactions necessarily affect the amount and composition of the ash, and should be borne in mind.

Extraneous Matters.—In addition to *meat-fibre*, the detection and estimation of which has already been described (page 319), other foreign matters are present in certain commercial preparations classed broadly as meat-extracts. In the table on page 306 there are several instances of preparations containing *glycerin*, and one in which a *vegetable extract* was present. *Albumin* and *gelatin* are often added as such. *Glucose* and *milk-sugar* are sometimes present, and may be detected as described on page 388.

Alcohol is an occasional constituent of "meat-juices."

It is not practicable to draw a sharp distinction between meat-extracts and the so-called "peptones" of commerce. Further information on articles undoubtedly belonging to the latter class will be found on page 383 *et seq.*

Boric Acid is sometimes added to meat-extracts as a preservative, and, some years since, was found by the author in notable quantity in a widely-used preparation. The presence of such a substance in an article intended for the use of invalids and persons whose digestion is impaired is very undesirable. Boric acid may be detected and determined by the methods employed for milk (page 175). A modified process recently proposed by C. Fresenius and Popp (abst. *Analyst*, 1897, p. 282) and applied by them to the examination of sausages, &c., may also be employed for the determination of boric acid in meat-extracts. An amount of the extract corresponding to about 3 grammes of dry substance should be concentrated to a syrup, if necessary, and mixed in a mortar with from forty to eighty grammes of recently-ignited sodium sulphate. The mixture is heated in the water-oven for about an hour, and as soon as the mass is dry some more sodium sulphate is added, and the whole reduced to a fine powder. This is digested with 100 c.c. of cold methyl alcohol for twelve hours, with frequent shaking, after which the alcohol is distilled off. As a rule the boric acid passes over completely in one distillation, but it is desirable to extract the residue a second time, using 50 c.c. of methyl alcohol. The distillate is made up to 150 c.c., and 50 c.c. treated with 75 c.c. of water and 25 c.c. of pure glycerin. The mixture is titrated with $\frac{N}{20}$ solution of caustic soda (free from carbonate), using phenolphthalein as an indicator. A pale-rose colour indicates the end of the titration. When it appears, some more glycerin should be added, and if the colour is not permanent the titration is continued till that

point is attained. The volume of alkali used (in c.c.) multiplied by 0·0031 gives the boric acid, H_3BO_3 (in grammes), in the volume of the distillate titrated. Borates will be dissolved out of the organic matters by the methyl alcohol, but will not pass over with the free boric acid. They may be determined in the usual manner in the methyl alcoholic extract, after evaporation, ignition, &c.

PROTEIDS OF DIGESTION.

In the process of digestion, the various constituents of food undergo changes which convert them into soluble and diffusible products capable of ready absorption and assimilation. Thus starch is hydrolysed to dextrin and maltose, native proteids are converted into proteoses and peptones, while fats are emulsified and partially saponified. These changes are due, for the most part, to certain principles known as digestive ferments, which are secreted at different points of the alimentary canal.

The Digestive Ferments belong to the class of soluble ferments or *enzymes*,[1] and their functions are quite distinct from the

[1] Ferments are defined by Halliburton as substances which produce chemical change in other substances, without apparently undergoing any change themselves, or at least without forming any constituent part of the final products. Ferments may be classified as *organised* or living (*e.g.*, yeasts, bacteria) and unorganised or soluble ferments, or enzymes (*e.g.*, pepsin, diastase).

The *enzymes* or unorganised ferments are soluble chemical compounds excreted by organised ferments (*e.g.*, invertin by yeast), or are the products of the activity of certain glands (*e.g.*, ptyalin, trypsin).

The proteid character of pepsin, fibrin-ferment, and diastase has been established, and it is probable that the other enzymes are also proteid, or of closely allied nature.

The action of the enzymes is in many cases hydrolytic. That is, the substances on which they exert a fermentative action are split up with assimilation of the elements of water. Myrosin, the ferment of mustard-seeds (Vol. III. Part iii. p. 101), appears to be an exception to this rule.

According to Bert and Regnard, the organised ferments are killed by hydrogen peroxide, but the unorganised ferments are not affected. On the other hand, Schützenberger and Dumas state that the activity of the organised ferments is destroyed by borax, by which the unorganised ferments are not affected. This action has a manifest bearing on the preservation of food by boric acid and borax.

The fermentative power of the enzymes is usually limited to the decomposition of compounds of a similar nature, no action being exerted on bodies of an essentially different chemical constitution. Thus:—pepsin, trypsin, and papain are proteolytic ferments—that is, they convert coagulable proteids into proteoses or peptones; ptyalin, amylopsin, and

action of the bacteria which play a part in the lower portions of the intestinal canal.

None of the digestive enzymes have been isolated in a state of purity, and their origin and constitution are doubtful; though they are known to be of proteid character, and in some cases closely related to the albumoses.

The digestive ferments act on food most energetically at about 40° C. At a lower temperature their activity is hindered, and at a higher temperature, varying from 50° to 75° C., and characteristic for each enzyme, it is destroyed. Moist trypsin and pepsin are rendered wholly inactive by exposure to a temperature of less than 100° C., but in a dry state they may be heated even to 170° without material change.

The digestive enzymes are readily soluble in water, and are precipitated from their aqueous solutions by excess of alcohol. Even prolonged contact with alcohol does not coagulate or otherwise materially change them, and when the alcohol is removed the solubility and digestive activity of the ferments are found to be substantially unimpaired.[1] On the other hand, the digestive

diastase convert starch, glycogen, &c., into sugar; while the ferments of bile and the pancreatic juice exert an emulsifying and saponifying action on fats. Emulsin hydrolyses salicin, amygdalin, &c., with formation of glucose, and the invertin of yeast and the intestinal canal inverts cane-sugar. Lastly, there are the enzymes which exert a coagulative action, such as fibrin-ferment, myosin-ferment, chymosin (the ferment of rennet), and some similar ferments of vegetable origin.

This selective action of the enzymes and the loss of their activity during the progress of the action distinguish them from inorganic hydrolytic agents (see G. Tammann, *Zeit. physiol. Chem.*, 1895, xviii. 426).

Hoppe-Seyler has classified the enzymes according as their action is similar to that of dilute acids or of alkalies employed at a higher temperature. Ptyalin and invertin are examples of the former, and trypsin of the latter class.

[1] A. Dastre (*Comp. rend.*, 1895, cxxi. 899) finds that the digestive ferments are quite insoluble in alcohol of 95 per cent. Trypsin is distinctly soluble in alcohol of 10 to 25 per cent., but the solubility diminishes rapidly up to 50 per cent., and becomes practically *nil* with 55 per cent. Pancreatic diastase is more soluble, the limit being at about 65 per cent. alcohol. With the blood-ferments, on the contrary, the solubility ceases to be recognisable with 4 to 5 per cent. alcohol. Combining these observations with those of others, Dastre arranges the enzymes in the following order of increasing solubility:—Blood-ferments, emulsin, ptyalin, trypsin, the ferment of *Gaultheria*, pancreatic diastase, myrosin.

The enzymes remain active in the alcoholic solutions, but their effects are greatly retarded, since the attraction of the alcohol for water is opposed to the essentially hydrolysing action of the ferments, and many of the digestible substances are insoluble in alcohol. With trypsin, digestive effects were observed up to 15 per cent. of alcohol, and with pancreatic diastase up to 20 per cent.

enzymes are destroyed by many chemical agents, and when in solution they are coagulated and rendered permanently inactive by boiling the liquid.

The following is a list of the ferments which, according to Sir Wm. Roberts,[1] have been found to occur in the alimentary canal :—

Digestive Juice.	Situation.	Ferments.	Action on Food Materials.
Saliva.	Mouth.	Ptyalin, or salivary diastase.	Changes starch to dextrin and maltose.
Gastric juice.	Stomach.	*a*. Pepsin.	Changes native proteids to peptones and other soluble proteid bodies in an acid medium.
		b. Curdling ferment; chymosin or rennin.	Coagulates the caseinogen of milk.
Pancreatic juice.	Pancreas (sweetbread).	*a*. Trypsin.	Changes proteids to peptones, &c., in neutral or alkaline media.
		b. Curdling ferment.	Coagulates the caseinogen of milk.
		c. Pancreatic diastase.	Changes starch to dextrin and maltose.
		d. Emulsive ferment.	Emulsifies and partially saponifies fats.
Bile.	Liver.	Emulsive ferment.	Assists in emulsifying fats.
Intestinal juice.	Intestinal canal.	*a*. Invertin.	Inverts cane-sugar.
		b. (?) Curdling ferment.	Coagulates the caseinogen of milk

From this table it appears that a long and complex series of ferment-actions are concerned in the process of digestion. Starch is attacked at two points,—in the mouth by *ptyalin*, and in the duodenum by the *pancreatic diastase*. These two ferments are commonly regarded as substantially identical, but there is evidence of weight in support of the view that the special function of the saliva is the formation of soluble starch, the conversion of which into dextrin and maltose at this stage is imperfect and subsidiary; whereas the much stronger hydrolytic action of the pancreatic diastase completes the conversion of the starch into these products. Proteid matters are also attacked at two points, namely, in the stomach by the *pepsin* of the gastric juice, and in the small intestine by the *trypsin* of the pancreatic juice, but these two ferments are quite distinct in their action. The curdling ferment, *chymosin* or *rennin*, the only known characteristic of

[1] The Lumleian Lectures, delivered before the Royal College of Physicians, 1880.

which is to curdle the casein of milk, is found in the gastric secretion, and an identical or closely allied ferment in the pancreatic juice. It is apparently present also in the juice secreted by the small intestine; but the last source has been disputed.[1] The bile is not known to possess any true ferment-action; but it assists, by its physical properties and its alkaline reaction, in emulsifying fatty matters and promoting their absorption. The secretion of *invertin*, the ferment which inverts cane-sugar, appears to be confined to the small intestine.

The changes produced in food by the digestive ferments affect the physical state of the principles more than their chemical composition. They are mainly processes of hydrolysis, whereby the substances operated on are rendered more soluble and diffusible. The changes produced by the enzymes can be produced more slowly in other ways. Thus by prolonged boiling in water, and more rapidly if the water be acidulated, starch is converted into dextrin and maltose, and proteids are changed into a body resembling peptone.

The Enzyme of Saliva.

The characters of saliva vary according to the glands from which the secretion is derived. Mixed human saliva, when perfectly fresh, is a transparent viscid fluid, which under the microscope shows epithelial cells and the so-called *salivary corpuscles* (probably altered leucocytes). The specific gravity of saliva ranges from 1·002 to 1·006, with an average of about 1·003. The reaction to litmus is usually faintly alkaline, but in some instances is distinctly acid, even when no fermentation has occurred.

Samples of mixed human saliva analysed by Jacubowitsch and by Frerichs gave the following results:—

	Per 1000 parts.	
	Jacubowitsch.	Frerichs.
Water,	995·16	994·10
Mucin, ptyalin, and soluble organic matters,	1·34	1·42
Epithelium,	1·62	2·13
Potassium thiocyanate,	0·06	0·10
Inorganic salts,	1·82	2·19
	1000·00	999·94

[1] A. Edmunds (*Jour. Physiol.*, 1896, xix. 466) has found that small quantities of a milk-curdling ferment similar to, if not identical with, chymosin can be obtained from many other parts of the body, namely:—testis, liver, lung, muscle, kidney, spleen, thymus, thyroid, brain, blood, small intestine, and ovary.

The *salts* of human saliva consist of about 62 per cent. of alkaline chlorides, 28 of sodium phosphate, and small quantities of sulphates, thiocyanates, &c.[1] Traces of ammonia and nitrites are also present, and urea, leucine, and lactic acid have been met with in disease.

The remarkable presence of *thiocyanates* in saliva can be readily proved by adding ferric chloride. A blood-red coloration is produced, which is not affected by dilute hydrochloric acid but is immediately destroyed by mercuric chloride. The proportion of thiocyanates present in the saliva is stated to average about 0·1 part per 1000.

PTYALIN.

Ptyalin, sometimes called salivary diastase, is the characteristic soluble ferment or enzyme of saliva. It has not been prepared in a state of purity, but may be obtained in a concentrated condition by macerating the finely-divided salivary glands of the pig in glycerin. The resultant liquid is strained and precipitated with alcohol, when a precipitate is obtained which is rich in ptyalin.

Cohnheim prepared a so-called ptyalin by treating human saliva with phosphoric acid and adding lime-water as long as a precipitate was thrown down. This was filtered off and treated with cold water, which dissolved the ferment, leaving a residue of mucin, &c., mixed with calcium phosphate. From the solution the ptyalin was precipitated by excess of alcohol, and the flocculent precipitate purified by re-solution in water and precipitation by alcohol. The product thus obtained still contained some phosphates. It was readily soluble in water, and the solution possessed strong amylolytic properties. It was precipitated by neutral and basic lead acetates, but not by tannin nor mercuric chloride, and did not give the xanthoproteic reaction.

Ptyalin possesses the power of converting starch into dextrin and sugar. Human saliva exerts no action on unboiled starch unless contact is prolonged for some days. Hence the farinaceous food of man requires to be cooked, in order to rupture the starch-corpuscles, but many of the lower animals are able to digest raw starch.[2] The digestion of starch by ptyalin takes place with

[1] The "tartar" deposited on the teeth consists mainly of calcium phosphate, with smaller quantities of iron phosphate, calcium carbonate, &c., and about 25 per cent. of organic matter.

[2] It is doubtful, however, if the digestion of starch in the lower animals is effected by the saliva. The saliva of man is stated to possess greater diastasic power than that of any other animal, while that of the herbivora is very weak, and especially so in the horse, in which the diastasic ferment is almost if not altogether wanting.

facility only in alkaline, neutral, or faintly acid solutions, an excess of acid materially impeding the reaction.

The first stage in the action of ptyalin consists in the formation of soluble starch. In presence of sufficient ferment, and at a suitable temperature, this change takes place almost instantaneously. The product still gives a blue reaction with solution of iodine. Erythro-dextrin, which yields a brown coloration with iodine, is next formed, and this is followed by achroo-dextrin and maltose, at which stage the mixture gives no colour-reaction with iodine. Thus the products of the conversion of starch by ptyalin are exactly parallel with those of its hydrolysis by diastase. Hence malt-extract is a valuable remedy in certain cases in which the nutritive power is defective.

Experiments by Chittenden and Griswold (*Amer. Chem. Jour.*, iii. 305), and more recently by Chittenden and Ely (*Pharm. Jour.*, [3], xiii. 367, 386, 429), have proved that human saliva,[1] in presence of an equal volume of gastric juice containing 0·05 per cent. of hydrochloric acid, is capable of forming, from a given quantity of starch, a much larger quantity of sugar than the same quantity of saliva can do alone under a like degree of dilution. This fact is the more remarkable since the same percentage of acid by itself greatly retards the hydrolytic action. These chemists further show that peptones—which are themselves products of gastric digestion—while destitute of diastasic action on starch, exert a decided influence on salivary digestion, stimulating the ferment to increased action, particularly in the presence of a proportion of acid which by itself completely prevents the conversion of starch into sugar. It appears probable, therefore, that salivary digestion continues in the stomach before the proportion of acid becomes excessive.

Enzymes of the Gastric Juice.

Normal gastric juice is a thin, colourless or faintly yellow liquid, having a strongly acid reaction, a peculiar taste and smell, and a specific gravity ranging from 1·001 to 1·010.

The most remarkable constituent of the gastric juice is the enzyme *pepsin*, which in presence of acids possesses the power of transforming native proteids into proteoses and peptones. In addition to pepsin, the gastric juice contains an enzyme known as *rennin* or *chymosin*, which possesses the property of curdling milk (see page 89).

No reliable complete analysis of normal human gastric juice is on record. A widely-quoted analysis of the juice obtained from a

[1] On the composition of human saliva, see R. H. Chittenden, *Amer. Jour. Physiol.*, i. ; abst. *Jour. Chem. Soc.*, 1898, ii. 241.

woman having a gastric fistula shows only a fraction of the free acid now known to be normally present.

The following figures represent the average of ten analyses by C. Schmidt of the gastric juice of the dog (obtained without admixture of saliva):—

Water,	973·062	parts per	1000.
Organic matters; including pepsin, mucin, and peptones,	17·127	,,	,,
Free hydrochloric acid,	3·050	,,	,,
Sodium chloride,	2·507	,,	,,
Potassium chloride,	1·125	,,	,,
Ammonium chloride,	0·468	,,	,,
Calcium chloride,	0·624	,,	,,
Calcium phosphate,	1·729	,,	,,
Magnesium phosphate,	0·226	,,	,,
Ferric phosphate,	0·082	,,	,,

The proportion of pepsin in the gastric juice varies considerably during the progress of digestion, at first diminishing and subsequently undergoing an increase. The acidity of the gastric juice is lowest at the commencement of digestion.

The gastric juice (of the dog) is not coagulated by boiling, but the proteolytic power of the pepsin is destroyed. Alcohol gives a precipitate which contains the enzyme, since on re-solution in water and addition of an acid it is capable of digesting albumin; but the addition of a large excess of strong alcohol is stated to destroy the pepsin. On saturating gastric juice with common salt, a precipitate is obtained containing albumoses and the greater part of the pepsin. Mercuric chloride and silver nitrate produce precipitates containing a portion only of the ferment. Lead acetate throws down the greater part of the ferment, but much of it dissolves out on washing the precipitate with water.

On adding sodium carbonate to gastric juice, a trifling precipitate is formed, consisting chiefly of earthy salts. A portion of the pepsin is carried down with the precipitate, but the filtrate, after acidulation, still possesses digestive properties.

Gastric juice possesses marked antiseptic properties, and may itself be kept for a long time without putrefying or losing its proteolytic power.

Pepsin.

The most important physiological property of the gastric juice is that of effecting the hydrolysis of natural proteid matters and converting them into the soluble products known as proteoses and peptones. This conversion is effected by the enzyme *pepsin*, the presence of which is essential to the digestive action. Its

activity is greatly promoted by the presence of free acid, hydrochloric acid being the most suitable, though other acids may be substituted.[1] Acid alone, of the strength met with in pepsic digestions, is not capable of effecting the complete digestion of proteids. In an alkaline medium pepsin is inactive, and in strictly neutral liquids its digestive action is insignificant.

Some experiments by Wroblewski (abst. *Pharm. Jour.*, 1896, i. 32) on pig's pepsin prepared by glycerin-extraction and subsequent filtration, and human pepsin obtained by the same process from the stomachs of infants that had died during birth, showed the former to be the more powerful. Fibrin stained with carmine was employed to ascertain the digestive power, the rate of the process being estimated from the relative depth of tint acquired by the liquids in a given time. The acids were in each case of equal neutralising power, that is, they were capable of neutralising $\frac{1}{20}$th normal alkali, using litmus as an indicator, except in the case of phosphoric acid, the strength of which was deduced from the specific gravity.

Wroblewski's experiments showed that digestion occurred most rapidly with oxalic acid, and, after that, hydrochloric, nitric, phosphoric, tartaric, lactic, citric, malic, paralactic, sulphuric, and acetic acids were active in the order named. With pig's pepsin and oxalic acid, digestion was complete in thirty minutes, and with child's pepsin in forty minutes. Acetic acid showed only a trace of red colour in the liquid after twenty hours' digestion with child's pepsin, and none after the same period with pig's pepsin. Caffeine hastened digestion, and the same was true in a less marked degree of theobromine and codeine. Veratrine delayed the process in a marked manner, and morphine and many other alkaloids less strongly.

The salts of the heavy metals and other agents which precipitate pepsin arrest gastric digestion. Many of the salts of the light metals (*e.g.*, magnesium and sodium sulphate, potassium iodide, alum, &c.) exert a similar action. Tannin arrests digestion, but phenol, when present in small quantity, has no inter-

[1] Experiments by A. Mayer (abst. *Pharm. Jour.*, [3], xii. 262) showed that the digestive action on coagulated egg-albumen of pepsin from the pig was favoured by elevation of temperature, the limit, however, being reached at about 55° C. Hydrochloric acid of a strength corresponding to about 0·2 per cent of real HCl was found preferable. Other acids were found active in the following order:—Nitric, oxalic, sulphuric, lactic, formic, succinic, acetic. Butyric and salicylic acids had no solvent action.

Hydrobromic and hydriodic acids are stated to hinder pepsic digestion. Sulphurous acid arrests it, while arsenious and hydrocyanic acids have little or no action.

fering action. Hence phenol is sometimes given to prevent abnormal bacterial fermentation. Salicylic acid, in small doses, appears to have but little effect, but in larger quantity interferes with pepsic digestion in a marked manner.

Alcohol, when present in considerable proportion, retards pepsic digestion, and hence it might be inferred that wine was an unsuitable menstruum for the administration of pepsin. It has, however, been shown by C. Symes (*Pharm. Jour.*, 1897, ii. 398) that the alcohol is rapidly removed by diffusion, so that he regards the objection as having no practical force.[1] On the other hand, Hermann-Peters has pointed out that wine is an unsuitable medium for the administration of pepsin, since (apart from the effect of the alcohol) the potassium acid tartrate reacts with the hydrochloric acid of the gastric juice to form free tartaric acid, which is not an efficient substitute for the hydrochloric acid converted in potassium chloride.

While pepsic digestion is much more vigorous and complete in presence of free hydrochloric acid, it is capable of taking place in cases where the acid present is insufficient to combine with the whole of the proteids present.[2] Not only albumin and its congeners, but also the albumoses and peptones, are capable of entering into combination with hydrochloric acid, and the acid present in these forms is less active, both as a digestive and as an antiseptic agent, than when in an uncombined form. The more degraded the proteid becomes, by the process of proteolysis, the larger the proportion of acid with which it combines, so that the peptone compounds contain more acid than the proteose compounds, and the latter more acid than is contained in syntonin, Peptone hydrochlorides are acid to litmus, but perfectly stable on evaporation. Each step of the process of proteolysis is indicated by the increasing proportion of acid which exists in combination with the products characteristic of the particular phase of the reaction.[3]

[1] See R. H. Chittenden, &c., *Amer. Jour. Physiol.*, 1898, i. 164.

[2] According to Schiff, when an insoluble aliment (such as coagulated egg-albumen, fibrin, or meat which has been deprived of the soluble extractive matters) is introduced into the stomach of a fasting animal no pepsin is secreted, and the proteid remains undigested; but that if with the proteid certain soluble aliments be introduced, pepsin is formed, and digestion immediately commences. Of these "peptogenes" the most effective were found to be solutions of dextrin, soup and extract of meat, infusion of green peas, bread, gelatin, and peptones. On the other hand, solutions of dextrose, soluble starch, fat-emulsion, and gum had no peptogenic effect, and milk and coffee but little.

[3] According to L. Sansoni (abst. *Jour. Chem. Soc.*, 1898, i. 233), albumin in aqueous solution has the property of combining with a certain quantity of

The normal acidity of the gastric juice is equal to about 0·2 per cent. of real hydrochloric acid, which is the free acid actually secreted with pepsin in the gastric juice, though it is probable that other acids may be set free from the food during the process of digestion. Hence water containing 1 per cent. of strong hydrochloric acid is a suitable medium for experiments with pepsin.

Pepsin may be dissolved out from the mucous membrane of the stomach of an animal by water or glycerin, and on adding a variety of reagents to the resultant extract a precipitate is obtained in which the presence of pepsin can be demonstrated by its proteolytic power. Attempts to purify the precipitate are apt to lead to great diminution in its digestive activity. Nor does the mucous membrane at all times yield an active extract. The pepsin appears sometimes to be absent or incompletely formed, existing as "pro-pepsin," "pepsinogen," or "pepsin-precursor," so that time, aided by atmospheric influences, may be necessary to develop the perfect ferment.[1]

hydrochloric acid, so that the acidity of the mixture is diminished, and the more concentrated the albumin solution, the greater the amount of acid is thus concealed. The loss of acidity can be proved either by the phloroglucol-vanillin reaction, or by means of phenol-phthalein. With peptone, it is only in the case of the first of these reagents that part of the acid is not recognisable, and the complete peptonisation of albumin seems to set free, or rather to cause to react with above reagents, the hydrochloric acid previously disguised by it. A mixture of albumin and hydrochloric acid does not recover the lost acidity even in presence of pepsin, provided the conditions are so arranged as to prevent the formation of much peptone. By the continued action of a temperature of 100° to 110°, the acidity is partly or wholly lost, but this does not occur with mixtures of peptone and hydrochloric acid.

Sansoni states that the combination of albumin with hydrochloric acid does not seem to take place in constant proportion, and when the mixture has not been heated to higher temperatures, the greater part of the hydrochloric acid can be separated from the albumin by dialysis. The foregoing observations show that peptone hydrochlorides, though acid to indicators of neutrality, are stable on evaporation, and that methods for the determination of free hydrochloric acid in gastric juice based on evaporation give erroneous results.

[1] According to Podwissotzky (abst. *Pharm. Jour.*, [3], xvii. 607, 664), the gastric mucous membrane of neither herbivorous nor carnivorous animals yields much pepsin to glycerin if treated immediately after the death of the animal, but if kept in a warm place for twenty-four hours, and then free from putrefaction, it will yield a much larger quantity. It has been stated that glycerin takes up nothing but pepsin from the gastric membrane, but Podwissotzky finds that it dissolves in addition a certain amount of propepsin or pepsinogen. After exhaustion with glycerin, the membrane will yield a further quantity of pepsin to hydrochloric acid, either with or without glycerin; whence it is inferred that the membrane originally contains two forms of propepsin, one of which is soluble in glycerin and the other insoluble.

The value of carefully-made preparations of pepsin in certain cases of functional disorder of the stomach is fully established. Some commercial preparations professedly contain all the digestive ferments; but it is evident that their real value depends solely on the proportion of pepsin, since all digestive ferments other than pepsin are destroyed or rendered inactive by the acid of the stomach.

R. H. Chittenden (*Digestive Proteolysis*, 1895) recommends the following method for the preparation of pure pepsin:—"The mucous membrane from the cardiac portion of a pig's stomach is dissected off and washed with water. The upper surface of the mucosa is then scraped with a knife until at least half the membrane is removed. These scrapings, containing the fragments of the peptic glands, are warmed at 40° C. with an abundance of 0·2 per cent. of hydrochloric acid [1] for ten or twelve days, in order to transform all the convertible albuminous matter into peptone. The solution is then freed from insoluble matter by filtration, and immediately saturated with ammonium sulphate, by which the pepsin, with some albumose, is precipitated in the form of a more or less gummy or semi-adherent mass. This is filtered off, washed with a saturated solution of ammonium sulphate, and then dissolved in 0·2 per cent. hydrochloric acid. The resultant solution is next dialysed in running water until the ammonium salt is entirely removed, thymol being added to prevent putrefaction, after which the fluid is mixed with an equal volume of 0·4 per cent. hydrochloric acid and warmed at 40° C. for several days. The ferment is then once more precipitated by saturation of the fluid with ammonium sulphate, the precipitate strained off, dissolved in 0·2 per cent. acid, and again dialysed in running water until the solution is entirely free from sulphate. The clear solution of the ferment obtained in this manner can then be concentrated at 40° C. in shallow dishes, and, if desired, the ferment obtained as a scaly residue.

Commercial pepsin was first prepared by mincing pigs' stomachs, macerating the fragments in slightly acidulated water, and evaporating the solution to dryness at a low temperature. Attempts were made to obtain an improved product by treating the filtered pepsin solution with basic acetate of lead, decomposing the

According to Von Herzer, but little pepsin is present in a fasting stomach in the morning, but there is abundance of propepsin.

[1] By "0·2 per cent. hydrochloric acid" is meant water containing 0·2 per cent. of real HCl. This can be prepared with a sufficient approach to accuracy by adding 5 c.c. of fuming hydrochloric acid, or 10 c.c. of acid of 1·11 sp. gravity to 1 litre of water.

precipitate with sulphuretted hydrogen, and evaporating the filtered liquid.

Various methods of manufacturing pepsin are employed, but none of them yield a perfectly pure product. The preparations vary very greatly in digestive power, but have greatly improved of late years, so that it is now expected that commercial pepsin shall dissolve, under certain conditions, as much as 3000 times its weight of hard-boiled egg.

The characters and methods of obtaining the preparations of pepsin met with in commerce in 1883 were described in a paper by A. Tscheppe (*Pharm. Jour.*, [3], xiv. 164). For the details of the methods now employed for the manufacture of commercial pepsin an anonymous paper in the *Pharmaceutical Journal* (for January 21, 1893) may be consulted with advantage.

For purifying the crude pepsins obtained by precipitation with salt, or by dissolving the inner skins of the stomachs and scaling the concentrated solutions, a useful method is to saturate the acidulated solution with crystallised sodium sulphate at a temperature of about 94° F. Sulphurous acid in saturated solution is then added until the liquid acquires a faint odour of the gas. The liquid is kept at the same temperature until the pepsin separates, an excess of sulphurous acid being kept constantly present in order to prevent decomposition. The precipitate of pepsin is removed and the saline liquid allowed to become cold, when the greater part of the sodium sulphate will crystallise out and may be used again. The precipitated pepsin is tolerably free from peptones, which remain in the saline solution, and when drained and pressed it yields a tolerably pure and active product. The remaining sodium sulphate may be removed by redissolving the pressed pepsin in water acidulated with hydrochloric acid, adding sulphurous acid, and dialysing the liquid into running water. The undiffusible portion is then evaporated *in vacuo*, the process being either carried to dryness or sufficiently far to allow of the ferment being scaled on glass plates. The scales so obtained are somewhat opaque, and have a slight bitter taste, which is probably due in part to adhering traces of sodium sulphate.

All specimens of commercial pepsin of good quality are colourless, or but very slightly coloured. The pulverulent forms occur as fine white or yellowish-brown amorphous powders. The scaled preparations are transparent or translucent, and of a pale greenish or lemon-yellow colour. Pepsin slowly absorbs water on exposure to the air, but should not be very hygroscopic (indicating the presence of peptone). Pepsin should be free from offensive odour, and have a peculiar, but not unpleasant, mildly acidulous or slightly

saline taste, usually followed by a suggestion of bitterness. An offensive or putrescent odour or deficiency in any other of the foregoing respects indicates faulty manufacture, or the presence of foreign matter, such as mucus, albumin, peptone, or inert animal tissue.

Pepsin usually has a slightly acid reaction. It may be neutral, but should never be alkaline. It should be soluble in cold water, with not more than slight opalescence, more readily soluble in water acidulated with hydrochloric acid, and soluble in about 10 parts of alcohol of 90 per cent.

Some forms of commercial pepsin are prepared by precipitating aqueous extracts of the mucous membrane of the stomach of the hog or sheep with common salt or sodium sulphate, and mixing the precipitate with powdered starch or milk-sugar.[1] The "Saccharated pepsin" of the United States Pharmacopœia (1890) contains 10 per cent. of pepsin and 90 per cent. of milk-sugar. Fluid preparations of pepsin are also much used.[2]

A sample of commercial pepsin examined in the author's laboratory was found to have the following composition (compare foot-note on page 352):—

Moisture,	5·00 per cent.
Insoluble matter,	none ,,
Coagulable albumin,	none ,,
Syntonin,	none ,,
Pepsin (and albumoses),	61·02 ,,
Peptones,	5·29 ,,
Starch,	none ,,
Non-nitrogenous organic matters (by difference),	27·69 ,,
Ash,	1·00 ,,
	100·00

Dry pepsin is not affected by exposure to a temperature considerably above the boiling point of water (F. A. Thompson, *Pharm. Jour.*, 1896, i. 86).

The formula of "Lactopeptine powder" is given by the manufacturer as follows:—"Sugar of milk, 40 oz.; pepsin, 8 oz.;

[1] Starch will be indicated by the blue coloration yielded with iodine, and milk-sugar by the reduction of Fehling's solution on boiling.

[2] Glycerin is an excellent solvent of pepsin, but unless used in such quantity as to render the preparation distasteful is not a good preservative. According to C. Symes, a solution of freshly-prepared undried pepsin in dilute glycerin, to which 10 per cent. of rectified spirit is added, forms, when filtered, an excellent medicinal preparation which may be flavoured to taste.

pancreatine, 6 oz.; ptyalin or diastase, 4 drachms; lactic acid, 5 fluid drachms; and hydrochloric acid, 5 fluid drachms."

L. A. Harding has published (*Bulletin of Pharmacy*, 1893) the results of his examination of twenty-four samples of commercial pepsin of American manufacture. Only one of these (made by Parke, Davis & Co.) had a dissolving power (by the U.S.A. test) exceeding 2500, and many were of very low quality. One saccharated pepsin for which a digestive power of 500 was claimed had an actual activity of 50.

H. W. Snow has also found that few of the so-called U.S.P. pepsins of commerce are really of U.S. Pharmacopœia quality. Only two out of fifteen samples examined had digestive powers of 1 : 3000, the remainder ranging from 1 : 2142 down to 1 : 535.

Assay of Pepsin.

The assay of commercial pepsin is always based on an attempt to ascertain its proteolytic power. This, however, varies greatly with the time and temperature employed, the condition of the proteid substance on which its action is exerted, and on other conditions more difficult to control. Further, in assays of pepsin as usually conducted, the peptones produced by action of the ferment retard and eventually arrest the action of the ferment, whereas, in the process as naturally carried on in the stomach, the soluble products are constantly removed as the digestion proceeds.[1] The process can, however, be caused to recommence by diluting the liquid with acidulated water. Seeing that the methods of

[1] In an experiment described by F. B. Benger, white of egg, acidulated water and pepsin were placed in a parchment-dialyser floated on a bowl of acidulated water. It was found that an enormously larger quantity of albumen could be dissolved under these conditions, where the products of digestion could diffuse away, than in a test-tube containing a similar mixture.

In another experiment described by Benger (*Lancet*, April 3, 1886), 1000 grains of hard-boiled white of egg and 10 ounces of acidulated water were placed in a beaker, while 100 grains of the same white of egg and 1 ounce of acidulated water were mixed in a test-tube which was immersed in the beaker. The liquids were then heated to 130° F., and one teaspoonful of an active fluid preparation of pepsin was then added to both beaker and test-tube, the temperature being maintained at 130° F., and the contents of both beaker and test-tube stirred occasionally. When the egg in the test-tube had all dissolved, which occurred in twenty-five minutes, the contents of the beaker were filtered through muslin, and the undissolved albumen was found to weigh 220 grains. Hence a small dose of pepsin had dissolved 780 grains of albumen in 10 ounces of liquid, while a similar dose had dissolved 100 grains in 1 ounce. The result is regarded by Benger as simply due to dilution, but the larger mass of hydrochloric acid in the case of the beaker mixture probably had a favourable influence on the digestion.

assaying pepsin are necessarily arbitrary, it is essential to lay down and adhere to certain precise conditions of procedure in order to render the results comparative.

Of the various tests for estimating the strength of samples of pepsin, there is no recognised one which, under all circumstances, will give uniform results, and slight variations in the methods of manipulation will often occasion a wide difference in results with the same sample of pepsin. The different official tests for ascertaining the digestive strength of pepsin are sufficient to show if a sample is above or below a required standard, but they do not give the actual strength.

The process of testing pepsin prescribed in the British Pharmacopœia of 1885 has been the subject of severe and richly-deserved criticism. The description given was deficient in the detail desirable in a process of a strictly empirical nature, and the low proteolytic activity required (50) was for years a direct premium on careless preparation or systematic adulteration.

The method of assaying pepsin prescribed in the United States Pharmacopœia of 1890 is in many respects preferable to the B.P. test of 1885, but the prolonged period during which the digestion is directed to be continued is inconvenient and open to objection in other respects. Mainly as a consequence of this lengthened period of digestion, the results obtained by the B.P. (1885) and U.S.P. processes, when applied to the same sample of pepsin, are widely different.

The British Pharmacopœia of 1898 requires pepsin to have a solvent power of 2500. The method of assay closely follows that of the U.S. Pharmacopœia, as is shown by the following table :—

	B.P. (1885) Test.	U.S.P. (1890) Test.	B.P. (1898) Test.
Pepsin, . .	2 grains.	0·00335 gramme.	0·005 gramme.
Albumen (moist),[1]	100 grains.	10 grammes.	12·5 grammes.
Water, . .	1 fluid ounce.	100 c.c.	125 c.c.
Hydrochloric acid,	5 minims.	2 c.c. of dilute acid (=0·21 gramme real HCl).	0·25 gramme HCl.
Time of digestion,	30 minutes.	6 hours.	6 hours.
Temperature, .	130° F.	100·4° to 104° F.	105° F.
Interval between agitations,	No definite time stated.	15 minutes.	No definite time stated.
Dissolving power required,	50	3000	2500

[1] By albumin is to be understood the soluble coagulable proteid of that name from any source, the term albumen being restricted to the unpurified albuminous mixture existing in white of egg.

C. D. Moffat (*Pharm. Jour.*, [3], xxv. 813) has compared the B.P. (1885) and U.S.P. methods of testing on three samples of commercial pepsin, with the following results :—

No.	Digestive Power Claimed.	Solvent Power Found.	
		By B.P. (1885) Test.	By U.S.P. Test.
1	1 : 3000	1 : 250	1 : 3000
2	1 : 3000	1 : 150	1 : 1800
3	1 : 2500	1 : 150	1 : 1800

From this it appears that the same sample of pepsin showed by the old B.P. test only one-twelfth of the dissolving power on albumen which is indicated by the U.S.P. test. But it will be observed that in the latter case the operation is prolonged to six hours, instead of being brought to an end in half an hour.

It should be borne in mind that the real digestive power of a pepsin is measured by the amount of peptone which it produces in a given time under certain conditions, whereas it is usual to observe the amount of albumin dissolved. A weak pepsin may dissolve all the albumin and convert it merely into syntonin, whilst a much stronger pepsin may carry the action further, even to the last stage, with production of peptone. As the albumin has been wholly dissolved in each case, to all appearance the two pepsins have done an equal amount of work, whereas in reality one may have a far greater activity than the other.

If pepsin is allowed to act on more coagulated albumin than it can fully digest, it is liable to spend and exhaust its activity in converting all the albumin into syntonin, with formation of little or no peptone. Owing to its colloid nature, the pepsin cannot penetrate the albumin, and therefore exerts its proteolytic power merely on the outer surface of the albumin particles. Hence the more finely the albumin is divided the more readily will it be attacked and digested. A weight of 100 grains of albumin requires about one ounce of acidulated water for solution. If less water is used, the solution is retarded.

When the albumin has undergone partial solution through the action of the pepsin, say for four hours, the undissolved portion is always in a more or less advanced state of digestion, and it is difficult to estimate it. It is, therefore, advisable to arrange the experiment so that in the end the albumin will be, as nearly as possible, completely dissolved. This is done by regulating the amount of

albumin used, and one or more preliminary tests will therefore be necessary.

In order to obtain uniform results, the experiments must be carried on each time under precisely similar conditions. For the preparation of the albumen, the eggs used must be fresh, and the time allowed for boiling must be the same in each case. The degree of fineness must be identical with every fresh sample of coagulated albumen, as has been pointed out. The proportion of albumen and acidulated water must not vary, and the degree of acidity must be the same in each experiment. The temperature of digestion, the time required to effect the solution of the albumen, and the agitation of the mixture during digestion must be precisely similar in each case. It will be found useful to compare the results obtained with a standard, this being the best pepsin obtainable. This will show how much of the pepsin under examination is required to produce the results given by a certain amount of the standard pepsin. The albumen may be conveniently prepared by placing fresh eggs in cold water, heating until the water boils, and then keeping the eggs in the boiling water for fifteen minutes. They are taken out and plunged into cold water, the coagulated white of egg separated from the yolk, and the white then rubbed and squeezed through a sieve having thirty meshes to the linear inch, rejecting the first portion.

The following is the method of assaying pepsin prescribed in the United States Pharmacopœia of 1890:—

A. To 294 c.c. of water add 6 c.c. of diluted hydrochloric acid (containing 10 per cent. of real HCl).

B. In 100 c.c. of solution *A* dissolve 0·067 gramme of the pepsin to be tested.

C. To 95 c.c. of solution *A*, brought to a temperature of 40° C. (= 104° F.), add 5 c.c. of solution *B*.

The resultant 100 c.c. of liquid will contain 0·21 gramme of absolute hydrochloric acid, 0·00335 gramme of the pepsin to be tested, and 98 c.c. of water.

To make the test, 10 grammes weight of recently-prepared, moist, finely-divided egg-albumen, prepared as above, is placed in a 200 c.c. flask, one-half of solution *C* added, and the liquid shaken well, so as to distribute the coherent albumin evenly through the liquid. The second half of the solution is then added, and the liquid again shaken. The flask is then kept at a temperature of 38° to 40° C. (= 100·4° to 104·0° F.) for six hours, and shaken gently every fifteen minutes. At the end of this time the albumin should have disappeared, leaving at most only a few thin, insoluble flakes.

The foregoing process will suffice to show whether the sample of pepsin has the standard dissolving power of 1 : 3000, but if this is not found to be the case, the true activity can only be determined "by ascertaining, through repeated trials, how much of solution *B* made up to 100 c.c. with solution *A* will be required exactly to dissolve 10 grammes of coagulated and disintegrated albumin under the conditions given above." This necessity renders the foregoing process of pepsin testing very tedious, and the results in the end are far from satisfactory. A valuable modification consists in doubling the quantity of pepsin employed, and reducing the time of digestion to three hours.

The British Pharmacopœia of 1898 states that:—"If 12·5 grammes of coagulated and firm white of fresh eggs (previously passed through a sieve having 12 meshes to the centimetre, and used before it has lost moisture), 125 c.c. of acidulated water, containing about 0·2 per cent. of hydrogen chloride (HCl), and 0·005 gramme of pepsin, be digested together at 105° F. (= 40·5° C.) for six hours, and shaken frequently, the coagulated white of eggs dissolves, leaving only a few small flakes, in an almost clear solution." This process closely resembles that of the United States Pharmacopœia, which it follows in the objectionably long time for which the digestion is continued. But the U.S.P. gives precise directions as to the frequency of stirring, which very important condition is ignored in the B.P.; and the U.S.P. employs a standard solution of pepsin, which allows of an exact quantity being readily taken for the experiment, whereas the B.P. apparently intends the very small quantity of 0·005 gramme of pepsin to be accurately weighed out, presumably with an error of not more than 0·0001 gramme! Nor is any correction made for the solvent action of the acid on the albumen, the entire effect being apparently credited to the pepsin.[1]

J. M. Lear (*Bulletin of Pharmacy*, x. 298) has pointed out that the percentage of water in egg-albumen varies considerably, both before and after coagulation, and that in the process of comminuting and weighing the albumen loss of moisture may occur, causing variation in the results, even when the same sample of pepsin and coagulated albumen from the same egg are employed. He therefore recommends that the whites of several eggs should be well mixed, diluted with nine times their weight of water, 25 grammes of the mixture made up to 250 c.c. with water, and the liquid boiled for five minutes. It is then cooled, any loss

[1] It is deplorable that those responsible for the production of the British Pharmacopœia should have adopted a process of pepsin-assay so unscientific in its principles and so defective in the details of its application.

by evaporation made good, and the liquid strained if necessary. 50 c.c. measure of the albumin solution is then digested with pepsin-hydrochloric acid in the usual way.

A similar process has been described by E. H. Bartley (*Pharm. Jour.*, [3], xxiv. 422).

In the opinion of the author the error due to the loss of moisture during manipulation of the albumen is exaggerated, and in such an empirical and unsatisfactory process as that of pepsin-testing as ordinarily practised may as well be left out of account. On the other hand, the proposal to substitute a solution of albumin for hard-boiled egg appears to be a good one.

The disadvantages attending the use of moist egg-albumen in pepsin-testing have also been pointed out by J. H. Stebbings, who recommends the use of scale-albumin (see below).

Instead of noting the digestive action of pepsin on coagulated white of egg, A. Ball (*Pharm. Jour.*, [3], xx. 227) has proposed to employ finely-divided fibrin from lean rump-steak. Any portion left undissolved can be filtered off and weighed with much greater ease and certainty than a residue of egg-albumen.

At the present time the whole subject of pepsin-assay is in a very unsatisfactory, not to say discreditable, condition, for which the unscientific and inadequately-described official methods of testing are largely responsible. There has commonly been no marked distinction drawn between the mere conversion of albumin into syntonin or other soluble form and the true peptonisation characteristic of the action of pepsin, both these changes being confounded under the term "digestion." Further, the effect of the hydrochloric acid used in effecting solution of the albumin has been generally ignored or considered insignificant, whereas it has been shown by L. A. Harding (*Pharm. Jour.*, [3], xxv. 914) that a material part of the solvent action observed is really due to the acid employed, and hence should be duly allowed for before coming to a conclusion as to the strength of the pepsin under examination. But the acid, under the conditions of a pepsin-assay, only converts the albumin into syntonin, the peptonisation of this product being due to the pepsin. Hence, as admitted by various authorities, the true gauge of the activity of a sample of pepsin is the amount of peptones, or of mixed peptones and albumoses, formed by its action under specified conditions. These facts have been pointed out and insisted on by J. H. Stebbings (*Analyst*, 1889, pp. 197, 210, 229), who, in an able criticism of the existing methods of assaying pepsin, recommends the following process of Kremel:—Egg-albumen in scales is dried at 40° C. and powdered, and 1 gramme treated in a 100 c.c.

flask with 0·1 gramme of the sample of pepsin to be tested, and 50 c.c. of 0·2 per cent. hydrochloric acid. The liquid is heated to 38° to 40° C., and kept at that temperature for three hours. It is then exactly neutralised with sodium carbonate, heated on the water-bath to 90° C., and cooled after coagulation has taken place. The liquid is then made up to 100 c.c., and 50 c.c. filtered off and evaporated to dryness over a water-bath. The residue is dissolved in hot distilled water, filtered through a moist filter, and the residue carefully washed. The solution is again evaporated to dryness and the residue weighed. The residue is ignited, and the resultant ash deducted to obtain the corrected weight of the albumoses and peptones formed.

This process actually determines the amount of digestion-products formed, and presents other points of advantage over the methods commonly employed. A great improvement might be effected by deducing the amount of albumoses and peptones from a determination of the nitrogen in the filtered liquid by Kjeldahl's process, either with or without previous precipitation with bromine. This would avoid the troublesome and uncertain desiccation of the residue of deliquescent peptones, &c., previously to weighing.

In digestion-experiments continued for three hours, in which a constant weight of 0·100 gramme of various samples of commercial pepsin was employed, Stebbings found the amount of peptones, &c., formed, to range from 0·1738 gramme (in a saccharated specimen) to 0·5844 gramme. With the pepsins of high activity now met with it would be desirable to reduce the amount used to 0·050 gramme.

The foregoing process does not allow of any distinction being drawn between the albumoses which may possibly result from the action of the acid used and the true peptone which is the peculiar and characteristic product of proteolysis by pepsin. Until recently, the determination of peptone formed in digestion-experiments was troublesome and uncertain, but by the application of a process devised by the author (*Analyst*, 1897, p. 258) this difficulty is in a great measure overcome, and the rational testing of pepsin has been rendered practicable. The process has been devised too recently to allow of any extended experience of its working, and some modifications of its details may be found desirable, but the following is the method as the author employs it at present. About 1 gramme of egg-albumen in scales is powdered and treated in a 100 c.c. flask with 20 c.c. of warm water. When the substance has dissolved, the liquid should be heated in a bath of boiling water to coagulate the albumen, and cooled to a temperature not exceeding 40° C. 0·100 gramme of the sample of pepsin to

be tested should next be added, followed by 25 c.c. of decinormal hydrochloric acid. The liquid is then warmed to 40° C., and kept at that temperature for three hours. A volume of decinormal sodium carbonate exactly equivalent to the acid previously used is then added, and the liquid heated on the water-bath to 90° C. for ten minutes. It is then cooled, diluted with water to 100 c.c., and passed through a dry filter. The precipitate will consist of syntonin and any unaltered albumin, while the filtrate contains any albumoses and peptones formed. 50 c.c. measure of the filtered liquid is now saturated in the cold with powdered zinc sulphate (of which about 60 grammes will be required), allowed to stand with occasional agitation for half an hour, and then filtered. The precipitate is washed with a cold saturated solution of zinc sulphate,[1] the filtrate diluted with water to 250 c.c., acidulated with hydrochloric acid, and treated with excess of bromine-water. The precipitate is treated as described on page 315, and the peptone formed deduced from the nitrogen found by Gunning's modification of Kjeldahl's process (page 21). An allowance must be made for the nitrogen contained in the pepsin employed. The albumoses may be deduced from the nitrogen found in the zinc precipitate, after washing it with a saturated solution of zinc sulphate. They may also be deduced from the nitrogen found by treating the remainder of the filtrate from the coagulated syntonin, &c., by Kjeldahl's process, either with or without previous precipitation with bromine.

Employing the foregoing process, the following results were recently obtained by A. B. Searle in the author's laboratory. One gramme of commercial egg-albumin in scales was used, with 0·100 gramme of pepsin.[2] The digestion was conducted at 40° C.

[1] Instead of washing the precipitate produced by zinc sulphate, the liquid still containing the precipitate in suspension may be weighed, passed through a dry filter, and the filtered liquid weighed before dilution. The result obtained on this filtrate (usually about four-fifths of the whole) is then calculated to the total weight of liquid previously ascertained.

[2] The following results were obtained when the sample of pepsin used was analysed by the same process :—

	Nitrogen.		×6·3=Proteids.	
	Per cent.		Per cent.	
Total (by Kjeldahl's process on original pepsin), .	10·57		66·59	
Precipitated by bromine (on original pepsin), . .	10·12	10·56	63·63	66·40
By Kjeldahl in filtrate from bromine-precipitate, .	0·44		2·77	
Precipitated by zinc sulphate (on original pepsin),	9·69		61·02	
By bromine in filtrate from $ZnSO_4$ precipitate, .	0·70	10·53	4·41	66·31
By Kjeldahl in filtrate from bromine-precipitate, .	0·14		0·88	
Precipitated by acetic acid and potassium ferrocyanide, ...	none		none	

The precipitates produced by bromine did not settle or adhere to the sides

and continued for three hours, though, in the experiment in which pepsin was used, all the albumin had dissolved after about an hour and a half.

	With Acid alone.		With Acid and Pepsin.	
	Nitrogen.	= Proteid.	Nitrogen.	= Proteid.
Total proteid (by Kjeldahl's process on albumin used, . . .	·0986	·621	·0986	·621
Pepsin proteid (by Kjeldahl's process on pepsin used), . . .	...	...	·0106	·067
A. Unchanged albumin (filtered off),	·0722	·455	trace	trace
B. Syntonin (precipitated on neutralising and boiling), . .	·0264	·166	·0387	·244
C. Soluble proteids (by bromine in portion of filtrate from syntonin), ...	...	trace	·0704	·444
D. Peptones (by Br in filtrate from $ZnSO_4$ precipitate), . . .	...	none	·0470	·296
E. Albumoses *plus* pepsin (by difference), ...	...	trace	·0234	·147
F. Albumoses (corrected for pepsin),	...	trace	·0128	·080
Sum of proteids (exclusive of pepsin), *i.e.*, A, B, D, and F, .	·0986	·621	·0985	·620

These results show that practically no albumoses or peptones are formed under the conditions of the experiment, when no pepsin is used, the change in this case going no further than the production of syntonin. It will be observed that in the absence of pepsin the greater part of the albumose remained undissolved at the end of the three hours, whereas in the presence of pepsin solution was complete in an hour and a half, and at the end of the experiment nearly half of the original albumin had been converted into true peptone; but the amount of this product was only about four times the weight of real pepsin employed.

Enzymes of the Pancreatic Juice.

The fluid secreted by the pancreas or sweetbread is a viscid fluid of saline taste and alkaline reaction. It contains from 1 to 2 per cent. of solid matter in solution, of which the greater part is organic and consists of proteid matter. Corpuscles and other morphological elements often occur in suspension. On cooling the fluid to 0° C., a gelatinous coagulum separates.

On heating to 75° C., the pancreatic juice is strongly coagulated. If treated with excess of alcohol in the cold, it yields an abundant

of the beaker nearly so readily as usual. Hence it was found impossible to obtain perfectly clear filtrates, the residual nitrogen in which was accordingly determined by Kjeldahl's process. The proximate analysis of the sample of pepsin, as deduced from the above figures, is given on page 344.

flocculent precipitate. If this be digested with absolute alcohol, the portion consisting of albumin is coagulated, but the greater part, including the enzymes of the juice, can be dissolved out by ice-cold water, and the resultant solution yields no precipitate with acetic acid.

The pancreatic juice is precipitated by strong mineral acids, by metallic salts, and by tannin. With chlorine- or bromine-water the fresh juice gives a white precipitate, but if the liquid has been exposed to warmth for some time a red coloration is produced.

Pancreatic juice undergoes putrefaction with great facility. This change may be prevented by adding chloroform, thymol, or salicylic acid—antiseptics which do not prevent the action of the ferments of the liquid.

Until recently, the ferments of the pancreas had not attracted much attention, and the remarkable proteolytic power possessed by the juice was scarcely suspected. At least three enzymes, having different functions, are now known to exist in the pancreas, and the presence of a fourth is maintained by some observers. Thus there is (*a*) the proteolytic ferment called *trypsin*, which converts rapidly native proteids into soluble products; (*b*) the amylolytic ferment or *pancreatic diastase*, which readily hydrolyses starch to dextrin and maltose; (*c*) a milk-curdling ferment of the nature of rennin or chymosin, of which but little is known;[1] and (*d*) a ferment called *steapsin* which emulsifies and partially saponifies fats (compare page 357).

While the enzyme of the saliva possesses the power of hydrolysing starch but has no proteolytic power, and the enzyme of the gastric juice dissolves proteids without affecting amylaceous matters, the mixed ferments of the pancreas exercise both functions, and that in a very active manner. Nor is the presence of any acid or alkali essential, though trypsin is considerably more active in presence of an alkaline carbonate.

The pancreatic enzymes may be extracted by methods analogous to those employed for the preparation of pepsin from the stomach. As in the latter case, the gland is liable to contain the ferments in an incompletely-formed condition, and these "zymogenes" are very difficult of extraction.

The mixed pancreatic enzymes may be extracted from the gland by means of glycerin, and such an extract may be preserved

[1] According to F. B. Benger (*Jour. Soc. Chem. Ind.*, 1887, p. 192), the milk-curdling ferment of the pancreas differs from that of the stomach in the fact that its activity is not impaired by the presence of 3 to 4 grains per ounce of alkaline bicarbonate, whereas as little as 1 grain per ounce wil suffice to prevent coagulation of milk by the latter.

indefinitely. Heidenheim obtains a very active glycerin extract of trypsin by pounding dog's pancreas with ground glass and allowing the mixture to remain at the ordinary temperature for twenty-four hours. It is then treated with dilute acetic acid (1 per cent.) in the proportion of 1 c.c. for each gramme of pancreas employed. To each part by weight of the acid mixture there are then added ten parts by weight of glycerin, and the liquid is filtered after three days.

A useful and permanent extract may be obtained by exhausting the finely-divided pancreas with water containing about 2 per cent. of boric acid and 1 per cent. of borax. A saturated solution of common salt in water also furnishes a useful extract.

Benger's "*Liquor Pancreaticus*" is stated to be prepared by digesting fresh, fat-free, finely-minced pancreas with four times its weight of dilute alcohol (rectified spirit 1 part, water 3 parts) for several days. The liquid is then faintly acidulated with acetic acid and filtered through paper. The product is a nearly colourless liquid, with very little taste or smell other than that due to the contained alcohol. It possesses both the amylolytic and proteolytic properties of the pancreas in a highly concentrated degree.

The *pancreatin* of the United States Pharmacopœia (1890) is defined as "a mixture of the enzymes naturally existing in the pancreas of warm-blooded animals, usually obtained from the fresh pancreas of the hog." It is described as "a yellowish, yellowish-white, or greyish amorphous powder, odourless, or having a faint, peculiar, not unpleasant odour, and a somewhat meat-like taste. Slowly and almost completely soluble in water, insoluble in alcohol." The test of digestive activity is thus described :—"If there be added to 100 c.c. of tepid water contained in a flask 0·28 gramme of pancreatin and 1·5 gramme of sodium bicarbonate, and afterwards 400 c.c. of fresh cow's milk previously heated to 38° C. (= 100·4° F.), and if this mixture be maintained at the same temperature for thirty minutes, the milk should be so completely peptonised that, if a small portion of it be transferred to a test-tube and mixed with some nitric acid, no coagulation should occur." It is added that "peptonised milk, prepared in the manner just described, or even when the process is allowed to go on to the development of a very distinct, bitter flavour, should not have an odour suggestive of rancidity."

Further information on pancreatised foods will be found on page 389.

Kühne prepares a purified pancreatic tissue from the fresh pancreas of the ox or other animal. This is freed from adhering fat and connective tissue, minced, and digested in cold alcohol.

It is then separated from the alcohol and extracted with boiling ether. The adherent ether is allowed to evaporate spontaneously, when the purified tissue is obtained as a white, friable mass, which may be preserved indefinitely. It is known in Germany under the title of Kühne's *Pancreaspulver*. By digesting it at 40° C. in ten parts of water containing 0·1 per cent. of salicylic acid, an extract of great activity may be obtained.

Pancreatic Diastase.

Cohnheim prepared from pancreatic juice, by the method employed by him for the isolation of ptyalin (page 336), an enzyme of high amylolytic power but which was free from proteolytic action. Von Wittich obtained an enzyme having similar characters by thoroughly dehydrating finely-divided pancreas with strong alcohol, and subsequently digesting it for some time in absolute alcohol. The product was then macerated in absolute glycerin, the solution treated with alcohol, and the precipitate dried at a low temperature.[1]

The product was purified by washing with alcohol, solution in absolute glycerin, and reprecipitation with alcohol.

The activity of *pancreatic diastase* commences at a temperature a little above the freezing-point, and increases gradually with the temperature until 30° C. is attained. It then remains constant to about 45°, but gradually declines with further increase of temperature until it becomes inert at 65° C.

Trypsin.

For the preparation of trypsin in a comparatively pure condition, Kühne treats the infusion or extract of pancreas with a large excess of alcohol. The precipitate (constituting the "pancreatin" of earlier investigators) is dissolved in ice-cold water, and the solution treated with absolute alcohol. The precipitate is digested for some time in absolute alcohol, and then treated with water. This leaves a residue of albumin, but dissolves the trypsin and a proteid called *leucoid*, which somewhat resembles native albumin. This body is precipitated by treating the aqueous liquid with acetic acid until it amounts to one per cent. The liquid filtered is rendered slightly alkaline with caustic soda, filtered, and the filtrate concentrated at 40° C. The tyrosine which separates out is removed by filtration, and the filtrate treated with alcohol, which throws down trypsin contaminated with leucoid, peptones, tyrosine, &c. The enzyme is then purified by repeated solution in water, dialysis, and reprecipitation with alcohol.

[1] Hüfner did not succeed in eliminating trypsin by the above means, his failure being possibly due to incomplete dehydration of the tissue before treatment with glycerin.

Trypsin is very readily soluble in water, but is insoluble in strong alcohol and in absolute glycerin. The aqueous solution is not decomposed by prolonged exposure to a temperature of 40° C., but is rendered inactive at about 75° C., and on boiling is said to yield about 20 parts of albumin and 80 of antipeptone for 100 parts of trypsin originally present.

On evaporation to dryness at a moderate temperature an aqueous solution of trypsin leaves a solid residue, which is amorphous, translucent, and of a yellowish colour.

Aqueous (preferably alkaline) solutions of trypsin dissolve raw fibrin with great rapidity, perceptible action occurring almost immediately.

The activity of trypsin increases gradually with the temperature up to 50° C., and then diminishes rapidly up to 75°, when the ferment is destroyed.

The successive changes undergone by proteids under the action of trypsin, and the characters of the products are described in the sequel (page 365).

Steapsin or Piolyn.

Both the tissue of the pancreas and extracts prepared from the perfectly fresh gland possess the property of emulsifying and partially saponifying neutral fats. The action is attended with the production of free fatty acid, which appears to react with the alkali of the pancreatic secretion to form a soap, the presence of which greatly facilitates the emulsification of the remainder of the fat.

The mode of action of the pancreas in causing these changes is, however, open to question, and the actual fact is denied by some observers,[1] while others assert that no emulsive action is exerted in the process of digestion by the pancreatic juice alone, the process not occurring until that secretion becomes mixed with the bile.

The emulsive action of the pancreas is evidently due to an enzyme and not to an organised ferment, since the power is possessed by perfectly clear glycerin extracts of the organ. Further, the action on fats is almost instantaneous, and takes place in presence of such proportions of thymol and other antiseptics as entirely inhibit the action of organised ferments.

The tissues and extracts of the pancreas also effect the hydrolysis of ethyl acetate, so that their power of saponifying esters is probably general.

[1] Sir W. Roberts and F. B. Benger have been unable to satisfy themselves of the action on fats commonly attributed to pancreas and extracts therefrom; but their high authority is outweighed by the positive evidence of numerous other observers.

Chemical Changes in the Digestion of Proteids.

The changes by which native proteids are split up, with successive formation of soluble and diffusible products, commence in the stomach, continue in the duodenum, and are completed in the small intestine.

These changes are apt to be retarded or entirely prevented by the presence of certain drugs and other foreign matters, such as colouring matters and antiseptics, or "food preservatives." Hence the influence of such bodies on the processes of digestion and assimilation is a matter of considerable practical importance. When present in excessive quantity, such additions can hardly fail to retard and interfere with the digestive action; but their effect in the small proportion generally employed, is less certain, and very little authentic information exists on the subject.[1]

[1] The Select Committee on Food Products Adulteration, which sat during the sessions of 1894, 1895, and 1896, reported that in their opinion the use of antiseptics in food "is one which deserves further investigation by recognised scientific authorities, with a view to the expression of an opinion that would be regarded as authoritative." The editors of the *Lancet*, accordingly, addressed a letter to various leading members of the medical profession, asking the following questions :—

(1) Is the presence of salicylic, boric, or benzoic acid, or of "formalin" in food, in quantities sufficient to preserve it, injurious to health?
(2) Should the use of antiseptics for this purpose be forbidden by law altogether?
(3) Should legislation be brought to bear on the restriction of the amount?
(4) Should the law insist that when preservatives are used the fact should be stated on the label?

In answer to these inquiries (*Lancet*, 1897, p. 56):—

Sir Henry Thompson writes that he has long held that the addition of antiseptics was undesirable, though unable to produce evidence that any one of them had given rise to deleterious action, owing to the impossibility of isolating the precise influence of the drug. He objects strongly to the *dietetic* use of drugs, and is of opinion that the name and quantity of the antiseptic employed should be on the label, or on a paper setting forth the maker's or vendor's name.

Dr F. W. Pavy writes that he does not consider our knowledge sufficiently extended to permit of its being taken for granted that no injury is producible, though there is no evidence of injury to health. He points out that it is the vendor, and not the consumer, that is benefited. He considers that notification of the fact of antiseptics being employed, and their nature and amount, would be sufficient; any deviation from the notification should be liable to prosecution. With the public interest thus safeguarded, he thinks that advantage might be taken of the power of antiseptics in preserving articles of food.

Dr F. J. Allan points out the possibility of daily accumulation of antiseptics quite sufficient to produce a gradual lowering of the standard of health and is of opinion that the fact of an antiseptic being added, and its nature, should be required by law to be announced at the time of sale.

The effect of boric acid on digestive ferments has been recently studied by R. A. Cripps (*Analyst*, 1897, 182), whose results agree with those previously obtained by Leffmann and Beam (*Analyst*, xiii. 103), and by O. Hehner (*ibid.*, xv. 221). According to Cripps, the action of amylolytic ferments is not retarded even in presence of such an amount of boric acid as is greatly in excess of that required for the preservation of food. The actions of proteolytic ferments and of the milk-curdling ferment chymosin were not found to be sensibly retarded, even when the proportion of boric acid was as high as one per cent.

R. H. Chittenden has shown that potassium permanganate, borax, ammonia-alum, sodium salicylate, quinine, and the salts of most alkaloids act antagonistically to the pepsic ferment (compare page 329).

F. D. Simons has found pepsic digestion to be retarded or inhibited by picric acid, tropæolin OOO, and metanile-yellow, while trypsic digestion is similarly retarded by essence of cinnamon,

Dr Sims Woodhead draws attention to idiosyncrasy and cumulative effect, and dwells upon our ignorance of the action of certain drugs (*e.g.*, formalin) on food stuffs. He points out that by the use of preservatives foods of inferior quality may be "doctored." He would make the use of antiseptics illegal unless their nature and quantity be made known.

Dr T. Lauder Brunton writes that "one must remember that poisons are formed in foods by spontaneous decomposition, which may take place after purchase. The question to be decided comes to be whether antiseptics are likely to be more injurious to health than the natural products of decomposition." His own belief is that preservatives are the less injurious. His answers are: "(1) The use of antiseptics should not be forbidden by law; (2) It is doubtful whether legislation should restrict the amount, as the makers will probably use the minimum amount found sufficient; (3) The fact of preservatives being used, and their amount, should be stated on the label."

Sir W. Roberts says that "there is no reliable information available, and an inquiry is needed."

Dr W. D. Halliburton is not able to give information as to injurious effects from his experience, but quotes F. J. Allan as mentioning cases of ill-health in children due to boric acid.

Dr J. B. Bradbury thinks that "it is not necessary to forbid antiseptics, but that the amount should either be restricted, or the fact of their addition stated on the label."

Dr Whitelegge cannot speak positively, though it is clear to him that the law should insist upon a plain statement on the label if any preservative be added.

The late Sir B. W. Richardson considered that antiseptics are not only necessary at this moment, but when used in proper form and quantity, cause no injury whatever. There ought to be a license given permitting a certain fixed, and not a dangerous, quantity of antiseptic, and it ought to be stated on the label what the antiseptic is and its quantity.

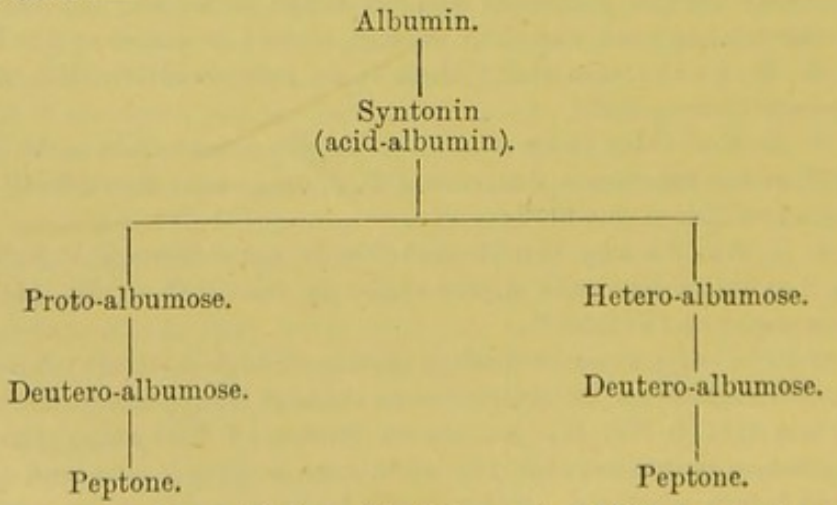
formalin, and Bismarck brown. Simons found salicylic acid and oil of wintergreen to retard peptic digestion in a less degree, while both pepsic and trypsic digestion proceeded normally in the presence of essence of peppermint, chrysoïdine, safranine or methylene-blue (*Amer. Chem. Jour.*, xix. 744).

H. A. Weber (*Amer. Chem. Jour.*, 1896, xviii. 1092; abst. *Analyst*, xxii. 39) has also studied the effect of certain coal-tar colours on pepsin- and trypsin-digestion. The ferments employed were Armour's pepsin and pancreatin, with blood-fibrin as the proteid to be digested. Experiments were made with methyl-orange, magenta, saffoline (acridine-red), and oroline or fast yellow (a mixture of sodium amido-azobenzene-disulphonate with sodium amido-azobenzene-monosulphonate). The results showed that, while none of these colours interfere with both gastric and pancreatic digestion, all of them interfere with either one or the other process and hence are very undesirable additions to food and drink.

Gastric or Pepsic Digestion.

By the action of the gastric juice or of pepsin-hydrochloric acid, proteids are first converted into acid-albumin or syntonin, and this body is changed by the further action of the ferment into the primary proteoses (proto-proteose and hetero-proteose). These bodies may then undergo further transformation, with production of secondary or deutero-proteoses. By continued action of the gastric ferment, peptones are formed.[1] This is the last stage of peptic digestion, but by the action of trypsin (the proteolytic enzyme of the pancreas) the process can be carried further.

The following is a representation of the general line of proteolysis as it occurs in the digestion of albumin by pepsin-hydrochloric acid :—



[1] According to Chandelon (*Berichte*, xvii. 2143), by treatment with nascent hydrogen peroxide egg-albumin undergoes hydrolysis, and yields much the same products as by pepsin-digestion. His results require confirmation.

In addition to the above products, a variable proportion of the original proteid is converted into an insoluble gelatinous body called *antialbumid*, which undergoes further change with difficulty.

The pepsin-digestion of other native proteids proceeds on similar lines to the above. The intermediate products are known as globuloses, myosinoses, caseoses, &c., according to the nature of the primary proteid, and may be conveniently called by the generic name of *proteoses*.

By the action of pepsin-hydrochloric acid, gelatin is converted into a body (gelatin-peptone or gelatone) very similar to albumin-peptone, but its nutritive value is open to question. Mucin, nuclein and keratin are not susceptible of pepsin-digestion. Hæmoglobin is split into acid-albumin and hæmatin, the former body then undergoing digestion while the latter remains unchanged. The albuminous envelopes of fat-cells undergo digestion in the stomach, but the fats themselves are unaltered. Starch is unaffected. Cane-sugar is stated to suffer partial inversion by the action of the mucin of the gastric juice.

Each one of the series of changes whereby native proteids undergo transformation into peptones is of a hydrolytic character. This is well shown by the following results, selected from a larger number given by R. H. Chittenden (*Digestive Proteolysis*, 1894).

	Mother Proteid.	Proto-proteose.	Hetero-proteose.	Deutero-proteose.	Peptone.
BLOOD-FIBRIN.					
Carbon, . . .	52·68	51·50	50·74	50·47	48·75[1]
Nitrogen, . . .	16·91	17·13	17·14	17·20	16·26[1]
Sulphur, . . .	1·10	0·94	1·16	0·87	0·77[1]
PARAGLOBULIN.					
Carbon, . . .	52·71	51·57	52·10	51·52	..
Nitrogen, . . .	15·85	16·09	16·08	15·94	..
EGG-ALBUMIN.					
Carbon, . . .	52·33	51·44	52·06	51·19	49·38[2]
Nitrogen, . . .	15·84	16·18	15·55	15·77	15·07[2]
Sulphur, . . .	1·81	2·00	1·63	2·02	1·10[2]
CASEIN.				α-caseose.	β-deutero-caseose.
Carbon, . . .	53·30	54·58	53·88	52·10	47·72
Nitrogen, . . .	15·91	15·80	15·67	15·51	15·97
MYOSIN.					
Carbon, . . .	52·82	52·43	..	50·97	..
Nitrogen. . . .	16·77	16·92	..	17·00	..
Sulphur, . . .	1·27	1·32	..	1·22	..
ELASTIN.					
Carbon, . . .	54·24	54·52	..	53·11	..
Nitrogen, . . .	16·70	16·96	..	16·85	..
GELATIN.					
Carbon, . . .	49·38	49·98	..	49·23	..
Nitrogen, . . .	17·97	17·86	..	17·40	..
Sulphur, . . .	0·71	0·52	..	0·51	..

[1] Amphopeptone. [2] Hemipeptone.

On examination of these figures it will be noticed that, with the curious exception of gelatin, each stage of the process of proteolysis is marked by a diminution in the percentage of carbon, and this is shown by Chittenden to be accompanied by a less distinct increase in the proportion of hydrogen. The loss of carbon appears to depend in part on the nature of the proteid itself, and probably in part upon the strength of the hydrolysing agent employed and the duration of the operation.[1]

In the proportion of sulphur there is a distinct diminution in the end-products, but in the case of nitrogen no constant difference can be traced. The results show generally that the process of gastric digestion is essentially one of hydrolysis, in which the proteid-molecule is gradually broken down or split apart into a number of simpler molecules, represented by the proteoses and peptones.

The dehydration of peptones, that is, their reversion to coagulable proteid, was apparently effected by Henninger by heating 10 parts of fibrin-peptone with 25 parts of acetic anhydride for one hour. The acid was distilled off, and the residue treated with hot water, in which the greater part dissolved. The aqueous solution was dialysed to remove the remaining acetic acid, when there remained in the dialyser a liquid which was coagulated by boiling, and was precipitated by nitric acid and by potassium ferrocyanide.[2]

By simply heating peptone to about 140° C., Henninger and Hofmeister obtained a substance the aqueous solution of which possessed many of the characters of a native proteid.

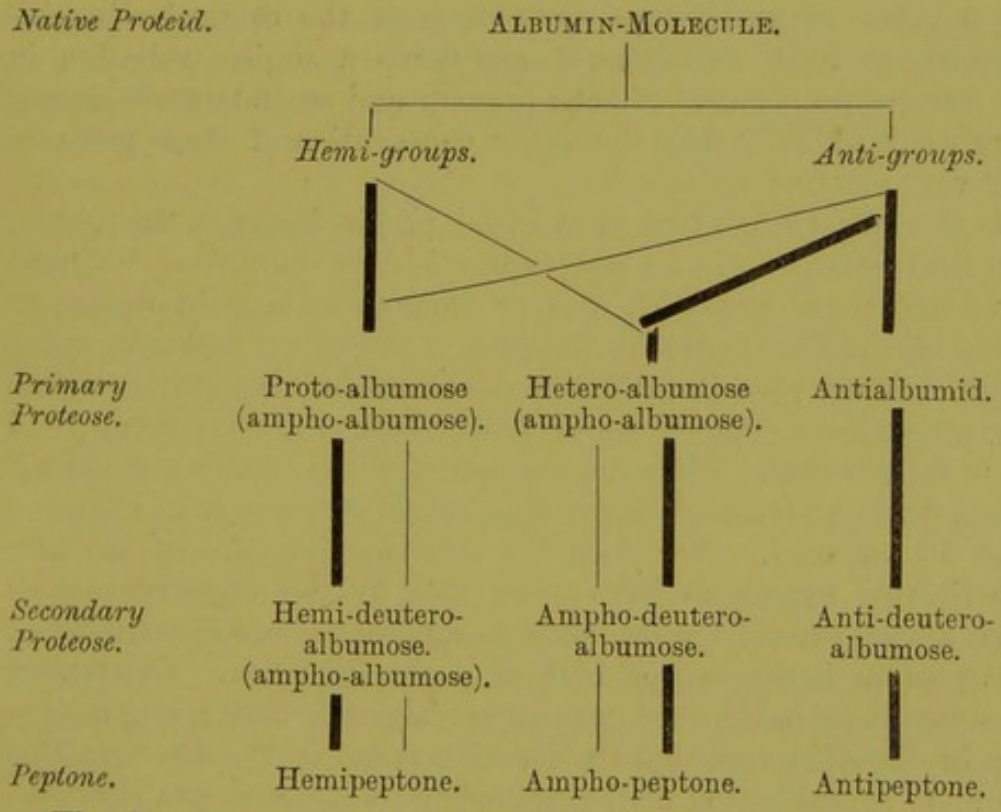
The diagram on page 360 by no means represents the whole of the known facts respecting pepsin-digestion. There is strong evidence in all the simple proteids of the presence of two distinct groups or radicals, namely the *hemi-* and the *anti*-group. These

[1] While accepting the view of Hoppe-Seyler that the process of peptonisation is one of hydrolysis, A. Gamgee considers that, in their attempts to purify and completely dry the peptones, Kühne and Chittenden adopted methods which were very liable to profoundly modify the unstable substances presented to them. "The decomposition of the barium and phospho-tungstic acid compounds with sulphuric acid, and the heroic methods employed to dry the bodies submitted for analysis, could scarcely be expected ultimately to furnish products of which the analyses would be concordant among themselves, or which would exhibit any definite relationship with the mother-substances from which they had been prepared."

[2] On the other hand, H. Schrötter states that the products are not regenerated albumin, as stated by Henninger, but simple acetyl-derivatives of albumoses. Hence he regards the assumption that peptones are products of the hydrolysis of albumin as no longer tenable.

are distinguished by their behaviour towards trypsin, the characteristic enzyme of the pancreatic juice. By this ferment, hemipeptone is split up with formation of leucine, tyrosine, and other crystallisable products of non-proteid character, while the antipeptone remains unchanged. This reaction furnishes a means of distinguishing anti-products from hemi-products, and of detecting the presence of trypsin, even in presence of pepsin.[1]

The following diagram, due to Chittenden, is similar to that on page 360, but differentiates between the hemi- and anti-groups. The thick lines indicate the main and the thin lines the subsidiary cleavages.



The diagram represents albumin as a conjugated body composed of hemialbumin and antialbumin. The first step of proteolysis converts the former into two primary albumoses—proto- and hetero-albumose—and the antialbumin into hetero-albumose and anti-albumid, the proportion of the last product being very variable. At the next stage the primary albumoses are converted into deutero-albumoses, and at the third step into the corresponding

[1] For the detection of leucine and tyrosine in the products of digestion-experiments, a portion of the fluid should be brought to a condition faintly acid to litmus, boiled, and filtered. A portion of the filtrate should be treated with a slight excess of basic lead acetate, again filtered, the lead removed by sulphuretted hydrogen and the filtered liquid concentrated to a syrup. If a drop of this liquid be placed on a slip of glass, covered, and allowed to stand

peptones. The proto-albumose has its origin mainly in the hemi-groups of the albumin-molecule, as shown by the thin line, though anti-groups are also concerned in its formation. Hetero-albumose, on the other hand, comes mainly from the anti-groups, but some hemi-groups are present. The two deutero-albumoses are both of an ampho- or mixed character, and will necessarily differ in the relative proportions of the hemi- and anti-groups they contain, while the peptones derived from them will also differ. Thus proto-albumose tends to yield an ampho-peptone in which the hemi-groups predominate, while the peptone derived from hetero-albumose contains an excess of anti-groups. Every variation in the number of anti-groups split off from the original albumin-molecule to form antialbumid represents a similar variation in the relative proportions of both primary and secondary albumoses. Hence it is evident that the inner constitution of these products may vary almost infinitely.

It is a curious fact that in artificial pepsin-digestion the conversion into peptone is in no case approximately complete. Chittenden found that, even with a large amount of active ferment, an abundance of free hydrochloric acid, a proper temperature, and a long-continued period of digestion (even five or six days), complete conversion into peptone never took place, the maximum yield being 60 per cent.; while the average of a large number of experiments made under most favourable conditions was somewhat less than 50 per cent. Nor does the deficiency of peptone in these experiments appear to have been due to the digestive action having been carried too far, since it appears to be well-established that peptone is the end-product of peptic digestion. Chittenden considers it probable that pepsin-proteolysis is only a preliminary step in digestion, and that its function is not in the direction of a complete peptonisation of the proteid-foods ingested, but is especially directed to the production of soluble products, proteoses, which can be further digested in the small intestine, or perhaps directly absorbed after they have passed the pylorus, or even from the stomach itself to a certain extent. He further concludes that the proteoses and peptones formed in the alimentary tract by pepsin-proteolysis must undergo some transformation (possibly with

for some time, crystals of tyrosine will gradually form, and spheroids of leucine may also be observed under the microscope.

Another portion of the filtrate from the coagulated proteids should be treated in the cold with excess of Millon's reagent, and filtered. The filtrate will turn red on boiling if tyrosine be present.

More detailed information respecting the detection and separation of leucine and tyrosine will be found in Vol. III. Part iii. page 299.

formation of syntonin) before reaching the blood-current, by which their peculiar physiological properties are modified.[1]

PANCREATIC OR TRYPSIC DIGESTION.

In trypsic digestion, proteids are exposed to the action of an enzyme having a much greater range of activity than pepsin, and hence the process of proteolysis as it occurs in the small intestine becomes a broader and more complex process than that in the stomach. In the case of trypsin no accessory body is necessary, a rapid transformation of insoluble proteids into soluble and diffusible products occurring by simple contact in presence of water. The primary and secondary products of pepsin-digestion are likewise subject to this change, and bodies of the simplest constitution may result from the hydrolytic changes produced by the action of trypsin. Thus hydrolysis does not stop, as in the case of pepsin-digestion, with the formation of soluble proteoses and peptones, but the hemi-portion of the latter is rapidly broken down into crystalline bodies, such as leucine, tyrosine, lysine, lysatine, &c.

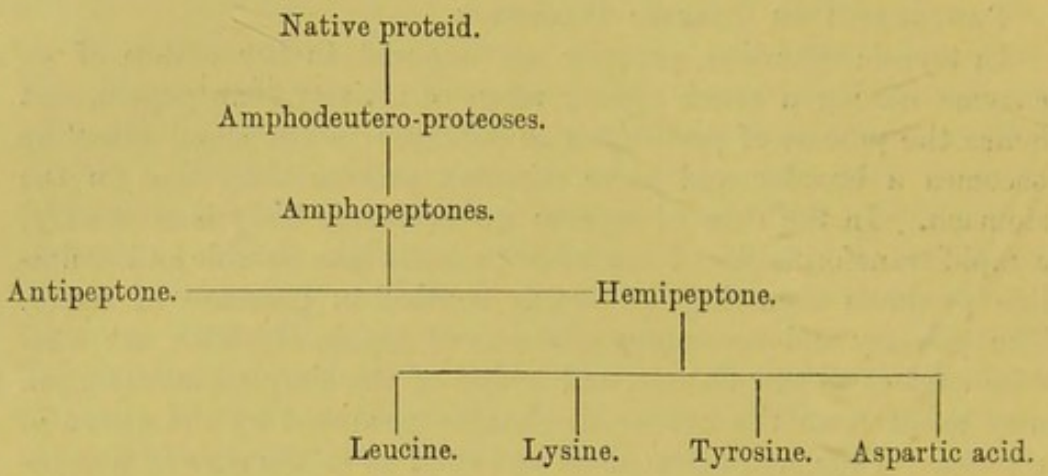
Under normal circumstances, pancreatic digestion is carried on in an alkaline medium containing from 0·5 to 1·0 per cent. of sodium carbonate, and the action of trypsin is manifested to the greatest advantage under such conditions. But trypsin will also act vigorously in a neutral fluid, and even in a fluid of faintly acid reaction, but free hydrochloric acid at once arrests trypsic digestion and destroys the ferment.[2]

In an alkaline trypsin-digestion, proto-proteoses and hetero-proteoses seldom make their appearance, the proteid matter being usually directly converted into soluble deutero-proteoses, which are then transformed by the further action of the ferment into peptones and other products. Thus the succession of changes

[1] R. H. Chittenden has pointed out (*Digestive Proteolysis*) that the proteoses and peptones formed in pepsin-digestion are more or less toxic when introduced into the blood, and they share this property with the proteoses formed by bacterial organisms, or by the enzymes to which they give rise. In other words, these primary cleavage-products of the proteid-molecule, however produced, are more or less poisonous, and, if introduced into the blood-current without undergoing previous change, may show marked physiological action.

[2] In experiments on blood-fibrin, Chittenden and Cummins found that, while a solution of trypsin containing 0·5 per cent. of sodium carbonate digested or dissolved 89 per cent. of the proteid in three to four hours at 40° C., a perfectly neutral solution of the ferment digested 76 per cent. under exactly the same conditions, and a 0·1 per cent. salicylic acid solution of the enzyme converted 43 per cent. of the proteid into soluble products.

occurring in a normal trypsic digestion may be represented as follows:—



The fact that deutero-proteoses are the primary products of trypsin-digestion shows that the natural function of trypsin is to take up the work at the point where it is left by pepsin and carry it a stage further. Trypsin is equally efficient in the digestion of all native proteids, but the products of its action are always deutero-proteoses, peptones, and crystallisable amido-compounds. But as the hemi-proteoses and peptones are those which yield the amido-compounds, the anti- varieties remaining unchanged, it follows that the peptone obtained as an end-product of a trypsin-digestion pushed to an extreme will consist substantially of *anti*-peptone. Hence when a native proteid, such as egg-albumin or fibrin, is subjected to a long-continued digestion with an alkaline solution of the pancreatic ferment, there is usually found about 50 per cent. of (anti)peptone, while the remaining 50 per cent. is represented mainly by amido-acids and other crystallisable compounds. The following analyses by Chittenden and his co-workers show the percentage composition of antipeptones free from proteoses and antialbumid, as obtained from various sources by alkaline trypsin-digestion. For comparison, the analysis of antialbumid from the digestion of myosin is also given.[1]

[1] ANTIALBUMID is a peculiar substance, which in some artificial trypsic digestions is formed in but very insignificant quantity, while in others it separates out as a gelatinous mass which may amount to nearly one-fourth of the total proteid matter. If dissolved in dilute caustic alkali, reprecipitated by neutralisation, and then again dissolved by dilute alkaline carbonate, it will yield some antipeptone by the further action of trypsin, though a considerable proportion is again liable to separate out as a gelatinous coagulum.

The amount of antialbumid formed in flask-digestions appears to be mainly dependent on the strength of the trypsin solution and the character of the

	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.
Antipeptone from blood-fibrin, ...	47·30	6·73	16·83	0·73	28·41
Antipeptone, . . .	49·59	6·92	15·79	..	..
Antipeptone from anti-albumin, ...	48·94	6·65	15·89	..	..
Antipeptone from casein, .	49·94	6·51	16·30	0·68	26·57
Antipeptone from myosin,	49·26	6·87	16·62	1·16	26·09
Antialbumid from myosin,[1]	57·48	7·67	13·94	1·32	19·59

From these data it appears that the peptones, though possibly distinct individuals, agree in their low content of carbon, and exhibit a contrast in this respect with the co-formed antialbumid.

As already stated, when either native proteids or the products of a previous pepsic digestion are subjected to pancreatic digestion for any length of time, the proteids of the hemi-group suffer further hydrolysis with formation of a series of crystallisable bodies, among the most prominent of which are the following amido-acids.

BUTALANINE. Amido-valeric acid, $\begin{cases} C_4H_7 \begin{cases} NH_2 \\ H \end{cases} \\ CO.OH \end{cases}$

LEUCINE. Amido-caproic acid, . $\begin{cases} C_5H_9 \begin{cases} NH_2 \\ H \end{cases} \\ CO.OH \end{cases}$

LYSINE. Diamido-caproic acid, . $\begin{cases} C_5H_9 \begin{cases} NH_2 \\ NH_2 \end{cases} \\ CO.OH \end{cases}$

TYROSINE. Para-hydroxyphenyl-amido-propionic acid, ... $\begin{cases} C_2H_3 \begin{cases} NH_2 \\ C_6H_4.OH \end{cases} \\ CO.OH \end{cases}$

ASPARTIC ACID. Amido-succinic acid, $\begin{cases} C_2H_3 \begin{cases} NH_2 \\ CO.OH \end{cases} \\ CO.OH \end{cases}$

GLUTAMIC ACID. Amido-pyrotartaric acid, ... $\begin{cases} C_3H_5 \begin{cases} NH_2 \\ CO.OH \end{cases} \\ CO.OH \end{cases}$

proteid undergoing digestion. If the proteid is in a fairly digestible form, and the enzyme solution reasonably active, scarcely any insoluble antialbumid may be formed.

[1] Chittenden gives the following figures in illustration of the percentage

In addition to these amido-acids, *ammonia*, *lysatine*, and *trypsophan* are normal and probably constant products of pancreatic digestion in cases where the possibility of bacterial fermentation has been rigidly excluded.

In connection with the study of the products of the decomposition of proteids, the speculations of P. W. Latham (*Croonian Lectures*, 1886) as to the constitution of albumin are of interest. Commencing with the fact that by the reaction of hydrocyanic acid on aldehyde, cyan-ethylic alcohol $CH_3.CH(CN).OH$ is formed, he points out that the cyan-alcohols are, as a class, very unstable bodies readily undergoing change, and when treated with ammonia form unstable cyan-amides, which easily undergo condensation with formation of imido-nitriles and elimination of ammonia. Latham thinks these facts suggest the enquiry: "Have we not in these cyanogen compounds substances possessing some properties that belong to living tissue, namely, those of undergoing intramolecular change and also condensation? And, further, if from these substances we can obtain the various products which result from the disintegration of albumin, may not albumin itself be simply a compound made up of these elements?"

In the laboratory, it is practicable to obtain from these cyan-alcohols the corresponding amido-acids, glycocine, leucine, etc.,[1] and all the acids of the acetic and lactic series. Latham suggests that, if it were found possible to reverse the process, albumin might be built up theoretically from such constituents.

Latham further instances the well-known molecular transposition by which ammonium cyanate is converted into urea, and quotes Pflüger's remark that the great molecular energy of cyanogen

composition of antialbumid produced by the action of dilute sulphuric acid at 100° C. on egg- and serum-albumin respectively.

	Carbon.	Hydrogen.	Nitrogen.
Antialbumid from egg-albumin, . .	53·79	7·08	14·55
Antialbumid from serum-albumin, . .	54·51	7·27	14·31

[1] $C_4H_9.CHO + HCN = C_4H_9.CH\begin{cases} CN \\ OH \end{cases}$

Valeric aldehyde. Pentyl cyan-alcohol.

$C_4H_9.CH\begin{cases} CN \\ OH \end{cases} + NH_3 = C_4H_9.CH\begin{cases} CN \\ NH_2 \end{cases} + H_2O$

Pentyl cyan-alcohol. Pentyl cyan-amide.

$C_4H_9.CH\begin{cases} CN \\ NH_2 \end{cases} + 2H_2O = C_4H_9.CH\begin{cases} COOH \\ NH_2 \end{cases} + NH_3$

Pentyl cyan-amide. Leucine.

compounds, suggests that the functional metabolism of protoplasm by which energy is set free, may be compared to the conversion of the energetic unstable cyanogen compounds into the less energetic and more stable amides. In other words, that ammonium cyanate is a type of living and urea of dead nitrogen, and that the conversion of the former into the latter is an image of the essential change which takes place when a living proteid dies.

In accordance with this view the group —CO.NH— is dead nitrogen and would, on becoming part of a living tissue, be transformed into =C:N.OH.

Cyanic acid, CNOH, is itself readily susceptible of polymerisation, with formation of cyamelide, $C_3H_3N_3O_3$.

Latham points out that the glycines are capable of uniting with each other, glycocine being not improbably glycocine amidoacetate, $H_3N\left\{\begin{matrix}O.CO.CH_2\\CH_2.CO.O\end{matrix}\right\}NH_3$; and that by the dehydration of this and allied bodies, cyan-alcohols might be formed.

Thus, a double molecule of glycocine by dehydration would give a substance which might contain either a CO.NH group or a C:N.OH group according to the arrangement of the atoms in the molecule.

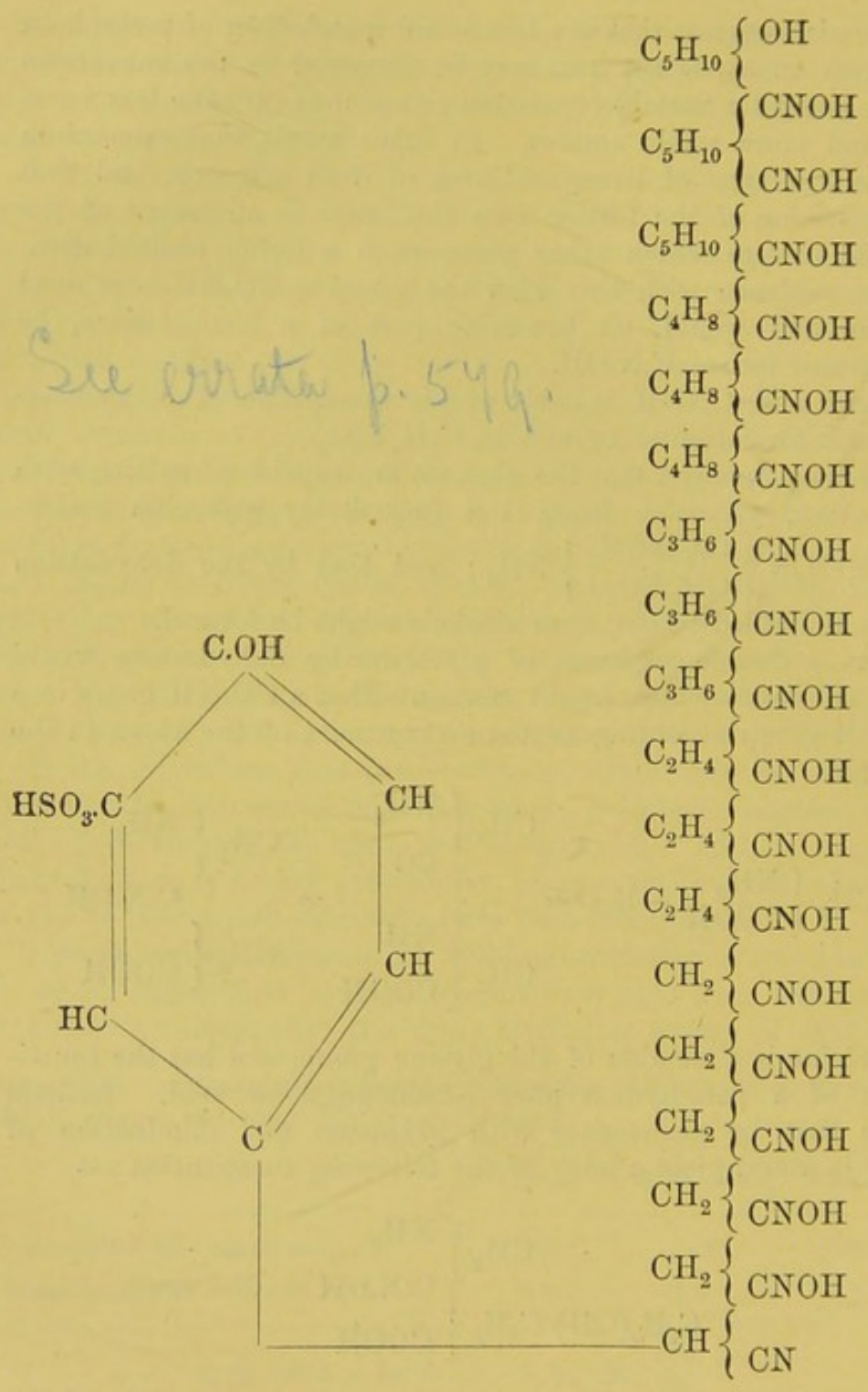
$$2CH_2\left\{\begin{matrix}NH_2\\COOH\end{matrix}\right. - H_2O = \begin{matrix}CH_2\left\{\begin{matrix}NH_2\\CO\end{matrix}\right.\\\\CH_2\left\{\begin{matrix}NH\\COOH\end{matrix}\right.\end{matrix} = \begin{matrix}CH_2\left\{\begin{matrix}NH_2\\\end{matrix}\right.\\C:N.OH\\CH_2\left\{\begin{matrix}\\COOH\end{matrix}\right.\end{matrix}$$

Tyrosine is a member of the glycine group, and has the constitution of a para-hydroxyphenyl-amidopropionic acid. Latham shows that by coalescence with glycocine and elimination of water it would yield a body of the following constitution :—

$$C_6H_4(OH)\,C_2H_3\left\{\begin{matrix}CH_2\left\{\begin{matrix}NH_2\\\end{matrix}\right.\\C:N.OH\\\left\{\begin{matrix}\\COOH\end{matrix}\right.\end{matrix}\right.$$

By similar ingenious reasoning Latham shows how such a body as taurine may be introduced into the molecule, and he finally builds up the following suggestive formula as possibly representing the constitution of albumin.

This formula contains $C_{72}H_{118}N_{18}O_{22}S$, and differs only by H_6 from Lieberkühn's formula for albumin, $C_{72}H_{112}N_{18}O_{22}S$.



The discovery of lysine, lysatine, and trypsophan or protein-chromogen (page 382) necessitates some modification of the above formula, which, of course, cannot be regarded as more than an ingenious and highly suggestive arrangement, which might be of service as a working hypothesis.

Characters and Separation of Digestion-Products.

The processes of pepsic and pancreatic digestion by soluble ferments must be clearly distinguished from that of bacterial decomposition, though both modes of change go on simultaneously in the intestinal canal, and some of the products are common to both kinds of fermentation. The bodies described on the following pages are unquestionably obtainable by enzyme-proteolysis under conditions which preclude any possibility of bacterial action.[1]

The separation of the various proteid-products formed in pepsin-digestion can be effected according to the principles of the tabular scheme on page 18. The following method, which is practically that prescribed by R. H. Chittenden, gives some additional working details :—

The liquid is strained through muslin to remove *undissolved matters*, and then boiled to coagulate unchanged *albumin* and prevent further action of the pepsin. The liquid, previously filtered if requisite, is then rendered exactly neutral to litmus, and the precipitated *syntonin* filtered off. The filtrate is then concentrated over the water-bath, and portions tested for *primary proteoses*. If these products be present, the neutral liquid will yield a more or less heavy precipitate on saturation with common salt. If primary proteoses be present in notable amount, the addition of nitric acid drop by drop to the neutralised fluid will produce a white precipitate, readily soluble on application of heat, but reappearing as the solution cools. If primary proteoses are entirely absent, no precipitate will be obtained by acid unless the liquid is saturated with common salt, in which case a portion of the deutero-proteose will be precipitated.

A similar method, which may be very simply and conveniently applied to the separation of the main products of pepsin-digestion, is described on page 351. See also a valuable paper on "The

[1] The process and the products of the bacterial decomposition of proteids are described in Vol. III. Part iii. pp. 322 *et seq.* It is characterised by the formation of indole, skatole, and phenols. Bacterial proteolysis occurring *under normal conditions* in the small intestine is distinguished from ordinary putrefaction by the entire absence of ptomaines from the contents of the intestine, even when a period of twenty-four hours has elapsed since death.

In experiments on trypsic digestion, especially when the process is prolonged, it is highly essential to avoid error and complication of the phenomena by the simultaneous occurrence of bacterial fermentation. This can be completely and readily prevented by the addition of a suitable antiseptic, such as salicylic acid or thymol. These, while preventing the development of putrefactive germs, and therefore of putrefaction, exert no inhibitory action on the enzymes of the pancreas.

Analysis of the Gastric Contents," by H. F. Hewes (*Amer. Jour. Pharm.*, 1898, page 25).

PROTEOSES.

As a class, the proteoses are characterised by far readier solubility in water than native proteids, by their much higher diffusibility, and by not being coagulated either by heat or alcohol, although they are *precipitated* by the latter agent. A peculiarity which is of service for the identification of proteoses is their extreme sensitiveness to increase of temperature, all proteose-precipitates tending to dissolve as the liquid is warmed and reappearing on cooling. The primary proteoses are precipitated by excess of picric acid, by potassium ferrocyanide and acetic acid, and by cupric sulphate, whereas deutero-proteoses are practically unaffected by these reagents.

The two primary proteoses are distinguished by differences of solubility. Thus, *proto-proteose* is readily soluble in pure water, while *hetero-proteose* is soluble only in salt solutions, dilute acids and alkalies. Hence, when the two proteoses are precipitated together by saturating the liquid with common salt, they may be readily separated by dissolving the precipitate in a little dilute salt solution, and then dialysing the solution in running water until the salt is entirely removed. *Hetero-proteose* will be precipitated, and may be filtered off, while *proto-proteose* remains in solution.

By prolonged contact with water, and even with concentrated salt solution, hetero-proteose tends to change into a semi-coagulated substance called *dysproteose*, which is insoluble in a dilute solution of common salt. Dysproteose can be reconverted into hetero-proteose, in part at least, by solution in dilute acid or alkali, and reprecipitation by neutralisation.

In the absence of peptones, *deutero-proteose* is best separated from primary proteoses by neutralising the liquid as nearly as possible, and then, after suitable concentration, saturating it with common salt. The precipitated primary proteoses are removed by filtration, and to the filtrate acetic acid saturated with common salt is added, drop by drop, as long as a precipitate continues to form. The precipitate consists of the primary proteoses not previously thrown down, together with a certain amount of deutero-proteose. From the filtrate, the remaining deutero-proteose can be obtained, unmixed with primary proteoses, by dialysing away the salt and acid, concentrating the fluid, and adding a large excess of alcohol.[1]

[1] In order to obviate the difficulty of removing large quantities of sodium chloride or ammonium sulphate by dialysis, and to avoid the actual loss of substance entailed in the separation of proteoses by Kühne's process, S. Fränkel

In presence of peptones, the proteoses must first be thrown down together by saturating the liquid with ammonium sulphate.[1] The precipitate is washed with a saturated solution of the precipitant, and dissolved in water for the separation of the proteoses. Kühne has pointed out that in order to effect the precipitation of the last traces of deutero-proteose, the liquid (saturated with ammonium sulphate) must be boiled for some time, and even then precipitation is seldom complete unless the reaction is made successively neutral, acid, and alkaline ; and the heating continued for some time after each change in reaction. Under these conditions, the last portions of deutero-proteose separate on the surface of the liquid as a gummy or oily mass, while any true peptone remains wholly unprecipitated.

For the separation of ampho-peptone from the filtrate,[2] Chittenden

proposes to make use of cupric sulphate (*Monatshaft f. Chemie.*, 1897, 433). This reagent, though it does not cause the slightest turbidity in solutions of pure deutero-albumose, gives a voluminous precipitate with solutions of 1 : 500, and a turbidity with solutions of 1 : 1000 of deutero-albumose contaminated with proto-albumose. Experiments were conducted with Witte's salt-free "peptone," with Finzelburg's "albumin and meat-peptone," and also with pepsin- and trypsin-peptones of Fränkel's own preparation.

A dilute solution of cupric sulphate was added to the solution of the peptone, when a tough coherent precipitate formed, leaving a turbidity which generally disappeared after a few hours' standing. Attempts to separate the copper from the solution by hydrogen sulphide or magnesium failed, but success was attained with barium ferrocyanide. A hot saturated solution of this salt was added until a filtered portion of the proteose solution only showed traces of copper. Before absolutely the whole of the copper was removed, the mixture was acidulated with acetic acid, warmed, filtered, and the filter washed. Solution of barium ferrocyanide was then added drop by drop to the warm filtrate as long as a red precipitate formed ; then barium acetate to remove sulphuric acid. With practice it was found easy to hit the exact point when all the cupric sulphate was removed. The solution was evaporated to a small bulk, poured into strong alcohol, dehydrated with absolute alcohol, and washed with ether.

Deutero-proteose prepared in this way gave no turbidity or precipitate with sodium chloride alone, but on the addition of acetic acid partial precipitation ensued ; ammonium sulphate also yielded an abundant precipitate. It gave neither turbidity nor precipitate with potassium ferrocyanide and acetic acid, nor with copper sulphate, and was therefore free from proto-albumose. Experiment showed that the method was suitable for the isolation of the deutero-albumose obtained in trypsin-digestions.

[1] The proposal of Bömer to employ zinc sulphate in place of ammonium sulphate has much to recommend it. It is fully discussed on page 322.

[2] H. Schrötter (*Monatsh.*, 1895, xvi. 609) states that both peptones and albumoses are precipitated by ammonium sulphate, which is therefore useless for their separation, but that, on the other hand, albumoses may be

recommends that the liquid should be concentrated somewhat, and set aside in a cool place for the crystallisation of a portion of the ammonium sulphate. The liquid drained from the crystals is then mixed with about one-fifth of its measure of alcohol, and allowed to stand for some time, when it separates into two layers—an upper one rich in alcohol, and a lower one rich in salts. The latter is tapped off and again treated with alcohol, by which a further separation of the salts is effected. The alcoholic strata containing the peptones are then united and exposed to a low temperature, to promote the crystallisation of the salt. The crystals are separated, the liquid concentrated, a little water added, and then boiled with barium carbonate until entirely free from ammonium sulphate. The liquid is filtered, any baryta removed by cautious addition of dilute sulphuric acid, the filtrate concentrated almost to a syrup, and poured into absolute alcohol.

Peptones.

The foregoing process suffices for the preparation of amphopeptone from the products of pepsic digestion. For the preparation of antipeptone from the products of trypsic digestion, the following method was employed by Kühne and Chittenden. Well-washed blood-fibrin was purified by boiling it in succession with water and alcohol, and then extracting it with ether. 300 grammes of the dry product thus obtained was again boiled with water, the excess of moisture expressed by the hand, and the moist substance treated with three litres of water containing 0·25 per cent. of caustic soda and 0·5 per cent. of thymol. An infusion obtained from 88 grammes of purified pancreas by digestion with

perfectly differentiated from peptones by their large percentage of nitrogen, their high molecular weight, and by the important fact that they contain sulphur, whereas peptones contain none. Further, according to Schrötter, the generally accepted view that, by the action of ferments or acids, albumin is first converted into albumoses and then into true peptones, is incorrect, the fact being that when albumin is heated with an acid a direct conversion into peptones, without the formation of albumoses, takes place. This view is stated to be confirmed by the fact that, when treated with an acid, albumoses are in a great measure decomposed, and give rise to no peptones.

Seeing that the essential feature of Kühne's method of separating albumoses from peptones consists in the non-precipitation of the latter by saturating the liquid with ammonium sulphate, it is difficult to see how Schrötter's position can be maintained.

Schrötter has described an albumose, prepared from Witte's commercial "peptone," which is soluble in and crystallises from alcohol, is practically ashless, and furnishes a hydrochloride of constant composition (*Monatsh.*, xiv. 612; abst. *Jour. Chem. Soc.*, 1894, i. 215).

thymol and caustic soda[1] was then added, and the whole digested at 40° C. for six days. Nearly the whole of the fibrin dissolved during the first day, but a little remained undissolved and floated on the surface of the liquid. The digested liquid was then slightly acidulated with acetic acid, boiled, filtered through cloth, and the filtrate concentrated to about a litre. The brownish syrup was drained off from the crystalline deposit of tyrosine and leucine which was formed on cooling, and alcohol added until the peptone began to precipitate. The liquid was then boiled to re-dissolve the precipitate, and set aside to crystallise. The filtrate from the second crop of crystals, now tolerably free from leucine, tyrosine, &c., was saturated with ammonium sulphate, which precipitated any albumose, the trypsin, and various accidental impurities. The filtrate from the precipitate formed by ammonium sulphate was then treated as in the preparation of amphopeptone (page 373). By the foregoing process, Kühne and Chittenden obtained a yield of 120 grammes of antipeptone (dried at 105° C.) from 300 grammes of purified fibrin.

Whatever their original source, peptones when purified possess very similar characters. When freshly-prepared, and in a still moist condition, a pure peptone forms a white amorphous substance resembling casein, but on heating to 80° or 90° C. it fuses to a pasty mass, which solidifies on cooling. When thoroughly dry, peptones form white or yellowish friable solids, which are extremely hygroscopic and dissolve in water with a hissing sound and considerable rise of temperature. Peptones are insoluble in ether, chloroform, or hydrocarbons. They are also insoluble in absolute alcohol, but dissolve in aqueous alcohol in greater proportion as the dilution increases.[2]

In aqueous solution, the peptones are neutral to litmus, lævorotatory, and diffusible through animal membrane with much greater facility than other proteids, but far less readily than salts. The diffusibility of peptones through parchment-paper occurs less

[1] 100 grammes of Kühne's powdered pancreas (page 356) is digested at 40° C., for twelve hours, with 500 c.c. of water containing 0·1 per cent. of salicylic acid, and the mixture filtered through gauze, neutralised with soda, and sufficient excess of soda added to bring the alkalinity to 0·25 per cent. of NaHO. The residue is suspended in 500 c.c. of water containing 0·25 per cent. of caustic soda and 0·5 per cent. of thymol, and again digested for twelve hours at 40° C. The residual matter consisting of the nuclein, collagen and undissolved elastin of the pancreas is drained off, pressed, and the filtered liquid added to the salicylic acid extract.

[2] According to A. Tscheppe (*Pharm. Jour.*, [3], xxi. 743), the presence of free acid or alkali prevents the precipitation of peptones by excess of alcohol.

readily than through animal membrane, and while much greater than that of other proteids is very small absolutely. This fact may be utilised to separate peptones from large admixtures of salts. Solutions of pure peptones are not precipitated by boiling nor by any mineral acid, nor are they affected by potassium ferrocyanide, either in presence or absence of acetic acid.[1] Neutral and basic lead acetates, ferric chloride, and cupric sulphate (5 per cent. solution) are similarly without action on peptones, or produce only insignificant turbidities, due in all probability to traces of other proteids present as impurities.

Peptones form no insoluble compounds with oxide of iron. The so-called iron "peptonates" are merely albuminates (Tscheppe). Stutzer's cupric hydroxide reagent has been employed as a precipitant of peptones, but it has been shown (by Szymanski, Weiske, and others) to effect a very imperfect separation.

Potassio-iodide of bismuth has been recommended by Dutto as a precipitant of peptones.

Peptones are precipitated by tannin, mercurous and mercuric nitrates, mercuric chloride, and potassio-mercuric iodide, but the perfection of the separation is impaired in some cases by the presence of dilute acid, alkali, and even of neutral salts (compare footnote on page 328).

L. A. Hallopeau (*Compt. rend.*, cxv. 356) has described a method of determining peptones based on their precipitation by an excess of mercuric nitrate. The solution of the peptone, previously freed from other proteids, is brought to a neutral or very faintly acid reaction, and treated with an equal measure of a solution of mercuric nitrate having a strength of from 10 to 15 per cent., and in which any free acid has been previously neutralised by cautious addition of sodium carbonate. The mercuric compound of the peptone forms a white, flocculent, voluminous precipitate, which is allowed to stand for twenty-four hours. The liquid is then passed through a tared filter, and the precipitate washed with cold water till the washings cease to darken on treatment with sulphuretted hydrogen. The filter and its contents are dried at 106° to 108° C. and weighed. The weight of the precipitate multiplied by 0·666 gives that of the corresponding peptone. Any notable quantity of chlorides interfere with the above process, by converting the mercuric nitrate into chloride, which reagent Hallopeau finds to precipitate peptones very imperfectly: hence

[1] Kühne and Chittenden find that, on first adding potassium ferrocyanide to the solution of a peptone, the liquid remains perfectly clear, but some opalescence is ultimately produced, however carefully the substance may have been purified.

mercuric nitrate must be employed in considerable excess over that required to react with the chlorides present.

When added to a solution of ampho-peptone, Millon's reagent (page 11) gives a voluminous white precipitate, which acquires a fine red colour on warming the liquid. With antipeptone it yields a white precipitate, changing to a dirty yellow or reddish colour on heating. The bright red coloration with Millon's reagent is also produced by tyrosine, which is probably formed in the reaction from ampho-peptone. Antipeptone, on the other hand, yields little or no tyrosine by the action of mineral acids and other powerful reagents, and hence gives no pronounced red colour with Millon's reagent.

Peptones are precipitated from their slightly acidulated solutions by phospho-molybdic and phospho-tungstic acids, but discrepant statements are made as to the perfection of the precipitation and the influence of a large quantity of free acid.[1] In the author's opinion, the value of these reagents for the separation of peptones has been over-rated.

In common with other forms of proteid and gelatinoid matters, peptones are completely precipitated from their acidulated aqueous solutions by excess of bromine-water (page 315).

As generally prepared, peptones possess an intensely bitter taste, but this character is due to the presence of some bye-product.[2]

The most characteristic reaction of the peptones is that with the biuret test (page 12). This is usually applied by adding to the solution a few drops of a very dilute solution of copper sulphate, and then a large excess of a concentrated solution of caustic potash or soda. In presence of a peptone a well-marked rose coloration is produced, free from the violet tint produced by other proteids (except albumoses). But if the copper solution be added in excess a violet coloration will result when only pep-

[1] According to Schützenberger (*Compt. rend.*, cxv. 764), phospho-tungstic acid, free from alkalies, precipitates only about half the fibrin-peptone prepared from horse-blood. The fraction precipitated contains a smaller proportion of oxygen than that not precipitated, which contains the excess of oxygen in the form of hydroxyl-groups. Schützenberger regards fibrin itself as a compound ether hydrolysable by pepsin, yielding two products which are both ureides. He considers that several homologous peptones exist.

[2] This bitter substance appears to be a constant product of gastric digestion, and is also developed in some cases in the later stages of pancreatic digestion of proteids. It is evidently a normal product of the digestion of proteids, and its presence probably accounts for the bitter flavour of the eructations complained of by dyspeptic persons and commonly attributed to the regurgitation of bile.

tones are present. This also occurs in presence of ammonia, unless the excess of fixed caustic alkali present is *very* large.

In the experience of the author, a preferable method of applying the biuret test is to add a large excess of caustic soda to the liquid, and then add a drop of diluted Fehling's copper solution.

According to J. A. M'William (*Brit. Med. Jour.*, 1892, i. 115), a very delicate test for peptones is to saturate the liquid with ammonium sulphate (filter from any albumoses, &c.), and add salicyl-sulphonic acid (sulpho-salicylic acid). A precipitate is obtained which readily dissolves on heating the solution, or on addition of a little water or glycerin.

Peptones are capable of combining both with metallic oxides and with hydrochloric acid. From the general mode of preparation of the peptones, and the stable character of their hydrochlorides, it appears probable that the products commonly described as peptones have been in many cases their *salts*.

C. Paal (*Ber.*, xxvii. 1827) has prepared a number of *peptone hydrochlorides* by the action of hydrochloric acid on egg-albumen under various conditions of concentration, temperature, &c. The products are colourless or pale yellow, brittle, amorphous, and extremely hygroscopic, the affinity for water increasing with the proportion of hydrochloric acid. They are miscible with water in all proportions, soluble in glacial acetic acid, and sparingly soluble in phenol. In the alcohols their solubility varies inversely with the molecular weight of the solvent. Peptone hydrochlorides are unaltered at 130° C., and have a sour cheesy flavour, with a bitter after-taste. They yield the biuret, xantho-protein, and Millon's reactions, are incompletely precipitated from aqueous solutions by phospho-tungstic acid, and yield soluble double salts with mercuric chloride. That they are true peptones is shown by the fact that little or no precipitate is produced by saturating their solutions with ammonium sulphate, and that the subsequent addition of an acid or alkali occasions no change. The aqueous solutions redden litmus, but no hydrochloric acid is lost by repeated evaporation of the liquid. The chlorine is only partially removed by silver nitrate.

Paal prepared the free peptones from their hydrochlorides by adding a slight excess of silver sulphate, filtering from silver chloride, removing the excess of silver by sulphuretted hydrogen, and the sulphuric acid by an exactly sufficient amount of baryta-water. The products amounted to about 80 per cent. of the theoretical yields. The free peptones thus obtained gave the usual reactions, were less hygroscopic than the salts, sparingly soluble in methyl alcohol, and almost insoluble in ethyl alcohol. Various

metallic peptonates were obtained. From the products of the gastric digestion of albumin, Paal also obtained peptones which in their general properties agreed with those resulting from the action of hydrochloric acid.

Paal determined the molecular weights of the peptones and their hydrochlorides by the boiling-point and cryoscopic methods, with results showing that the free peptones have a molecular weight of about 400, and that in the hydrochlorides one molecule of peptone is combined with one of hydrochloric acid.

The composition and characters of commercial peptones are described on page 383 *et seq.*

AMIDO-COMPOUNDS AND BASES FORMED IN DIGESTIVE PROTEOLYSIS.

The chief compounds of amido or basic character resulting from the cleavage of proteid matters by digestive enzymes are enumerated and formulated on page 367.

The properties and methods of isolating leucine, tyrosine, and aspartic and glutamic acids have already been fully described (Vol. III. Part iii. pp. 211–227). Ammonia requires no special description.

LYSINE, $C_6H_{14}N_2O_2$, is an interesting base discovered, together with lysatine, a few years since by E. Drechsel in the products of the action of hydrochloric acid and stannous chloride on casein. E. Fischer subsequently separated these bases from the products of the decomposition of gelatin, and Siegfried from those of the action of hydrochloric acid in presence of stannous chloride on conglutin, gluten-fibrin, hemiprotein, and egg-albumin. From these modes of formation it might be inferred that the bodies in question were direct products of the hydrolytic cleavage of the proteid-molecule, and hence that they would be formed also in trypsic digestions. Hedin has proved the correctness of this assumption, and has shown that the amount of these bases thus formed is not inconsiderable. Thus, from the trypsic digestion of 3000 grammes of moist blood-fibrin he obtained 28 grammes of pure lysine platinichloride, together with sufficient lysatine to establish its identity.

Lysine has the constitution of a diamido-caproic acid, and may hence be regarded as an amido-leucine. It is homologous with diamido-valeric acid or ornithine $(NH_2)_2C_4H_7.COOH$, a base of which the benzoyl-derivative ornithuric acid, replaces hippuric acid in the urinary excrement of birds (compare Vol. III. Part iii. page 386).

Lysine is a powerful base, absorbing carbon dioxide from the air. It forms a crystalline sulphate and two crystallisable hydrochlorides, the latter salts containing respectively B,HCl and B_2,HCl.

Lysine in solution is dextro-rotatory, but when heated to 150° with baryta, it is said to be converted, without decomposition, into an optically inactive isomer, which forms a platinichloride which, according to Siegfried, crystallises free from water or alcohol and contains B,H_2PtCl_6.

Lysatine, or Lysatinine, $C_6H_{13}N_3O_2$, is a base allied to creatine (Vol. III. Part iii. page 285), with which body or with creatinine it appears to be homologous.[1] It forms a double salt with silver nitrate, having the composition $C_6H_{13}N_3O_2,HNO_3,AgNO_3$. By boiling this salt with baryta-water, Drechsel obtained about 10 per cent. of urea nitrate. This reaction is similar to that by which creatine yields sarcosine and urea, and is of special interest as establishing for the first time the direct line of cleavage along which proteids can be split up with formation of urea. Drechsel estimates that about one-ninth of the urea daily excreted may come from the direct decomposition of lysatine formed in pancreatic digestion.

For the preparation of lysine, Drechsel and Krüger (*Ber.*, 1892, p. 2452) employ a modification of the process of Hlasiwetz and Habermann, which consists in boiling casein with ordinary hydrochloric acid and stannous chloride for several days in a flask furnished with a reflux condenser. They add metallic zinc to keep up a constant evolution of hydrogen, and the apparatus is arranged to exclude atmospheric air. When the decomposition of the proteid is complete, the liquid is diluted and the tin precipitated by sulphuretted hydrogen. The filtrate is concentrated to its original bulk, and treated while hot with a hot saturated solution of crystallised phospho-tungstic acid. This is stated to precipitate all the bases, while the amido-acids are unaffected. The precipitate is filtered off and washed free from chlorides by water containing 5 per cent. of sulphuric and the same proportion of phospho-tungstic acid. It is then suspended in boiling water, and decomposed by a slight excess of baryta-water. The liquid is boiled to expel ammonia, filtered, and the excess of barium exactly precipitated by sulphuric acid. The filtrate is acidulated with hydrochloric acid and concentrated to a syrup on the water-bath. This is dissolved in proof-spirit, and the (filtered) solution treated with an excess of an alcoholic solution of platinic chloride. The precipitate of potassium platinichloride is filtered off and the filtrate treated with more alcohol, which

[1] It is uncertain whether the formula of lysatine should be written $C_6H_{13}N_3O_2$, or $C_6H_{11}N_3O+H_2O$. If the former be correct the base is analogous to creatine and will be appropriately called *lysatine*. If the latter formula be adopted the analogy to creatinine renders *lysatinine* the more appropriate name.

precipitates the platinum salt of lysine, the corresponding compound of lysatine remaining in solution. On repeated recrystallisation, lysine platinichloride is obtained in fine yellow needles containing $C_6H_{14}N_2O_2,H_2PtCl_6 + C_2H_6O$. This is dissolved in water, the solution boiled to expel alcohol, the platinum precipitated by sulphuretted hydrogen and the acid solution of lysine dihydrochloride thus obtained evaporated to the crystallising point. The resultant salt is readily soluble in water and dilute spirit, but insoluble in absolute alcohol. If boiled with freshly-precipitated lead hydroxide and water, the free base is obtained in solution; or the hydrochloride may be heated on the water-bath with an equivalent quantity of sulphuric acid, the resultant sulphate exactly decomposed by baryta-water, and the filtered liquid evaporated to the crystallising point. The crystals contain $2C_6H_{14}N_2O_2+CO_2$, but this compound loses its CO_2 when heated at 110° C. in a current of air free from carbon dioxide.

Lysine may be isolated from the products of the decomposition of casein by hydrochloric acid by converting it into its dibenzoyl-compound lysuric acid. This is then purified by the recrystallisation of its acid barium salt, $2C_6H_{12}Bz_2N_2O_2+Ba(C_6H_{11}Bz_2N_2O_2)_2$. From this pure lysine can be readily obtained.

Lysatine was first isolated by Drechsel from the mother-liquor from which lysine platinichloride had been separated (see above). It may be obtained from this liquid by diluting it largely with water, and distilling it *in vacuo*, to get rid of alcohol and ether. The platinum in the aqueous solution is precipitated by sulphuretted hydrogen, and the filtrate heated on the water-bath to drive off free hydrochloric acid. The liquid is then evaporated to the consistency of a syrup, diluted with water, and a strong solution of silver nitrate added from a burette in amount exactly sufficient to combine with the chlorides present. The filtrate and washings from the silver chloride are again evaporated to a syrup, and the same volume of silver nitrate solution added as was previously used. Five or six volumes of alcohol and some ether are added to this liquid, when it becomes very turbid. As the turbidity disappears, a thick oil separates out at the bottom of the liquid. The clear mother-liquor is decanted off, and treated with ether in small quantities at a time, until crystals, which adhere to the sides of the vessel, separate. A large excess of ether is now added, and the mixture set aside in a cool place over night. Snow-white needles and flakes of the silver nitrate compound of lysatine will then be found to have separated. This may be recrystallised from a small quantity of water to which alcohol and ether are added, when the compound is obtained perfectly pure.

According to Siegfried, lysine and lysatine may be readily separated from the precipitate produced when phospho-tungstic acid is added to the products of decomposition of the proteids. The precipitate, freed from chlorine, is dissolved (almost completely) in boiling water, and decomposed by the addition of a very slight excess of baryta-water. The filtrate from the barium precipitate is saturated with carbon dioxide, boiled for half-an-hour, and filtered. When cold, silver nitrate solution is added until no more precipitate is produced. The bulky precipitate formed is separated and well washed with water. The filtrate is evaporated to a thin syrup, and treated gradually with small quantities of absolute alcohol, which gives a dense semi-crystalline precipitate, containing lysine, which may be isolated by decomposing the precipitate by sulphuretted hydrogen, concentrating the filtrate, treating it with platinic chloride, and then with alcohol and ether as described above. The filtrate from the lysine silver-nitrate precipitate may be treated with more alcohol when a fine crystalline precipitate of the lysatine silver-nitrate compound is obtained.

Trypsophan, or Protein Chromogen, is an interesting body which is not only produced in trypsic digestion but is a constant product of the breaking down of proteids. It is presumably derived from the hemi-portion of the proteid. Its most characteristic reaction is the production of *proteinochrome*, a substance of an intense violet colour. This is produced whenever trypsophan is brought in contact with bromine or chlorine, and hence has been termed the "bromine-body." The colouring matter is readily formed by adding bromine-water to a liquid containing trypsophan, and can be separated from proteoses and peptones by solution in 90 per cent. alcohol. It is also soluble in ether, the absorption-spectrum of the ethereal solution showing a well-marked band in the green. Two specimens of the bromine-compound were found by Stadelmann to have the following composition :—

	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.	Bromine.
I., . .	49·00	5·28	10·99	3·77	11·01	19·95
II., . .	48·12	5·09	11·92	3·10	12·00	19·77

From the average composition of its bromine-derivative, Stadelmann concludes that trypsophan, which has not itself been isolated in a form sufficiently pure for analysis, must contain approximately :—carbon, 61·02 ; hydrogen, 6·89 ; nitrogen, 13·68 ; sulphur, 4·69 ; and oxygen, 13·71 per cent. Stadelmann regards trypsophan as a true proteid body, in some respects allied to

peptones, but in other particulars widely differing from compounds of that class.

The most interesting point concerning trypsophan is the very large proportion of sulphur contained in it. This precludes the possibility of the body being a simple product of the cleavage of native proteids. It appears probable that in trypsophan is concentrated the sulphur eliminated by the formation of leucine and other sulphur-free bodies from hemipeptone.

Commercial Peptones and Peptonised Foods.

Various preparations have appeared in commerce of late years which are produced by treating meat, blood-serum, white of egg, &c., in such a manner as to convert the natural proteid into soluble compounds. This is most rationally effected by subjecting meat or other proteid matter to a process of digestion outside the system by means of pepsin or pancreas-ferment, whereby the coagulable proteids are converted more or less completely into albumoses and peptones. The "peptone" thus obtained is claimed to be eminently suited for the sustenance of invalids and persons whose digestive organs are defective, since it relieves the digestive organs of part of their necessary work of peptonisation, and provides the patient with a food which is susceptible of direct conversion into serum-albumin by the cells which line the digestive tract. Syntonin is stated by some to have no advantage for this purpose over unchanged albumin, and the presence of both albumose and peptone appears to be indispensable to this process of direct assimilation.[1]

The utility of employing peptones in therapeutics, and even in the diet of invalids, has been questioned by several authorities, but Denaeyer contends that they have been misled by employing preparations which consisted of syntonin or albumoses only, and therefore were not true peptones. On the other hand, it is maintained by B. Oppler and others that albumoses and peptones present no advantage over suitable soluble albumins, and are very liable to cause irritation of the intestinal canal.

According to A. Denaeyer, the so-called peptones which are produced by the action of superheated steam on meat contain no true peptone.[2] These products may be termed "pseudo-peptones,"

[1] According to Hofmeister, true soluble albumoses, such as the deutero-albumose of Kühne, are not alone capable of direct assimilation. In fact, it is strongly denied that the albumoses are products intermediate between coagulable albumin and peptones, since by further treatment with hydrolysing agents they are at once degraded into non-proteid decomposition-products, without first passing through the stage of peptone.

[2] See footnote 2, page 301.

and are typified by the preparations of Kemmerich, who has himself shown that they do not yield the rose-coloured biuret reaction characteristic of true peptone. They are stated by Denaeyer to consist essentially of acid-albumin or syntonin, and give an abundant precipitate on acidulating their solutions with acetic acid and adding potassium ferrocyanide.

The preparation known as "Somatose" is an article of German origin. It is stated to be prepared from fresh meat, and is a light, white or greyish powder, almost tasteless, and readily soluble in water. It is claimed that somatose consists of soluble albumoses, directly assimilable, and that it is free from the disagreeable taste and tendency to cause vomiting and irritation of the digestive canal sometimes occasioned by true peptones. According to an analysis published by the manufacturers, somatose contains:—water, 10; albumoses, 78; peptones, 2 to 3; and salts, 6 per cent.; with 12 per cent. of total nitrogen (equivalent to 75·6 per cent. of possible proteids).

O. Hehner found a sample of Somatose to contain:—water, 14·16; fat, 0·41; albumoses, 62·13; peptones, 5·87; meat-bases (calculated from excess-nitrogen by factor 6·3), 3·50; ash, 5·26; and difference, 8·67 per cent. Proteid nitrogen, 10·88; total nitrogen, 11·54 per cent.

According to Denaeyer, Somatose is neither an albumose nor a peptone, but has in fact the characters of an alkali-albumin. This statement is partially correct, as is shown by the following results recently obtained by A. R. Tankard in the author's laboratory, by the bromine-process described on page 315:—

	Per cent.	
Water,	14·25	
Alkali-albumin (precipitated from the cold aqueous solution by acetic acid in slight excess),	21·83	
Coagulable albumin (precipitated from the filtrate by boiling),	3·40	62·25%
Albumoses (from the filtrate by zinc sulphate),	33·96	
Peptones (from the filtrate by bromine),	3·06	
Meat bases (calculated from excess-nitrogen by factor 3·12),	2·62	
Ash (having an alkalinity equivalent to 1·91 per cent. Na_2CO_3),	5·30	
Difference,	15·58	
	100·00	

Total nitrogen (by Kjeldahl's process),	10·78 × 6·3 = 67·91%
Proteid nitrogen (precipitated by bromine),	9·94 × 6·3 = 62·62%

The following analytical results have been published by Rideal and Stewart (*Analyst*, 1897, p. 230).

	Somatose.	Witte's Peptone.[1]
	Per cent.	Per cent.
Water,	7·30	7·62
Ash,	1·06	2·46
Organic matter,	91·64	89·92
	100·00	100·00
Total nitrogen,	13·72	14·67
×6·33=	86·75	92·91
N in ammonium sulphate precipitate,	12·44	10·13
N in phospho-tungstic acid precipitate,	12·65	12·65
N in Stutzer's copper precipitate,	6·64	4·20

The practically identical amounts of nitrogen precipitated by ammonium sulphate and by phospho-tungstic acid prove the substantial absence of true peptone from somatose.

"Iron Somatose" is a light brown powder, which is practically tasteless, readily soluble in aqueous liquids, not coagulated by heat, and not precipitated by dilute acids or alkalies. It contains exactly 2 per cent. of iron, and appears to present advantages over other preparations of iron in the treatment of chlorosis and other forms of anæmia.

"Nutrose" is a preparation consisting of a neutral compound of casein with fixed alkali (page 93). It is said to be free from the tendency to disorder the stomach and to occasion diarrhœa which is observed with some products containing true peptones.

The preparation known in commerce as Carnrick & Co.'s "Beef Peptonoids" is described as "prepared from the nitrogenous or flesh-forming principles of beef, wheat, and milk, constituting a purely nitrogenous food of the highest value, and showing a presence of between 80 and 90 per cent. of flesh-producing matter." The preparation is stated to be five times more nutritious than beef, mutton, fowl, or oatmeal. A sample of "beef peptonoids" analysed in the author's laboratory by A. R. Tankard was found to have the following composition :—

[1] See a paper, by E. P. Pick (*Zeit. Physiol. Chem.*, 1887, xxiv. 246), on the "Fractional Precipitation of Witte's Peptone by Ammonium Sulphate."

	Per cent.	
Water,	2·13	
Insoluble proteids,	12·22	Total proteids = 16·27 per cent.
Albumoses,	3·17	
Peptones,	0·88	
Meat-bases (excess-N × 3·12),	2·87	
Starch,	23·64	
Milk-sugar (by difference),	48·52	
Fat,	2·00	
Ash,	4·57	
	100·00	
Total nitrogen,	3·50	
Proteid nitrogen,	2·58 × 6·3 = 16·25 per cent.	

"Peptarnis," manufactured by the Liebig's Extract of Meat Company according to Kemmerich's method, is stated to be "prepared from pure beef only, by means of heat and pressure, and contains no addition whatever of acids, common salt, or any other substances." According to the manufacturers, it contains:—Water, 28·95; gelatin, 3·92; albumin, 1·85; albumoses, 23·42; peptone, 23·06; meat-bases, 8·94; fat, 0·18; sodium chloride and phosphates, 9·68 per cent. Total nitrogen, 9·95 per cent.

An analysis of Peptarnis in the author's laboratory gave the following results:—Water, 26·82 per cent.; albumoses, 21·28; peptones (by bromine-process, page 315), 7·67; and ash, 11·50 per cent. Total nitrogen, 9·49.

There are not a few preparations met with in commerce masquerading as concentrated and peptonised foods, but which analysis shows have little right to such description. Some of these are so dilute as to have practically no nutrient value, and their substitution for true foods in cases of sickness and debility is liable to result in deplorable consequences. As examples, the three following analyses (recently made in the author's laboratory) may be quoted[1]:—

	A.	B.	C.
	Per cent.	Per cent.	Per cent.
Water,	67·92	86·40	85·76
Alcohol,	15·60		
Coagulable proteids,	none	none	none
Albumoses (by $ZnSO_4$),	trace	0·48	0·09
Peptones (in filtrate by bromine),	0·43		0·52
Meat-bases (excess-N × 3·12),	1·54		0·14
Saccharine and other non-nitrogenous organic matters (by difference),	13·58	12·55	13·04
Ash,	0·93	0·57	0·45
	100·00	100·00	100·00
Total proteids,	0·43	traces	0·61
Total nitrogen,	0·56	0·08	0·14

[1] The commercial names of these preparations are purposely omitted, in

Sample A. had a strongly acid reaction, and barium chloride produced a considerable precipitate insoluble in dilute hydrochloric acid. On evaporating the liquid to dryness at 100° C. the residual solids *charred*.

The true peptones of commerce are commonly produced by the action of tartaric or hydrochloric acid and pepsin on meat. They are characterised by containing, in addition to soluble albumoses, true peptone, as defined by Kühne. When free from syntonin, such products yield no precipitate on addition of potassium ferrocyanide to their solutions previously acidulated with acetic acid (page 10), and give a very strong and pure rose-coloured biuret reaction, especially after separation of the albumoses by saturation with ammonium sulphate.

In one modification of the method of preparing commercial peptones, minced meat is treated at a digestion-temperature with pepsin and tartaric acid for a prolonged period (twenty-four hours). The product occurs in commerce as a very light, white powder, having a faintly urinous taste and stronger after-taste. It is very hygroscopic, readily soluble in water, and the solution gives a strong and pure peptone biuret reaction. The reactions show such a product to contain the true peptone of Kühne, together with deutero-albumose. Unfortunately, the tartaric acid method of preparing peptones unavoidably results in the production of a considerable proportion of decomposition-products, which contaminate the finished preparation. Consequently, peptones produced by this process never give an alcohol precipitate (representing the peptone and albumose present) higher than 30, or at the outside 35 per cent.

By employing hydrochloric acid instead of tartaric acid, a much better product is obtainable. In the products of digestions which have been well-regulated with respect to time, temperature, and proportions of pepsin and acid, the alcoholic extract will be small, while the precipitate may be as high as 70 per cent. A long digestion is to be avoided, the practice being based on the erroneous belief that by further treatment the albumose first formed underwent conversion into peptone. Denaeyer states that the best results are obtained (*i.e.*, 70 per cent. of albumose and peptone) by digestions of six hours' duration with pepsin and 2 per cent. of hydrochloric acid.[1]

deference to the well-known maxim of English law, "The greater the truth, the greater the libel."

[1] The details of the method of preparing commercial peptones on a small scale, and the characters of the products when successfully made, will be found in a paper by A. Catillon (*Pharm. Jour.*, [3], xi. (1881) 759).

The decomposition-products which result from excessive digestion have no alimentary value, and are formed at the expense of the proteids and gelatinoids, which are correspondingly deficient. Thus from the proteids there are formed, by the prolonged action of acid, leucine, lysine, tyrosine, and amidobutyric, aspartic, and glutamic acids; while gelatinoids yield leuceïnes, glycocine, alanine, and other amido-compounds. All these various decomposition-products, as well as the natural extractive principles of meat (*e.g.*, creatine, creatinine, carnine, &c.), are stated by Denaeyer to be soluble in strong alcohol, which precipitates coagulable albumin, acid-albumin, albumoses, peptones, and gelatin. On this fact, A. Denaeyer (*Jour. Pharm. Anvers*, Nov. 1891; abst. *Analyst*, xvi. 234) has based the following method of assaying commercial peptones[1]:—

An amount of the sample containing 2 grammes of dry matter is dissolved in 10 c.c. of water, and this solution is treated with 100 grammes of alcohol (of 95 per cent.). The mixture is allowed to stand for twenty-four hours in a cool place, when the clear liquid is decanted, the precipitate washed with alcohol, the filtrate evaporated, and the residue dried at 105° C. till constant. The alcohol precipitate is similarly dried and weighed. Treated in this manner, a well-prepared meat-peptone should not yield more than 30 per cent. of matters soluble in alcohol, though preparations are met with in commerce which give as much as 60 per cent. Such extracts contain, in addition to creatine and normal extractive bases, considerable quantities of the decomposition-products of the proteids and gelatinoids.[2]

As stated on page 313, the distinction of the constituents of commercial peptones into those soluble and those insoluble in alcohol of about 90 per cent. strength, is by no means so sharp as asserted by Denaeyer, whose process has no value beyond that of a rough technical method of assay.

The methods of analysing meat-extracts (page 310 *et seq.*) and differentiating the various nitrogenised constituents are applicable to the examination of commercial peptones.

L. Hugounenq states that he has examined two peptones adulterated with 32 per cent. of milk-sugar.

For the detection of *glucose* and *milk-sugar* in adulterated peptones, L. Ruizand heats the solution containing the peptone under examination with neutral copper acetate, when a reduction results,

[1] The process is inapplicable to the so-called peptones produced by the action of superheated steam on meat (page 301).

[2] If a few drops of the alcoholic solution be evaporated on a glass slide, leucine, tyrosine, &c., can be recognised by their microscopic characters.

if glucose alone is present. To detect milk-sugar, 5 grammes of the peptone should be dissolved in 45 c.c. of water, 5 c.c. of hydrochloric acid added, and the whole heated on the water-bath for two hours at 70°. After neutralisation with caustic soda solution, the liquid is treated with 12 grammes of sodium acetate and 8 grammes of phenyl-hydrazine hydrochloride. The liquid is filtered boiling hot to remove glucosazone, whilst galactosazone separates from the filtrate on cooling. The galactosazone is filtered off, washed with cold water, and purified by repeated crystallisation, when it can then be identified by its melting-point, which should be 188°–191° C.

Pancreatised Foods.

Articles of diet which have been peptonised or partially peptonised by the action of pancreatic or allied enzymes are extensively used by invalids and others unable to digest ordinary food.[1] Pancreatised milk, cream, soup, beef-tea, and gruel are prominent among various similar preparations now met with in commerce.[2]

In the case of *milk*, when the process of peptonisation is complete, the product has a somewhat bitter taste, which, if considered objectionable, may be disguised by the addition of coffee. Partially peptonised milk scarcely differs in taste or appearance from ordinary milk, but has undergone considerable modification in constitution, and is especially suited for the feeding of infants. It possesses the great advantage that the casein is not curdled by the

[1] Papaïn (sometimes called vegetable trypsin), the interesting ferment of the juice of the Papaw or Papaya tree (*Carica Papaya*) of Java, has attracted much attention of late years (see *Pharm. Jour.*, [3], xx. 227 ; xxiv. 183, 207, 633, 705, 757, 758, 831, 845, 1005, 1888 ; xxv. 183). Critical researches show the great probability that papaïn-digestion does not go further than the formation of deutero-proteoses, no true peptone being formed. Thus Gordon Sharp (*Pharm. Jour.*, [3], xxiv. 634) finds simple papaïn-digestion to produce, besides dysalbumose and undigested matters, globulose, proto-albumose, and hetero-albumose in traces, and deutero-albumose in abundance, while peptone was entirely absent. These conclusions have been traversed by R. H. Chittenden and others (*Amer. Jour. Physiol.*, 1898, i. 255), who find true peptone to be produced. Papaïn is rendered inactive or is destroyed by acid in the proportion usually present in the gastric juice, and hence can be of no practical value as an aid to digestion. Commercial papaïn appears usually to owe to an admixture of pepsin the greater part of such digestive activity as it may possess.

Cibil's "Fluid Extract of Beef" is said to be manufactured by the aid of papaïn, and the "Esco Beef Juice" to be peptonised by a ferment similar to papaïn (see page 310).

[2] The manufacture of peptonised food-products by fermentation with the enzyme of pine-apple juice forms the subject of English Patent No. 19,178 of 1890 (see *Jour. Soc. Chem. Ind.*, x. 477).

gastric juice with production of tough compact lumps, but on addition of acid forms light flakes which are much more readily digested.

If milk be treated with a pancreatic extract at about 38° C. in an open vessel, the tendency to form a skin is diminished or altogether absent, a slight brittle and perfectly smooth pellicle being formed instead. The milk next becomes gradually but softly curdled. The curds gradually redissolve for the most part, and the milk resumes its original appearance, but some of the curds remain undissolved for a lengthened period. If the milk be diluted with about one-third of its measure of water before treatment, this separation of curds does not occur, or is represented only by a slight and transient thickening. In the next stage, the milk loses its glossy white appearance, and gradually assumes a dull yellowish-grey shade, which is characteristic but not very conspicuous. At the same time the milk acquires a pure bitter taste, which is not disagreeable unless the process is pushed to an extreme.

The gradual transformation of the casein into peptone may be followed by testing the milk periodically with acetic acid. At first, the addition of the acid to a portion withdrawn from the main quantity of the liquid occasions an abundant precipitation of curdy matter, but this reaction gradually diminishes in intensity, and finally ceases. At this point the conversion into peptone may be regarded as complete. These reactions, namely, the coagulation on boiling in a neutral medium and precipitation in the cold by acetic acid, appear to be due to the formation of a substance called metacasein. When milk is rendered alkaline by sodium bicarbonate, no precoagulation on boiling occurs during its digestion by pancreatic extract, but the formation of metacasein may be detected by carefully neutralising the alkalinity and then boiling. These reactions should be borne in mind when preparing peptonised milk, which, on the small scale, is best effected as follows :—

One pint of the liquid should be diluted with four ounces of water, and heated to about 60° C. From 10 to 20 grains of sodium bicarbonate should then be added, together with a suitable quantity of pancreatic extract. The mixture is maintained at about 60° for a time varying from thirty minutes to ninety minutes, according to the strength of the pancreatic preparation and the degree of peptonisation desired. The time of treatment should be adjusted so that the product may have a somewhat bitter taste, without this being so pronounced as to be disagreeable. Unless intended for immediate consumption, the liquid should then be boiled to destroy the ferment and prevent further action. By previously

skimming the milk and restoring the cream after the final boiling, the product obtained is more palatable and milk-like in appearance.

HÆMOGLOBIN AND ITS ALLIES.

Hæmoglobin is the substance to which, in one or other of its modifications, the blood owes its red colour.

The red colouring matter of the blood is contained in the red corpuscles, which, together with a relatively small number of white corpuscles,[1] are suspended in the fluid portion or plasma.

The corpuscles may be separated from the plasma of the blood by the means indicated on page 37. The corpuscles may also be conveniently separated by defibrinating the blood, by agitation with twigs or glass rods, and then rotating the liquid in a centrifugal apparatus (page 140). The separated corpuscles are treated with a 1 per cent. solution of common salt, and the rotation repeated until the serum is effectually removed. If the product be then treated with about five parts of water and a little ether, the red corpuscles will burst and dissolve, and the white corpuscles may be removed by rotating the liquid.

[1] In leucocythæmia, the number of the white corpuscles of the blood is enormously increased, so that they sometimes amount to one-sixth, or even one-third, of the number of the red corpuscles (instead of about 1 : 330, as in normal blood). Charcot's crystals (Vol. III. Part iii. page 200) appear in the blood of leucocythæmic persons.

The white corpuscles of the blood consist of more or less granular, nucleated masses of protoplasm. They are capable of amœboïd movements and migration from the blood to the tissues. Migration takes place to a great extent in inflamed parts, and may cause the formation of an abscess, which is a collection of pus or white blood-corpuscles suspended in a liquid of the nature of serum. Such information as exists respecting the chemical composition of the white corpuscles is derived from the examination of such abnormal formations.

According to W. D. Halliburton the best-known chemical constituent of the nucleus of the white corpuscles is *nuclein*, a compound proteid which in its general characters resembles mucin, but is distinguished by its high content of phosphorus (see page 35). It may be isolated by treating the cells with artificial gastric juice, by which the investing protoplasm is dissolved, leaving the nuclein unaffected.

A body, called by Rovida the *hyaline substance* and probably belonging to the class of nucleo-albumins, forms the chief constituent of the protoplasm. Halliburton has also described an albumin and two globulins. Fat, glycogen, cholesterin, lecithin, and mineral matters are likewise present.

The mean composition of male and female *human blood* is stated by Becquerel and Rodier to be as follows :—

	Male.	Female.
Density of defibrinated blood,	1060·0	1057·5
Density of serum,	1028·0	1027·4
Water,	779·00	791·10
Fibrin,	2·20	2·20
Fatty matters,	1·60	1·62
Serolin,	0·02	0·02
Phosphorised fat (lecithin),	0·49	0·46
Cholesterin,	0·09	0·09
Saponified fat,	1·00	1·04
Albumin,	69·40	70·50
Blood-corpuscles,	141·10	127·20
Extractive matters and salts,	6·80	7·40
Sodium chloride,	3·10	3·90
Other soluble salts,	2·50	2·90
Earthy phosphates,	0·33	0·35
Iron,	0·57	0·54

The following table shows the relative composition of the blood-*corpuscles* and *plasma* of normal human blood, according to Schmidt and Lehmann :—

		Corpuscles.	Plasma.
Specific gravity,		1088·5	1028·0
Water,		688·00	902·90
Organic Solids,		303·88	88·55
Containing	Hæmoglobin (oxy) and proteids of the stroma,	298·97	..
	Fibrin,	..	4·05
	Serum-albumin, globulin, &c.,	..	78·84
	Fat,	2·31	1·72
	Extractive matters,	2·60	3·94
Mineral Matters,		8·12	8·55
Chlorine,		1·686	3·640
Sulphuric acid (SO_3),		0·066	0·115
Phosphoric acid (P_2O_5),		1·134	0·191
Potassium,		3·328	0·323
Sodium,		1·052	3·341
Oxygen,		0·667	0·403
Calcium phosphate,		0·114	0·311
Magnesium phosphate,		0·073	0·222

In the above analysis of the mineral matters of blood-corpuscles the iron is excluded, since it exists in organic combination as hæmoglobin.

Gamgee (*Physiol. Chem.*) has shown that the above curious difference in the distribution of the potassium and sodium does not hold good in the case of the blood of most animals.

The following is the composition of dried *corpuscles* of normal blood, according to Hoppe-Seyler and Jödell :—

	Human Blood.		Dog's Blood.	Goose's Blood.
	I.	II.		
Proteids (*i.e.*, albumin, globulin), &c., . .	12·24	5·10	12·55	36·41
Hæmoglobin, ...	86·79	94·30	86·50	62·65
Lecithin, ...	0·72	0·35	0·59	0·46
Cholesterin, ...	0·25	0·25	0·36	0·48

P. Manasse (*Zeit. physiol. Chem.*, xiv. 452) gives the proportion of lecithin and cholesterin in red corpuscles at 1·867 and 0·151 per cent. respectively. They are extracted together on treating the corpuscles with ether.

From the foregoing figures it appears that hæmoglobin is the chief solid constituent of the blood-corpuscles. The proportion is fairly constant in the red corpuscles of normal blood, but in certain diseases, and especially in chlorosis and other forms of anæmia, not only are the number (usually) and size of the corpuscles diminished, but the proportion of hæmoglobin contained in them is considerably less than the normal. Thus, taking the maximum proportion of hæmoglobin in healthy blood as 100, it may fall as low as 85 without the health being sensibly impaired. But in anæmia the hæmoglobin varies between 66 and 12½, so that in cases of moderate intensity the amount is only one-half to one-fourth of the normal; while in extreme cases the proportion is not more than one-seventh or one-eighth of the normal amount.[1]

The *diameter* of the red corpuscles in normal human blood are stated to range from 8·5 μ to 6·5 μ. Corpuscles of these diameters each form about 12 per cent. of the total number, while the remaining 75 to 76 per cent. are of medium size (about

[1] These facts render intelligible the therapeutic value of preparations of iron in all cases of anæmia, and especially of chlorosis. The following analyses of blood by Andral and Gavarret illustrate the improvement effected by the administration of iron in two cases of chlorosis. The number of red corpuscles increased coincidently with the improvement in the complexion and general condition of the patients. The figures are parts per 1000.

	Normal Woman's Blood.	Blood in Chlorosis. A.		Blood in Chlorosis. B.	
		Before using Iron.	After using Iron.	Before using Iron.	After using Iron.
Water, ...	791·1	866·7	818·5	852·8	831·5
Fibrin, ...	2·2	3·0	2·5	3·5	3·3
Blood-corpuscles, .	127·2	46·4	95·7	49·7	64·3
Solids of serum, . .	79·5	83·9	83·3	94·0	100·9

7·5 μ). In anæmia, the blood contains an increased proportion of small corpuscles, together with some very small (2·2 to 6 μ) and frequently some very large corpuscles, having a diameter of 10 to 12 μ, or even 14 μ. The diameter of the red corpuscles varies in different mammals, and affords an indication (though not one of a very positive kind) of the origin of the blood. (Compare chemico-legal detection of blood, in sequel.)

The *weight* of a red corpuscle has been calculated by Welcker to average 0·00008 milligramme.

The *enumeration* of the red corpuscles of the blood is of considerable importance from a physiological standpoint, and can be effected simply and with tolerable accuracy. The method consists in largely diluting the blood to be examined with a suitable fluid, taking a known measure of the resultant liquid, and counting the corpuscles in a certain measure by the aid of a micrometer.

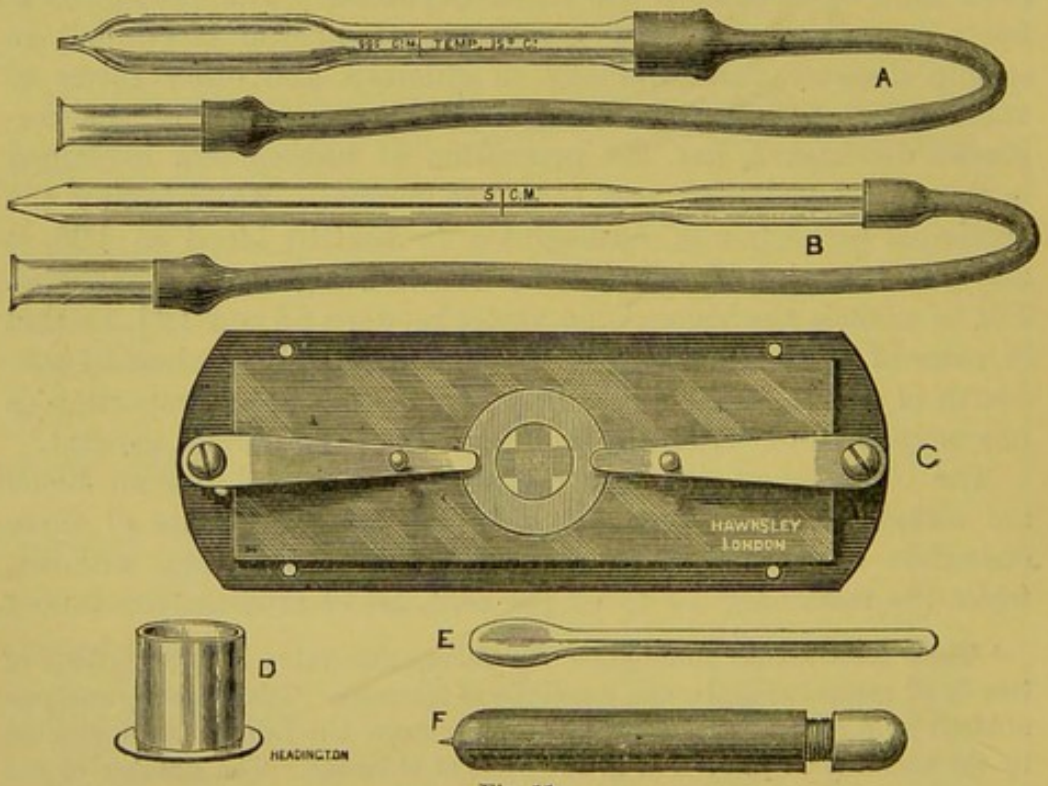


Fig. 11.

The enumeration of the blood-corpuscles can be conveniently effected by the *hæmacytometer* (fig. 11), an apparatus devised by Sir Wm. Gower (*Lancet*, Dec. 1, 1877).[1] A is a small pipette, which, when filled to the mark on its stem, holds exactly 995 cubic millimetres; B, a capillary tube marked to contain exactly

[1] Gower's Hæmacytometer is made and sold by Mr Thos. Hawksley, 357 Oxford Street, London, W.C., to whom the author is indebted for the illustration in the text.

5 cubic millimetres; and C, a brass stage-plate, carrying a glass slip, on which is a cell 0·2 mm. deep. The bottom of this is divided into squares 0·1 mm. in diameter. Upon the top of the cell rests a cover-glass, which is kept in its place by the presence of two springs proceeding from the ends of the stage-plate. A small glass jar, D, and glass stirrer, E, with a sharp spear-pointed lancet or needle, F, and some minor accessories complete the equipment.

In employing the hæmacytometer, 995 c.m. of an aqueous solution of sodium sulphate of 1025 specific gravity is taken by the pipette, A, and blown into the mixing jar, D. The finger is then pricked with the lancet and 5 c.m. of blood sucked into the capillary pipette, B, and blown into the diluting liquid. The two liquids are well mixed by rotating the glass stirrer, E, between the finger and thumb, and a small quantity of the diluted blood placed in the centre of the glass-cell, a covering glass gently placed on the top, and secured with the brass springs, which should be *lifted* and not slid into position.[1] The plate is then placed on the stage of a microscope. In a few minutes the corpuscles will sink to the bottom of the cell, and on focussing the objective can be seen at rest on the squares. The corpuscles in ten of the squares towards the centre of the cell are then counted, and their number, multiplied by 10,000, gives the number in a cubic millimetre of the blood examined.

The number of red corpuscles present in normal human blood

[1] The blood should not be drawn until the diluting liquid has been placed in the mixing jar, and the capillary pipette is ready for use. The blood should be obtained by a puncture with the point of the lancet sufficient to permit the escape of a drop without much pressure. If the finger be much pressed, squeezed, or ligatured, the relative amounts of serum and corpuscles will be disturbed. It is better to draw somewhat more than the required quantity into the capillary pipette, then remove the blood adhering to the exterior of the point with a soft cloth, and keep the cloth in contact with the point while the excess of blood is blown out. The small end of the stirrer may be used to remove the drop of diluted blood from the mixer to the cell. The drop must be placed in the middle of the cell, and care must be taken not to rub the stirrer on the bottom of the cell. The covering glass should be placed on the cell as nearly horizontally as possible. When this is done the drop of solution should appear as a disk in the middle of the cell, or nearly so; its edges must not touch the sides of the cell. A weak diluting liquid causes many corpuscles to swell to twice the size of the others, and care must be taken not to mistake these swollen red corpuscles for white corpuscles. By raising the objective out of focus, the white cells may be readily distinguished by their greater refractive power. A magnifying power of 300 diameters is the most suitable, and the light should be oblique and not too intense.

was found by Malassez to range from 4,000,000 to 4,600,000 per cubic millimetre, with an average of about 4,500,000. The average number of corpuscles in healthy blood was found by Vierordt and Welcker to be 5,000,000 per cubic millimetre, and later results agree with this sufficiently nearly to justify the adoption of this number as the standard, the number in man's blood being somewhat above, and in woman's somewhat below the average. By employing Gower's hæmacytometer, a more convenient mode of statement is obtained than that of the number of corpuscles per cubic millimetre. Taking 5,000,000 as the average per cubic millimetre for healthy blood, the average number in two squares of Gower's cell is 100. These two squares contain 0·00002 cubic millimetre of blood, and Gower takes this quantity as the "hæmic unit." The number per hæmic unit, *i.e.*, in two squares (ascertained by counting a larger number, 10 or 20, and taking the mean), thus expresses the percentage proportion of the corpuscles to that of health. The proportion of white corpuscles to the red, or their number per hæmic unit, is best ascertained by observing the number of squares visible in the field of the microscope, and noting the number of white corpuscles in a series of ten or twenty fields. The number of red corpuscles corresponding to the ten or twenty fields is easily observed, and thus the proportion of white corpuscles to red is ascertained. The normal *maximum* of white per two squares (hæmic unit) is 0·3 per 100 of red corpuscles.

Hæmoglobin.

Hæmoglobin is the pigment to which the blood of vertebrate animals owes its colour. Its most characteristic and physiologically important property is that of absorbing and combining with free oxygen to form oxyhæmoglobin, to the presence of which body the bright red colour of oxygenated or arterial blood is due. In normal arterial blood, hæmoglobin proper, sometimes called "reduced hæmoglobin," exists only in traces. It occurs in larger proportion in venous blood, and exists comparatively free from admixture with oxyhæmoglobin in the blood of asphyxiated animals.

Hæmoglobin is found in the blood-corpuscles of all vertebrate animals, except, according to Lancaster, *Amphioxus* and *Leptocephalus*. It is likewise present in certain of the muscles, especially the red muscles of rodents. Hæmoglobin also occurs in certain invertebrate animals, including crustaceans, insects, molluscs, and various worms. In the case of these lower animals, with a few exceptions, the hæmoglobin is not enclosed in corpuscles but dissolved in the blood plasma.

Hæmoglobin is peculiar in containing a notable proportion of iron (0·42 per cent.) in an organic form of combination.[1]

In consequence of the great readiness with which hæmoglobin absorbs free oxygen with conversion into the stable body oxy-hæmoglobin, the investigation of the parent substance is attended with peculiar difficulty, and hence hæmoglobin itself has been less completely studied than its oxy-compound.

Hæmoglobin has been prepared in definite crystals, but its extreme solubility in water renders it difficult to obtain in this condition. According to Hüfner the crystals are best prepared by sealing up a concentrated aqueous solution of oxyhæmoglobin in glass tubes from which the air has been displaced by hydrogen. On long-keeping the putrefactive decomposition which ensues causes the absorption of the combined oxygen, and ultimately, on exposure to a low temperature, crystals of hæmoglobin are deposited.[2] The crystals of hæmoglobin are strongly pleochromatic. In colour they are dark red, with a strong purplish or bluish tint, and contrast in a marked manner with the bright scarlet colour of crystals of oxyhæmoglobin.

The absorption-spectrum of hæmoglobin is described on page 426.

The most remarkable character of hæmoglobin is that of uniting directly with certain gases to form more or less stable com-

[1] The iron which forms an essential constituent of the blood of the vertebrata is, in some of the lower animals, replaced by copper. Thus, the blood of the *octopus* contains *hæmocyanin*, a copper-containing colouring matter which in an oxidised state is blue, but which becomes colourless on reduction. Hæmocyanin is contained in the blood-plasma of the octopus, and apparently unassociated with any other proteid. It is colloidal, uncrystallisable, and the spectrum exhibits no definite absorption-bands. On treatment with acids it yields a substance allied to hæmatin, crystallising in prisms.

F. Heim states that copper is present in the blood of the lobster, but absent from that of many crustacea, including the crab, hermit-crab, and crayfish. The bluish blood of *Limulus cyclops* and of *Helix pomatia* and certain other mollusca has been found to contain copper. Oysters sometimes contain a notable quantity of copper.

Chlorocruorin is a green colouring matter occurring in the green blood of certain annelids of the genus *Sabella*. Like hæmoglobin, it exists in an oxidised and a reduced condition. In the former state the absorption-spectrum exhibits two bands; one well-marked between C and D, and a second much fainter, almost midway between D and E. On treatment with a reducing agent, the latter band disappears and the former becomes fainter and slightly displaced. The original spectrum is restored by agitation with air.

Turacin is a cupreous pigment contained in certain red feathers (page 7).

[2] A similar separation of crystals of hæmoglobin sometimes occurs in microscopic specimens of oxyhæmoglobin which have been sealed up under a cover-glass with canada-balsam (see also footnote 1, page 399).

pounds. The compounds thus formed with oxygen, carbon monoxide, and nitric oxide are definite and of constant composition. They crystallise in characteristic forms, and their aqueous solutions exhibit characteristic absorption-spectra. The compounds of hæmoglobin with carbon dioxide, hydrogen cyanide, and acetylene are less stable and definite in character, and the existence of the last two has been denied.

OXY-HÆMOGLOBIN.

On exposing hæmoglobin to the air it rapidly unites with a molecule of oxygen to form oxy-hæmoglobin, or hæmatocrystallin, the characteristic red colouring matter of the corpuscles of arterial blood.

To obtain oxyhæmoglobin in a pure state, Kühne recommends that blood should be defibrinated and strained through muslin. It is then transferred to a flask, and ether gradually added (about one volume to 16 of blood), with frequent agitation, until the mixture begins to appear transparent. The flask is then immersed in a freezing mixture (ice and salt), when, in a somewhat variable time, the contained liquid will become almost pasty from the formation of crystals of oxyhæmoglobin. These are separated from the mother-liquor by rotation in a centrifugal apparatus, dissolved in the smallest possible quantity of water, the solution filtered, cooled to 0° C., one-fourth of its volume of alcohol added, and the liquid again immersed in a freezing mixture. The crystals of oxyhæmoglobin which separate may be further purified by re-solution in water, addition of alcohol, and recrystallisation at a temperature at or below 0° C. The product may be dried *in vacuo* at 0° C. over strong sulphuric acid, and when moisture-free is fairly stable.

When it is merely required to prepare a specimen of oxyhæmoglobin for microscopic examination, a drop of the blood may be placed on a glass slide, together with a minute drop of water, and the liquid allowed to evaporate spontaneously until a ring of dried substance appears round the edge. If a cover-glass be now applied and the specimen kept at a low temperature, crystals of oxyhæmoglobin will usually form after a short time.

The readiness with which crystals of oxyhæmoglobin are obtainable varies greatly according to the origin of the blood under treatment. Thus, crystals are yielded with great facility from the blood of the rat, squirrel, and guinea-pig; somewhat less readily from the blood of the cat, dog, mouse, and horse; with difficulty from the blood of man, rabbit, and sheep; and with still greater difficulty by that of the calf, pig, pigeon, and frog.

The solubility of the oxyhæmoglobin from different sources

also varies considerably, and inversely as the facility with which it is obtained in crystals. The readiness with which oxyhæmoglobin is decomposed by treatment with acids and alkalies is stated to be greater in the case of the product from the blood of the dog or man than in that from herbivorous animals. The crystals vary also in the proportion of water of crystallisation they contain, and also in their crystalline form.[1] Thus oxyhæmoglobin from human blood, and from most other animals, crystallises in columnar prisms or rhombic plates (fig. 12) (*a*); from the guinea-pig (*c*) and certain birds in rhombic tetrahedra; and from the squirrel (*d*) and hamster in hexagons.[2] Quadrant *b* represents the crystals from the heart-blood of the cat.

Fig. 12.—Blood Crystals.

It has been supposed that the variations in crystalline form of the oxyhæmoglobin from different sources was dependent on the proportion of combined water—in other words, that several hydrates of oxyhæmoglobin exist, and that these have different crystalline forms. Experiment shows, however, that while the crystalline form is practically constant for the crystals from the same source the proportion of combined water is variable.[2] Nor does the form of the crystals appear to depend on any constituent of the serum, for if the blood-corpuscles of any animal be separated

[1] S. M. Copeman finds that when crystals are obtained from human blood by the addition of putrid serum, they are rectangular, and consist of hæmoglobin, while the crystals from the blood of all other animals, except the monkey, consist of oxyhæmoglobin (compare page 439).

[2] The oxyhæmoglobin from the blood of the mouse is commonly stated to crystallise in hexagons, but W. D. Halliburton (*Proc. Physiol. Soc.*, 1886, page 2) obtained it in rhombic needles. The crystals from squirrel's blood he found to be hexagons, and from the fact that they exhibited no double refraction when viewed between crossed Nicol's prisms they were apparently true hexagons, and not rhombic plates with "hexagonal habit."

By repeated recrystallisation, the hexagonal constitution of the oxyhæmoglobin from squirrel's blood could be broken down, the crystals ultimately obtained being a mixture of rhombic needles and tetrahedra. The reverse experiment of mixing the hæmoglobin crystals from the rat (rhombic needles) and guinea-pig (tetrahedra), and thus allowing crystallisation to take place, yielded no needles or tetrahedra, but rhombic crystals simulating hexagons. Halliburton considers that these variations in crystalline form are probably dependent on the varying amounts of water of crystallisation present.

from the serum by a centrifugal apparatus and then mixed with the serum of some animal the blood-crystals of which have a different form, it is found on crystallisation that the form of the oxyhæmoglobin is unchanged.[1]

On the other hand, the oxyhæmoglobin from all sources appears to be identical in its chemical composition, in its absorption-spectrum, and in its decomposition-products.[2]

The following figures were obtained by Hoppe-Seyler by the analysis of crystallised oxyhæmoglobin from different sources, after drying at 100° C.

	Source of Blood.			
	Dog.	Goose.	Guinea-pig.	Squirrel.
Crystalline form,	Rhombic prisms.	Rhombic prisms.	Rhombic tetrahedra.	Hexagons.
Water of crystallisation,	3-4	9·4	7	9·4
Carbon,	53·85	54·26	54·12	54·09
Hydrogen,	7·32	7·10	7·36	7·39
Nitrogen,	16·17	16·21	16·78	16·09
Oxygen,	21·84	20·69	20·68	21·44
Sulphur,	0·39	0·54	0·58	0·40
Iron,	0·43	0·43	0·48	0·59
	100·00	99·23[3]	100·00	100·00

[1] According to V. P. Cervera (abst. *Chem. News*, xlii. 258), on mixing blood with a little bile, crystals are formed which vary with the origin of the blood. Thus from human blood he obtained right rectangular prisms; from the blood of the horse, cubes; from the ox, rhombohedra; from the sheep, rhombohedral tablets; from the dog, rectangular prisms; from the rabbit, tetrahedra; from the mouse, octahedra; and from the blood of common poultry, cubes modified at their angles.

[2] F. Hoppe-Seyler (*Zeit. Physiol. Chem.*, xiii. 477; abst. *Jour. Chem. Soc.*, 1889, page 787) distinguishes between the pigment as it exists in the red corpuscles and the pigments oxyhæmoglobin and hæmoglobin separated from the corpuscles, and he suggests the names *arterin* and *phlebin* for the oxidised and reduced pigments respectively as they exist in the corpuscles. He considers these bodies to be probably compounds of lecithin with oxyhæmoglobin and hæmoglobin respectively. He points out that while these latter bodies are soluble in the plasma and serum of the blood, the corpuscular pigments, arterin and phlebin, are insoluble. The corpuscular pigments do not crystallise, give off oxygen readily in a vacuum, and readily decompose hydrogen peroxide, whereas the reverse of this is true of oxyhæmoglobin and hæmoglobin. Further, arterin is not affected by a weak solution of potassium ferricyanide, whereas oxyhæmoglobin is converted into methæmoglobin.

The group which unites with respiratory oxygen is regarded by Hoppe-Seyler as probably hæmochromogen (page 418), but at any rate is the same both in the corpuscular pigment and in hæmoglobin.

[3] Hoppe-Seyler also found 0·77 per cent. of P_2O_5 in the hæmoglobin of the blood of the goose.

From these analyses, and others by C. Schmidt, Preyer calculated the following as the average composition of oxyhæmoglobin :—

Carbon.	Hydrogen.	Nitrogen.	Oxygen.	Sulphur.	Iron.
54·00	7·25	16·25	21·45	0·63	0·42 per cent.

This composition corresponds to the following empirical formula, which, for convenience of comparison, is placed in juxtaposition with those of Hüfner, Zinoffsky, and Jutt :—

Preyer, . . .	$C_{600}H_{960}N_{154}O_{179}S_3Fe$
Hüfner, . . .	$C_{550}H_{852}N_{149}O_{149}S_4Fe$
Zinoffsky, . .	$C_{712}H_{1130}N_{214}O_{245}S_2Fe$
Jutt, . . .	$C_{648}H_{1040}N_{178}O_{177}S_2Fe$

The most remarkable fact observable in the analyses of oxyhæmoglobin is that the substance contains iron as an essential constituent of the molecule. This peculiarity distinguishes hæmoglobin and its allies from ordinary proteids.

The moist crystals of oxyhæmoglobin form a pasty mass of a vermilion-red colour, and when dried at 0° C. over strong sulphuric acid are rapidly converted into a brick-red powder. When thoroughly dry, oxyhæmoglobin may be heated to 100° C. without change; but if a trace of moisture be present, the substance becomes brown, is no longer completely soluble in water, and acquires optical properties indicative of the formation of methæmoglobin.

The crystals of oxyhæmoglobin may be preserved in alcohol for a long time without change of form; but they lose their colour, brilliancy, and bi-refractive character.

Oxyhæmoglobin is insoluble in alcohol, amylic alcohol, ether, chloroform, carbon disulphide, and volatile oils.

Oxyhæmoglobin is sparingly soluble in water, with blood-red colour; but, as already stated, the facility with which it dissolves varies with the source from which it is prepared. At 5° C. the solubility in water of oxyhæmoglobin from pig's blood is about 2 per cent. In feebly alkaline solutions, such as blood-serum, oxyhæmoglobin dissolves far more readily than in pure water. Aqueous solutions of oxyhæmoglobin decompose on keeping, the colour changing to brown by reflected and green by transmitted light, and the liquid becoming gradually more acid, and containing formic and butyric acids, methæmoglobin, &c. At 0° C. these changes are very gradual, but the rate increases with the temperature and the concentration of the solution. A feebly-alkaline solution of oxyhæmoglobin is more stable than one in pure water,

and a dilute solution containing sodium carbonate can be kept without material change for several months. When heated to about 80° C., aqueous solutions of oxyhæmoglobin are rapidly decomposed, with production of a clot of globin coloured brown by hæmatin, and formation of traces of volatile fatty acids. On boiling, the decomposition is immediate. Hot alcohol causes the same change.

Solutions of oxyhæmoglobin in syrup or glycerin are more permanent than those in pure water, but the solutions gradually undergo change, with formation of methæmoglobin (page 409).[1]

Alkalies (unless very dilute) and acids (more readily) split up oxyhæmoglobin in solution, without precipitation, the change being the more rapid with concentration, increase of temperature, and amount of the reagent.

The products obtained by the action of acids and alkalies on oxyhæmoglobin are described on page 411 *et seq.*

The reduction of oxyhæmoglobin to hæmoglobin may be effected by boiling its solution *in vacuo*; by passing a current of hydrogen, nitrogen, or carbon dioxide through the solution; or by the addition of a chemical reducing agent (page 437).

A solution of pure hæmoglobin combines with a volume of oxygen to form oxyhæmoglobin equal to that which is absorbed by a volume of blood containing the same quantity of hæmoglobin. Thus, 1 gramme of hæmoglobin absorbs 1·56 c.c. of oxygen at the standard pressure and temperature, and as the average proportion of hæmoglobin in blood is about 14 per cent., it follows that $1{\cdot}56 \times 14 = 21{\cdot}8$ c.c. of oxygen will be retained by 100 c.c. of blood. This result is in fair agreement with the fact that 20 volumes of oxygen can be obtained from 100 volumes of aërated blood.[2]

[1] "Soluble syrup of hæmoglobin" has been strongly recommended as a remedy for anæmia. A syrup of this class, prepared by M. Adrian & Co. of Paris, gave the following analytical results on examination in the author's laboratory :—

	Per cent.
Water,	41·56
Coagulable proteid,	14·29
Peptonoid bodies (by bromine),	none.
Ash,	0·46; containing Fe, 0·22 per cent.
Total nitrogen,	$2{\cdot}32 \times 6{\cdot}3 = 14{\cdot}62$ per cent.

The preparation was neutral to litmus. The proportion of iron is much larger than corresponds to the possible hæmoglobin present. The diluted syrup exhibited the spectrum of methæmoglobin.

[2] The proportion of oxygen absorbed by blood is not materially increased

When it is desired to ascertain the composition of the gases contained in blood it is essential that the sample should be collected without permitting any contact with air. This is effected by connecting the blood-vessel from which the blood is drawn by a narrow caoutchouc tube with the tap of a Lunge's nitrometer or similar arrangement filled with mercury, and then gradually drawing the blood into the graduated tube. From 30 to 40 c.c. of blood is a suitable quantity to employ. The blood is then warmed to about 45° C. and the gases removed by a mercurial pump. The last trace of carbon dioxide is difficult to remove, but its evolution may be effected by introducing about 2 c.c. of a thoroughly-boiled solution of phosphoric acid into the blood-receptacle when the operation is nearly at an end. The gas evolved from the blood may be analysed by the usual methods, the carbon dioxide being absorbed by a solution of caustic potash, the oxygen by an alkaline solution of pyrogallol, and carbon monoxide (if present) by an acid solution of cuprous chloride.

Gamgee and Berthelot have independently shown that a sensible quantity of heat is evolved on the combination of hæmoglobin with oxygen.

Solutions of oxyhæmoglobin are precipitated by most metallic salts, including cupric and ferrous sulphates, ferric chloride, and mercuric chloride, but are said not to be precipitated by silver nitrate nor lead acetate, even after addition of ammonia, though these reagents cause the rapid decomposition of oxyhæmoglobin.

Oxyhæmoglobin gives the usual reactions of proteids (page 9).

If a solution of oxyhæmoglobin or blood be diluted till the liquid has a pink colour, it exhibits an absorption-spectrum characterised by two well-defined bands in the green, closely resembling those produced by an ammoniacal solution of cochineal. By treating the liquid with acids, deoxidising agents, carbonic oxide, &c., various changes in the spectrum are produced. These afford a certain and delicate means of recognising blood and hæmoglobin, and are fully described in the sequel.

On mixing a dilute solution of oxyhæmoglobin, either in the pure state or as existing in blood, with a freshly-made tincture of guaiacum-resin, adding a few drops of hydrogen peroxide, and shaking, a fine blue colour is produced. This reaction is of extraordinary delicacy, but it is produced by so many substances besides the colouring matter of blood as to render the test of very

by raising the pressure. Even under a pressure of ten atmospheres the oxygen absorbed is only increased from 20 to 23 per cent. by volume.

The tension of oxygen in the blood and in solutions of hæmoglobin has been investigated by G. Hüfner (*Zeit. physiol. Chem.*, xii. 568).

limited practical value.[1] The following is the best method of procedure :—

A tear of guaiacum-resin is washed with a little rectified spirit to remove the exterior portion, and the remainder crushed and dissolved in alcohol to form a solution of about 10 per cent. strength. Eight to ten drops of the liquid to be tested for blood should be placed in a flat porcelain dish or the cover of a porcelain crucible, and two or three drops of the tincture added. This should not of itself produce any coloration, but on then adding a drop of peroxide of hydrogen, either aqueous or ethereal, a fine blue coloration is developed if blood be present. On subsequently adding sufficient ether or alcohol, the colouring matter will be dissolved to a sapphire-blue liquid. Oil of turpentine, previously shaken with water and air, may be substituted for the hydrogen peroxide. The guaiacum test is serviceable for the detection of blood in urine, but is useless for the examination of milk, which itself gives a blue coloration. For the detection of blood on dyed fabrics, Woodman and Tidy recommend that the reagents should be added to the stain, and the fabric then pressed between two pieces of white blotting-paper, which will be coloured blue. Another plan is to moisten the fabric with water, press it for some time between blotting-paper, and then apply the reagents to the paper.

Carbonyl-Hæmoglobin.

If a current of carbon monoxide be passed through a solution of hæmoglobin or venous blood, a definite compound of the gas with hæmoglobin is formed. This compound contains equal molecules of its components, and is formed with such facility and is so stable that, on passing carbon monoxide through a solution of oxyhæmoglobin or of aërated blood, the oxygen is displaced and carbonyl-hæmoglobin formed.[2] The reverse action on treatment with oxygen does not occur. It follows that blood-corpuscles which have been exposed to carbon monoxide gas are permanently changed in character, and can no longer serve as carriers of oxygen to the system. The well-known poisonous effects of carbon monoxide, and of blast-furnace gases, producer-gases, lime-kiln gases, charcoal fumes, and other mixtures containing carbonic oxide are due to this cause. Blood-corpuscles, the hæmoglobin of which has become converted into the carbon monoxide compound, may be

[1] Among these are gluten, flour, gum-arabic, milk, various metallic chlorides, a mixture of hydrocyanic acid and cupric sulphate, the juice of cherries, currants, and raspberries, certain fungi, &c.

[2] The carbonic oxide displaces an equal measure of oxygen, CO taking the place of O_2.

regarded as at least temporarily inert and poisoned, and when they form a considerable proportion of the total corpuscles of the blood the result is fatal.

Although the carbonic oxide compound of hæmoglobin is more stable than the oxygen compound, the carbonic oxide can be expelled by boiling the blood in a vacuum, or by passing hydrogen or air through the liquid for a long time.

According to N. Gréhant (*Compt. rend.*, cvi. 289), blood absorbs carbon monoxide from an atmosphere containing only 0·02 per cent. of this gas. 100 c.c. of the blood of a dog poisoned by an atmosphere containing 0·1 per cent. of carbon monoxide contained only 14·2 c.c. of oxygen, against 27·0 c.c. obtained from normal blood.[1] On treating 100 c.c. of the poisoned blood with acetic acid at 100°, it evolved 14·4 c.c. of carbonic oxide. Hence it appears that if the air contain 0·1 per cent. of carbonic oxide, one-half the hæmoglobin of the blood will enter into union with it.[2] The analysis of the gases from the blood is evidently of great value in cases of poisoning by carbonic oxide.

The blood of animals poisoned with carbonic oxide has an

[1] This volume of oxygen is larger than has been found by other observers to be absorbed by normal blood.

[2] Experiments by J. S. Haldane (*Jour. Physiol.*, 1895, xviii. 430) on himself show that the symptoms caused by carbonic oxide depend on the extent to which the hæmoglobin has been saturated. During rest the symptoms do not become sensible till the corpuscles are about one-third saturated. With half saturation, the symptoms (headaches, respiratory distress, &c.) become urgent. About one-half of the carbonic oxide contained in the respired air is absorbed.

The time required for the production of sensible symptoms depends on the time taken for the inhalation of about 660 c.c., or the absorption of about half this volume of pure carbonic oxide. This time varies in different animals with the respiratory exchange per unit of body-weight, and is about twenty times as long in a man as in a mouse. Hence a mouse can be used as an indicator of the respirability of a vitiated atmosphere before men venture into it.

Haldane finds the affinity of hæmoglobin for carbon monoxide to be about 140 times greater than its affinity for oxygen. With a given percentage of carbonic oxide in air, a certain percentage saturation of the blood is reached in about 150 minutes, and is not afterwards exceeded, however long the breathing of air vitiated to the same extent is continued. Distinct symptoms of poisoning, appreciable during rest, are produced when the proportion of carbonic oxide reaches 0·05 per cent., and with about 0·2 per cent. urgent symptoms are produced. The disappearance of the carbonic oxide from blood when fresh air is again breathed is always much slower than its absorption, and is chiefly due to the dissociation of carbonyl-hæmoglobin by the mass-influence of the oxygen in the pulmonary capillaries.

intensely florid, cherry-red hue, which differs from that of normal arterial blood by its permanence. This is due to the difficulty with which carbonyl-hæmoglobin undergoes putrefactive change.

Carbonyl-hæmoglobin can be obtained in crystals by passing carbonic oxide gas through a solution of oxyhæmoglobin and adding alcohol. On exposing the mixture to cold, crystals of carbonyl-hæmoglobin separate.

Carbonyl-hæmoglobin crystallises in forms isomorphous with those of oxyhæmoglobin, but the crystals are of a bluer shade, less soluble, and more stable than those of the latter compound. According to Hoppe-Seyler, carbonyl-hæmoglobin is unaffected either by pancreatic ferment or by putrefactive change. Lachowitz and Nencki find that, if kept under alcohol, the crystals remain unchanged for months; but in the dry state they become amorphous.

For the detection of carbonyl-hæmoglobin in cases of suspected poisoning by carbonic oxide, Hoppe-Seyler adds to the blood twice its measure of caustic soda of 1·3 specific gravity. If the carbonic oxide compound be present, a precipitate of brilliant red colour will be formed, quite different from the brownish-green mass produced when oxyhæmoglobin is alone under treatment. Salkowski modifies this test by diluting the blood with twenty measures of water, and treating the resultant liquid in a test-tube with an equal volume of solution of caustic soda of 1·34 specific gravity. In a few seconds carbonic oxide blood becomes whitish, then red; and on standing red flocculi separate and finally rise to the surface of a faintly rose-coloured liquid. Normal blood merely yields a dirty brown coloration.

Numerous other reagents change the colour of normal blood to a dirty green, grey, or brown, without materially affecting the bright red colour of blood containing carbonic oxide. Various tests for carbonyl-hæmoglobin have been based on these reactions. Thus Salkowski shakes the diluted blood with sulphuretted hydrogen water, which turns normal blood dirty green in a few minutes. Katayama finds a similar change to be produced by yellow ammonium sulphide and acetic acid. Richter substitutes formic acid for acetic acid. Zaleski adds a few drops of a solution of a salt of copper to 4 c.c. of the sample, previously diluted with an equal measure of water. Normal blood gives a chocolate-brown precipitate, carbonic oxide blood a brick-red. Rubner finds a similar reaction to be produced by shaking the blood with four or five measures of lead acetate solution.

A Wetzel has recorded a number of similar reactions (abst. *Jour. Chem. Soc.*, 1890, pp. 432, 1200), but gives the preference

to the following test :—Ten c.c. of the sample of blood, 15 c.c. of a 20 per cent. solution of potassium ferrocyanide, and 2 c.c. of acetic acid (made by mixing 1 volume of glacial acid with 2 of water) are mixed and gently shaken. Coagulation ensues, the mixture gradually becoming solid. If normal blood only is present, the coagulum is dark brown, but in presence of carbonic oxide it is light red. In the latter case, the mass becomes gradually dark brown at the top, the change proceeding gradually to the bottom. If the blood available is limited in quantity, it should be diluted with 5 to 10 measures of water, and 10 c.c. of the diluted liquid treated with 5 c.c. of the ferrocyanide solution and 20 drops of the acetic acid.

Another test described by Wetzel is to dilute the blood with three measures of a 3 per cent. solution of tannin. At the end of twenty-four hours normal blood has a grey colour, while carbonic oxide blood has become carmine-red. The reaction is described as being very delicate and well-suited for the detection of traces of carbonic oxide in air.

The most delicate and satisfactory test for carbonyl-hæmoglobin is, undoubtedly, the observation of its absorption-spectrum. This has a general resemblance to that of oxyhæmoglobin, but the position of the bands is slightly different. A sharp distinction between the two is afforded by the fact that reducing agents produce no change in the spectrum of carbonyl-hæmoglobin. (Compare page 430.) In the case of blood still containing a notable proportion of oxyhæmoglobin, the characteristic spectrum of carbonyl-hæmoglobin may be observed by reducing the former colouring matter with ammonium sulphide, which will probably produce a broad absorption-band extending from about D to E. If the liquid be then gradually diluted, this band (due to reduced hæmoglobin) will fade, while the two bands due to carbonyl-hæmoglobin will appear and persist until a much greater degree of dilution has been reached.

The two bands characteristic of oxyhæmoglobin are only exhibited by normal blood when nearly fresh, since methæmoglobin is soon formed and putrefaction at once causes the reduction of the colouring matter to hæmoglobin. As carbonyl-hæmoglobin resists putrefaction far more strongly, the exhibition of two absorption-bands by blood which has been long kept is regarded by Hoppe-Seyler as a proof of poisoning by carbonic oxide.

If an aqueous solution of carbonyl-hæmoglobin be heated to boiling, it is coagulated, but the red precipitate still shows the two typical absorption-bands. These remain unaffected if the solution

of carbonyl-hæmoglobin be treated in the cold with dilute sulphuric acid, but on heating hæmatoporphyrin is formed.

If an aqueous solution of carbonyl-hæmoglobin be heated to boiling with caustic soda in absence of oxygen, reddish-black crystals of carbonyl-hæmochromogen are deposited. Their solution exhibits the same absorption-spectrum as carbonyl-hæmoglobin. Hoppe-Seyler gives the following measurements of the bands in wave-lengths:—

	Band α.	Band β.
Carbonyl-hæmoglobin,	582·5–561·6	550·5–522·2
Carbonyl-hæmochromogen,	582·5–561·6	550·0–522·2
Hæmochromogen,	565·3–547·4	526·9–513·9

H. W. Vogel (*Ber.*, x. 792) finds that the formation of carbonyl-hæmoglobin affords a very delicate and simple means of detecting carbonic oxide in air. For this purpose he dilutes fresh normal blood with water till the liquid is only faintly tinged with red. About 3 c.c. of this diluted blood is then poured into a 100 c.c. flask containing the air to be examined, and shaken for one minute. In the presence of carbon monoxide the reagent will become of a brighter rose tint, and will show a slightly modified absorption-spectrum. On adding ammonium sulphide, the two absorption-bands characteristic of normal blood will be replaced by a single broad faint band, but the two similar bands of the carbonyl-hæmoglobin spectrum will remain unchanged. The delicacy of the test may be increased by employing a larger volume of air.

A. P. Fokker (*Chem. Centralb.*, xx. 380; *Jour. Soc. Chem. Ind.*, 1884, p. 579) has described the following process, by which it is claimed that it is possible to detect carbonic oxide in a single drop of blood. From 1 to 2 c.c. of the blood to be examined is placed in a small deep beaker, clamped between three bent brass wires. The upper ends of these wires support a watch-glass containing a solution of palladious chloride, while the lower ends are soldered to a round brass plate. The arrangement is placed in a porcelain vessel filled with water, and covered with a narrow bell-glass. About two-thirds of the water is then sucked out of the bell-jar by means of an india-rubber tube. The water rises in the bell-jar, and the beaker, kept upright by the brass plate, swims on its surface. The water is then heated to boiling, when the blood coagulates, and the palladious chloride is reduced by the liberated carbonic oxide. If only traces of this gas are present, the reduction is not complete, and the arrangement must be allowed to stand for twenty-four hours. Free ammonia, if present, will produce a yellow amorphous compound with the palladium

chloride, but carbonic oxide forms a brilliant black metallic mirror. A similar reaction is produced by sulphuretted hydrogen, which is, however, rarely present in fresh blood.

Nitrosyl-Hæmoglobin.

The compound formed by the union of hæmoglobin with nitric oxide is even more stable than the carbonic oxide compound. As a consequence, nitrosyl-hæmoglobin is formed when nitric oxide gas is passed through blood the hæmoglobin of which has already been converted into the carbon monoxide compound. It may also be obtained from ordinary blood, but this should first be freed from oxygen by a current of hydrogen, and the nitrous acid neutralised as it is formed.

The absorption-spectrum of nitrosyl-hæmoglobin exhibits two bands somewhat similar to, but less refrangible than, those of oxy-hæmoglobin. They are unaffected by reducing agents.

The fatal effects which occasionally result from exposure to nitrous fumes are probably due to the formation of nitrosyl-hæmoglobin. A very fatal form of pneumonia results from the inhalation of nitrous fumes.

Carbon Dioxide Hæmoglobin.

If any true chemical compound of hæmoglobin and carbon dioxide exist, it is certainly of a character different from those formed by hæmoglobin with oxygen, carbon monoxide, and nitric oxide. A solution of hæmoglobin certainly takes up much more carbon dioxide than can be explained on the assumption of mere physical absorption. It is probable that the so-called carbon dioxide hæmoglobin is a compound of the gas with hæmochromogen, a coloured substance formed by the action of carbon dioxide on hæmoglobin. This compound, whatever may be its true nature, is stated to exhibit an absorption-spectrum having a single band, the centre of which lies more to the violet than that of hæmoglobin.

Decomposition-Products of Hæmoglobin.

Hæmoglobin readily undergoes change by exposure to air, or by treatment with ferments or chemical reagents. The following are the most important and best-known products of its decomposition.

Methæmoglobin.

If blood or a solution of oxyhæmoglobin be exposed to the air for some time it undergoes a change, which is indicated by a marked difference in its absorption-spectrum, owing to its conversion into methæmoglobin.[1]

[1] The spectrum of methæmoglobin is frequently exhibited by the fluids from ovarian cysts, &c , and by urine containing blood.

The nature of methæmoglobin, and its relation to hæmoglobin and oxyhæmoglobin, have been the subject of much controversy. Methæmoglobin is produced by the action of oxidising agents on oxyhæmoglobin, and hence has been regarded as a more highly oxygenated compound than the latter body. On the other hand, the fact that methæmoglobin may be obtained by the action of hydrogenised palladium on oxyhæmoglobin suggested that it is a compound intermediate between that body and (reduced) hæmoglobin. The question cannot be considered closed, though the view now most generally adopted is that methæmoglobin has the same composition as oxyhæmoglobin, but that the oxygen, which in the latter is only loosely combined, in the former is in a more stable form of combination, so as not to be removable by a vacuum, by carbonic oxide, or by a current of hydrogen. Methæmoglobin crystallises in the same form as oxyhæmoglobin, and may be converted into that body by reduction and subsequent exposure to air.

W. D. Halliburton states that crystals of methæmoglobin, in quantity sufficient for microscopic examination, may be obtained by shaking a few c.c. of defibrinated blood with a few drops of amyl nitrite for about a minute. If a drop of the mahogany-coloured liquid thus obtained be placed on a microscope-slide, it yields an abundant crop of crystals in a few seconds. In the guinea-pig these are tetrahedra, but in the product from rat's and squirrel's blood they are a mixture of hexagons and rhombic prisms, the former largely predominating.

Methæmoglobin may be obtained in sufficient quantity for spectroscopic purposes by adding a few drops of a dilute (0·5 to 1·0 per cent.) solution of potassium ferricyanide to 10 c.c. of a moderately strong solution of oxyhæmoglobin, and warming the liquid very gently.[1] The spectrum is then observed, and if the absorption-bands of oxyhæmoglobin are still strongly marked, another drop or two of the ferricyanide solution should be added, and the warming repeated. If the liquid be now examined with a spectroscope it will exhibit the appearance described on page 428.

[1] Various other agents are capable of effecting the conversion of oxyhæmoglobin into methæmoglobin. Among these are metallic nitrites, amyl nitrite, permanganates and chlorates, iodised potassium iodide, bromine, sodium fluoride, osmic acid, kairine, quinol, catechol, ether, terebenthene, and certain acids.

According to G. Hayem (*Compt. rend.*, cii. 698), amyl nitrite and kairine convert the hæmoglobin into methæmoglobin without any other alteration of the corpuscles or the serum, whereas most of the other substances destroy the blood-corpuscles more or less completely. Ferricyanides, however, act only on dissolved hæmoglobin, and exercise no action on the pigment as it exists in the corpuscles (compare footnote on page 400).

Hæmatoïdin was the name given by Virchow to a substance crystallising in reddish or orange rhombohedra, which is often found in old blood-clots, cerebral hæmorrhages, hæmaturia, &c. Though evidently a decomposition-product of hæmoglobin, the exact nature of hæmatoïdin was long in dispute. It is, however, established that the substance is extremely similar to, if not absolutely identical with *bilirubin* (Vol. III. Part iii. page 404), a fact which indicates the intimate relationship between hæmoglobin and the pigments of bile.[1]

Hæmatin. $C_{32}H_{32}N_4FeO_4$.

On adding either acid or alkali to a solution of oxyhæmoglobin, the bright red colour changes to brown. This alteration is due to the decomposition of the original molecule into a brown ferruginous pigment called hæmatin, and a proteid called globin.

Globin is produced from hæmoglobin, either by the action of heat, or by treatment with an alkali or an acid. In the first case the globin is coagulated, and in the latter is converted into the condition of alkali-albumin and syntonin respectively. In all cases, hæmatin is simultaneously formed. Globin belongs to the globulin class of proteids (page 3). Its solutions are precipitated by saturation with sodium chloride or magnesium sulphate.

Decomposition of oxyhæmoglobin into hæmatin and globin often occurs in old blood-clots or extravasations, and may be readily produced by the action of either gastric or pancreatic juice on oxyhæmoglobin. Hence, hæmatin is often found in the alimentary canal and in the fæces, and occurs in the urine after poisoning by sulphuric acid or arseniuretted hydrogen.

It has been suggested that hæmoglobin is not a true chemical unit, but consists of hæmatin mixed with a crystallisable proteid. A careful review of the facts affords little support to this theory. The discrepancies in the ultimate analysis of oxyhæmoglobin are sufficiently explained by the difficulty of obtaining the substance thoroughly water-free and in a condition of absolute purity.

Zinoffsky states that on decomposition hæmoglobin yields one molecule of hæmatin with 34 atoms of carbon, and two molecules of globin, each containing 339 atoms of carbon and 1 atom of sulphur.

According to J. A. Menzies (*Jour. Physiol.*, 1885, xvii. 402),

[1] $\underset{\text{Hæmatin.}}{C_{32}H_{32}N_4O_4Fe} + 2H_2O = \underset{\text{Bilirubin.}}{C_{32}H_{36}N_4O_6} + Fe.$

Whether hæmatin is converted in the liver into bilirubin, or whether the reverse change occurs, is uncertain, but Nencki and Sieber consider the latter reaction the more probable.

when methæmoglobin is produced by the spontaneous decomposition of oxyhæmoglobin the solution becomes acid, and if decomposition proceeds further, whereby the reaction changes to alkaline, a reconversion to hæmoglobin takes place. If the decomposition be caused by reagents, their further action gives rise to hæmatin, which is precipitated unless held in solution by acids. When potassium ferricyanide is employed, the hæmatin passes into the condition of cyanhæmatin.

Hæmatin may be prepared in moderate quantity by making defibrinated blood into a thin paste with potassium carbonate, and drying the mixture at 100° C. The residue is powdered, and extracted with about four times its bulk of boiling methylated spirit. The deeply coloured liquid is poured off, and the residue again extracted with boiling spirit, and the extracts mixed and filtered. The product is a solution of hæmatin in alkaline alcohol, and gives the single-banded absorption-spectrum of alkaline hæmatin (page 425). On gradually adding sulphuric acid till the reaction is strongly acid, potassium sulphate is precipitated, and may be filtered off. The solution now gives the spectrum of acid hæmatin. To obtain the dissolved hæmatin free from salts, the acid liquid should be treated with ammonia in excess, evaporated to dryness at 100°, and the salts dissolved from the residue by repeated boiling with water. The residual hæmatin may be washed successively with alcohol and ether, and dried at 130° to 150°.

Hæmatin is readily obtained in a pure state by boiling hæmin crystals (page 415) with strong acetic acid, and washing the residue successively with water, alcohol, and ether. It is then dissolved in dilute caustic alkali, the solution treated with hydrochloric acid, and the precipitate washed with boiling water till free from chlorides. The hæmatin is dried by prolonged exposure to a temperature of 130° C.

C. A. MacMunn (*Jour. Physiol.*, vi. 22) extracts clotted blood with rectified spirit containing 1 part in 18 of strong sulphuric acid. The resultant solution is filtered, diluted with an equal measure of water, and shaken with chloroform. The latter, which is coloured reddish-brown, is separated, filtered, and agitated with water to remove acid. On evaporating the chloroform, the hæmatin is left as a dark brown pigment, which dries up to a bluish-black powder. If the chloroform solution of hæmatin is allowed to stand at rest for a few hours, it deposits hæmatin in crystals resembling in shape and colour those of hæmin (page 416).

As obtained by the foregoing methods, hæmatin forms a non-crystalline, bluish-black, scaly substance resembling iodine. The powder is brown. Hæmatin is quite insoluble in either hot or

cold water, or in alcohol alone, but is dissolved by hot alcohol acidulated with sulphuric acid. It is commonly stated to be insoluble in ether or chloroform, but when a solution is strongly acidulated with acetic acid and agitated with ether, the latter liquid dissolves sufficient hæmatin to exhibit the absorption-spectrum of that body, and MacMunn's process of preparing hæmatin is actually based on its extraction from an acid liquid by chloroform. Hæmatin dissolves tolerably readily in strong acetic acid, especially if warm, and is also soluble in acidulated alcohol, but not in acidulated water. It is readily soluble in water or alcohol containing alkali.

Hæmatin is a remarkably stable substance. It is not susceptible of putrefaction, and is unaffected by the gastric and pancreatic juices. It is unaltered by treatment with caustic alkalies, even when heated, and is not decomposed by cold hydrochloric or nitric acid. By treatment with reducing agents in presence of an alkali, hæmatin is converted into hæmochromogen (page 417). When treated with strong sulphuric acid, hæmatin is dissolved with violet-red coloration and conversion into an iron-free colouring matter called hæmatoporphyrin (page 418). The same substance is formed by the action of fuming hydrochloric acid.

By the action of tin and hydrochloric acid, hæmatin is converted mainly into hexahydro-hæmatoporphyrin, a reddish-brown pigment, soluble in alcohol, but insoluble in water and alkalies.

Hæmatin does not combine with carbonic oxide, unless an alkali and reducing agent be added, when it is converted into carbonyl-hæmochromogen.

Hæmatin may be exposed to a temperature of 180° without change, but when more strongly heated is decomposed with evolution of hydrocyanic acid. On ignition in the air it leaves 13·51 per cent. of ferric oxide.

Solutions of hæmatin in water or alcohol containing alkali appear olive-green when examined in thin layers by reflected light, but by transmitted light they appear of a red colour, and absorb powerfully the portion of the spectrum in the region of the D line (see further, pages 425, 429).

Hæmin.—When hæmatin is heated to about 80° with glacial acetic acid and a fragment of sodium chloride, and the liquid rapidly cooled, crystals are deposited which are known as hæmin or Teichmann's crystals. Their formation affords a delicate and characteristic test for hæmatin and hæmoglobin, but their composition and crystalline form vary somewhat with the relative pro-

portions of hæmatin and acetic acid, and are also dependent on the temperature. The crystals contain chlorine as an essential constituent, but by substituting a bromide or iodide for a chloride, exactly similar compounds containing bromine or iodine are obtained.[1]

According to Hoppe-Seyler, the formula for hæmatin is $C_{68}H_{70}N_8Fe_2O_{10}$, the hæmin formed therefrom by reaction with hydrochloric acid being an additive-compound containing $C_{68}H_{70}N_8Fe_2O_{10},2HCl$. On the other hand, the researches of Nencki and Sieber (*Ber.*, xvii. 2267; xviii. 392) confirmed by those of Bialobrzeski and of Küster[2] (*ibid.*, xxix. 821; xxx. 105), appear to prove definitely that the reaction of hæmatin with the halogen-acids occurs with elimination of the elements of water —that is, a hydroxyl-group is exchanged for chlorine, bromine, or iodine. Nencki and Sieber ascribe to hæmatin the formula $C_{32}H_{31}N_4FeO_3.OH$, and regard the compound formed with hydrochloric acid as *chlor-hæmatin*, containing $C_{32}H_{31}N_4FeO_3.Cl$.

The study of the hæmins is complicated by the facility with which they unite with acetic acid, ethylic alcohol, amylic alcohol, &c., to form compounds of considerable stability. Further, the researches of W. Küster point to the existence of several homologous hæmatins.[3]

Nencki and Sieber give the formula $(C_{32}H_{31}N_4FeO_3.Cl)_4,C_5H_{12}O$ for chlor-hæmatin deposited from iso-amylic alcohol, and this is confirmed by Bialobrzeski; but Küster found twice the above proportion of amylic alcohol in the crystals. The compound is unaltered by digestion with alcohol, or by heating to 110° C., but at 130° to 135° it loses its amylic alcohol. The crystals are

[1] Compounds formed by the reaction or union of hæmatin with hydrocyanic acetic, valeric, and tartaric acids have also been described.

[2] Küster considers that a substance found by Cloëtta to contain nitrogen and iron in the atomic ratio of 3 : 1 had undergone alteration, owing to the action of the concentrated sulphuric acid used in its preparation.

H. Struve has published researches the conclusions of which are at variance with the results of other observers (abst. *Jour. Chem. Soc.*, xlviii. 71).

[3] Küster obtained a hæmatin acetate containing :—

$$(C_{32}H_{31}N_4FeO_3.OAc)_4,CH_3.COOH.$$

He also obtained two specimens of chlor-hæmatin containing $C_{32}H_{33}N_4FeO_3.Cl$ (one of these crystallised with one-sixth molecule of amylic alcohol, and another with an additional CH_2), whilst a brom-hæmatin prepared by him contained $C_{31}H_{31}BrN_4FeO_3,EtOH$. By the action of hydrobromic acid on hæmatin in solution in absolute alcohol, the compound $C_{32}H_{31}N_4FeO_3.Br,EtOH$ was obtained in small, dark-coloured, rhombic crystals (*Ber.*, xxvii. 572).

not changed in composition by digestion with dilute hydrochloric acid, but on dissolving the compound in dilute soda solution amylic alcohol is liberated, and on subsequently acidulating the liquid with hydrochloric acid, hæmatin, $C_{32}H_{31}N_4FeO_3.OH$, is precipitated. On boiling hæmatin or hæmin for some time with acetic anhydride, a change in composition is effected, probably from the introduction of acetyl-groups.

For the preparation of hæmin crystals in quantity, Nencki and Sieber (*Ber.*, xvii. 2267) mix freshly defibrinated blood with a solution of common salt, and allow it to stand for twenty-four to forty-eight hours in shallow dishes. Two measures of absolute alcohol should then be added, and the mixture stirred well till thoroughly coagulated. After standing twenty-four hours, the coagulated mass is filtered off and spread on blotting paper. After partial drying by exposure to the air for about twenty-four hours, the mass is powdered, and 400 grammes heated to boiling with 1600 grammes of amylic alcohol. 25 c.c. of hydrochloric acid (sp. gr. 1·12) should then be added, and the liquid boiled for ten minutes and filtered. On cooling, the chlor-hæmatin is deposited in glittering rhombic plates, which should be washed with alcohol and ether, and dried at 105° C. The yield is only from $1\frac{1}{2}$ to 3 grammes of pure crystals from three litres of blood. The crystals were of the same composition, whether derived from human blood, or that of the ox, horse, or dog.

For the preparation of hæmin crystals, Schalféeff (abst. *Jour. Chem. Soc.*, 1885, p. 566) adds defibrinated and filtered ox-blood to an equal measure of glacial acetic acid previously heated to 80° C., and reheats the mixture to that temperature.[1] On cooling, an abundant deposition of crystals occurs. The crystals are repeatedly washed with water, collected on a filter, and again washed successively with water, alcohol, and ether. According to M. Bialobrzeski, the composition of the hæmin crystals thus obtained is best represented by the following formula :—

$$(C_{32}H_{31}N_4FeO_3.Cl)_3 + C_{32}H_{31}N_4FeO_3.OAc + HOAc.$$

No acetic acid is liberated by simple boiling with water, but by treating the crystals with dilute caustic soda, sodium acetate is formed.

[1] If the mixture is heated above 80°, the crystalline deposit is diminished, or the crystals redissolve and do not separate again on cooling. The amount of hæmin crystals obtained by Schalféeff's method is stated to be from 83 to 90 per cent. of the theoretical yield, and never less than 5 grammes per litre of blood.

Chlor-hæmatin or hæmin, obtained by the foregoing methods, forms thin rhombic plates (fig. 13) of a brownish-red or dirty brown colour, having a dark violet-blue metallic reflection. The crystals are bi-refractive and pleo-chromatic. They make a brown mark on porcelain.

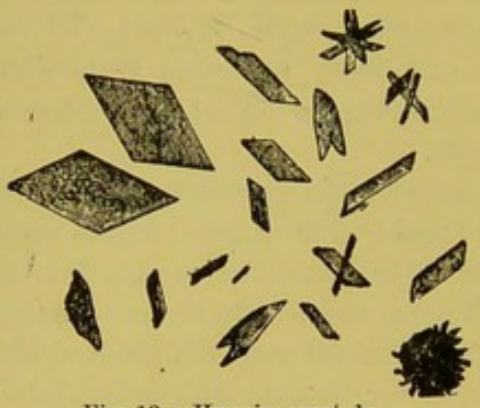


Fig. 13.—Hæmin crystals.

Hæmin is insoluble in water, alcohol, ether, or chloroform. It is also insoluble in cold dilute hydrochloric or acetic acid, but dissolves without decomposition in either of these acids when concentrated. Cold dilute sulphuric acid does not affect hæmin, but the concentrated acid dissolves it with violet-red coloration (compare page 418), and hydrochloric acid gas is evolved on heating.

Nitric acid of 1·2 specific gravity does not attack hæmin at the ordinary temperature, but at 100° C. it oxidises and completely decomposes it.

Hæmin is not affected by an aqueous solution of sodium carbonate, but is dissolved by caustic alkalies and by ammonia. It is reprecipitated from these solutions by dilute acids or by calcium salts.

Hæmin is substantially unaltered by exposure to a temperature of 200° C. When ignited in the air it leaves a residue of ferric oxide (13·10 per cent.).

Teichmann's Test for Blood.—The formation of hæmin crystals affords one of the most delicate and characteristic tests for the colouring matter of blood, and hence is utilised for the chemico-legal recognition of blood-stains.

To obtain microscopic crystals of hæmin, a small drop of blood should be allowed to evaporate on a glass microscopic slide.[1] A minute crystal of common salt is then added, and this is followed by a drop of glacial acetic acid. A cover-glass is then applied, and the liquid cautiously heated until it becomes brown and begins to boil.[2] The slide is then cooled, and examined with

[1] It is important that the blood should be evaporated. If the test be performed on the original blood, or an aqueous solution of the colouring matter, the result is liable to be unsatisfactory, probably owing to the dilution of the acetic acid.

[2] V. Schwartz recommends that, instead of heating the slide, the acetic acid should be allowed to evaporate spontaneously.

S. M. Copeman makes the mixture in a watch-glass, over which

a microscopic power of 300 diameters. Amid a brown amorphous mass, numerous minute crystals of hæmin ("Teichmann's blood-crystals") will be observed.[1] These assume the form of dark reddish-brown or nearly black rhomboids (fig. 14), sometimes aggregated into radiating bundles, while other crystals present the form of a cross. Occasionally the hæmin crystals are oval, and not unlike crystals of uric acid deposited from urine, except for the orange colour of these latter.

Fig. 14.—Crystals of hæmin.

In applying this test to the examination of suspected blood-stains, the red stain—whether on a textile fabric, wood, stone, or metal—should be cut out, scraped, or chipped away from its surroundings, and dissolved in a little cold distilled water, with the addition of ammonia if necessary to effect solution. A portion of the extract thus obtained is then evaporated to dryness with a minute fragment of sodium chloride, and the residue treated on a glass slide with glacial acetic acid in the manner already described.

If potassium iodide be substituted for common salt in the above test, exactly similar crystals of *iodhæmatin* are obtained. This modification of the reaction is preferred by Husson and Hénocque for the examination of blood-stains.

Bufalini (abst. *Jour. Chem. Soc.*, 1886, p. 184) heats the aqueous extract of the blood-stain with a drop of tincture of iodine, and adds a very little acetic acid. A drop of the liquid is placed on a glass slide, and this is repeatedly passed through a flame, whilst eight to ten drops of acetic acid are gradually added. By this treatment, crystals of iodhæmatin are stated to be obtained with absolute certainty in a few minutes.

HÆMOCHROMOGEN. $C_{32}H_{30}N_4FeO_4$ (or $C_{34}H_{37}N_4FeO_5$).

Hæmochromogen is obtained, instead of hæmatin, when hæmoglobin is treated with caustic alkali solution in the absence of oxygen. It is readily obtained by treating hæmoglobin solution or diluted blood with caustic soda and ammonium sulphide or other reducing agent (page 430). By oxidation it can be converted into hæmatin, and hence was called by Stokes reduced

another is inverted. The arrangement is heated to such a degree that the glasses are not too hot to be held between the fingers, when a drop of the liquid is placed on a slide, covered, and examined under the microscope.

[1] Hæmin crystals must not be confounded with those of oxyhæmoglobin, sometimes called "blood-crystals," described and illustrated on page 399.

hæmatin. Hoppe-Seyler has suggested that the iron in hæmochromogen is in the ferrous state, while that in hæmatin is in the ferric condition. His formula for hæmochromogen shows two atoms of hydrogen more than are present in the molecule of hæmatin. Hæmochromogen solutions exhibit a highly characteristic absorption-spectrum (page 425), the production of which affords one of the most delicate and certain means of recognising old blood-stains as such (see page 429).

Carbonyl-hæmochromogen is stated by Hoppe-Seyler (*Zeit. physiol. Chem.*, xiii. 477) to be formed when carbonic oxide is passed through a solution of hæmochromogen, or a solution of carbonyl-hæmoglobin heated to boiling with caustic soda in the absence of oxygen. The absorption-spectra of carbonyl-hæmoglobin and carbonyl-hæmochromogen are identical.

Hoppe-Seyler considers it probable that it is hæmochromogen which in the red corpuscles and in oxyhæmoglobin is combined with respiratory oxygen, and in carbonyl-hæmoglobin with carbonic oxide (compare footnote, page 400).

Hæmochromogen also forms a compound with nitric oxide.

Hæmatoporphyrin is produced by the action of strong sulphuric acid on hæmatin or hæmin.[1] The hæmatin should be well mixed with concentrated sulphuric acid, and the liquid filtered through asbestos. The filtrate is of a purple-red colour, and on diluting it with a large quantity of water the greater part of the hæmatoporphyrin is thrown down as a brown flocculent precipitate. If the acid solution be nearly neutralised by an alkali, further precipitation occurs. The iron of the hæmatin remains in solution as a ferrous salt, the reaction, according to Nencki and Sieber, being represented by the equation :—

$$C_{32}H_{32}N_4FeO_4 + H_2SO_4 + O_2 = C_{32}H_{32}N_4O_5 + FeSO_4 + H_2O.$$

According to Nencki and Sieber's first accounts, hæmatoporphyrin contains $C_{32}H_{32}N_4O_5$, but in a more recent research (*Monatsh.*, ix. 115) they regard the substance of this formula either as a mixture or as an anhydride of true hæmatoporphyrin, which they prepare by acting on hæmin with a saturated solution of hydrobromic acid gas in glacial acetic acid. To the body thus obtained they ascribe the formula $C_{16}H_{18}N_2O_3$, identical with that of anhydrous bilirubin, which body hæmatoporphyrin resembles in many of its properties.

True hæmatoporphyrin is stated by Nencki and Sieber to be insoluble in water and dilute acetic acid, and only slightly soluble

[1] Hæmatoporphyrin is also obtained by heating hæmatin or hæmin with fuming hydrochloric acid in sealed tubes at 130° C.

in amyl alcohol, ether, or chloroform. It is easily dissolved by alcohol, dilute mineral acids, and solutions of caustic and carbonated alkalies. The substance is reddish, amorphous, and very unstable, turning brown and becoming insoluble in alkalies or hydrochloric acid after being heated to 100° C. *Hæmatoporphyrin hydrochloride*, $C_{16}H_{18}N_2O_3$,HCl, crystallises in tufts of needles, and the *sodium salt*, $C_{16}H_{17}NaN_2O_3 + H_2O$, in microscopic prisms.

When introduced into the system, hæmatoporphyrin is partly expelled in the urine,[1] but the greater portion is retained, and probably utilised in the formation of hæmoglobin.

For the detection of hæmatoporphyrin in urine, Hammarsten precipitates the liquid with barium acetate and filters. The filtrate is precipitated alternately with barium acetate and sodium carbonate, until a small filtered portion no longer gives a white precipitate with each of these reagents. The hæmatoporphyrin is carried down in the precipitates, which are washed well and extracted with acidified alcohol. The acid alcoholic solution is diluted with several times its measure of water, and shaken with chloroform, which extracts most of the colouring matter. The chloroformic layer is rapidly tapped off from the upper stratum, washed well with water, and evaporated in shallow basins in the dark. According to Hammarsten, the brown residue left after evaporation is soluble with splendid purple colour in chloroform, insoluble in cold water and in very dilute acids, sparingly soluble in cold alcohol, but soluble in hot alcohol, from which it crystallises in needles resembling those of the hæmatoporphyrin hydrochloride of Nencki and Sieber. On spectroscopic examination, the absorption-bands were found to be slightly nearer to the red end of the spectrum than those of the hæmatoporphyrin obtained by Nencki

[1] Hæmatoporphyrin has been shown by A. E. Garrod (*Jour. Physiol.*, 1894, 349; abst. *Jour. Chem. Soc.*, 1895, ii. 55) to be present in small amount in normal urine. For its isolation, Garrod directs that 20 c.c. of a ten per cent. solution of sodium hydroxide be added to every 100 c.c. of the urine, and the precipitated phosphates washed with water. The precipitate is then dissolved in rectified spirit to which sufficient hydrochloric acid has been added to dissolve the phosphates. The solution thus obtained shows the spectrum of *acid* hæmatoporphyrin. Ammonia is next added to neutralise the hydrochloric acid, and the precipitated phosphates redissolved by acetic acid. Chloroform extracts the whole of the hæmatoporphyrin from this liquid, and the chloroformic solution exhibits the spectrum of *alkaline* hæmatoporphyrin.

O. Hammarsten (*Jour. Chem. Soc.*, lxii. 649 and 1136) has pointed out that hæmatoporphyrin is very frequently present in dark-coloured urine excreted after the administration of sulphonal, and his suggestion that sulphonal is the cause of the appearance of hæmatoporphyrin in the urine has been fully confirmed by Salkowski and others.

and Sieber. In only one case out of the four did the substance appear to be absolutely identical with their product. In another case the chromogen of a similar colouring matter was met with.

Hæmatoporphyrin exhibits different spectra according as it is in neutral, acid, or alkaline solution.[1] The spectrum of acid hæmatoporphyrin is characterised by two absorption-bands, one of which adjoins D on the red side of the line, whilst the other, which is very strongly marked, lies midway between D and E. To obtain the spectrum of alkaline hæmatoporphyrin, the precipitate produced on neutralising the diluted sulphuric acid solution obtained in the preparation of hæmatoporphyrin from hæmatin should be dissolved in dilute alkali. The spectrum of alkaline hæmatoporphyrin, as shown by this solution, is distinguished by four absorption-bands,—one half-way between C and D, two between D and E, and one very wide band extending from *b* nearly to F (see fig. 15, page 425). Neutral hæmatoporphyrin shows a five-banded spectrum.

A solution of hæmatoporphyrin in ammonia and zinc chloride also exhibits four absorption-bands. The two lying between C and D and between *b* and F disappear within twenty-four hours, the former first. The other two bands are permanent.

Hæmatoporphyrin occurs naturally in many invertebrates. Thus it exists in the dorsal streak of the earth-worm. It is also found in the egg-shells of some birds. A substance which has received the name of *polyp-erythrin*, a pigment present in various actiniæ and deep-sea polypes, is probably identical with hæmatoporphyrin.

A. Gamgee (*Proc. Roy. Soc.*, 1896, 339 ; abst. *Jour. Chem. Soc.*, 1896, i. 714) has shown that turacoporphyrin and hæmatoporphyrin give similar spectra and are essentially identical.

Hæmatolin is the name given by Hoppe-Seyler to a substance which is produced in small amount along with hæmatoporphyrin by the action of sulphuric acid upon hæmatin out of contact with the air. Unlike hæmatoporphyrin, it is insoluble in sulphuric acid and in caustic alkalies.

Urohæmatoporphyrin is regarded by MacMunn as solely a reduction-product of hæmatin, which has been produced in the organism by the reduction of effete hæmoglobin or effete histohæmatin. It has been found in certain diseased conditions, such as acute rheumatism, pneumonia, peritonitis, &c.[2]

[1] Hæmatoporphyrin may be quickly obtained for spectroscopic purposes by adding a small quantity of blood to a large quantity of sulphuric acid.

[2] Urohæmatoporphyrin may be obtained from urine containing it by the method used for the isolation of urobilin. The pigment is mainly precipitated when neutral and basic lead acetate are added to the urine until no

Urohæmatoporphyrin can be prepared artificially by the action on hæmatin of sodium-amalgam, zinc and sulphuric acid, and other reducing agents. In acid solutions, the spectrum exhibits a narrow absorption-band almost coincident with the D line, and another darker band between D and E; besides a feeble shading between these two, and a band at F closely resembling that of urobilin. If the alcoholic solution of the isolated colouring matter be treated with ammonia, the liquid shows a five-banded spectrum closely resembling that of neutral hæmatoporphyrin.

Hæmatoporphyroïdin is a product obtained by Le Nobel from the decomposition of hæmatoporphyrin.

Determination of the Colouring Matter in Blood.

The proportion of hæmoglobin in blood averages 14 per cent. The exact amount may be deduced from a determination of the iron, or from a colorimetric comparison of the depth of colour with that of a solution of pure oxyhæmoglobin or other standard solution.

For the determination of the iron, at least 25 (and preferably 50) grammes of blood should be evaporated on the water-bath, and the residue treated as described on page 294. When the greater part of the organic matter is consumed, the ash should be boiled with hydrochloric acid, and the liquid diluted and filtered. In the filtrate the iron is determined by one of the ordinary volumetric methods.[1] Dry oxyhæmoglobin contains 0·42 per cent. of iron. Hence the amount of the metal found, multiplied by the factor 238·1 ($=\frac{100}{0·42}$), gives the weight of oxyhæmoglobin in the quantity of blood employed for the experiment.[2]

A. Jolles (abst. *Analyst*, 1897, page 164) has described a method, well-suited for clinical purposes, of colorimetrically determining the iron in blood. 0·05 c.c. of the blood, drawn by suction with a capillary pipette from the tip of the finger, is placed in a platinum crucible with a little water. The liquid is evaporated to dryness, and the residue ignited. 0·1 gramme of dry powdered acid potassium sulphate is then added, and the contents

further precipitate is formed. The precipitate is extracted with alcohol acidified with sulphuric acid. From this solution chloroform extracts urobilin, together with any urohæmatoporphyrin which may be present.

[1] Reduction of iron to the ferrous state by means of zinc, and titration with an $\frac{N}{50}$ solution of potassium permanganate or bichromate is a suitable method.

[2] The blood of an average man weighs about 4536 grammes, or approximately 10 lbs. Taking the percentage of hæmoglobin in blood at 14 per cent., the entire blood of the body will contain 593 grammes of hæmoglobin, or about 21 ounces. The iron in this quantity, representing the total blood of a man, will be only 2·67 grammes, or about 41 grains.

of the crucible fused. After cooling, the mass is dissolved in hot water, and the iron in solution determined colorimetrically by potassium ferrocyanide.

Several colorimetric methods of determining the actual colouring matter in blood have been devised. When carefully conducted, they afford very fair approximations to the truth, and, the errors being constant, they are well-suited for comparative determinations.

A standard solution of oxyhæmoglobin is first made, and the depth of colour of the diluted blood compared with it. The standard solution may contain 0·1 per cent. of oxyhæmoglobin, and the blood may be primarily diluted with ninety-nine times its measure of water. The oxyhæmoglobin should be recrystallised, dissolved in ice-cold water, and the solution filtered. Instead of weighing out a definite quantity of the dry substance, an approximately equivalent amount of the moist crystals may be dissolved, and the strength of the solution ascertained by evaporating 50 c.c. to dryness at 100° and weighing the residue. Oxyhæmoglobin does not keep well in the solid state, but Hoppe-Seyler states that a saturated aqueous solution may be kept in a hermetically sealed tube for an indefinite time, without undergoing any change beyond the reduction of the colouring matter to hæmoglobin. On opening the tube the solution rapidly absorbs oxygen, with re-formation of oxyhæmoglobin.

Instead of employing hæmoglobin as a standard, it is more convenient in practice to substitute an ammoniacal solution of picro-carmine,[1] which, if perfectly neutral, may be kept without change in closed bottles for a long time. This solution, if once standardised against pure oxyhæmoglobin, can be used in its place in future experiments.

The comparison of the colour of the diluted sample of blood with that of the standard should be carried out in two tubes of about one centimetre in diameter, and graduated in tenths of a centimetre.[2]

[1] For the preparation of "picrocarmine," 1 gramme of carmine should be dissolved in hot water, with the aid of ammonia, and the solution added to 100 c.c. of a boiling hot saturated solution of picric acid in cold water. The mixture is evaporated to dryness, the residue dissolved in 100 c.c. of water, and the solution filtered. If the solution is not clear, more ammonia should be added, and the evaporation and re-solution repeated. This solution is then gradually added to glycerin diluted with an equal measure of water containing a little phenol, until the tint corresponds exactly with that of the standard solution of oxyhæmoglobin it is desired to imitate. If the shade is too yellow, a little perfectly neutral solution of carmine should be added.

[2] The tubes must be of exactly equal calibre, which will be the case if the same measure of water (say 25 c.c.) fills them both to the same height.

Ten c.c. of the standard solution should be placed in one tube, and an equal measure of the diluted sample in the other. The latter is then further diluted with water until its tint corresponds exactly with that of the standard. The comparison is best made with a Lovibond's tintometer, but perfectly good results can be obtained by placing a piece of wet blotting-paper behind the tubes and holding them, side by side, up to a good light—preferably a window having a north light.

It is evident that when the tints are equalised the proportions of colouring matter in the two liquids are inversely as their volumes. Thus if 10 c.c. of the diluted blood has required to be further diluted to 13·8 c.c. to reduce its tint to that of the standard solution containing 0·1 per cent. of oxyhæmoglobin, the proportion of colouring matter in the original sample of blood was 13·8 per cent. Hence, if the foregoing directions as to the preparation of the standard solution and the dilution of the blood sample have been followed, the volume occupied by the diluted sample, after adjustment of its tint, gives directly the percentage of colouring matter in the original blood.

Hoppe-Seyler prefers to convert the oxyhæmoglobin of the sample of blood into hæmatin, and compare the colour of the liquid with that of a standard solution of hæmatin. This is prepared by dissolving 0·050 gramme of hæmatin (dried at 120°) or 0·525 of hæmin crystals in 500 c.c. of water containing a little ammonia. For the determination, 10 c.c. of defibrinated blood is exactly weighed, and at least one-tenth of its measure of strong acetic acid added. The liquid is then heated on a water-bath for some minutes, allowed to cool, rendered alkaline with dilute ammonia, and diluted to 100 c.c. Ten c.c. of this solution may be further diluted to 1 litre, and the colour of the solution compared with the standard, in the manner employed in nesslerising. If the tubes are viewed transversely, as described above, more concentrated solutions should be employed.

In a more recent publication (*Zeit. physiol. Chem.*, xvi. 505), Hoppe-Seyler has proposed to use carbonyl-hæmoglobin as a standard of comparison. He recommends that a large quantity should be prepared, as it will remain unchanged for years if kept in well-stoppered bottles. The sample of blood to be examined should be treated with a stream of carbonic oxide, to convert its colouring matter into carbonyl-hæmoglobin.

A glass of suitable red tint, such as is supplied with Lovibond's tintometer, will enable all liquid standards to be dispensed with, and possesses other practical advantages.

Gower (*Lancet*, ii., 1878, p. 822) has described a "hæmoglo-

binometer" based on the foregoing principles, which is very suitable for clinical use.[1]

J. Jutt (*Chem. Centralb.*, 1895, ii. 683) has based a method of determining the colouring matter of blood on the formation of compounds of oxyhæmoglobin with zinc and copper salts.

Other methods of estimating the colouring matter of blood have been based on the estimation of the light as viewed in a spectroscope or polarimeter, on the amount of oxygen absorbed, &c., but they mostly require the use of special apparatus, and are not superior in accuracy to the processes already described.

By ascertaining the richness of a sample of blood in corpuscles, by means of the hæmacytometer (page 394), and then determining the proportion of colouring matter, a comparison is obtained between the number of corpuscles and the amount of hæmoglobin. By dividing the percentage of colouring matter by the percentage of corpuscles, the average value of the corpuscles is obtained. Thus if a sample of blood contain 60 per cent. of the normal proportion of colouring matter and 90 per cent. of the average proportion of corpuscles, the value of each corpuscle is only two-thirds of the normal.

Absorption-Spectra of Hæmoglobin and its Allies.

One of the most valuable and delicate means of detecting hæmoglobin, and of studying its decomposition and conversion into allied products, consists in the observation of its absorption-spectrum.[2]

Spectrum of Oxyhæmoglobin.—On placing a flat glass cell containing fresh blood diluted with water in front of the slit of a spectroscope, the spectrum is modified according to the strength of the blood solution employed. With a strong solution, the whole of the yellow and green light from the Fraunhofer-line D to a point about midway between *b* and F is cut out. With gradually increasing dilution the region of absorption becomes narrower and light passes through the centre. This effect

[1] The apparatus is made by Mr Thomas Hawksley, 357 Oxford Street, London, W.C. The standard of comparison, which is sold with the apparatus, is a glycerin jelly tinted with picrocarmine so as to correspond with the colour of normal blood diluted to one-hundredth with water.

[2] The method was applied in 1862 by Hoppe-Seyler to the detection of blood in chemico-legal cases. In 1864, Stokes observed that two distinct spectra were yielded by blood, according to the condition of oxidation of the colouring matter; and shortly afterwards H. C. Sorby improved the method of manipulation, and adapted the spectroscope to the examination of microscopic quantities of blood (*Quart. Jour. Science*, 1865, xi. 198).

increases until, with the most suitable dilution, there will be observed a somewhat narrow black absorption-band with well-

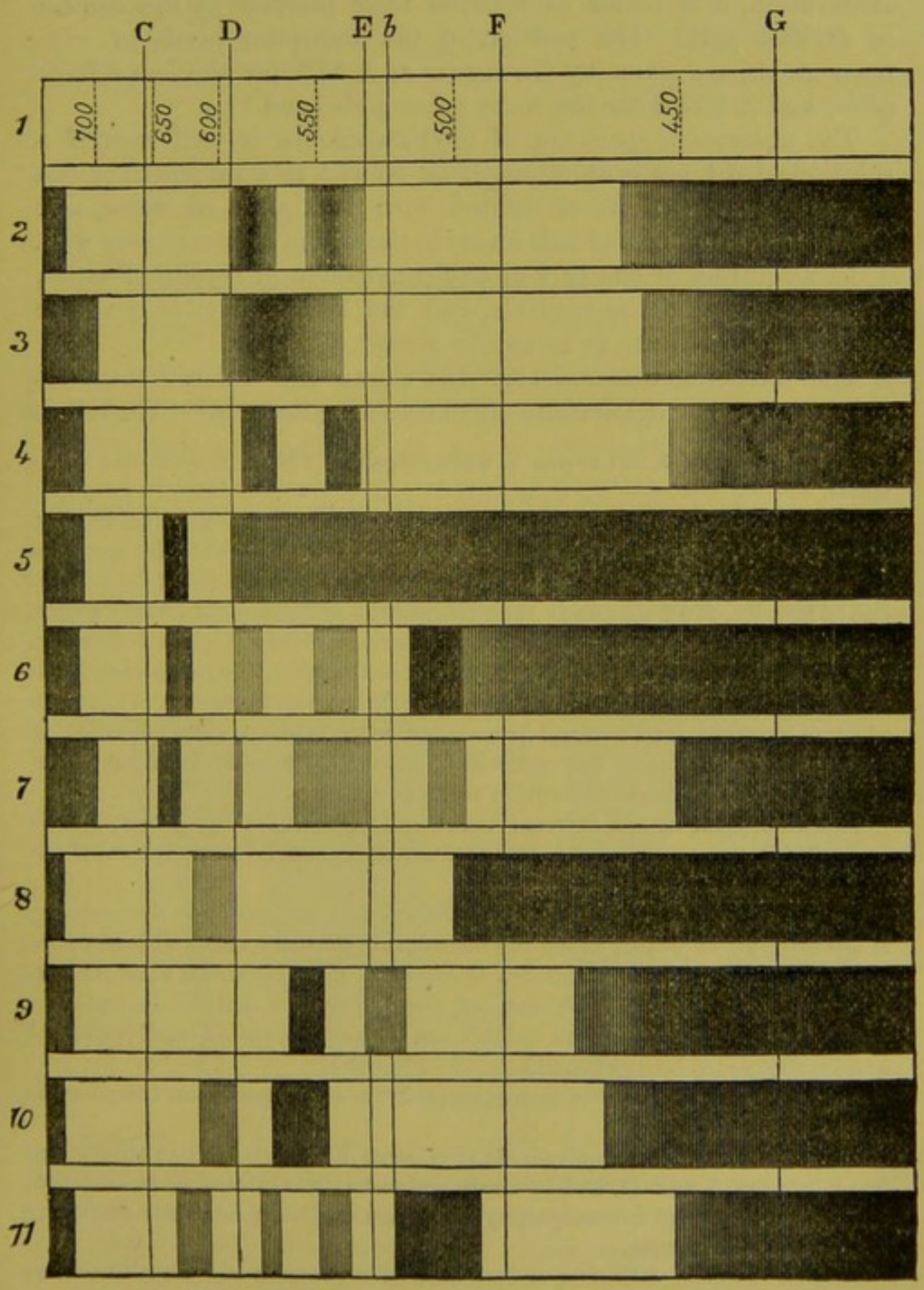


Fig. 15.—Absorption-spectra of Blood Colouring Matters.

defined edges having its centre near the D line, and a second, wider and fainter, band having its greatest intensity on the less

refracted side of the E line. As the widths of these and similar absorption-bands depend on the strength of the solution under observation, it is better to describe their position by the central or darkest part. The position of the absorption-bands of oxyhæmoglobin are given by Gamgee as λ 579 for the less refrangible, and λ 553·8 for the more refrangible band.[1]

The absorption-spectrum of oxyhæmoglobin is well seen if a solution of 0·1 per cent. strength be viewed in a thickness of one centimetre. Fresh blood diluted with 100 parts of water, and viewed in a thickness of half an inch, shows the spectrum very well.

In a solution of one part of oxyhæmoglobin in 10,000 of water, both bands are still perceptible, and the less refrangible is visible in solutions so dilute as to appear almost colourless.

The foregoing diagram (fig. 15) shows the absorption-spectra exhibited by hæmoglobin and allied colouring matters.[2] The letters

[1] The expression λ 500 means a wave-length of 500-millionths of a millimetre.

[2] The diagram only relates to the visible spectrum of the colouring matters. The absorption of the extreme violet and ultra-violet rays of the solar spectrum by hæmoglobin and its allies has been studied by A. Gamgee (*Proc. Royal Soc.*, 1896, lix. 276), who finds that compounds of hæmoglobin with oxygen, carbonic oxide, and nitric oxide present, even in highly dilute solutions, an absorption-band between the Fraunhofer-lines G and H, the mean absorption in the case of *oxyhæmoglobin* coinciding with the wave-length λ 414, and in those of *carbonyl-* and *nitrosyl-hæmoglobin* with λ 420·5. By reduction or ebullition in a vacuum, the molecule of oxygen is removed from oxyhæmoglobin, and the centre of absorption shifts to λ 426.

The absorption of the extreme violet and ultra-violet by *methæmoglobin* indicates that the substance is the product of a partial decomposition of the oxyhæmoglobin molecule.

Acid solutions of *hæmatin* exhibit an absorption-band exactly on the boundary of the ultra-violet proper, extending further into the ultra-violet with increased concentration. *Alkaline* solutions of *hæmatin* exert a general absorption of the extreme violet and ultra-violet, but show no trace of definite absorption.

Solutions of *hæmochromogen* exhibit an intense absorption-band between *h* and G, the centre being at λ 420.

The band characteristic of hæmoglobin in no way depends on the presence of iron in the molecule.

Acid solutions of *hæmatoporphyrin* of extreme dilution exhibit an absorption-band between *h* and H, and by more concentrated solutions K is absorbed. Alkaline solutions of hæmatoporphyrin behave similarly, but their absorptive action is more intense.

Neither *urobilin*, *bilirubin*, or *hydrobilirubin* presents any definite absorption-band in the region of the spectrum where the absorption-bands of hæmoglobin and its derivatives occur.

Ammoniacal and caustic alkali solutions of *turacin*, so dilute as to be almost colourless, absorb the rays of the region in question in a manner so

refer to the Fraunhofer-lines of the solar spectrum. The figures are wave-lengths (according to the observations of MacMunn) ex-

Diagram.	Colouring Matter.	Strength of Solution.	Position of Bands in Wave-lengths.			
			First.	Second.	Third.	Fourth.
2	Oxyhæmoglobin, .	0·37 per cent.	589–564	555–517	..	..
3	Hæmoglobin, . .	..	597–535	..	..	..
4	Carbonyl-hæmoglobin,	..	583–564	547–521	..	..
5	Methæmoglobin, . .	Concentrated.	..	..	..	..
6	Methæmoglobin, . .	Dilute.	647–622	587–571	552–532	514–490
7	Acid hæmatin, . .	Ethereal solution.	656–615	597–577	557–529	517–488
8	Alkaline hæmatin, .	..	630–581	..	..	..
9	Hæmochromogen, .	..	569–542	535–504	..	..
10	Acid hæmatoporphyrin,	..	607–593	585–536	..	..
11	Alkaline hæmatoporphyrin,	..	633–612	589–564	549–529	518–488

pressed in millionths of a millimetre. Thus the wave-length of the D ray is λ 589. The figures in the above table also indicate

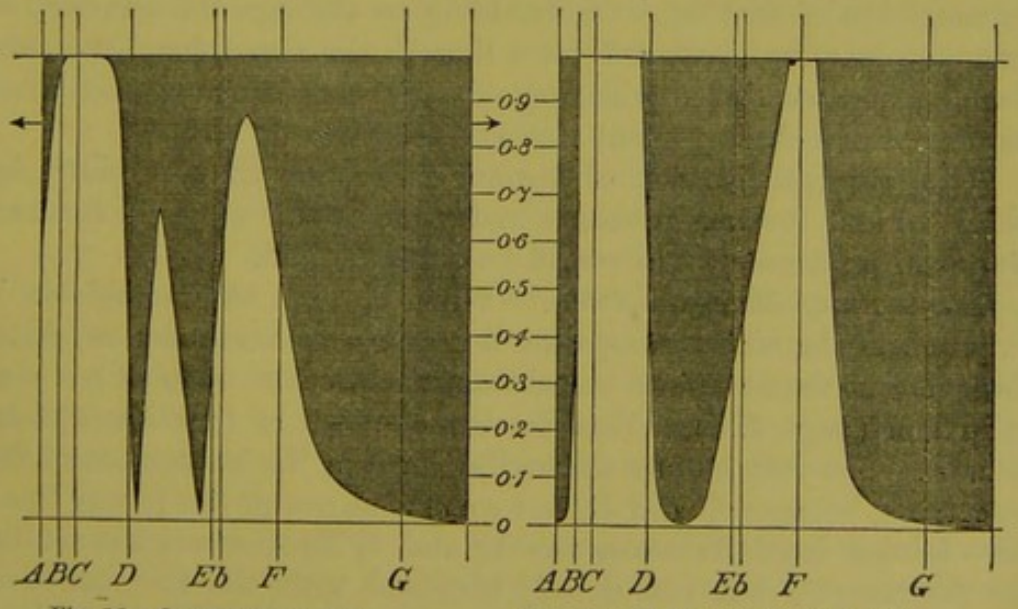


Fig. 16.—OXYHÆMOGLOBIN.

Fig. 17.—HÆMOGLOBIN (reduced).

like acid solutions of hæmatin that it is impossible to tell from the photographs which colouring matter has been employed. *Turacoporphyrin*, formed by the action of strong sulphuric acid on turacin, gives to the ultra-violet an intense absorption-band identical with that yielded by hæmatoporphyrin obtained in a similar manner.

The violet and ultra-violet absorption-spectra of blood have also been studied by J. L. Soret, *Compt. rend.*, xcvii. 1269.

approximately the position and extent of the absorption-bands, but must not be relied on too strictly, since the width of the bands varies materially with the strength of the solutions employed.

Figures 16 and 17 (page 427) are diagrams of the absorption-spectra of oxidised and reduced hæmoglobin. The letters on the base-line show the position of the Fraunhofer-lines, while the figures between the diagrams show the percentage strength of solutions of the colouring matter, observed in a thickness of one centimetre, required to produce the absorption shown by the shading of the diagrams. Thus a solution containing 0·3 per cent. of oxyhæmoglobin exhibits a dark but somewhat narrow absorption-band, commencing at the Fraunhofer-line D, and a second wider band extending from a point between D and E to E. A solution of reduced hæmoglobin of the same strength exhibits a single broad band extending nearly from D to E.

Spectrum of Hæmoglobin (reduced).—If a solution of fresh blood, or of oxyhæmoglobin of such strength as to show to advantage the two-banded absorption-spectrum above described, be treated with a deoxidising agent, the two bands disappear and are replaced by a single broad band, extending from about λ 597 to λ 535, and having its darkest part at a point somewhat nearer to D than E. This is the spectrum of h æ m o g l o b i n (reduced hæmoglobin of Stokes). On bubbling air through the solution, or even on leaving it exposed for a time to the atmosphere, the two-banded spectrum of oxyhæmoglobin will reappear, provided that an excess of reducing agent was avoided.

The absorption-band of hæmoglobin disappears rapidly by dilution, and becomes invisible under conditions in which the two bands of oxyhæmoglobin would be quite distinct.

Spectrum of Methæmoglobin.—When blood or oxyhæmoglobin is exposed to the air for some time it acquires an acid reaction, turns brown, and then exhibits the absorption-spectrum of m e t h æ m o-g l o b i n (page 425). This is characterised by four absorption-bands :—one very intense and well-defined in the red portion of the spectrum, between C and D, but somewhat nearer the former line ; two fainter bands between the D and E lines, somewhat similar in position to, but not strictly identical with, the two bands of oxyhæmoglobin ; and a fourth broad band, seen in dilute solutions only, about midway between E and F.[1] On addition of ammonia,

[1] H. B e r t i n - S a n s (*Compt. rend.*, cvi. 1243, abst. *Jour. Chem. Soc.*, 1888, p. 858) has examined the spectrum of acid methæmoglobin, in order to decide whether it consists properly of four bands, or whether the two bands between D and E are due to the presence of oxyhæmoglobin. He concludes these two bands are always present, whatever the proportion of reagent used

the two least refrangible bands shift slightly towards the violet end of the spectrum. On treatment with ammonium sulphide or other suitable reducing agent, avoiding excess, the spectrum of methæmoglobin changes to that of hæmoglobin, and on subsequent exposure to air the two-banded spectrum of oxyhæmoglobin appears. This behaviour distinguishes methæmoglobin from hæmatin, which cannot by similar treatment be made to yield the bands characteristic of oxyhæmoglobin.

Spectrum of Hæmatin.—On treatment with acids and certain other reagents, hæmoglobin is decomposed with formation of hæmatin (page 411). For spectroscopic purposes, this change may be effected instantaneously and at the ordinary temperature by addition of citric acid to the solution. The liquid thus obtained contains *acid hæmatin*, sometimes called hæmatoïdin. On shaking the acid liquid with ether, hæmatin is dissolved and colours the upper layer. The spectrum of acid hæmatin exhibits four absorption-bands—one in the red, between the lines C and D, but somewhat less refrangible than the similar line of methæmoglobin; a narrow and faint band over the D line; and two faint lines in the green. None of these lines are very dense or characteristic.

On adding to a solution of acid hæmatin sufficient ammonia to render it alkaline, the solution contains *alkaline hæmatin* and shows one faint and ill-defined band overlapping the line D and extending some distance towards the red end of the spectrum.

The spectrum of alkaline hæmatin can be obtained instantaneously by adding caustic soda to a solution of oxyhæmoglobin, methæmoglobin, hæmoglobin, or blood.

Spectrum of Hæmochromogen (reduced hæmatin).—On adding a reducing agent to a solution of alkaline hæmatin, the liquid exhibits a spectrum traversed by a deep, well-defined band about midway between the D and E lines, having a mean wave-length of 557, and a second fainter band between E and *b*. The latter may escape notice if the solution be dilute, but the former is the

to convert the oxyhæmoglobin into methæmoglobin, and that they only disappear when the solution is largely diluted. The two bands are also different in character and position from the bands of hæmoglobin. According to Bertin-Sans, the mean wave-lengths of the methæmoglobin bands are 633, 580, 538, and 500 respectively. The first is the most intense; the second is about the same breadth, but is very feeble; the third is more intense than the second, and about twice as broad; the fourth is seen only in a dilute solution, and is broader and more intense than either the second or the third. This spectrum has a general resemblance to that of hæmatin in an acidulated alcoholic solution, but is more distinct.

deepest and best-defined band yielded by hæmoglobin and its allies. On exposure to air, these bands disappear and are replaced by the single faint band of alkaline hæmatin. The absorption-spectrum of hæmochromogen can be observed, even if the blood under examination be putrid, by simply adding excess of caustic soda, followed by sodium hyposulphite ($Na_2S_2O_4$).

G. Linossier has observed that the absorption-bands of hæmochromogen disappear on heating the liquid to about 50° C., reappearing on cooling if air has been excluded.

Spectrum of Carbonyl-hæmoglobin.—This compound, which results from the action of carbon monoxide on blood or oxyhæmoglobin, exhibits an absorption-spectrum characterised by two bands somewhat similar to those of oxyhæmoglobin, but slightly more refrangible. Their centres are respectively at λ 572 and λ 534–538 (according to concentration). The spectrum of carbonyl-hæmoglobin is readily differentiated without exact measurement of the bands by the fact that it is unaffected by ammonium sulphide or other reducing agents (see page 407).

If potassium ferricyanide be added to a solution of carbonyl-hæmoglobin, a spectrum is obtained identical with that of methæmoglobin (pages 425, 428), and if the liquid be then treated with ammonia and ammonium sulphide, the spectrum of carbonyl-hæmoglobin reappears.

Katayama has observed that, on treatment with acetic acid and ammonium sulphide containing free sulphur, normal blood yields a greenish-grey or reddish-grey colour, whereas with blood containing carbonic oxide a fine rose-red coloration is produced. The spectrum of the latter liquid exhibits one band between C and D, and two others between D and E, this effect being due, according to Hoppe-Seyler, to the joint absorption produced by carbonyl-hæmoglobin and sulphur-methæmoglobin.

The spectra of *hæmatoporphyrin* and allied compounds are described on pages 420 and 427.

Construction and Use of the Micro-spectroscope.[1]

Although almost any form of spectroscope may be employed for the examination of the absorption-spectrum of blood, the micro-spectroscope possesses great practical advantages. The arrangement devised by H. Clifton Sorby (fig. 18) was the first instrument of the kind.[2] It consists of a train of prisms arranged for

[1] The author's personal experience of the micro-spectroscope includes the use of all three forms of instrument described in the text, in addition to frequent verification of the various reactions described.

[2] The Sorby micro-spectroscope is made by R. & J. Beck, Limited, to whom the author is indebted for the illustrations.

direct vision, fitted in a removable brass tube. Below the prisms is an achromatic lens, the focus of which can be adjusted by rack-work. The slit is placed in the focus of the eye-piece, in the position usually occupied by the diaphragm, and its width can be adjusted by a screw, so that the best effect may be obtained with the illumination available. As a rule the slit should be very narrow, and the lens adjusted so as to show the Fraunhofer-lines clearly. In order to allow of exact comparison of the liquid or substance under examination with others of known nature, the instrument is furnished with a second stage on which the comparison-object can be placed. By means of a right-angled prism, D, the light from this is reflected through one-half of the main slit. A supple-

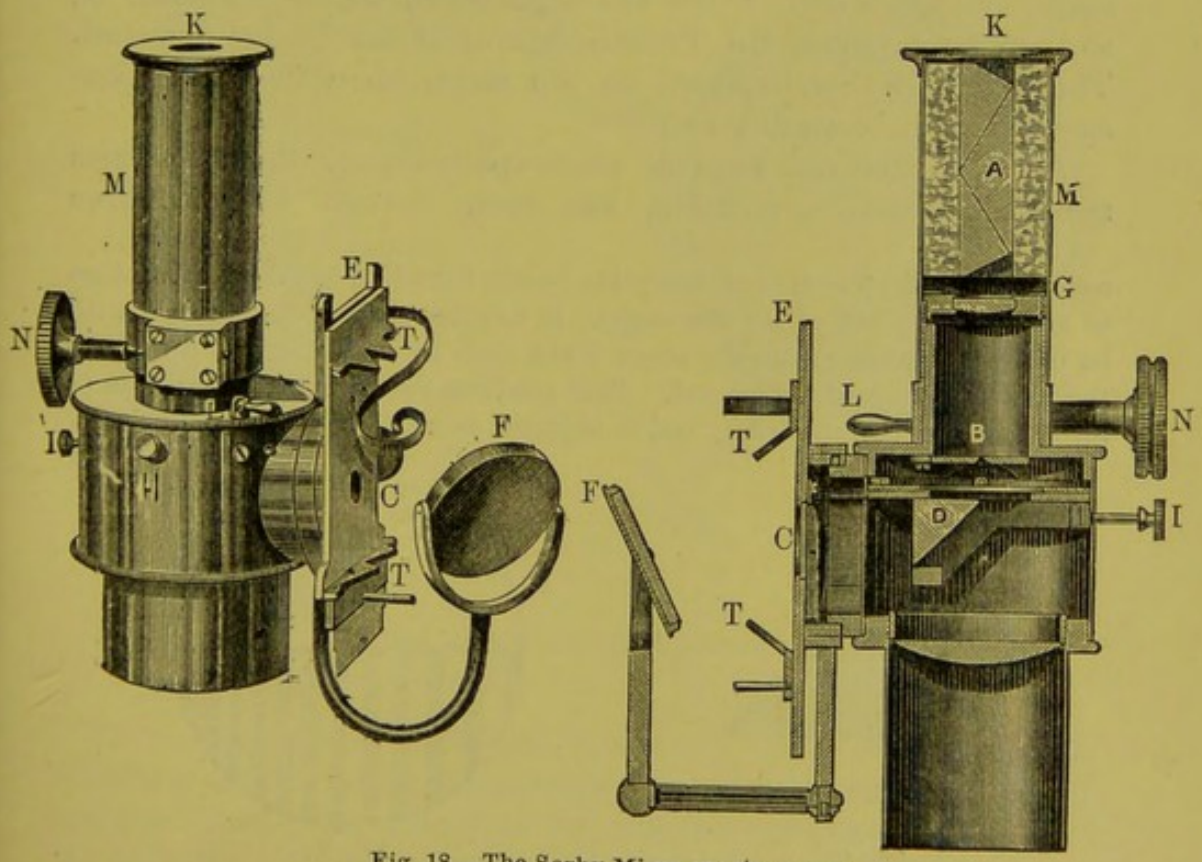


Fig. 18.—The Sorby Micro-spectroscope.

mentary arrangement—consisting of a mirror, micrometer-scale, and collimating lens (not shown in the illustration)—allows the position of the absorption-bands to be read off and recorded. In the practical examination of blood-stains, however, the author has found this arrangement of little value.[1]

[1] Details of the methods of employing Browning's bright-spot micrometer and similar devices will be found in works on microscopic manipulation.

H. C. Sorby has devised an interference-scale (fig. 19) (made by J. Browning and by R. & J. Beck, Limited) which is very convenient for observing

To use the instrument, the ordinary eye-piece of the microscope is removed and replaced by the spectroscope. The upper tube, M, containing the prisms, is then removed, and the sliding half of the slit drawn back by the milled head, H, so that one-half of the field of view is clear. The object to be examined is next placed on the stage, brought into focus, and the centre adjusted to the remaining edge of the slit. The side-shutters are then adjusted by the levers, L, so as to shut out all light but that passing through the object, and the sliding half of the slit pushed back to its position by the milled head, H. The slit is next brought into focus by means of the rack and pinion, N, and the prism-tube, M, replaced. The object is then removed from the stage, and the width of the slit adjusted by the milled head, H, so that, by daylight, the Fraunhofer-line D can be distinguished. The object is then replaced on the stage, when the absorption-spectrum will be readily seen.

Another excellent form of micro-spectroscope, which possesses several practical advantages, has been devised by C. Zeiss

and recording the positions of absorption-bands, but which has the disadvantage of monopolising one of the two stages. It consists of two Nicol-prisms, with an interposed plate of quartz, about 0·043 inch in thickness, cut parallel to the principal axis of the crystal. This produces twelve interference-lines or black bands in the spectrum, and is adjusted so that the D line of the solar

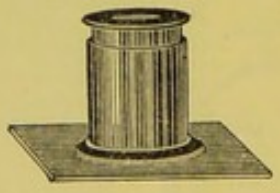


Fig. 19.

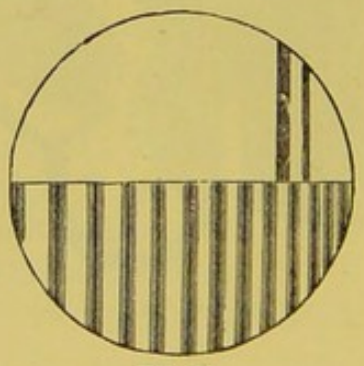


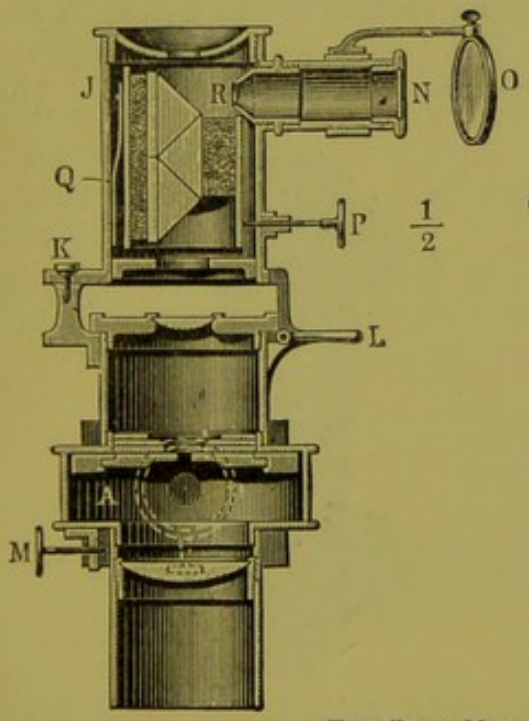
Fig. 20.

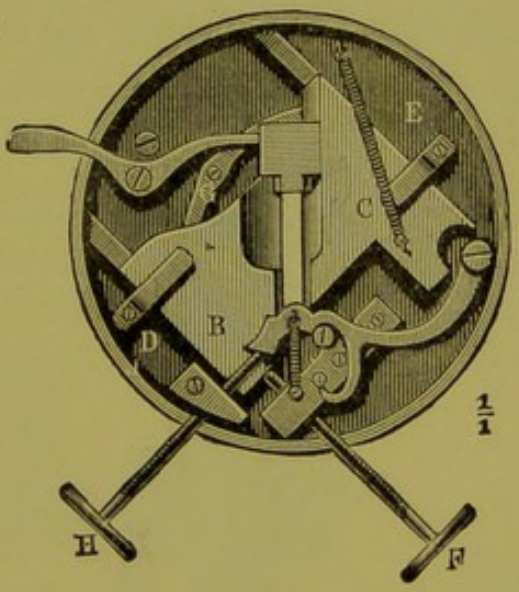
spectrum is situated exactly midway between the third and fourth bands, reckoning from the red end. The bands are shaded off gradually on each side, so that the shaded portions are about equal to the intermediate bright line spaces. On Sorby's interference-scale, the positions of the principal Fraunhofer-lines are about as follow :—

A	B	C	D	E	*b*	F	G
¾	1½	2⅜	3½	5⅛	6⅞	7½	10⅜

Fig. 20 shows the appearance of the field when the lower half is occupied by the standard scale, and the upper by the object under examination.

(fig. 21).[1] The direct-vision prism can be moved away from or brought over the eye-piece at will, and is secured in position by means of a catch (L). By means of a screw and spring the micrometer-scale can be readily adjusted to the standard position with regard to the spectrum, namely, that in which the Fraunhofer-line D corresponds with the division 58·9. This arrangement allows the wave-lengths of all other lines and bands to be read off at once without the necessity of referring to a table. The length and width of the slit can be adjusted by the screws F, H.





THE ZEISS MICRO-SPECTROSCOPE.

Fig. 21.—Section of instrument. Fig. 22.—Mechanism of slit.

In a modified form of instrument (fig. 23), devised by H. Clifton Sorby, the spectroscope is placed between the object and the object-glass, instead of between the eye-piece and the eye.[2] An ordinary binocular microscope is used with an object-glass of 3 inch focus, corrected for looking through glass an inch thick, the correcting lenses being at the top, so as to be as far as possible from the slit. This is placed at the focus of the object-glass, and between it and the lenses, is a direct-vision prism (composed of

[1] The Zeiss micro-spectroscope can be obtained from Chas. Baker, 244 High Holborn, London, W.C., to whom the author is indebted for the illustration in the text.

[2] This form of micro-spectroscope is made by R. & T. Beck, Limited, of 68 Cornhill, London, E.C., to whom the author is indebted for the illustrations (fig. 23).

a rectangular prism of flint-glass between two of crown-glass having an angle of 61°). A small right-angled prism is placed over one-half of the slit, to allow of the comparison of two spectra side-by-side. When the two spectra are in focus, their line of junction is some distance within it. To correct this inconvenience, a cylindrical lens, of about two feet focal length, is placed below the prism so as to bring the spectra and their line of contact to the same focus. In front of the slit is a stop with a circular opening, to shut out lateral light, and a small achromatic lens of about ½ inch focal length, which gives a better field and counteracts the effect of the concave surface of the liquids in the tube cells if they are not quite full. This lens merely receives the light from the object, and does not form an image of it. The

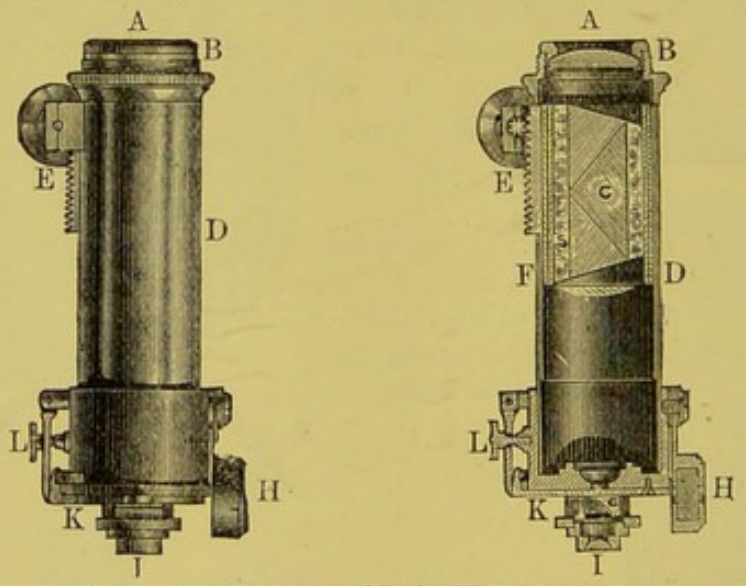


Fig. 23.—Modified form of Sorby Micro-spectroscope.

width of the slit and the focus of the object-glass are capable of adjustment, and a Sorby interference-scale, in which herepathite plates are substituted for Nicol's prisms (see footnote, p. 432), is attached in such a position that it can be instantaneously adjusted when it is desired to record the position of the absorption-bands under view.[1]

[1] In using the instrument, the object-glass (A) should be first screwed into the body of the microscope. The rest of the apparatus is then slid into the object-glass, and turned so that the sides of the spectrum are seen square or upright and not rhomboidal. The outer tube is then adjusted by the rack-and-pinion movement, E, so as to obtain a clearly-defined image of the slit, K, the width of which is then regulated by the small milled head, L, so that in daylight the Fraunhofer-line D can be seen. The whole body of the microscope is focussed so that the small lens, I, just touches the object to be examined.

If it be desired to register the position of the absorption-bands, the small

The author's personal experience includes all three forms of micro-spectroscope above described.

In observing the absorption-spectra of liquids and transparent objects, such as solutions of blood, etc., the object should be illuminated in the ordinary manner by transmitted light. Opaque objects require to be illuminated very strongly by the condensing lens, or preferably by a lieberkuhn, which gives a light of much greater intensity. Either daylight or artificial light may be employed, the former being preferable for observing the more refrangible end of the spectrum, and the latter for bands in the red and green.

For rough purposes, a liquid to be examined may be contained in a narrow test-tube, but the great delicacy and advantage of dealing with minute quantities of colouring matter, which give the micro-spectroscope its chief value, are wanting in this mode of operating.

By far the best method of examining liquids with the micro-spectroscope is to make use of the glass tube-cells devised by H. C. Sorby (fig. 24). These are constructed from pieces of barometer-

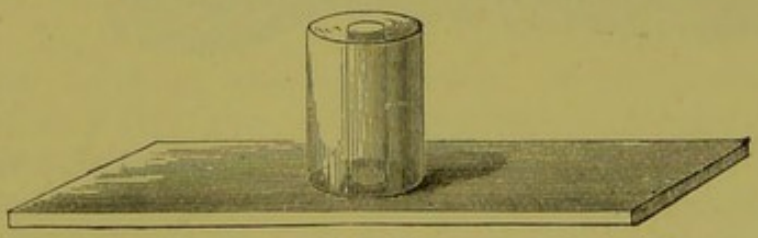


Fig. 24.

tubing, about $\frac{3}{4}$ inch in length and $\frac{1}{8}$ in diameter, both ends being ground. One end of the tube is cemented by pitch, sealing-wax, or purified gutta-percha to a microscope-slide.[1] Cells of this size can be inverted and deposits removed without any liquid being lost; and they can be used either open or closed by a cover of thin glass.

A valuable adjunct to the micro-spectroscope is shown in fig. 25. It consists of a mahogany support for the slide holding the tube-cell, and may be employed either on the main or the comparison-

reflecting-prism, G, should be pushed home, the small box, H, containing the standard scale, turned down, and the light thrown through it.

[1] In some cases Sorby inserts between the plate and the cell a diaphragm of platinum-foil, having in it a circular hole about two-thirds the internal diameter of the tube, and fixed so that its centre may correspond with that of the cell. This prevents any light which has not penetrated through the whole length of the solution from passing upward—a precaution which is very important when using direct concentrated sunlight to penetrate through turbid or very opaque liquids.

stage. The support is so constructed that when the slide is adjusted in the groove intended for it the cell is exactly in the proper position relative to the microscope. If the absorption be found too great, the slide may be placed in such a position that the light passes through it transversely (as shown in the illustration).

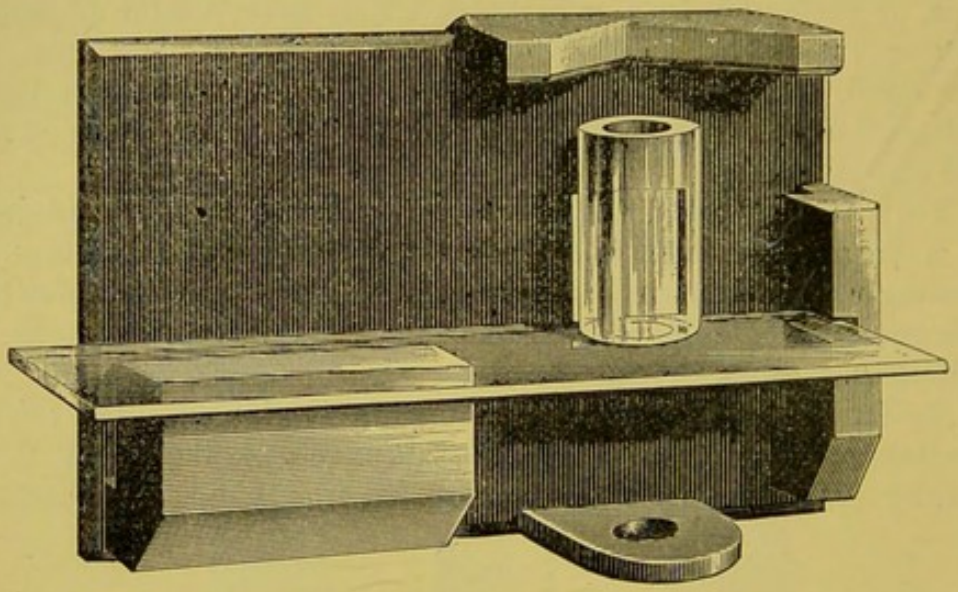


Fig. 25.

In order to test the capability and adjustment of a micro-spectroscope, a drop of fresh blood should be diluted with water in a test-tube of about $\frac{1}{4}$ inch diameter, until the liquid appears pinkish rather than red when held up to the light. A portion is then transferred by a small pipette to a Sorby's tube-cell, which must be completely filled. The cell is then closed by a cover-glass, taking care that no bubble of air is included. The excess of moisture is then removed by cautious application of blotting-paper, and the slide bearing the cell is placed vertically on the main stage of the microscope, and illuminated by transmitted light. The spectroscope is then adjusted and the slit focussed as described on page 432. The mirror of the comparison-stage is now adjusted, and the reflecting prism placed in position, so that one-half of the field of vision is filled by the normal spectrum, and the other by the spectrum modified by the passage of the light through the diluted blood.[1] If the conditions are favourable this spectrum

[1] The mode of manipulation described in the text assumes that a monocular microscope is employed and that the spectroscope replaces the eye-piece. When the microscope is binocular, the inclined tube can be conveniently used as a finder. For this purpose, the reflecting prism is pushed in so as to permit the passage of light up the inclined tube, which is fitted with an

will be seen to be traversed by the two dark absorption-bands characteristic of oxyhæmoglobin (see fig. 15, p. 425). If the solution is too strong the two bands will coalesce, in which case the cell should be turned on its side (fig. 25, p. 436), and the light allowed to pass through it transversely. If the bands are faint and ill-defined, even after further narrowing the slit, the solution is too weak. In this case a stronger solution must be made, or a longer tube-cell used, so that a greater thickness of the liquid may be traversed.

Another portion of the same blood-solution should be placed in a similar cell and examined on the comparison-stage, when, if the illumination be properly adjusted, the two adjacent spectra will appear similar in every respect.

A well-defined spectrum of oxyhæmoglobin having been obtained, the effect of reagents may be tried. These reagents should, when their nature permits, be used in the solid state. A particle about the size of a pin's head should be added to the contents of the tube-cell, and crushed by means of a stout platinum wire turned up at the end to form a kind of hoe. A vertical motion of this will also aid the rapid solution of the reagent. Liquid reagents, such as caustic soda, ammonia, and ammonium sulphide, should be added in single small drops by means of a thin glass rod or capillary pipette, and the liquid mixed by means of the hoe. As reducing agents, ferrous sulphate, ammonium sulphide, and sodium hyposulphite ($Na_2S_2O_4$)[1] are the most convenient; as an acidifier, citric acid answers all purposes;

ordinary eye-piece. When the object is brought on the field the reflecting prism is withdrawn, so that the whole of the light may pass up the right-hand body which carries the spectroscope. In practice the object-glass is merely used to concentrate the beam of light and throw it on the slit of the spectroscope. Hence there is no occasion for it to be accurately focussed on the object.

[1] The use of sodium hyposulphite was first suggested by Linossier (*Bul. Soc. Chim.*, xlix. 691); but the proposal has been widely misunderstood, the reagent being described in certain leading text-books as "hyposulphite of soda," and no hint given that the substance intended was other than ordinary "hypo," more properly called sodium thiosulphate, $Na_2S_2O_3$. The nature of the reagent intended to be used has been misunderstood in cases within the author's knowledge. Sodium thiosulphate has no reducing action on neutral or alkaline solutions of oxyhæmoglobin.

Sodium hyposulphite, $Na_2S_2O_4$, was discovered by Schützenberger, and may be readily extemporised by agitating a strong solution of sodium hydrogen sulphite (bisulphite) with zinc filings or turnings. The bottle should be nearly filled with the solution, and the reagent carefully excluded from the air. Even then it is very unstable.

and as an alkali, ammonia or caustic soda is employed according to circumstances.

The spectra of hæmoglobin and its allies are described and illustrated on p. 424 *et seq.*

The spectrum of (reduced) *hæmoglobin* is obtained by adding a drop of sodium hyposulphite or ammonium sulphide to the diluted blood-solution. Another very efficient reducing agent is ferrous sulphate, but in order to avoid precipitation its addition must be preceded by that of some potassium-sodium tartrate (Rochelle salt). Stannous chloride may be substituted for the ferrous salt, and has the advantage of yielding a colourless oxidation-product.

If an excess of the reducing agent has been avoided, the two-banded spectrum of oxyhæmoglobin may be restored by agitating the liquid, or even by merely exposing it to air for a time. Dilution with water previously shaken with air has the same effect.

The spectrum of *methæmoglobin* will be developed on adding a minute fragment of potassium ferricyanide or sodium nitrite to the diluted blood-solution. Subsequent treatment with a reducing agent will change the spectrum to that of *hæmoglobin*.

If the solution of oxyhæmoglobin be treated with a sufficiency of caustic soda the liquid will show the spectrum of *alkaline hæmatin*. This will be changed to that of *acid hæmatin* on acidulating the liquid with citric acid. If, instead, a drop of a reducing agent be added, the highly-characteristic spectrum of *hæmochromogen* (reduced hæmatin) will be developed.

If the blood-solution be treated with citric acid, the colour becomes paler, and the spectrum changes to that of *acid hæmatin*, the absorption-bands of which are weak and ill-defined. But if the acid liquid be now treated in succession with potassium-sodium tartrate, ferrous sulphate, and ammonia, the strongly-developed absorption-bands of *hæmochromogen* will appear.

By oxidation, hæmochromogen may be reconverted into hæmatin, but it is not possible to restore it to the condition of hæmoglobin.

It will be seen that the conversion of oxyhæmoglobin into hæmochromogen may be effected by several distinct routes, the stages of which vary considerably. Thus :—

OXYHÆMOGLOBIN.	1. Methæmoglobin, hæmoglobin, oxyhæmoglobin, acid hæmatin, alkaline hæmatin,———————	HÆMOCHROMOGEN.
	2. Hæmoglobin, alkaline hæmatin,—	
	3. Acid hæmatin, alkaline hæmatin,—	
	4. Alkaline hæmatin, ———————	

With care, the spectra of all the stages of route 1 can be observed with the contents of a single tube-cell, representing about 0·002 gramme of blood.

Whatever the original condition of the blood colouring matter, it can always be converted into hæmochromogen by the use of caustic soda and a reducing agent. The production of such intermediate products as acid and alkaline hæmatin have little value in practice.

When serum in a certain stage of putridity is added to a drop of blood on a microscope-slide, and the specimen covered, crystals appear after a variable time. S. M. Copeman (*Brit. Med. Jour.*, ii., 1889, 190) finds that in the case of human blood and of monkey's blood these crystals always consist of hæmoglobin, being rectangular in the former case and diamond-shaped in the latter. In the case of all other animals, the blood-crystals, if obtained at all, invariably consist of oxyhæmoglobin (readily distinguished from hæmoglobin by the spectrum). Besides putrefying serum, Copeman found that human blood could be made to crystallise by treatment with a solution of bile-salts, by agitation with ether, or by semi-digestion in the stomach of the common leech, though the use of serum is preferred. Copeman has obtained crystals from blood-stains by the same method, but with greater difficulty. He suggests that the differences observed afford a means of distinguishing human blood from that of the lower animals. Unfortunately, the blood of many "domestic" animals cannot be made to yield crystals by the method found practicable with human blood, so that while the formation of rectangular crystals of hæmoglobin proves the blood to be human, the non-formation of crystals leaves its origin uncertain. Better results are sometimes obtainable by agitating the blood with ether (compare page 398), but the process does not appear to be applicable to very small quantities.[1]

Spectroscopic Examination of Blood-Stains.[2]

The absorption-spectra exhibited by the colouring matter of blood

[1] Copeman's experiments are interesting and suggestive, but they do not appear to afford a practical solution of the problem. The conversion of the colouring matter into hæmatin would be fatal to the success of the process. Another practical difficulty is the necessity of obtaining a crystal of such a size that the image formed by the object-glass will be large enough to cover the slit of the spectroscope, which compels the use of a very high power.

[2] The directions given in the text for the spectroscopic examination of blood-stains are largely taken from the writings of H. Clifton Sorby, to whom the author is also indebted for information personally communicated. Sorby's recommendations are, in most cases, fully confirmed by the author's own experience.

in its different modifications afford the most delicate and certain means of detecting blood in chemico-legal cases ; and one especially suited for the examination of blood-stains in cases where only a very small quantity of material is available. The only serious practical difficulty attending the method of examination lies in the tendency of the stains to become insoluble in water or other available solvents by lapse of time or the action of mordants, which change necessitates the adoption of special precautions.

Broadly speaking, the older a blood-stain the darker and less soluble it will be. If the stain be recent, and on an article not capable of combining with either of the proteids of the blood, the colouring matter will be chiefly in the form of oxyhæmoglobin, which will readily dissolve with red or reddish-brown colour on treating the stain with cold water.

On exposure to air in a damp place, a blood-stain may become mouldy, with complete destruction of the colouring matter ; but if kept dry, the hæmoglobin gradually changes into a complex mixture of methæmoglobin, hæmatin, and hæmatoporphyrin. If moderately recent, such a stain will be brown, and will dissolve with difficulty and probably incompletely in cold water, yielding a dirty brown solution. The alteration of blood-stains takes place far more rapidly in the atmosphere of a town, and especially in a room where gas is burnt, than in the pure air of the country. Contact with sweat may cause the hæmoglobin of a blood-stain on a dirty garment to undergo very rapid alteration into hæmatin. Such facts should be taken into consideration in forming any opinion as to the age of a blood-stain from its colour, solubility, and spectroscopic examination.[1]

The colouring matter of very old blood-stains is insoluble in water, but it may be extracted with dilute citric acid or ammonia. In the case of a white fabric, somewhat diluted ammonia should be used, but this reagent is apt to dissolve an undesirable amount of dye-matter from coloured fabrics (especially reds), and citric acid should be employed for treating these.[2] It may readily happen

[1] Pfaff has proposed the use of a 1 per cent. solution of arsenious oxide in water for judging the age of blood-stains, solution occurring with decreasing facility with the age of the stain. So many conditions come into play besides actual age that no reliable opinion can be based on the results.

[2] Before actually treating a suspected stain on a dyed fabric, it is desirable to submit a portion of the material, free from stain, to the action of water, citric acid, and ammonia, with a view of ascertaining whether colouring matter is dissolved which will interfere with or modify the blood-spectra subsequently observed. In the event of blood not being recognised in a suspected stain on such fabric, a portion of the material should be treated with blood, and the process applied to the authentic blood-stain so produced.

that a solution made with the aid of citric acid or ammonia has very little colour, and yet contains abundance of the colouring matter of blood to allow of its subsequent recognition by the spectroscope. This is due to the fact that the absorption-bands of hæmatin, whether in acid or alkaline solution, are not well-defined. But on subsequently treating the liquid with a reducing agent, and, if necessary, rendering it alkaline, the very deep and persistent band of reduced hæmochromogen will be readily observed. The production of this band, as the result of the treatment of a liquid with a reducing agent and ammonia, is in itself ample evidence of the presence of blood in the substance treated.

In order to examine a supposed blood-stain to the best advantage, it is desirable to remove as much of the surrounding material as possible. Thus if the stain be on cotton, linen, silk, wool, &c., a portion of the fabric on which the spot exists should be cut out. If on a porous material, such as wood, brick, or stone, the stained substance should be scraped away for some depth, and reduced to fine powder. Stains on metal, especially on iron or steel, are most difficult of treatment, since the colouring matter is readily mordanted and rendered insoluble by the iron. Sometimes, on drying the article thoroughly, the incrustation will peel off, but if not, it must be removed by scraping.

On whatever material the stain may have been, when the suspected portion has been more or less separated in the above manner from the surrounding material, a portion of the coloured substance should be treated in a watch-glass with a few drops of cold water, and allowed to stand at rest for a short time. If the stain is at all recent, and the colouring matter has not been mordanted in any way, the liquid will acquire a more or less distinct red or brown colour. In this case it is probable that the colouring matter of the blood is, in part at least, in a soluble form, and the subsequent examination will be simple. By preference, the liquid should be decanted from any insoluble residue. If necessary, the liquid may be filtered, but this operation should be avoided if possible. If the decanted or filtered liquid be perfectly colourless, it is useless to examine it for blood, but, of course, evidence of blood may still be obtained from the insoluble portion. In such cases, special means must be employed to effect the solution of the colouring matter. If a blood-stain has been strongly dried before a fire, or otherwise heated, so as to coagulate the albumin, the colouring matter is rendered insoluble, and cannot be extracted by treatment with water, citric acid, or cold ammonia. On heating the stain in dilute ammonia or caustic soda, the hæmatin is readily dissolved, and may be detected by

reducing it to hæmochromogen, either with or without previous concentration of the solution.

The same method may be employed for extracting the colouring matter from a blood-stain which has been washed, the liquid being subsequently concentrated by evaporation or neutralised and precipitated with zinc acetate.

In cases where the colouring matter remains obstinately fixed on a fabric, probably from combination with the mordant, Sorby recommends that the stain should be digested in dilute ammonia (in a watch-glass), and the liquid squeezed out repeatedly by a pair of forceps, and ultimately between the finger and thumb. The thick, turbid, unfiltered liquid is then deoxidised in the ordinary manner, and examined for the reduced hæmochromogen band, using lime-light or concentrated sunlight if necessary.[1] By operating in this manner, Sorby succeeded in detecting blood in a stain six years old on brown cloth. When the tube containing the turbid liquid was kept in such a position as to allow the suspended matter to settle out, no absorption-band was produced by the supernatant fluid, the colouring matter evidently existing in the insoluble deposit. Hence, in such cases any process of deposition or filtration is inadmissible. The effect of the insolubility must be overcome by increasing the intensity of the light, and not by removing the deposit.

The spectroscopic recognition of blood on *rusty iron* is somewhat difficult.[2] For reasons not thoroughly understood, ammonia and citric acid sometimes fail to dissolve the colouring matter. In such cases glacial acetic acid aided by heat will sometimes be found successful, but a more certain plan is to heat the spot to 50° C. with a cold-saturated solution of borax. The solution obtained should be treated with acetic or citric acid, filtered if necessary, reduced, rendered alkaline, and examined for the spectrum of hæmochromogen.

In some cases the difficulty due to insolubility of the colouring matter may be overcome by examining the stain by reflected light, for which purpose a Lieberkuhn's parabolic mirror is the most suitable means of illumination.

The recognition of blood in suspected stains on *leather* presents peculiar difficulties, since the presence of tannic acid so mordants the blood that neither water nor citric acid will dissolve the colouring matter, and ammonia produces an inconveniently dark

[1] A cell-tube furnished with a diaphragm of platinum or tin-foil should be employed (see footnote, page 435).

[2] The test for blood-stains on rusty iron described on page 445 may be advantageously employed.

solution. If the stain is on the surface of the leather, and has never been wetted, a thin shaving should be cut off, so as to obtain as much blood and as little leather as possible. The shaving should then be bent with the stained side outwards, and placed on the mouth of a cell-tube filled with water, in such a manner that the suspected blood-stain shall be in contact with the solvent without the rest of the leather becoming wetted. In this manner the dissolved colouring matter sinks to the bottom of the cell without coming in contact with the rest of the leather. After removing the shaving, the liquid in the cell may be examined in the usual manner.

If a blood-stain on leather has been wetted, the foregoing method is inapplicable. In such cases, Sorby recommends that the stained leather should be digested for a considerable time in water containing 2 per cent. by measure of hydrochloric acid. The liquid is then poured off (not filtered) and treated with ammonia in excess, by which it acquires a purple or neutral tint. This colour is intensified by the iron reagent which Sorby uses for reduction, but it is evident that the presence of tannin renders this treatment unsuitable. Stannous chloride or ammonium sulphide can be substituted with advantage, and there will then be little difficulty in recognising the absorption-band of reduced hæmochromogen.

Earth and soil-stained clothes render blood completely insoluble. Sorby recommends extraction with ammonia, and examination of the turbid solution with an intense light. A saturated solution of borax, as recommended by Dragendorff, may be advantageously substituted for ammonia. One part of blood absorbed in 200 of peat can thus be detected.

Very few colouring matters yield absorption-spectra which can be mistaken for that of blood, even by the inexperienced observer, and these can be absolutely distinguished by the application of reagents.

The petals of the red variety of *Cineraria* contain a colouring matter the spectrum of which exhibits two absorption-bands in the green, similar in position to, but differing in relative width from, those of oxyhæmoglobin. The bands of *cineraria* are completely altered on adding ammonia, while those of oxyhæmoglobin are unaltered.

Turacin, the copper-containing colouring matter present in the feathers of the turaco or plantain-eater (page 6), exhibits a spectrum similar to that of blood, but it does not yield the spectrum of hæmochromogen on reduction.

A solution of *cochineal* in alum shows absorption-bands some-

what resembling those of oxyhæmoglobin, and are rendered more intense on adding ammonia. On subsequently adding excess of boric acid, the cochineal bands shift to the blue end of the spectrum, whereas those due to blood are unaffected by this treatment.

A solution of *soluble indigo* when *hot* exhibits a spectrum not unlike that of blood, but it is decolorised by treatment with alkali and a reducing agent.

Lac-dye, *alkanet*, *madder*, and *alizarin*, when dissolved in alum, exhibit absorption-bands which distantly resemble those produced by the colouring matter of blood. The spectra are all modified by treatment with ammonia, and the bands are destroyed on adding sodium sulphite, whereas the absorption-bands of blood are unaffected by such treatment. *Rosaniline* (magenta, fuchsine), also, is completely decolorised by sulphites, and the colour is not restored by exposure to air.

Chemico-Legal Detection of Blood.

The examination of stains supposed to be due to blood is often of the first importance. Hence the utmost precaution must be taken to avoid the possibility of error, and special care must be taken in preserving and identifying samples and articles submitted for examination.[1]

The appearance of blood-stains is very variable. The shape should, however, be carefully observed, so that an opinion can be formed as to whether the spot is a smear, a drop, or a splash. In the case of a fabric a knowledge of the side on which the blood fell may be of importance. Blood-stains on fabrics are generally dull, but on polished metal they have the appearance of dark shining spots, and are readily removed.

The microscopic characters of any animal structures or products associated with blood in a suspected stain should be carefully observed, and their nature ascertained if possible. Blood from the stomach often contains epithelium cells and *sarcinia;* and that from abscesses, fat, cholesterin, and pus-corpuscles. The presence

[1] On making a preliminary inspection of any article supposed to be stained with blood, careful record should be made of the position on which suspicious marks occur. In the case of articles of clothing, the positions of these marks should be indicated by inserting safety-pins. Spots on wood or metal may be surrounded with a circular mark made with a black-lead pencil, and analogous means should be adopted in other cases to facilitate the subsequent operations. A convenient plan in many cases is to make a sketch of the article and mark on it with red ink the positions of the stains on the original article. Means must also be taken to allow of the certain recognition of the article on subsequent occasions, as in the witness-box.

in suspected stains of spermatozoa, vaginal epithelium, hairs of different kinds, fæcal or biliary matter, brain-tissue, &c., may often have great significance.

F. Gantter (*Zeitschrift f. Anal. Chem.*, 1895, ii. 159 ; abst. *Analyst*, xx. 186) has suggested the use of the reaction of blood with hydrogen peroxide as a means of recognising blood-stains, especially on *rusty iron*. A small quantity of the rust should be scraped off and placed on a microscope-slide having a black background. The specimen is then moistened with a drop of water made very feebly alkaline, and a drop of hydrogen peroxide added. If the slightest trace of blood be present, numerous comparatively-large bubbles of gas are developed, and these shortly unite, forming a fine snow-white scum which persists for some hours. It is characteristic of the scum that it forms from the outside of the drop inwards, so that it is surrounded by a ring of clear fluid. The development of gas occurs only on those particles of the specimen to which blood is attached. Stains on rust six months old respond to the test as sharply as when fresh. If air-bubbles are formed on moistening the specimen, they should be dissipated by touching them with a thin glass rod before adding the hydrogen peroxide. Gantter considers that a negative reaction proves the absence of blood ; but the formation of bubbles is not absolute proof of its presence, since pus and other animal fluids behave in a similar manner with hydrogen peroxide. Gantter's test has proved very satisfactory in the author's laboratory in the hands of A. R. Tankard.

The blood of flea- and bug-spots can be recognised by the spectrum, but no fibrin or blood-corpuscles can be detected.

When a suspected stain has yielded no evidence of the presence of blood, it is often important to ascertain its true nature. It is not possible to prescribe a systematic method for the recognition of every kind of stain, but the following hints will be found useful for the identification of the stains most likely to occur.

On treating the solution obtained as described on p. 441 with a slight excess of ammonia, the colouring matter of blood will remain unchanged or be slightly intensified and reddened. On addition of more ammonia the colour will darken to brown. Fruit-stains, flower-stains, and archil are turned green or blue by ammonia; cochineal, logwood, camwood, brazil-wood, madder, alizarin, &c., acquire a crimson colour ; annatto, tannin matters, rust, iron-moulds, and spots of red paint are unchanged; while sanguinaria is decolorised.

Tannin matters are recognised by the blue or green coloration produced by ferric chloride.

Iron-moulds are not soluble in cold water, but dissolve in hydrochloric acid, and the solution contains a ferric salt. Spots on *rust* may be similarly examined, after being scraped off from the metal. The direct treatment of the iron or steel with acid would cause error, owing to the solution of the metal.

Red paint, containing iron, should be separated as far as possible from its surroundings, boiled with ether to remove the oil, and then dissolved in acid.[1]

Stains of *grease* can usually be recognised as such under a lens. On covering the stain with a piece of filter-paper and pressing a hot iron on it, the grease will be partially transferred to the paper, making a spot which can be removed by ether.

Tar and *pitch* evolve a characteristic odour when warmed, and can be dissolved by benzene, ether, or turpentine.

The chief methods which are available for the detection of blood in chemico-legal inquiries are :—

1. The production of hæmin crystals, already fully described (p. 416).
2. The guaiacum test (p. 403), open to many fallacies.
3. The spectroscopic examination of the colouring matter (p. 439).
4. The microscopic recognition of the blood-corpuscles (p. 446).

Each of these methods has advantages of its own. Whenever possible, the indications obtained by one method should be checked by the application of the others.

The very important problem of the origin of the blood in a suspected stain cannot be said to have been satisfactorily solved. Such approaches to its solution as are at present possible are discussed on pages 439 and 452.

Microscopic Characters of Blood-Corpuscles.[2]

The microscopic detection of blood-stains, apart from the hæmin test, is dependent almost entirely on the recognition as such of the red corpuscles. The white corpuscles are too few in comparison, and too variable in appearance, to be of much value in this respect.

When viewed by a power of 400 to 1000 diameters, the red corpuscles of human blood appear as nearly transparent, concave, circular discs of a faintly yellowish colour (fig. 26). Generally

[1] Dark-coloured dyes are often mordanted with iron, which fact must be borne in mind before concluding that a stain on a fabric has been caused by iron-mould or ferruginous paint.

[2] The author is much indebted, in the compilation of this section, to the admirable description of the microscopic characters of blood-corpuscles given by the late T. G. Wormley in his *Micro-Chemistry of Poisons*.

they are seen with a bright central spot, which, by a slight change of focus, will appear shaded, and in the mammalia is apparently not a true nucleus. The concavity is destroyed by immersion in water (fig. 27) or other fluid of less density than blood-serum, the

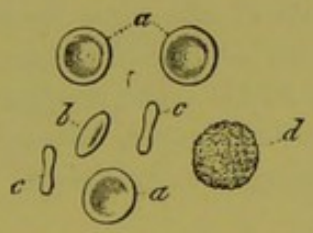


Fig. 26.—Human blood-corpuscles. *a* to *c*, red corpuscles; *d*, colourless corpuscle.

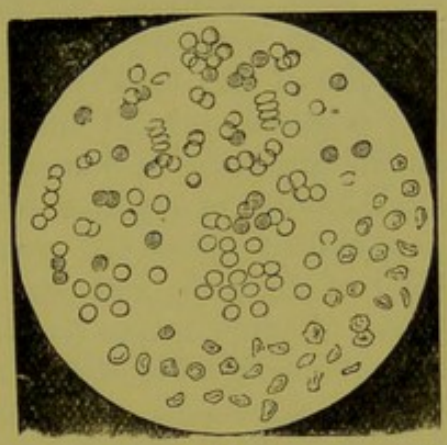


Fig. 27.—Human blood-corpuscles (×300) as seen in water.

corpuscles swelling up and ultimately disintegrating. On the other hand, when immersed in a liquid of greater density than ordinary blood-serum, the corpuscles become serrated or crinkled, presenting the appearance shown in *b*, fig. 28. When human blood is left

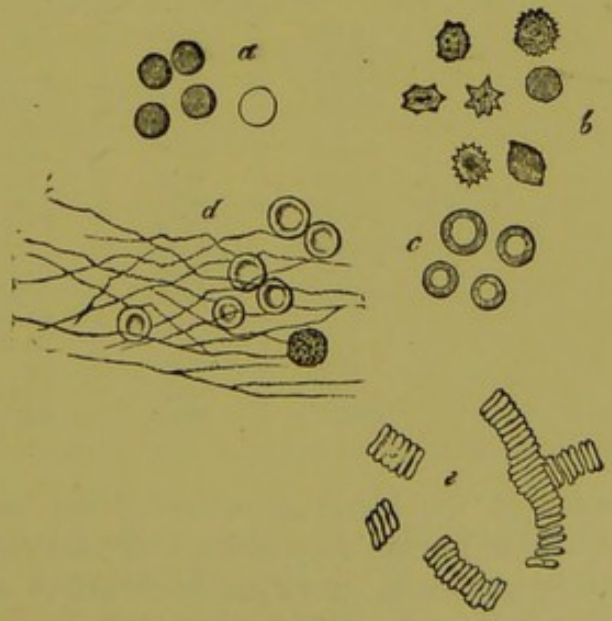


Fig. 28.—Human blood-cells. *a*, after the action of water; *b*, in evaporating blood; *c*, dried up; *d*, in coagulated blood; *e*, arranged in rouleaux.

at rest, the corpuscles have a remarkable tendency to collect together in a manner suggestive of rouleaux of coins (*e*), but they immediately separate on agitation.

The red corpuscles of the blood of all the mammalia, except

the camel tribe, are circular. In the camel tribe, the corpuscles are about the same size as those of other mammalia, but of an oval shape. The corpuscles of the blood of birds, reptiles, batrachians, and fishes are oval (except in a single family of the last), distinctly nucleated, and generally of large size, especially in the case of the batrachia.

The size of the blood-corpuscles from each source is approximately constant, even in different individuals of the same species. Thus the corpuscles of human blood have an average diameter of about 1-3200th of an inch (compare page 394). The corpuscles of the human fœtus are usually considerably larger, averaging 1-3000 of an inch, and occasionally reaching nearly twice the diameter of adult human blood.

The following table shows the diameter of the red corpuscles of various mammalia, expressed both in vulgar fractions of an inch and in decimals of a millimetre. The blood was in each case allowed to dry in a thin layer on the slide.

Source of Blood.	T. G. Wormley.		Gulliver.	
	Inch.	Millimetre.	Inch.	Millimetre.
Man,	1-3250	·0078	1-3200	·0080
,, fœtus,	1-3000	·0085	...	...
Cat,	1-4372	·0058	1-4404	·0058
Dog,	1-3561	·0071	1-3532	·0072
Fox,	...	...	1-4177	·0061
Pig,	1-4268	·0059	1-4230	·0060
Elephant,	1-2738	·0093	1-2745	·0092
Horse,	1-4243	·0059	1-4600	·0054
Ass,	1-3620	·0070	1-4000	·0064
Ox,	1-4219	·0060	1-4267	·0059
Sheep,	1-4912	·0052	1-5300	·0048
Goat,	1-6189	·0041	1-6366	·0039
Deer,	...	...	1-7060	·0036
Hare,	...	...	1-3560	·0071
Rabbit,	1-3653	·0069	1-3607	·0070
Mouse,	1-3743	·0067	1-3814	·0066
Rat,	1-3652	·0069	1-3754	·0067

The corpuscles of the blood of some of the quadrumana are very similar in size to human corpuscles. Gulliver has recorded the following measurements, in vulgar fractions of an inch. The numerator, being 1 in each case, is omitted.

Lemur,	3976; 4003; 4440.
Monkey,	3368; 3342; 3412.
Ape,	3512; 3602.

The following are measurements by Gulliver of the corpuscles

of the blood of certain oviparous vertebrate animals. The figures are fractions of an inch.

Source of Blood.	Longer Diameter.	Shorter Diameter.
1. Fowl,	1-2102	1-3466
2. Turkey,	1-2045	1-3598
3. Pigeon,	1-1973	1-3643
4. Duck,	1-1937	1-3424
5. Goose,	1-1836	1-3839
6. Turtle (green),	1-1231	1-1882
7. Frog,	1-1108	1-1821
8. Toad,	1-1043	1-2000
9. Trout,	1-1524	1-2460
10. Pike,	1-2000	1-3555
11. Eel,	1-1745	1-2842

The corpuscles of the blood of the lamprey are circular, but exhibit a well-marked nucleus.

The oval shape of, and presence of a distinct nucleus in, the blood-corpuscles of oviparous animals afford a fairly sharp indication of their origin. But if the blood from such a source be treated with a liquid of less density, the characteristic oval corpuscles are apt to become nearly or completely spherical in outline; though under these circumstances the nuclei still remain visible, since they are usually much more strongly marked than the outlines of the corpuscles themselves. In cases where these outlines are indistinct or invisible, they may often be brought out by adding a little carmine or iodine solution, or simply by changing the incidence of the light reflected from the microscope-mirror.

A useful method of developing the outlines and nuclei of corpuscles from oviparous animals, when the stain is not very old, is to treat the specimen with a little freshly-prepared tincture of guaiacum. On then adding a drop of ether which has been shaken with a solution of hydrogen peroxide, the nuclei will appear sharply-defined and of a dark blue colour, while the surrounding portions of the corpuscles will remain uncoloured or acquire a delicate violet hue.

It is sometimes loosely stated that the corpuscles of human blood range in diameter from $\frac{1}{2000}$ to $\frac{1}{4000}$ of an inch, thus implying that their size is very variable. This is not the case, the fact being that the majority of the corpuscles are remarkably uniform in size. T. G. Wormley, whose investigations on the microscopic characters of blood-corpuscles are of a most complete and convincing kind, has recorded the following observations respecting the diameter of the corpuscles of three distinct specimens of human blood. A and B were fresh blood, but C was

thirteen months old. In each case, *five hundred* corpuscles on a well-spread slide were measured in the order presented by a mechanical stage, every corpuscle of normal form being included. The measurements are the denominators of vulgar fractions of an inch, the numerator (1) being omitted.

	A.	B.	C.
Magnifying power employed (diameters), .	2,300	1,150	..
Micrometry, ...	40,000	20,000	40,000
Mean diameter of corpuscles, . .	3,255	3,242	3,266
Corpuscles larger than 2857, . .	1	1	1
,, from 2817 to 2898, . .	4	6	4
,, from 2898 to 3077, . .	49	65	22
,, from 3077 to 3389, . .	385	361	406
,, from 3389 to 3636, . .	42	56	59
,, from 3636 to 4000, . .	20	12	9
,, less than 3846, . . .	2	1	1

Wormley states that slides prepared with human blood sometimes show no corpuscles of less diameter than 1-3600 of an inch; but that, on the other hand, different slides from the same blood, and even certain portions of the same slide, show corpuscles uniformly smaller in size than are usually found in the same blood. This contraction is likely to occur when the blood absorbs moisture before drying. Although the blood-discs may, under the above conditions, *diminish* in size, Wormley states that they *never increase*, while under examination, at above their normal diameters. In fact, the remarkably-constant figures recorded by various observers for the diameters of the blood-corpuscles, not only of human blood but of that of other mammals, show that the contraction is not of much consequence in practice.[1]

For the microscopic detection of blood-corpuscles in dried stains or coagula, it is necessary to soak the specimen in a liquid which will soften the corpuscles without dissolving or otherwise affecting their microscopic characters. Of the various reagents proposed a mixture of glycerin (one part of glycerin with seven of water) is

[1] In leucocythæmia the red corpuscles are diminished in number, but remain substantially unaltered in diameter. In chronic anæmia the corpuscles are stated by Hayem to be always diminished in both number and size, their average diameter being sometimes only 1-3900 of an inch, while, according to Eichhorst, in progressive pernicious anæmia, the corpuscles have an average diameter of 1-3000 of an inch.

From experiments on different animals, Manassein concluded that the corpuscles were diminished in size in septicæmic poisoning, by a high temperature, and by carbon dioxide; whilst they were enlarged by the action of oxygen and of agents lowering the temperature of the body (as alcohol and quinine), and in acute anæmia.

generally to be preferred. This liquid should have a specific gravity of 1030, which is approximately that of the serum of blood.[1]

Whenever possible, a particle of dried clot, even if extremely small, should be chosen for examination rather than a fragment of stained fabric. Even in a minute stain a clot may frequently be found by examining the specimen under a lens. If found, the clot—or, in the absence of a clot, the scrapings or a few fibres of the stained fabric—should be placed on a microscope-slide, gently crushed, moistened with a small drop of glycerin-water, covered with a thin glass, and examined with a power of not less than 400 diameters.

When the stain is comparatively fresh, the corpuscles soon become apparent, but if the stain be old some hours may be required for its disintegration. In such cases the breaking up of the mass may be much facilitated by gently moving the cover-glass. A cautious addition of caustic alkali often assists the process of disintegration. Wormley recommends, as a good method of examining old stains, that the moistened clot should be placed on the cover-glass, and this inverted over a glass-slide having a slightly concaved centre.

The disintegration having been satisfactorily accomplished, the micrometric measurement of the corpuscles should be conducted under a higher power.[2] Wormley employs a magnifying power of 1150 diameters, and in some cases twice this power. If sufficiently numerous, not less than forty corpuscles should be measured, their diameters carefully recorded, and the average size calculated.

It is sometimes contended that while the measurement of fresh corpuscles may afford an indication of their origin, they are likely to be so altered in size by drying and keeping as to render the observation worthless. T. G. Wormley (*Micro-Chemistry of Poisons*) gives the following measurements of corpuscles from old blood-stains and clots, which show that the above objection is ill-founded.

[1] Other liquids recommended for the purpose are :—a 10 per cent. aqueous solution of chloral hydrate ; a 6 per cent. aqueous solution of sodium sulphate ; and a mixture of 30 grammes of white-of-egg, 40 of sodium chloride, and 270 of water. For preliminary examination, a particle of the clot or stain may be moistened with turpentine.

[2] If, during the examination, any of the corpuscles become entirely decolorised and spherical in form, they are evidently abnormal, and should not be included in the measurements. Some of the corpuscles will be found to resist the action of the glycerin-water better than others.

No.	Animal.	Age of Specimen.	Nature of Specimen.	Average size of Corpuscles.	
				Dried Specimen.	Fresh Blood from similar Animal.
1	Man, .	2 months,	Stain (unknown origin),	1-3358 inch	1-3250 inch
2	Man, .	2½ ,,	Stain,	1-3236 ,,	1-3250 ,,
3	Man, .	3 ,,	Stain,	1-3384 ,,	1-3250 ,,
4	Man, .	19 ,,	Clot,	1-3290 ,,	1-3250 ,,
5	Elephant,	13 ,,	Clot,	1-2849 ,,	1-2738 ,,
6	Dog, .	4 ,,	Minute spot,	1-3626 ,,	1-3561 ,,
7	Rabbit, .	18 ,,	Clot,	1-3683 ,,	1-3653 ,,
8	Ox, .	16 ,,	Stain,	1-4544 ,,	1-4219 ,,
9	Ox, .	32 ,,	Stain (unknown origin),	1-4495 ,,	1-4219 ,,
10	Ox, .	4½ years,	Clot,	1-4535 ,,	1-4219 ,,
11	Buffalo, .	18 months,	Clot,	1-4312 ,,	1-4351 ,,
12	Goat, .	17 ,,	Stain,	1-5897 ,,	1-6189 ,,
13	Ibex, .	18 ,,	Clot,	1-6578 ,,	1-6445 ,,

In every instance but that of No. 6 not less than forty corpuscles were measured. In this case the spot was so minute as to be barely visible to the naked eye, and its nature was unknown at the time of examination. In the case of No. 10 (ox-blood, 4½ years old), the corpuscles were readily obtained, and two closely concordant series of observations were made.

The microscopic recognition of blood-corpuscles is a valuable confirmation of the spectroscopic indications, but undoubtedly derives its chief value from the possibility it affords of identifying the blood as of human origin. Many of the highest authorities deny that any safe conclusion can be formed on this point, and discountenance the expression of any opinion as to the probable origin of the blood detected. In the opinion of the author, this attitude is unjustified. Although it is not possible, as the result of a microscopic examination, to affirm positively that a stain contains human blood, it is quite possible to differentiate it to such an extent that, by a process of elimination, other sources are practically excluded. Thus if on measurement of not less than thirty corpuscles they be found to be approximately equal in size, and to have an average diameter of 1-3350 of an inch, it is impossible that they can have been derived from the blood of a cat, pig, horse, ox, sheep, or goat. If the circumstances are such as to exclude the possibility of the blood being that of an elephant or monkey, there remain (among the commoner animals) only the dog, the ass, and the rodents as possible sources, and the corpuscles of all these are sensibly smaller than those of human blood. A person accused of crime will often give an explanation of the origin of a blood-stain which the size of the corpuscles will enable the expert to confirm or refute.

In a case within the author's personal experience, a man accused of murder asserted that certain stains were caused by the blood of a sheep, an explanation which the size of the corpuscles showed to be false.[1] In another similar case the stain was attributed to the blood of a fowl, an origin which the circular shape of the corpuscles proved to be impossible.

In the plate on page 454 the differences in the size of the blood-corpuscles from various animals, as viewed under a magnifying power of 1,150 diameters, is very apparent.[2]

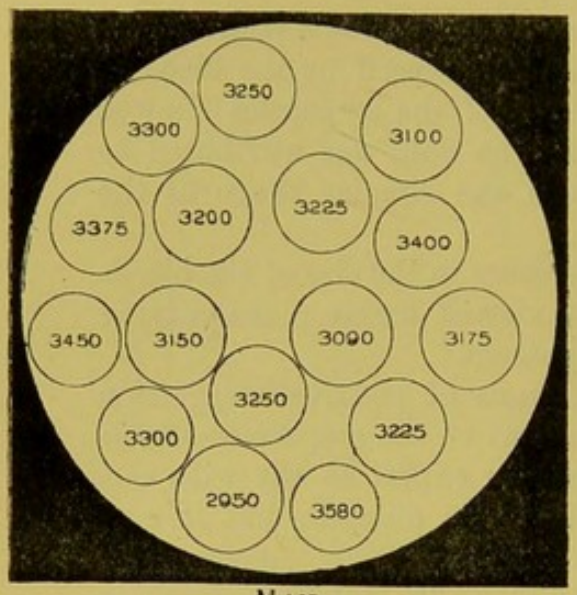
Hence, while not furnishing the means of positive recognition of human blood, the careful measurement of the corpuscles affords a valuable presumption, and enables the expert to say with certainty that the blood is *not* that of certain animals, but is consistent in its characters with human blood.

In examining blood-stains microscopically, before concluding that the discs observed are actually blood-corpuscles, a little solution of iodine should be allowed to run under the slide. *Starch-granules*, which sometimes present a close resemblance to blood-corpuscles, will be turned blue, and are thus readily differentiated. The application of iodine or magenta will generally cause the appearance of a true nucleus in *sporules* of certain fungi which somewhat closely resemble blood-corpuscles.[3] The discs met with in deal, cedar, and other coniferous woods sometimes present a resemblance to blood-corpuscles; but their arrangement in rows, the double ring which surrounds them, and other peculiarities, prevent them from being mistaken for blood-discs by a careful observer.

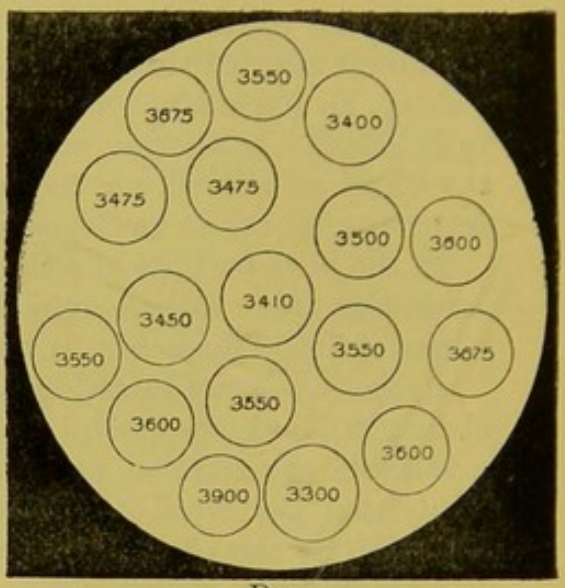
[1] Wormley mentions a case in which the clothes of the accused presented numerous stains which he claimed were due to the slaughter of sheep, and his statement with regard to killing sheep was confirmed by several witnesses. A close examination of the clothes indicated the existence of two kinds of stains, and subsequent observations by three independent persons showed that while some of the stains contained corpuscles similar in size to those of sheep's blood, others contained corpuscles wholly inconsistent with the theory of the accused, and of a size corresponding with the corpuscles of human blood.

[2] The illustrations are from the late T. G. Wormley's *Micro-Chemistry of Poisons*, to the publishers of which work, Messrs J. B. Lippincott & Co., the author is indebted for the use of the plate.

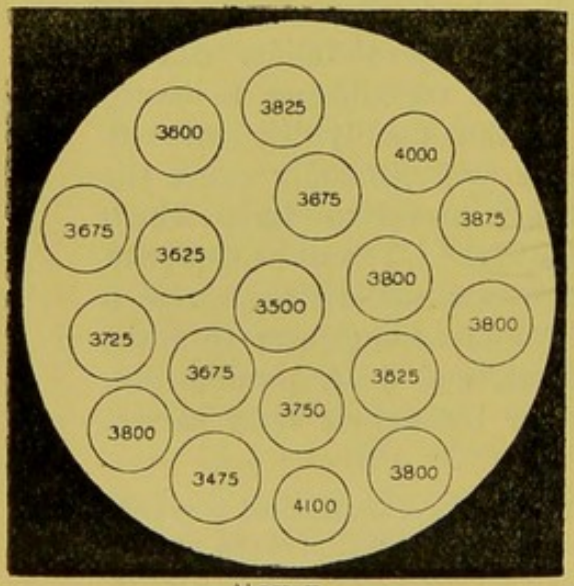
[3] Gorup-Besanez (*Physiol. Chem.*, 1867, p. 350) mentions a case in which a specimen of earth was, from its red colour, suspected to be strongly impregnated with blood. On examination by Erdmann, it was found to contain minute circular bodies which might readily be mistaken for blood-corpuscles, but which were in fact the spores of the alga *Porphyridium cruentum*. An examination of the spectrum would have shown at once the absence of blood.



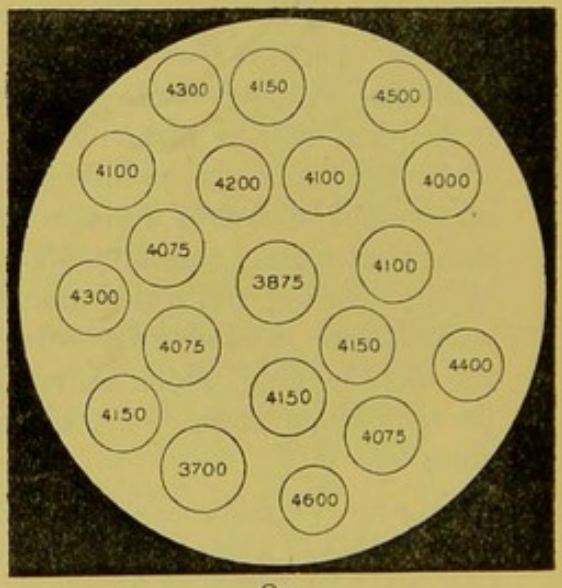
MAN.



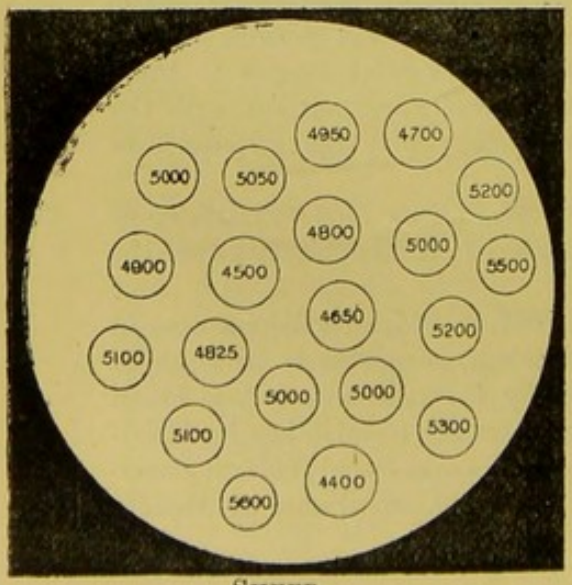
DOG.



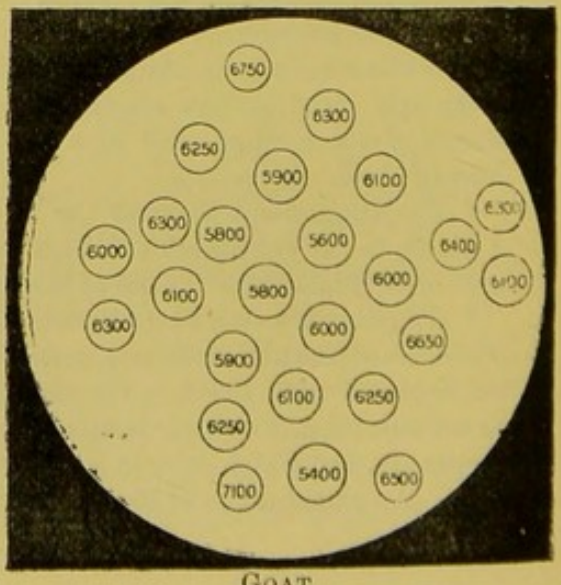
MOUSE.



OX.



SHEEP.



GOAT.

Detection of Blood in Refuse Liquids.

For the examination of *soapy water* and similar refuse-liquids supposed to contain blood, the sample should be rendered distinctly acid with acetic acid, and any liberated fatty acids removed by agitation with ether. The aqueous liquid is then separated, neutralised, and treated with zinc acetate as long as precipitation occurs. The precipitate is filtered off, washed with cold water, dissolved in soda or ammonia, a reducing agent added, and the liquid examined spectroscopically. The hæmin test is also available. *Urine* may be examined in a similar manner.

Struve has described a method of detecting blood in *urine*, based on the preparation of hæmin crystals from the precipitate produced by tannin. A small quantity of the washed precipitate is placed, while still moist, on a microscope-slide, and allowed to dry spontaneously. It is then treated with sodium chloride and acetic acid in the manner described on page 416. C. Rosenthal (abst. *Jour. Chem. Soc.*, l. 956) regards this method as uncertain, but considers the presence of iron in the ash of the tannin-precipitate to be satisfactory evidence of the presence of the colouring matter of blood in the urine.

Sorby examines the untreated urine with the micro-spectroscope, or after filtration and dilution of the urine to an extent sufficient to prevent marked absorption of the less refrangible portion of the green of the spectrum. The urine to be examined is contained in a 10-inch tube closed by glass plates. Obviously, the 2-decimetre tube of a polarimeter would be suitable for the purpose. Sorby states that as little as one drop of blood in a pint of urine can thus be detected.

PROTEOÏDS OR ALBUMINOÏDS.

The term "albuminoid" was formerly used to designate not only albumin and its congeners, but also gelatin and its allies. As previously stated (page 1) there has recently been a tendency to restrict the term "albuminoid" to bodies allied to gelatin and keratin, while distinguishing albumin and its more immediate allies as "proteids."

It is undesirable and confusing to divert a name from its original or commonly-accepted signification, and hence it is better to avoid the term "albuminoid" whenever possible, and distinguish the bodies of the two classes as *proteids* and *proteoïds*. The more important members of the first of these classes have already been considered. To the proteoïds belong gelatin and its congeners, keratin, fibroïn, chitin, &c.

The proteoïds are a group of highly-complex nitrogenised compounds, which are all strictly amorphous. They are insoluble in cold water, but some members of the class are dissolved by boiling with water (with or without pressure), to solutions which, if not too dilute, gelatinise on cooling. These compounds may be conveniently grouped together under the name of collagenes or gelatoïds. A distinction between the proteids and gelatoïds, which has been very generally insisted on, is that when compounds of the former class are split up by the action of somewhat diluted mineral acids, tyrosine and other compounds of the aromatic series are prominent among the products of their decomposition; whereas gelatin and its congeners yield no appreciable quantities of aromatic compounds on similar treatment.[1] This

[1] The accuracy of these generally-accepted statements, and the deductions therefrom, have been challenged by R. Maly (*Monatsh. Chem.*, x. 26; abst. *Jour. Soc. Chem. Ind.*, 1889, p. 468). From experiments based on the oxidation of the substances by potassium permanganate, he concludes that albumin and gelatin behave similarly. He regards the formation of iso-glyceric acid from the former substance and its absence from the products of the oxidation of gelatin as of little importance, and probably due to differences in the condi-

distinction does not hold good for bodies of the keratin and fibroïn classes, tyrosine being present to a sensible extent in the products of their decomposition.

In the absence of definite knowledge as to their constitution, the proteoïds scarcely admit of classification, but the better-known members may be provisionally grouped as follows :—

A.	COLLAGENES or GELATOÏDS. *Collagen* and *Gelatin;* from bones, skin, &c. Pages 458, 460. *Chondrigen* and *Chondrin;* from permanent cartilages. Page 494. *Isinglass;* from swimming bladders of fishes. Page 476. *Sericin* (silk-gum) from silk. Page 502. *Hyalins.* Page 498.	Dissolved more or less readily by boiling water. The solutions gelatinise on cooling. Contain little or no sulphur.
B.	FIBROÏDS. *Elastin*, from elastic ligaments. Page 499. *Fibroïn*, from silk and spiders' webs. Page 503.	Unacted on by boiling water or very dilute boiling alkali. Dissolved by stronger alkali. Unaffected by dilute acids. Contain no sulphur.
C.	CHITINOÏDS. *Chitin*, from external coatings of invertebrata. Page 528. *Conchiolin*, from shells of mollusca. Page 529. *Spongin*, from sponges. Page 529.	Unacted on by boiling water or alkalies. Contain no sulphur.
D.	KERATOÏDS or KERATIN SUBSTANCES. *Keratin;* from hoofs, horns, feathers, hair, wool, &c. Page 530. *Neurokeratin*, from brains. Page 531.	Unacted on by boiling water. Dissolved by boiling with dilute caustic alkali. Contain sulphur.

tions of the experiments. According to Maly, the supposed distinction between albumin and gelatin, based on the occurrence of compounds of the aromatic series among the products of decomposition of the former substance, does not hold good, since benzoic acid is formed by the oxidation of gelatin, though no indole nor tyrosine can be detected. Maly considers that this difference of behaviour does not prove constitutional difference, since oxyprotosulphonic acid (*Monatsh. Chem.*, 1885, page 91), which is simply oxidised albumin, yields benzoic acid, but not tyrosine, indole, or phenol, when boiled under pressure with baryta-water. Hence gelatin behaves exactly like oxidised albumin, and oxyprotosulphonic acid is a compound intermediate between albumin and gelatin, as shown by the following table :—

	Carbon.	Hydrogen.	Nitrogen.	Oxygen.	Sulphur.
Albumin, ...	52·98	7·09	15·70	22·41	1·82
Oxyprotosulphonic acid, . .	51·21	6·89	14·59	25·54	1·77
Gelatin, ...	50·2	6·7	17·9	25·0	

Oxyprotosulphonic acid does not give lead sulphide when boiled with lead

COLLAGENES or GELATOIDS.

The bodies of this group are insoluble in cold water. By boiling with water they are converted more or less readily into soluble products, the solutions of which *gelatinise* on cooling. Thus collagen or osseïn, the gelatinoid proteoïd of ligaments and bones, undergoes hydration by boiling with water, and is thereby converted into *gelatin*. Similarly, chondrigen, the proteoïd of permanent cartilage, yields *chondrin* by boiling with water. Sericin, the collagene of silk ("silk-glue"), when boiled with water, yields a solution which, if sufficiently concentrated, forms a jelly on cooling.

Collagen. Osseïn.

The white fibres of connective tissue consist essentially of a proteoïd called collagen, which, by prolonged boiling with water, is converted into gelatin.

Collagen may be prepared by first soaking finely-divided tendons in water to remove soluble proteids, and then for some days in lime-water to dissolve the mucoid cementing-substance between the fibres. The matter left insoluble is washed with water, then with dilute acetic acid, and lastly again with water. The product con-

acetate and caustic alkali, but yields a sulphite when fused with caustic soda or heated under pressure with baryta-water.

Maly concludes that gelatin is as much an albuminoid (proteid) as fibrin or casein, and that the classification into albuminoids (proteids) and gelatinoids cannot be maintained.

By the action of alcoholic hydrochloric acid upon gelatin, Buchner and Curtius (*Berichte*, xix. 850; *Jour. Chem. Soc.*, l. 635) obtained a diazo-compound which had all the characteristic properties of a fatty diazo-compound. The action of caustic alkali upon this compound results in the formation of ethyl alcohol, from which result it is probable that the compound has the constitution :—$CHN_2.CH(OH).CO.O(C_2H_5)$. Albumin treated in the same manner gave a similar result.

According to P. Schützenberger (*Compt. rend.*, cii. 1296; abst. *Jour. Chem. Soc.*, l. 818) when gelatin or collagen is heated at 200° C. with barium hydroxide, one-fifth of the total nitrogen is converted into ammonia. Carbonic and oxalic acids are also formed (in the ratio of the products of decomposition of urea and oxamide) together with amido-acids of the acetic series, including glycocine, alanine, leucine, &c. In addition to the above products, Schützenberger obtained acids of the series $C_nH_{2n}N_2O_5$, the value of n varying from 8 to 10. From these results it appears probable that these acids contain nitrogen in the form of the imido-group, and that collagen is formed, with elimination of water, by the combination of one molecule of urea or oxamide, two $C_nH_{2n}N_2O_5$ groups, and four $C_nH_{2n+1}NO_2$ groups (n being 2, 3, 4, or 6, with a mean value of 3·5).

sists of collagen, mixed with small quantities of nuclein and elastin.

A better product may be obtained by digesting carefully-cleansed tendons with trypsin, which dissolves all the tissue-elements except the true collagenous fibrils.

The collagenous matter of bones, often called osseïn, consists of collagen. It may be obtained by digesting bones for some days in cold dilute hydrochloric acid, which dissolves the calcium phosphate, &c., without affecting the osseïn. After soaking in cold water to remove residual acid and calcium salts, the osseïn remains as a swollen elastic mass which retains the shape of the original bone.[1]

As thus prepared, collagen or osseïn is insoluble in water, saline solutions, or dilute alkalies. In dilute hydrochloric acid it swells up to a transparent gelatinous mass without undergoing solution, and on exact neutralisation of the acid returns to its original condition.

By prolonged boiling with water at the ordinary pressure, but more readily under increased pressure, collagen or osseïn dissolves with conversion into gelatin, the solution of which gelatinises on cooling. The conversion occurs more rapidly in presence of an acid, but unless the operation be carefully regulated the gelatin first formed undergoes further change into gelatin-peptones or gelatones, the solutions of which do not form a jelly on cooling.

The change of collagen successively into gelatin and gelatin-peptones is doubtless a process of hydrolysis, and is represented by Hofmeister by the following equations :—

(1) $$\underset{\text{Collagen.}}{C_{102}H_{149}N_{31}O_{38}} + H_2O = \underset{\text{Gelatin.}}{C_{102}H_{151}N_{31}O_{39}}$$

(2) $$\underset{\text{Gelatin.}}{C_{102}H_{151}N_{31}O_{39}} + 2H_2O = \underset{\text{Semiglutin.}}{C_{55}H_{85}N_{17}O_{22}} + \underset{\text{Hemicollin.}}{C_{47}H_{70}N_{14}O_{19}}$$

The change from collagen to semiglutin and hemicollin is

[1] Bones vary considerably in composition, especially with regard to the proportions of water and fat, the ratio of osseïn to mineral matters being fairly constant. According to Hoppe-Seyler, the average composition of undried bone, without separation of marrow or blood, is as follows :— Water, 50·00 ; fat, 15·75 ; osseïn, &c., 11·40 ; and mineral matters, 21·85 per cent.

Approximately, two-thirds of the dry solids of bone consist of inorganic matters and one-third of organic substances. *Bone-ash* contains, on the average, Ca, 38·49 ; Mg, 0·44 ; PO_4, 54·46; CO_3, 6·24 ; Fl, 1·28 ; and Cl, 0·19 per cent. *Bone-black* or *Animal Charcoal*, obtained by heating bones in closed retorts, contains about 84 per cent. of calcium phosphate, 6 of calcium carbonate, and 10 per cent. of carbon. On exposure to air, it absorbs from 7 to 10 per cent. of water.

accompanied by an increase of 2·22 per cent. in weight, doubtless due to the assimilation of the elements of water.

On the other hand, Hofmeister states that gelatin can be re-converted into collagen by heating it to 130° C. Hence collagen appears to be an anhydride of gelatin.

The characters of gelatin-peptones are fully considered on page 465 *et seq.*

Isinglass appears to be a collagen which is converted into gelatin with great facility. Its characters are fully described on page 476.

Gelatin.[1]

Gelatin does not appear to exist ready-formed in nature,[2] but is a proteoïd resulting from the hydrolysis of collagen or osseïn by boiling with water or dilute acids.

For the preparation of *pure gelatin* the best quality of the commercial article should be soaked in successive quantities of cold distilled water for some days to remove salts, dissolved in boiling water, and the hot solution filtered into strong alcohol (90 per cent.). The gelatin is precipitated in white stringy masses, which are collected, re-dissolved in hot water, and re-precipitated by alcohol. The product still contains about 0·6 per cent. of ash.

The composition of gelatin is approximately:—Carbon, 50·2; hydrogen, 6·7; nitrogen, 17·9; oxygen and sulphur, 25·0. The following formulæ have been ascribed to gelatin:—

Schützenberger and Bourgeois, .	$C_{76}H_{124}N_{24}O_{29}$
Hofmeister, ...	$C_{102}H_{151}N_{31}O_{39}$

The recorded statements respecting the presence and proportion of sulphur in gelatin show great discrepancies. Schlieper found from 0·12 to 0·14 per cent. of sulphur in gelatin from bones and ivory, and von Bibra always found very appreciable quantities of sulphur in bone-gelatin. Hammarsten (*Jour. Pharm. et Chim.*, ix. 273) found about 0·7 per cent. of sulphur in fine commercial gelatin yielding 1·74 per cent. of ash. An analysis of Nelson's gelatin in the author's laboratory showed the presence of 0·17 of sulphur, while Brazilian isinglass was found to contain 0·38 per cent.

The constitution of gelatin is discussed on page 456.

[1] Gelatin is sometimes called glutin, but it does not appear desirable to encourage the use of this synonym, owing to its close resemblance to gluten, the proteid of wheat (page 69).

[2] According to this view, isinglass is not actual gelatin, but an anhydride which is converted into gelatin with great facility by the action of water.

Pure gelatin is an amorphous, more or less transparent, brittle substance, having a vitreous appearance. It is free from colour, taste, and smell.

When heated, gelatin softens without actually melting, swells up, and decomposes with an odour resembling that of burning hair or feathers, leaving a difficultly-combustible charcoal.

In the dry solid state, gelatin is unalterable in the air, but when moist or in solution it putrefies with extreme facility, the solution first becoming acid and subsequently ammoniacal. The formation of putrefaction-products of acid reaction is characteristic of gelatin.

When immersed in cold water, gelatin gradually swells up and softens, taking up from five to ten times its weight of water without undergoing solution to any sensible extent. The amount of water absorbed is sufficient to dissolve the gelatin completely on warming it to 30° C.

In boiling water, gelatin dissolves readily to form a solution neutral to litmus. If the solution of gelatin in hot water be very dilute, it remains unchanged on cooling, but if somewhat stronger it becomes viscous. When the proportion of gelatin exceeds about 1 per cent., the solution sets to a jelly on cooling, the consistency of the jelly formed increasing with the concentration of the liquid, but being also dependent on the origin and purity of the gelatin. The jelly liquefies again completely at the boiling-point of water. Repeated heating and cooling destroy the property of gelatinising, but this character is said to be restored by precipitating the gelatin from its solution with excess of alcohol. Long-continued boiling at the ordinary pressure prevents a solution of gelatin from gelatinising on cooling, and the property is lost instantly by heating the solution under increased pressure to 140° C. (compare page 465).

If potassium bichromate be added to a solution of gelatin in hot water, the jelly which forms on cooling becomes insoluble in warm water after exposure to light. This fact is largely utilised in photo-lithography.[1]

[1] The exact *modus operandi* varies materially according to circumstances. Broadly speaking, it consists in obtaining a sensitive film by pouring a solution of bichromated gelatin on to a glass plate. This is exposed under a photographic negative, and then treated with warm water. The gelatin dissolves from those portions of the film which were protected from the light by opaque parts of the negative, while the rest remains insoluble. In this manner a reproduction of the photograph is obtained in relief, and after being hardened by alum can be used in a printing-press. In practice, however, the gelatin film is not used direct, the impression being transferred to a lithographic stone.

Solutions of gelatin are eminently undiffusible,—in fact, the generic term "colloid," applied to such liquids, is founded on this circumstance.

Gelatin exhibits a strong lævo-rotation. The value of $[a]_D$ at 30° C. is stated by Hoppe-Seyler to be −130°.

Gelatin is practically insoluble in ice-cold water containing 10 per cent. of alcohol. This fact is employed for its determination. In strong alcohol, gelatin is insoluble at any temperature, and on adding excess of alcohol to its aqueous solution, the gelatin is thrown down as a white, coherent, elastic mass. No true coagulation occurs, for the precipitate has all the characters of the original gelatin, swelling up in cold water and dissolving on heating.

Gelatin is wholly insoluble in alcohol, ether, chloroform, benzene, carbon disulphide, and fixed and volatile oils.

Strong acetic acid dissolves gelatin to form a solution which does not gelatinise on cooling, but yet possesses powerful adhesive properties. "Coagulin" and similar commercial preparations are of this nature. Dilute nitric acid forms a similar product soluble in cold water ("marine glue"; "soluble glue"; compare page 490). When heated with strong nitric acid, gelatin is destroyed with formation of oxalic acid and other products.

Boiling with hydrolysing agents converts gelatin into a mixture of gelatin-peptones or gelatones (page 465).

By treatment with moderately concentrated sulphuric acid, or by boiling or careful fusion with caustic potash, gelatin is broken up with formation of leucine and glycocine as the chief products. No tyrosine is produced, a fact which distinguishes gelatin from albumin and other products (compare, however, page 456).

On dry distillation, gelatin gives a highly offensive oil (Dippel's oil; bone oil), containing a considerable proportion of pyridine bases; together with ammonia, permanent gases, and other ordinary products of the decomposition by heat of nitrogenised organic matters.

On adding gallotannic acid to an aqueous solution of gelatin, a white or buff-coloured precipitate is formed. A solution of gelatin in 5000 parts of water is at once rendered turbid by gallotannic acid or other variety of tannin. Nevertheless, the precipitate is not wholly insoluble in pure water, especially if hot, but is quite insoluble in presence of excess of tannin.

The reaction of gelatin with tannin is utilised for the detection of both bodies, and is employed for the assay of tannin matters. It is also utilised for tanning, the gelatinoids of the skin being thereby converted into leather.

The compound of gelatin with tannin is sometimes called tannate of gelatin, but it is doubtful if it has a constant composition. According to Mulder, several compounds of gelatin with tannin exist. Gallotannic acid has been found to combine with dry gelatin in the proportion of 135 parts to 100. E. Davy states that the compound of gelatin with oak-bark tannin contains 54 per cent. of gelatin and 46 of quercitannic acid. Schiebel obtained nearly similar results, and states that 100 parts of gelatin treated with a large excess of a 10 per cent. solution of oak-bark combines with 118·5 parts of quercitannic acid. On the other hand, when a dilute tannin solution was employed, and the gelatin was present in excess, a slowly-subsiding precipitate was obtained which contained 59·25 per cent. of tannic acid.

Gelatin is not thrown down from its aqueous solution by the ordinary mineral or organic acids, by alkalies,[1] or by most metallic salts. Phospho-molybdic and phospho-tungstic acids precipitate it, as does mercuric chloride (if used in excess); but no precipitate is produced by alum, ferric chloride or sulphate, salts of copper, or by neutral or basic lead acetate. Saturation of its aqueous solution by ammonium sulphate, magnesium sulphate, or zinc sulphate completely precipitates gelatin.

On adding a saturated aqueous solution of picric acid to a cold aqueous solution of gelatin, a precipitate is produced which re-dissolves on shaking. On gradually continuing the addition of picric acid, the precipitate becomes permanent; but dissolves on heating, and reappears as the solution cools. The precipitate coagulates on shaking, producing a yellow sticky mass, and leaving the liquid nearly clear (Allen and Tankard).

Solutions of silver and gold are stated not to precipitate gelatin, but by the action of sunlight some of the metal becomes reduced.

Platinic chloride solution reacts with gelatin in much the same way as picric acid, except that the precipitate does not appear to be so readily soluble in excess of gelatin.

Platinic sulphate solution also gives a precipitate with solutions of gelatin, which rapidly coagulates on standing, even without shaking. In other respects, the reaction of gelatin with platinic sulphate is similar to its behaviour with the chloride.[2]

[1] Addition of caustic soda or ammonia to a solution of commercial gelatin often produces a considerable precipitate of calcium phosphate.

[2] According to E. Davy, platinic sulphate precipitates gelatin in the form of brown, viscous flakes, which blacken on the filter and are afterwards easily pulverised. He regards the reaction as an infallible test for gelatin,

L. Crismer recommends an acid solution of chromic acid as a precipitant for gelatin (compare page 328).

With the biuret test (page 12) gelatin gives a violet coloration. No precipitation of cuprous oxide occurs on boiling the liquid.

When a current of chlorine is passed through a solution of gelatin of about one per cent. strength, the liquid remains clear for a time, but subsequently froths strongly, each bubble becoming encased in a white pellicle. When the chlorine is in excess, as indicated by the yellow colour of the liquid, the frothing subsides, the liquid becomes clear, and the whole of the gelatin is thrown down as a white granular precipitate. When this is thoroughly washed with cold water and dried *in vacuo* over sulphuric acid, the substance is obtained as a pale yellowish-white powder, which is odourless, tasteless, and imputrescible, and insoluble in water or alcohol, but soluble in alkalies.[1]

Contrary to the general statement, that bromine and iodine give no compound with gelatin similar to that yielded with chlorine, the author, in collaboration with A. B. Searle (*Analyst*, 1887, p. 258), has proved bromine to act in a very similar manner to chlorine, and this observation has been independently made and extended to iodine by Hopkins and Brook (*Jour. Physiol.*, xxii. 184). The method of operating employed by the author is that described on page 315, but the process as applied to gelatin is not at present capable of successful employment.[2]

applicable even in presence of albumin and in solutions so dilute as to give no indication with tannin. The author has been unable to confirm Davy's observation.

[1] The observations described in the text are those of Rideal and Stewart (*Analyst*, 1897, p. 228). The reaction of chlorine on solutions of gelatin was first described by Mulder in 1840, and was regarded by him as an argument in favour of his protein theory, the precipitate itself being termed protein-chlorous acid (*Berzelius' Jahresber.*, xix. 734; *Jour. f. Chem.*, xliv. 489). Considerable controversy occurred as to the chemical nature of the compound, but all agreed as to its properties, its constancy of composition, and its insolubility. The proportion of nitrogen found in the compound by the early observers is in close agreement with the percentage found by Rideal and Stewart in specimens prepared by them and analysed after drying at 80° C.

[2] Experiments made under the author's direction by A. R. Tankard, on various specimens of commercial gelatin and glue, yielded results which at present are incapable of interpretation. The completeness of the precipitation of gelatin by bromine-water is affected by conditions not at present understood. In some cases the precipitation was very complete, while in other experiments, in which the conditions were but very slightly varied, much nitrogen remained unprecipitated. The author has the subject still under investigation.

The recognition of gelatin in animal fluids is attended with some difficulty. The reaction with tannin is fairly delicate, but is also given by all the proteids. The behaviour of gelatin with picric acid (page 463) is peculiar, and may be occasionally useful for its recognition. As a rule, the property of gelatinising on cooling is the only test from which the presence of gelatin in a complex animal liquid can be safely inferred (compare page 326).

Gelatin is distinguished from albumin and its allies by not yielding a precipitate with potassium ferrocyanide or ferricyanide. Reactions distinguishing gelatin from chondrin and mucin are described on page 496.

Gelatin is very readily digested, but it is doubtful how far, when given alone, it has a true alimentary value. Pure gelatin certainly does not rank with the proteids as a flesh-former, notwithstanding its high percentage of nitrogen, and animals rapidly waste when fed solely on gelatin. On the other hand, according to Voit, in conjunction with a small quantity of proteid food gelatin is capable of maintaining nitrogenous equilibrium as well as if the food taken were wholly proteid in nature. Voit distinguishes between circulating and organic albumin, and considers that while gelatin can never replace the latter it may replace the former so far as it prevents the conversion of organic into circulating albumin ; and it also diminishes the waste of fat in the body. The question has great practical importance, in view of the widely-prevalent practice of feeding invalids on gelatinous preparations.

Gelatin-Peptones. Gelatones.

By the action of dilute acids, of certain neutral salts (*e.g.*, the chlorides and iodides of the alkali-metals), of the gastric and pancreatic ferments, and of certain microbes, gelatin loses its characteristic property of forming a jelly when its hot, tolerably concentrated aqueous solution is cooled. The prolonged action of boiling water alone induces the same change, and it is stated that contact with warm water always modifies gelatin to some extent, though the change only becomes pronounced when the treatment is prolonged. The change is accompanied by an assimilation of the elements of water and an increase in weight, the whole action being apparently closely allied to that by which albumoses and peptones are produced from coagulable proteids by similar treatment. In fact the product (or products) of the reaction may be conveniently called gelatin-peptone or gelatone.[1] Two distinct

[1] P. Tatarinoff (*Compt. rend.*, xcvii. 713) prepared gelatin-peptone by digesting pure gelatin with pepsin-hydrochloric acid at 40° C., and when solution was complete neutralising the liquid with calcium carbonate, boiling,

gelatones are recognised by Hofmeister, who terms them respectively *semiglutin* and *hemicollin* (see page 459). Semiglutin is sparingly soluble in 70 to 80 per cent. alcohol and is precipitated by platinic chloride, whereas hemicollin is soluble in 70 to 80 per cent. alcohol, and is not precipitated by platinic chloride.

C. Paal (*Ber.*, xxv. 1202) has shown that when gelatones are formed by the action of dilute mineral acids on gelatin they unite with the acid to form salts, which are not only soluble in water, but, unlike the free gelatones, in ethyl and methyl alcohols. For their preparation, Paal warms 100 parts of the purest commercial gelatin on the water-bath with 160 parts of water and 40 of concentrated hydrochloric acid, until a sample of the product is completely soluble in a large quantity of absolute alcohol. The whole is then poured into four to five volumes of absolute alcohol, the precipitated salts filtered off, the filtrate treated with ether, the precipitate redissolved in alcohol, and the resultant solution evaporated under reduced pressure. The gelatone hydrochloride thus obtained forms a brittle, white, vesicular mass, which is readily soluble in water, methyl alcohol, ethyl alcohol, and acetic acid; but is insoluble in ether, benzene, or carbon disulphide. It is very hygroscopic, is unaltered at 130° C., and gives a violet coloration with the biuret test. It is lævo-rotatory, the value of $[a]_D$ in aqueous solution being about $-60°$. The proportion of hydrochloric acid found in different preparations ranged from 10·5 to 12·5 per cent., while the ash was only about 0·5 per cent. When stronger hydrochloric acid was employed, or the heating continued longer, compounds containing a higher percentage of acid were obtained. This fact tends to show that the products are a mixture

filtering, and precipitating the concentrated liquid by alcohol. After standing twenty-four hours, the precipitate was dissolved in cold water, and the filtered liquid dialysed after addition of a few drops of hydrochloric acid. The dialysate was concentrated, and the peptone again precipitated by alcohol. Two preparations obtained in this manner had the following percentage composition :—

	Carbon.	Hydrogen.	Nitrogen.	Difference.
I., ...	50·00	7·26	17·57	25·17
II., ...	49·53	7·00	17·69	25·78

A similar product was obtained by digesting gelatin with stronger hydrochloric acid alone, at a somewhat higher temperature. It seems probable that Tatarinoff's products consisted of gelatone hydrochlorides and not of free gelatone.

of compounds representing different degrees of peptonisation. The hydrochlorides containing high proportions of acid are more diffusible and soluble than those containing small percentages of HCl. Thus, by dialysing a salt containing 10·56 per cent. of acid, Paal obtained from the diffusate a compound containing 14·19 per cent. of HCl, whereas the residue contained only 5·79 per cent. and was insoluble in absolute alcohol.[1]

A similar separation was effected by treating the alcoholic solution with mercuric chloride, whereby two mercurichlorides were obtained. One of these separated out at once, and the second on addition of ether.

On warming gelatin with a weaker acid than was used in the foregoing experiments a salt was obtained which contained 6·85 per cent. of HCl, and was insoluble in ethyl alcohol, but soluble in methyl alcohol; while the product obtained by means of pepsin and very dilute hydrochloric acid was not completely soluble in cold methyl alcohol, and could be separated by dialysis into two fractions, one of which contained 2·97 per cent. of HCl and was almost insoluble in methyl alcohol, while the other contained 11·13 per cent of HCl.[2]

In order to prepare the free gelatones from their hydrochlorides, a slight excess of silver sulphate should be added to the solution, the excess of silver removed from the filtrate by hydrogen sulphide, and the sulphuric acid by an equivalent amount of baryta-water. The gelatones thus obtained are soluble in all proportions in water, but are insoluble in alcohol or ether. Their aqueous solutions are acid to litmus, but do not turn congo-red to blue. The percentage of carbon in gelatones is somewhat less and that of hydrogen rather higher than in gelatin, which fact is in agreement with the

[1] It was found that only those salts containing 10 per cent. or upwards of HCl were soluble in absolute alcohol, and those fractions which were only just dissolved by ethyl alcohol were much less soluble in propyl alcohol, and insoluble in amyl alcohol; while the salts with less than 10 per cent. of acid were all readily soluble in methyl alcohol, and dissolved also in the ethyl alcohol solutions of the salts containing more acid.

[2] From determinations by the cryoscopic method in aqueous solution, and by the boiling-point method on solutions of gelatone hydrochlorides in water, methyl alcohol, and ethyl alcohol, Paal concludes that the molecule is less as the percentage of acid increases, the molecular weight of the free gelatones being about 300 in three cases, and 215 in a fourth; while gelatin itself has a molecular weight of about 900. (Hofmeister's formula for gelatin, $C_{102}H_{151}N_{31}O_{39}$, corresponds to a molecular weight of 2433.) Paal's results further show that the gelatone hydrochlorides are stable in solution in ethyl alcohol, but that when in solution in water or methyl alcohol they are dissociated into equal molecules of gelatone and hydrochloric acid.

view that they are products of the hydrolysis of the latter body. Paal considers that the gelatin-molecule is resolved by hydrolysis into gelatones of gradually decreasing molecular weight, till a point is reached at which they are split up with formation of amido-acids, lysine, lysatine, &c. (page 379); and that, as the molecule of gelatin, like that of albumin, consists of two proteid atom-complexes which suffer hydrolysis with different degrees of facility (the salts containing a high proportion of acid being the more readily converted into amido-acids and other non-proteid products), the simpler products of decomposition are always mixed with unaltered gelatones.

In a later paper (*Ber.*, xxxi. 956), Paal states that the hydrochlorides containing the lower proportions of acid are precipitated by saturating their solutions with ammonium sulphate, and hence are salts of *gelatoses*, analogous to the proteoses (page 372). The portions not precipitated by ammonium sulphate consist of salts of gelatin-peptone or *gelatone*. On the average, these latter salts contain, when anhydrous :—ash, 0·18 to 0·58 per cent.; HCl, 10·38 to 13·14; C, 43·28 to 45·95; and H, 6·43 to 7·13 per cent. By dialysis, Paal effected a further fractionation of these salts. Thus from the diffusate of a hydrochloride which was hardly soluble in ethylic alcohol, but which dissolved readily in cold methylic alcohol, Paal obtained a yield of more than 60 per cent. of the original substance, containing :—ash, 0·89; HCl, 13·44; and carbon and hydrogen corresponding to C, 48·86, and H, 7·31 per cent. in the free gelatone. On dialysing this last product into water, a further separation was effected, the portion diffusing during the first twenty-four hours being an extremely hygroscopic yellow mass, which was very soluble in alcohol, contained 16·06 per cent. of HCl, and yielded 1·21 per cent. of ash on ignition. The portion which diffused during the second period of twenty-four hours was much less soluble in alcohol, less hygroscopic, and contained only 9·38 per cent of HCl.

Paal finds these salts to be partially precipitated by phospho-tungstic acid, with liberation of the gelatone, while the unprecipitated portion gives a distinct biuret reaction.[1] By treating the phospho-tungstic precipitate with barium hydroxide in excess, a *barium-gelatone* was obtained; and this reacted with ferrous sulphate, yielding an aqueous solution of *ferro-gelatone*. This latter body absorbed atmospheric oxygen, especially on warming, forming free gelatone, which remained in solution, and ferric hydroxide, which was quantitatively precipitated.

[1] Paal attributes this result to the formation of products intermediate in nature between gelatones and amido-acids.

Paal finds that when gelatone salts are dissolved in absolute alcohol and the solution saturated with hydrochloric acid gas, whilst warmed on the water-bath under a reflux-condenser, merely a trifling increase occurs in the amount of combined hydrochloric acid, further peptonisation only taking place in presence of water.

Chittenden and Solley (*Jour. Physiol.*, xii. 23; abst. *Jour. Chem. Soc.*, 1891, p. 949) have investigated the products of the digestion of gelatin by the gastric and pancreatic enzymes. They obtained three distinct products, two of which are related to the albumoses, and the third to Kühne's albumin-peptone. The two former substances, *proto-gelatose* and *deutero-gelatose*, are formed both in gastric and in pancreatic digestion, and are distinguished from the third product, which is a true gelatin-peptone or *gelatone*, by being (like albumoses) precipitated on saturating the liquid with ammonium sulphate. Proto-gelatose is partially precipitated by saturating its neutral solution with sodium chloride, and is completely precipitated on subsequently adding a little acetic acid. Proto-gelatose yields a heavy precipitate with hydrogen platinichloride. Deutero-gelatose is not precipitated by either of these reagents. By further ferment action, proto-gelatose is converted into deutero-gelatose, and finally into gelatin-peptone or gelatone. No trace of hetero-gelatose was obtained. Chittenden and Solley give the following analytical figures representing the percentage composition of the gelatin employed, and of the gelatoses obtained :—

	Gelatin used.	Products of Gastric Digestion.		Products of Pancreatic Digestion.	
		Proto-gelatose.	Deutero-gelatose.	Proto-gelatose.	Deutero-gelatose.
Carbon, . . .	49·38	49·98	49·23	49·45	49·07
Hydrogen, . . .	6·81	6·78	6·84	6·61	6·66
Nitrogen, . . .	17·97	17·86	17·40	17·81	17·52
Sulphur, . . .	0·71	0·52	0·51	0·57	0·65
Oxygen, . . .	25·13	24·86	26·02	25·56	26·10
	100·00	100·00	100·00	100·00	100·00
Ash, ...	1·26	1·98	1·08	1·75	1·08

Bacterial Decomposition of Gelatin.

The products of the decomposition of gelatin under the action of bacteria have been studied by Rideal and Stewart (*Analyst*,

xxii. 255). The following is a tabulated summary of the results obtained, the figures being grammes per 100 c.c. of the liquid [1]:—

	Total Nitrogen.	Gelatin and Albumoses.	Albumoses.	Ammonia and Volatile Bases.	Bases and Extractives.	Peptone N. unaccounted for.	Gelatin.	Albumin and Cellulose.
Original gelatin, . .	1·242	1·109	0·514	0·003	0·061	0·069	0·595	..
Original gelatin, . .	1·246	..	0·528	0·004	..	..	0·581	..
After incubation at 20° to 21° C. with—								
B. prodigiosus, 14 days,	1·232	1·024	0·409	0·049	..	..	0·615	..
B. fluor. liquefaciens, 1 day,	1·33	1·141	0·402	0·028	..	..	0·739	..
,, ,, 2 days,	1·256	0·682	0·297	0·045	0·105	0·424	0·385	..
,, ,, 3½ ,,	1·267	0·242	0·135	0·063	0·486	0·476	0·107	..
,, ,, 16 ,,	1·26	0·216	0·081	0·168	0·742	0·124	0·135	0·01

It will be noticed that the original dry gelatin contained roughly 50 per cent. of matter precipitable by Stutzer's reagent (so-called albumoses). In the early stages these albumoses seem to have been first attacked by the organism; but subsequently their decomposition proceeded at an equal rate with that of the true gelatin.

A notable feature is the small production of ammonia and volatile bases, in view of the fact that substances such as trimethylamine, indole, and skatole have been generally stated in analyses by other observers to be constant accompaniments of the liquefaction of proteids.[2]

The figures for the nitrogen unaccounted for, which would be almost entirely peptones, are interesting. The original gelatin

[1] The gelatin and albumoses were determined by precipitating the solution with saturated ammonium sulphate solution, dissolving the precipitate in warm water, and dividing. The total nitrogen was determined in one-half of the solution, and in the other, very exactly, the SO_3, the nitrogen due to the corresponding ammonium sulphate being deducted from the total ammonium sulphate.

The albumoses were determined separately by precipitating a portion of the original solution with Stutzer's reagent, washing, and estimating the nitrogen in the precipitate.

The volatile bases were determined in a portion of the original liquid by distillation with caustic soda and titration of the distillate. A further portion of the original was precipitated with alcohol, filtered, and the nitrogen-content of the filtrate regarded as existing as bases and extractives.

[2] That this was not due to the volatilisation of such bodies during the incubations was proved by the comparative absence of odour and of strong alkaline reaction in the air of the flasks, and in such loss not being indicated by the total nitrogen. Previous investigations by Rideal and Stewart with proteids showed that the quantity of ammonia produced was insignificant, amounting, even after sixteen days' incubation with *B. fluorescens liquefaciens*, to only 0·168 gramme of nitrogen per 100 c.c., corresponding to 0·204 gramme of ammonia.

solution contained 0·069 gramme per 100 c.c., but this gradually increased as the liquefaction proceeded, until, with *B. fluorescens liquefaciens*, it reached the maximum figure of 0·476, when the medium was just entirely liquefied, and then diminished, after sixteen days, to 0·124, showing that the bacillus, after bringing about the decomposition of nearly all the gelatin and albumoses, subsequently attacked the peptone, which had meanwhile been produced, with a corresponding increase of bases and extractives soluble in alcohol.

It is known that certain bacteria produce peptonising enzymes, and in the case of the *B. fluorescens liquefaciens* the organism appears, as shown above, to have subsequently fed on the peptone produced by its enzyme.

FORMO-GELATIN.

On adding a solution of formaldehyde—such as commercial formalin—to an aqueous solution of gelatin, no change occurs unless the gelatin solution be very concentrated or contains free alkali, but on evaporating the liquid to dryness, the gelatin is converted into an insoluble substance known as formo-gelatin. To effect complete conversion of the gelatin into this body, any free acid should be previously neutralised by agitation with precipitated calcium carbonate or other means. On treating the residue left on evaporation with boiling water any trioxymethylene formed from the formaldehyde is dissolved, while the formo-gelatin is unaffected.

From the small proportion of formaldehyde required to produce formo-gelatin, it is very doubtful whether that body is a definite compound. Acrylic aldehyde is stated to yield a similar product, but acetic aldehyde only reacts in the absence of water.

Formo-gelatin is quite insoluble in either cold or boiling water, but is completely dissolved by treatment with diluted sulphuric acid (1·34 sp. gravity) for twelve hours, whereas the corresponding compound from casein (page 95) remains undissolved.

According to E. O. Beckmann (*Chem. Centr.*, 1896, ii. 930) the same insoluble substance is obtained by the action of formaldehyde on gelatin which has lost its power of gelatinising by prolonged heating,[1] but he states that gelatin-peptone is not similarly

[1] To prepare an insoluble film of formo-gelatin from altered gelatin, A. Zimmermann (*Eng. Patent*, 1894, No. 23,585) dissolves 75 grammes of gelatin in 500 c.c. of water, and boils the liquid for two days, replacing the water as it evaporates. He then adds 4·2 grammes of commercial formalin, containing 40 per cent. of formaldehyde. The liquid remains clear for a prolonged time, but on evaporation yields insoluble formo-gelatin. Gelatin altered by boiling with dilute acids or alkalies acts in the same manner.

acted on. "Albumin-peptone" and "tryptone" are also said to be unaffected. Beckmann has based on these facts a method for the determination of gelatin in meat-extracts (page 325).

According to A. Zimmermann, methylene-blue colours formo-gelatin but not ordinary gelatin.

Formalin-gelatin has received several practical applications. Schröder prepares it (abst. *Pharm. Jour.*, 1896, ii. 63) by adding 2 per cent. of commercial "formalin" (40 per cent. aqueous solution of formaldehyde) to a warm solution of gelatin in its own weight of water. The liquid is stirred, the resulting mass covered with "formalin," and allowed to stand for some time. The product is then powdered, well washed with water, and dried. H. K. van Vloten (*Jour. Soc. Chem. Ind.*, 1896, p. 553), who prepared the compound in a somewhat similar manner, states that powdered formo-gelatin can be introduced into wounds without producing irritation.

The following method has been proposed by G. Romyn (abst. *Jour. Soc. Chem. Ind.*, 1896, p. 679) for the detection of unaltered gelatin in formo-gelatin. About 0·5 gramme of the sample is mixed with 10 c.c. of water and heated for ten minutes in the water-bath with frequent shaking. The mass swells considerably, but particularly so in the presence of unaltered gelatin, which goes into solution. If the liquid is now passed whilst hot through a dry filter, it will gelatinise on cooling if unaltered gelatin be present. The separation of the gelatin may be accelerated by placing the filtrate in a freezing mixture and allowing it to melt slowly.

Half a gramme of the sample is mixed with 5 c.c. of water and 1 c.c. of caustic soda solution. The mass swells considerably, but much more strongly in the presence of unaltered gelatin. If now a mixture of 2 c.c. of normal silver nitrate solution and 0·5 c.c. of ammonia be added, the mass should begin to darken within one or two minutes, and after five or ten minutes the liquid should have acquired a violet-brown colour. If much free gelatin is present the colour makes its appearance more slowly, and assumes a pure brown shade, without any violet. Pure gelatin does not produce any coloration until after the lapse of a few hours.

Commercial Gelatin.

Various kinds of gelatin occur in commerce, ranging in quality from the finest colourless gelatin to the coarsest kinds of size and glue. Isinglass and "fish-gelatin" are collagenous substances which readily undergo conversion into gelatin.

Commercial gelatin is prepared by subjecting fragments of

hoofs, horns, hides, bones,[1] calves' feet, &c., to the action of boiling water or steam.[2] The resulting liquor is skimmed and strained, to effect the removal of fatty matters and any deposit which may be formed. The resultant solution is then allowed to gelatinise by spontaneous cooling.[3]

Hartshorn shavings give a tasteless gelatin, which is quite free from fatty matter. Ivory-dust also yields a superior product.

A tasteless, inodorous, nearly colourless gelatin, fit for all purposes, is prepared by purifying the solution of the product from hoofs, &c., with a mixture of animal- and wood-charcoal.

The gelatin of commerce varies greatly in quality, and is often far from being so pure as is generally assumed. In addition to containing sensible amounts of *calcium carbonate* and *phosphate*, it sometimes contains a considerable proportion of *chondrin* (*q.v.* page 494) and of *gelatin-peptones* (page 465). The presence of these bodies is the probable cause of the great variation in the quality of commercial gelatin.[4]

[1] Bones are stated to yield about one-third of their weight of gelatin when treated in this manner.

[2] According to H. Weiske (*Bied. Centralb.*, 1883, p. 673), the gelatin obtained by boiling bones (freed from mineral matter) with successive quantities of water, differs materially from the product obtained by heating the residue from the foregoing treatment with water under pressure. The difference is probably due to the presence of gelatin-peptones in the latter product.

[3] Nelson's gelatin is manufactured from the cuttings or parings of skin, hide, parchment, &c. These are macerated in cold dilute caustic alkali till thoroughly softened, which is generally accomplished in about ten days, washed, treated with sulphurous acid gas, freed from water by pressure, and dissolved at a temperature of about 70° C. The solution is strained, allowed to settle, poured on slabs of slate or marble, and, when sufficiently solidified, cut into strips and washed to remove all trace of acid. It is then redissolved at a moderate temperature, re-solidified, and desiccated by exposure to dry air or placed *in vacuo* on nets.

[4] The following results were obtained by A. R. Tankard in the author's laboratory, by the analysis of samples of commercial gelatin, &c.

	Brazilian Isinglass.	Nelson's Gelatin.	Superior Glue.	Common Glue.
Moisture,	15·05	17·90	16·64	17·00
Sulphur,	0·38	0·17	0·58	0·46
Nitrogen precipitated by zinc sulphate,	14·00	13·09	9·59	11·06
×5·4% = gelatin,	75·88	70·95	51·98	23·71
Total nitrogen (by Kjeldahl),	14·56	14·10	14·00	14·42
Ash,	1·30	3·70	3·40	2·40
Containing:—				
Lime,	moderate	considerable	small	small
Magnesia,	..	..	trace	trace
Ferric oxide,	faint trace	trace	small	very small
Phosphoric acid,	present	present	present	trace
Carbonic acid,	none	considerable	none	none

The solution of a sample of Nelson's gelatin examined in the author's laboratory, gave a considerable precipitate with ammonium oxalate, and had an alkalinity to methyl-orange corresponding to 2·8 per cent. of CaO.

The suitability of commercial gelatin for alimentary use is readily tested by pouring boiling water over it, and digesting the two together for a short time. If the gelatin be pure and wholesome, its colour will remain unaltered, and it will evolve no disagreeable odour while dissolving. The resulting solution and jelly will also be odourless, free from unpleasant taste and acid reaction to litmus, and perfectly transparent. If the jelly is a yellow, gluey-looking mass of offensive odour, the gelatin is of inferior quality and unfit for culinary purposes.

The gelatin of the British Pharmacopœia (1898) is described as "the air-dried product of the action of boiling water on such animal tissues as skin, tendons, ligaments, and bones." It is required to be free from chondrin, since it is described as giving no precipitate with acids, alum, lead acetate, or ferric chloride (5 per cent.). The solution in 50 parts of hot water is described as inodorous and solidifying to a jelly on cooling.

Photographic Gelatin.—The following table by W. W. Abney (*Photography with Emulsions*) shows the characters of commercial gelatins intended for photographic purposes :—

Description of Gelatin, &c.	Ash, per cent.	Drachms of Water absorbed by 50 grains of Gelatin.
Coignet's gold-label,	nearly 1	7
Coignet's special,	,, 1	7
Nelson's No. 1 photographic,	,, 2	5½
Nelson's opaque,	,, 2	8
Nelson's amber,	,, 1	4
Ordinary French (not branded),	,, 2	6
Swinburne's No. 2 patent isinglass,	,, 1	5¾
Cox's gelatin, in packets,	,, 1	4¾
Gelatin supplied through Henderson,	,, 2	8
"Swiss" gelatin supplied through Houton,	,, 2	5

In judging of the suitability of gelatin for photographic purposes, Abney considers : (1) The proportion of ash of minor importance, specimens of excellent quality sometimes containing 2½ per

cent. (2) A good photographic gelatin will take up from 5 to 10 times its weight when soaked in cold water. (3) The solubility of commercial gelatin varies considerably. Thus Nelson's gelatin will dissolve in the ordinary "cold" water in warm weather, and scarcely sets at 75° F. (=24° C.), whereas Coignet's gold-label gelatin only melts at about 110° F. (=43·3° C.), and sets rapidly. For ordinary photographic emulsions, Abney recommends a mixture of hard and soft gelatins in proportions dependent on the weather, a good mixture containing 1 part of the former to 3 of the latter. (4) Fatty matters may be determined in the usual manner, or removed by skimming the solution of the sample or converting it into jelly and removing the top. (5) The colour of the solution, the tenacity of a jelly of known strength, and the presence of acid (which is sometimes present in sufficient proportion to be recognised by the taste) should also be noted.

Gelatin containing much chondrin is of inferior quality, and unsuitable for the preparation of photographic emulsions, the solution having less gelatinising power than that of pure gelatin. When present in notable proportion, chondrin may be detected by making a solution of the sample of gelatin in ten parts of hot water. To this, a strong solution of chrome-alum is added, which in presence of much chondrin will cause the liquid to set to a jelly while still hot.

Gelatin photographic emulsions are of very variable composition, and occasionally it is desirable to examine them quantitatively.

For the estimation of the silver, the sample should be dissolved in or diluted with water, and digested with excess of dilute nitric acid at 100° C. for some hours. The precipitate will probably consist simply of silver bromide, but may also contain chloride and iodide.[1]

Addition of silver nitrate to the filtrate causes a precipitation of silver bromide, &c., corresponding to any potassium bromide present in the original emulsion. For the estimation of the water, the emulsion is dried at 100° C. Air-dried gelatin emulsions generally lose from 8 to 15 per cent. of water at 100°. The proportion of gelatin in the sample is ascertained by subtracting from the dry residue the amounts of silver and potassium salts previously found.

Haloid salts of potassium and silver may, with gelatin, be regarded as the normal constituents of gelatin emulsions. Some samples contain foreign ingredients, for the detection of which

[1] Where great exactness is not required the silver chloride may be dissolved out by ammonium carbonate, and the bromide separated from iodide by strong ammonia.

a large quantity of the emulsion should be pressed between canvas, and 50 grammes macerated in cold water for twelve hours. The soluble salts diffuse into the water, and may be detected therein by ordinary methods. For the detection of alcohol, the original sample should be distilled, while phenol and thymol may be detected by the odour developed on warming with sulphuric acid. Salicylic acid may be detected by precipitating the aqueous solution with three measures of alcohol, evaporating the filtrate to a small bulk, agitating with ether, treating the ethereal layer with dilute caustic soda, faintly acidulating with hydrochloric acid, and adding ferric chloride solution, which will give a violet coloration if salicylic acid be present. Excess of silver nitrate may be detected by neutral potassium chromate, and may be determined by titration with standard sodium chloride, employing the chromate as an indicator.

For the analysis of collodio-gelatin emulsions, the sample should be treated with a large quantity of water, which precipitates the silver bromide and pyroxylin. After drying at 100°, the precipitate is weighed, moistened with nitric acid, and ignited, the loss of weight being the pyroxylin. The aqueous filtrate contains the gelatin. The acetic acid is determined by titrating a portion of the liquid, and the alcohol by distillation of the neutralised portion. Ether does not occur in this last form of emulsion.

ISINGLASS.

Isinglass is a variety of collagen prepared from the "sound" or swimming bladder of the sturgeon and other fishes. It should be white, with a yellowish tinge, opaline or semi-transparent, fibrous, and tenacious. When pure, isinglass has no odour, and but a faint taste. It contains from 15 to 20 per cent. of moisture.

According to Mulder, isinglass contains:—Carbon, 50·76; hydrogen, 6·64; nitrogen, 18·32; and oxygen and sulphur, 24·69 per cent.

Isinglass consists of nearly pure collagen. It is not gelatin, but is converted into that body by boiling with water. It consists of fibres or threads, which when immersed in cold water swell up but retain their organised structure, and it is to this property that the use of isinglass for clarifying wine, beer, and other liquids is due. The best qualities of isinglass dissolve almost entirely in boiling water. The solution gives a remarkably strong jelly on cooling.

Russian isinglass, which is obtained from the sturgeon, is the kind most valued. Its solution has a higher viscosity than that of any other variety. Brazilian isinglass, sometimes called "Cayenne isinglass," is obtained from *Silurus Parkerii*, and occurs in leaves an inch or more in thickness. Rat's-tail isinglass is made

from the swimming bladder of the cod, hake, and other fishes. It is opaque, and incompletely soluble in water.[1]

Isinglass which has been bleached by sulphurous acid generally retains traces of sulphates, and hence the solution is precipitated by barium chloride.

Isinglass is not infrequently adulterated with bone-gelatin, gut, and inferior kinds of fish-gelatin.

On treating Russian isinglass with hot water the substance swells uniformly, producing a whitish opaline jelly, which gradually dissolves entirely. Gelatin, on the contrary, swells irregularly, and gives a nearly transparent solution.

On ignition, Russian isinglass usually leaves from 0·4 to 0·9 per cent. of reddish ash, containing a little calcium carbonate. Gelatin yields at least 1·5 per cent. of white ash, consisting of calcium phosphate or carbonate, with traces of chlorides and sulphates.

On treating isinglass with hot water, any admixture of ordinary intestinal membrane will be left insoluble, or, at any rate, not more than 30 per cent. of the adulterant will pass into solution. Inferior fish-gelatins leave from 20 to 30 per cent. of insoluble residue, and give solutions of a very strong and disagreeable odour.

F. Prollius (abst. *Jour. Chem. Soc.*, 1884, p. 647) has examined a number of samples of isinglass and fish-gelatin from different sources. The insoluble matter was determined by weighing the residue obtained on treating the sample with hot water. The gelatinising power was ascertained by dissolving 1 part of the sample in 90 parts of water, filtering, and determining the viscosity of the resultant solution. The following were the results obtained :—

Source of Isinglass.	Ash, per cent.	Water, per cent.	Residue insoluble in hot water; per cent.	Viscosity; Seconds.
Astracan, from Schmidt and Dihlmann, Stüttgart,	0·20	16·0	2·8	507
Astracan, from a collection.	0·37	18·0	0·7	485
Astracan, fine iridescent Russian quality, Tübingen collection,	1·20	17·0	1·0	500
Astracan, Russian, from Gehe, of Dresden,	0·80	19·0	3·0	491
Astracan, in laminæ, from Gehe,	0·50	19·0	0·4	480
Astracan, in threads (Hamburg threads),	0·40	17·0	1·3	477
Hamburg isinglass,	1·30	19·0	2·3	470
Do., another quality,	0·13	19·0	5·2	..
Rolled northern fish-bladder,	3·20	..	10·8	467
Icelandish bladder,	0·60	17·0	21·6	463
Indian isinglass,	0·78	18·0	8·6	437
Yellow, unknown quality,	2·30	17·0	15·6	360

[1] Interesting information on the origin, preparation, and applications of isinglass will be found in the *Jour. Soc. Chem. Industry*, 1887, page 764.

Prollius recommends the microscopic examination of isinglass as an indication of its purity. Isinglass exhibits a very pronounced fibrous structure, which is never observed in the case of ordinary gelatin.

The commoner kinds of isinglass, especially coarse Brazilian, are employed for clarifying wine, &c. *Beer-finings* are usually prepared by treating isinglass or fish-gelatin with sour beer, or with acetic or sulphurous acid. The last reagent is preferred by brewers, since the antiseptic properties of the sulphurous acid are of value. One pint of finings containing $\frac{1}{8}$ oz. of isinglass, "cut" by sulphurous acid, should fine a barrel (36 gallons) of beer. The finings are not actually dissolved, but simply softened or "cut" by the acid. The clarifying action appears to be purely mechanical, the particles causing turbidity becoming entangled in the gradually-sinking network of gelatinous matter. Hence an actual solution of any kind of gelatin would be useless for this purpose.

Isinglass is employed by cooks for thickening soups, jellies, &c., but in this case its clarifying properties are not called into play, and hence gelatin is often substituted under the name of "patent isinglass."

Isinglass, though free from chondrin, is not suitable for the production of photographic films, on account of its ready solubility and inferior tenacity.

Size is a kind of coarse gelatin employed in paper-making, distemper-painting, and for weighting and imparting stiffness and gloss to cotton goods, &c.

Bone-size is pale, clear, and forms solid, semi-transparent cakes or masses.[1] Its adhesive power is inferior to glue-size.

Glue-size is a dark brown, semi-fluid, very adhesive mass. It frequently contains hairs and animal refuse, and it often gives strongly the reactions for chondrin (page 496).

When exposed to warmth and moisture, size is liable to mildew, with disastrous results to the goods on which it has been applied. Salts of magnesium and zinc and other bodies of antiseptic properties are largely employed to prevent this change. Common salt is sometimes present in large proportion.

The feel and apparent tenacity are not reliable guides in judging of the quality of size, and the specific gravity is equally misleading.

[1] According to Wagner, the best bone-size is made from the "sloughs" of the horns of oxen. Four cwts. of these are boiled with water for ten hours, and the liquid strained. Three lbs. of powdered alum and 2 lbs. of powdered zinc sulphate are then added to the hot liquid with vigorous stirring. The liquid is run into shallow tubs and allowed to cool. The yield of size is about 10 cwts., and it will keep eighteen months.

In assaying size, a portion should be dissolved in water, and a quantity of the solution representing a known weight of the sample should be evaporated to dryness in a shallow dish to ascertain the proportion of solid matter. Another portion of the solution should be treated with sulphuric acid, evaporated to dryness, and the residue ignited to obtain the sulphated ash, which will represent the mineral additions to the size.

GLUE.

Glue is an impure desiccated gelatin prepared by boiling bones and other collagenous substances—such as horns, hoofs, hides, tendons, parchment, &c.—with water.

The chief commercial varieties of glue are skin-glue (leather-glue) and bone-glue.[1] Fish-gelatin and size may also be regarded as varieties of glue.

Glue is valued commercially by its colour, which ranges from pale yellow to brownish-black, and by its freedom from cloudy or black spots when held up to the light.[2] Glue obtained from bones usually has a milky appearance, owing to the presence of a small quantity of calcium phosphate, which remains on igniting the glue. Opaque white substances are sometimes added to simulate this appearance. Unless in very thin sheets, glue should be hard, and difficult to break with the hammer; but when broken it should yield suddenly and present a sharp vitreous fracture.

Glue of good quality is not affected by ordinary atmospheric changes, and its aqueous solution is neutral to litmus.

[1] In the manufacture of glue from bones, the coarsely-ground substance is first treated with cold hydrochloric or sulphurous acid, until they become soft and transparent. The insoluble residue is drained, washed with cold water till free from acid, and then subjected in iron digesters to the action of superheated steam for three or four hours, when the product is run out into settling tanks. The fat is skimmed off while the liquid is still hot, and the latter is then treated with sulphurous acid if a fine pale product is desired. The liquid is next strained through wire-gauze, and concentrated to the desired strength.

The manufacture of glue is described at length in Wagner's *Manual of Chemical Technology*, translated by Wm. Crookes.

[2] For clarifying glue and removing impurities therefrom, P. C. Hewitt (*Eng. Patent*, 1894, No. 13,369) employs a solution of casein, prepared by treating skimmed milk with rennet and dissolving the resultant curd in lime-water, any excess of which may, if desired, be neutralised by phosphoric acid. This liquid is added to the solution of the glue. In some cases, albumin (preferably blood-albumin) is also added. Hewitt states that the resultant precipitate is sufficiently hard to allow the filtration to proceed without much hindrance. The glue thus clarified is finally bleached by sulphurous acid or other bleaching agent in the usual manner.

Inferior glue absorbs water far less readily than the better qualities, and this fact has been utilised by Schattenmann for its assay.[1] A known weight of the dry glue is immersed in water at the ordinary temperature for twenty-four hours. In this time, the finest quality of white glue, or that made from white bones, is stated to absorb from twelve to thirteen times its weight of water, forming a thin elastic jelly. Glue from dark bones takes up less water, and gives a soft, brown jelly, devoid of consistency or elasticity, and falling to pieces when handled. Common glue, made from animal refuse, will absorb only from three to five parts of water in twenty-four hours.

Although the best glues take up more water than inferior qualities when immersed in the liquid, well-made and well-dried glues are much less hygroscopic than badly-made specimens, or than those prepared from inferior materials. The latter are also liable to undergo putrefaction on exposure to damp.

The *odour* of glue varies with its source, and affords a valuable criterion of its quality (see page 481).

Concentrated acetic acid renders glue transparent, and then dissolves it. The resultant solution does not gelatinise, but retains its adhesive properties.

The *ash* of glue varies considerably, both in amount and composition. In the case of bone-glue, the ash consists essentially of phosphates, while in certain other kinds it is chiefly calcium carbonate. Some glues of American manufacture contain a notable admixture of fine chalk, which gives the glue an opalescent appearance and is said to improve the quality in a marked manner. Barium sulphate, zinc oxide, and lead sulphate and carbonate are occasionally present, especially in Russian glue. White lead is alleged to increase the adhesive power of the glue.

R. Kissling (*Chem. Zeit.*, xi. 691, 719; abst. *Jour. Soc. Chem. Ind.*, 1887, 565) has published the following scheme for the systematic examination of glue :—

Water.—From 2 to 3 grammes of glue-shavings are dried at 110° to 115° C. till constant in weight.

Ash.—The residue is burned in a covered platinum crucible, if necessary with the addition of a drop of nitric acid. The ash

[1] S. Rideal soaks 10 grammes of the coarsely-powdered sample of glue in water at 15° C. in a weighed beaker for forty-eight hours. At the end of this time the water is carefully decanted, and the increase of weight ascertained, the character and odour of the jelly being also noted. Good glues give a firm jelly and absorb from five to nine times their weight of water. In other cases, the product is not a jelly but a slimy liquid. According to Kissling the amount of water absorbed by glue affords no indication of its cohesive power, and Fels describes it as uncertain.

from bone-glue fuses by the heat of the bunsen burner; its aqueous solution is neutral, and it contains phosphoric acid and chlorides, whereas the ash from leather-glue does not fuse, owing to the presence of lime. It has an alkaline reaction, and is generally free from phosphoric acid and chlorides.

Free Acid.—30 grammes weight of glue is suspended in 80 c.c. of water and allowed to stand for several hours. The volatile acids are then driven over by a current of steam. As soon as the distillate amounts to 200 c.c., the distillation is arrested and the contents of the receiver titrated with standard alkali. Sometimes the distillate contains sulphurous acid, in which case a known volume of standard alkali should be previously placed in the receiver.

Drying Capacity.—The solution of glue freed from volatile acids is made up with water to 150 grammes and heated on the water-bath. 10 c.c. of the liquid is then spread on a watch-glass, and allowed to stand in a room which is free from dust and not exposed to frequent changes of temperature. The change of the glue-jelly is observed for several days, and if possible the behaviour of this jelly is compared side-by-side with jellies from glues of known quality, as the temperature and amount of moisture in the air has an influence on the consistency of the jelly.

Foreign Matter.—The rest of the glue solution from the last test is diluted with hot water and transferred to a graduated cylinder holding one litre. After filling up to the mark with water, the contents of the cylinder are allowed to subside for twenty-four hours, and the deposit recorded as "foreign matter."

Odour.—The smell of glue varies very much, and furnishes one of the most useful indications of its quality. Leather-glue has the least odour. It often happens that a sample of glue is odourless in the solid state, though its jelly has a marked unpleasant smell. Kissling recommends that samples of glue of known quality and origin should be rasped to a fine powder and preserved in well-stoppered bottles. These standards will retain their characteristic odours for years.

Fat.—For the estimation of fat in glue, &c., Kissling dissolves 20 grammes of the sample in 150 c.c. of water containing 10 c.c. of hydrochloric acid of 1·19 specific gravity. The liquid is heated for three or four hours on a water-bath under a reflux condenser. The solution is cooled, 50 c.c. of petroleum-ether added, the liquid well shaken, and, after standing until clear, a known measure of the solvent is drawn off, evaporated, and the residue weighed.

The following results, obtained by the above process, have been recorded by Kissling :—

No.	Kind of Glue.	Price in Shillings per 100 kilos.	Water; per cent.	Ash.					Volatile Acids per cent.	Foreign Matter.	Capacity of Drying.	Odour of Glue.	
				Per cent.	Phosphoric Acid.	Chlorine.	Reaction of Extract.	Appearance.				Solid Blocks.	Hot Jelly.
1	Leather-Glue.	92	15·7	3·05	none	none	strongly alkaline	powder	none	trace	very good	very good	very good
2		92	...	2·68	none	trace	alkaline	,,	none	trace	very good	very good	very good
3		92	15·6	1·40	none	trace	alkaline	,,	0·022	trace	very good	very good	very good
4		92	18·1	1·40	trace	none	alkaline	,,	0·015	3	very good	good	good
5	Bone-Glue.	66	17·0	2·46	much	none	neutral	molten	0·110	40·5	bad	bad	medium
6		66	15·6	1·26	much	much		,,	0·831	...	good	medium	medium
7		66	14·9	1·43	...	...		,,	0·262	...	medium	medium	medium
8		50	...	2·80	much	much		,,	0·487	6	bad	very bad	very bad
9		50	...	2·63	much	much		,,	0·172	trace	good	medium	bad
10		68	16·41	2·66	very much	trace		,,	0·038	trace	medium	very good	very good
11		70	16·00	1·93	very much	little		,,	...	...	...	...	...
12		68	13·24	2·00	very much	little		,,	0·068	15	very bad	good	good
13		60	17·70	5·07	much	much	alkaline	compact but unmolten	0·082	20	bad	medium	bad
14		66	...	3·04	much	much	neutral	molten	none	...	medium	very good	good
15		66	12·28	1·80	much	none		,,	0·056	trace	bad	good	bad
16		67	13·56	2·80	much	little		,,	0·113	20	good	good	good

Kissling has also described (*Chem. Zeit.*, xiii. 1667 ; abst. *Jour. Soc. Chem. Ind.*, 1890, 399) an apparatus for measuring the tensile strength of glue. It consists of two solid cylinders of nickel-plated iron. These are fastened together with the aqueous glue solution (1 : 2) and left overnight, being kept pressed together by a weight. The glued cylinders are then hooked on to a lever, carrying a scale-pan, and weights added until the cylinders part at the glued joint. Kissling's figures thus obtained show that the tensile strength bears no relation to the price of the glue.

W. Kalmann (*Jour. Soc. Chem. Ind.*, ix. p. 113) confirms the value of Kissling's method for the determination of the origin of glues (see page 480) by noting the composition of the ash of the glue and its behaviour on heating. For the determination of the *free organic acid*, Kalmann dissolves one gramme of the coarsely-powdered sample of glue in water on the water-bath, tests the solution with blue litmus-paper, and then titrates the liquid with decinormal alkali, using phenol-phthalein as the indicator. The liquid is then cooled, a little starch solution added, and a decinormal solution of iodine added until the liquid becomes blue, the amount of iodine required being equivalent to the *sulphurous acid* of the sample. The blue colour should then be discharged by adding a drop of a solution of sodium acid sulphite, and then again titrated with decinormal alkali and phenol-phthalein. The volume required indicates the amount of hydriodic acid formed, according to the equation :—$M_2SO_3 + I_2 + H_2O = M_2SO_4 + 2HI$. The result should agree with that of the iodine titration, and affords a proof that the organic matter of the glue has not affected the accuracy of that determination.

The presence of mineral acids and of normal sulphites renders glue unsuitable for woollen manufacturing, since any notable proportion of these impurities will produce light patches on dyed wool. On this account, Kalmann states that Nos. 2, 4, 6, and 9 in the following table were found unsuitable :—

No.	Variety of Glue.	Reaction with Litmus.	Na_2O equivalent to amount of Free Acid present.	SO_2.	Ash.
			Per cent.	Per cent.	Per cent.
1	Gelatin.	Faintly acid.	0·46	0·03	..
2	Bone glue.	,, ,,	1·32	0·46	3·55
3	,, ,,	,, ,,	0·81	0·20	3·09
4	,, ,,	Acid.	1·56	0·96	..
5	,, ,,	Neutral.	0·44	0·11	..
6	,, ,,	Acid.	2·02	1·41	1·66
7	,, ,,	Neutral.	0·67	0·09	2·21
8	,, ,,	Faintly acid.	0·95	0·36	2·37
9	,, ,,	Acid.	1·99	1·03	1·92

R. Williams applies Löwenthal's tannin process (Vol. III. Part i. p. 110) to the assay of glue. The sample is employed in one per cent. aqueous solution, a known measure of "pure tannic acid" added, the liquid filtered, and the excess of tannic acid determined in the filtrate by a decinormal solution of potassium permanganate. Williams gives the following figures obtained by the examination of three samples of glue of very similar quality, and points out that the proportions of water and mineral matter are much less than have been recorded by other observers.

	No. 1.	No. 2.	No. 3.
Organic matter, . . .	91·04 per cent.	89·70 per cent.	90·72 per cent.
Mineral matter, . . .	1·43 ,,	1·48 ,,	2·11 ,,
Water, ...	7·53 ,,	8·82 ,,	7·17 ,,
	100·00 ,,	100·00	100·00
Tannin (calculated as gallo-tannic acid) precipitated, .	77·5 ,,	77·9 ,,	78·6 ,,

F. Jean (abst. *Analyst*, 1897, p. 162) has described a similar process, the excess of tannin being determined by a standard solution of iodine. Carles (*Analyst*, 1897, p. 275) has criticised the process, and states that the precipitating power of commercial gelatin on pure tannin is very variable.

W. Fahrion (*Zeit. angew. Chem.*, 1898, p. 529) has described the following method of analysing glue and glue-yielding substances. The sample to be examined is finely rasped, and two portions of from 3 to 5 grammes each weighed out. In one, the moisture is determined by drying at 110° to 120° C. until the weight is constant, the residue being then used for the determination of the ash. The second portion is mixed with 15 to 25 c.c. of an 8 per cent. solution of alcoholic soda, and evaporated to dryness on the water-bath. The residue is taken up with alcohol, and the liquid again evaporated to dryness. The residue is then washed with hot water into a separating-funnel, the liquid acidified with hydrochloric acid, and on cooling shaken out with ether. The solid oxy-acids, which are left undissolved, may be estimated by dissolving them in warm alcohol, evaporating the solution, and weighing the residue. On evaporating the ethereal extract, a mixture of unsaponifiable matter, fatty acids, and fluid oxy-acids is obtained. The residue is weighed, and treated with petroleum-spirit, in which the fluid oxy-acids are insoluble. On shaking the petroleum-spirit solution with caustic soda solution containing some alcohol, the fatty acids are removed, while the unsaponifiable

matter remains in solution, and may be weighed after evaporating the petroleum-spirit. The alkaline solution containing the fatty acids is heated on the water-bath to remove alcohol, the residue diluted with water, decomposed with hydrochloric acid, and shaken out with petroleum-spirit, which on evaporation leaves the fatty acids. Fahrion applies the same process to the analysis of leather,[1] and has recorded the following results. The sum of the constituents of the horn is 101·20. In the other cases the sum is exactly 100·00 :—

	Water.	Ash.	Unsaponifiable Matter.	Fatty Acids.	Fluid Oxy-acids.	Solid Oxy-acids.	Proteid Substance (by difference).
Glue (fine white),	13·74	1·80	0·49	0·08	0·04	0·27	83·58
Hide-powder,	19·15	0·25	0·72	0·18	0·08	0·37	79·25
Purified sheep's leather,	11·23	10·06	9·74	0·99	0·46	1·01	66·51
Sheep's horn,	9·09	1·00	0·68	1·03	0·29	1·49	87·62
Bone belonging to the horn,	10·00	53·87	4·81	4·23	0·19	1·52	25·38
Chamois leather (sheep), I.,	18·66	8·28	0·49	4·15	0·37	0·53	67·52
Chamois leather (sheep), II.,	17·95	1·38	0·10	1·17	Trace.	0·69	68·71
Chamois leather (sheep), III.,	17·60	4·38	0·30	3·10	0·28	1·00	73·34
Chamois leather (doe),	15·15	6·03	0·49	4·16	0·45	1·37	72·35
Chamois leather (goat),	15·18	3·83	3·03	0·28	0·61	0·56	76·51
Chamois leather (buffalo),	20·54	3·21	0·10	0·46	0·11	0·56	75·02
Glacé kid,	11·69	7·12	3·66	6·58	0·66	0·75	69·54

Fahrion finds that the action of the alcoholic soda on albuminoid bodies results in the formation of an amorphous nitrogenised acid of the formula $C_8H_{14}N_2O_5,H_2O$, which he regards as probably identical with Schützenberger's *proteic acid*, obtained by

[1] F. Gantter (*Zeit. Anal. Chem.*, xxxii. 413 ; abst. *Jour. Chem. Soc.*, 1893, ii. 610) employs the following method for the valuation of hide-clippings for glue-making. A weight of 100 grammes of the clippings is boiled with 1 litre of water containing a few drops of caustic soda until dissolved. Any sand or other *mineral impurity* is allowed to settle out, and *fat* removed in the usual way. The clear solution is then made up to 2 litres, 20 c.c. evaporated, and the residue dried at 105°, weighed and ignited. This gives the *total soluble organic matter*. Another measure of 10 c.c. is diluted with 30 c.c. of water, neutralised with acetic acid, and treated with a solution of tannin till no further precipitation takes place. The liquid is then made up to 100 c.c., filtered, the excess of tannin removed by hide-powder, and the *organic matter not precipitable by tannin* determined in the filtrate by evaporating, weighing, and igniting as before. The difference between the total soluble organic matter previously determined and that not precipitable by tannin is regarded as *gelatin*.

heating albumin with baryta-water under pressure. The same compound is yielded by the action of alcoholic soda on glue, casein, human hair, horn, wool, and silk.

In the examination of *dégras*, the presence of proteic acid in the portion of the fatty acids insoluble in petroleum-ether is indicative of the dégras being derived from chamois-leather. The acid may be distinguished from solid hydroxy-fatty acids by its content of nitrogen.

Kissling considers (*Chem. Zeit.*, 1896, xx. 697; abst. *Jour. Soc. Chem. Ind.*, xv. 729) that Fahrion's saponification process (page 484) for the valuation of glue may possibly throw some light upon the constitution of gelatin, but that it is not adapted for commercial work. He regards Lipowitz' test, based on the consistency of the glue-jelly (page 487), as unsatisfactory, since it is very difficult to carry out, whereas the viscosity-method suggested by him may lead to some reliable results being obtained. He directs that a solution of 1 part of glue in 3 parts of water should be examined at a temperature of 20° C.

Since the value of glue depends upon its adhesive power, a determination of the non-gelatinous substances present is of some value. C. Stelling (*Chem. Zeit.*, xx. 461; abst. *Analyst*, 1896, p. 239) estimates these constituents by the following method:— A weight of fifteen grammes of the sample is soaked overnight in 60 c.c. of water in a 250 c.c. flask. Next day the jelly is dissolved on the water-bath, and any loss by evaporation made up. The flask is then filled to the mark with 96 per cent. spirit, and the liquid thoroughly shaken. After standing for six hours, 25 or 50 c.c. of the liquid is filtered off, evaporated to dryness, and the residue, consisting of the non-gelatinous substances, dried at 100° C. and weighed. Stelling gives the following figures, which, though only of approximate accuracy, are comparable with each other:—

Description.	Number of Samples.	Non-gelatinous Substances.		
		Maximum.	Minimum.	Average.
		Per cent.	Per cent.	Per cent.
Gelatin (various kinds),	5	4·53	2·53	3·39
Glue,	5	4·70	2·00	3·49
Leather-glue,	3	7·60	4·30	5·73
Bone-glue from acid bones,	4	11·84	9·24	10·33
Bone-glue from acid bones,	3	16·78	13·16	15·15
Pressed glues from neutral bone-powder,	17	32·10	14·30	20·66
Whale-glue,	1	...	...	22·00
Substance used for clarifying wine,	1	...	...	33·20
Substance used for clarifying wine,	1	...	...	59·30

In criticising Stelling's process, Kissling (*Chem. Zeit.*, xxii. 171) points out that pure gelatin is not completely insoluble in alcohol of 72 per cent., while the non-gelatin consists of fat and other impurities besides the decomposition-products of gelatin. Nevertheless, Kissling considers that the method affords a partial indication of the value of a glue. He found the "non-gelatin" determined by Stelling's method in twelve samples of commercial glue to range from 7·6 per cent. in a skin-glue to 23·2 in a very inferior specimen of bone-glue. Kissling considers that if a buyer of glue desires a freedom from bad odour, good gelatinising power, and freedom from acidity, a superior skin-glue must be selected; but if only the adhesive properties of the glue are in question a cheap bone-glue will answer the purpose.

J. Fels (*Chem. Zeit.*, 1897, pp. 56, 70) has published some valuable criticisms of various methods of testing glue, none of which he regards as wholly satisfactory.[1] He describes a method of Lipowitz (*Neue Chem. techn. Unters. Berlin*, 1861, p. 37), based on the consistency of the jelly and its capacity for bearing a weight, as "correct as a comparative method."[2] Fels gives the preference to the following process, based on the viscosity of a solution of the sample:—The glue is reduced to powder, and dried

[1] Fels condemns Gráger's process, based on titration of the glue solution by tannin, because "the tanno-gelatin is of uncertain composition." Moffat's method, dependent on the determination of the nitrogen by combustion with soda-lime, is vitiated as a measure of the gelatin, since "other nitrogenous" compounds are present. Stelling's method, based on the determination of the "matters not glue," after precipitation by tannin, Fels describes as "inaccurate." Schattenmann's test, dependent on the increase in the weight of the glue by soaking in cold water, Fels regards as "uncertain," and Cadet's plan of noting amount of water absorbed on exposure in damp air as "unreliable." Weidenbusch's method of observing the breaking strain of a rod made of glue and plaster of Paris is described as "uncertain," while that based on the weight required to separate two pieces of wood glued together is "dependent on time."

[2] The value of Lipowitz' method as a practical test of the quality of glue has been confirmed by Heinze. Ten grammes of the sample should be dissolved in 90 c.c. of warm water, and the solution kept in a beaker at 18° C. for twelve hours, to allow the glue to set thoroughly. A saucer-shaped piece of tinned iron, 1 inch in diameter, is soldered on its concave side to a stout iron wire having a small tin funnel fixed to the other end. The saucer, wire, and funnel together weigh 10 grammes. The arrangement is placed vertically in the beaker so that the cup rests on the jelly, and the whole is supported by a slip of metal placed across the top of the beaker. By placing shot in the funnel a gradual increase of weight on the jelly is produced, until the cup breaks through. The weight it is found necessary to use to rupture the jelly is a measure of the tenacity of the glue.

at 100° C. for two hours for the determination of the moisture. A weight of 100 grammes of the powdered glue is soaked in about 400 c.c. of cold water for twenty-four hours, and then dissolved by heating on the water-bath. The amount of liquid is then adjusted so that the solution contains 15 per cent. of dry glue. It is then tested in an Engler's viscosimeter[1] at 30° C., the time taken for 500 c.c. to run out being recorded and compared with that required by an equal volume of water at the same temperature taken as 100.

Fels records the following results yielded by five samples of glue[2] when treated in the above manner :—

No.	Description of Sample.	Moisture.	Time of Efflux of 500 c.c. at 30° C., water requiring 90 seconds.	Viscosity (water=100).
1	Light yellow, transparent thick plates, ...	16·3 per cent.	149	165
2	Brown, transparent glue, . .	14·0 ,,	125	136
3	Sherry-coloured, transparent glue, ...	15·4 ,,	171	191
4	Light yellow, brittle plates, .	18·2 ,,	150	160
5	Muddy (trüber) glue, . .	15·2 ,,	199	221

The figures for the viscosity express the same differences in the quality of the samples as were deduced from the determination of the consistency of the jellies by Lipowitz' method. They also confirm the behaviour of the samples when soaked in cold water. Thus, No. 2 became completely slimy in a few hours, and fused into a single lump, whereas No. 5 kept its shape, and on scraping with the finger showed scarcely any gelatinisation. Nos. 3 and 5 in twelve hours gave a thick jelly, while No. 2 in twenty-four hours yielded only a thin and poor jelly. The superior quality of No. 5 sample appears to justify the ordinary preference for a dark glue. Light-coloured glues are apt to have been bleached at the expense of tenacity.

Although Fels prescribes the use of Engler's viscosimeter, the employment of this instrument is not compulsory, since the figures are not absolute but compared with water taken as unity or 100. In fact, S. Rideal[3] prefers a modification of Slotte's

[1] Engler's viscosimeter is described and illustrated in the *Jour. Soc. Chem. Ind.*, 1890, p. 654.

[2] Stanford states that a 5 per cent. solution of algin (page 493) would have a viscosity ten times as great.

[3] The author is indebted to Dr Rideal for the communication of a number of valuable notes on the testing of glue.

apparatus,[1] as being simpler and giving data in terms of absolute measurement. Rideal uses a one per cent. solution of the glue,[2] and determines the viscosity at 18° C., obtaining figures ranging from 1·19 to 1·60.[3]

Methods of testing glue have been based on the weight required to forcibly separate two surfaces of nickel-plated steel, wood, or stone which had been glued together by a solution of the samples. Rideal found wood and metal quite unreliable, and the results with stone-china far from constant. He considers that the rigidity of the stone blocks gives them an advantage over wood, the elas-

[1] This instrument is described and figured in the *Jour. Soc. Chem. Ind.*, 1891, p. 615.

[2] The solution of the glue must be strained successively through muslin and through a capillary tube under pressure, to separate lumps, which are frequently present in "muddy" glues, and are apt to clog the orifice of the viscosimeter.

[3] The following practical tests were communicated to S. Rideal by a large firm of glue manufacturers in America :—

1. *Viscosity or "Body" Test.*—One ounce of glue is soaked thoroughly in 10 ounces of water, melted in the water-bath, poured into a "testing tube" kept at a standard temperature, and the time taken for the glue to run through observed. Water takes 37 seconds, while the very weakest glue takes from 40 to 43 seconds. The ratio 100 : 116 is the "body test" indicating the consistency of the glue and extent of surface it will cover, and therefore its economy in use and good-working and water-taking qualities. More importance is attached to this test than to the following.

2. *Consistency of the Jelly, or Shot Test.*—The solution used in the previous test is kept in an ice-box for three or four hours till the jelly is firmly set. A tube, which can be loaded with shot, is then placed on the jelly and the weight which the jelly will support without breaking observed. Care must be taken that the glue has not been over-heated, so as to cause the formation of a skin on the surface.

3. *Reaction to Litmus.*—A slightly acid or alkaline reaction is of no importance, though a neutral glue is preferred. Customers prefer a glue which shows a little lime, though an acid-made glue is not nearly so apt to decompose as a lime-made glue.

4. *Foam Test.*—The solution used for the previous tests is beaten or stirred vigorously with a small glass-rod for three or four seconds, the height of the foam measured in inches, and the rate of its disappearance noted. "Some glues show ½ inch of foam, some ¼, ⅛, and some none at all. It does not necessarily follow that there is anything wrong with the glue, but a great many customers object to foam."

5. *Grease Test.*—"To a solution of the glue add a little lamp-black or Turkish-red, thoroughly mix with a brush, and paint it on some pieces of paper. If there is abundance of grease it will produce little round, white, smooth surfaces on the red or black paint." A negative result by this test is specially required by the paper-trade and some others.

ticity and compressibility of which often brings about the rupture prematurely.

Casein-glue is prepared by coagulating separated milk, as free as possible from fat, with dilute acetic acid. The curd is well washed, pressed, and dissolved in a strong solution of borax or sodium silicate. The resultant viscous fluid has strongly adhesive properties, and is well-suited for use by joiners, bookbinders, &c.

Gluten- or albumin-glue is prepared from partially-decayed gluten, obtained in the manufacture of starch from wheaten flour.

Liquid glue is prepared by dissolving 5 parts of glue in its own weight of water, and adding 1 part of nitric acid of 1·31 sp. gravity. When the evolution of nitrous fumes is at an end, the liquid is cooled. By the above treatment, the glue loses its character of gelatinising without its adhesive properties being impaired. A preferable preparation is obtained by dissolving gelatin or fine glue in moderately strong acetic acid, with the aid of heat, and then adding to the solution some powdered alum and one-fourth of its measure of alcohol.[1]

Coagulin is a strong solution of gelatin in concentrated acetic acid. It becomes fluid on warming, but gelatinises partially on cooling.

Gelatin Substitutes.

Various lichens and sea-weeds yield a highly gelatinous product on boiling with water, and some of them are employed as articles of food and in medicine.

The following table (from Thorpe's *Dictionary of Applied Chemistry*) shows the composition of various *British algæ*:—

Alga.	Water.	Nitrogen in Dry Substance.
	Per cent.	Per cent.
Chondrus crispus, bleached,	17·92	1·534
,, ,, bleached,	19·79	1·485
,, ,, unbleached,	21·47	2·142
,, ,, unbleached,	19·96	2·510
Gigantina mamillosa,	21·55	2·198
Laminaria digitata (dulse tangle),	21·38	1·588
Rhodymania palmata,	16·56	3·465
Porphyra laciniata,	17·41	4·650
Sarcophyllis edulis,	19·61	3·088
Alaria esculenta,	17·91	2·424

From the above results it is evident that the jellies from these

[1] Knaffl prepares a superior liquid glue by treating 3 parts of glue with 8 of water, 0·5 of hydrochloric acid, and 0·75 of zinc sulphate. The whole is heated for twelve hours to a temperature not exceeding 85° C. The product keeps for a long time and is largely employed for joining horn, wood, and mother-of-pearl.

sources, as a rule, contain but little nitrogen, and resemble gelatin merely in their physical characters.[1]

GELOSE is a gelatinous substance contained in *Gelideum corneum*, an alga known as Chinese moss or Japanese isinglass. The sea-weed contains only traces of soluble matters, but swells up in cold water and dissolves wholly in boiling water with the exception of 2 to 3 per cent. of nitrogenised corpuscles. The solution coagulates on cooling to a colourless translucent jelly. Payen prepared gelose by treating *Gelideum corneum* (or certain other algæ) with cold dilute acetic acid, water, and dilute ammonia; washing thoroughly, dissolving the substance thus purified in boiling water, and drying the jelly which forms on cooling.

Gelose occurs in commerce in bundles of long, very slender threads, resembling isinglass. It is said to contain C, 42·77; H, 5·77; and O, 51·45 per cent.[2] Gelose has ten times the gelatinising power of isinglass, and will set to a jelly with 500 times its weight of water. It is not, however, a suitable substitute for isinglass, since the melting-point of the jelly is above the temperature of the mouth. The aqueous solution of gelose is

[1] CETRARIN or LICHENIN is the gelatinous substance of *Cetraria Islandica*, a native of the north of Europe, commonly known as Iceland moss or Iceland lichen. The lichen was official in the British Pharmacopœia of 1885, which described it as almost odourless when dry, but having a feeble sea-weed-like odour when moistened with water. The taste is mucilaginous and bitter. A strong decoction gelatinises on cooling. By prolonged boiling with dilute sulphuric acid, the jelly from Iceland moss yields a crystallisable sugar, having an optical activity of $[a]_D = +46{\cdot}85°$. *Lichenstearic acid*, $C_{43}H_{76}O_{13}$, and *cetraric acid*, $C_{30}H_{30}O_{12}$, have been obtained from Iceland moss (abst. *Jour. Chem. Soc.*, 1890, p. 600). Cetraria is omitted from the B. Pharmacopœia of 1898, but is official in most foreign Pharmacopœias. It is described as demulcent, nutritious, and slightly tonic. In Iceland, the bitter principle is removed by washing in cold water and sodium carbonate, and the lichen then made into jelly, or dried, ground, and made into bread.

CARRAGEENIN is the gelatinous substance contained in *Chondrus crispus* or Irish moss, a sea-weed which is only exposed at low spring tides. It contains about 80 per cent. of dry matter, containing 1½ to 2 per cent. of nitrogen. The greater part of the moss consists of carrageenin, a gelatinous substance apparently allied to pectin. The decoction or jelly is employed as a demulcent and emollient in pulmonary affections, &c., and is also used as a substitute for isinglass. It also finds employment as a size, and, to a limited extent, for thickening colours in calico-printing and for stiffening silk. A thick mucilage of Irish moss, suitably scented, is employed as "bandoline" or "fixature." On the west coast of Ireland, where it abounds, the moss is used as an article of food. Carrageen moss is official in many foreign Pharmacopœias.

[2] This composition corresponds approximately to the formula $C_{11}H_{18}O_{10}$. On the other hand, the formula $C_6H_{10}O_5$ is sometimes attributed to gelose.

precipitated by alcohol. After drying, gelose is insoluble in cold water, dilute acids or alkalies, and Schweitzer's ammonio-cupric solution. Gelose loses its property of gelatinising when heated with water under a pressure of six atmospheres, or when boiled with dilute acids (including acetic acid), and the product reduces Fehling's solution on boiling. When heated with nitric acid, gelose yields mucic and oxalic acids. A 10 per cent. aqueous solution of gelose exhibits a specific rotatory power of $-4{\cdot}25°$; but by boiling the acidulated solution, the liquid gradually acquires a nearly equal dextro-rotation, and is no longer precipitated by alcohol. On the other hand, by treating gelose with water at 100° C., Porumbaru (*Compt. rend.*, xc. 1081) obtained a *lævo*-rotatory sugar containing $C_6H_{12}O_6,H_2O$.

The gelatinous principle of *agar-agar*, an edible sea-weed found in Malacca, Borneo, Ceylon, etc., is probably identical with gelose.

Alginic Acid or Algin is a gelatinous substance present in certain sea-weeds, especially *Laminaria*. It was first isolated by E. C. C. Stanford (*Jour. Soc. Chem. Ind.*, iii. 297; iv. 518, 595; *Jour. Soc. Arts*, 1862), who has observed that the whole of the alkaline salts, together with a considerable quantity of extractive matter containing dextrin and mannite, can be extracted from the Laminaria or "dulse tangles" by simple maceration in cold water. This treatment removes about 33 per cent. of the air-dry weed, of which from 20 to 22 per cent. consists of salts of potassium, sodium, and magnesium, including the whole of the iodine. The residue insoluble in cold water consists substantially of the above-mentioned nitrogenised body alginic acid, together with the algic cellulose or algulose which represents the cellular fabric of the plant. The alginic acid or algin is removed by digestion in a hot dilute solution of sodium carbonate, which dissolves it as sodium alginate, leaving the algulose in an extremely fine state of division, which renders it very difficult to remove by filtration. On treating the filtrate with hydrochloric acid, the alginic acid is precipitated as an amorphous substance of light amber colour, which is washed and bleached.[1]

[1] Stanford gives the following analyses of *Laminaria*. In addition to the three main constituents, the aqueous solution contains salts, mucilage, and mannite, while in the sodium carbonate solution a modified dextrin is present.

	Water.	Alginic Acid.	Cellulose.	Salts and Undetermined Matters.
Laminaria digitata, stem, . .	37·04	21·00	28·20	13·76
,, ,, frond, . .	44·00	17·85	11·00	27·15
Laminaria stenophylla, stem, .	34·50	25·70	11·27	28·53
,, ,, frond, .	40·02	24·06	15·06	20·86

Alginic acid is stated to have the formula $C_{76}H_{80}N_2O_{22}$. When precipitated by adding a mineral acid to the solution of its salts, alginic acid forms a very gelatinous precipitate, which when dry resembles albumin or horn. It has a specific gravity of 1·5. Alginic acid is insoluble in either hot or cold water.

Alginic acid acts on sodium carbonate with evolution of carbon dioxide and formation of *sodium alginate* or soluble algin.

Soluble algin dissolves in water to form a very viscous solution. Stanford states the viscosity of algin at fourteen times that of starch, and thirty-seven times that of gum-arabic. Solutions of sodium alginate are precipitated or coagulated by alcohol, acetone, and collodion, but not by amylic alcohol, ether, glycerin, or sugar. Alginic acid is precipitated from the solution of its sodium salt by most mineral acids, and by picric, oxalic, tartaric, and citric acids. A two per cent. aqueous solution is rendered semi-solid by acidulating it with hydrochloric acid. On the other hand, no precipitation occurs on treating a solution of sodium alginate with acetic, formic, benzoic, succinic, carbolic, tannic, arsenious, or boric acid. Soluble algin yields no precipitate with silicates of alkali-metals, chromates, permanganates, tungstates, or molybdates. It is not precipitated by hydrogen peroxide, chlorine, bromine, or iodine.

Sodium alginate gives no precipitate with the caustic alkalies or with ammonia, but insoluble alginates are thrown down by lime-water and baryta-water. Precipitates are also produced by most metallic salts, some of the alginates formed being of curiously complex composition. No precipitates are produced by salts of magnesium, the alginate of this metal being soluble. By bringing magnesia or magnesium carbonate in contact with alginic acid and water, the two insoluble bodies react to form soluble *magnesium alginate*.

Solutions of sodium alginate are distinguished from those of albumin by not coagulating on heating and by yielding no precipitate with mercuric chloride, potassium ferrocyanide, or tannic acid; from mucin, by not being precipitated by acetic acid; from gelatin, by giving no precipitate with tannin; from starch, by yielding no blue colour with iodine; from gelose, by containing nitrogen and not gelatinising on cooling. Sodium alginate differs from dextrin, gum-arabic, gum-tragacanth, and pectin by its insolubility in dilute alcohol and dilute mineral acids.

A number of ingenious applications of alginic acid and its salts have been proposed by Stanford (*Jour. Soc. Chem. Ind.*, 1885, p. 518). Some of these salts are likely to prove valuable as envelopes for certain medicines—such as mercury, bismuth, etc.—since they pass through the stomach unaltered, and hence delay the absorption of the medicine till it has reached the duodenum.

Chondrigen and Chondrin.

Chondrigen is the principal proteoïd constituent of the matrix of hyaline cartilage.[1] It is an elastic, semi-transparent substance, which is insoluble in hot or cold water and does not swell up materially by treatment either with water or with dilute acetic acid. By prolonged treatment for some hours with water under pressure (at 120° C.), chondrigen is gradually dissolved with formation of *chondrin*, the solution of which gelatinises on cooling. Hence the hyaline matrix of cartilage appears to bear the same relation to chondrin that the ground-substance of connective tissue bears to gelatin.

For the preparation of chondrin in a state of approximate purity, costal cartilage should be boiled with water for a few minutes, and the perichondrium removed by scraping. It is then cut into fine slices and boiled with water at the ordinary pressure for twenty-four hours; or preferably heated with water under pressure for three or four hours to 120° C. The solution is filtered while hot—to remove elastin, cellular elements, &c.—and treated with a large excess of alcohol. The precipitated chondrin is washed successively with alcohol and ether, and if necessary purified by re-solution in hot water and precipitation by alcohol.

Chondrin so prepared forms a hard, transparent mass, free from taste or odour, and insoluble in cold water. It dissolves in hot water, and the solution gelatinises on cooling, but less strongly than a solution of gelatin of the same strength.

The following is the percentage composition of chondrin according to various authorities :—

[1] The following results by Hoppe-Seyler show the composition of two typical kinds of human hyaline cartilage :—

	Costal Cartilage.	Articular Cartilage.
Water,	67·67 per cent.	73·59 per cent.
Organic solids,	30·13 ,,	24·87 ,,
Inorganic solids,	2·20 ,,	1·54 ,,
	100·00 ,,	100·00 ,,

The *inorganic solids* of costal cartilage in 100 parts of ash consisted of :— K_2SO_4, 26·66 ; Na_2SO_4, 44·81 ; NaCl, 6·11 ; Na_3PO_4, 8·42 ; $Ca_3(PO_4)_2$, 7·88 ; and $Mg_3(PO_4)_2$, 4·55 per cent.

The *organic solids* in the cells are mostly of proteid nature. The ground substance of the matrix, which forms the greater part of the organic material of cartilage, consists substantially of chondrigen

	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.
Mulder, ...	49·3	6·6	14·4	0·4	29·3
Fischer and Bödeker, .	50·0	6·6	14·4	0·4	28·6
Schützenberger and Bourgeois, ...	50·16	6·58	14·18	none	29·08
Von Mering, . . .	47·74	6·76	13·87	0·6	31·04

It will be seen from the above figures that the elementary analyses of chondrin present considerable discrepancies, and suggest that the substance dealt with is not a definite substance but liable to variations in composition. The results obtained by Morochowetz, and confirmed by Landwehr, Krukenberg, and Mörner, strongly support this view. Morochowetz found that on treating cartilage from various sources with lime- or baryta-water, a 0·5 per cent. solution of caustic soda, or a 10 per cent. solution of common salt, mucin is dissolved out, and may be thrown down from the solution by acetic acid; while the substance left undissolved is readily convertible by boiling with water into perfectly normal gelatin. According to these observations, chondrin is a mixture of gelatin and mucin, while chondrigen is a mixture of collagen with mucin or hyalogen, the latter component masking its true nature. By micro-chemical examination of the cartilage of the trachea, and the use of staining agents, Mörner detected the existence of a network of collagen, enclosing spherical masses termed "chondrin balls." By treating sections of cartilage with very dilute hydrochloric acid (0·1 to 0·2 per cent.), and then with very dilute solution of caustic potash (0·1 per cent.), he succeeded in dissolving out the chondrin balls and leaving the network, which by treatment with dilute acids or superheated water was in great part converted into typical gelatin. The chondrin balls were found to consist of a mixture of free chondroitic acid and a mucin called chondromucoid, which on decomposition yielded proteid matter and chondroitic acid.

Chondroitic acid, $C_{28}H_{51}SN_3O_{30}$, has the characters of a hyalin (page 498), though Mörner was unable to verify the existence of the corresponding hypothetical hyalogen. Chondroitic acid yields a reducing sugar when boiled with dilute sulphuric acid. It is interesting on account of its low percentage of nitrogen, and from the fact that the sulphur of the molecule is wholly in the form of an ethereal sulphate.

According to Schützenberger and Bourgeois, the

products of the decomposition of chondrin by boiling with baryta-water differ from those yielded by gelatin under the same treatment in a much larger (three times) production of acetic acid, and in the entire absence of glycocine. The latter of these results, if correct, is difficult to reconcile with Morochowetz' view of the complex nature of chondrin.

The behaviour of chondrin with solvents and reagents corresponds exactly with a mixture of gelatin and mucin. Thus :—

	Gelatin.	Chondrin.	Mucin.
Solubility, . . .	Insoluble in cold water, alcohol, or ether. Soluble in hot water; such solutions set into a jelly when cold.	Insoluble in cold water, alcohol, or ether. Soluble in hot water; such solutions set into a jelly when cold.	Insoluble in cold water, alcohol, or ether. Insoluble in hot water.
Reaction with:—			
Acetic acid, . .	No precipitate.	Precipitate; insoluble except in large excess.	Precipitate; insoluble except in large excess.
Mineral acids, . .	No precipitate.	Precipitate; readily soluble in excess.	Precipitate; readily soluble in excess.
Tannic acid, . .	Precipitate.	Precipitate.	No precipitate.
Mercuric chloride, .	Precipitate.	Precipitate.	No precipitate.
Lead acetate, . .	No precipitate.	Precipitate.	Precipitate.
Alum, . . .	No precipitate.	Precipitate.	Precipitate.
Boiling dilute mineral acids,	No reducing substance formed.	1. Syntonin. 2. Body reducing cupric oxide.	1. Syntonin. 2. Body reducing cupric oxide.

The reducing substance formed by boiling chondrin with dilute acids has been called chondriglucose, but it is said to contain nitrogen, and it is doubtful if it is a true sugar. It is probably identical with the reducing substance resulting from the hydrolysis of mucin by acids.

Mucins.[1]

Under the general name of mucin are grouped various closely-allied substances which give to many animal secretions, such as snail-slime, synovia, saliva, &c., their characteristic viscid or ropy consistency. A mucin is the characteristic principle of *mucus*, the thick, slimy liquid which covers mucous membranes. Mucins are widely distributed in the animal kingdom, in many cases probably playing a part at present very imperfectly understood.

[1] The mucins are compound proteids of the gluco-proteid class, and are classed with the albuminoids or proteoids only for the sake of convenience.

Mucins are contained in many of the tissues, especially the umbilical cord, the tendons, and the submaxillary gland. Mucins are especially abundant in the snail, the mantle and foot containing two distinct varieties.

The method used for the preparation of mucins varies with the material treated, but is based on the solubility of mucins in dilute alkaline liquids, and their precipitation therefrom by acetic acid. Precipitation by alcohol, or saturation of the solution by a neutral salt, may also be employed for the preparation of mucins.

When freshly precipitated, the mucins are glutinous substances which mix with water without undergoing real solution. They dissolve in dilute solutions of the caustic alkalies, in lime-water, and in baryta-water. On addition of an acid to either of these solutions, the mucin is precipitated. If acetic acid be used, the precipitate is practically insoluble in excess, nor does hydrochloric acid of a strength ranging from 0·1 to 1·0 per cent. of real HCl dissolve most varieties of mucin, but solution is generally effected by acid of 5 per cent. strength.

In their natural condition, the mucins are soluble in a 10 per cent. solution of common salt, but they become insoluble after precipitation by an acid. By saturating their solutions with common salt, ammonium sulphate, and certain other neutral salts, the mucins are completely precipitated, but not coagulated. The mucins are not coagulated either by boiling with water or by precipitation with alcohol.

The mucins behave like ordinary proteids with Millon's and Adamkiewitz' reagents (page 12). With the biuret test, mucins yield a rose-red coloration. No reduction occurs on boiling.

Lead acetate completely precipitates mucins from their neutral or faintly alkaline solutions. Alum also precipitates them, but negative reactions are yielded by most other metallic salts (including mercuric chloride); though all mucins do not behave in the same manner with such reagents. The mucins are not precipitated by tannin.

Such varieties of mucin as have been examined in that respect are not dissolved by pepsin-hydrochloric acid, but are digested by alkaline solutions of trypsin.

By prolonged boiling with concentrated sulphuric acid, the mucins yield leucine and tyrosine.

By boiling for twenty to thirty minutes with dilute sulphuric acid, mucins are decomposed into a proteid substance which is apparently s y n t o n i n, and a p s e u d o - s u g a r which reduces Fehling's solution on boiling, is optically inactive, does not undergo the alcoholic fermentation, and is said to contain nitro-

gen. This decomposition shows the mucins to belong to the class of compound proteids (page 3).

The following analyses of mucins from different sources have been recorded :—

Origin of Mucin.	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen.	Observer.
1. Mucous contents cyst, . . .	52·17	7·01	12·64	...	28·18	Scherer.
2. Snails, . . .	50·30	6·84	13·62	1·74	27·53	Hammarsten.
3. Snails (*Helix pomatiæ*), . . .	48·94	6·81	8·50	...	35·38	Eichwald.
4. Tendon, . . .	48·3	6·44	11·75	0·81	...	Loebisch.
5. Submaxillary gland,	52·31	7·22	11·84	...	28·63	Obolensky.
6. Submaxillary gland,	48·84	6·80	12·32	0·84	31·20	Hammarsten.

No. 2 yielded 0·33, and No. 6, 0·35 per cent. of ash. Hammarsten has found that the mucin from the foot of the snail is distinct from that present in the mantle.

The mucin of urine is described on page 63.

Hyalogens and Hyalins.—Hyalin is a term originally applied to the leading constituent of the walls of hydatid cysts, but it was subsequently extended by Krukenberg to allied substances obtainable from other animal structures. These substances, in their natural condition, are insoluble, and are termed hyalogens, but by the action of alkalies or water under pressure, they are converted into soluble bodies which are generically called hyalins. Thus chondrosin is a hyalogen contained in the sponge, *Chondrosia reniformis*; spirographin is obtained from the cartilage and skeletal tissues of the worm, *Spirographis*; and neossin is the chief constituent of the edible bird's nest. The hyalins corresponding to these hyalogens are called respectively chondrosidin, spirographidin, and neossidin. Similar hyalogens have been described as existing in the vitreous humour of the eye and in hyaline-cartilage (page 494). They agree in their leading characters with the mucins, of which they appear to be simply varieties.

FIBROÏDS.

The proteoïds of the fibroïd class are insoluble in water, and are not readily acted on by dilute acids. They are dissolved by not too dilute solutions of caustic alkalies, especially on heating.

The fibroïds are represented by elastin and the fibroïn of silk and of spiders' webs. They contain no sulphur.

Elastin.

This proteoïd is the characteristic constituent of the elastic fibres which remain after the removal of more soluble compounds (mucin, gelatin, fat, &c.) from the ligaments of the neck of herbivorous animals,[1] by successive treatment with boiling water, caustic alkali, dilute acids, alcohol, and ether.

Thus obtained, elastin forms a pale yellowish substance, in which the shape of the fragments of the original elastic fibres may be distinguished under the microscope. When moist, it is yellow and elastic, but on drying becomes brittle, and may, with some difficulty, be reduced to powder.

Elastin is insoluble in water or any menstruum which does not act on it chemically.

The following analyses of elastin have been published :—

Origin.	C.	H.	N.	S.	O.	Ash.	Authority.
Neck-band (*ligamentum nuchæ*),	54·24	7·27	16·70	0·30	21·79	0·90	Chittenden and Hartwell.
Aorta, ...	53·95	7·03	16·67	0·38	21·97	0·72	Schwarz.

When treated with strong alkalies or mineral acids at 100°, elastin dissolves, and in the latter case, if sulphuric acid be used, leucine (30 to 40 per cent.) and tyrosine (0·25 per cent.) are found among the products of its decomposition. When boiled with strong hydrochloric acid and stannous chloride, the same crystalline products are obtained, together with ammonia, glycocine, and an amidovaleric acid ; but no aspartic or glutamic acid. This behaviour distinguishes elastin from both proteids and gelatin, since the former yield aspartic and glutamic acids but no glycocine, and the latter never yields any trace of tyrosine (page 456).

Silk.[2]

Silk is the filament which the silkworm winds round itself before assuming the condition of a chrysalis.

[1] For the preparation of elastin, the *ligamentum nuchæ* of an ox should be cut into thin slices and boiled with water for several days, then treated for some hours at 100° C. with a 1 per cent. solution of caustic potash, and afterwards again boiled with water. This is poured off and the ligament boiled with water containing 10 per cent. of acetic acid. It is next treated in the cold with water containing 5 per cent. of hydrochloric acid, washed with water, boiled with strong alcohol, and finally digested for several weeks with ether.

[2] The author is indebted to Mr F. W. Richardson for perusal and criticism of this section.

There are many varieties of silkworms and the silks they produce are of very different quality. The silkworm which lives principally upon the leaves of the white mulberry tree (*Moria alba*), known as *Bombyx mori*, is the chief producer of silk; but much attention has of late been given to other silk-producing larvæ, and especially to the Tussah worm, *Antheræa mylitta*, and *A. pernyi*, which feeds on the leaves of the oak, &c. (see *Jour. Soc. Dyers &c.*, iv. 155).

The Bombyx worm secretes a thread from twin orifices situated near the head,[1] and communicating with sacs running one on each side the whole length of the body. The glutinous secretion rapidly coagulates in the air. As it flows from two distinct apertures, the thread produced is double, the twin fibres being enclosed in a thin, perfectly smooth case, known as "silk-glue," "silk-gelatin," "silk-gum," or "bast" (in France, *grès*). This gelatin case is readily soluble in soap solution, dilute caustic alkalies, and weak chromic acid, the application of these reagents effecting a separation of the enclosed twin threads. The internal fibre, freed from the gelatin case, consists essentially of *fibroïn*, while the coating is composed substantially of *sericin*. According to P. Bolley, in the silk-producing and secreting gland of the worm, glutinous semi-fluid fibroïn occurs without admixture with sericin, the latter compound being a product of the subsequent aërial oxidation of fibroïn.

Raw commercial silk from the mulberry silkworm is generally regarded as containing 11 per cent. of moisture, 66 of fibroïn, 22 of sericin, and 1 per cent. of mineral and colouring matters.[2] The mineral matters are chiefly potash, lime, magnesia, and phosphoric acid, the ash containing about 10 per cent. of the last-named constituent. The greater part of the mineral matters of raw silk are simply adherent to the fibre, and are removed together with the

[1] Chappe triturated the contents of the glandular organs of silkworms with about one-third their weight of water, and was thus enabled to blow permanent globes and diversely-shaped vessels (*Ann. de Chimie*, xi. 113).

[2] The colouring matter of yellow silk has been investigated by L. Dubois (*Compt. rend.*, cxi. 482), who has isolated from it several bodies agreeing in many respects with vegetable carrotene (Vol. III. Part i. page 356). They are yellowish-red and crystallisable, alterable by exposure to light and air, soluble in alcohol, ether, chloroform, and benzene to golden-yellow solutions, and in carbon disulphide to a brownish-red solution. They give absorption-spectra free from bands, and dissolve in strong sulphuric acid, with blue coloration changing to green, destroyed by addition of water. In addition to these colouring matters, Dubois isolated a deep greenish-blue pigment, which is probably crystallisable but is present in very small quantity.

sericin by prolonged boiling with soap-solution. The residual fibroïn retains only about 0·6 per cent. of mineral matter.[1]

H. Silbermann (*Chem. Zeit.*, 1895, No. 27) gives the following analyses of typical samples of *mulberry silk*:—

Kind of Silk.	Fibroïn.	Ash of Fibroïn.	Sericin.	Wax and Fat.	Salts.
White; cocoons, . .	73·59	0·09	22·28	3·02	1·06
White; raw, . .	76·20	0·09	22·01	1·36	0·30
Yellow; cocoons, .	70·02	0·16	24·29	3·46	1·92
Yellow; raw, . .	72·35	0·16	23·13	2·75	1·60

A good specimen of *mulberry silk* was found by F. W. Richardson to contain:—

	Air-dry.	Dried at 100° C.
	Per cent.	Per cent.
Water, ...	12·50	...
Fatty substances, ...	0·14	0·16
Resinous substances, ...	0·56	0·64
Sericin, ...	22·58	25·81
Fibroïn, ...	63·10	72·11
Mineral matters, ...	1·12	1·28
	100·00	100·00

Raw Tussah silk has been analysed by Bastow and Appleyard (*Jour. Soc. Dyers &c.*, iv. 88) with the following results, the sample being reeled and previously dried at 100° C.

Soluble in hot water, ...	21·33 per cent.
Subsequently dissolved by alcohol (chiefly fatty acid), .	0·91 ,,
Subsequently dissolved by ether, ...	0·08 ,,
Total loss by boiling off with 1 per cent. solution of curd soap, ...	26·49
Mineral matters, ...	5·34[2]

[1] J. Carter Bell states that the ash of a genuine silk (purified) varies according to the district in which it is produced, and averages 0·5 per cent. He found 0·35 per cent. of ash in a pure white silk, while a Chinese salmon-coloured silk contained only 0·25 per cent. In the raw state, the ash of silk varies very much. In good Italian silk it is about 1 per cent. Yama-mai silk sometimes yields as much as 8 per cent. of ash, which is greatly reduced by successive treatments of the fibre with alcohol, dilute sulphuric acid, and boiling soap solutions.

[2] The *ash* of Tussah silk was found by Bastow and Appleyard to have the following centesimal composition:—K_2O, 31·68; Na_2O, 12·45; CaO, 13·32; MgO, 2·56; Al_2O_3, 1·46; SiO_2, 9·79; P_2O_5, 6·90; SO_3, 8·16; CO_2, 11·14; Cl, 2·89; less O equivalent to Cl, 0·65; total, 99·70. As sulphur is not a constituent of the organic compounds of mulberry silk, the occurrence of sulphates in the ash of Tussah silk is remarkable. The presence of a notable proportion of alumina is still more extraordinary, as aluminium is not a generally-recognised constituent of animal products, and is contained in

Silk Conditioning.—In a damp atmosphere, raw silk will take up a large proportion of moisture (25 to 30 per cent.) without any indication of the fact other than the increase in weight. To ascertain the proportion of moisture in raw silk, a number of hanks of the sample are dried at 110° in a current of air until constant in weight. To the weight thus obtained it is usual to add 11 per cent., the result being regarded as the weight of the silk in a normal condition of humidity.

Silk Scouring.—In the process of scouring silk, the silk-gum or sericin is removed more or less completely. This is effected by heating the hanks of raw silk in a solution of soap at 90 to 95° C., the process being sometimes repeated with a fresh soap-bath. The silk at first swells up, but as the glue dissolves, it acquires a soft and silky feel. The waste liquid, known as "boiled-off liquor," is a useful addition to the dye-bath in dyeing silk with coal-tar colours. The last traces of sericin are removed by washing the silk in water at 60°, to which some soap and sodium carbonate have been added. The silk is sometimes again boiled with soap-solution, rinsed with warm sodium carbonate solution, and lastly washed with cold water. If the silk is to remain undyed or to be dyed a pale colour, it is exposed whilst moist to sulphur dioxide gas for five or six hours. This operation, which has for its object the bleaching of the silk, is, in many cases, repeated several times.

Besides the above process, which is employed to produce completely-ungummed silk, involving a loss of 22 to 30 per cent. of the original silk, *souple silk* is produced when only from 8 to 12 per cent. of sericin is removed;[1] and when *écru* silk is required, the hanks are simply washed with hot water containing a little soap, whereby a loss of only 3 to 4 per cent. results.

Sericin, obtained by treating silk with water under pressure, is a yellowish, transparent substance, resembling gelatin. It dissolves in hot water, hot soap solutions, and weak caustic alkali. The hot aqueous solution forms a strong jelly on cooling. Aqueous solu-

so few plants that its presence in the secretion of the silkworm is difficult to account for. It is probable that the greater part of the ash found by Bastow and Appleyard in Tussah silk was derived from adhering impurities, especially as the fibroïn prepared from the same silk contained only 0·226 per cent. of ash.

[1] Soupling may be regarded as a morphological alteration of the raw silk fibre, whereby the sericin is rendered soft, pliant, and lustrous. Souple silk is stated by Silbermann to possess the property of taking up vegetable and mineral weighting materials in a greater degree than either raw or boiled-off silk. For coloured silks, the soupling is often carried out in a boiling bath of cream of tartar or acid sodium sulphate. For black silk, the soupling is combined with the weighting and dyeing processes.

tions of sericin are precipitated by alcohol, tannin, basic lead acetate, stannous chloride, chlorine, and bromine. Potassium ferrocyanide in presence of acetic acid gives a greenish precipitate.

By the action of dilute sulphuric acid on sericin, Cramer obtained 5 per cent. of tyrosine (distinction from gelatin) and 10 per cent. of serin, a substance having the constitution of an amido-glyceric acid, $C_2H_4O(NH_2).COOH$.[1]

According to Mulder, sericin has the following empirical formula and ultimate composition. For convenience of comparison, the formula and composition of fibroïn (according to Mulder) are also given :—

	Sericin, $C_{15}H_{25}N_5O_8$	Fibroïn, $C_{15}H_{23}N_5O_6$
Carbon,	42·60	48·80
Hydrogen,	5·90	6·23
Nitrogen,	16·50	19·00
Oxygen,	35·00	25·00
	100·00	100·00

P. Bolley attributes to sericin the composition :—Carbon, 44·32 ; hydrogen, 6·18 ; nitrogen, 18·30 ; and oxygen, 31·20 per cent.

The excess of oxygen in sericin over that in fibroïn is probably due to the greater oxidation of the surface of the fibre, the conversion of fibroïn into sericin being supposed to take place by assimilation of water and oxygen :—

$$\underset{\text{Fibroïn.}}{C_{15}H_{23}N_5O_6} + H_2O + O = \underset{\text{Sericin.}}{C_{15}H_{25}N_5O_8}$$

Fibroïn is related in character to elastin (page 499), and is

[1] Serin resembles glycocine (Vol. III. Part iii. page 206) in its power of uniting with both bases and acids. It differs from alanine or amido-propionic acid by an atom of oxygen. By the action of nitrous acid, alanine yields lactic acid, while serin gives glyceric acid when similarly treated.

$C_2H_3\begin{cases}NH_2\\H\\CO.OH\end{cases}$ Alanine. $\qquad$ $C_2H_3\begin{cases}OH\\H\\CO.OH\end{cases}$ Lactic acid.

$C_2H_3\begin{cases}NH_2\\OH\\CO.OH\end{cases}$ Serin. $\qquad$ $C_2H_3\begin{cases}OH\\OH\\CO.OH\end{cases}$ Glyceric acid.

obtained by treating silk successively with boiling water, alcohol, ether, and acetic acid. The resultant fibroïn retains the fibrous character of the original silk, but is quite white, destitute of lustre, and soft to the touch.[1] On ignition, fibroïn evolves an odour recalling that of burnt horn. Fibroïn does not dissolve in ammonia or solutions of the carbonates of alkali-metals, and is not affected by a one per cent. solution of caustic potash or soda, but is dissolved by stronger ley, especially if hot. On dilution, a precipitate is obtained which is said to consist of unaltered fibroïn. Fibroïn is soluble in hot glacial acetic acid, and is also soluble in strong hydrochloric, nitric, sulphuric, or phosphoric acid. It is likewise dissolved by alkaline solutions of nickel, zinc, copper, &c.[2]

The proportion of fibroïn in the silk from *Bombyx mori* has been variously stated according to the method employed for its determination. Thus Mulder, who prepared it by boiling the raw silk with acetic acid, gives it as 53 to 54 per cent. Städeler, by acting on the silk with a 5 per cent. solution of caustic soda in the cold, obtained from 42 to 50 per cent. of fibroïn. Cramer, by heating the silk with water at 133°, obtained 66 per cent.; and Francézon, by boiling the silk twice with soap, and then treating it with acetic acid, found 75 per cent. of fibroïn. Vignon, as the result of an improved method of determination,[1] states the average proportion of fibroïn in raw silk at 75 per cent.

[1] For the preparation of pure fibroïn, L. Vignon (*Compt. rend.*, cxv. 17, 613) recommends that a skein of raw white silk weighing about 10 grammes should be boiled for thirty minutes in 1500 c.c. of water containing 15 grammes of neutral soap. The silk is then rinsed successively in hot and in tepid water to remove the soap, hydro-extracted, and submitted to a repetition of this treatment, a fresh soap-bath being used in which the silk is immersed for twenty minutes. The silk is then rinsed with water, passed through dilute hydrochloric acid, again rinsed with water, and then washed twice with 90 per cent. alcohol. The fibroïn thus obtained is very brilliant, white, and soft, and leaves only 0·01 per cent. of ash on ignition. It was found by Vignon to have the elementary composition: — C, 48·3; H, 6·5; N, 19·2; and O, 26·0 per cent.

[2] Bastow and Appleyard (*Jour. Soc. Dyers &c.*, iv. 89) have pointed out that the fibroïn of Tussah silk is acted on by solvents far less readily than the fibroïn of mulberry silk, and they consider the two products to be chemically distinct. Tussah fibroïn was prepared by repeatedly boiling the silk with a 1 per cent. solution of curd soap, washing with water, extracting with hydrochloric acid, again washing with water, drying, and extracting successively with alcohol and ether. Thus purified, Tussah fibroïn had the following elementary composition (exclusive of 0·226 per cent. of ash):— Carbon, 47·18; hydrogen, 6·30; nitrogen, 16·85; and oxygen, 29·67 per cent.

These higher figures doubtless refer to silk previously dried at 100° C.

The formula of silk-fibroïn is uncertain. Mulder attributed to it the composition $C_{15}H_{23}N_5O_6$. Mills and Takamine adopt the expression $C_{24}H_{38}N_8O_8$. Schützenberger and Bourgeois, by the analysis of the fibroïn prepared by Francézon's process (see last page), arrived at the formula $C_{71}H_{107}N_{24}O_{25}$;[1] Cramer, $C_{15}H_{23}N_5O_6$; while F. W. Richardson (*Jour. Soc. Chem. Ind.*, xii. 426) suggests for fibroïn the empirical formula $C_{60}H_{94}N_{18}O_{25}$. The fibroïn prepared by Vignon's process has a composition corresponding to the formula $C_{29}H_{47}N_{10}O_{12}$.

M. P. Richard has attempted to prove the presence of the amidogen group in silk by diazotising the fibre with a solution of sodium nitrite containing hydrochloric acid. After twenty-four hours' exposure to the nitrous acid the silk acquired a characteristic pale yellow colour, and on being then washed and plunged into the alkaline solutions of different phenols (phenol, resorcin, pyrogallol, and α- and β-naphthol) it was dyed differently with each (page 545).

From the results of the action of alcoholic potash, F. W. Richardson considers the fibroïn of mulberry-silk to be more probably an amido-anhydride than an amido-acid.[2]

T. Weyl (*Ber.*, xxi. 1529) found that by boiling purified white silk under a reflux condenser for eighteen hours with dilute sulphuric acid (1 : 5), the whole dissolved to a yellowish-

[1] When heated with baryta-water under pressure, Silbermann found fibroïn to undergo decomposition with formation of oxalic, carbonic, and acetic acids, and an amido-mixture containing :—$C_{68}H_{141}N_{21}O_{43}$.

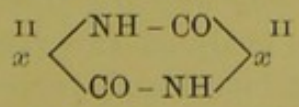
The amido-mixture is stated to have undergone further decomposition as follows :—

$$C_{68}H_{141}N_{21}O_{43} = C_9H_{11}NO_3 + 7C_2H_5NO_2 + 7C_3H_7NO_2 + 2C_4H_9NO_2 + 4C_4H_7NO_2$$

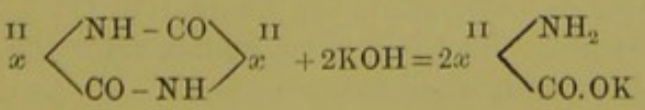
Amido-mixture.	Tyrosine.	Glycocine.	Alanine.	Amido-butyric acid.	Amido-acid of the acrylic series.

(See also H. Silbermann, *Chem. Zeit.*, xvii. 1693.)

[2] Richardson suggests for fibroïn the following constitutional formula, in which x represents a hydrocarbon residue :—



The equation depicting the decomposition which takes place on saponification with potash would then be :—



brown liquid, with the exception of a few globules of a fatty acid. From the product Weyl isolated 5·2 per cent. of tyrosine, 7·5 per cent. of glycocine, and 15 per cent. of a crystalline sublimable compound which was apparently α-alanine. The remaining products (72 per cent.) resisted enquiry.

Silk-fibroïn resembles proteids in its behaviour with Millon's and Adamkiewitz' reagents, and with the biuret test.

F. W. Richardson found combed and well-purified mulberry-silk to absorb 30 per cent. of iodine when treated with Hübl's reagent (Vol. ii.). The product retained a deep yellow colour even after washing with potassium iodide and water. Attempts to acetylise fibroïn gave entirely negative results.

Nitro-Silk.—Silk is acted on very peculiarly by dilute nitric acid. When immersed for about a minute in nitric acid of 1·133 sp. gr., at a temperature of about 45° C., and then washed thoroughly with water, silk acquires a characteristic yellow colour, fast to light and air. Pure nitric acid free from nitrous compounds does not produce the colour, which varies in intensity with the proportion of nitrous compounds present. Nitrous acid, produced by adding hydrochloric acid to a solution of sodium nitrite, dyes silk a pale yellow colour which is not fast; but if the silk thus treated be then immersed in dilute nitric acid or in a mixture of hydrochloric acid and potassium permanganate, the yellow colour becomes deeper and permanent to air and light, proving that the action of nitric acid is that of an oxidising agent. If "nitro-silk" be treated with an alkali, the yellow colour is considerably deepened, the shade obtained varying with the base used, being palest with ammonia and approaching red with baryta. Alkaline carbonates produce the same intensity of colour as the corresponding caustic alkalies. The alkali cannot be removed by washing. Nitro-silk is slowly decolorised when boiled in a concentrated acid solution of stannous chloride, but alkaline reducing agents appear to have no action. Nitro-silk behaves like ordinary silk with solvents, except that when treated with concentrated sulphuric acid it swells up and gives a sticky mass similar to ordinary egg-albumen. Vignon and Sisley, to whom the above observations on nitrated silk are due (*Compt. rend.*, 1891; *Jour. Soc. Dyers &c.*, viii. 14), analysed two samples of white Canton silk, which had been degummed and purified by successive treatments with boiling soap solution, distilled water, hydrochloric acid, water and alcohol. One of the samples thus purified was dyed with nitrous nitric acid, by which treatment it gained 2 per cent. in weight. The following figures show the composition of the dyed and undyed fibroïn:—

	Undyed Silk.	Dyed Silk.
Carbon,	48·3 per cent.	46·8 per cent.
Hydrogen,	6·5 ,,	6·5 ,,
Nitrogen,	19·2 ,,	21·6 ,,
Oxygen,	26·0 ,,	25·1 ,,
	100·0 per cent.	100·0 per cent.

Action of Solvents on Silk.—Silk is gradually dissolved by cold concentrated sulphuric acid to form a slightly coloured solution.[1] Hydrochloric acid dissolves it without colour to a perfectly limpid liquid. Zinc chloride and alkaline solutions of copper and nickel also dissolve silk. Cold concentrated solutions of caustic potash and soda dissolve silk completely, but 2 per cent. solutions dissolve the sericin only, leaving the fibroïn. The solutions are not precipitated by dilution with water, but evolve ammonia when heated.

The different varieties of silk differ materially in their behaviour with reagents. Von Höhnel effected a quantitative separation of a mixture of real silk, Yama-mai silk, sheep's wool, and cotton into its four constituents, by boiling the mixture with hydrochloric acid for half a minute, whereby the real silk only was dissolved; while on continuing the boiling for two minutes with concentrated hydrochloric acid, the Yama-mai silk passed into solution. On heating the residue with caustic potash solution, the wool was dissolved, while the cotton remained unchanged. The analysis of mixed fibres is described in detail on page 513 *et seq.* Bastow and Appleyard (*Jour. Soc. Dyers &c.*, iv. 89) observed the following differences between mulberry and Tussah silk :—

Treated with	Mulberry Silk. (*Bombyx mori.*)	Tussah Silk. (*Antheræa mylitta.*)
A hot 10 per cent. solution of caustic soda,	Completely dissolved in twelve minutes.	Required fifty minutes for complete solution.
Cold concentrated hydrochloric acid (sp. gr. 1·16),	Dissolved almost instantly.	Only partially dissolved in forty-eight hours.
Cold concentrated nitric acid,	Dissolved in five minutes with light yellow colour.	Dissolved in ten minutes to a light brown solution.
Neutral solution of zinc chloride of 1·725 sp. gr.,[2]	Dissolved almost instantly.	Required considerable time for complete solution.
Saturated aqueous solution of chromic acid mixed with an equal measure of water,	Dissolved immediately.	Slowly acted on.

[1] By diluting the solution of silk in cold concentrated sulphuric acid with water, boiling, neutralising with milk of lime, and evaporating the filtered solution to dryness, F. W. Richardson obtained 15 per cent. of glycocine, leucine, and tyrosine, and 85 per cent. of a substance having the odour and appearance of glue, readily soluble in water, but quite insoluble in alcohol.

[2] J. Persoz (*Jour. Soc. Dyers &c.*, iii. 128) also states that mulberry silk is

F. Filsinger (*Chem. Zeit.*, 1896, xx. 324) states that mulberry silk is readily soluble in an alkaline solution of copper hydroxide in glycerin, whereas Tussah silk is scarcely affected by this reagent.

Optical Activity of Silk.—Solutions of silk are lævo-rotatory. L. Vignon (*Compt. rend.*, cxiii. 802, cxiv. 129; abst. *Jour. Soc. Chem. Ind.*, xi. 427) removed the colouring matter from silk (from *Bombyx mori*) by repeated treatment with hot alcohol acidulated with hydrochloric acid. The silk thus purified was immersed in a cold 3 per cent. aqueous solution of caustic soda, when a yellowish, limpid solution of *sericin* was obtained having an optical activity of $[\alpha]_D = -39{\cdot}2°$. Silk from the same source was boiled twice with a 10 per cent. solution of soap, which was washed away by water after each boiling, the residual silk washed with acidulated water (0·1 per cent. of hydrochloric acid), and finally with alcohol. The white *fibroïn* thus obtained was dried and dissolved in moderately strong hydrochloric acid, when a clear viscous solution was obtained having after dilution an optical activity corresponding to $[\alpha]_D = -40°$, which was materially altered by further dilution, or by the addition of excess of ammonia. Solutions obtained by Vignon in a similar manner from other varieties of silk showed a value for $[\alpha]_D$ from $-9°$ to $-43{\cdot}6°$, mean $-29{\cdot}6°$, for the alkaline solution of the sericin; and from $-39{\cdot}4°$ to $-50°$, with a mean of $-44°$, for the hydrochloric acid solution of the fibroïn (abst. *Jour. Soc. Chem. Ind.*). The fibroïn of *Yama-mai* was found to be insoluble in hydrochloric acid; but soluble after some time in cold concentrated sulphuric acid.

Microscopical Characters of Silk.—The silk from *Bombyx mori* (mulberry silkworm) and *Saturnia spina* is a homogeneous, hyaline, formless fibroïn thread resembling glass-rod, and rarely exhibits signs of striation. The fibre of real silk is readily distinguished from its substitutes by the microscope. Its fibre is round, of less diameter than that of the substitutes, and is not very much coloured.

On the other hand, the fibres of Tussah and other "wild" or exotic silks, now much used on account of their low price, consist of bundles of circular threads varying in diameter from 0·0003 to

almost immediately (in less than thirty seconds) dissolved by a boiling solution of zinc chloride of 1·45 specific gravity, while Tussah silk is only very slowly acted on; and a very fair separation of the two silks may be effected by immersing the mixture in the hot reagent for one minute, and then removing and washing it, when the residue will consist of Tussah silk only.

0·0015 millimetre, which give the main fibre a striated appearance. The fibrillæ are agglutinated by a matter not greatly differing from their own substance, and not capable of being sharply distinguished and separated from the fibroïn, as the silk-gum of mulberry silk is from the enclosed fibroïn. Many of the twin fibres of the exotic silks are flattened to a marked extent.

F. Filsinger (*Chem. Zeit.*, xx. 324) describes the cross-section of Tussah fibres as larger and flatter than that of mulberry silk, and as showing many fine air-tubes. Characteristic bands frequently cross the fibres in an oblique direction, giving them a microscopic appearance similar to that of cotton.

F. von Höhnel (*Dingl. polyt. Journ.*, cxlvi. 465; *Jour. Soc. Chem. Ind.*, ii. 172) has recorded the following characters of raw silk from different sources. The figures refer to the largest diameter of single threads and are expressed in thousandths of a millimetre :—

Kind and Origin of Silk.	Diameter. μ.	Colour and Microscopic Appearance.	
		Broad Side.	Narrow Side.
Mulberry silk from *Bombyx mori.*	20 to 25.	White or yellowish-white; shining.	White or yellowish-white; shining.
Senegal silk from *Bombyx Faidherbi.*	30 to 35.	Shining yellowish or brownish-white, or pale yellow, grey, brown, and occasionally bluish-white.	Grey, brown, or black, with occasionally lighter shades.
Ailanthus silk from *Bombyx cynthia.*	40 to 50.	Shining yellowish-white, with yellow, brown, brownish-grey spots.	Dirty grey or brown to black, with green, yellow, red, violet, or blue spots.
Yama-mai silk from *Antheræa yama-mai* (Japan).	40 to 50.	Bluish-white, with dark blue, blue, and black shades.	Glaring and fine colours with dark or black shades.
Tussah silk from *Actius selene* (?).	50 to 55.	Irregular in thickness. Thickest parts with grey and blue spots; thinner parts, bluish-white, yellow, or orange-red.	Dark grey, with pink or light green spots.
Tussah silk from *Antheræa mylitta.*[1]	60 to 65.	Similar to *A. selene*, but spots orange-red, red, or brown.	Similar to *A. selene.*

[1] The cocoons of the larvæ of *Antheræa mylitta* are firm and hard, of a silvery drab colour, egg-shaped, and much larger than those of the true silk-

DETECTION OF SILK. ANALYSIS OF MIXED FABRICS.

The foregoing observations refer to the microscopic appearances of different varieties of silk, but the microscope also serves to distinguish silk from cotton, wool, and other textile fibres. The following illustrations show the appearance of silk and other fibres under a magnifying power of three hundred diameters:—

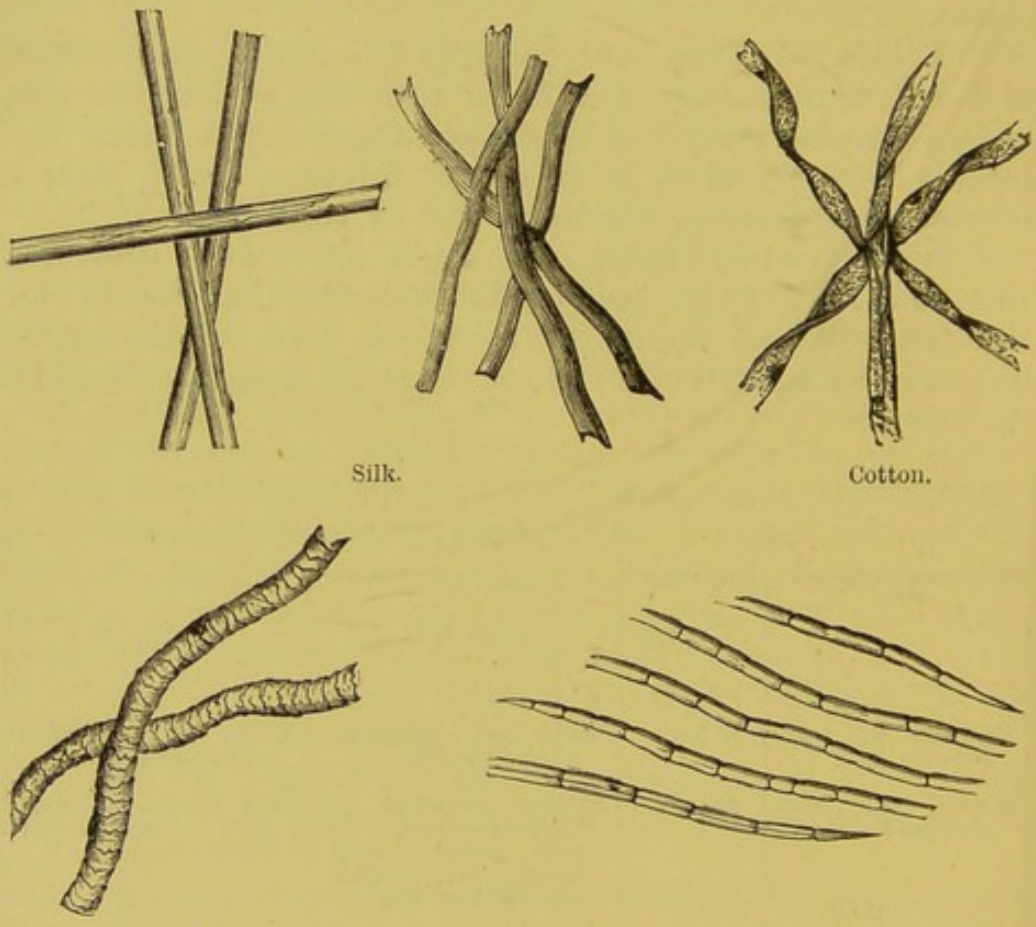


Fig. 29.—TEXTILE FIBRES, magnified 300 diameters.

worm. The product is used largely for the manufacture of silk plush and buff-coloured Indian silks.

Other wild silks are the *Eria* silk of India, the *Muga* silk of Japan, the *Fagara* or *Atlas* silk of China, &c.

Woodcuts illustrating the magnified appearance of a number of varieties of silk have been published by T. Wardle (*Jour. Soc. Dyers &c.*, i. 196); and details of the tension, strength, and diameter of the fibres have been recorded by the same author (*Jour. Soc. Arts*, xxxiii. 671).

The microscopic appearance of Tussah silk has been described and illustrated by F. H. Bowman (*Jour. Soc. Dyers &c.*, iv. 90).

Interesting particulars respecting wild silk have been given by C. Grosseteste (*Bulletin Soc. Ind. Mulhouse*, 1888; *Jour. Soc. Dyers &c.*, iv. 155).

The following are illustrations of the appearance of mixed fibres under the same magnifying power:—

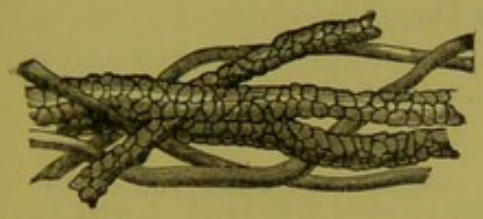


Fig. 30.—Mixed wool and silk.

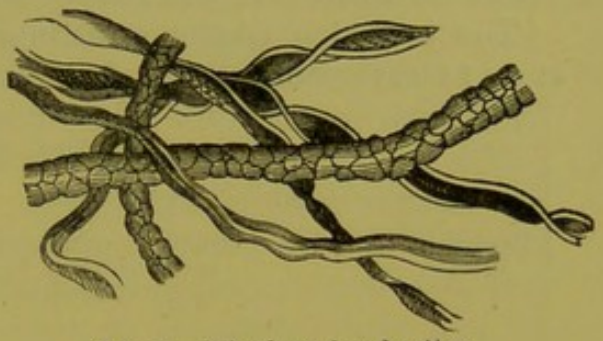


Fig. 31.—Mixed wool and cotton.

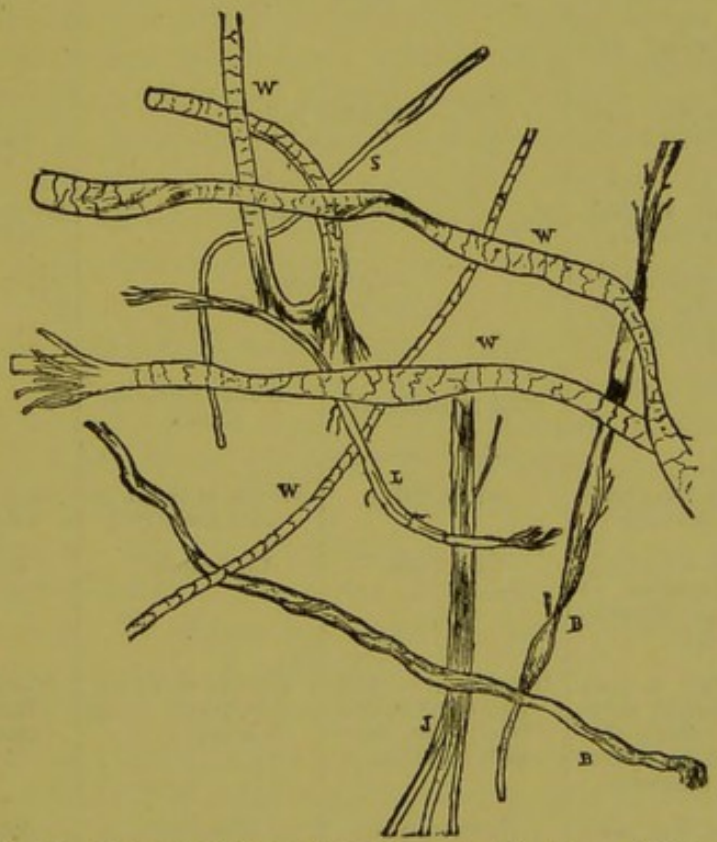


Fig. 32.—Complex Mixture of Fibres.—B, cotton; J, jute; L, linen; S, silk; W, wool.

Under the microscope, *silk* appears as a slender, homogeneous, solid cylinder, free from either scales or medullary substance. Sheep's *wool* appears thicker than silk, and has a perfectly circular stalk with tile-shaped scales. *Linen* (flax) is cylindrical and never flat; it is not stiff or twisted, and is characterised by the narrowness of its medullary tube. *Hemp* resembles linen-fibre, being easily broken; its ends branch out stiffly, and its tube is open. *Cotton* fibres are long, flat, and resemble a twisted band.

Animal fibres, such as silk, wool, and hair, contain nitrogen, and

evolve an odour of burnt feathers when ignited. They are dyed by magenta and picric acid without a mordant, and are coloured yellow by nitric acid and red by Millon's reagent.

The following reactions distinguish silk qualitatively, and to some extent quantitatively, from other fibres.

	Silk, Wool, Fur, or Hair.		Cotton or Linen.
Heated in small test-tube,	Brittle, carbonaceous residue, and smell of burnt feathers. Condensed moisture is alkaline to litmus.		Charring and smell of burning wood. Condensed moisture is acid to litmus.
Boiled in a saturated aqueous solution of picric acid, and rinsed in water,	Dyed yellow.		Unchanged.
Treated with cold nitric acid of 1·2 sp. gravity,	Coloured yellow.		No change of colour.
Boiled with Millon's reagent (page 11),	Red coloration.		No change of colour.
Moistened with dilute hydrochloric acid and dried at 100° C.,	Unchanged.		Becomes rotten.
	Silk (Mulberry).	Wool, Fur, or Hair.	
Heated to boiling with hydrochloric acid,	Dissolved.	Swells, without at once dissolving.	Mostly undissolved.
Boiled with a concentrated solution of basic zinc chloride (page 514),	Dissolved.	Unchanged.	Unchanged.
Treated with a cold concentrated solution of ammonio-cupric oxide (Schweitzer's reagent),	Dissolved. Not precipitated from the solution by salts.	Undissolved. Dissolves on heating.	Dissolved. Solution precipitated by salts.
Treated in the cold with 10 per cent. caustic soda,	Undissolved.	Dissolved.	Undissolved.
Boiled with 2 per cent. caustic soda solution (page 513).	Dissolved. Solution not darkened by lead acetate; negative reaction with sodium nitroprusside.	Dissolved. Solution gives black or brown precipitate with lead acetate, and violet colour with sodium nitroprusside.	Unchanged.
Behaviour with Molisch's test (page 513),	Dissolved; with little coloration.	Undissolved; yellow or brown coloration.	Dissolved; deep violet colour.

Reactions for distinguishing mulberry silk from Tussah silk are given on page 507.

According to W. Molisch (*Dingler's Polyt. Journ.*, 21st July 1886), a convenient method of distinguishing animal from vegetable fibres is as follows :—The material is first boiled and washed several times with water, to remove dressing materials. A quantity of about 0·010 gramme is then treated in a test-tube with 1 c.c. of water and two drops of a 15 to 20 per cent. alcoholic solution of α-naphthol. About 1 c.c. of strong sulphuric acid is then added and the mixture well shaken. Any vegetable fibres rapidly dissolve, and the liquid acquires a deep violet colour. With fibres of purely animal origin, solution occurs in the case of silk, but wool remains undissolved ; a yellow or brownish colour may be developed, but there will be no violet tint. In most cases dyes and mordants do not interfere with the reaction.

Liebermann has pointed out that if a fibre be boiled with a solution of magenta, to which sufficient caustic soda has been previously added to cause decolorisation, and then well washed in water, it will acquire a deep pink colour if of animal origin, while cotton and linen will be unaffected. The test is rendered more delicate by immersing the fibre in water acidulated with acetic acid, after washing it in pure water.

This reaction and that with picric acid may be conveniently employed to render visible the animal fibres in a mixed yarn or fabric. Wool (and hair) fibres may be rendered visible by immersing the material in a dilute boiling solution of caustic soda, to which a little lead acetate has been added. The wool will be turned brown or black from the formation of lead sulphide, while silk and vegetable fibres are unaffected.

Of course, the foregoing tests are of value chiefly for the preliminary examination of undyed fibres.

Various methods of *determining the proportion of silk* in mixed fabrics have been proposed, but most of them are very imperfect, and the best are not free from errors, owing to the impossibility of finding any reagent which will dissolve either cotton, silk, or wool, without at the same time more or less attacking the other two fibres.

In France, silk is sometimes determined in plush by boiling the fabric with a 2 per cent. solution of caustic soda, but this reagent acts to a serious extent on cellulose, especially in presence of air. A reagent sometimes recommended for the solution of silk is prepared by dissolving 16 grammes of copper sulphate in 140 to 160 c.c. of water, and adding from 8 to 10 grammes of pure glycerin. A solution of caustic soda is then gradually poured in until the precipitate first formed redissolves, excess of soda being avoided. It is said that this solution has no action on

wool and cotton, while it readily dissolves silk, but Richardson found that when heated with the soda-copper solution for twenty minutes (the time necessary to dissolve silk from plush), purified cotton lost from 1 to 1½ per cent. of its weight, and became very friable and dusty; while woollen fabrics, treated in a similar manner, lost from 9 to 16 per cent. of their weight. Hence the reagent is useless for the analysis of fabrics containing wool.

Persoz observed that silk dissolved very readily in a boiling solution of oxychloride of zinc, and on this reaction Von Remont has based the following method of analysing mixed fabrics containing silk, wool, and cotton. Four quantities (A, B, C, and D) of two grammes each of the air-dried material are weighed out. One of these (A) is kept, and each of the other three boiled for fifteen minutes in 200 c.c. of water containing 3 per cent. of HCl. The liquid is decanted, and the boiling repeated with more dilute acid. This treatment removes the size, and in most cases the colouring matter. Cotton is decolorised very quickly, wool less readily, and silk but imperfectly. Light coal-tar dyes on silk can be neglected, but the black dye on silk often forms a considerable part of the weight of the fabric. Its incomplete removal is indicated by the ferruginous ash left on igniting the fabric, a test which is more reliable than the incomplete decolorisation of the stuff, since this may occur when only an insignificant amount of colouring matter remains. The acid is removed from the material by washing and pressing, and portion B is then laid aside. To remove the silk, portions C and D are then next placed for two minutes in a boiling solution of basic zinc chloride of 1·72 specific gravity,[1] then thrown into water, and washed first with water acidulated with 1 per cent. of hydrochloric acid and then with pure water until the washings are free from zinc. Portion C is pressed and laid aside. For the separation of the wool and cotton D is boiled gently for fifteen minutes with 60 to 80 c.c. of caustic soda solution of 1·02 sp. gravity, and then washed very carefully in water, taking care not to destroy the vegetable fibre. The four portions, A, B, C, and D, are then dried for an hour at 100° C., and left till the following day fully exposed to the air, so that they may absorb the normal amount of hygroscopic moisture. If *a*, *b*, *c*, and *d* are the weights of the portions represented by the corresponding capital letters, then :—

[1] The reagent is prepared by dissolving 1000 grammes of zinc chloride in 850 c.c. of hot water, adding 40 grammes of zinc oxide, and heating the mixture until solution is complete.

$a-b$ = dye and finish ; $b-c$ = silk ; $c-d$ = wool ; and d = vegetable fibre. Some operators make an allowance of 5 per cent. on the weight of d, to compensate for loss by the action of the alkali. In the case of a black silk resisting the action of dilute acid, the composition can be arrived at by the method described on page 522.

The foregoing process has been critically examined by F. W. Richardson (*Jour. Soc. Chem. Ind.*, xii. 430). He considers the plan of working on the air-dry material, and subsequently exposing the treated portions over-night to the air, is apt to lead to very misleading results. The material should be thoroughly dried at 100° C. before being weighed out, and the treated portions weighed after being dried at the same temperature.[1] Boiling with water containing three per cent. of real hydrochloric acid acts too greatly on the wool and cotton to render such treatment desirable. Boiling for ten minutes with 1 per cent. hydrochloric acid is to be preferred. Richardson plunges the material two or three times into the boiling zinc chloride solution, taking care that the total time of immersion does not exceed one minute. It is necessary that the zinc solution should be sufficiently basic and concentrated to obtain moderately good results. Even then, purified cotton loses about 0·5 per cent., and purified wool from 1·5 to 2·0 per cent. of its weight.

An ammoniacal solution of nickel oxide has been recommended by F. W. Richardson (*Jour. Soc. Chem. Ind.*, xii. 430) as a reagent for separating silk from wool and cotton in mixed fabrics.[2] Silk dissolves rapidly even in the *cold* solution, and after treatment for two minutes, which is the time necessary to dissolve the silk from fabrics other than plush, purified cotton lost 0·45, and purified wool 0·33 per cent. In order to dissolve silk from plush, it is necessary to boil the material with the nickel solution for ten minutes under a reflux condenser. Cotton loses 0·8 per cent. of its weight when thus treated.

For the analysis of *plush*, Richardson recommends brief exposure of the fabric to a boiling solution of basic zinc chloride, as already described, but for the determination of silk

[1] As textile fibres are very hygroscopic, suitable precautions must be adopted to prevent the material from re-absorbing water during weighing.

[2] The reagent is best prepared by dissolving 25 grammes of crystallised nickel sulphate in about 80 c.c. of water, 36 c.c. measure of a 20 per cent. solution of caustic soda is then added, and any excess of alkali carefully neutralised by dilute sulphuric acid. The precipitate of nickel hydroxide is redissolved in 125 c.c. of strong ammonia, and the solution made up with water to 250 c.c.

in light fabrics, especially when wool is also present, exposure from one to three minutes (according to the nature of the material) to a cold solution of ammoniacal nickel oxide is to be preferred.

In illustration of the action of the separating reagents, Richardson mentions the case of a plush which contained rather more silk than cotton, and gave the following results :—

	By Ammoniacal Nickel Solution.	By Basic Zinc Chloride Solution.	By Copper-Glycerin Reagent.
Moisture, dye, and finish, . . .	11·34	11·00	10·04
Silk, ...	45·60	45·00	47·06
Cotton, ...	43·06	44·00	42·90
	100·00	100·00	100·00

Plush backs are much harder than most cotton fabrics, and with them successive treatment with acid and with the copper-glycerin reagent gives good results ; but the copper reagent is not suited for use with other cotton goods, and wool is dissolved by it to a very serious extent. Thus Richardson found a mixed woollen and silken fabric, after being thoroughly cleansed from extraneous matter and dried, gave with the nickel reagent 25·4 per cent. of silk, and with basic zinc chloride 27·0 per cent. He considers the former result the more accurate, as the zinc solution acts excessively on the wool.

	Fibres actually present ; per cent.	Percentage obtained by		
		Ammoniacal Nickel Solution.	Basic Zinc Chloride Solution.	Copper-Glycerin Reagent.
Silk, ...	5·84	5·92	5·52	18·80
Wool, ...	76·31	76·58	80·08	64·05
Cotton, ...	17·85	17·50	14·40	17·15
	100·00	100·00	100·00	100·00

In fabrics containing silk, wool, and cotton, the silk should first be dissolved by treatment with the nickel reagent. The portion left insoluble is first treated with very dilute hydrochloric acid (1 per cent.), and the wool then dissolved by boiling for seven minutes with a two per cent. solution of caustic soda. The

foregoing results were obtained by Richardson from a material which had been very imperfectly dyed, and the constituent fibres of which were easily separated and weighed. It will be observed that fairly accurate results were obtained by the nickel reagent, but not by the copper nor the zinc solution.[1]

The ammoniacal nickel solution has been employed in the author's laboratory with very satisfactory results. The following are the details of the manipulation, which differ in certain respects from those of Richardson :—The yarn or fabric is cut up very fine with a pair of scissors, and thoroughly dried at 100° C. One gramme of the material thus prepared is treated with 40 c.c. of the cold ammoniacal nickel solution for two minutes. The liquid is then filtered, and the residue, consisting of the wool and cotton of the sample, is digested for two or three minutes in boiling dilute hydrochloric acid containing one per cent. of real HCl. It is then washed free from acid, dried at 100° C., and weighed. To separate the wool from the cotton, the residue from the above process is boiled with about 50 c.c. of a one per cent. solution of caustic potash for ten minutes, and the solution filtered. The residue, which consists of *cotton*, is washed free from alkali, dried at 100° C., and weighed.

The foregoing description assumes that silk-gum, dyes, and weighting materials have been previously removed. To remove the silk-gum the material may be boiled in a solution of soap, but Richardson considers that its complete removal is best effected by treatment in the cold with a 2 per cent. solution of caustic potash. This plan has the advantage of decomposing prussian blue, and facilitating the subsequent solution of the iron by water containing one per cent. of real hydrochloric acid. After this last treatment the material should be thoroughly washed, and dried. Metallic mordants are apt to be imperfectly removed, and their amount must be deduced from the ash left on ignition and subtracted from that of the other constituents. It is advisable to boil some dyed fabrics successively with methylated spirit and with ether, to remove certain dyes and oily matters.

No general method for the removal of colouring and weighting materials from fabrics can be prescribed. Much information on the subject will be found in the sequel.

Dyeing and Weighting of Silk.

Silk has a great affinity for the coal-tar colours, which dye it without the use of any mordant, though it is customary to use a

[1] Richardson considers the solvent action of basic metallic solutions on silk to be due to dissociation of the amido-anhydride of which the fibre is composed, with formation of soluble metallic amido-compounds.

soap-bath (boiled-off liquor), with or without the addition of acetic or other weak acid. Indigo-sulphuric acid is used for indigo tints. For black, the silk is treated with ferric acetate, washed and immersed in a bath of potassium ferrocyanide, followed by another iron bath, washing, and immersion in a catechu or other tannin bath, followed by one of logwood and soap. Alizarin-black is much used for dyeing mohair goods (astrachans).

A systematic method devised by B. Martinon (*Jour. Soc. Dyers &c.*, iii. 124), for recognising the nature of dyes on silk, is reproduced in Vol. III. Part i., page 394 *et seq.*, where other processes are also given.

In addition to legitimate processes of dyeing, silk is subjected to operations having for their object the introduction of weighting materials. This practice of adulteration, which serves no good object, is carried out systematically and to an enormous extent, the adulterants frequently amounting to several times the weight of the true silk.[1]

J. Carter Bell (*Jour. Soc. Chem. Ind.*, 1897, p. 304) gives the following analyses of unweighted and weighted silk.[2] The

[1] The history, objects, and effects of silk-weighting have been fully discussed by Sir Thos. Wardle (*Jour. Soc. Chem. Ind.*, 1897, p. 297).

M. J. Langdon (*Jour. Soc. Chem. Ind.*, 1897, p. 405) has suggested that the adulteration of silk by weighting should be limited by arrangement between the dyers to the comparatively moderate amounts of 16 to 84 ounces for 1 lb. of silk, according to the nature of the material.

J. Carter Bell (*Jour. Soc. Chem. Ind.*, 1897, p. 303) has described a case in which 100 lbs. of silk were sent to the dyer with the request that he would weight it to 1000 lbs. This being done, the weighted silk contained 9 per cent. of moisture, less than 2 per cent. of nitrogen (corresponding to less than 10 per cent. of silk), and yielded 43 per cent. of ash.

In April 1897, the silk-weavers of Zurich entered into an agreement for one year with the Swiss silk-dyers, forbidding, on pain of heavy fines, the weavers to import or use, and the dyers to dye, any silk, whether of home or foreign origin, that had been weighted to such an extent as seriously to impair the strength of the fibre. Following this example, the silk-dyers of Crefeld (Germany) agreed that the maximum degree of weighting in the future should be 30 to 40 per cent. for taffetas, and 50 to 60 per cent. for other kinds of silk (*Jour. Soc. Chem. Ind.*, 1897, p. 532).

Highly-weighted silk does not inflame when heated, but smoulders slowly away, leaving the original form intact. Instances of the spontaneous combustion (smouldering) of black silk are on record (see E. Königs, *Dingler's Polyt. Jour.*, ccxxxvii. 73 ; abst. *Jour. Chem. Soc.*, 1880, p. 935).

[2] The practice of weighting silk probably had its origin in the desire to make up the loss of weight (amounting to about 25 per cent.) which occurs in the boiling-off process, and competition has increased the amount of weighting materials introduced till they have reached the present enormous proportions.

standard taken for silk was pure boiled-off silk, dried at the ordinary temperature, and assumed to contain 18 per cent. of nitrogen.

	Moisture.	Ash.	Silk.
1. White silk,	5·1	0·349	100
2. Salmon-coloured Shanghai,	5·5	0·246	100
3. Black,	7·9	18·7	25
4. White,	8·2	50·0	25
5. Shot silk,	6·2	34·7	39
6. —	10·2	33·7	32
7. Pink silk,	8·5	43·2	54
8. ,,	9·4	52·4	43
9. ,,	8·1	49·5	46
10. Blue,	6·5	35·4	60
11. Gold colour,	5·8	41·7	52
12. Cream colour,	9·1	45·7	46
13. Pink and green,	8·6	34·2	50
14. Pink,	8·6	28·2	60
15. Cream,	9·3	8·9	87
16. Yellow,	8·6	9·1	86
17. Black,	8·5	42·9	12
18. Sky-blue,	9·1	48·9	37
19. —	8·1	45·9	36

The observation of the specific gravity of silk has been proposed by Vignon (*Jour. Soc. Chem. Ind.*, xi. 1002) as a means of ascertaining the proportion of weighting materials present, but the practical utility of the method is lessened by the necessity of knowing the composition of such weighting materials. Vignon ascertains the specific gravity of the fibre, in its ordinary air-dry condition, in benzene, after removing occluded air by means of the air-pump. The specific gravities of some textile fibres were thus determined by L. Vignon and by H. Silbermann (*Chem. Zeit.*, xviii. 744; abst. *Jour. Soc. Chem. Ind.*, 1894, p. 907) with the following results: — Wool, 1·28 to 1·33; cotton, 1·50 to 1·55; mohair (combed), 1·30; hemp (carded), 1·48; ramie, 1·51 to 1·52; linen (spun), 1·50; jute (spun), 1·48; silk (raw), 1·30 to 1·37; boiled-out silk, 1·25. The specific gravity of silk varies in course of chemical treatment by dyeing, bleaching, and weighting, and in course of mechanical treatment by stretching, soupling, &c.

In order that useful conclusions as to the approximate amount of weighting present in any given silk may be deduced from its specific gravity, the nature of such weighting and the process of dyeing used must be known,—at least qualitatively.

The materials used for weighting silk are of a very varied character. The general practice is to immerse the silk in some metallic solution, and then transfer it to a bath of salt which will react to form an insoluble precipitate on or in the silk-fibre. Thus iron is precipitated as ferrocyanide, gallotannate, and

catechu-tannate; tin as catechu-tannate, tungstate, phosphate, silicate, hydroxide, &c. The use of tin in the form of ammonium stannichloride, $(NH_4)_2SnCl_6$, commonly called "pink salt," and its precipitation in the fibre as phosphate and silicate, has increased enormously of late years. Chromium compounds are also used, besides sulphates of sodium, magnesium, and barium, and various organic matters (*e.g.*, gelatin, glucose, logwood, and tannin matters).[1]

In some cases it is requisite to identify and separately determine the various weighting materials present in a sample of silk, but in other cases it is sufficient to know their joint weight. For the determination of such additions as oil, sugar, soap, &c., 5 grammes of the sample of silk should be dried at 100°, and then exhausted in succession with ether, alcohol, and boiling water; drying and noting the loss of weight after each treatment.[2]

[1] Salts of nearly all the commoner metals have been proposed, and protected by patent, for use as weighting materials. Detailed information on the methods of weighting silk will be found in an article by H. Silbermann (*Farb. Zeit.*, viii. 34, 51, 68; abst. *Jour. Soc. Chem. Ind.*, 1897, p. 326).

[2] H. Silbermann (*Chem. Zeit.*, xviii. 744; abst. *Jour. Soc. Chem. Ind.*, 1894, p. 907) recommends the following method for the recognition of the nature of the weighting and dyeing materials present in silk:—Readily-soluble materials, such as cane-sugar, glucose, glycerin, magnesium salts, &c., are estimated directly by boiling the silk with water, and testing the extract with Fehling's solution, &c. From 2 to 3 grammes of the silk are ignited, and the ash tested for tin (present in the fibre as basic chloride and stannic acid), chromium, iron, &c. Fatty matters, wax, and paraffin are detected by extraction with ether or benzene. The silk is soaked in warm, dilute hydrochloric acid (1 : 2). If the fibre be almost decolorised by this treatment, only a slight yellow tint remaining, whilst the solution assumes a deep brownish colour *not* changed to violet by lime-water, it is safe to conclude that the silk has been dyed by alternate passage through baths of iron and tannin. The yellow colour of the fibre is due to a residuum of tannin, and the precise shade (from greenish to brownish-yellow) enables some idea to be formed as to the nature of the tanning material used (sumach, divi-divi, catechu, &c.). Decolorisation of the fibre, the acid extract being pink, changed to violet by lime-water, indicates a logwood-black (so-called "English black"). If the fibre retain a deep greenish tint and the solution be yellow and unaffected by lime-water, the black is died on a prussian blue ground. If the latter, as is often the case, has been produced during the final stage of dyeing, this will be shown by its solubility in the acid. A green fibre and pink solution, altered to violet by lime-water, point to a logwood-black on a Berlin-blue ground. In the hydrochloric acid solution, metals, such as lead, tin, iron, chromium, and aluminium, may be determined. Blacks produced by artificial dyes on an iron-tannin or iron-blue catechu ground, are recognised by the coloration imparted to acid and caustic soda solutions. As regards blacks produced solely by the agency of aniline-dyes, &c., treatment with a hydrochloric acid

The percentage of weighting materials is usually expressed on the raw silk, and not on the conditioned silk. As the raw silk loses 25 per cent. of silk-gum in the process of conditioning, it follows that 75 parts of conditioned silk represent 100 of raw, and this fact must be allowed for in stating the proportion of weighting materials found.

It has been shown by Ponci that the increase of the volume of silk by dyeing is in practice quite as important as the increase of weight, and that the desired effect is produced better by the use of tannin than by the introduction of excessive proportions of metallic oxides (*Jour. Soc. Dyers &c.*, iii. 195).

In examining *white silk*, the sum of the *soluble weighting materials* should be determined by treating a known weight of the sample four or five times with hot water, washing it thoroughly, and redrying it. In consequence of the great and variable hygroscopic character of silk, to obtain reliable results it is desirable to make the test side-by-side with a similar one on a standard silk, as nearly as possible equal in quality to the sample to be examined. After treatment with water and redrying, the samples are placed together under a clock-glass, and when the standard silk has regained its original weight the washed sample is reweighed, the loss representing the matters soluble in water. An aliquot part of the solution may be evaporated to dryness and the residue weighed, to obtain a direct estimation of the matters dissolved. Unweighted silk loses on the average 25 per cent. of its weight on boiling off, so that only the loss in excess of this must be regarded as due to weighting, unless the additions be actually identified and determined by other means. *Glucose* may be directly determined in the solution by Fehling's solution, and *cane-sugar* after inversion. *Sulphates* and *chlorides*, *magnesium*, &c., may be detected and determined as usual. *Stannic oxide* will be left on igniting the

solution of stannous chloride does not affect aniline and alizarin blacks; naphthol-black is altered to a reddish-brown colour, whilst wool-black becomes yellowish-brown. Aniline and alizarin blacks may be distinguished by means of sulphurous acid, which attacks only the former, turning it greenish. Tannin substances in general may be extracted by alkaline solutions and subsequently precipitated and distinguished by ferric acetate. To remove the whole of the weighting material and dye, it is recommended to boil the silk with acid potassium oxalate, wash with dilute hydrochloric acid, and finally treat with soda solution. When iron and tin are both present in the fibre, it is well to extract the tin previously by means of an alkali-metal sulphide. In conclusion, Silbermann gives a number of experimental results, which exhibit the relation existing between the density of silk and the percentage and nature of the loading material. See also L. Vignon, *Bull. Soc. Chim.*, 1892, 249).

silk in porcelain after the above treatment. In its presence, the material burns with difficulty, and the residue retains the shape of the original silk. The weight of the ash (assuming it to be wholly SnO_2) may be calculated to the form in which the tin exists in the weighted silk, namely SnO_2,H_2O, by multiplying it by the factor 1·12.[1] It may be identified as stannic oxide as described on pages 295 and 523.

For the further examination of white silk, H. Silbermann (*Chem. Zeit.*, xx. 472 ; *Jour. Soc. Chem. Ind.*, 1896, p. 563) recommends the following procedure :—

A weighed portion of the silk is boiled with dilute hydrochloric acid to dissolve any tannin-lakes of tin or other metals, and in the solution tannin is tested for by the addition of an excess of sodium acetate and a ferric salt. If tannin-lakes be present the determination of the weighting materials consists in :— (1) Precipitation of tannin from the aqueous solution with gelatin ; (2) estimation of tannin in this precipitate, and of sugar, &c., in the filtrate ; (3) successive treatment of the silk with dilute hydrochloric acid and sodium carbonate, and precipitation of tannin from both solutions by means of "gum" solution (? gelatin) ; (4) ignition of the silk and determination of metallic weighting. If the ash be not completely soluble in hot, moderately concentrated hydrochloric acid, it may contain barium sulphate or silica.[2]

To calculate the percentage of weighting material, W, in the silk examined, Silbermann employs the following formula, in which a is the weight of the sample before treatment, b the weight after extraction with water, p the SnO_2 left on ignition, and d the loss of weight during the boiling of the fibre itself. This is taken at 20 to 25 for boiled-out silk ("cuit"), 5 to 9 for souple silk, and 0 to 2 for écru :—

$$W = \frac{a(100-d)}{b-1{\cdot}13p} - 100$$

Dark-coloured and black silk may contain hydroxides of tin, iron, and chromium, fatty matters, tannin, prussian blue, and various other colouring matters. Fatty matters may be removed and determined by treatment with ether. Treatment of the silk

[1] A simpler, and perhaps preferable, method for the detection of tin is to heat the silk with just sufficient hydrochloric acid to effect its complete solution, dilute the liquid with water, filter, and pass hydrogen sulphide, when any tin will be precipitated as yellow stannic sulphide.

[2] The ash may also contain stannic phosphate and tungstic oxide. The author doubts if treatment with hydrochloric acid can be relied on to remove stannic oxide (compare page 294).

with hydrochloric acid (1·07 sp. gr.) at 50 to 60° C. dissolves the logwood and leaves the silk of a maroon colour in the absence of prussian blue, or black-blue if it be present. In the latter case, on then treating the silk with dilute caustic soda, a solution containing ferrocyanide will be obtained, which will yield a precipitate of prussian blue when acidulated with hydrochloric acid and treated with ferric chloride. The metallic oxides will be contained in the ash left on igniting the silk, and are best examined by fusing the residue in platinum or silver with nitre and sodium carbonate and treating the product with water, when the tin and chromium will be dissolved as stannate and chromate respectively, and the iron will remain insoluble. From the acidulated filtrate the tin may be precipitated by sulphuretted hydrogen, and the chromium thrown down from the filtrate by ammonia, or determined by other known means. For the detection of *tannin*, a portion of the sample should be boiled with water, and a small quantity of ferric acetate solution added, when a blue-black liquid is produced in presence of tannin. Or the sample may be boiled with very dilute hydrochloric acid, and the liquid tested with gelatin solution or ferrous sulphate. Tannin may be determined by dissolving it from the silk by passing it through an alkaline soap bath, and finding the loss of weight on redrying.[1]

To determine the *total proportion of weighting materials*, a known quantity of the silk, previously dried at 110° C., should be boiled for an hour with a 2 per cent. solution of caustic soda and then with dilute hydrochloric acid (250 grammes of the commercial acid per litre). This treatment is repeated four times, washing the material between each bath. The silk, which will now have become very brittle and must be carefully handled, is dried at 110° C., and weighed.

If souple or écru silk be under examination it should be subjected to a final washing with soap before drying.

By the foregoing treatment all foreign substances are removed,

[1] The following is an outline of the method of analysing weighted silk recommended by E. Königs, Director of the Silk-Conditioning Establishment at Crefeld:—(1) Estimate moisture by drying. (2) Fatty matters by extraction with ether. (3) Boil out silk-glue with water. (4) Dissolve out prussian blue with alkali, re-precipitate with acid and ferric chloride, and ignite precipitate with addition of HNO_3; one part of $Fe_2O_3 = 1·5$ parts of prussian blue. (5) Estimate SnO_2 present in ash of silk and calculate as catechu-tannate of tin; one part of $SnO_2 = 3·33$ parts of catechu-tannate. (6) Estimate total Fe_2O_3, subtract that present as prussian blue, and the amount naturally in the silk (0·4 to 0·7 per cent.), and calculate the remainder to tannate; one part of $Fe_2O_3 = 7·2$ parts of ferric tannate (or 5·1 per cent., if present as a ferrous compound).

except mere traces of tannin and colouring matters, and in some cases small quantities of metallic compounds. The weight of the ash left on igniting the treated silk, if multiplied by 1·25, will represent pretty nearly the hydrated metallic oxides retained in the washed silk.

A certain loss of silk-substance occurs in the treatment, and hence the proportion of weighting materials found in the sample is somewhat in excess of the truth. But the chief source of error lies in the uncertainty of the allowance to be made for loss in the weight of the silk by boiling off. For boiled-off silk this (d) is taken by Silbermann at 25 per cent.; for souple silk at 8 per cent.; for écru at 0; and for fancy silks at 10 per cent. If p be the original weight, and D the weight after boiling, the percentage of weighting, W, may be found by the following equation:—

$$W = \frac{(100 - d) \times (p - D)}{D}$$

In cases where the treated silk leaves a sensible quantity of ash, a, on ignition the following equation must be substituted:—

$$W = \frac{(p - D + 1{\cdot}25a) \times (100 - d)}{D - 1{\cdot}25a}$$

The foregoing process is tedious and not very accurate. A preferable plan is to determine the total nitrogen by Kjeldahl's process (page 20), after removing any gelatin, prussian blue, or other nitrogenised matters. This is effected by boiling a weighed quantity of the silk (1 or, preferably, 2 grammes) with a 2 per cent. solution of sodium carbonate for half an hour.[1] The silk is then removed, washed, and heated to 60° C. for half an hour in water containing 1 per cent. of real hydrochloric acid, and well washed with hot water. The treatment with sodium carbonate and hydrochloric acid should be repeated until the silk no longer retains a blue colour. In the case of souple and écru silks, ammonia or ammonium carbonate should be substituted for sodium carbonate, and the discharged silk should be subjected to a final boiling for an hour and a half with a soap solution containing 25 grammes of soap per litre.

Air-dried silk with 11 per cent. of water contains 17·6 per cent. of nitrogen, and hence the true silk in a sample free from prussian blue, &c., can be found by multiplying the percentage of nitrogen by 5·68.

[1] Prussian blue may be removed by boiling the silk in a solution of acid oxalate of potassium.

The determination of the nitrogen must be conducted with great care to render the results of value.

Silk fabrics should be separated into warp and weft, and these analysed separately, since the weft is usually much more heavily-weighted than the warp.

It is a frequent practice to express the proportion of weighting materials on 100 parts of the silk treated, and not on 100 parts of the product. This plan is adopted in the following statement of results obtained by Gnehm and Blumer (*Jour. Soc. Dyers &c.*, 1898, p. 114) by a modification of the foregoing process.[1]

	Weighting above Pari.	
	Actual.	Found.
A. Japanese trame,[2]	96 per cent.	95·71 per cent.
B. Japanese trame,[2]	50 ,,	49·66 ,,
C. Yellow Italian organzine,[2] . . .	53 ,,	52·81 ,,

In the case of sample A the loss of weight in discharging (*d*) was taken at 18 per cent., and in that of C at 22 per cent. Notwithstanding the close concordance of the above results, the varying amounts of loss assumed to occur detract much from the accuracy of the process. Indeed, Gnehm and Blumer state that the results obtained differ from the truth in some cases by 5 to 10 per cent.

Collodion Silk. Artificial Silk. Lustro-cellulose.[3]

The production from nitro-cellulose of an artificial textile fibre resembling silk[4] has been the subject of various patents, of which

[1] Gnehm and Blumer treat the silk first with hydrochloric acid at 60° C., and subsequently with sodium carbonate at 80°, repeating both the treatments several (up to seven) times. (See also Gnehm and Schwartz, *Revue Gén. des Mat. Col.*, 1898, p. 2; abst. *Jour. Soc. Chem. Ind.*, xvii. 495.)

[2] *Trame* or *tram*, usually employed for wefts, is the product of the union of two or more single untwisted threads, which are then doubled and slightly twisted. *Organzine*, generally used for warp-silk, is produced by the union of two or more single threads separately twisted in the same direction, and then doubled and re-twisted in the opposite direction.

[3] The products described by these names are quite distinct from that resulting from the deposition on cotton-fibre of silk from its solution in alkali or in ammoniacal copper or nickel oxide (see *Jour. Soc. Dyers &c.*, ix. 180).

[4] These products do not appear to be capable of replacing real silk in the warp of woven goods, but for the weft, and especially in decorative and mixed fabrics, it appears to be capable of being used with lustre and effect equal to silk itself, and costs considerably less.

those of Chardonnet, Du Vivier, and Lehner are the chief. All these processes have a close general resemblance, though differing in certain details which have much importance in their chemical application. Some of the processes first patented have been superseded, so that, broadly speaking, artificial silk is now manufactured by:—(*a*) nitrating cellulose by treatment with a mixture of sulphuric and nitric acids, taking care not to carry the process of nitration too far; (*b*) solution of the resultant, thoroughly washed, nitrated cellulose in a mixture of alcohol and ether; (*c*) forcing or drawing the viscous collodion (after careful filtration) through minute orifices of glass; (*d*) solidification of the fine thread by immersion in water (Lehner) or evaporation of the solvent (Chardonnet); (*e*) denitration of the fibre by an ammonium sulphide or other reducing agent.

A specimen of lustro-cellulose examined by Cross and Bevan (*Jour. Soc. Chem. Ind.*, xv. 318) was found to contain:—carbon, 43·77 per cent.; hydrogen, 6·40; and nitrogen, 0·19 per cent. On boiling with dilute hydrochloric acid (sp. gravity, 1·06) the lustro-cellulose yielded only traces of furfuraldehyde, and on boiling with dilute soda and Fehling's solution no cuprous oxide separated. By prolonged boiling with a one per cent. solution of caustic soda it lost 9·14 per cent., which shows a much higher resistance to alkaline hydrolysis than might have been expected.

From the foregoing results, Cross and Bevan conclude that no oxycellulose results from the treatment of the cellulose, and that the permanent hydration-changes which take place are of minor importance.

According to Cross and Bevan, the proportion of moisture in lustre-cellulose is much higher than that present in unaltered cellulose, which usually contains from 10 to 12 per cent.; the tensile strength is about two-thirds of that of true silk; and the elasticity somewhat less.[1] Its dyeing capabilities are considerable.

According to H. Silbermann (*Jour. Soc. Dyers &c.*, ix. 163) a concentrated solution (40 per cent.) of caustic potash dissolves mulberry silk and Chardonnet's artificial silk in a few minutes, while tussah silk and Vivier's artificial silk are comparatively unaffected. Boiling with ammonia or soap for fifteen minutes has no appreciable effect on collodion-silk, and boiling dilute acids have no immediate action. Lustro-cellulose from wood-pulp gives no reaction with iodine, whereas that prepared

[1] These statements are in accordance with those of W. M. Gardner (*Jour. Soc. Dyers &c.*, xi. 166).

from cotton-waste is stated to take up a considerable proportion.

P. Truchot (abst. *Analyst*, 1897, p. 248) states that artificial silk, or lustro-cellulose, has a specific gravity of 1·490, whereas real silk ranges in gravity from 1·357 to 1·367. When the fibres of collodion-silk are moistened with water they become very weak, whereas the fibres of true silk, whether moist or dry, withstand great tensile strain.

A sample of collodion-silk examined by Truchot yielded 1·52 per cent. of ash on ignition, and contained 0·25 of nitrogen, corresponding to 1·63 of trinitro-cellulose.

On treatment with ammonio-cupric oxide (Schweitzer's reagent) both true silk and collodion-silk swell considerably before dissolving. On adding hydrochloric acid to the resultant solution of collodion-silk, or even on merely diluting it with water, a white precipitate of cellulose is thrown down.

Collodion-silk dissolves in sulphuric acid of 1·71 specific gravity with deep yellow colour, and if diphenylamine sulphate be then added, a deep blue coloration is produced. The reaction, which is due to the presence of unreduced nitro-cellulose, may be obtained, without previously dissolving the fibre, by simply immersing the material in a solution of diphenylamine sulphate, when the fibres of the artificial substance will be coloured dark blue, whereas those of natural silk remain colourless.

The behaviour of collodion-silk in dyeing is the subject of very contradictory statements. According to some observers, it behaves like animal fibres, and according to others like ordinary cellulose. The truth probably lies between these two extremes.

CHITINOIDS.

The body known as chitin occurs throughout the invertebrata in the form of an investment to the outermost cellular layer or ectoderm. In many cases, a chitinous composition has been ascribed to structures solely on account of their insolubility in caustic alkalies and in dilute acids, or even in only one of these reagents. At least two distinct compounds, chitin and conchiolin, have been confounded in this manner. They exhibit essential differences in characters and composition, and, together with spongin, may be conveniently classed together as "chitinoïds." The following table shows the ultimate composition attributed to these three substances:—

	Chitin.[1]	Conchiolin.	Spongin.
Carbon, ...	46·32	50·7	47·44
Hydrogen, ...	6·40	6·5	6·30
Nitrogen, ...	6·14	16·7	16·15
Oxygen, ...	41·14	26·1	30·11
	100·00	100·00	100·00

Chitin.

Chitin, as it occurs in nature, is frequently impregnated with calcareous matter, as in the shells of the crustacea, or with silica, as in the radula of the higher mollusca.

Chitin was prepared by Hoppe-Seyler by boiling the wing-cases of the cockchafer with dilute solution of caustic soda until they became colourless. The product was then boiled in succession with water and dilute hydrochloric acid, and finally exhausted with boiling alcohol and with ether.

Chitin may be obtained by a similar process from the shell of the crab or lobster, but in this case the substance should be previously digested with hydrochloric acid, to dissolve the earthy matters deposited in the chitinous tissue.

Chitin is a colourless, amorphous substance, which, when prepared in the foregoing manner, retains the form of the parts composed of it. It is insoluble in water, alcohol, ether, acetic acid, and in dilute mineral acids. Chitin resists in a remarkable manner the action of alkalies, and can be subjected to a prolonged treatment with their boiling concentrated solutions without undergoing decomposition.

Chitin is dissolved by strong mineral acids. If the chitin prepared as above described be treated with cold, concentrated hydrochloric acid, and the solution diluted with a large excess of water, the chitin is precipitated in a colourless, gelatinous form.[2]

[1] This analysis of chitin differs materially from that on page 529, which latter is to be preferred.

[2] C. F. W. Krukenberg (*Zeit. Biol.*, xxii. 480; abst. *Jour. Chem. Soc.*, 1886, p. 808) finds that the action of cold hydrochloric acid on chitin for one hour is not one of simple solution. A chlorinated compound is first formed, which swells up in the acid, and on filtering, a cloudy filtrate is obtained, from which about 2 per cent. of chitin is precipitated by water or baryta. The filtrate from this precipitate does not contain glycosamine or other decomposition-products. After longer action of hydrochloric acid, the chlorinated body suffers partial or complete dissociation, and the filtered liquid

The composition of chitin has been investigated by Ledderhose, under the direction of Hoppe-Seyler and Baumann (*Zeit. physiol. Chem.*, 1878, ii. 213), who, as the mean result of twelve analyses, found it to contain :—Carbon, 45·69 ; hydrogen, 6·42 ; nitrogen, 7·00 ; and oxygen, 40·89 per cent. These figures, which are materially different from those given on page 528, correspond to the formula $C_{15}H_{26}N_2O_{10}$.

Ledderhose finds that chitin undergoes hydrolysis when heated with acids, with formation of glucosamine and acetic acid, according to the following equation :—

$$2C_{15}H_{26}N_2O_{10} + 6H_2O = 4C_6H_{13}NO_5 + 3C_2H_4O_2.$$

According to Berthelot (*Compt. rend.*, xlvii. 227), when chitin is dissolved in concentrated sulphuric acid, a fermentable sugar is formed, but this observation has not been confirmed.

Conchiolin.

This substance is obtained by macerating the shells of mussels or snails in dilute hydrochloric acid, and then boiling with caustic soda.

Conchiolin closely resembles chitin, with which substance it was formerly supposed to be identical. It is distinguished from chitin by its much larger content of nitrogen, and by yielding leucine when boiled with dilute sulphuric acid, without any sugar-like substance being simultaneously formed.

Spongin.

Spongin, or spongiin, is the characteristic proteoïd body of sponges.[1] It has been compared to the collagenes and to the

contains a dextrinoid substance of feeble reducing power, together with very small quantities of chitin (precipitable by water) and glucosamine hydrochloride. By the action of 5 and 10 per cent. solutions of potassium or sodium carbonate saturated with chlorine, chitin was converted into a substance corresponding with amidulin. After twelve days a small quantity of chitin had dissolved, and after filtering and removing the salts by dialysis a substance was obtained which dissolved readily in cold water, reduced Fehling's solution on heating, and gave, both with neutral and with basic lead acetate, copious precipitates insoluble in excess of the reagent. These characters and its indiffusibility distinguish the substance from glucosamine hydrochloride.

[1] An interesting description of the natural history of sponges has been published by E. M. Holmes (*Pharm. Jour.*, [3], xvii. 991). Information on the methods of *bleaching* sponges will be found in the *Pharmaceutical Journal*, [3], xiv. 88, and in the *Chemist & Druggist*, xxx. 643. Potassium permanganate is commonly employed. According to a recipe in *Le*

fibroïn of silk, but on the whole appears to be best classed with the chitinoïds.

Spongin is obtained when sponge is boiled in succession with dilute hydrochloric acid, dilute caustic soda, water, alcohol, and ether. The product contains 16·15 per cent. of nitrogen (compare page 528).

Spongin is unaffected by the reagents employed for its preparation, and is also insoluble in ammonia. It is dissolved slowly by strong alkalies, and is reprecipitated on neutralising the solution. Dilute acids have no action on spongin in the cold, but it is dissolved by concentrated acids.

When boiled with water under pressure, spongin yields no gelatin. Boiling dilute sulphuric acid is stated by Städeler (*Annal. Chem. Pharm.*, cxi. 12) to decompose it with formation of leucine and glycocine, but not tyrosine.[2]

KERATIN SUBSTANCES. KERATOIDS.

When cuticular and allied tissues—including nails, horns, hoofs, feathers, whalebone, scales, &c.—are treated in succession with boiling ether, alcohol, water, and dilute acids, the insoluble

Moniteur de la Teinture (abst. *Jour. Soc. Dyers &c.*, iv. 63), sponges may be conveniently bleached by immersing them in saturated bromine-water for several hours. This treatment is repeated till the desired tint is obtained. The sponges are then passed through a bath of dilute sulphuric acid, and finally washed with cold water. It is stated that the treatment in no way injures the quality of the sponge.

[2] The constitution of spongin has been studied by Zalocostas (*Compt. rend.*, 1888, p. 252; abst. *Pharm. Jour.*, [3], xix. 165). Sponge, after being washed with dilute hydrochloric acid and benzene, was submitted to the action of baryta-water under pressure. The ammoniacal nitrogen formed in the decomposition was equal to about one-fourth of the total nitrogen, as in the case of albumin. Also, for each molecule of carbon dioxide and oxalic acid were found two atoms of ammoniacal nitrogen, as in all proteid matters. Zalocostas represents the reaction by the following equation:—

$$\underset{\text{Spongin.}}{2C_{40}H_{64}N_{12}O_{17}} + 24H_2O = 6NH_3 + 2CO_2 + \underset{\substack{\text{Oxalic}\\\text{acid.}}}{C_2H_2O_4} + \underset{\substack{\text{Acetic}\\\text{acid.}}}{C_2H_4O_2} + \underset{\text{Fixed residue.}}{2C_{37}H_{76}N_9O_{24}}.$$

This residue consisted of fixed nitrogenous principles. Zalocostas points out that the molecules of water fixed in the reaction are equal to the number of nitrogen atoms, and that the relation of carbon to hydrogen in the fixed residue is as 1 : 2·66, whereas the relation in the case of proteids and collagenous substances is as 1 : 2. The analysis of this mixed fixed residue showed the presence of leucine, butalanine, tyrosine, glycalanine, &c.

residue retains the shape of the original tissue and is known as keratin. The product varies somewhat in its characters and composition with its origin, so that a number of allied substances are comprehended under the general name of keratin. The following table illustrates the composition of the "keratin" obtained from different sources :—

Source of Keratin.	Carbon.	Hydrogen.	Nitrogen.	Sulphur.	Oxygen (by difference).	Authority.
Feathers,	52·46	6·94	17·74	?	22·86	...
Quills,	51·7	7·2	17·9	...	...	Scherer.
Wool,[1]	50·65	7·03	17·71	4·61	20·00	Scherer.
Hair (man's),[1]	50·65	6·36	17·14	5·00	20·85	Van Laer.
Fur (white rabbit's),	49·45	6·52	16·81	4·02	23·20	Kühne and Chittenden.
Nails,	51·00	6·94	17·51	2·80	21·75	Mulder.
	51·09	6·82	16·90	2·80	22·39	Scherer.
Horn (cow's),	51·03	6·80	16·24	3·42	22·51	Tilanus.
Hoof (horse's),	51·41	6·96	17·46	4·23	19·94	Mulder.
Epithelium,	51·53	7·03	16·64	2·18	22·32	Hoppe-Seyler.
Epidermis,	50·28	6·76	17·21	0·74	25·01	Hoppe-Seyler.
Whalebone,[2]	51·86	6·87	15·70	3·60	21·97	Van Kerckhoff.
Tortoise-shell,	54·89	6·56	16·77	2·22	19·56	Mulder.
Neuro-keratin(brain),	56·99	7·53	13·15	1·87	20·46	Kühne and Chittenden.

E. Bourquelet (*Pharm. Jour.*, [3], xix. 1035) gives the following limits of composition of keratoids, so far as has been at present recorded :—Carbon, 50·3 to 52·5 per cent. ; hydrogen, 6·4 to 7·0 ; nitrogen, 16·2 to 17·7 ; oxygen, 20·7 to 25·0 ; and sulphur, 0·7 to 5·0 per cent.

From the foregoing results it appears that while the keratin from different sources is tolerably constant in the proportions of carbon and hydrogen contained in it, the percentages of sulphur and of oxygen vary within wide limits. This fact is further illustrated by the following results recorded by P. Mohr (*Zeit. Physiol. Chem.*, xx. 403 ; abst. *Jour. Chem. Soc.*, 1895, i. 255) :—

[1] Other analyses of the keratin from wool are given on page 541.

[2] Substitutes for whalebone have been protected by various inventors including the following English patents :—J. Baier, 1895, No. 10193 ; W. Hunkemöller, 1896, No. 1214 ; and Martin & Levy, 1895, No. 1820 ; 1896, No. 4718.

Source of Keratin.	Sulphur, per cent.	Source of Keratin.	Sulphur, per cent.
Woman's hair; dark blonde, .	4·95	Pig's hair, ...	3·59
Girl's hair; dark brown, . .	5·34	Sheep's wool, ...	3·68
Boy's hair; red blonde, . .	4·98	Goose feathers, ...	2·59–3·16
Boy's hair; red, ...	5·32	Pig's hoof, ...	2·69
Rabbit's hair, ...	4·01	Calf's hoof, ...	3·57
Calf's hair, ...	4·35	Ox hoof; white, . . .	3·49
Horse's hair, ...	3·56	Ox hoof; black, . . .	3·45

All the keratoids yield more or less ash on ignition, the proportion in some cases being considerable. The nature of the ash varies with the source of the keratin. Thus hoofs and horns give an ash consisting chiefly of calcium phosphate, while the ash of wool is largely composed of potassium and sodium sulphates (compare page 541). The ash of hair consists chiefly of sulphates of alkali-metals, ferric oxide, and silica (compare page 534). The silica in the ash of feathers is stated by von Bibra to range from 27 to 40 per cent.

The greater part of the sulphur of keratoids is only loosely-combined, so that on boiling hair, wool, or feathers with lead acetate and excess of caustic soda, the liquid blackens from formation of lead sulphide. Hoppe-Seyler found that by heating horny substances with baryta-water in sealed glass-tubes, nearly the whole of the sulphur is obtained as barium hydrosulphide.

When heated in the dry state, keratoids swell up, char, and evolve a characteristic odour of burnt feathers.

Many of the keratoids are very hygroscopic, taking up a large proportion of water without affording any indication of its presence beyond the increase in the weight of the substance.

The keratoids are quite insoluble in alcohol or ether, and are not much affected by boiling with water at the ordinary pressure; but, when heated under pressure for a long time to 150° to 200° C., they dissolve with evolution of hydrogen sulphide to a turbid solution which does not gelatinise on cooling, and gives on evaporation a residue insoluble in water.

When treated with alkalies keratin substances swell up, and are entirely dissolved by boiling alkaline solutions.[1] Ammonia

[1] H. E. Smith (abst. *Jour. Chem. Soc.*, 1884, p. 1398) states that a half to one per cent. solutions of caustic potash or soda have no action on keratin;

acts similarly but less strongly. On adding excess of acid to the solution of keratin in an alkali, a white flocculent precipitate is formed and hydrogen sulphide is evolved.

When treated with cold glacial acetic acid, horny substances swell up, and on boiling are largely dissolved. Whalebone is converted into a gelatinous substance by boiling with concentrated acetic acid, but tortoise-shell is little changed by such treatment.

Nitric acid turns keratoid bodies yellow, and on application of heat dissolves them with formation of oxalic acid and other products.

On treatment with cold concentrated sulphuric acid, the keratoids swell up, and on heating dissolve more or less completely. The solution appears to contain syntonin, for on dilution with water and addition of potassium ferrocyanide it yields a flocculent precipitate, and also gives a white flocculent precipitate when exactly neutralised.

When chlorine is passed into water containing a finely-divided keratoid, or when a keratoid is treated with bromine-water, the substance undergoes no change in external appearance, but after drying it is harsh to the touch, and then dissolves in ammonia with evolution of nitrogen.

The keratoids give the Millon and Adamkiewitz reactions for proteids (pages 11, 12).

When treated with fuming hydrochloric acid, most keratoid bodies swell up to a jelly and subsequently dissolve; with the exception of hair, which is unaffected by such treatment.

According to H. E. Smith (abst. *Jour. Chem. Soc.*, 1884, p. 1398) keratin is unacted on either by pepsin or trypsin. The first of these statements is correct, but the second is probably erroneous, since keratoids undergo digestion in the small intestine.[1]

When keratoid substances are boiled with dilute sulphuric acid, they undergo decomposition with formation of aspartic acid, volatile fatty acids (including propionic acid), ammonia, leucine, and from 3·5 to 4·0 per cent. of tyrosine. The formation of the last substance distinguishes keratoids from the collagenes. Keratoids also differ from the collagenes in not yielding gelatin by the action of superheated water or dilute acids, and in containing a notable proportion of sulphur in a loose form of combination (see page 544).

"Keratin" is official in the German Pharmacopœia (3rd edition).[1]

that solutions of 20 per cent. dissolve it, whilst 40 per cent. solutions have a weaker action.

[1] Keratin is employed for coating pills intended to act on the small intestine. Its resistance to the gastric juice and solubility in the pancreatic juice

It is directed to be prepared by digesting shavings of quills with a mixture of equal parts of ether and spirit, and subsequently with an acidulated solution of pepsin at 40° C. The residue is dissolved in acetic acid by prolonged boiling (thirty hours), the solution strained, evaporated to a syrup, and dried on plates. The product forms a brownish-yellow, tasteless, and odourless powder or scales. It should not yield anything to water, spirit, ether, dilute acids, or pepsin-hydrochloric acid; and should not yield more than 1 per cent. of ash, nor contain more than 3 per cent. of matter insoluble in acetic acid or ammonia.

Hair.

Hair is one of the most stable of the keratoïd substances, resisting the action of reagents very strongly. It is remarkable for the very high proportion of sulphur contained in it (see page 532), and for the presence of a large proportion of silica in the ash left on ignition. The *ash* of hair ranges from 0·5 to 2·0 per cent., and consists chiefly of sulphates of alkali-metals, ferric oxide, and silica, the last constituent sometimes forming 40 per cent. of the total ash.[1] Vanquelin states that the iron and manganese present in dark hair are replaced in fair hair by magnesia, but this result requires confirmation.

Hair consists of a cylindrical keratinous tube covered with minute scales, the points of which are directed towards the free extremity. Hence three morphological elements are distinguishable :—the cuticle, the cortex, and the medullary substance.

Hair is closely allied in structure and chemical composition to fur and wool, and no clearly-defined distinction can be drawn between them. The fine hair on some animals closely resembles wool, while the coarse wool on others simulates hair. Wool is peculiar in the wavy or curling nature of the fibre, and is distinguished by the comparatively loose attachment of the outer scales. In hair, these scales usually lie so close to the stem that the serrations are scarcely perceptible, whereas in wool they are strongly in evidence (see fig. 34, page 538). The structure of wool and hair has been fully described by F. H. Bowman (*Jour. Soc. Dyers &c.*, 1885, p. 109 *et seq.*).[2]

render it very suitable for this purpose (see Bourquelet, *Pharm. Jour.*, [3], xix. 1035).

[1] Gorup-Besanez (*Ann. Chem. und Pharm.*, lxvi. 321) found the hair of the lower animals to contain from 0·12 to 0·57 per cent. of silica, whereas human hair contained only from 0·11 to 0·22 per cent.

[2] "The difference between wool and hair is rather one of degree than kind, and all wool-bearing animals have the tendency, when their cultivation is

The following illustrations (taken from A. Swaine Taylor's *Medical Jurisprudence*) show the microscopic appearance of hair and fur from different sources. The magnifying power is 300 diameters in each case, except in the case of No. 4, which is shown only 70 times the natural size.

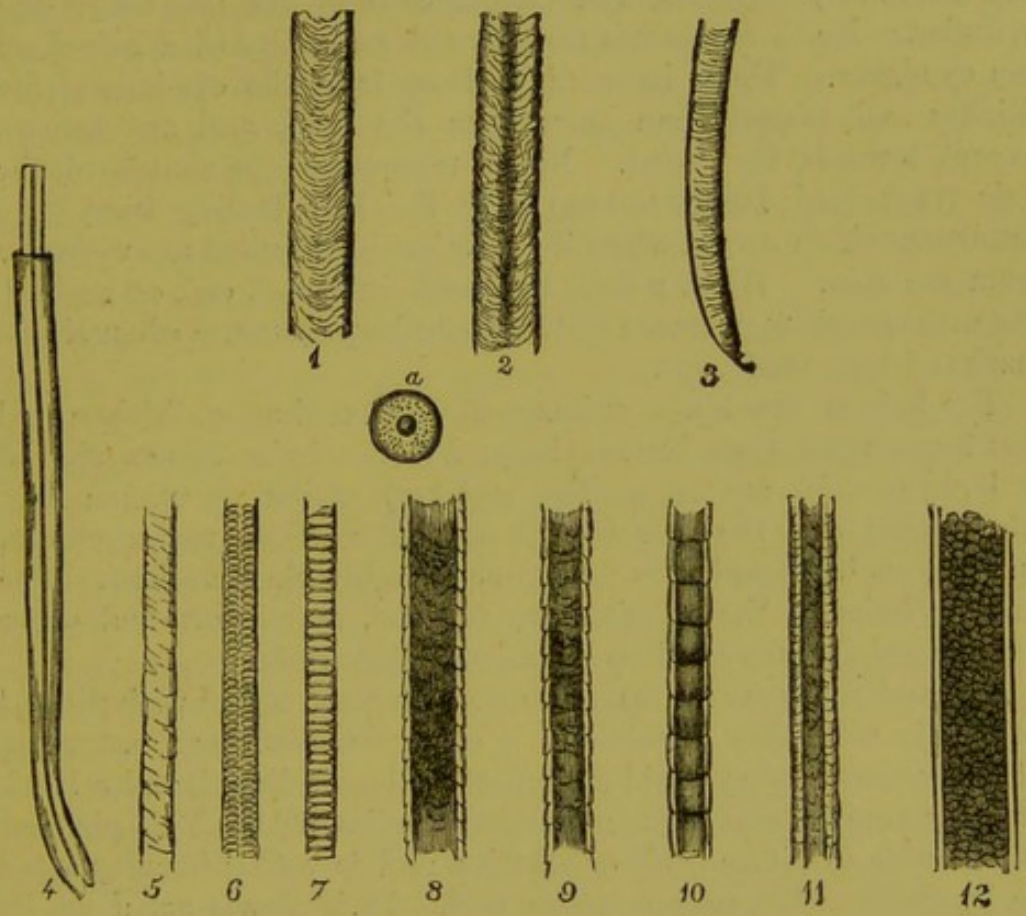


Fig. 33.—MICROSCOPIC CHARACTERS OF HAIRS.

1, Hair of child; 2, hair of adult (*a*, transverse section); 3, conical hair from eye-brow; 4, human hair (×70) with tubular sheath, as torn out by force; 5, hair of spaniel; 6, fur of rabbit; 7, fur of hare; 8, horse-hair; 9, goat's hair; 10, hair of fox; 11, cow's hair; 12, hair of fallow deer.

The cells and the linear markings on the cortical portion afford the chief distinctions between hairs from different sources.

The hair of the human head appears under the microscope as

neglected, to produce hair rather than wool. Wool and hair, fur being intermediate, are simply modifications of the same root-substance, and the scales of the wool-fibre have a much larger free margin than is the case with hair, being only attached to the stem by about one-third of their length, and in many cases the ends are more or less turned outwards, so as to present a much more serrated edge than is the case with hair. The interior of the fibre portion, however, does not differ in the least from that of hair, and can neither be distinguished from it chemically or microscopically."—F. H. Bowman.

transparent cylinders of various colours, and exhibits markings resembling (but less distinct than) those of wool. As a rule, the hair of women is finer and longer than that of men, and the hair of children finer and more silky than that of an adult. No. 2*a*, representing a transverse section of human hair, shows the cortical and medullary portions, and the air-cells in the interior of the cylinder. No. 3 represents the pointed extremity of a hair from the eye-brow. These hairs (and those from the eye-lashes) are thicker and coarser than hairs from the head, and are opaque, except towards the point. No. 4 represents the sheath of the hair (magnified 70 diameters) with the hair issuing from it, a condition which occurs when the hair has been pulled out violently from the skin. When a hair has been indented, cut, or bruised, the microscopic appearance of the medullary structure often shows marks of such treatment.

The hair of the lower animals is coarser, shorter, thicker, and less transparent than human hair; but the hairs of the spaniel and skye-terrier are long, silky, and very similar to human hair. The diameter of the hair from a particular animal varies greatly, and cannot be regarded as more than roughly characteristic. The furs or hairs of the rabbit, hare, squirrel, mouse, rat, and other rodents are characterised by dark, transverse cells.

When colourless (white) hairs or feathers are boiled with diluted sulphuric acid, they dissolve to a colourless solution; but when black or brown, they yield a similarly coloured liquid, and a black or brown amorphous pigment remains undissolved. This pigment is insoluble in dilute acids or alkalies, and is with difficulty acted on by strong acids, except nitric acid. It is slowly acted on by bromine, forming a substance soluble in water, and is said to yield only traces of ammonia when ignited with soda-lime. The black pigment is present to the extent of about 1 per cent. in the feathers of the rook. A mean of ten analyses of the black pigment from the feathers of several species of *Corvus* showed:—Carbon, 55·4; hydrogen, 4·25; and nitrogen, 8·5 per cent. (Hodgkinson and Sorby, *Jour. Chem. Soc.*, 1877, page 427).

The pigment of black hair and feathers is changed to yellow by treatment with hydrogen peroxide, an oxidising agent which is extensively employed for bleaching hair and feathers.[1]

Hair-Dyes generally contain some compound of a heavy metal which forms a black sulphide. This, by reaction with the loosely-

[1] To produce "golden hair," the grease is first thoroughly removed by washing the hair in soft soap and water, with the addition of a little ammonia. Hydrogen peroxide (of 20 volumes strength = 3 per cent. of available oxygen) is then applied to the hair twice a day.

combined sulphur present in the hair, occasions the desired darkening in colour. Solutions of lead, bismuth, copper, and silver are those most generally used for the purpose.

Many of these so-called hair-dyes are objectionable, and some are actually dangerous. Erysipelas and inflammatory swellings are liable to result, and well-authenticated cases of lead-poisoning and paralysis have occurred from the use of preparations containing lead. On the label of one of the worst of these liquids the statement is made that the preparation is free from lead and other injurious ingredients.

A widely-used article is composed of lead acetate, suspended sulphur, rose-water, and glycerin. In another, said to be capable of dyeing any shade from black to light brown, the free sulphur is replaced by sodium thiosulphate ("hyposulphite"). A chestnut-brown dye contains cupric nitrate and pyrogallic acid, while a black dye is composed of silver and copper nitrates, with ammonia. A preparation which dyes hair almost instantaneously consists of a solution of ammonio-nitrate of silver. This is applied to the hair first, and is followed by a solution of pyrogallic acid.[1] A hair-dye consisting essentially of a solution of potassio-citrate of bismuth has been recommended by Hager, and one containing ammonio-citrate of bismuth, with the addition of sodium thiosulphate, by Naquet (*Year-Book Pharm.*, 1873, p. 360; 1883, p. 326).

Of course the statement that such preparations as the above are "restorers" and not dyes is untrue. Were it correct, they would restore hair which was formerly red to its original colour.

[1] An article in the *Lancet* for January 13, 1877, states that of eighteen samples (three American and the rest English) of so-called hair-restorers, including all the best-known, fourteen were found to contain suspended sulphur and lead in varying but always in very considerable quantity. Many were described as "perfectly harmless," "free from injurious substances," &c., and only one was plainly stated on the label to be poisonous if taken internally.

Two other samples contained sodium thiosulphate instead of free sulphur. The handbill which accompanied one of these warned purchasers against dangerous hair-restorers containing lead, as likely to lead to paralysis and insanity, and recommended that all such preparations should be tested for lead by potassium iodide. This reagent gives no precipitate of lead iodide in presence of excess of sodium thiosulphate, so that the lead which existed in considerable proportion in the preparation in question would escape detection by the test recommended!

Another sample contained a considerable quantity of lead in solution, but no sulphur or thiosulphate. The remaining preparation was that referred to in the text as containing ammonio-nitrate of silver and pyrogallic acid in separate bottles.

Amido-phenols have recently been employed as hair-dyes. These bodies, especially the salts of meta-phenylene-diamine and diamido-phenol, resemble pyrogallol in undergoing rapid oxidation when their solutions are exposed to the atmosphere, with the formation of coloured bodies which dye the hair. According to a patent by Lumière, the dye is prepared by dissolving the hydrochloride or other salt of diamido-phenol in dilute alcohol, and adding sodium sulphite to prevent too rapid oxidation. By varying the strength, various shades of dye are obtained.

Wool.

Wool is closely allied in composition and general characters to fur and hair (page 534), but is usually more elastic, flexible, and curly; and the flattened scales, which are observed under the microscope to cover its surface (figs. 29, 34), give it the property of matting with greater readiness than is the case with either fur or hair. This difference is less apparent in raw wool than in that which has been scoured and treated with a dilute acid.

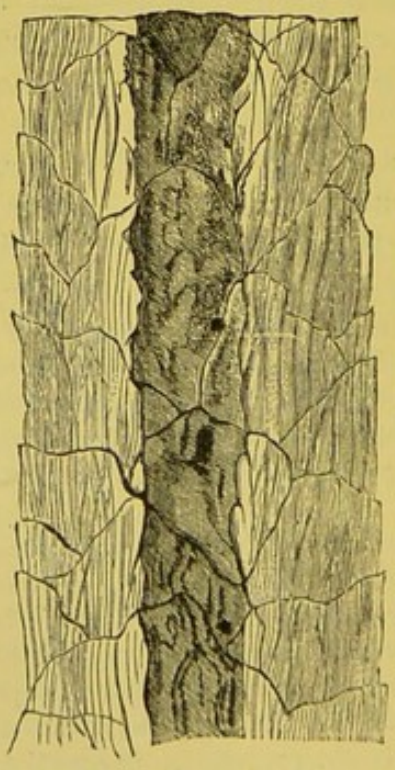


Fig. 34.—SHEEP'S WOOL (highly magnified), showing the cortical scales and the medullary tube.

The microscopic characters of wool have been described and

illustrated at length by F. H. Bowman (*Jour. Soc. Dyers &c.*, i. 86) and Watson Smith (*ibid.*, v. 12).[1]

Raw wool is far from being an approximately pure keratoid substance. Besides moisture and accidental dirt it contains a considerable proportion of *suint* or wool-soap (page 541), sometimes called "yolk."

J. J. Hummel (*The Dyeing of Textile Fabrics*) gives the following method for the analysis of raw wool :—

Moisture is determined by drying the wool at 100° C. in a current of hydrogen or other inert gas.

Wool-fat is determined by extracting the sample with ether. The solution also contains more or less of the oleates of the wool, which are separated from the wool-fat by agitating the ethereal solution with water.

The residual wool is washed repeatedly with cold distilled water, by which more *oleates* are extracted. The solution is added to the water separated from the ether. The wool is next washed with alcohol, and the oleates dissolved added to those recovered from the aqueous solution. The wool is then treated with dilute hydrochloric acid, which removes earthy oleates not soluble in water.[2] The wool is washed with cold water to remove acid, dried, and treated in succession with ether and alcohol. On evaporating these solvents to dryness, a residue of oleic acid is obtained, from which the amount of pre-existing *earthy oleates* can be calculated.

The wool, freed in the foregoing manner from fatty and soapy matters, is dried and teased out over paper to remove dirt, sand, &c. The purified *wool-fibre* is then carefully washed on a fine sieve, dried at 100° and weighed, the amount of *sand* and *dirt* being taken by difference.

The following analyses, by Märcker and Schulze, were made

[1] Sheep's wool varies enormously in the diameter of the fibres and in other characters. The chief varieties of wool from animals other than the sheep are :—*Cashmere wool*, from the fine downy hair of the Cashmere goats. *Vicuna wool*, now little used, from the very slightly curly hair of the llama or vicuna goat. *Alpaca wool*, similar to but not quite so fine as vicuna wool, from the alpaco or pako goat of Peru. *Mohair*, or so-called "camel's wool," from the long, slightly curly, silky hair of the angora goat.

A mixture of ordinary sheep's wool with the hair of hares or rabbits is sometimes substituted for vicuna wool. The term "viguna" or vicuna wool is now applied in the wool trade to a mixed fabric of silk and cotton.

[2] In the case of very dirty wool a considerable quantity of lime is dissolved which has its origin in calcareous dust and not in lime-soap.

by the foregoing method, and illustrate the results yielded by typical raw wool.

	Wool of Lowland Sheep.	Wool of full-bred Rambouillet Sheep.	"Pitchy" Wool.
Moisture, ...	23·48	12·28	13·28
Wool-fat extracted by ether, . . .	7·17	14·66	34·19
Wool-soap extracted by subsequent treatment:—			
By water (wool-sweat), . . .	21·13	21·83	9·76
By subsequent treatment with alcohol, ...	0·35	0·55	0·89
By subsequent treatment with dilute hydrochloric acid, ...	1·45	5·64	1·39
By subsequent treatment with ether and alcohol, ...	0·29	0·57	...
Pure wool-fibre, ...	43·20	20·83	32·11
Dirt (by difference), ...	2·93	23·64	8·38
	100·00	100·00	100·00

The following analyses by Faist further illustrate the general composition of sheep's wool (air-dried).

Description.	Mineral Matter.	Suint and Fatty Matter.	Pure Wool.	Moisture.	Pure Air-Dry Wool.
Raw Wools:—					
Hohenheim, with little suint, . . .	6·3	44·3	38·0	11·4	49·4
Hohenheim, with much glutinous suint, .	16·8	44·7	28·5	7·0	35·5
Washed Wools:—					
Hohenheim, . . .	0·94	21·00	72·00	6·06	78·06
Hohenheim, with difficultly soluble suint, .	1·3	40·0	56·0	2·7	58·7
Hungarian, very soft, .	1·0	27·0	64·8	7·2	72·0
Würtemburg, less soft, .	1·2	16·6	77·7	3·5	82·2

Wool-Fat is remarkable for containing a large proportion of *cholesterin* and *isocholesterin* (Vol. ii. page 311). It takes up a considerable proportion of water. Purified wool-fat forms the lanolin of pharmacy.

Wool-Sweat, Wool-Soap, or Suint is the portion of the wool-

soap soluble in water. It has a very complex composition, and has been the subject of numerous researches. Suint consists chiefly of the potassium salts of the higher fatty acids, including cerotic acid; with smaller quantities of valerate, butyrate, propionate and acetate; and phosphates, sulphates, and chlorides.[1] Ammonium compounds are present in small proportion.

The *mineral matter* of purified sheep's wool ranges from one to two per cent., and consists largely of sulphates of the alkali-metals. It is remarkable for containing a notable proportion of silica.[2]

Wool-Gelatin, according to Gardner and Carter (*Jour. Soc. Dyers &c.*, xiv. 167), is present in small proportion (1·65 per cent.).

Wool-Keratin, according to F. H. Bowman (*Jour. Soc. Dyers &c.*, 1885, p. 136), has the following composition:—

	Carbon.	Hydrogen.	Nitrogen.	Oxygen.	Sulphur.	Loss.
Lincoln Wool, . .	52·0	6·9	18·1	20·3	2·5	0·2 [3]
Irish Wool, . . .	49·8	7·2	19·1	19·9	3·0	1·0 [3]
Northumberland Wool,	50·8 [3]	7·2	18·5	21·2	2·3	...
Southdown Wool, .	51·3 [3]	6·9	17·8	20·2	3·8	...
Mean, . .	50·27	7·05	18·37	30·40	2·90	...

[1] A. and F. Buisine (*Comp. rend.*, cvii. 789) have isolated from suint glycollic acid, $CH_2(OH).COOH$, and normal pyrotartaric (propylene-dicarbonic) acid, $COOH.(CH_2)_3.COOH$, homologous with succinic acid. Oxalic, succinic, lactic, malic, benzoic, hippuric, and uric acids, with glycocine and leucine, are also present.

[2] A sample of Lincoln wool which had previously been scoured by soap, thoroughly washed with pure water, and dried, was found by W. H. Wood (*Jour. Soc. Dyers &c.*, 1885, p. 139) to yield one per cent. of *ash* of the following composition:—

	Percentage Composition.		
	Total Ash.	Ash soluble in Water.	Ash insoluble in Water.
K_2O, ...	31·1	42·3	trace
Na_2O, ...	8·2	17·3	trace
CaO, ...	16·9	4·5	51·2
Al_2O_3 and Fe_2O_3, ...	12·3	3·6	37·7
SiO_2, ...	5·8	4·1	11·1
SO_3, ...	20·5	24·8	trace
CO_2, ...	4·2	3·4	...
P_2O_5, ...	trace	trace	trace
Cl, ...	trace	trace	...

F. H. Bowman found 2 per cent. of magnesia in the wool-ash from sheep which had pastured on a magnesian-limestone district.

[3] The "loss" in the first two analyses represents the mineral matter. In the last two analyses this is included in the carbon.

The mean of Bowman's analyses of wool-keratin correspond to the empirical formula :—$C_{43}H_{71}N_{13}SO_{13}$.[1]

When wool is heated to about 140° C. it begins to decompose, and on heating more strongly it yields an oily substance of intolerable odour, together with large quantities of pyrrol, much sulphuretted hydrogen, a small quantity of carbon disulphide, and mere traces of oily bases (*Ann. Chem. Pharm.*, cix. 127).

Wool is affected to an appreciable extent by prolonged boiling with water, sensible traces of sulphuretted hydrogen being evolved. Bowman states that wool which looked quite bright when well washed with tepid water, became quite dull and lustreless under treatment with boiling water, and the appearance under the microscope was materially altered. In presence of only a very small quantity of alkali, the prolonged action of boiling water gelatinises wool more or less completely.

Wool is not much affected by solutions of soap, borax, ammonium carbonate, or carbonate of alkali-metal, but is acted on by caustic alkalies even when very dilute. Knecht found that when wool was boiled for three hours with water containing caustic soda in amount equal to 0·3 per cent. of the weight of the wool, the fibre was not disintegrated, but when the alkali was increased to 0·6 per cent. complete disintegration and almost complete solution of the wool took place.

Schützenberger obtained the following products by decomposing purified wool with an aqueous solution of baryta, under pressure, at 170° C. :—

		Per cent.
Nitrogen evolved as ammonia,		5·25
Carbon dioxide (separated as $BaCO_3$),		4·27
Oxalic acid (separated as BaC_2O_4),		5·72
Acetic acid (by distillation and titration),		3·20
Pyrrol and other volatile products,		1 to 1·5
Percentage composition of fixed residue ; containing leucine, tyrosine, and other volatile products,	C,	47·85
	H,	7·69
	N,	12·63
	O,	31·83

[1] Mills and Takamine (*Jour. Chem. Soc.*, 1883, xliii. 142) regard wool-keratin as a substance of definite composition, having the empirical formula $C_{42}H_{157}N_5SO_{15}$. This is based on the mean of the analyses of wool-keratin recorded in Gmelin's *Handbook of Chemistry* (xviii. 351) ; but R. L. Whiteley (*Proc. Chem. Soc.*, 1886, p. 142) has pointed out that by a misprint the respective percentages of nitrogen and hydrogen were transposed. The corrected average composition of keratin, according to the average of the analyses in question, is C, 49·96 ; H, 7·11 ; N, 16·65 ; S, 3·39 ; and O (by difference), 22·89 per cent. These figures correspond to the formula :—$C_{41}H_{71}N_{12}SO_{14}$.

By distilling wool (flannel) with strong caustic potash, Williams obtained a distillate containing a large quantity of ammonia, besides butylamine and amylamine.

A fairly accurate separation of wool from cotton and linen can be effected by boiling the mixed fibres for some time with a solution of caustic soda of 1·05 specific gravity. This dissolves the wool, leaving the vegetable fibres unaffected.

On the contrary, the vegetable fibres are affected by acids far more readily than wool, which fact is extensively employed for the recovery of the wool from waste fabrics of mixed nature.[1]

Lanuginic Acid.—By dissolving carefully-washed wool in concentrated baryta-water, passing carbon dioxide, and treating the filtered liquid with lead acetate, a precipitate is obtained which, when washed, suspended in water, and decomposed by hydrogen sulphide, gives a solution which, after filtration and evaporation to dryness, yields a dirty yellow, non-deliquescent, acid substance. This body E. Knecht (*Jour. Soc. Dyers &c.*, 1889, p. 72) calls lanuginic acid. It dissolves slowly in cold, but readily in hot water, is sparingly soluble in alcohol, and insoluble in ether. The aqueous solution of lanuginic acid is not coagulated by boiling, but it precipitates both acid and basic colouring matters, forming lakes, and it also yields precipitates with tannin and with potassium bichromate. The compounds of lanuginic acid with colouring matters appear to be perfectly definite in composition, and precipitation by night-blue or picric acid may be conveniently employed for the determination of the acid. With phospho-tungstic acid and with Millon's reagent lanuginic acid behaves like the proteids.

When heated to 100°, lanuginic acid becomes soft and plastic, and this property is shared by its lakes, most of which actually melt at the temperature of boiling water. Lanuginic acid becomes anhydrous at 110°, and on ignition leaves a considerable quantity of ash, consisting chiefly of barium carbonate. By substituting soda for baryta in the preparation of lanuginic acid, Knecht obtained a product identical in external properties and all other characters with that previously prepared, but leaving a much smaller quantity of ash on ignition. Knecht finds lanuginic acid to have the following ultimate composition:—C, 41·61; H, 7·31; N, 16·26; S, 3·35; and O, 31·44 per cent.

By heating wool with five times its weight of water to a tem-

[1] The goods are exposed in a closed chamber to the action of hydrochloric acid gas for a number of hours. When taken out and passed through suitable machinery the cotton falls to a fine dust, while the wool-fibre or shoddy remains practically unchanged. A similar method (in which sulphuric acid is commonly preferred) is employed for freeing raw wool from the "burrs" frequently contained in it.

perature of 200°–230° C. for four hours, the substance almost wholly dissolved, with liberation of sulphuretted hydrogen, &c., and the solution contained lanuginic acid. Very similar products were obtained by Knecht by heating horn and human epidermis with water to 200° C. under pressure.

When wool is boiled with a solution of caustic soda to which a little lead acetate has been added, it dissolves with brown or black coloration, due to the formation of lead sulphide. It appears, however, that the whole of the sulphur of wool does not enter into this reaction, a portion being in a more stable state of combination. Thus Chevreul found that by treating wool with alkalies the greater part of the sulphur is removed, but he was unable to extract the whole of it in this manner. By steeping wool twenty-eight times in lime-water for twenty-four hours each time, and washing with hydrochloric acid between each treatment, he succeeded in reducing the proportion of sulphur to 0·46 per cent. The wool treated in this manner was no longer blackened by boiling with lead acetate and excess of caustic alkali. Knecht finds that lanuginic acid also gives a negative reaction with the alkaline lead reagent, from which fact he considers it probable that the residual sulphur of the wool exists as lanuginic acid, or as some substance which readily yields lanuginic acid as a first product of its decomposition by reagents.

The blackening of wool by boiling with an alkaline solution of lead affords a ready means of distinguishing it from silk, cotton, and linen, neither of which fibres contains sulphur. The recognition of wool in mixed fibres may also be readily effected by the microscope [1] (see further, page 511).

[1] *Mixed Fabrics* of wool and cotton are frequently met with in commerce, and are not unfrequently described as "all wool." The microscopic examination, by E. G. Clayton (*Analyst*, xx.174), of ten samples of "flannelette" obtained in various neighbourhoods, and supplied at different prices, showed that six consisted entirely of cotton, and that the other four did not contain more that five per cent. of wool. Of nineteen samples of "sanitary flannel" similarly examined, sixteen were found to be all wool, one nearly all wool, one chiefly cotton, and one wholly cotton. Eighteen samples of "flannel" were found to consist entirely of wool.

S. Kapff (abst. *Analyst*, September 1898) has described the following method of analysing semi-woollen textile fabrics:—The material is extracted with ether, to remove *fat*, and then immersed in boiling water containing three per cent. of hydrochloric acid. The source of heat is then removed, and the liquid well-stirred while cooling. After thirty minutes, the fabric is removed and boiled in water for fifteen minutes, washed free from acid, dried for two hours at 100° C., and then exposed to the air to allow the fibres to recover their original moisture. The loss of weight gives the amount of *colouring matter, weighting material*, &c. The material thus purified is

E. Knecht has shown (*Jour. Soc. Dyers &c.*, iv. 72, 104) that when wool or silk is dyed with coal-tar colouring matters of basic nature, a complete decomposition of the dye takes place, the base uniting with the fibre to form an insoluble coloured lake, while the acid remains in the dye-bath. Knecht has further shown that when wool is dissolved in moderately dilute sulphuric acid, a solution is obtained which possesses the property of precipitating any of the acid colouring matters from their solutions, and that when wool is boiled with very dilute sulphuric acid, and then extracted repeatedly with distilled water until all free acid has been removed, the wool can subsequently be dyed a full shade in neutral solutions of the acid colouring matters. From these observations it appears evident that by the action of the sulphuric acid there is produced in the fibre a substance having the property, not previously possessed by the wool, of forming lakes with acid colouring matters.

The behaviour of wool with coal-tar colouring matters suggests strongly that wool-keratin has the constitution of an amido-acid, in which case it should be possible to obtain the corresponding diazo-compound. This reaction has apparently been effected by P. Richard (*Jour. Soc. Dyers &c.*, 1888, p. 154), though the characters of the product did not agree in every respect with those of known diazo-compounds, and E. Knecht considers the theory untenable (*Jour. Soc. Dyers &c.*, 1889, p. 75). A very similar body is yielded by silk (page 505).

If moistened wool be treated with chlorine-gas in excess, the chlorine is absorbed with great evolution of heat, hydrochloric acid is formed in large amount, and the wool is converted into a white pulpy substance. By using a limited quantity of chlorine diluted with air, the wool remains intact but becomes more transparent, acquires a glossy, silk-like appearance, a crackling feel, and an increased capacity for absorbing dyes. When chlorinated in the foregoing manner, wool does not readily acquire a yellow colour by subsequent treatment with soap or alkalies (*Jour. Soc. Dyers &c.*, 1898, page 175).

The chlorination of wool is the subject of a recent English patent (1897, No. 11,917).

then immersed in 250 c.c. of water containing 5 grammes of caustic soda. The liquid is raised to the boiling point, the material removed, and the residual cotton collected on a filter and washed successively with hot water, water slightly acidulated with hydrochloric acid, and again with pure water till free from acid. The cotton is then dried at 100° C. as before, exposed to the air for twelve hours, and weighed. The addition of 4·5 per cent. to the weight obtained gives the amount of *cotton-fibre* in the mixture, the difference between this and the weight of the purified fibre previously found giving the *wool*.

APPENDIX.

Analyses of Proteids, &c., from R. H. Chittenden's *Digestive Proteolysis.*

Substance.	C.	H.	N.	S.	O.	P.	Ash.	Origin.	Authority.
Serum-albumin,	53·05	6·85	16·04	1·77	22·29	..	{0·57	Serum from horse blood.	Hammarsten.
Serum-albumin,	52·25	6·65	15·88	2·27	22·95	..	{1·84	Pleural exudation.	Hammarsten.
Egg-albumin,	52·25	6·90	15·25	1·93	23·67	..	..	Non-coagulated.	Hammarsten.
Egg-albumin,	52·33	6·98	15·89	1·83	22·97	..	1·11	Non-coagulated.	Chittenden and Bolton.
Lact-albumin,	52·19	7·18	15·77	1·73	23·13	..	..	Cows' milk.	Sebelien.
Vegetable albumin,	52·25	6·76	16·07	1·48	23·44	..	0·70	Corn or maize.	Chittenden and Osborne.
Vegetable albumin,	53·02	6·84	16·80	1·28	22·06	..	0·32	Wheat.	Osborne and Voorhees.
Proteose, animal,	52·13	6·83	16·55	1·09	23·40	..	0·79	Hemi-albumose, urine.	Kühne and Chittenden.
Proteose, vegetable,	50·60	6·68	16·33	1·62	24·77	..	2·99	Corn or maize.	Chittenden and Osborne.
Proteose, vegetable,	51·86	6·82	17·32	..	..	..	0·25	Wheat.	Osborne and Voorhees.
Proteose, vegetable,	49·98	6·95	18·78	..	..	..	1·80	Flax-seed.	Osborne.
Proteose, vegetable,	46·52	6·40	18·25	..	..	..	2·20	Cocoanut meat.	Chittenden and Setchell.
Vitellin, spheroidal,	51·71	6·84	18·12	0·85	22·48	..	1·20	Corn or maize.	Chittenden and Osborne.
Vitellin, crystalline,	51·60	6·97	18·80	1·01	21·62	..	0·30	Squash-seed.	Chittenden and Hartwell.
Vitellin, amorphous,	51·81	6·94	18·71	1·01	21·53	..	0	Squash seed.	Chittenden and Hartwell.
Vitellin, crystalline,	51·48	6·94	18·60	0·81	22·17	..	0·54	Flax-seed.	Osborne.
Vitellin, spheroids,	51·03	6·85	18·39	0·69	23·04	..	0·49	Wheat.	Osborne and Voorhees.
Vitellin, crystalline,	51·63	6·90	18·78	0·90	21·79	..	0·56	Hemp-seed.	Chittenden and Mendel.
Vitellin, crystalline,	51·31	6·97	18·75	0·76	22·21	..	0·03	Castor-bean.	Osborne.
Vitellin, crystalline,	52·18	6·92	18·30	1·06	21·54	..	0·20	Brazil nut.	Osborne.
Vitellin, semi-crystalline,	51·23	6·90	18·40	1·06	22·41	..	0·25	Cocoanut meat.	Chittenden and Setchell.
Myosin, mean of thirteen samples,	52·82	7·11	16·77	1·27	21·90	..	1·45	Muscle-tissue.	Chittenden and Cummins.
Myosin, vegetable,	52·68	7·02	16·78	1·30	22·22	..	0·63	Corn or maize.	Chittenden and Osborne.

Substance.	C.	H.	N.	S.	O.	P.	Ash.	Origin.	Authority.
Myosin, vegetable, crystalline,	52·18	7·05	17·90	0·53	22·34	..	0·10	Oats.	Osborne.
Paraglobulin,	52·71	7·01	15·85	1·11	23·24	..	0·30	Blood of horse.	Hammarsten.
Fibrinogen,	52·93	6·90	16·66	1·25	22·26	..	1·75	Blood of horse.	Hammarsten.
Zein,	55·23	7·26	16·13	0·60	20·78	..	0·43	Corn or maize.	Chittenden and Osborne.
Gliadin,	52·72	6·86	17·66	1·14	21·62	..	0·51	Wheat.	Osborne and Voorhees.
Gliadin,	53·01	6·91	16·43	2·26	21·39	..	..	Oats.	Osborne.
Glutenin,	52·34	6·83	17·49	1·08	22·26	..	..	Wheat.	Osborne and Voorhees.
Coagulated proteid,	52·33	6·98	15·84	1·81	23·04	..	0·27	Egg-albumin.	Chittenden and Bolton.
Coagulated proteid,	51·58	6·88	18·80	1·09	21·65	..	0·25	Vitellin, hemp-seed.	Chittenden and Mendel.
Fibrin,	52·68	6·83	16·91	1·10	22·48	..	0·56	Blood of horse.	Hammarsten.
Oxyhæmoglobin,	53·85	7·32	16·17	0·39	21·84	..	0·43 Fe.	Blood of dog.	Hoppe-Seyler.
Oxyhæmoglobin,	54·71	7·38	17·43	0·48	19·60	..	0·39 Fe.	Blood of pig.	Hüfner.
Mucin,	50·30	6·84	13·62	1·71	27·53	..	0·33	Snail.	Hammarsten.
Mucin,	48·84	6·80	12·32	0·84	31·20	..	0·35	Submaxillary gland.	Hammarsten.
Chondromucoid,	47·30	6·42	12·58	2·42	31·28	..	..	Cartilage.	Mörner.
Nuclein,	50·60	7·60	13·18	..	..	1·89	..	Human brain.	V. Jaksch.
Nuclein,	49·58	7·10	15·02	..	..	2·28	..	Pus.	Hoppe-Seyler.
Casein,	52·96	7·05	15·65	0·71	22·78	0·84	..	Cow's milk.	Hammarsten.
Casein,	53·30	7·07	15·91	0·82	22·03	0·87	0·98	Cow's milk.	Chittenden and Painter.
Nucleo-histon or leuco-nuclein,	48·41	7·21	16·85	0·70	24·41	2·42	..	Leucocytes.	Lilienfeld.
Gelatin,	49·38	6·81	17·97	0·71	25·13	..	1·26	Connective tissue.	Chittenden and Solley.
Elastin,	54·24	7·27	16·70	0·30	21·79	..	0·90	Neck-band.	Chittenden and Hartwell.
Elastin,	53·95	7·03	16·67	0·38	21·97	..	0·72	Aorta.	Schwarz.
Keratin,	49·45	6·52	16·81	4·02	23·20	..	1·01	White rabbit's hair.	Kühne and Chittenden.
Neuro-keratin,	56·99	7·53	13·15	1·87	20·46	..	1·35	Human brain.	Kühne and Chittenden.
Reticulin,	52·88	6·97	15·63	1·88	22·30	0·34	2·27	Reticular tissue.	Siegfried.

ADDENDA.

Page 1. A. Wroblewski (*Ber.*, 1898, p. 3045; abst. *Analyst*, xxiii. p. 106) defines proteids as bodies which on completed decomposition with acids yield as final products:— Ammonia, nitrogenous, organic basis (such as lysine, arginine, &c.), and amido-acids (such as leucine, tyrosine, &c.). He groups the proteids as albuminous substances, compound albuminous substances, and albuminoids. In the last group he distinguishes three classes: — Structural substances, including keratins, elastins, and collagenes; albumoses and peptones; and enzymes.

Page 3. *Thyro-iodin* or *iodothyrin* is the name of a remarkable compound first observed by Baumann (*Zeit. physiol. Chem.*, xxi. 319) in the thyroid glands of sheep. It appears to be of proteid nature, but cannot be arranged in any of the recognised classes. Thyro-iodin is peculiar in containing nearly 10 per cent. of iodine, and about 0·5 per cent. of phosphorus, besides a considerable percentage of nitrogen. It is described as an amorphous brown powder, which, when heated, swells up and evolves an odour of pyridine. It is almost insoluble in water, but readily soluble in alcohol and in dilute caustic alkalies, being precipitated in an apparently unchanged condition from the latter solution by addition of an acid.

The human thyroid gland, when in a normal condition, contains the same or a closely-related iodised organic compound, but in cases of goitre the proportion of iodine is stated by Baumann to be smaller. On the other hand, A. Oswald (*Zeit. physiol. Chem.*, 1897, xxiii. 265), as the result of numerous determinations of iodine in the thyroid glands of persons who died in different Swiss cantons from various diseases, concludes that the proportion of iodine is not inversely proportional to the prevalence of cretinism, but is directly proportional (both in human subjects and in the lower animals) to the amount of colloid matter in the acini.

According to E. Gley (*Compt. rend.*, 1897, cxxv. 312), the

parathyroid glandules of rabbits contain from 2½ to 3 times as much iodine as the thyroid glands of the same animals. In dogs, the percentage of iodine in the parathyroid glandules is much higher than in the thyroid.

Iodothyrin gives none of the reactions of albumin. The gland retains its activity after being exposed to 100° C., and after treatment with dilute mineral acids, so that the substance is not of the nature of an enzyme. According to R. Hutchison (*Jour. Physiol.*, 1896, xx. 476), thyro-iodin yields no reducing substance on treatment with mineral acids, and no nuclein bases, and hence is neither a gluco-proteid nor a nucleo-proteid. On gastric digestion it readily splits into a proteid and a non-proteid part, both of which, but especially the latter, contain iodine. The non-proteid part contains all the phosphorus of the original substance. In addition to iodothyrin, the thyroid gland contains a nucleo-proteid, ordinary extractives in fair abundance, and from 0·5 to 0·8 per cent. of inosite.

The discovery of iodothyrin appears to throw light on the use of compounds of iodine in the treatment of affections of the thyroid gland, and it offers an explanation of the fact that preparations of the thyroid gland are effective much more rapidly than inorganic compounds of iodine.

Under the name of *thyroglandin*, E. C. C. Stanford (*Pharm. Jour.*, [4], vii. 166) has described a preparation which he states contains all the active principles of the thyroid gland, and hence is preferable to iodothyrin, &c. The characters of various preparations of the thyroid gland have been reviewed by V. Coblentz (*Jour. Soc. Chem. Ind.*, August 1898).

The successful employment of preparations of the thyroid gland in medicine has led to the production of various artificial products of an allied nature. The *iodocasein* of A. Liebrecht (*Ber.*, 1897, xxx. 1824), obtained by the action of iodine on casein, is a substance of this kind. It is a white powder containing about 8·7 per cent. of iodine, has acid properties, gives the biuret reaction, and is said to resemble iodothyrin in its physiological action (compare page 551).

Page 6. O. Schmiedeberg (abst. *Jour. Chem. Soc.*, 1898, ii. 342) has studied the dark brown or black animal pigments known as *melanins*, and incidentally gives the *formulæ of various proteids*.

Page 6. A. Krüger (abst. *Jour. Chem. Soc.*, 1889, p. 528) has studied the *proportion and mode of occurrence of sulphur in proteids*. He distinguished, as Liebig had previously done, between the loosely-combined sulphur, removable by treatment with caustic alkali, and that more firmly combined.

Certain native proteids, of which casein and Ritthausen's legumin are examples, appear to contain all their sulphur in the firmly-combined condition, since they do not blacken when boiled with lead acetate and caustic alkali. In white of egg and fibrin Krüger found :—

	White of Egg.	Fibrin.
Total sulphur, . . .	1·66 per cent.	1·20 per cent.
Loosely-combined sulphur, .	0·44 ,,	0·38 ,,
Ratio of total to loosely-combined sulphur, . . .	4 : 1·06 ,,	3 : 0·95 ,,

The fibrin and albumin which had been treated with caustic alkali for the removal of the loosely-combined sulphur were found to be converted into amorphous peptonoid bodies, the solutions of which by saturation with ammonium sulphate (page 17) gave precipitates having the reactions of an albumose, while the filtrates contained bodies agreeing in characters with true peptones. On analysis, the parent proteids and these products were found to have the following composition :—

	Carbon.	Hydrogen.	Nitrogen.	Oxygen.	Sulphur.	Ash.
Egg-albumin, .	52·98	7·09	15·70	22·41	1·6–1·8	..
Proteose, . .	55·76	6·93	14·46	21·57	1·28	(0·31)
Peptone, . .	48·06	6·73	11·70	33·04	0·47	(2·88)
Fibrin, . . .	52·50	6·95	16·57	22·76	1·22	..
Proteose, . .	55·26	6·75	15·46	21·74	0·79	(0·21)
Peptone, . .	52·58	6·60	14·43	25·92	0·47	(0·85)

In the proteoses there was, in both cases, a fall in the percentage of nitrogen and sulphur, and this is still more marked in the peptones. The sulphur in the proteoses is approximately equal to the firmly-combined sulphur of the parent proteids, and the composition of the two proteoses is nearly the same. On the other hand, the composition of the two peptones is very different. The loss of sulphur is regarded by Krüger as probably due to the formation of another sulphurised organic body (? trypsophan, page 382) which escaped detection. No sulphates were formed.

Krüger compares the firmly-combined sulphur of proteids to that in thio-ether, mercaptan, and sulphinic compounds; and the loosely-combined sulphur to that in thio-acids, cystin, and compounds in which either the group :C:S or the group ⋮C.S.S.C⋮ is present.

Page 10. N. C. H. Schjerning (*Zeit. Anal. Chem.*,

xxxvi. 643; xxxvii., 73; abst. *Analyst*, 1898, pp. 104, 185) has determined the amounts of *proteid-nitrogen precipitated* from solutions of diastase (Merck's), commercial peptones, meat-extracts, egg-albumen, and from beer, milk, urine, and decoction of yeast by the addition of various *metallic salts*. Schjerning considers that he has established the following facts :—

1. The sulphates and chlorides of the metals employed precipitate at most only the true albumins, and these very incompletely. 2. The acetates of the metals of the magnesium group and of the extended magnesium group precipitate only true albumins. The precipitating power of the metal appears to rise with its atomic weight. 3. The acetates of lead and its analogues precipitate all the proteids up to the albumoses. 4. Ferric and manganic acetates precipitate all proteids up to the real peptones. 5. Uranyl acetate and phosphotungstic acid precipitate all the proteids. In presence of phosphoric acid, uranyl acetate partially precipitates ammoniacal nitrogen. 6. Mercuric chloride precipitates all proteids up to the albumoses—that is, the same amount as is precipitated by lead acetate. Mercuric acetate precipitates all proteids, and in addition more or less of the amido-nitrogen present. Hence Schjerning considers mercuric acetate less suitable than cupric acetate for the quantitative precipitation of proteids.

Schjerning also obtained results which indicate that whilst beer-wort contains only four proteids, milk contains a fifth, which is not precipitable by stannous chloride, but is precipitated by all the other reagents, including magnesium sulphate. To this substance Schjerning gives the name albumin II. It appears to agree in amount with the lactalbumin and lactoglobumin of König, whilst the albumin I., precipitable by stannous chloride, agrees closely with that of casein. Witte's peptone and Liebig's meat-peptone contain albumoses, but no peptones.[1] Liebig's meat extract shows 11 per cent. of the nitrogen in the form of peptones (see König and Bomer, page 313). Only traces of proteids could be detected in normal urine.

Page 10. Several iodine compounds or derivatives of albumin have been recently prepared and described by F. G. Hopkins and by F. Hofmeister (abst. *Jour. Chem. Soc.*, 1898, i. 54, 99, 390). Compounds of this character have been introduced into commerce under the names of "Alpha-eigon," "Beta-eigon," &c.

[1] E. P. Pick (*Zeit. Physiol. Chem.*, xxiv. 246; abst. *Jour. Chem. Soc.*, 1898, i. 288), after many experiments on fractional precipitation with ammonium sulphate, concludes that Witte's peptone contains several albumoses and peptones.

Page 21. A. Atterberg (*Chem. Zeit.*, 1898, xxii. 505 abst. *Analyst*, Sept. 1898) has examined various modifications of Kjeldahl's process. He employs 20 c.c. of strong sulphuric acid and a little mercury, and adds 15 to 18 grammes of potassium sulphate when the nitrogenous substance is dissolved.

Page 33. A method of examining commercial egg-albumin has been published by P. Carles (*Jour. Pharm. Chem.*, 1897, vi. 102; abst. *Jour. Soc. Chem. Ind.*, 1897, p. 769).

Page 33. Hopkins and Pinkus (*Jour. Physiol.*, 1898, xxiii. 130) state that if an equal volume of a saturated solution of ammonium sulphate be added to white of egg, and acetic acid added in faint excess, *crystals of albumin* are obtained, even without evaporation. Their affinity for carbol-magenta and methylene-blue prevent any confusion with crystals of ammonium sulphate.

Page 34. A very complete analysis of the oil of egg-yolk has been published by M. Kitt (*Chem. Zeit.*, 1897, xxi. 303; abst. *Jour. Soc. Chem. Ind.*, 1897, p. 448).

Page 47. Gordon Sharp (*Pharm. Jour.*, Aug. 13, 1898, p. 197) regards *egg-albumin* as calcium albuminate, and *serum-albumin* as sodium albuminate (with some of the potassium compound). He suggests that this difference of composition accounts for the difficulty with which pepsin acts on egg-albumin in the absence of hydrochloric acid.

Page 60. Bourceau (abst. *Analyst*, 1898, p. 44) points out that *coagulable albumin* and *urinary proteoses* have not the same clinical significance. He finds that a drop or two of a solution of oxyphenyl-sulphonic acid containing a trace of salicyl-sulphonic acid gives an opaque white precipitate when added to 1 c.c. of a urine containing albumin or alkali-albumin, but that no precipitate occurs with proteoses, peptones, alkaloids, antipyrine, salicylates, urates, or phosphates.

Page 67. The *proteids of the pea, lentil, horse-bean, vetch, and soya-bean* form the subjects of papers by Osborne and Campbell (*Amer. Chem. Jour.*, xx. pp. 348, 393, 406, 410, and 419).

Page 68. A further research on the *proteids of maize* has been published by T. B. Osborne (*Amer. Chem. Jour.*, 1897, xix. 525; abst. *Jour. Chem. Soc.*, 1898, i. 391).

Page 69. E. Fleurent (*Compt. rend.*, cxxvi. 1374; abst. *Jour. Soc. Chem. Ind.*, 1898, p. 685) has extended his researches on the *proteids of cereals* to *leguminous seeds*, and especially to horse-beans, which contain from 25 to 32 per cent. of nitrogenous matters. A. Livache (abst. *Jour. Soc. Chem. Ind.*, 1898, p. 685) has discussed in detail the practical results of Fleurent's researches.

Page 74. A chemical method for the determination of crude

gluten in wheat-flour for bread-making has been described by E. Fleurent (*Compt. rend.*, 1896, cxxiii. 755; abst. *Jour. Soc. Chem. Ind.*, 1897, p. 59).

Page 75. The composition of the *gluten-flour of commerce* is the subject of a paper by V. G. L. Fielden (*Pharm. Jour.*, [4], vii. pp. 170, 184). The following is a tabulated statement of his analytical results :—

	A.	B.	C.	D.	E.
Gluten, per cent., . . .	76·0	60·0	65·0	8·5	66·0
Starch and sugar, per cent., .	7·6	16·7	13·26	68·8	11·63

Sample "D" is stated to be of American origin, and is sold as "crude gluten." Fielden refers to an article in the *British Medical Journal* for July 16, 1898, on "The Chemistry of Diabetic Foods," in which results by F. Kraus, jun., are mentioned, showing that the diabetic breads sold both in Germany and in Britain often contain very large proportions of starch.

Page 86. The *proteids of cows' milk* have been studied by K. Storch (*Monatsh.*, 1897, xviii. 244; abst. *Jour. Chem. Soc.*, 1897, ii. 420; *Analyst*, 1897, p. 211), who confirms Hammarsten's view that there is only one kind of casein present in cows' milk.

Page 88. The cause of the *coagulation of heated milk* has been studied by R. Bardach (abst. *Analyst*, 1897, p. 212). He controverts the view of Cazeneuve and Hadden, that the coagulation of heated milk is due solely to the acids produced by the oxidation of lactose, and attributes it to the alteration of the casein by heat, so that it can be precipitated by the small amount of acid derived from the lactose, which is otherwise incapable of affecting it.

Page 90. R. Benjamin (abst. *Jour. Chem. Soc.*, 1897, ii. 63) concludes that *rennet* acts only on the caseinogen of milk and on no other proteid of either animal or vegetable origin. Solutions of casein fermentable in this way are, like milk itself, alkaline to lacmoïd and acid to phenolphthalein. A caseinogen solution is stated to be coagulable only in the presence of soluble calcium salts.

Page 97. *The structure of the fat-globules in cows' milk* has been studied by V. Storch (*Analyst*, 1897, p. 198). He concludes that the fat-globules in milk are coated with a mucoid substance which forms a membrane round each globule, these membranes or semi-fluid envelopes being retained when the fat-globules are washed free from milk-serum. The membranes can be stained and seen under the microscope. The mucoid substance

of the membranes occurs in butter in minute liquid drops, and forms over 60 per cent. of the total proteid matter of butter. From the composition of butter-serum and of butter-milk serum, Storch considers that it can be proved that butter-serum contains an aqueous proteid substance, differing from other known proteids in its low proportion of nitrogen (14·76 per cent.) and high proportion of sulphur (2·20 per cent.). Storch regards butter as a conglomerate of the fat-globules of milk, interspersed with numerous minute liquid drops, of which the smallest consist of mucoid substance, and the largest of butter-milk. Richmond has confirmed the existence of Storch's mucoid body, but doubts the existence of the envelope enclosing each fat-globule. The high proportion of sulphur in Storch's body and its resistance to reagents, suggests its analogy to the keratoids (page 530).

Page 100. Figures illustrating the composition of *sows' milk* have been published by E. Petersen (abst. *Jour. Chem. Soc.*, 1898, ii. 85).

Page 100. A. Pizzi (abst. *Jour. Chem. Soc.*, 1896, ii. 120) has published analyses of the *milk of sheep, goats, buffaloes*, and *rabbits*. He also gives some data obtained by the examination of the fats from these sources.

Page 103. According to M. A. Siegfried (*Zeit. Physiol. Chem.*, 1897, xxii. 575; abst. *Jour. Chem. Soc.*, 1897, ii. 220) the phosphorus of the nucleon accounts for only six per cent. of the total phosphorus of cows' milk; whereas in human milk, in which the proportion of nucleon is twice as great,[1] it accounts for 41·5 per cent. of the total phosphorus. The remainder of the phosphorus in human milk is stated to exist as casein, there being, according to Siegfried, practically no inorganic phosphorus (?).

Page 103. L. Vaudin (abst. *Analyst*, 1897, p. 282) has studied the proportion of *mineral matters and earthy phosphates* in cows' milk.

Page 106. See an analysis of *human milk* by Söldner and Camerer (abst. *Jour. Chem. Soc.*, 1896, ii. 378).

Page 107. V. and J. S. Adriance (abst. *Jour. Soc. Chem. Ind.*, 1898, p. 186) have published the results of the analysis of two hundred individual specimens of *human milk*, taken at periods of lactation varying from two days to fifteen months. The following was the range of composition in 120 samples regarded as normal, that is, having no ill-effect on the child:—Fat, 3 to 4

[1] According to K. Wittmaack (*Zeit. Physiol. Chem.*, 1897, xxii. 567) the percentage of nucleon (phosphorcarnic acid) in cows' milk averages 0·056; in human milk, 0·124; and in goats' milk, 0·11.

per cent.; carbohydrates, 6 to 7; proteids, 1 to 2; salts, about 0·20; and total solids, 12 per cent. The reaction of the milk was uniformly alkaline.

The average percentage composition of human milk during the course of lactation is shown in the following list selected from a fuller table given in the paper :—

Period.	Fat.	Carbo-hydrates.	Proteids.	Ash.	Total Solids.	Water.
2 days, . . .	3·83	5·80	2·77	0·27	12·20	87·80
3 ,, . . .	3·83	5·90	2·20	0·26	12·20	87·80
7 ,, . . .	3·83	6·22	1·90	0·24	12·20	87·80
14 ,, . . .	3·83	6·63	1·70	0·20	12·20	87·80
4 weeks, . . .	3·83	6·68	1·58	0·19	12·20	87·80
6 months, . . .	3·83	6·78	1·25	0·16	12·20	87·80
9 ,, . . .	3·83	6·84	0·04	0·16	12·04	87·96
12 ,, . . .	3·83	6·90	0·83	0·15	11·77	88·23
15 ,, . . .	3·83	6·96	0·63	0·14	11·50	88·50

Page 108. A number of analyses of human milk have been recorded by Carter and Richmond (*Brit. Med. Jour.*, 1898, i. 199). They find the proteids, ash, and sugar to decrease as lactation advances. The composition of the fat (as indicated by the refractive index) also varies, while the proportion of volatile acids increases as lactation proceeds.

Page 112. A. Schlossmann (*Zeit. Physiol. Chem.*, 1897, xxiii. 258; abst. *Analyst*, xxiii. 38) has published the results of his analyses of sixteen samples of *asses' milk* obtained from one establishment in Dresden. The specific gravity ranged from 1·031 to 1·036. The average of the total solids of the samples was 11·15 per cent.; of the ash, 0·399; and of the sugar, 4·94 per cent. The total nitrogen varied from 0·22 to 0·27 per cent., of which 86 per cent. existed as proteid matters precipitable by trichloracetic acid or salicyl-sulphonic acid. The fat-globules were very small, and the percentage of fat ranged from 0·15 to 0·60 per cent. When the milk was titrated with standard sulphuric acid with phenol-phthalein as indicator, 100 c.c. of the milk required 6 c.c. of decinormal acid, against 40·4 c.c. when the indicator was methyl-orange or turmeric.

Page 115. The *average composition* of 12,907 samples of *milk*

taken in 1897, on arrival from the farms at the depôts of the Aylesbury Dairy Company, was as follows:—

	Spec. Gravity.	Total Solids.	Fat.	Solids not Fat.
Morning Milk, . .	1·0324	12·54	3·60	8·94
Evening Milk, . .	1·0320	12·98	4·03	8·95
Average, . . .	1·0322	12·76	3·82	8·94

Page 118. H. Wing (abst. *Jour. Chem. Soc.*, 1897, ii. 220) states that the addition of fat to the fodder of cows increases neither the yield of milk nor the proportion of fat contained in it.

Page 118. P. Dornic (abst. *Jour. Chem. Soc.*, 1897, ii. 402) states the effect of working cows on the quality of the milk yielded is very slight. The solids and acid increased a little, whilst the yield of milk diminished slightly. The milk produced during the period of work frequently curdled at 45°, whilst that yielded during the period of rest, curdled at 70° to 75°.

Page 122. R. Eichloff (abst. *Jour. Chem. Soc.*, 1897, ii. 511) has described a sample of *colostrum fat*. It had a deep golden colour, an unpleasant smell and taste, was of almost waxy consistency, and melted at 35° C.

Page 130. Scott-Smith and Searle (*Analyst*, 1898, p. 3) have called attention to the faulty graduation of certain *Leffmann-Beam bottles*.

Page 134. J. Froidevaux (abst. *Analyst*, 1898, p. 6), has described a method of determining fat in milk which does not curdle readily (*e.g.*, human, humanised, and condensed milk) by adding a solution of calcium phosphate in acetic acid, separating the clot, and extracting it with ether after drying.

Page 157. An improved form of *Richmond's milk-scale* is described in the *Analyst*, vol. xxiii. p. 2.

Page 167. From an examination of the books of the Aylesbury Dairy Company for many years past, H. D. Richmond (*Analyst*, 1898, p. 90) finds that the milk believed to be genuine which contained less than 8·3 per cent. of *non-fatty solids*, numbered only 0·59 per 1000, and those with less than 8·1 per cent., only 0·1 per 1000.

Page 168. L. Vaudin (abst. *Analyst*, 1898, p. 6) agrees with Duclaux that the decolorisation of indigo-carmine is due to the presence of microbes in the milk, and he suggests that the indigo test should be applied to all milk offered for sale.

Page 168. H. D. Richmond (*Analyst*, 1898, p. 90) strongly supports the author's recommendation of 0·5 per cent. of *total nitrogen*, as an additional limit in forming an opinion on milk supposed to be watered.

Page 169. For the determination of *added water in milk*, H. D. Richmond (*Analyst*, 1898, p. 169) proposes to add the difference between the specific gravity of the sample and 1000 to the figure representing the percentage of fat. Thus, if a milk have the specific gravity of 1029·2, and contain 3·27 per cent. of fat, the figure from which the water is calculated is $29{\cdot}2 + 3{\cdot}27 = 32{\cdot}47$. Genuine milk gives the mean figure, 36·0, but Richmond considers 34·5 as a safer limit. Accepting this figure, the percentage of added water in the example given above would be found by the proportion:—34·5 : 32·47 :: 100·0 : 94·1 per cent. Experiments by Richmond on milks which had been diluted with known proportions of water, showed that the proposed method of calculating the added water gave nearer approximations to the truth than figures calculated from the non-fatty solids.

Villiers and Bertault (abst. *Analyst*, 1898, p. 175) have proposed a method of detecting *added water in milk*, which is based on the fact observed by them, that a peculiar relation exists between the lactose and the salts in milk-whey, so much so that the refractive power of the latter is virtually a constant.

Page 171. A method for the direct detection of *nitrites in milk* has been described by E. Riegler (abst. *Analyst*, 1897, p. 235).

Page 171. According to Cotton (abst. *Analyst*, 1898, p. 37) *cane-sugar* may be conveniently detected in milk, as follows:—Ten c.c. measure of the sample is mixed with 0·5 gramme of powdered ammonium molybdate, and 10 of dilute hydrochloric acid (1 : 10) added. In a second tube, 10 c.c. measure of milk of known purity, or 10 c.c. of a 6 per cent. solution of milk-sugar is similarly treated. The tubes are then placed in a water-bath, and the temperature gradually raised to about 80°C.

If saccharose be present, the milk assumes an intense blue colour, while genuine milk or milk-sugar solution remains unaltered, unless the temperature be raised to the boiling point. Cotton states that with as little as one gramme of cane-sugar per litre of the milk, the reaction is well-marked, but that less than 6 grammes of sugar per litre is not used for the purpose of adulteration.

Page 173. A. W. Stokes (*Analyst*, 1897, p. 221) has called attention to the use of a solution of commercial *dextrin* for the adulteration of milk.

Page 173. On the *addition of gelatin to cream*, see A. W. Stokes (*Analyst*, 1897, pp. 320, 322).

Page 175. For the detection of *annatto* in *milk*, A. Leys (abst. *Analyst*, 1898, p. 174) shakes 50 c.c. of the suspected sample with twice its measure of a mixture of 240 c.c. of alcohol of 93 per cent., 320 c.c. of ether, 20 c.c. of water, and 8 c.c. of ammonia of 0·92 sp. gravity. After standing for twenty minutes, the lower layer, which in presence of annatto will have a greenish-yellow tint, is tapped off, and gradually treated with half its measure of a ten per cent. solution of sodium sulphate, the separator being inverted without shaking after each addition. By this treatment, the casein separates in flakes, which conglomerate and rise to the surface, when the subjacent liquid is tapped off, strained through wire-gauze, and placed in four test-tubes. To each of these amylic alcohol is added, and the tubes shaken and immersed in cold water, which is gradually raised to 80° C. This causes the emulsion to break up, and the amylic alcohol, holding the annatto in solution, to rise to the surface. The amylic alcohol layers are separated from the lower strata, evaporated to dryness, and the residue dissolved in warm water containing a little alcohol and ammonia. A bundle of white cotton fibre is then introduced, and the liquid evaporated nearly to dryness on the water-bath. The fibre, which is coloured a pale yellow even with pure milk, is washed and immersed in a solution of citric acid, when it will be immediately coloured rose-red if the milk contained annatto. Saffron, turmeric, and the colouring matter of marigolds give no similar reaction.

Page 175. J. Froidevaux (abst. *Analyst*, Sept. 1898) has described methods for detecting saffron, Poirrier's "Orange III," and other *added colouring matters in milk*.

Page 184. A. Jorissen (abst. *Analyst*, 1897, p. 282, and 1898, p. 41) has summarised various tests proposed for the *detection of formalin* in milk, but objects to all of them except Hehner's reaction (page 184) on the ground that they are not peculiar to formalin. Jorissen points out that on placing a little morphine hydrochloride in a porcelain basin, adding a few drops of concentrated sulphuric acid, and touching the mixture with the end of a glass rod previously dipped in a solution of formaldehyde, even if exceedingly dilute, a purple colour is produced which changes to an indigo-blue.

E. Rimini (abst. *Jour. Soc. Chem. Ind.*, 1898, p. 697) has described two new reactions of *formaldehyde*, suitable for its detection in milk.

Page 189. The manufacture of *sterilised and humanised milk* is described in the *Pharmaceutical Journal*, June 12th, 1897.

Page 193. R. Dupouy (abst. *Analyst*, 1897, p. 211), de-

scribes several colour-tests for *unboiled milk*, with each of which boiled milk gives negative reactions. The best reagent appears to be para-phenylene-diamine (1 : 4 diamido-benzene). From twenty to thirty millegrammes of this substance should be dissolved in one c.c. of hot water, and when cold, an equal measure of milk, and one drop of hydrogen peroxide solution are added. With raw milk, a very dark violet colour is produced. Watered and skimmed milk, if not previously heated, also give the reaction, but with less intensity than normal milk. The value of the test has been confirmed by H. Leffmann (*Analyst*, 1898, p. 85), who points out that the solution of the reagent must be freshly-prepared, since after standing an hour or so it gives a slight blue colour, both with raw and with boiled milk, even without the addition of hydrogen dioxide. Leffmann finds that the colour is produced in a marked degree when the milk has been heated to 170°F. (= 76·5° C), but that the property is lost if the milk be heated to 180° F. (= 82° C).

Leffmann found that the blue coloration was produced with substantially equal distinctness by whole milk, skimmed milk, and separated milk, and also by the liquid obtained by treating raw milk with magnesium sulphate in excess, and filtering from the precipitate formed.

Leffmann states that when *amidol* is employed in place of diamido-benzene, a red colour is obtained with raw milk on adding hydrogen dioxide; but eikonogen and various amido-, hydroxy-, and carboxy-derivatives of benzene and naphthalene were found to be inactive.

Page 194. The *changes* which take place in *milk* either spontaneously or during culinary processes have been discussed at length by A. Béchamp (*Bul. Soc. Chim.*, 1896).

Page 195. A. Devarda has described a special apparatus for determining the *acidity of milk* (*Chem. Centralb*, 1896, ii. 1003; abst. *Jour. Chem. Soc.*, lxxiv. ii. 58).

Page 195. *To stain bacteria in milk*, it is recommended (*Jour. R. Micro. Soc.*, 1892, p. 291) that a loopful of milk should be mixed on a cover-glass with a loopful of distilled water, dried, and fixed at a gentle heat. The cover-glass is then placed in a watch-glass containing chloroform - methylene - blue (prepared by mixing 12 to 15 drops of a saturated alcoholic solution of methylene-blue with 3 to 4 c.c. of chloroform. After being moved to and fro in this solution for a few minutes the cover-glass is removed, the chloroform allowed to evaporate, and the preparation washed with water. On examination (in water) under the microscope, if fresh milk or cream was employed, the bacteria are alone stained blue.

If the milk was curdled, the flakes of casein are coloured a pale blue, but this does not interfere with the recognition of the bacteria, which are stained deep blue.

Page 211. A *legal distinction between skimmed milk and separated milk* has been established by the decision of the Court for the consideration of Crown cases reserved, who, in the case of Petchley *v.* Taylor, refused an appeal from a conviction of the appellant by a Metropolitan Police Magistrate. According to the case as stated by him, the appellant sold to the respondent a tin containing a substance described as "Cup Brand Condensed Milk." On the tin were the words "This tin contains skimmed milk with nothing added but the finest sugar." It was proved that the substance in the tin was separated milk, and that 97 per cent. of the original fat had been abstracted. It was also proved that the term "skimmed milk" meant milk from which a portion of the fat had been removed by skimming the surface of the milk, and that the greatest amount of fat that could be thus removed was 63 per cent. (*Analyst*, xxiii. 168; reprinted from the *Times* of May 2nd, 1898).

Page 218. In the determination of *fat* in *cream* and *clotted cream*, H. D. Richmond (*Analyst*, 1898, p. 91) employs amylic alcohol.

Page 218. M. Weibull (abst. *Analyst*, 1897, p. 213) confirms the observation of Richmond that the *ratio of water to non-fatty solids* is the same in separated cream as in the original milk.

Page 231. A. M'Gill (*Analyst*, 1898, page 128) suggests that the author's formula for calculating the *concentration of condensed milk* would be improved by basing the calculation on the non-fatty milk-solids (8·5) instead of on the total milk-solids (at 12·5 per cent.). The difference is insignificant in the case of whole-cream milk, but when the fat of the condensed milk has been partly or largely removed the error becomes very sensible. M'Gill finds the specific gravity of sweetened condensed milk to be usually between 1·31 and 1·33. Hence, instead of assuming the density of the condensed milk to be 1·28, M'Gill prefers to determine it by diluting 50 grammes to 250 c.c. with water, and from the ascertained density of this calculating that of the original condensed milk by the following formula :—

$$\text{Density of condensed milk (G)} = \frac{1}{6 - \text{density of diluted milk}}.$$

M'Gill also takes into account the specific gravity of the uncondensed milk, and proposes the following formula :—

$$W = \frac{\text{Non-fatty milk-solids in condensed milk (N)} \times \text{sp. gr. (G)}}{8{\cdot}755}$$

$$\text{Fat} = \frac{8{\cdot}5 \times \text{fat in condensed milk (F)}}{\text{Non-fatty milk-solids in sample (N)}}.$$

Page 240. Russell and Babcock regard the ripening of hard cheese as due to the joint action of bacteria and enzymes (*Nature*, lvii. 373).

Page 247. C. Besana (*Chem. Zeit.*, xxi. p. 265; abst. *Analyst*, 1897, p. 159) has described a sample of Parmesan cheese which was covered with deep black marks resembling ink-stains, and had an odour like that of garlic. Nothing unusual was detected by the microscope, but chemical analysis showed the stains to be due to ferrous sulphide, the source of which was uncertain.

Page 249. The *determination of fat and water in cheese* is described in a paper by A. Devarda (*Zeit. anal. Chem.*, 1897, p. 751; abst. *Analyst*, xxiii. 75).

Page 252. R. Hefelmann has pointed out (abst. *Analyst*, 1897, p. 159) that the refractive index of the lower fatty acids gradually increases on continued heating, so that *refractometer figures for cheese-fat* must not be interpreted too rigidly. The same conclusion follows from the observations of Forster and Riechelmann (abst. *Analyst*, 1897, p. 235).

For the preparation of the cheese-fat for examination in the refractometer, they cut up the sample into strips the size of matches, and introduce from 3 to 5 grammes into the lower and wide part of a Gerber's butyrometer, with both ends open. The lower end is then closed with a caoutchouc stopper, and 6·5 c.c. of boiling water introduced. After shaking, 6·5 c.c. of sulphuric acid, of 1·820 to 1·825 sp. gravity, should be introduced, and the whole shaken. When the solution of the cheese is complete, which is usually the case in about a minute, the butyrometer is filled to the top of the graduated tube with hot water, and allowed to rest. When the fat has risen to the surface, with the assistance of rotation if necessary, a drop of the fat is removed and examined in the refractometer.

The following are the differences, according to Wollny's thermometric adaptation of Zeiss' refractometer, shown by cheese fat from different sources, as observed by Forster and Riechelmann. The figures agree closely with those of von Raumer and of Bresner.

Description of Cheese.	C.
Swiss,	−1·5
Edam,	+0·3
Cream,	−1·7
Gorgonzola,	−1·6
Camembert,	−3·2
Limburg,	−2·1
Margarine-Romadour,	+6·5
Brie,	−2·0

Page 262. Le Wall and Pursel (*Amer. Jour. Pharm.*, lxx. 343) have pointed out that milk-sugar of good quality is liable to give a brown coloration when sprinkled on strong sulphuric acid unless care be taken that the portion taken for examination is free from fragments of the thread on which the sugar was crystallised. Le Wall and Pursel recommend the optical activity as the most certain test of the purity of milk-sugar.

Page 264. M. A. Siegfried (*Ber.*, xxvii. 2762; abst. *Jour. Chem. Soc.*, 1895, pages 76, 313) states that carnic acid is present in muscle in the form of a phosphorus compound, *phosphorcarnic acid*. The same substance has been observed among the products of tryptic digestion. Carnic acid is stated to have the formula $C_{10}H_{15}N_3O_5$. Its barium and calcium salts are readily soluble and the solutions decompose when boiled with the deposition of the corresponding phosphate. Carnic acid forms a ferric compound, *carniferrin*, the production of which is used by Siegfried as a means of separating carnic acid from muscle. Carniferrin is soluble in alkalies, and only gives the reactions for iron after being in contact with the reagents for some time. Carnic acid is said to be monobasic; but the silver salt contains $C_{10}H_{13}N_3O_5Ag_2$, the second atom of silver probably replacing the hydrogen atom of an imido-group. By the action of baryta-water phosphorcarnic acid is split up with formation of phosphoric and carnic acids.

In testing for carnic acid, the formation of ammonium thiosulphate from ammonium sulphide and carnic acid has been suggested by Siegfried, but he points out that the ammonium sulphide must be colourless and recently prepared, since the yellow sulphide itself gives ammonium thiosulphate on evaporation. Siegfried concludes that carnic acid is identical with anti-peptone.

Nucleon is the name suggested by Siegfried for compounds allied to the nucleïns, but containing peptone instead of albumin. It is also necessary, as pointed out by Kossel, to distinguish between true nucleons and paranucleons. Phosphorcarnic acid belongs to the paranucleons, and may be termed muscle-nucleon.

Page 290. For the determination of *boric acid* in sausages and other meat-products, see page 331.

Page 294. Janke (abst. *Jour. Chem. Soc.*, 1898, ii. 257) has described a method of determining *zinc in foods*, according to which the sulphated ash is dissolved in water, and the filtered solution neutralised by sodium carbonate. Any iron is then precipitated as phosphate by means of sodium acetate, and the zinc thrown down as sulphide from the filtrate by passing sulphuretted hydrogen.

Page 299. The following arrangement (fig. 35) for *collecting gases contained in canned foods* has been devised by C. A. Doremus (*Amer. Chem. Jour.*, 1897, xix. 733). A bevelled, hollow steel needle is attached to the upper arm of an adjustable clamp. The point and lower part of the shaft are covered by a rubber stopper,

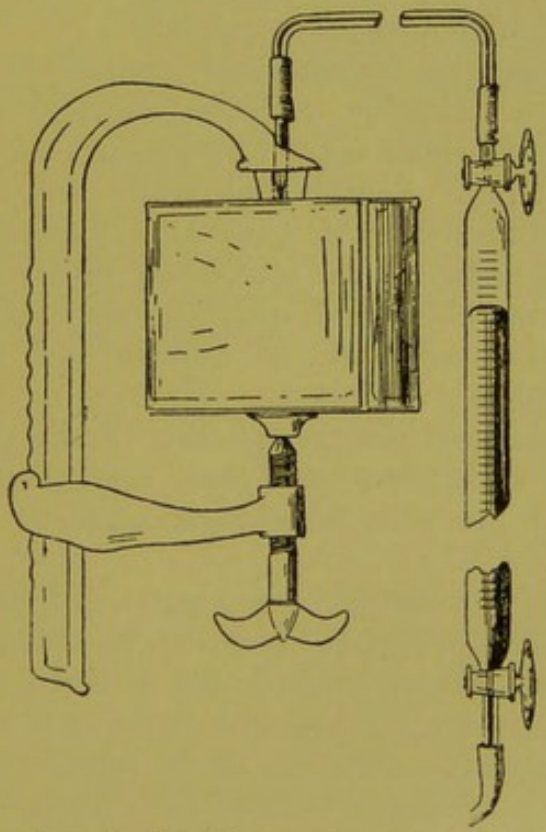


Fig. 35.—Apparatus for collecting the gases of canned foods.

which serves as a soft pad. The lower arm is moved along the body of the clamp until the can to be pierced is held between the rubber stopper and the head of the screw. The upper part of the needle is connected by means of a capillary tube, filled either with water or mercury, with a receiver also filled with either of these liquids. The receiver may be a stop-cock eudiometer or a nitrometer.

The apparatus adjusted, a turn or two of the screw clamps the can tightly between the rubber-pad at the top and the screw-head below. The rubber yields to the pressure, making a tight joint around the needle. When the latter pierces the tin the contained gases of the can escape gently into the eudiometer.

Page 315. *The halogen-derivatives of albumin* have been independently studied by Hopkins and Brook (*Jour. Physiol.*, 1887, p. 184) and by Blum and Vaubel (*Jour. prakt. Chem.*, lvii. 365). Blum has patented certain compounds (*Eng. Patent*, 1897, No. 12,817).

Page 323. Baumann and Bomer (abst. *Analyst*, 1898, p. 134) have further investigated the behaviour of proteids with zinc sulphate, and have arrived at the following conclusions :—

1. If 1 c.c. of dilute sulphuric acid (1 : 4) be added to 50 c.c. of the proteid solution, the albumoses are precipitated as well with zinc sulphate as with ammonium sulphate.

2. Ammonium salts, tyrosine, and creatine are not precipitated by zinc sulphate. Leucine is thrown down in small quantities, but considering the small amount present in meat-preparations the error is not material. Leucine and tyrosine are, on the other hand, both separated in considerable quantities by ammonium sulphate.

3. The meat-bases are as completely separated by phosphomolybdic acid in the filtrate from the zinc sulphate precipitate as they are in the absence of zinc sulphate, and the peptones are even more completely separated.

4. The filtrate from the precipitation of the albumoses can be treated directly with phosphomolybdic acid, thereby avoiding the error caused by the different nitrogen-contents of albumose and gelatose.

5. Ammonia and creatine are separated from solutions with almost quantitative exactness by sodium phosphomolybdate.

Baumann and Bomer propose the following method for the determination of the albumoses and peptones in meat-extracts and peptone-preparations :—Fifty c.c. of the solution, containing about 1 gramme of dry substance, is freed from insoluble and coagulable albumin, and mixed with 1 c.c. of dilute sulphuric acid (1 : 4), and sufficient zinc sulphate to supersaturate it. The precipitate is filtered off and washed with a slightly acidified saturated solution of zinc sulphate, and the nitrogen determined by Kjeldahl's method. In the filtrate, the peptones, meat-bases, and ammonia are precipitated by sodium phosphomolybdate. The precipitate is filtered off, and the nitrogen of this also determined by Kjeldahl's method. The ammoniacal nitrogen is determined by distillation with magnesia, either in the aqueous solution,

or, preferably, in a second phosphomolybdate precipitate. The amount so found, when deducted from the total nitrogen in the phosphomolybdate precipitate, gives by difference the nitrogen present as peptones and meat-bases, with, under certain circumstances, small quantities of leucine.

Page 335. The composition and chemical examination of *saliva* is discussed in a paper by E. Gerard (abst. *Analyst*, 1898, p. 79).

Page 335. The composition of human saliva has been investigated by R. H. Chittenden (*Proc. Amer. Physiol. Soc.*, 1898, 3; abst. *Jour. Chem. Soc.*, 1898, ii. 241). He found that although human saliva is ordinarily alkaline to litmus or lacmoïd, it is acid to phenol-phthalein. The alkalinity to litmus is therefore due to alkali-metal phosphates. The average alkalinity was found to be equal to 0·14 per cent. of sodium carbonate, and the acidity was such that 1 gramme of saliva requires 0·06 milligramme of sodium hydroxide to neutralise it. The alkalinity, acidity, and amylolytic power are greater in saliva coming from glands after a long rest than in that secreted an hour after a meal. The increased amylolytic power is due to an increase in the organic substances, including the enzyme. Alcoholic drinks increase the amylolytic activity of saliva.

Page 358. Gordon Sharp (*Pharm. Jour.*, Aug. 13, 1898, p. 197) has investigated the products of the *putrefactive digestion of egg- and serum-albumins.*

Egg-albumin gives:—	Serum-albumin gives:—
Unaltered albumin.	Unaltered albumin.
Alkali-albumin.	Alkali-albumin.
Proto-albumose.	Proto-albumose.
Hetero-albumose (little).	Hetero-albumose (much).
An alkaloidal substance.	Deutero-albumose (little).
Crystals (probably leucine and tyrosine).	An alkaloidal substance.
No peptone.	Crystals (probably leucine and tyrosine).
	No peptone.

The products of *peptic digestion* of both egg- and serum-albumin consisted chiefly of deutero-albumose, with only traces of true peptone. The digestion had therefore gone past the proto- and hetero-albumose stage, and just reached the peptone stage.

The products of *papain digestion* were found in both cases to consist of traces of proto- and hetero-albumoses, and abundance of deutero-albumose. Peptone was absent. The ferment acted much more readily upon serum-albumin than upon the egg-albumin.

The products of *digestion in presence of yeast* of milk-albumin, as in the maturing of koumiss, consists most probably of the higher

proteids (proto- and hetero-albumoses), but the peptone stage is never reached.

Page 358. According to A. L. Gillespie (*Proc. Royal Soc.*, 1897, lxii. 4), the *contents of the alimentary canal* in the dog, calf, and probably in man, are acid throughout. When the food leaves the stomach, it becomes more concentrated by absorption of water, and hence increasingly acid. It still contains hydrochloric acid combined with proteid (page 378), but the increased proportion of metallic chlorides shows that this acid is being rapidly acted on by the soda of the pancreatic fluid. The organisms present may be classed as those which produce acids and those which produce alkaline substances. The former are generally in excess, and do not, as a rule, liquefy gelatin. The latter are the ordinary putrefactive organisms. The ammonia formed by the action of these unites with the lactic acid formed by the first class, and the salt so formed is advantageous for the further development of both kinds. Excess of hydrochloric acid in the matter leaving the stomach causes a relatively great destruction of the alkali-forming organisms, and thus lessens decomposition in the intestine. Trypsin, although it is slowly destroyed by organic acids, is yet capable of considerable proteolytic action in their presence. The absorption of fluids is greatest in the duodenum and lower ileum. Salol exerts its antiseptic power chiefly in the lower part of the intestine, and on the acid-forming organisms; while calomel acts principally on the alkali-producing organisms, and in the upper region of the bowel.

Page 368. At a meeting of the International Congress of Applied Chemistry held at Vienna in August 1898, Dr Leo Lilienfeld made a communication in which he claimed to have effected the *synthesis of peptone*, or, according to some accounts, of actual albumin, and is said to have demonstrated each step of the process on the spot, and then and there proved by tests the absolute identity of his synthetic product with natural albumin or peptone. The process is said to consist in the condensation of phenol with glycocine (amido-acetic acid) by means of phosphorus oxychloride. The reaction is stated to occur with great readiness, and to yield quantitative results. Bearing in mind the ingenious suggestions of Latham (page 368) it is not improbable that Lilienfeld has made a substantial advance in the direction of the synthesis of proteids, but a process which does not involve the employment of sulphur in any form is obviously incapable of yielding either peptone or true albumin. The hearers of the paper must have been very complaisant if the results of such simple tests as could alone be performed at a congress convinced

them of the identity of the synthetic product with natural albumin; and if peptone was the alleged product its absolute identification as such would be still more difficult.

Pages 403 and 408. In determining small quantities of *carbon monoxide in normal blood*, L. de Saint Martin (*Compt. rend.*, 1898, cxxvi. p. 1036) insists on the necessity of exhausting the liquid twice, the first time to eliminate all gases other than carbon monoxide, consisting of the greater portion of carbon dioxide, oxygen, nitrogen, traces of hydrogen, &c., then, after an interval, a second time in presence of tartaric acid to remove the carbon monoxide mixed with carbon dioxide and traces of nitrogen.

Page 405. Desgrez and Nicloux (*Compt. rend.*, 1898, cxxvi. p. 1526) have recognised the presence of *carbon monoxide as a normal constituent of the blood of animals* living in Paris, and the same observation has been made by Saint Martin. By examining the blood of a country dog, Nicloux has attempted to decide whether the carbon monoxide is slowly fixed by the hæmoglobin of the blood from the air, or generated in the organism. The amount of carbon monoxide found was as great as that in town animals, but Nicloux does not regard the experiment as conclusive.

Page 459. According to J. J. Andeer (*Compt. rend.*, cxxvi. p. 1295), an aqueous solution of phloroglucinol acts as a powerful decalcifying agent on the bones of animals, but is without action on the most delicate organic tissue. If, in addition to this, the bones are treated with hydrochloric acid, the residual ossein will contain no trace of calcium phosphate or carbonate.

Page 468. C. Paal has continued his researches on the *salts of gelatone* (*Ber.*, xxxi. 956; abst. *Jour. Chem. Soc.*, 1898, i. 456).

Page 492. Several patents have recently been obtained for obtaining "*tang-acid*" and other organic products from *sea-weed* (see *English Patents*, 1896, No. 11,538; 1898, Nos. 12,275 and 12,277).

Page 493. E. C. C. Stanford (*Pharm. Jour.*, [4], vii. 199, 209) has proposed the use of *alginates of the heavy metals* (*e.g.*, iron, bismuth, mercury, antimony) in medicine, and claims the advantage that they pass through the stomach unaltered, but are absorbed on reaching the duodenum. Ferric alginate exerts no irritating effect on the stomach, even in presence of gastric ulcer, and has proved valuable in obstinate cases of anæmia.

Page 534. Normal human hair contains no iodine or bromine; but, according to W. Howald (*Zeit. physiol. Chem.*, xxiii. 209), very soon after the administration of the usual medicinal doses of potassium iodide or bromide, iodine or bromine can be detected in the hair.

INDEX.

ERRATA.

Page 138, last line but one, *for* "half the length" *read* "the same length."
Page 138, *delete the words* "and increasing the amount of milk from five c.c. to ten c.c."
Page 173, line 33, *for* "milk" *read* "cream."
Pages 339, 340, 341 and 360, page headings, *for* "Pepsic" *read* "Peptic."
Page 359, line 15; p. 360, l. 3 and 17; p. 367, l. 14; p. 371, l. 2; and p. 374, l. 19, *for* "pepsic" *read* "peptic."
Page 360, line 3; p. 365, ll. 3, 4, and 24; p. 366, l. 1; p. 371, footnote, l. 7; p. 374, l. 20; and p. 382, l. 20, *for* "trypsic" *read* "tryptic."
Page 366, page heading, *for* "Trypsic" *read* "Tryptic."
Page 370, in the structural formula for albumin the CNOH groups should be opposite to the junctions of the brackets.

ERRATA.

Volume III. Part III.

Page 13, line 18, *for* "Its" *read* "It has an," and after "reaction" *insert* "and."
Page 13, line 19, *for* "volumes" *read* "parts."
Page 18, formula of Eseridine *should be* "$C_{15}H_{23}N_3O_3$."
Page 35, footnote 1, last line, in two places, *for* "Non-" *read* "Nor-."
Page 37, line 3, *delete* the comma after "potassium."
Page 81, footnote, line 11, *for* "acid" *read* "baryta."
Page 211, page heading, *for* "Eucine" *read* "Leucine."
Page 274, footnote, line 16, *for* "cyanate" *read* "cyanide."
Page 285, last line but one of text, *for* "dehydration" *read* "hydration."
Pages 317, 319, in several places, formula for hypoxanthine *should be* "$C_5H_4N_4O$."
Page 323, line 27, *for* "phenyl-amidohydoxylactic" *read* "phenyl-amido-hydroxylactic."
Page 324, line 2, *for* "Hydroparaparacoumaric" *read* "Hydroparacoumaric."
Page 332, line 10, *for* "Monomines" *read* "Monamines."

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