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NOTES ON
PRACTICAL PHYSIOLOGY

BY

JOHN MALCOLM, M.D.

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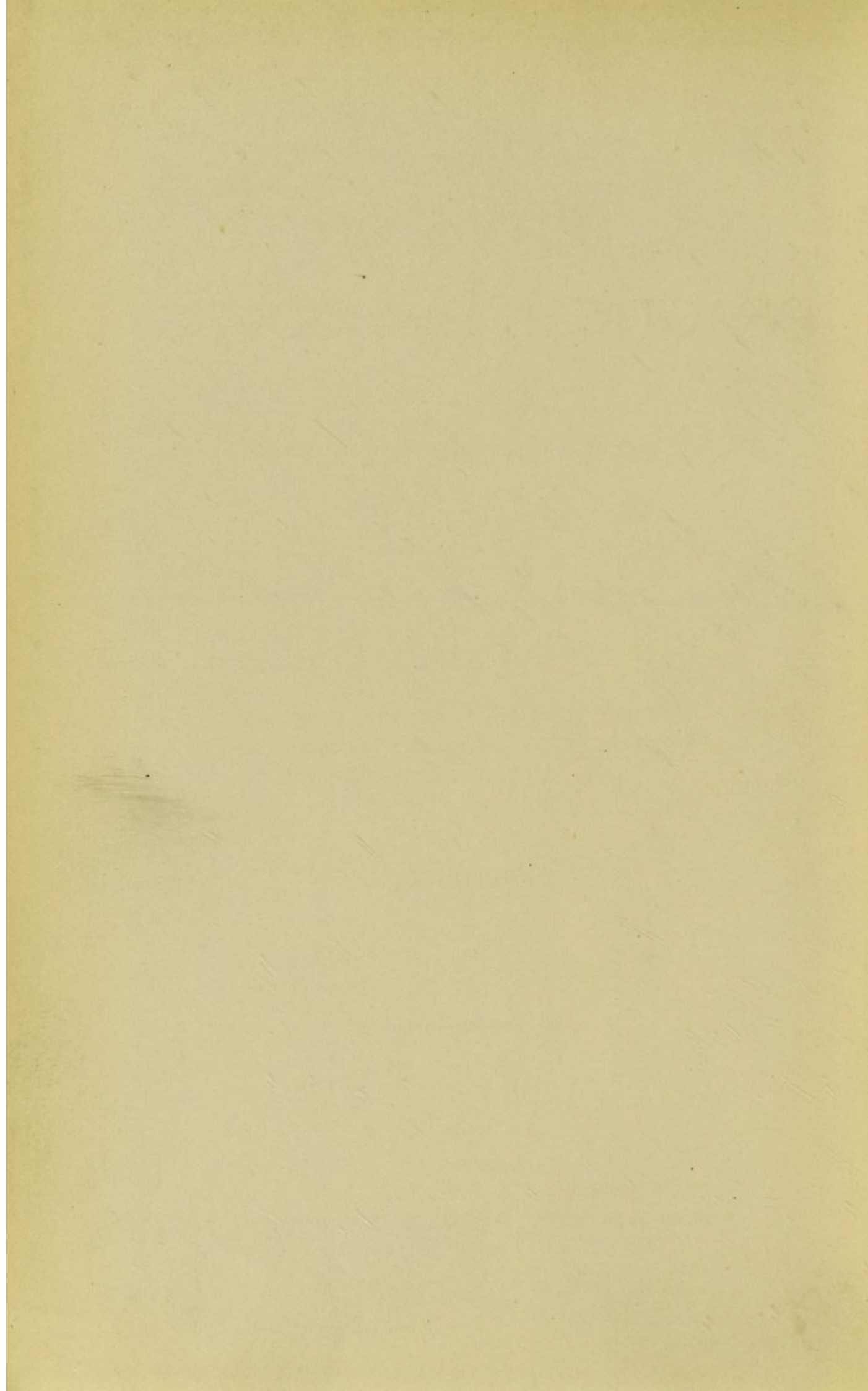
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Professor W. D. Halliburton
with the author's compliments.



NOTES ON PRACTICAL PHYSIOLOGY

For the use of Students of Medicine,

BY

JOHN MALCOLM, M.D. (Edin.),

Professor of Physiology in the University of Otago, and formerly
an Assistant to the Professor of Physiology in the
University of Edinburgh.

Illustrated.

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PREFACE.

This short description of the experiments usually performed in classes of Practical Physiology has been written specially for students attending the class of Practical Physiology at the University of Otago.

The subject is treated in the order of the systematic lectures to enable students to study the theoretical and practical sides of the subject together.

Inasmuch as Physiology, besides being an excellent ground for general scientific training, is a subject of direct importance in Medicine, due attention has been paid to those methods of clinical research that can be profitably pursued by medical students of the second year, but it is almost unnecessary to say that many of the experiments described are not directly applicable to the problems of disease.

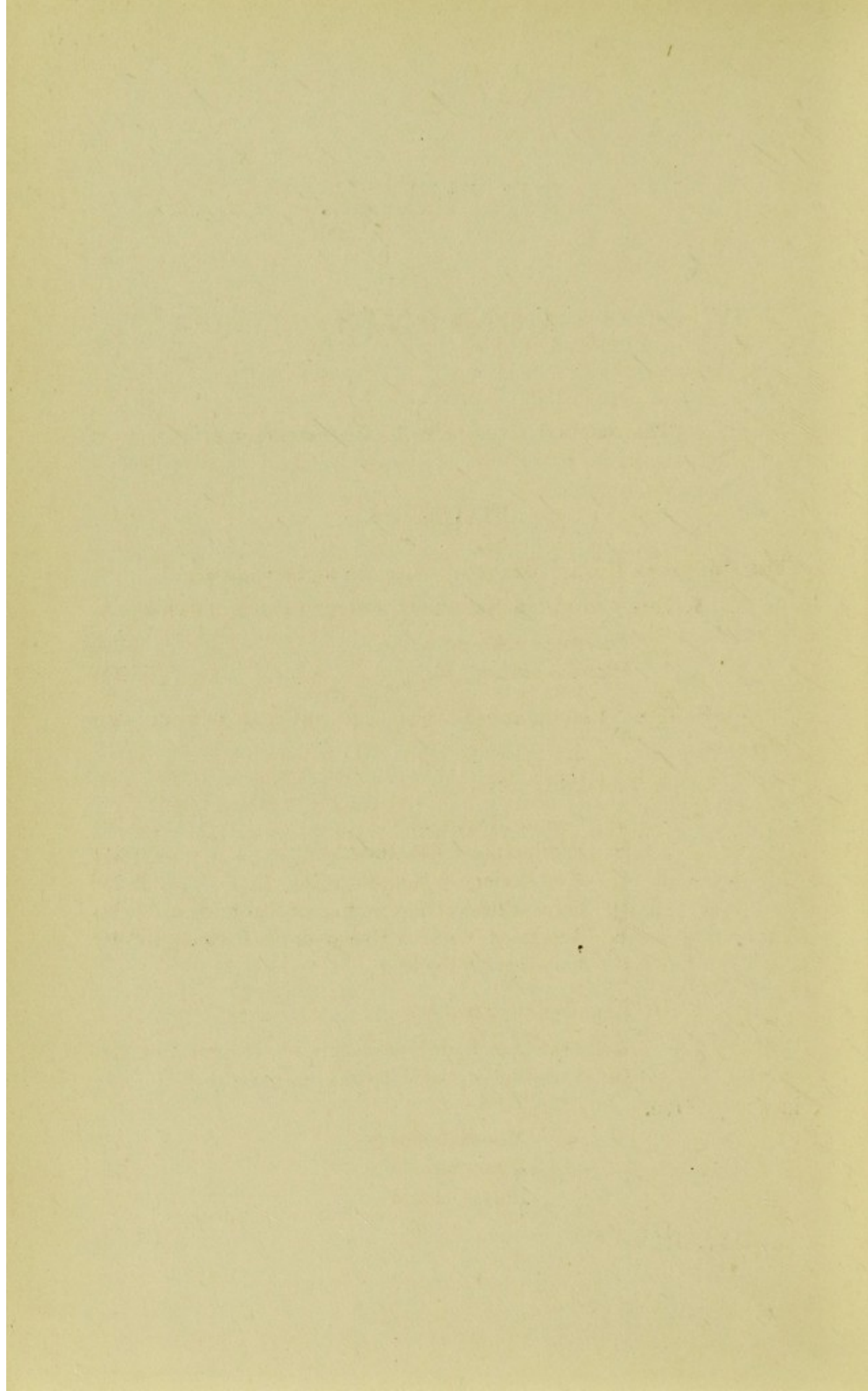
It would be difficult to give a list of all the works consulted in the preparation of these exercises, but for the convenience of students who wish to extend their knowledge, the author can recommend the following books to which he himself is much indebted, viz., the Practical Exercises in Stewart's Manual of Physiology; Milroy's Practical Physiological Chemistry; Porter's Introduction to Physiology; and Practical Physiology by Beddard, Edkins, Hill, Macleod, and Pembrey. For those who can read German, Thierfelder's edition of Hoppe-Seyler's Handbuch der phys. u. path. chem. Analyse, and the Handbuch der phys. Methodik edited by Tigerstedt, now appearing in parts, are the best authorities on chemical and experimental work respectively.

I am indebted to my friend, Mr. J. Brown, for the drawings in perspective.

J.M.

UNIVERSITY OF OTAGO,
DUNEDIN, N.Z.,

MAY, 1909.



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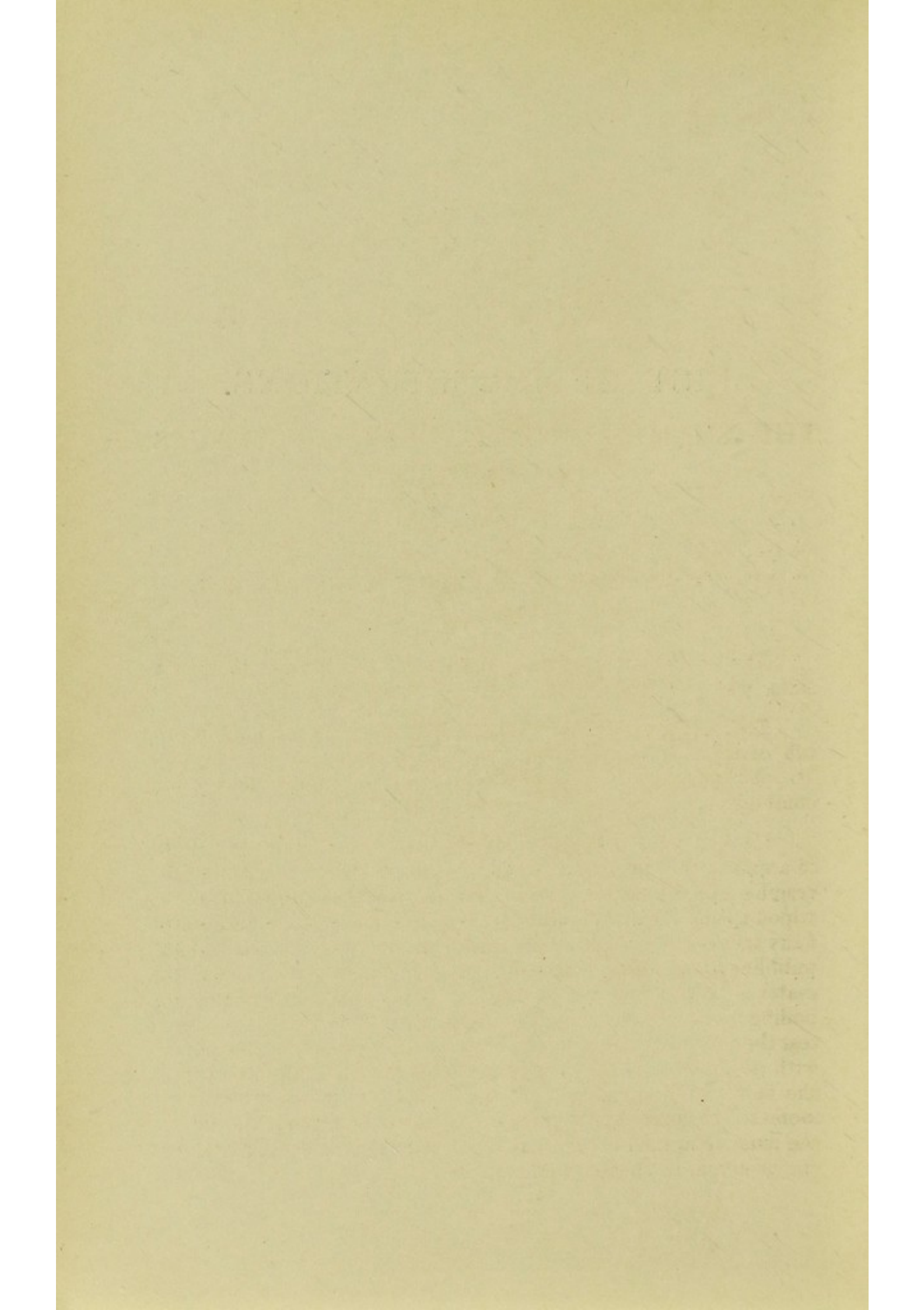
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CHAPTER I.

THE CHEMICAL CONSTITUENTS OF TISSUES IN GENERAL.

(A) THE CHEMICAL ELEMENTS FOUND IN THE TISSUES.

The chief constituents of dead cells are certain inorganic salts, proteins, fats, carbohydrates, extractives and water.

THE INORGANIC CONSTITUENTS may be examined in the ash of any cellular organ such as thymus, pancreas, muscle, etc. To obtain the ash one must destroy the organic matter by combustion or incineration in a crucible.

(1) Put some of the minced and dried tissue, about the bulk of a walnut, in a porcelain crucible. Put on the lid and place the crucible upright on a pipeclay triangle which is supported on a tripod over a bunsen flame. Begin with a small flame and gradually increase it till the whole mass is completely charred and all bubbling has ceased. Allow the crucible to cool, extract the black material with a few c.c. of distilled water previously heated to boiling in a test tube, filter through an ash-free filter paper, and test the filtrate for chlorides (2). Meanwhile, place the filter-paper with its contents in the crucible and, leaving the lid off, re-apply the flame, at first gently to drive off the moisture; when dry, increase the flame and incinerate completely, placing the lid of the crucible in such a way as to direct a current of heated air over the contents, as shown in Fig. 1.

When no more black carbon remains, cool the ash and extract it with 10-20 c.c. of hot distilled water containing a small quantity of HCl. Note the effervescence which sometimes occurs on adding this, due to the presence of carbonates. Filter and use the filtrate for tests (3), (4), (6), (7), (8), (9), (10).

(2) **Chlorides.**—Addition of silver nitrate gives AgCl as a white, curdy precipitate insoluble in nitric acid, soluble in ammonia.

(3) **Phosphates.**—Ammonium Molybdate solution and nitric acid give a yellow precipitate of phospho-molybdate of ammonia, more marked when the mixture is heated nearly to boiling point.

(4) Uranium nitrate and sodium acetate solution give a whitish precipitate when heated. The precipitate is soluble in free nitric acid [see later under estimation of phosphates (269)].

(5) **Carbonates.**—CO₂ results from the combustion of the organic matter and forms carbonates if sufficient alkali is present. This occurs rarely in animal, though usually in vegetable tissues. Effervescence on adding HCl indicates its presence.

(6) **Sulphates.**—Sulphates arise in a similar way from oxidation of the organically bound sulphur of proteins. Barium chloride and HCl give a dull white precipitate. HCl is added to keep in solution the barium phosphate which would otherwise be precipitated.

(7) **Sodium** may be detected by the flame test; and

(8) **Potassium** by addition of sodium-hydrogen-tartrate—fine crystalline precipitate. (N.B.—The solution must first be neutralised).

(9) **Calcium.**—When testing for calcium in an acid fluid such as this, ammonia must first be added till the reaction to litmus is slightly alkaline; then render the fluid slightly acid with acetic or citric acid, and add ammonium oxalate—a precipitate of calcium oxalate forms.

(10) **Magnesium.**—Filter off the precipitate of calcium oxalate obtained in (9) and make the filtrate strongly alkaline with ammonia. Since phosphates are practically always present,

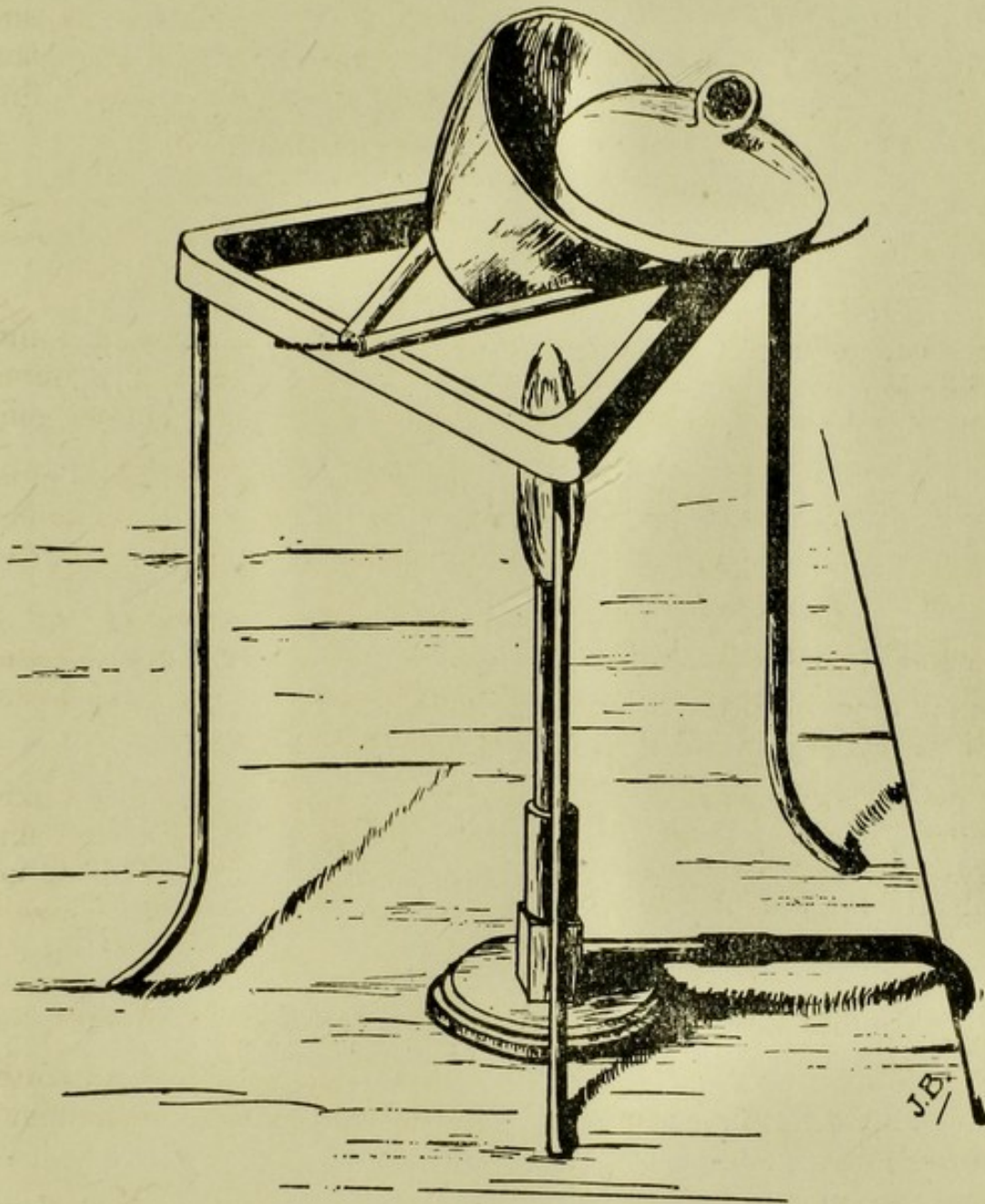


Fig. 1.

Method of using the crucible and lid when completing incineration.

a precipitate of triple phosphate (MgNH_4PO_4) forms if there is sufficient magnesium.

Iron is also present in the ash of most tissues, but is especially abundant in the red blood corpuscles (see 160 and 296).

Of the foregoing, the *chlorine, phosphorus, sodium, potassium, calcium* and *magnesium* form the main bulk of the inorganic constituents.

THE ORGANIC CONSTITUENTS, on the other hand, are *carbon, oxygen, hydrogen, nitrogen, sulphur, phosphorus*, with occasionally other elements such as *iron, iodine, fluorine*, etc. On complete incineration of the tissue many of these form volatile products such as CO_2 , H_2O , SO_2 , and are therefore driven off, unless sufficient alkali is present.

The presence of **carbon** is sufficiently indicated by the charring which occurs on incineration.

(11) **Nitrogen** (Lasseigne's test).—Place in a dry test tube a small amount of the dried tissue such as mince, add a small piece of metallic sodium, hold the end of the tube in the flame till it becomes red hot and then plunge it into a small quantity of distilled water in a porcelain basin; the tube breaks and its contents partially dissolve in the water. Filter, and test some of the filtrate for the presence of a cyanide by adding a few drops of weak ferrous sulphate solution, acidifying with HCl , and then adding a ferric salt, such as ferric chloride:—A precipitate of Prussian Blue forms if a cyanide is present, and indicates the presence of nitrogen in the material originally taken.

(12) **Sulphur** may also be tested for in the same filtrate. Add sodium nitro-prusside—a reddish-violet colour indicates the presence of sulphur as a sulphide.

(13) Another method of demonstrating the presence of sulphur or of phosphorus is to incinerate the tissue in a porcelain crucible with the addition of a considerable amount of dry sodium carbonate. To hasten the oxidation small quantities of potassium nitrate may be added from time to time. After cooling dissolve the residue in distilled water and test for sulphates (6) and for phosphates (3).

(14) **Hydrogen** and **Oxygen** are shown to be present by heating the thoroughly dried material in a dry test tube. Watery vapour forms and condenses in the upper part of the tube.

Iodine occurs in appreciable amount in the thyroid gland (see test 310).

(B.) THE "PROXIMATE PRINCIPLES" OF THE TISSUES AND FOOD.

The elements just mentioned (C, O, H, N, S, P,) are combined with one another so as to form definite molecules of organic substances which can be isolated in more or less pure form. They are the *proteids* (or *proteins*), *carbohydrates*, and *fats*. The tissues also contain small quantities of substances derived from these, such as creatin, xanthin, lactic acid, lecithin, cholesterolin, etc. These are generally spoken of as "extractives."

(I) **PROTEINS** or **PROTEIDS**.—These have certain colour reactions in common, and the majority are precipitated by certain reagents, but there are individual differences which, along with our growing knowledge of the constitution of the protein molecule, form the basis of the classification.

(A) Colour reactions for Proteins in general.

(15) *Xanthoproteic Test*.—To a solution or suspension of protein in water add about an equal amount of nitric acid. Heat to near boiling, cool under the tap, and add ammonia cautiously to the upper part of the solution. If a yellow colour appears on adding the ammonia, or if a yellow colour is produced by heating with the nitric acid and is darkened to orange by the alkali—the result is positive. The presence or absence of a precipitate on adding the nitric acid is to be disregarded.

Apply the test to the following proteins if available: egg-white, gelatine, albumose, keratin, and make notes of the result in each case.

(16) *Millon's Reaction*.—Millon's reagent is prepared by dissolving metallic mercury in strong nitric acid and then diluting with water. It consists of a mixture of mercurous and mercuric

nitrites in nitric and nitrous acid. Add some of the reagent to the protein solution (a precipitate may or may not appear). Heat the mixture. A brick-red colour indicates a positive result. Apply the test to the proteins mentioned above and to 5% carbolic acid (phenol) and note the results.

(17) *Biuret Reaction* (or *Piotrowski's Test*).—To a protein solution add one drop cupric sulphate solution and then some KOH sufficient to make strongly alkaline. A fine violet or pink colour develops if the result is positive. If the result is doubtful add a little more cupric sulphate cautiously, but avoid excess of copper and do not mistake the blue colour of a solution of cupric hydrate in the alkali for the *fine* colour of the reaction. Repeat the test with the proteins available and compare test (252) on the substance biuret itself.

(18) *Glyoxylic or Hopkins' Reaction*.—To the protein solution add an equal amount of glyoxylic acid solution; mix and add concentrated sulphuric acid so as to form a layer at the bottom of the tube. A bluish violet colour develops at the plane of contact (this may take some time to appear).

(19) *Liebermann's Test*.—To some concentrated hydrochloric acid in a test tube add one drop of undiluted white of egg—heat, at first gently then up to boiling—a violet colour appears.

(20) *Molisch's Test*.—To a protein solution add a few drops of a solution of alpha-naphthol in methyl alcohol. Mix, and add some concentrated sulphuric acid. A reddish to violet colour develops at the plane of contact.

The last three tests are not generally used for the detection of proteins. Tests (15), (16), (18), and (19), are due to the presence of an aromatic radicle in the protein molecule and are therefore weak or negative with gelatine solutions and some other proteins which have little or no aromatic radicle. Tests (19), and (20) are due to a carbohydrate radicle, and test (17) to a peculiar grouping of the atoms which is found in practically all proteins.

(B) Precipitation Tests for Proteins in general.

(21) *Coagulation by Heat* may be included under these. Heat some egg white solution and note clouding and formation of a coagulum; this result is facilitated by the presence of neutral salts and a faintly acid reaction. In the absence of these two conditions a dilute solution of protein may become opalescent but does not form a distinct coagulum. Test this statement with a very dilute egg white solution as follows:—Into each of two clean test tubes put about the same quantity of the diluted protein given you; to one add a little sodium chloride; heat both tubes to boiling and note the difference between them. The influence of the reaction may be tested in a similar way.

The temperature at which a coagulum forms is distinctive of some varieties of protein, see (32). Apply the heat test to solutions of the following and note the results—egg-white, globulin, hæmoglobin, gelatine, albumose, caseinogen.

(In testing urine for albumin a few drops of acetic acid should always be added to the urine *after* heating it to boiling. This serves the double purpose of facilitating coagulation and redissolving the precipitate of earthy phosphates which sometimes appears on heating that fluid).

(22) *Mineral Acids*.—To some egg white solution add a few drops of 20% mineral acid (nitric, sulphuric, or hydrochloric) a white precipitate appears in the cold. The “ring” method of application is useful here—hold the test tube containing the protein solution at an angle of about 45° to the vertical and pour the acid slowly down the lower side of the tube. It falls to the bottom, and on holding the tube upright the two fluids will be found one above the other with a white ring or disc of precipitated protein on the surface of the acid.

(Heller’s test consists in the application of nitric acid in this way. It is very frequently used in testing urine).

Repeat the tests with gelatine, caseinogen, and albumose.

(23) *Salts of the Heavy Metals*.—The majority of these combine with and precipitate proteins from their solutions, *e.g.*, mercuric chloride, copper sulphate, ferric chloride, etc. One test

used clinically may be put under this heading—acidify the solution of protein with acetic acid and add a few drops of potassium ferrocyanide, a precipitate forms if albumin or globulin is present. If only traces of these are present the precipitate may take several hours to develop. Gelatine gives this reaction if the solution is not too concentrated.

(24) *Other Precipitants*.—There are many other good precipitants of proteins which are used as qualitative tests, and sometimes for quantitative estimation (in Esbach's tube), sometimes also they are used to rid a solution of all protein matter. Some of these are *tannic acid*, *picric acid*, *trichloroacetic acid*, *salicyl-sulphonic acid*, *potassio-mercuric iodide with hydrochloric acid*, etc. Test as many of these reagents as are available. *Alcohol* is also used in the same way. When the precipitate first forms it can be redissolved in water, but after long contact with the alcohol it becomes insoluble in water, *i.e.*, coagulated.

This illustrates the difference between the terms “precipitation” and “coagulation” of proteins.

(C) The action of the Neutral Salts on Protein.

These have an important action on proteins in solution, and they are used to test for the presence of a protein by precipitation but to a larger extent to remove one or all proteins completely from solution. The precipitated protein can be recovered unchanged from the precipitate, so that the action is more physical than chemical.

The salts are used in the following ways:—

(a) *Full Saturation*.—For this it is necessary to add the solid crystals of the salt to the protein solution and shake till no more crystals dissolve. The fluid must not be heated if it contains proteins which are coagulable thereby, otherwise it may.

(b) *Half Saturation*.—For this a fully saturated solution of the salt is required and is mixed with an equal quantity of the fluid to be treated. The result, on thorough mixing, is half saturation of the whole fluid. This half (or 50%) saturation is the degree of partial saturation most commonly employed, but the solubility of proteins in partially saturated solutions of salts has

been more carefully worked out, *e.g.*, if we take serum-globulin solution and add fully saturated ammonium sulphate solution, precipitation of the globulin begins when the mixture contains 29% of the saturated salt solution, and becomes more and more marked as the percentage of salt solution increases till 46% is reached, at which point all of this globulin is thrown down, so that in this case half saturation (50%) is more than enough to precipitate all the substance. "The limits of concentration necessary to initiate and to complete precipitation of different proteins are as characteristic of each special protein as is the solubility of a crystalline substance." (Cohnheim).

Test the truth of the following statements with the reagents and material available :—

(25) *Ammonium Sulphate in full Saturation precipitates all proteins except peptones.*—To some egg white solution add crystals of ammonium sulphate and shake vigorously till no more of the salt will dissolve. A precipitate forms—filter and test the filtrate for protein (15 or 16) no result—because egg white contains no peptone.

Repeat the test on an albumose and peptone mixture. A precipitate of albumose forms and the filtrate gives a positive result when tested for protein by (17).

[In applying the biuret test to a fluid containing an ammonium salt, sufficient alkali must be added to displace the ammonia, therefore make a very concentrated solution of KOH or NaOH by dissolving some of the solid alkali in a small quantity of water, cool, and add this instead of the ordinary KOH.]

Repeat with a gelatine solution and note how the precipitate frequently floats up to the surface of the heavy solution of Am. SO_4 .

(26) *Ammonium Sulphate in half saturation precipitates globulins, primary albumoses, caseinogen, and some other proteins.*—To some 1 in 5 egg white solution add an equal amount of saturated Am SO_4 , a precipitate of egg globulin forms, filter and test the filtrate for egg albumin by (15), (21), or other test, *e.g.*, full saturation with Am SO_4 (25). Repeat the half-saturation with a mixture of albumoses (primary and secondary). Filter

and test the filtrate in this case by some of the colour tests (15), (16), or (17): the secondary albumose which is present is not precipitated by the half-saturation with AmSO_4 and is found in the filtrate.

Repeat the half saturation with caseinogen and any other protein available.

(27) *Sodium Sulphate* in full saturation at a temperature of 37°C acts in the same way as full saturation with AmSO_4 at room temperature.

Test this on egg white solution using the water bath to warm the mixture.

(28) *Magnesium Sulphate* in full saturation at room temperature acts in the same way as half-saturation with AmSO_4 .

Test this as in (26). The solution must be shaken for a considerable time with the MgSO_4 to ensure full saturation.

(29) *Sodium Chloride* in full saturation precipitates all globulins, caseinogen, and nucleo-proteins.

It may be noted here that while certain proteins are precipitated by concentrated solutions of salts, a small percentage of neutral salt is required to keep some proteins in solution, especially the globulins, and it is chiefly sodium chloride which seems to play this part in the body.

(D) Some Physical Properties of Proteins.

Indiffusibility.—Most proteins are unable to diffuse through animal or parchment membranes, *i.e.*, they belong to the class of bodies known as colloids.

(30) Place some blood serum (1 in 5) in a parchment tube, tying one end securely to form a test tube of it, and suspend it in a jar of water. After a few days test the water in the jar. It will be found to contain most of the salts, *e.g.*, chlorides, but no protein. The tube will contain the proteins and some of the globulin (eu-globulin) is seen to be precipitated due to removal of the salts.

Filter some of the contents of the dialyser, and half-saturate with AmSO_4 —a precipitate (pseudo-globulin) will appear.

In spite of their colloid nature some proteins are *crystallisable* *e.g.*, Hæmoglobin.

(31) To a drop of rat's or of guinea pig's blood on a slide add a similar amount of distilled water, cover and allow the fluid to evaporate slowly at room temperature. Crystals of Hæmoglobin appear at the edges—note their shape. Some proteins appear naturally in crystalline form in seeds, and others can be crystallised by partial saturation of their solutions with ammon. sulphate and adding acetic acid till a precipitate just begins to be visible. Examine crystals of serum albumin of horse's blood which have been made in this way.

(32) *Temperature of Heat Coagulation.*—The point at which this occurs varies with the amount of protein in solution, the salts present, and the degree of acidity. The more marked these conditions are, the lower is the temperature of coagulation, but in spite of these difficulties the temperature of coagulation is of value in recognising some classes of proteins, *e.g.*, those found in muscle.

Make the fluid (*e.g.*, egg white 1 in 5) faintly acid with acetic acid, put a thermometer in the test tube, and place this within a second larger test tube half full of water, and then put the whole into a water bath heated by a moderate-sized flame. Allow the temperature to rise very gradually, stirring the protein solution occasionally with the thermometer. Note the position of the mercury when distinct cloudiness occurs; try to keep the fluid at this temperature for a little till a distinct flocculent coagulum forms. Filter into a fresh tube and repeat the experiment with the filtrate; another coagulum may be obtained at a higher temperature than the first. Since egg white is a mixture of several proteins, coagula may occur at 50°—60°, at 64° (albumins) and about 75° (ovo-globulin).

(E) Action of various reagents on Proteins.

Alkalis.—Strong alkalis (NaOH or KOH) added to concentrated protein solutions cause swelling and formation of a jelly of alkali-albumin (Lieberkuhn's Jelly).

(33) Make this jelly by adding 23% NaOH to undiluted egg white. If this be now diluted with an equal amount of water and heated, or

(34) If 10 % KOH be added to egg white and heat applied, there results a decomposition of the proteins and ammonia is evolved—test for this by holding moist red litmus paper over the mouth of the test tube.

(35) If the contents of the tube be now cooled and a few drops of dilute sodium nitroprusside added, a reddish violet colour appears indicating the presence of a sulphide.

(36) Or the sulphide may be tested for by the addition of neutral lead acetate (black ppt.). This shows that the action of strong alkalis and heat on proteins is a very penetrating one, some nitrogen is split off as ammonia and some sulphur which combines with the alkali to form a sulphide (“loosely combined sulphur”). Less energetic action of heat (40°C) and weak alkali cause formation of alkali-albumin as shown in the next experiment.

(37) To some 1 in 5 egg white add several drops of 10% KOH. and place in the water bath at 40° C. for ten to twenty minutes, The proteins become changed into alkali-albumin.

(38) Test this solution as follows:—boil—no coagulation results; neutralise with acetic acid after adding a drop of litmus solution to act as indicator—a precipitate comes down which dissolves in excess of the acid.

(39) *Acids*.—Concentrated acids also cause formation of a jelly when acting on concentrated protein solutions. To some undiluted egg white add some glacial acetic acid—a jelly results which is more opaque than Lieberkuhn's.

(40) To some 1 in 5 egg white add some 10% acetic acid and keep at 40° C. (as in 37 above) for twenty minutes—acid-albumin is formed.

(41) Test this in similar fashion to (38):—boil—no coagulation; add litmus solution and neutralise—acid-albumin is thrown down and redissolves in excess of the alkali.

On the whole the action of acids and heat on protein is less penetrating than that of alkalis, but the result of long continued action is the same—the meta-protein is converted into proteoses,

peptones, and finally into the amido acids, hexone bases, etc., which are the ultimate decomposition products of proteins.

Action of Ferments.—Two classes of ferments act on proteins :—

(a) Some proteins are coagulated by specific ferments, *e.g.*, caseinogen (by rennin), fibrinogen (by fibrin ferment), myosinogen and paramyosinogen (ferment doubtful).

(b) All proteins are split by the digestive ferments pepsin, trypsin, erepsin, into their meta-protein derivatives and ultimately into the amido acids, etc.

These will be studied in connection with milk, blood, digestion, etc.

(F) Varieties of Proteins.

(a) *Protamines.*

(b) *Histones.*

(c) *Albumins*, found free in nature, *e.g.*, egg albumin (present in egg white) serum albumin of blood, lact-albumin, etc. These give all the typical protein reactions and can be isolated from globulins by neutral salts.

(d) *Globulins* generally occur along with albumins, are insoluble in distilled water (except the pseudo globulins) and insoluble in concentrated solutions of neutral salts.

Repeat experiments (26) and (28) with an egg white or serum.

(e) *Sclero-proteins* (albuminoids) *e.g.*, collagen gelatin, keratin, etc. See under Connective Tissue, tests (81), (82), (78).

(f) *Phospho-proteins* (nucleo albumins), *e.g.*, caseinogen, vitellin, see under milk and eggs.

(g) *Conjugated proteins*, or protein united to a prosthetic group, *e.g.*, nucleo-protein (nuclein + protein), gluco-protein such as mucin (protein + a carbohydrate), chromo-protein such as haemoglobin (protein + hæmatin).

(h) Derivatives of Protein.

(1) *Meta-proteins* or albuminates, *e.g.*, acid and alkali albumin, Cu- Fe- and Hg-albuminates.

(2) *Proteoses* or albumoses.

(3) *Peptones*.

(4) *Polypeptides*.

These varieties of protein will be considered under the different tissues and fluids, and under digestion.

II. CARBOHYDRATES.—The chief carbohydrates found in the body are glucose, lactose, maltose, glycogen, and dextrin. Cane sugar and starch are important food stuffs, and will also be considered here.

(A) **Tests for reducing Sugars in general.**—Glucose or dextrose is the aldehyde of a hexatomic alcohol. Owing to the presence of the aldehyde group, CHO, it can absorb oxygen from substances capable of yielding it and so form acids, COOH. This occurs most readily in hot alkaline solutions, and the newly formed acids unite with the alkali. Consequently all “reduction” tests are done in alkaline solution [except Barfoed’s test, (44)], .

(42) *Trommer’s Test.*—To some glucose solution add some KOH and a few drops of cupric sulphate solution. Flakes of cupric hydrate appear, which are dissolved by the glucose to form a blue solution. Continue adding the CuSO_4 till no more flakes will dissolve and then heat. A yellow coloured ppt. which changes to red appears just below boiling temperature. This indicates “reduction,” the cupric hydrate, $\text{Cu}(\text{OH})_2$, being reduced to cuprous hydrate, $\text{Cu}_2(\text{OH})_2$, and from this red cuprous oxide, Cu_2O , separates out. In applying the test to some solutions, *e.g.*, starch, black flakes of cupric oxide (CuO) may appear—this of course does not indicate reduction.

(43) *Fehling’s Test.*—

[Solution A., 34.64 grm. crystals of cupric sulphate in 500 c.c. water; solution B., 173 grm. sodium potassium tartrate + 60 grm. sod. hydrate in 500 c.c. water. Mix exactly equal amounts of A. and B. If kept for some time Fehling’s solution may reduce of itself on boiling, therefore test before use.]

Take some Fehling's solution in a test tube, heat to boiling. If no reduction occurs add about an equal amount of the solution to be tested and heat again to boiling—if glucose is present in sufficient amount reduction appears just at or before boiling point. In this case the red oxide frequently separates out more readily than in Trommer's Test.

If ammonia is added to the Fehling's solution in sufficient amount, it holds the reduced oxide of copper in solution so that the result is a clear colourless fluid which gradually deposits Cu_2O as the ammonia boils off (cf. Pavy-Fehling 276b).

(44) *Barfoed's Test* (cupric acetate in acetic acid solution). This is the only reduction test commonly employed where the reaction is acid. Glucose is able to reduce it. Mix equal quantities of the reagent and the carbohydrate solution and heat to boiling point, but do not prolong the boiling for more than one minute. Cuprous oxide appears when the result is positive.

Maltose and lactose give negative results with this test, and even with glucose the reduction is not very marked. Apply the test to solutions of these three sugars.

(45) *Boettger's Test*.—Add a little bismuth subnitrate powder to the glucose solution and about double that amount of dry sodium carbonate. Boil for a short time. A black or grey deposit indicates reduction of the bismuth salt.

Nylander's Solution is sometimes employed instead of the dry bismuth subnitrate. It bears the same relationship to Boettger's Test that Fehling's does to Trommer's.

(46) *Ammoniacal Silver Nitrate Solution* gives a mirror of reduced metallic silver on heating with a reducing sugar.

(47) Several organic pigments are reduced and show colour changes or loss of colour when heated with glucose in alkaline solution. To some *saffranin* solution in a test tube add several drops of KOH and some of the solution to be tested; mix, heat to boiling—the red colour changes to a yellow if a reducing sugar is present. Cool, shake up with air, and note return of the colour.

Repeat the test with *indigo-carmin*e solution. In many of the reduction tests the substance reduced may be re-oxidised by shaking with air or oxygen. This occurs easily in the foregoing test, and it can also be shown with the copper reductions if the test tube is allowed to stand till next day. The oxidised sugar is unaffected.

(48) *Moore's Test*.—Heat the glucose solution with some caustic alkali, a yellow to brown colour appears due to formation of caramel. This test is given by all reducing sugars.

(49) *Phenyl-hydrazine Test* (Neumann's Method).—Mark the level of 3 cc. on a large wide test tube by running in that amount of water from a burette. Empty the tube. Put into it 5 c.c. of the suspected fluid, 2 c.c. of 50% acetic acid saturated with sodium acetate, and two drops of fluid phenyl-hydrazine. Heat the mixture on the open flame and keep it nearly boiling until the volume of fluid is reduced to 3 c.c. Cool, examine the deposit with a microscope for yellow needle-like crystals of the osazone arranged in feather or double fan shapes. If the deposit is amorphous heat again to boiling point and allow to cool slowly. The crystals of the various osazones (phenyl-glucosazone, phenyl-lactosazone, phenyl-maltosazone, etc.) are recognisable under the microscope, but more exactly by determination of the melting point of the purified crystals. The older method of applying the test is to add a pinch of crystalline phenyl-hydrazine hydrochloride and double that amount of sodium acetate and heat on a boiling water bath for half an hour or more. Crystals of the osazone separate out on cooling.

(50) *Fermentation Test*.—Add some baker's yeast (or cake yeast which has been soaked in water to expel entangled air) to the glucose solution and place the mixture in some form of tube which will allow of the detection of gas formation (Southall's ureometer or a special fermentation tube). Keep in a warm place, preferably at 40° C., for several hours, and examine for gas (CO₂) formation. Two control experiments should also be set on, one with yeast and a fermentable sugar (glucose) to prove whether the yeast is active, and one with yeast and water to see whether the added yeast contains enough adherent sugar to cause gas formation on being fermented.

(51) The *Polarimeter* is used to detect the presence of optically active substances such as sugars, and also to estimate the amount present where the nature of the sugar is known.

The solution must be sufficiently transparent to allow light to penetrate the whole length of the tube, and for this it is generally necessary in the case of urine to add some solid lead acetate, shake thoroughly, and filter through a dry filter paper into a dry vessel.

The method of manipulation depends on the form of polarimeter used.

In using Fleischl's *Spectro-polarimeter* direct the instrument towards a bright part of the sky, place the "null-punkt" or empty tube in the body of the apparatus and examine the field through the eyepiece. Two spectra, each crossed by a dark band, will be seen, one above the other. Turn the analyser till these two are exactly over each other and read the zero point, which may or may not coincide with the zero of the scale. Repeat several times and take the average. Now substitute for the empty tube the tube filled completely with the solution to be tested and examine the field again. If the bands have shifted one past the other the substance is optically active, and according as the analyser has to be turned to the right hand or to the left to make the bands coincide, the substance is respectively dextro-rotatory or laevo-rotatory. When the bands are exactly superimposed the percentage of glucose present can be directly read off on the scale in this form of polarimeter. Use the vernier to get the first decimal place, and take the average of several readings.

(B) Varieties of Carbohydrates important in Physiology.

I. *Monosaccharides* with the general formula $C_6H_{12}O_6$ (= hexoses) and $C_5H_{10}O_5$ (= pentoses).

(a) *Glucose* has been already studied (42)—(51).

(b) *Laevulose* occurs in the alimentary tract from inversion of cane sugar in food. It gives results similar to glucose, with tests (42—50)., but rotates the plane of polarised light to the left.

(52) On heating a laevulose solution with an equal amount of HCl and a few grains of resorcin, the fluid becomes a deep red and a brownish red ppt. separates out.

(c) *Galactose* occurs along with glucose as a result of the inversion of lactose.

(d) For *Pentoses* see under urine (278).

II. *Disaccharides*, general formula $C_{12}H_{22}O_{11}$.

(a) *Cane Sugar*.—A food substance. Does not reduce if pure, try any or all of tests (42—48). Forms no osazone (49).

(53) On boiling a solution of cane sugar with a few drops of HCl, or under the influence of inverting ferments at body temperature, cane sugar splits into dextrose and laevulose, and the fluid will now give all the tests for these. Repeat the reduction tests and (52) on this solution. Remember to neutralise the HCl present before applying the reduction tests.

(54) Concentrated sulphuric acid when applied by the “ring” method causes a black (charred) band to appear at the plane of contact with the cane sugar solution.

(55) Cane sugar slowly ferments with ordinary yeast. This is an indirect effect due to an inverting ferment in the yeast which splits the sugar into monosaccharides; these then undergo alcoholic fermentation with development of CO_2 .

(b) *Maltose*.—A di-saccharide resulting from the digestion of poly-saccharides such as starch. It gives positive results with all the reduction tests, but is slower and less powerful in this respect than glucose. It does not give Barfoed's Test (44). It forms phenyl-maltosazone (49) the crystals of which are coarser than those of glucosazone. It ferments easily (50). Test the truth of these statements on a solution of maltose. On inversion one molecule of maltose yields two of glucose.

(c) *Lactose*.—A di-saccharide found in milk. It gives all the reduction tests except Barfoed's. With phenyl-hydrazine it forms lactosazone, the crystals of which are finer and more

closely matted together into balls than those of glucosazone are. It does not ferment with ordinary yeast. On inversion it splits into glucose and galactose.

III. *Poly-saccharides*—general formula $(C_6H_{10}O_5)^N$.

(a) *Starch*.—The chief form of carbohydrate in food.

(56) Examine microscopically starch grains in a scraping from potato or in wheat, flour, etc.

(57) Add some powdered starch to cold water and note its imperfect solubility. Heat to boiling; an opaque or opalescent appearance results. The grains burst and swell up forming a mucilage or imperfect solution. Cool and use this mucilage for tests (58) (62).

(58) *Iodine test*.—To starch mucilage add iodine solution (dissolved in KI) a deep blue colour results which appears black if concentrated starch mucilage is used, so that to see the blue colour it may be necessary to dilute with water. This blue iodide of starch is dissociated by heating and re-forms on cooling if all the iodine has not been driven off. Alkali, if added, combines with the iodine and a colourless solution results: the test must therefore be done in the cold and in neutral or acid solution.

(59) Pure starch solutions do not reduce cupric hydrate. do Trommer, Fehling or other reduction test.

(60) *Tannic Acid* precipitates starch.

(61) Starch is colloid, does not diffuse through a parchment dialyser and is precipitated by neutral salts. Fully saturate a starch solution with ammonium sulphate. Note precipitate. Filter and test filtrate with iodine solution.

(62) On *hydrolysis* starch mucilage yields reducing sugars.

(a) Add some hydrochloric acid to starch mucilage and boil for several minutes—the solution becomes clear; cool, neutralise the acid present and test for a

reducing sugar. The starch has been converted into dextrins, maltose, and this again into glucose.

- (b) To starch mucilage add some saliva and keep in the water bath at body temperature (37°C) for a few minutes. It will now reduce $\text{Cu}(\text{OH})_2$ owing to the presence of maltose (and dextrins).

(b) *Dextrins*.—A series of bodies with the same general composition as starch, but of smaller molecule.

The two chief varieties are Erythro-dextrin and Achroö-dextrin. They result from the hydrolysis of starch and glycogen by acids or digestive ferments and the steps in the process are as follows:—The starch is first converted into *amidulin*, or “soluble starch”; this is shown by the previously opalescent mucilage becoming clear while at the same time iodine gives a blue colour with the solution; *erythrodextrin* is next formed and the fluid now gives a red colour with iodine; *achroö-dextrin* then results and iodine now gives no colour reaction; last of all the sugars are formed. If the hydrolysis has been carried out with diastatic ferments (ptyalin, amylopsin, etc.) *maltose* is the end product, but if HCl is used the maltose is converted further into *glucose*. It ought to be mentioned, however, that some maltose appears early in the hydrolysis, that it is accompanied by traces of another disaccharide, iso-maltose, and that in digestion experiments *in vitro* it is difficult to convert the whole of the dextrins into the maltoses unless these products are removed as they are formed.

Test some commercial dextrin as follows:—

(63) Add iodine—note the reddish violet to reddish brown colour—test the effect of adding KOH to this and of heating as in (58).

(64) Dextrin solutions reduce cupric hydrate (43).

(65) Hydrolyse some dextrin solution (62a) and note the increased reduction power.

(66) Basic lead acetate gives no precipitate with dextrin solutions.

Note also the smell and taste of dextrin.

The different varieties of dextrin can be isolated by their insolubility in alcohols of different strengths and by precipitation with neutral salts.

(c) *Glycogen*.—"Animal Starch" found in liver, muscles, leucocytes and other tissues of the body. It forms an opalescent solution in water.

(67) Pure glycogen solutions do not reduce. Do Fehling's or Trommer's test.

(68) On hydrolysis with weak HCl as in (62a)—glucose results—Repeat the test for reduction employed in (67).

(69) Iodine solution gives a mahogany red colour with glycogen solutions which behaves like iodide of starch, when heated or if the reaction is changed (58).

(70) Glycogen is completely precipitated when its solution is saturated with ammonium or magnesium sulphate. This serves to distinguish it from Erythro-dextrin, which is not completely precipitated. Compare the results of saturating a dextrin solution and a glycogen solution with AmSO_4 . In the one case the filtrate gives a red reaction with iodine, which does not occur in the case of glycogen.

(71) Basic lead acetate precipitates glycogen but this cannot be depended on as a distinction between it and dextrin.

III. FATS.—Fats are combinations of glycerine and fatty acids the chief varieties of which are palmitic, stearic, and oleic. These occur in varying proportions in the fat of the body and food, and these variations account for their differences in fluidity—the more olein there is, the softer is the fat and the lower its melting point.

The fatty acids may be tested for by saponification of the fat.

(72) *Saponification*.—To some melted fat in a test tube add an excess of caustic potash in alcohol, and keep the mixture in

a boiling water bath till all the oiliness has disappeared. The contents of the tube should now be perfectly miscible with water for, by the action of the alkali and heat, the fat has taken up water and has split into glycerine, and fatty acid which unites with the alkali to form a soap.

Add some of the solution to water in a test tube and shake—a froth forms.

Add some of the solution to an excess of warm 20% sulphuric acid in a test tube. The soap is decomposed and the free fatty acid which results floats to the top in the form of oily globules which harden on cooling, and then resemble wax.

Add some of this to caustic potash, it dissolves, again forming a soap.

(73) The glycerine part of the fat may be tested for by the *Acrolein* reaction. To some fat in a dry test tube add more than the same bulk of potassium bisulphate crystals and heat strongly. A vapour which is very irritating to the nose and eyes is given off. The cause of the irritation is a volatile aldehyde, acrolein. Like other aldehydes this is a reducing substance and if a piece of filter paper moistened with ammoniacal silver nitrate is held in the vapour it is blackened, cf. test (46).

CHAPTER II.

THE ANIMAL CELL AND SIMPLE TISSUES.

Slides illustrating the structure of typical cells and simple tissues are to be examined at this stage, and experiments on amoeboid and ciliary movement performed (See Schäfer's Essentials of Histology).

(A) CHEMISTRY OF PROTOPLASMIC CELLS AND SIMPLE TISSUES.

When extracts are made of very cellular organs such as the thymus gland, pancreas, lymphatic glands, the most characteristic substances obtained are **nucleo-proteins**.

(74) Minced thymus gland or pancreas is to be extracted over night with ammoniacal water, then strained several times through flannel. From this solution the nucleo-protein may be precipitated by the cautious addition of acetic acid; avoid excess of acid as the precipitate is soluble therein. Allow the nucleo-protein to settle, pipette off the supernatant fluid, add some artificial gastric juice to the deposit, and place it in an incubator at 40°C to digest.

Next day an insoluble residue of **nuclein** will be found—draw some of this up in a pipette and note that, like the nucleo-protein itself, it dissolves in alkalis and is precipitated by acids.

Filter the solution through an ash-free paper and incinerate the nuclein and paper together in a porcelain crucible, first adding a few pinches of pure sodium carbonate. Cool, dissolve the ash in weak nitric acid and test for phosphates (3) (4).

Besides nucleo-proteins, dead cells contain albumins and globulins (though probably these are combined with nuclein during life), inorganic salts, and two substances, lecithin and cholesterin, which are constantly present in protoplasm, besides traces of metabolic products and food materials.

Lecithins are complex fats which on hydrolysis yield glycerine (1 mol.), fatty acid (2 mol.), phosphoric acid (1 mol.), and a poisonous basic substance, choline.

Note the physical appearance of a lecithin, its solubility in chloroform and ether, and the presence of phosphorus (demonstrated).

Cholesterin is a crystallisable substance of large molecule ($C_{27}H_{46}O$) occurring in bile, medullary sheath of nerves, etc.

It is soluble in ether and in hot alcohol. The *crystals* are rhombic plates, frequently having one of the acute angles defective.

(75) Examine these dry, and then allow some 1 in 5 sulphuric acid to run in under the coverglass—the crystals become red and then violet. If a little iodine solution be added to the drop of H_2SO_4 before applying it to the crystals, a play of colours results—violet, blue, green, and red.

(76) *Salkowski's Reaction*.—To a chloroform solution of cholesterin in a dry test tube add an equal volume of concentrated sulphuric acid. The upper (chloroform) layer becomes red while the lower layer shows a green fluorescence. On pouring off the chloroform layer into a shallow basin the colour changes to blue, green, and finally yellow.

(77) Evaporate gently on open flame some of the cholesterol solution on a piece of porcelain after adding a few drops of nitric acid. A yellow residue remains which becomes red on the addition, before cooling, of a drop of ammonia. This reaction resembles the murexide test for uric acid. (See 261).

(B) CHEMISTRY OF EPITHELIAL TISSUES.

Besides the constituents common to all cells, the epithelial tissues contain Keratin and Mucin.

(78) **Keratin.**—A sclero-protein or albuminoid is the chief constituent of the epidermic scales, hair, nail, hoof, feathers, etc.

With some horn shavings verify the following:—

The Keratin is insoluble in water and acids; it swells in cold alkali and partially dissolves in hot; it gives the test for “loosely combined sulphur” very distinctly (35), (36): also Millon’s and the xanthoproteic reactions for protein (the particles take on the characteristic colour) (15) (16).

(79) **Mucin.**—A conjugated protein of the gluco-protein class, produced by “goblet” cells, and present in saliva and mucous secretions generally.

Filter some saliva, add a drop of acetic acid—note the white precipitate of mucin insoluble in excess of the acid. If a sufficient amount of this precipitate can be obtained, boil it with weak HCl for some time—cool, neutralise, and test for reducing sugar.

(C) CHEMISTRY OF CONNECTIVE TISSUES.

The **Mucin** of connective tissue differs in some particulars from that of saliva. It can be extracted from tendons by lime water or weak alkali, which destroy salivary mucin.

(80) Precipitate the mucin from a lime water extract of tendon with *acetic acid*—collect the precipitate and hydrolyse as

above. Acid albumin will be present, formed from the protein part and a reducing sugar from the prosthetic part of the mucin.

Collagen.—A sclero-protein, is a very insoluble substance.

(81) It gives the protein colour reactions (15), (16), and (17); it swells up and becomes clear on being soaked in acetic acid; and on boiling with water it forms gelatine.

(82) **Gelatine.**—The hydrate of collagen; in strong solutions sets to a jelly—under $\frac{1}{2}\%$ it remains fluid at room temperature. On a gelatine solution perform the usual protein reactions, and note the peculiarities. (17) Biuret—violet colour; (16) Millon's, faint or absent; (15) Xantho-proteic weak, usually only a faint yellow appearing after addition of the ammonia; (18)-(20) glyoxylic, Liebermann's and Molisch's tests—negative; (conclusion—the aromatic radicle only feebly represented in the gelatine molecule); (21) boiling does not coagulate gelatine; (22) nitric acid causes no precipitate; (23) Potassium ferrocyanide and acetic acid cause a precipitate in weak solutions; it is precipitated by full saturation with ammonium sulphate and magnesium sulphate, (25) and (28).

Elastin.—A sclero-protein from elastic tissue is insoluble in water—it gives the protein colour reactions (15), (16). etc.

Bone and Dentine consist of two-thirds inorganic and one-third organic matter.

(83) Take a piece of dried bone about the size of a large pea and incinerate it in a porcelain crucible arranged as shown in Fig. 1. Note the blackening which occurs indicating the presence of carbonaceous matter. Continue the incineration till this completely disappears, cool, add about half full of the crucible of hot distilled water and drop in nitric acid gradually till no more of the residue dissolves. Filter to remove unconsumed carbon, and test the filtrate for phosphates (3), calcium (9), magnesium (10). Note the great preponderance of the first two of these.

(84) Decalcify some of the same bone in diluted nitric acid or in sulphurous acid and examine the residue for collagen (81).

(The chemistry of muscle is considered at the end of Chapter III.).

CHAPTER III.

MUSCLE AND THE ACTION OF STIMULI IN GENERAL.

(Examine slides showing the structure of striped, cardiac, and non-striped muscle).

The physical properties of muscle which require special study are **EXTENSIBILITY** and **ELASTICITY**.

(85) **Prepare a Recording Drum** for taking a tracing by covering it with a special glazed paper. First place the paper on the desk with the smooth surface downwards and the gummed edge at the far end facing you. Remove the cylinder from the apparatus and place it across the paper with the upper end to the right hand side; wrap the paper firmly round the cylinder and secure it by overlapping and fixing the gummed edge. Light a piece of camphor placed on a porcelain slab and rapidly rotate the paper in the flame or use a fish-tail gas burner applied to the drum revolving *in situ*. Aim at getting a slight uniform coating of soot all over the paper. If the first attempt fails, wipe off the whole of the blackening and repeat the process. Next, prepare the myograph lever for this experiment by reversing the lever in the elbow-piece so that it can fall clear of the wooden part of the myograph (Fig. 2); also, see that the scale pan and series of weights are in readiness.

(86) **Pith a Frog** by holding it in a cloth and inserting a stout pin into the spinal canal at the junction of the skull with the vertebral column. The correct point to insert the pin is in a line with the posterior margin of the tympanic membrane of **each** side. Move the pin quickly from side to side so as to separate the brain from the spinal cord, then without withdrawing the pin push it forwards into the cranial cavity and move it about so as to destroy the brain completely, and then push it down into the spinal canal and work it backwards and forwards so as to destroy the spinal cord. If properly pithed, the limbs will be perfectly limp and no reflex movements can be elicited on pinching the skin.

(87) Now dissect out the muscle—in this case the sartorius. A good routine method in all muscle work is to **prepare the lower limbs and pelvis** as follows:—Hold the frog by the hind limbs (in a cloth to prevent heating the parts with the hand). Keep the dorsal aspect uppermost and the head and spinal column will droop downwards so that the upper ends of the iliac bones form two small projections, thus marking the upper limits of the pelvis. Cut through the vertebral column with scissors about half an inch above the prominences and continue the incision through skin and abdominal wall obliquely towards the pubis on each side. Allow the viscera including the kidneys to fall forwards and cut the rectum across close to its lower end. You have now the hind limbs and pelvis with the lower dorsal vertebræ. Remove the skin by pulling it right off from above downwards.

(88) The **Sartorius Muscle** will be found on the front of the thigh arising from the symphysis pubis and ending on the tibia by a short tendon. Pass a thread under this tendon, tie tightly, making sure first that it is round the tendon and not round the muscle which is easily divided by the tightening of the ligature. Cut the tendon from the tibia making the incision as far away from the ligatured point as possible, and then dissect the muscle up to its origin. Repeat the same dissection on the other side and then remove the whole front of the pelvis by two cuts with the scissors through the acetabula. The symphysis can then be split into two, and so you obtain two preparations, each consisting of sartorius muscle, its tendon with thread attached,

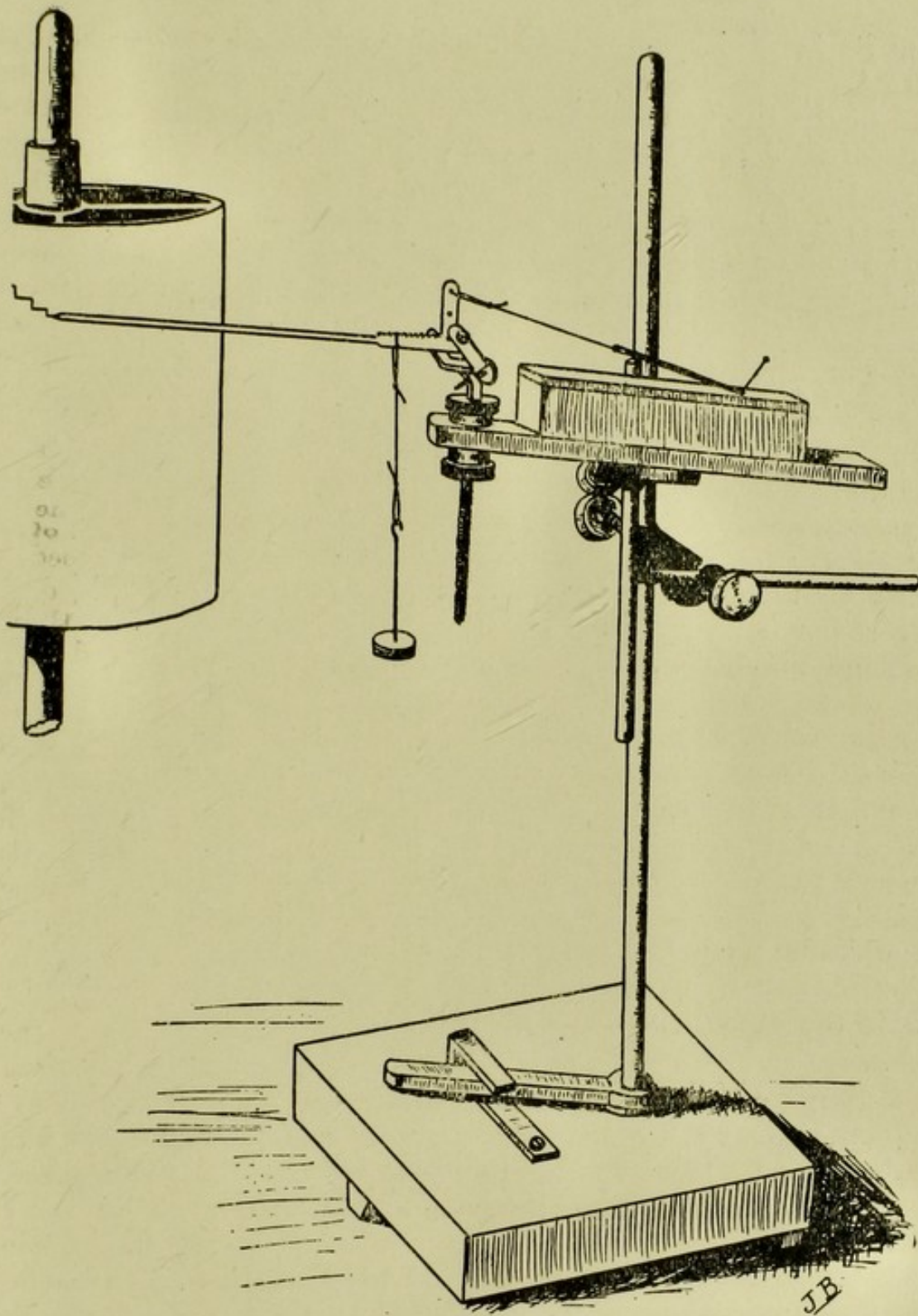


Fig. 2.

Arrangement of the apparatus for the experiment
on the extensibility of muscle.

and its bony origin. Pass a pin through this bony part down into the cork of the myograph and tie the thread to the upper end of the short arm of the crank lever. Suspend the scale pan from the lever at a point near its axis and adjust the level of the lever so that it is horizontal or even points slightly upwards: this may be done by sliding to or fro the elbow-shaped piece which carries the lever and clamping it at the proper place. Next bring the lever point against the blackened surface.

(89) Note that the vertical rod of the myograph moves as a whole on its own axis, and that the movement is limited by the "**lever stop**," a short arm of brass at the foot of the vertical rod, which comes in contact with a brass guard. Before applying the lever of the myograph to the blackened surface see that this stop is close up against that side of the guard which is next to the drum (A in Fig. 3). The writing point of the lever can now be removed from its contact with the paper without moving the foot of the stand (B in Fig. 3), and it can be replaced with exactly the same degree of pressure against the writing surface and therefore with the same position of the point. Practice the use of this arrangement and note the use of the T-piece to which the platform of the myograph is clamped in correcting any tendency of the lever to leave the paper, or to press too much against it as it rises or falls. Keep the wooden part of the myograph as close up to the vertical rod as practicable since the apparatus is more stable in that position. The writing lever, the T-piece, and the lever stop should all lie in the same plane or in parallel planes.

(90) **Experiment on Extensibility.**—Use a stationary drum, *i.e.*, disconnect it from the shafting and arrange it so that it can be rotated by hand for a short distance at a time, and see that it remains motionless at any point to which it has been rotated.

Apply the writing point to the blackened surface near the top of the paper, using the lever stop as just described. Rotate the drum gently so as to produce a fine horizontal base-line or abscissa about four inches long, and then reverse the drum so that the writing point is at the left hand end of this line.

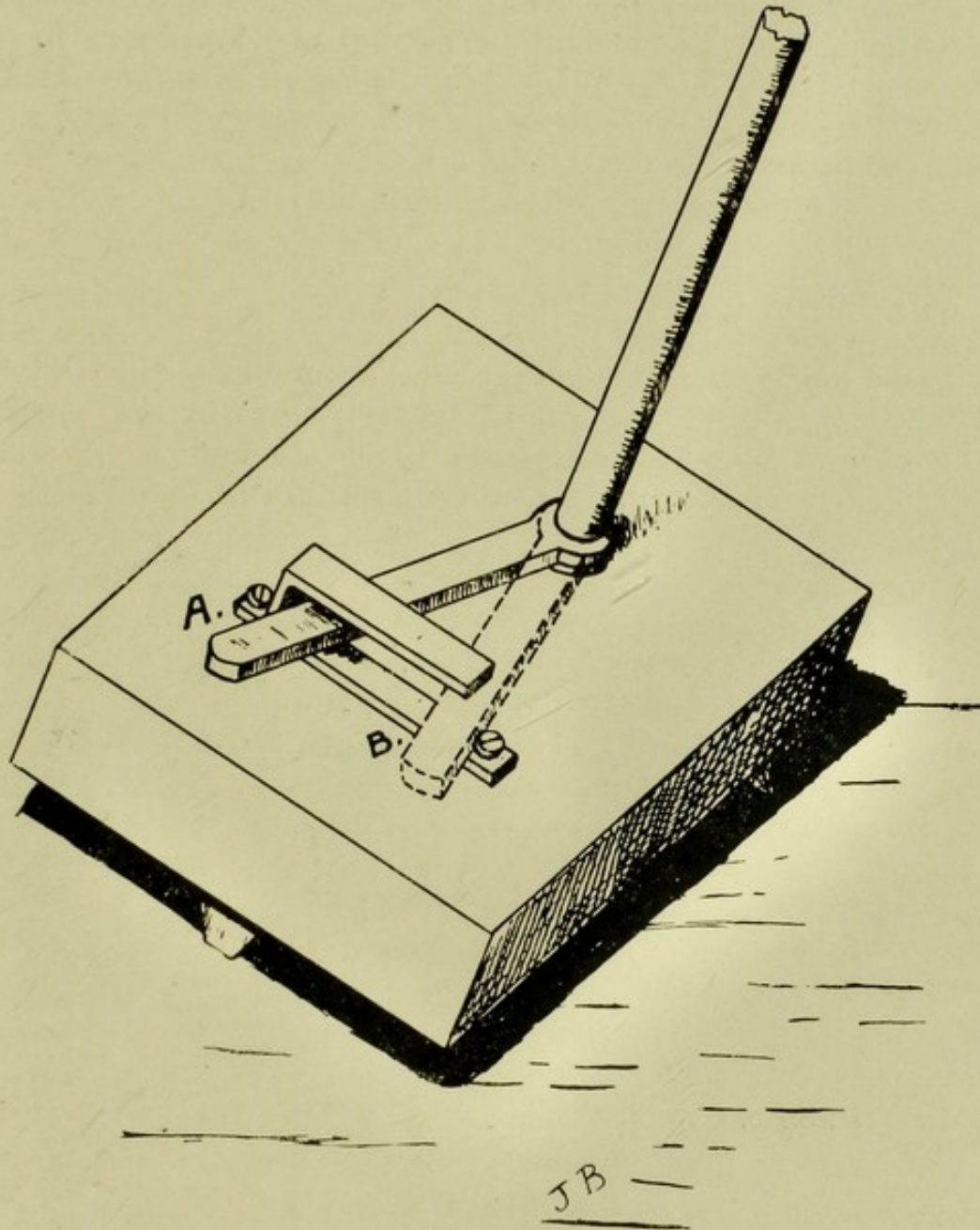


Fig. 3.
The use of the lever stop.

Place one of the weights gently on the scale pan and allow it to extend the muscle and so trace a vertical line. After a definite short interval of time rotate the drum so that a short horizontal line is traced; this must be of a definite length, say three-quarters of an inch; then place a second weight over the first and allow it to act for the same time as the first, and then rotate the drum again for three-quarters of an inch. Proceed in this way to produce a stair-like tracing till no further extension occurs. The line joining the bases of the vertical extensions (ordinates) is the curve of extensibility.

(91) It is convenient to take at the same time the curve of **elasticity** (or recoil). To do this, simply reverse the process, taking off one weight after the other: allow equal time intervals between each removal, and rotate the drum for an equal distance each time.

(92) **To preserve the tracing.**—Remove the cylinder from the apparatus. Cut the paper along the seam and remove it without smudging the tracing; place it flat on the desk and write neatly on it—the object of the experiment (*e.g.* extensibility and elasticity of muscle)—the preparation used (*e.g.* frog's sartorius)—the date—your name, and any other markings that may be necessary, *e.g.*, in this case put in the curves of extensibility and elasticity as dotted lines joining the lower ends of the vertical lines. Now varnish it—hold the paper by its ends with blackened surface uppermost, allow the centre to dip into the varnish and move it to and fro till the whole surface has been covered. Pin it up by one end to drain off excess of varnish, and let it dry for several hours.

ACTION OF ARTIFICIAL STIMULI IN GENERAL.

All living tissue possess excitability, that is, they respond in some definite way to a stimulus; in the case of muscular tissue, the effect of applying a stimulus to the nerve is a visible shortening of the organ—so that a nerve-muscle preparation is convenient for the study of stimuli in general.

A stimulus may be described as a change of condition or environment of a tissue, suddenly produced and generally consisting in the application of some form of energy, *e.g.*, mechanical, thermal, chemical, photic, electrical. They imitate the effects of natural stimuli, *e.g.*, the result of stimulating with electricity the nerve supplying an organ is activity (or inhibition of activity) of the organ; but in some cases the result of artificial stimulation is not quite the same as that of the natural mode.

The effect of a stimulus depends on (*a*) the strength of the stimulus, and (*b*) the excitability of the tissue, so that a series of results varying in intensity may be obtained, although there are some exceptions to this rule, *e.g.*, cardiac muscle.

(93) **Make a Gastrocnemius-sciatic preparation** from a frog in the following way. After pithing the frog (86), divide the vertebral column above the pelvis and remove the skin from the hind limbs as already described (87). Tie a short length of thread round the tendon of the gastrocnemius muscle of one side and divide the tendon beyond the ligature. Separate the muscle from the leg bones up to the knee and cut the leg off below that point. Expose and separate carefully the sciatic nerve where it lies among the muscles on the back of the thigh, taking care not to grasp the nerve with forceps or to damage it in any way. Then turn the frog, place one blade of the scissors from above downwards deeply into the cavity of the pelvis and cut through the symphysis pubis, and divide the frog into two by cutting up along one side of the urostyle as far as the end of the vertebral column which is also to be divided into two equal parts. Grasp one of these with forceps and proceed to dissect out the lumbar plexus and sciatic nerve of the side on which the muscle is already prepared. Dissect out the whole length of the nerve down to the knee joint; lay the nerve over on the gastrocnemius and cut through the femur and thigh muscles above where the nerve is bent over. The result is that you have a nerve-muscle preparation consisting of the lumbar plexus and sciatic nerve with a piece of vertebral column to act as a handle to the nerve, the gastrocnemius muscle with its bony attachments at the knee, and the tendo Achillis with thread for fixing to the writing lever.

(94) Now **prepare the myograph stand**.—Cover the cork platform with a piece of blotting paper moistened throughout with normal saline. This should be exactly the same size as the platform. Replace the crank lever in its usual position (Fig. 4), if it has just been used for the extensibility experiment and tie the thread to the short arm of the lever—the best kind of knot to use here is a loop knot so arranged that the thread can be tightened up afterwards if necessary. There are usually two holes on the short arm of the lever, and the one selected will depend on circumstances:—the lower will give the larger curve but gives more work to the muscle during contraction.

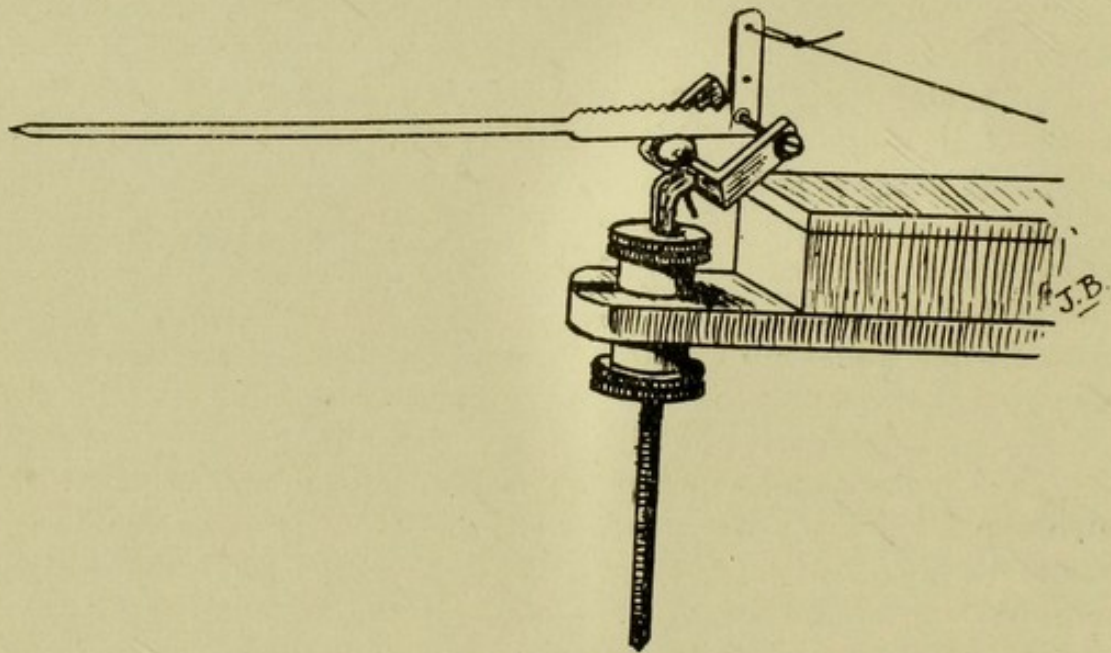


Fig. 4.

The upper end of the muscle is now to be fixed by pinning the knee joint to the cork platform of the myograph stand: the position of the pin will depend on the length of thread from tendon to lever. When properly arranged the thread should be moderately taut, the lever should be horizontal or should point slightly downwards, and the head of the small screw on the elbow piece which carries the lever should be raised so that, while it does not support the lever, it prevents it from descending. These preliminary manipulations (93 and 94) are required in practically all experiments on nerve-muscle work, and should be carried out

as rapidly as possible, care being taken not to allow the tissue to become warmed by contact with the hands, or by breathing on to the preparation. Various methods of stimulation may now be applied to the preparation, *e.g.*, electric, mechanical, thermal, chemical. The most universally employed of all these is the electric method because of the ease with which its strength can be graduated and the accuracy with which it can be applied to one definite spot. As the other methods are destructive to the tissue they may best be done at the end of some lesson after the preparation has been used for other experiments. For convenience, however, they are described at this stage.

(95) **Mechanical Stimulation** is brought about by clipping through the nerve with blunt scissors, pinching or tapping it. It may be carried out more systematically by an arrangement whereby drops of mercury fall from a given height on to the nerve.

(96) **Thermal Stimulation.**—Apply a hot wire to the cut end of the nerve.

(97) **Chemical Stimulation.**—Apply crystals of sodium chloride in the same way.

(98) **Experiment to show Stimulation with various strengths of Galvanic Electricity.**—Set up a Daniell cell as follows—Rinse out one of the porous inner cell with water and almost fill it with weak sulphuric acid; place the zinc plate in this and set the whole in the glass jar containing the copper and copper sulphate solution. Attach wires to the zinc and copper elements; bring one to a mercury key and the other to the binding screw at one end of the wire of a monocord; connect the mercury key to the other end of the monocord wire; attach one wire of a pair of pin electrodes, also to one end of the monocord and the other limb of the electrodes to the movable iron rider; (Fig. 5). Then push the pin electrodes slantwise into the myograph cork near the broad end and lay the nerve across the pins:—the nerve should lie on the moist blotting paper and should if necessary be moistened from time to time with salt solution. It will be noticed that one end of the monocord wire (*a*) in Fig. 5) has two other wires connected with it, one from the source

of the electricity, and an electrode wire. Place the rider on the monocord wire near this end of it and close and open the key:—Usually no result follows because only a small fraction of the available current will pass by the electrodes in that position of the rider. Place the rider further along the monocord wire and again close and open the key.—If the muscle does not contract, move the rider

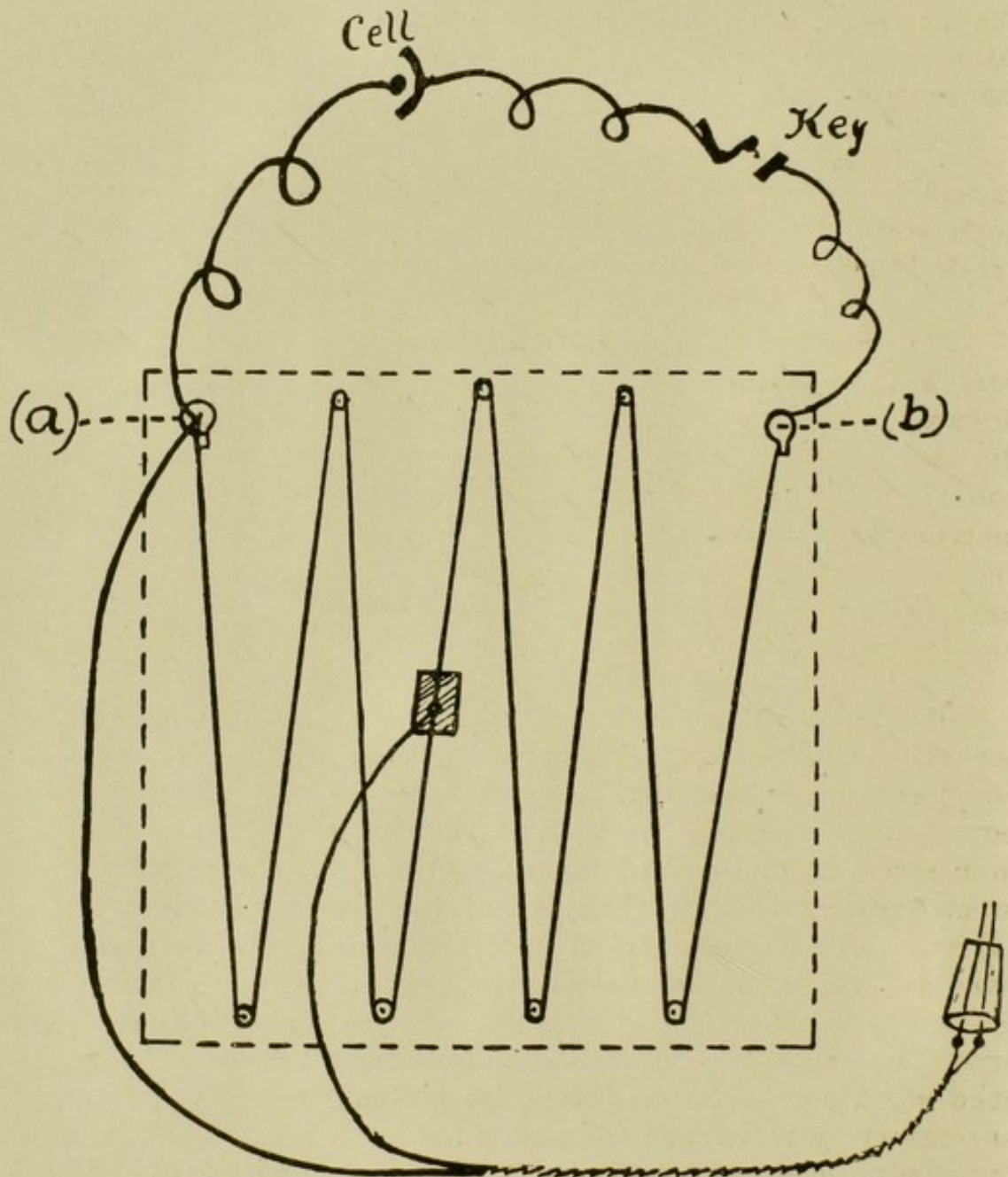


Fig. 5. (Exp. 98).

still further along till contraction is obtained. Note that the contraction occurs on closing and not on opening the key. With full strength of the current, however, (rider at *b*), (Fig. 5) contraction may occur on both closing and opening the key; during the flow of the current there is no contraction, at least not with weak currents.

This shows how a stimulus may be graduated in strength; that the result may vary with the strength; and that the stimulation occurs during the *change of condition* and not during its maintenance.

This last statement is more fully illustrated by the next experiment in which the galvanic current can be sent into the nerve almost instantaneously, or can be set up so slowly and gradually that no stimulation results.

(99) For this purpose the **Rheonome** (Fig. 6) is used. It consists of a base-board with a circular trough to hold a conducting fluid such as saturated solution of zinc sulphate; into this there dip two strips of metal with binding screws to carry the wires coming from the cell, a simple key being interposed. Two metallic arms, which can be rotated on a vertical axle set in the centre of the apparatus, also dip into the zinc sulphate solution, and have the electrodes attached one to each; the nerve is laid across the electrodes as in the foregoing experiment.

When the arms are placed in line with the connections to the cell (AA^1 in Fig. 6) a considerable amount of the electric current will pass to and through the nerve because the electrodes are now in communication with points which have a wide difference in electric potential; but when the arms are rotated so that they lie at right angles to the line joining the battery communication (BB^1) no current passes through the electrodes because they are now in communication with points of equal potential (compare to Wheatstone's bridge, in position BB^1 all four arms are equal). If now the movable arms are rotated suddenly from position BB^1 to position AA^1 the current is as suddenly sent through the nerve and contraction results, but if the same movement be done slowly and steadily there is no contraction.

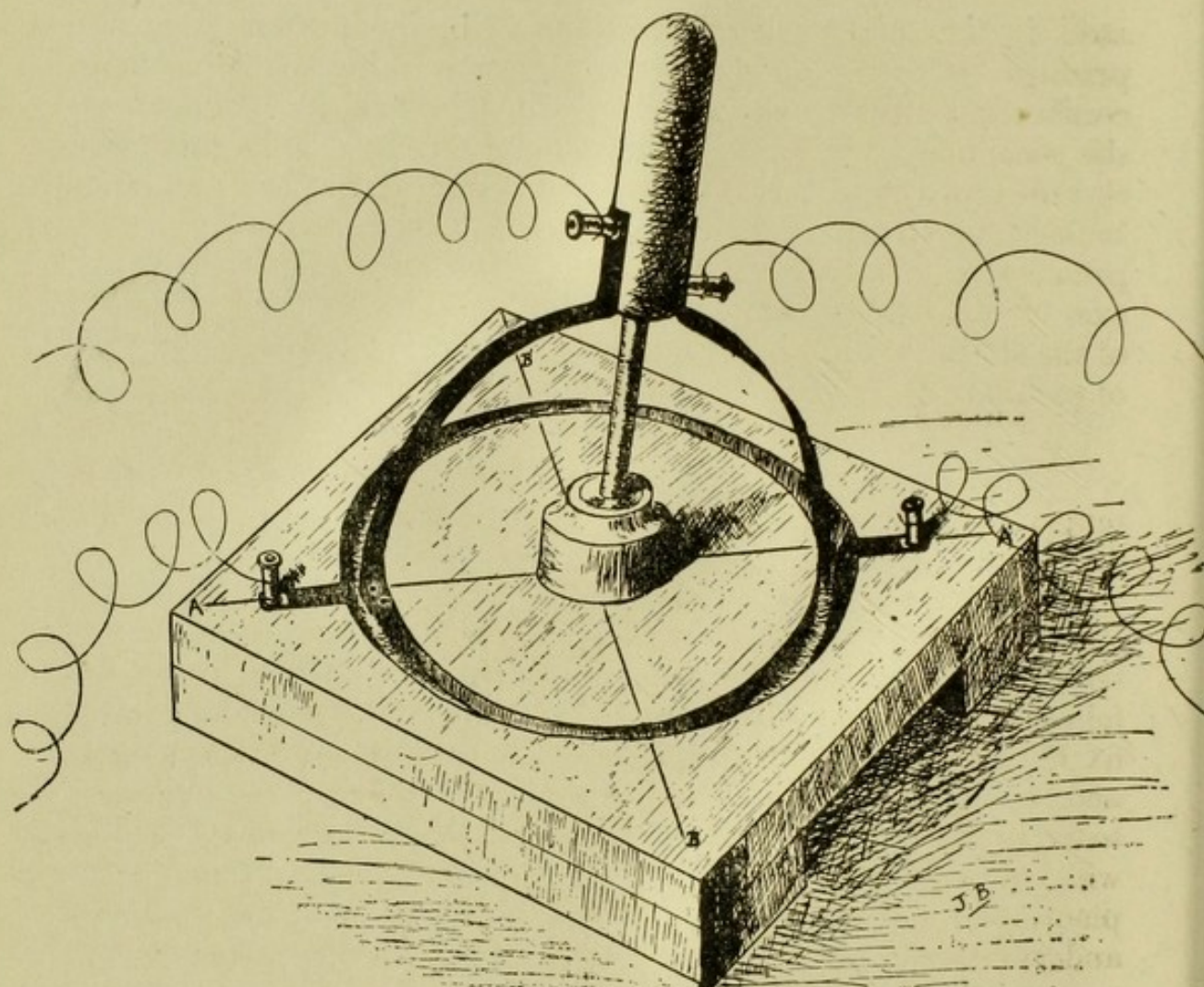


Fig. 6. (Exp. 99).

The Rheonome (von Fleischl's).

(100) **Galvanic Stimulation of Sensory Nerves.**—Clean the electrodes, apply them to the tip of the tongue and send the current through. A warm prickling sensation is felt during the whole time of the flow and occurs whether the stimulus is slowly applied or not.

(103) **Experiment to show Stimulation with various strengths of Faradic Electricity.**—The form of induction coil used in practical physiology is generally known as the sledge inductorium (du Bois Reymond) because the strength of the shock is gradu-

ated by sliding the secondary closer to or further away from the primary coil. In some forms (*e.g.*, Porter's) this movement is combined with and supplemented by a rotatory movement of the secondary coil which has the same effect. The principle of the apparatus is, that when a galvanic current is set up or broken, increased or diminished in amount in the wire of the primary coil, it induces a "shock" or current of short duration in the closed circuit of the secondary coil, and the strength of the shock depends on the proximity of the coils and the degree of parallelism between the turns of wire in them.

If a nerve or excitable tissue forms part of this secondary circuit, it is stimulated by the passage of the electricity through it.

Connect a Daniell cell through a simple mercury key to the terminals of the primary coil. Fit up the secondary circuit as follows:—Attach a wire to each terminal of the secondary coil; fix the other ends of these to each side of a short-circuiting key, and fix the ends of the pin electrodes to the same key, also one to each side (Fig. 7). Apply the electrodes to the tongue which will thus complete the circuit. Open the short-circuit key, place the secondary coil about 12 c.m. from the primary, close and open the mercury key—a sharp twinge or shock will be felt on opening, possibly a lesser shock or nothing on closing the key. Repeat this with the short-circuit key closed, and note the difference. Trace out the two separate circuits in this stimulating arrangement. The primary circuit consists of the wire from one pole of the cell to the mercury key, another wire from there to the one terminal of the primary coil, the wire of the coil itself, its other terminal, and the wire thence to the other pole of the cell. The secondary circuit consists of the piece of tissue between the pins of the electrodes (tongue in this case), one pin and its attached wire, one side of the short-circuit key, a wire thence to one terminal of the secondary coil, the long thin wire which forms the secondary coil, its other terminal, a wire to the short-circuit key, one side of that key, and the other electrode wire and pin. Note how the closure of the short-circuit key diminishes the size of this secondary circuit and, by providing an easier path for the "shock," prevents the stimulus from reaching the tissue.

These arrangements exemplify what must be present in every case of stimulation by induced electricity, viz.—a complete secondary circuit which involves the tissue to be stimulated; this must be in proximity to a complete primary circuit through which a galvanic stream can be sent in an interrupted or varying manner so as to produce shocks in the secondary.

Unipolar induction is an apparent exception in regard to the completeness of the secondary circuit, see (104).

Note especially the means of varying or of interrupting the galvanic stream; in the above experiment it is a simple mercury in other experiments it will be found to consist of an automatic key—(Neef's Hammer), or of a vibrating spring, or of the apparatus for obtaining a graphic record of muscle contraction—drum, pendulum, or spring myograph.

Repeat this experiment with the secondary coil at different distances from the primary and note how finely the stimulus can be graduated.

(102) Now instead of the tongue (sensory nerves) apply the electrodes to the sciatic nerve of a gastrocnemius-sciatic preparation (93), and take a tracing of the effects of opening and closing the key in the primary circuit. Begin with the coils so far apart (say 35 c.m.) that the stimulus is insufficient ("sub-minimal"). Gradually approximate the secondary coil to the primary till the smallest appreciable effect is obtained ("minimal" stimulus). Allow the muscle to record the height of its contraction on a stationary drum, having first taken a base-line or abscissa. Write the distance of the coils from each other under each attempt at stimulation. At first the closure of the mercury key gives no effect but some mark should be put on the abscissa to indicate that it was tried. As the coils are approximated the opening shocks cause larger contractions till by and bye no further increase in height is obtained, the stimulus is now said to "maximal," while those intermediate between minimal and maximal are "submaximal." It will now be found that the closing shocks begin, increase in efficiency and become maximal in just the same way as the opening shocks but with the coils closer together. During this part of the experiment the

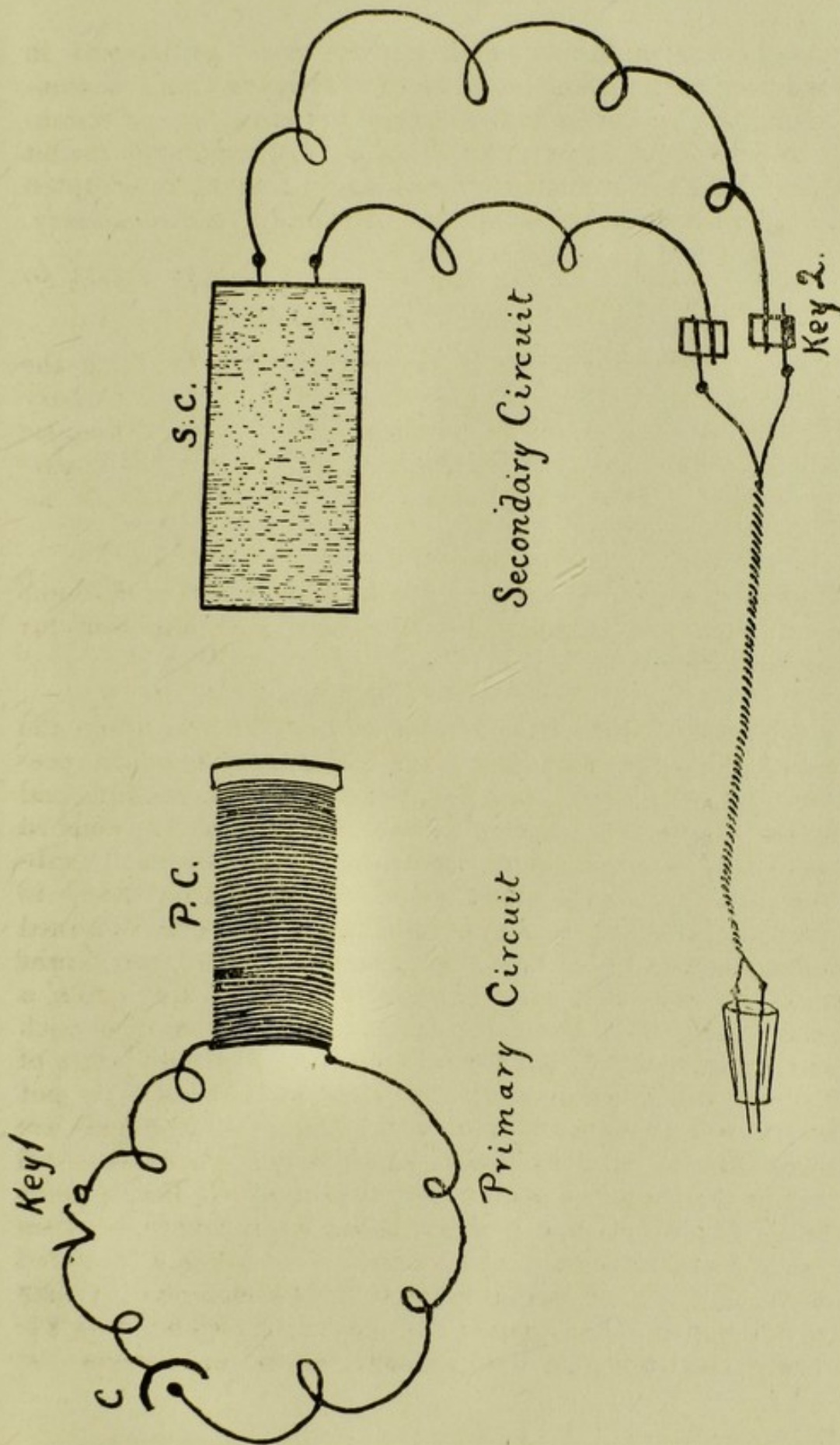


Fig. 7.

Simple stimulating arrangement.

C., cell; *Key 1*, mercury key; *P.C.*, primary coil; *S.C.*, secondary coil; *Key 2*, short-circuiting key.

opening shocks should not be allowed to reach the nerve otherwise the preparation will rapidly become fatigued; they must be short-circuited by the key in the secondary circuit.

(103) **Neef's Hammer** (Fig. 8).—It is frequently necessary to stimulate a nerve by a number of rapidly recurring shocks and as this would be inconvenient and too slow if done by hand, an automatic arrangement known as Neef's Hammer is commonly used. Its mechanism is similar in principle to that used in ringing an electric bell—that is, the galvanic current generated on completing the circuit magnetises a piece of soft iron (D) which then draws a spring away from contact with a platinum point (C). This contact is an essential part of the circuit and therefore the current ceases, the spring is no longer attracted by the soft iron, it returns to its contact with the platinum point and therefore the current is re-established. The number of make and break shocks depends on the rate of vibration of the spring which is usually about 30 per sec. If the secondary coil is sufficiently near the primary there will be a stimulus at both make and break, consequently doubling the above rate.

Connect the cell through a simple key with the two binding screws on the middle and left hand pillars of Neef's Hammer (BB¹ Fig. 8). Adjust the height of the screw which carries the platinum point till the spring vibrates continuously. Connect the secondary circuit through the short-circuit key to the pair of electrodes and try the effect on the tongue and on the nerve muscle preparation. Trace out the course of the current in the primary circuit and note how the automatic 'make' and 'break' is caused.

(104) **Unipolar Induction**.—Fit up Neef's Hammer as in (103) and bring a single stout wire from one terminal of the secondary coil to the nerve of the nerve muscle preparation which for this experiment should be placed on a porcelain slab or glass plate. Connect another wire to the gas piping or water tap and bring its other end in contact with the muscle. Now set Neef's Hammer agoing and in most cases the muscle will contract. The reason is that some free static electricity is generated in the incomplete secondary circuit, and this, if discharged into the earth through a living tissue, causes stimulation. It

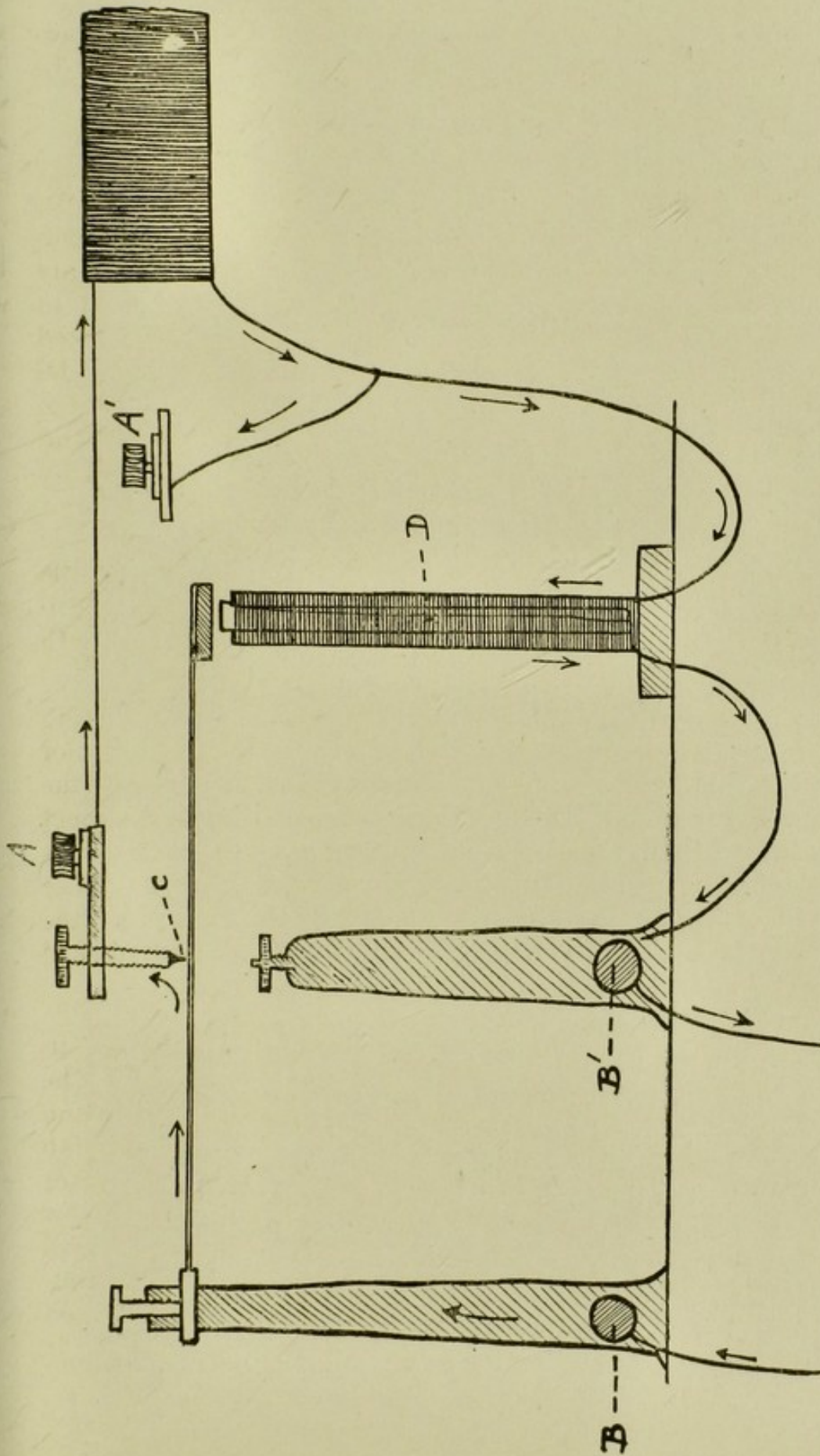


Fig 8.

AA^1 represent the simple terminals of the primary coil; BB^1 , the terminals for the interrupted current (Neef's hammer). The course taken by the current is indicated by the arrows; C , is the point where the current is broken when the spring descends; D , the electro-magnet.

is therefore useless to employ a simple key in the secondary circuit because in such case there would still be one complete wire from the coil to the preparation at the end of which an electric charge might accumulate and might find its way to the earth through the muscle by means of the myograph stand, etc. The short-circuit key, on the other hand, completely protects the preparation.

THE CONTRACTION OF MUSCLE.

So far, we have used the nerve-muscle preparation to illustrate the action of stimuli in general. We shall now study more closely the nature of the **response** to an artificial stimulus and or this the nerve-muscle is the most suitable because the result is a mechanical one which can be graphically recorded. When as muscle or other organ is *directly* stimulated, the stimulus is applied to the organ itself; in *indirect* stimulation the stimulus is applied to the nerve supplying the muscle. (That muscle fibres are themselves capable of responding to a stimulus is shown by various facts including those of *curara* poisoning which may at this stage be demonstrated).

(105) **Graphic record of a muscle twitch or single contraction.**—This requires, in addition to the writing lever and means of stimulation, some form of moving surface, such as a revolving cylinder, pendulum myograph, or spring myograph, and in each case, if it is desired to find the latent period, the moving part must act as a key and cause stimulation at one definite point in its motion.

Examine the “drums” used for muscle work in this laboratory. They are of two kinds—(1) de Burgh Birch’s and (2) Sherrington’s. In the former, note how the motion is conveyed from the grooved pulley to the revolving cylinder, how the brake can be adjusted so that the cylinder is stopped immediately the friction pulley leaves the under surface of the circular base-plate. This pulley can be shifted nearer to or further from the central axis, and so the speed can be varied; the other means of varying the speed is by using different combinations of pulleys on shafting and on drum, always remembering that to increase the speed the cord must come from a large pulley on the shafting to a small one

on the drum., and *vice versa* for diminished speeds. For this experiment use such a speed as will cause the cylinder to revolve about once per second, or half that speed. Note the presence of the two binding screws on the iron foot of the drum, one of these is insulated; note further that in its revolution the pin which projects from the circular plate makes contact with an insulated strip of brass which also has a binding screw. Connect the insulated binding screw on the foot of the drum with this brass strip by a wire; it will now be obvious that once in each revolution the whole apparatus will act as a key, first closing and then opening the primary circuit as the pin makes and breaks contact with the strip of brass. Now make the following connections, zinc or copper of cell to simple mercury key, thence to one terminal of the primary coil; copper or zinc of cell to one binding screw on base plate of drum and from the other to the remaining simple terminal of primary coil. Fit up the secondary circuit with a short-circuit key and pair of electrodes. (Fig. 9). The mercury key in the primary circuit is useful but not absolutely necessary. Prepare a blackened cylinder (85) and a gastrocnemius-sciatic preparation (93) as already described; attach the thread to the writing lever and adjust the level of the lever; fix the electrodes in the cork near the muscle and lay the nerve across them. Find the correct position of the secondary coil to give only a break shock: this may best be done by allowing the pin to make contact with the brass strip so that the circuit through the drum is closed, then open and close the mercury key, with the short circuit key open. Bring the secondary coil as close to the primary as is possible without producing a make shock and then close the short-circuit key. Close the mercury key and keep it closed for the rest of the experiment. Place the writing lever against the blackened surface of the drum, with the lever stop hard up against its guard (Fig. 3, A). Allow the drum to revolve once to trace an abscissa, about a couple of inches from the top of the paper. Remove the writing lever from the paper, open the short-circuit key and allow the drum to revolve once and give a stimulus. If the muscle contracts well, stop the drum, replace the lever which should touch the abscissa already taken, start the drum and after one revolution when it has gained its uniform speed open the short-circuit key till just one twitch

occurs, then close it immediately and stop the drum. Now mark the *point of stimulation*; to do this revolve the drum by hand till the projecting pin is just about to leave or break contact with the brass strip, raise the writing lever for half an inch or less at this point on the abscissa. It is obvious that if the drum had been moving with exceeding slowness the curve would have begun and ended there, but owing to the rapid rate at which the curve was taken the lever did not begin to rise till a short time afterwards. The distance between the point of stimulation and the beginning of the curve allows us to measure the period of latent stimulation or *latent period*. To obtain the length of this

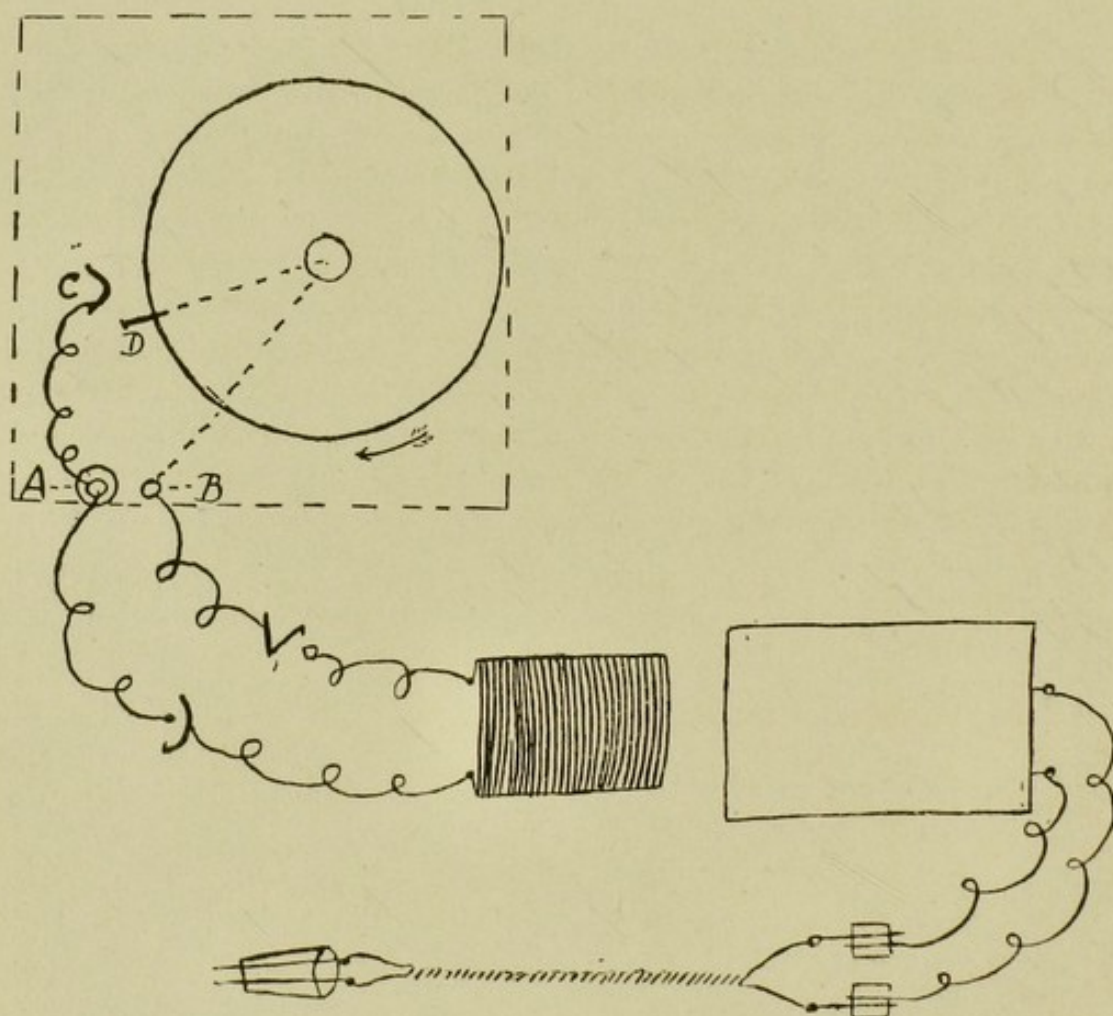


Fig. 9. (Exp, 105).

A, B, the binding-screws on the drum, *A* is insulated and connected by a wire to *C*, the brass strip with which the pin *D* makes contact.

and of the other periods of the curve it is necessary to find the time value of the moving surface. This may be done by counting the exact number of revolutions per minute, finding the time occupied by one revolution and measuring the length of the paper or the circumference of the cylinder—but a more convenient way is to allow a tuning fork to write its vibrations beneath the abscissa. Clamp the tuning fork on a myograph stand, arrange it with the lever stop so that the style just touches the paper when the stop is against the guard. Remove the fork by means of the lever stop, nip, and suddenly release it, and return it to its contact with the drum which should be revolving at its full speed as when the muscle curve was being taken.

The latent period has already been discussed, the other periods are those of *contraction* and of *relaxation*. To measure these the writing lever should be raised and made to touch the very summit of the curve, then allow it to drop and trace the arc of a circle which will cut the abscissa at a point. This line need not be traced all the way down, it is sufficient if it touches the summit and the abscissa. Draw vertical lines downwards from this point, from the point of stimulation, from the point where the curve begins to leave the abscissa, and from where it returns to it and estimate how much time is occupied by each period. The tuning fork vibrations are 100 per second. Label and varnish the tracing as in (92).

The influence of various conditions on the form of the muscle curve may now be considered (106-111).

(106) **Influence of Load.**—Take a simple muscle curve as above (105) with an unweighted lever which is supported by the “screw-stop” when the muscle is at rest. Then load the muscle by suspending the scale pan from the lever at a point as far from the axis of the lever as is the attachment of the thread from the muscle. In that way the muscle has no leverage and lifts the actual weight employed (plus the weight of the lever itself). Add one or more weights to the scale pan sufficient to affect the height of the contraction, and take another curve over the first with the same point of stimulation as before. Repeat with further increments of weight till the muscle cannot lift the load, and find out the actual weight lifted at each contraction including the weight

which prevented contraction altogether ("absolute contractile force"). It may be found that the first increase in weight causes a higher contraction than in the un-weighted muscle, due to the beneficial effect of tension on the excitability of muscle.

From the above experiment the *work* done by the muscle can be approximately calculated, if the actual amount of shortening in m.m. is deduced from the height of the curve by taking into account the magnification of the lever; this shortening multiplied by the weight lifted expressed in grammes gives the number of gramme-millimetres of work. The diminished rise of the lever is due to the contracted muscle being simultaneously extended by the weight employed. An instructive experiment on the extensibility of contracted muscle as compared to uncontracted may be done as follows:—

(106a) *Extensibility of Contracted Muscle and the work done by a Contracting Muscle.*—Dissect out the sartorius muscle of a frog as described in (88) and arrange the myograph lever, muscle preparation and scale pan as described in (90). Fit up the induction coil to give an interrupted current (103) and fix the electrodes in contact with the pelvic end of the muscle. Place the writing point against the drum, using the lever stop as usual. Rotate the drum by hand so as to produce a short length of abscissa. Begin at the left end of this abscissa and record first the height of a contraction when the muscle is weighted by the scale pan alone, using an interrupted current, of a strength just sufficient to cause maximal contraction, for say three seconds. Rotate the drum about half an inch, add a weight to the scale pan, record the resulting extension, and again stimulate and record the height of the contraction. Move the drum again for a similar distance, apply another weight, record the extension and again stimulate. Continue these operations till full extension is obtained, and be careful in each case to move the drum the same distance, to allow the extending weight to act for the same time, and to stimulate with the same strength and for the same length of time.

A dotted line joining the bases of the ordinates gives the curve of extensibility of muscle at rest, while a similar line joining the tops of the ordinates gives the curve of extensibility of contracted muscle, for the application of the weight to the muscle before contraction may be assumed to have the same effect as if the muscle were kept contracted and then had the weight applied to it. Note that the two curves approach each other, *i.e.*, the extensibility of contracted muscle is greater than that of muscle at rest, and consequently it may be assumed that at a certain point

beyond where the curves cut, stimulation of the loaded muscle would produce elongation (Weber's paradox). From the same tracing the work done by the muscle may also be calculated by estimating the magnification of the lever and multiplying the actual shortening of the muscle by the weight lifted as mentioned above.

One or other of the adductor muscles of the thigh may be used in the above experiment instead of the sartorius.

(106b) *After-load*.—In experiment (106) the weights are supported by the head of the screw-stop in the intervals between contraction. If this screw be raised so as to slacken the thread, the muscle contracts to an appreciable extent before it begins to lift the weight; this is termed "after-loading." If the slackness of the thread is increased it will be evident that the load will be applied to the muscle at a later phase of the contraction. On a drum revolved by hand allow the lever to trace a short abscissa with the whole weight of the lever and scale pan with a moderate load supported by the muscle, *i.e.*, with the screw-stop screwed far down. Stimulate with a single break shock (102) and take the height of the contraction, the drum being stationary. Now raise the screw stop so as to support the lever and weight at a higher level and trace another short abscissa; repeat the stimulation. Raise the screw still further, trace yet another abscissa and again stimulate. Continue this process till the contraction of the muscle no longer lifts the lever. It will be found that in the later contractions the weight is jerked up to a greater height than in the initial contraction, and if the work done at each contraction be estimated, it will be found to be greatest with a certain small amount of after-load.

(107) **Influence of varying temperatures on the muscle curve.**—Take a simple muscle curve as above described (105) at room temperature and mark the point of stimulation. Then cool the muscle by placing a small block of ice in contact with it for a few minutes and then take another curve with the same point of stimulation. Remove the excess of water and raise the temperature of the muscle by dropping on to it some normal saline warmed to 30°C. in a test tube. Repeat the curve using the same point of stimulation. Where two students are working together it is convenient to insert two projecting pins into the base-plate at points diametrically opposite so that two curves are obtained at each revolution of the drum.

Put on a tuning fork tracing and mark off the periods as in (105). Note the advantage of the lever stop in this experiment.

(108) **Influence of repeated stimulations.**—The same arrangement may be used for this. Allow the muscle to cool to room temperature or use a fresh preparation, and on a new abscissa record successive contractions, allowing the drum to rotate continuously with the short-circuit key open; or remove the writing point from time to time and record only every tenth contraction till complete **fatigue** sets in. The latter method shows the effects more clearly, but in the former, the “staircase” effect may sometimes be obtained, the first few stimulations causing successively larger contractions. Two stimulating pins may be used here also.

(109) Examine the arrangement of the **ergograph** experiment for the study of voluntary fatigue in human muscle.

(110) **Influence of Veratrin on the Muscle Curve.**—Make a hyoglossus preparation by removing the whole lower jaw, and the floor of the mouth from a frog. This should include the tongue and hyoid bone. Tie a thread to the tip of the tongue, attach the thread to the writing lever and fix the preparation to the platform of the myograph by pushing the pin electrodes through the floor of the mouth with one pin on each side of the hyoglossus where it arises from the concavity of the hyoid plate. Arrange the other parts of the apparatus as in (105) and find the position of the secondary coil which will give a break shock. Record a single contraction on a drum moving slowly—one revolution in five to ten seconds. Remove the writing lever from the paper, inject into the tongue a few minims of veratrin solution and repeat the curve after waiting a minute or two. The injection is to be made under the mucous membrane of the tongue near the electrodes. The usual result is a very much prolonged curve. With successive stimulation the effect wears off and then returns on allowing the muscle to rest.

(111) **Effects of a Second Stimulus applied soon after the first.** (*a*) **Summation.**—Arrange the rate of drum and apparatus as for (105). Use a gastrocnemius sciatic preparation, and take a simple muscle curve using a very weak stimulus so that the contraction is minimal or sub-maximal. Insert a second screw pin into the base-plate close to the first or fixed one, and repeat the curve. The new curve will also be a simple curve but a maximal one. Here the stimuli have been summated.

(b) **Superposition.**—Move the secondary coil nearer to the primary till a maximal break stimulus is obtained. Take a simple curve on a new abscissa, using one pin only. Insert a second pin in the same position as in the foregoing experiment, *i.e.*, close to the first one, and repeat the curve. Then move the pins successively further and further apart, and so take curves showing the effect of two stimuli separated by successively longer intervals of time. The actual length of these intervals depends on the rate of the drum. Mark on the abscissa the second point of stimulation in each case. These curves may all be superimposed or they may be recorded on a new abscissa each time, in which case a simple curve resulting from the first stimulus should be put in for the sake of comparison. Take time tracing as usual.

(112) **Tetanus, or Compound Contraction.**—In this experiment a larger number of stimuli following each other at definite intervals of time are applied to the nerve-muscle. Use the same arrangement as above (105) but instead of the drum which in these experiments acted as a key, insert in the primary circuit a vibrating spring (Fig. 10). The spring has a point attached

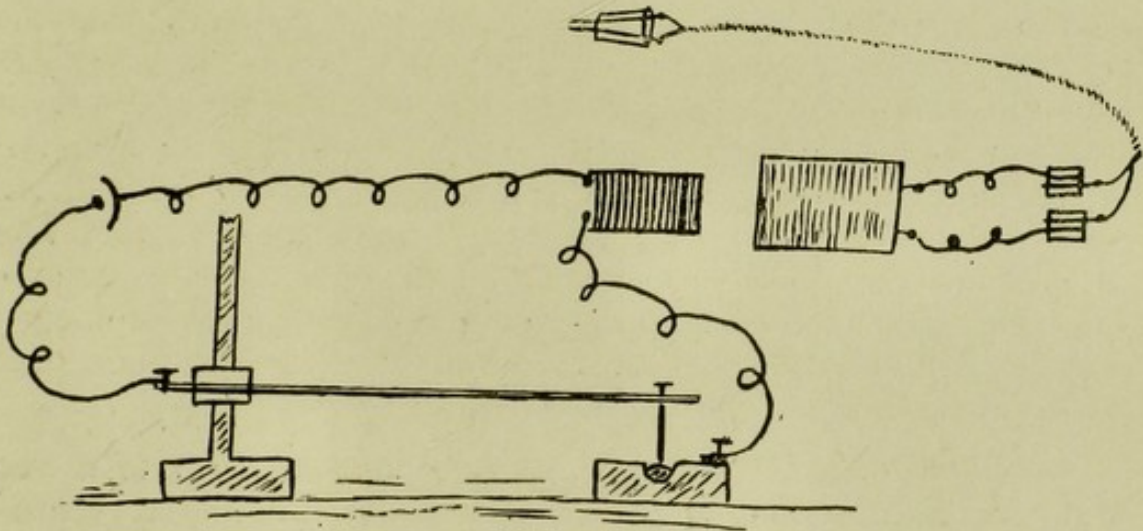


Fig. 10. (Exp. 112).

which is made to dip into and out of the mercury cup of the simple key. Arrange the rate of the drum to go as slowly as one revolution in twenty to thirty seconds. Place the secondary coil

so as to give only break shocks. Clamp the spring at the end away from the contact-making point. Bring the writing lever against the surface of the paper with the usual precautions (89). See that the level of the spring is so adjusted that the contact-making point is just clear of the surface of the mercury when at rest. While the drum is revolving slowly depress the spring with the finger, open the short-circuit key, and when the writing point is at a suitable place on the abscissa, release the spring and allow it to vibrate, and give about half a dozen stimuli, then close the short-circuit key. Probably in this position of the spring, where the vibration is at a slow rate, each stimulus will cause a complete separate contraction. If two students are working together, repeat this on another abscissa before changing the rate of vibration of the spring. Then clamp the spring about an inch to an inch-and-a-half nearer the contact-making end and repeat the process. In this way a series of tracings may be obtained showing all degrees of tetanus up to complete fusion. Avoid fatiguing the preparation by not taking more than about six contractions each time. If complete fusion of the contractions cannot be obtained with the spring, owing to difficulty in approximating the stand of the spring to the mercury cup, put on a tracing with the Neef's Hammer (103). Finally, take a time tracing with the seconds clock and note how many stimuli per second were sent into the nerve at each position of the clamp.

(113) **Reaction of Muscle after Contraction.**—Stimulate the gastrocnemius muscle of one side till complete fatigue has set in. Cut it across and express a drop of the muscle juice on to a piece of glazed blue litmus paper. Place alongside it a similar drop from the un-fatigued muscle of the other side. Allow both to act for a few minutes; wash off, and compare the acidity. Usually the fatigued muscle is distinctly more acid.

Various other experiments on the contraction of muscle, *e.g.*, that on the rate of propagation of the wave of contraction, on the nature of sustained voluntary contraction, on the muscle sound, on the use of the dynamometer, etc., may be done by the class or demonstrated at this stage.

THE CHEMISTRY OF THE MUSCLE.

Muscle consists of about 75% water, 20% proteins, 2% fat, 2% extractives, and 1% inorganic salts. Some ordinary butcher meat has been freed from adherent fat, minced, and extracted for twenty-four hours with 5% magnesium sulphate.

(114) Strain some of the extract through flannel till the fluid is as clear as can be obtained. Determine the heat coagulation temperatures of the proteins present (32). Since we are dealing with muscle which has undergone rigor mortis, **myosin** is the chief protein present. It coagulates about 56° C., but the muscle and therefore the extract usually contains some **para-myosinogen** which has not been converted into myosin, and a coagulum due to this protein appears about 47° C. The other antecedent of myosin, **myosinogen**, has the same heat coagulation temperature as myosin so that the one cannot be distinguished from the other by this method; probably both are present. Further coagula in small amounts may be found at 63° C. and 73° C., due to traces of serum globulin and serum albumin respectively.

(115) Allow some of the MgSO_4 extract to drop into distilled water, a cloud forms due to the precipitation of the myosin. It is also precipitated on dialysis and by full saturation with MgSO_4 and is therefore classified with the globulins.

(116) Dilute some of the MgSO_4 extract with three to five times the volume of water, and keep it for some time on the water bath at 40° C. A clot forms which is loosely coherent, and resembles a precipitate more than a true clot. It may be due to a formation of myosin from myosinogen and para-myosinogen which have not previously formed myosin and which have been extracted by the magnesium sulphate solution.

(117) Boil some of the MgSO_4 extract, after diluting it with water if there is much protein present. Filter, and test the filtrate for protein by biuret or other colour test (15-17). There is no result because all the proteins of muscle are coagulable by heat. This filtrate contains the extractives and salts, and when

concentrated by evaporation it resembles the common "extract of meat" of commerce.

(118) A solution of "extract of meat" is put out. Test it for protein (15-17) as above, and for carbohydrate (42-43); only traces of these, if any, will be found. Fat is also practically absent except for lecithin, so that meat extract is of little value as a food, although the presence of certain extractives, (nuclein bases, creatinin, etc.) and salts make it extremely useful as a stimulant.

(119) **Lactic Acid** (sarcolactic).—Test the reaction of the solution of meat extract with litmus paper. It is almost always acid. Place some of the semi-solid extract in a dry test tube, add some ether, and rub the extract and ether together against the side of the tube with a glass rod. Allow it to extract for ten minutes or longer; then warm a porcelain basin containing a very little water and pour the ethereal extract into it, keeping the ether from the flame. The ether soon evaporates, leaving a watery solution of lactic acid. Test this as follows:—

(120) *Uffelmann's Test*.—To a test tubeful of 5% carbolic acid add one or two drops of dilute ferric chloride and mix. The result is a fine blue solution known as Uffelmann's Reagent. Add a few drops of the watery solution of lactic acid to the upper part of the tube, the blue colour is replaced by a yellow.

(121) **Phosphates**.—Add some magnesia mixture and some ammonia to a solution of meat extract. Ammonio-magnesium phosphate is thrown down; filter, and keep the filtrate for (122). Wash the precipitate on the filter paper with one in three ammonia, then run some dilute nitric acid through the paper and collect the acid solution, add ammonia to this till a precipitate just appears, again render acid with nitric acid, add ammon. molybdate and heat—a yellow precipitate of phospho-molybdate of ammonia forms.

(122) **Nuclein Bases**.—To the phosphate-free filtrate as obtained above add some silver nitrate solution, and if a whitish precipitate forms (AgCl) add ammonia till it dissolves. A dark, gelatinous precipitate forms of a silver-magnesium compound

of hypoxanthin and xanthin. This may take some time to appear (cf. 262).

(123) Remove the phosphates from another portion of meat extract solution by adding some calcium chloride and ammonia; filter, and add a few drops of ferric chloride to the filtrate. A dark precipitate of carniferrin forms, being a compound of iron with **phospho-carnic** acid. Carnic acid has the composition of anti-peptone.

(124) **Creatin.**—This is the most characteristic of the extractives of muscle. It can be recognised by the form of its crystals (transparent rhombic plates). In acid solution it readily passes over into its anhydride, **creatinin**, which is more easily tested for. Some meat extract solution has been treated as follows.—Basic lead acetate was added till no more precipitate appeared, the fluid was then filtered, and the excess of lead removed by passing H_2S and filtering. The resulting solution was then decolourised with animal charcoal and again filtered. By this means the phosphates and many of the other constituents are removed, and the solution can now be tested for the creatinin, which has formed from the creatin owing to this and to the previous manipulation of the extract of meat.

(125) *Weyl's Test for Creatinin.*—Add a few drops of freshly prepared dilute sodium nitro-prusside and then render the fluid alkaline with NaOH or KOH—a deep red colour results which becomes yellow on acidifying with acetic acid, and if the yellow fluid is heated, it becomes green, and a blue deposit (Prussian Blue) forms on standing.

(126) *Jaffe's Test for Creatinin.*—To the solution add a few drops of picric acid solution, and then make alkaline with KOH—a deep reddish brown colour results, more marked on warming. Glucose solutions give a similar result.

(127) If mince was used for the examination of the ash of a tissue, see (1)-(10) it will be unnecessary to consider further the **inorganic constituents** of muscle.

CHAPTER IV.

NERVE TISSUE AND ELECTRO-PHYSIOLOGY.

Examine slides illustrative of the structure of nerve fibres and their mode of termination, and of nerve cells stained by various methods.

That nerve fibres, in common with all other living tissues, possess excitability and respond to a stimulus is shown by the experiments already described under "Action of Artificial Stimuli in General," and those experiments on contraction of muscle where the stimulation is indirect, *i.e.*, applied to the nerve. The response obtained on stimulating a nerve fibre is origination and conduction of a nerve impulse which spreads as a wave along the fibre, and which manifests its presence by the effect on the end organ. Its presence can also be detected by the electric change which accompanies it (142). That these two properties *excitability* and *conductivity*, are distinct from each other is shown by various experiments in which one of these properties can be affected while the other is not.

(128) Make a gastrocnemius-sciatic preparation (93) and fit up the Neef's hammer stimulating arrangement (103). Connect two pairs of electrodes to the terminals of the secondary coil through a switch-commutator without crossed wires so that the shock can be sent at will through either pair. A small chamber has been made for the myograph stand in which the middle portion of the nerve can be enclosed, and through which a stream of CO₂ can be passed (fig. 11.) One of the pairs of electrodes (*b*) passes through the wall of the chamber so as to touch the

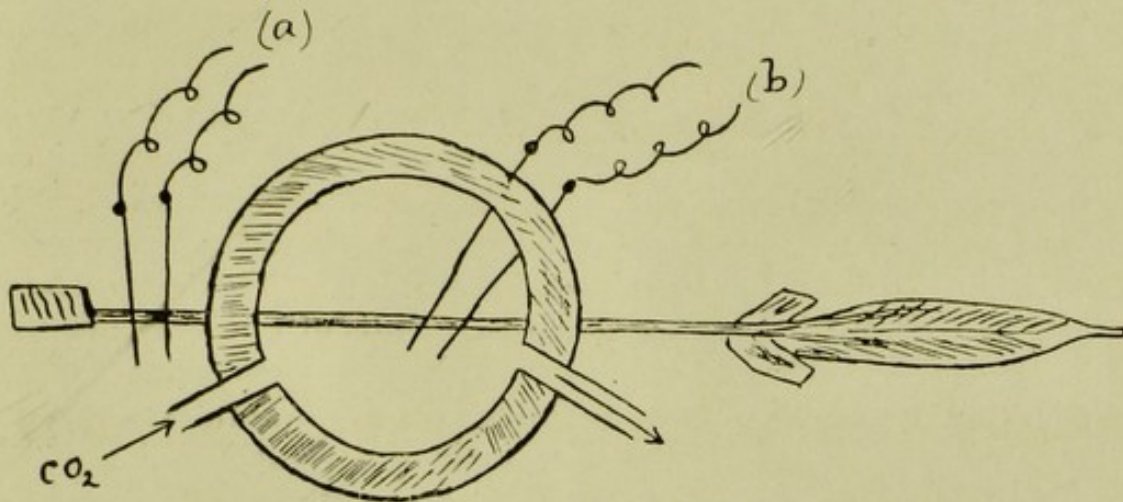


Fig. 11. (Exp. 128).

enclosed part of the nerve; the other pair (*a*) is to be placed on the nerve outside the chamber at the spinal end. In this way one can stimulate the nerve outside or inside the chamber before and after passing the gas. Make these arrangements and find the position of the secondary coil which is just sufficient to cause a slight tetanus of the muscle when the stimulus is applied at (*a*) or at (*b*).

Now connect the chamber with the bottle containing the marble chips, add HCl and allow the CO_2 to pass through the chamber for several minutes; then repeat the stimulation at (*a*) and (*b*). If the experiment is successful, no contraction follows the stimulation at (*b*), the inference being that the excitability of the portion of nerve exposed to the gas has been abolished while the conductivity has been unaffected. Vapour of alcohol may be tried in the same way. It abolishes conductivity while excitability is unaffected.

When a galvanic current of moderate strength is sent through a nerve we have seen that contraction of the muscle follows on closing the key and sometimes also on opening. When the current is strong, contraction may continue during the flow, but even where this does not occur there are marked changes in excitability and conductivity of the nerve trunk. These changes, collectively spoken of as **electrotonus** will now be considered. The positive and negative poles of the battery have almost diametrically opposite effects on living tissues, and the influence of the positive pole is known as **anelectrotonus**, that of the negative as **katelectrotonus**.

These effects are well shown on the beating heart of the frog.

(129) Examine the still beating heart of a pithed frog after removing the sternum and pericardium. Note that during contraction (systole) the ventricle becomes pale and rigid, while between the beats the muscle substance relaxes and becomes of a dark red colour due to the contained blood. Connect a Daniell cell to a simple key and use the bare ends of two ordinary wires as electrodes. Place one of these ends, viz., that connected with the zinc (negative pole or cathode) on some indifferent part of the animal, *e.g.*, under the liver or in the mouth, and place the other (positive pole or anode) on the contracting ventricle and note what occurs when the current is switched on. During contraction the muscle substance in the region of the anode remains relaxed and a red flush can be seen around the spot in contact with the wire. The result of application of the cathode in a similar way is that during relaxation of the ventricle the area around the wire remains pale and contracted. In each case removal of the electrode causes momentarily the reverse effect.

The inference to be drawn from this is that the anode depresses the excitability of living tissue while the cathode increases it, and that the same is the case with nerve may now be shown :—

(130) **Influence of the positive and negative poles on the excitability of nerve.**—Arrange a nerve-muscle preparation on the myograph and connect it to the writing lever in the usual way (94). Fit up (1) a simple stimulating arrangement (101), viz., one Daniell cell and mercury key connected to the simple terminals of the primary coil, and pin electrodes connected to the terminals of the secondary coil through a short-circuit key (fig. 7); (2) a battery to give a galvanic current of varying strength and direction (fig. 13), viz., connect two Daniell cells in series (zinc of one to copper of the other); connect one pole of this battery through a mercury key to one of the binding screws of the monocord, the other pole directly to the other binding screw; two “leading-off” wires are then taken from the monocord to the commutator, one of these is to be fixed to one of the binding screws of the monocord (A in fig. 13)—this binding screw will therefore have two wires attached besides the wire of the monocord itself—

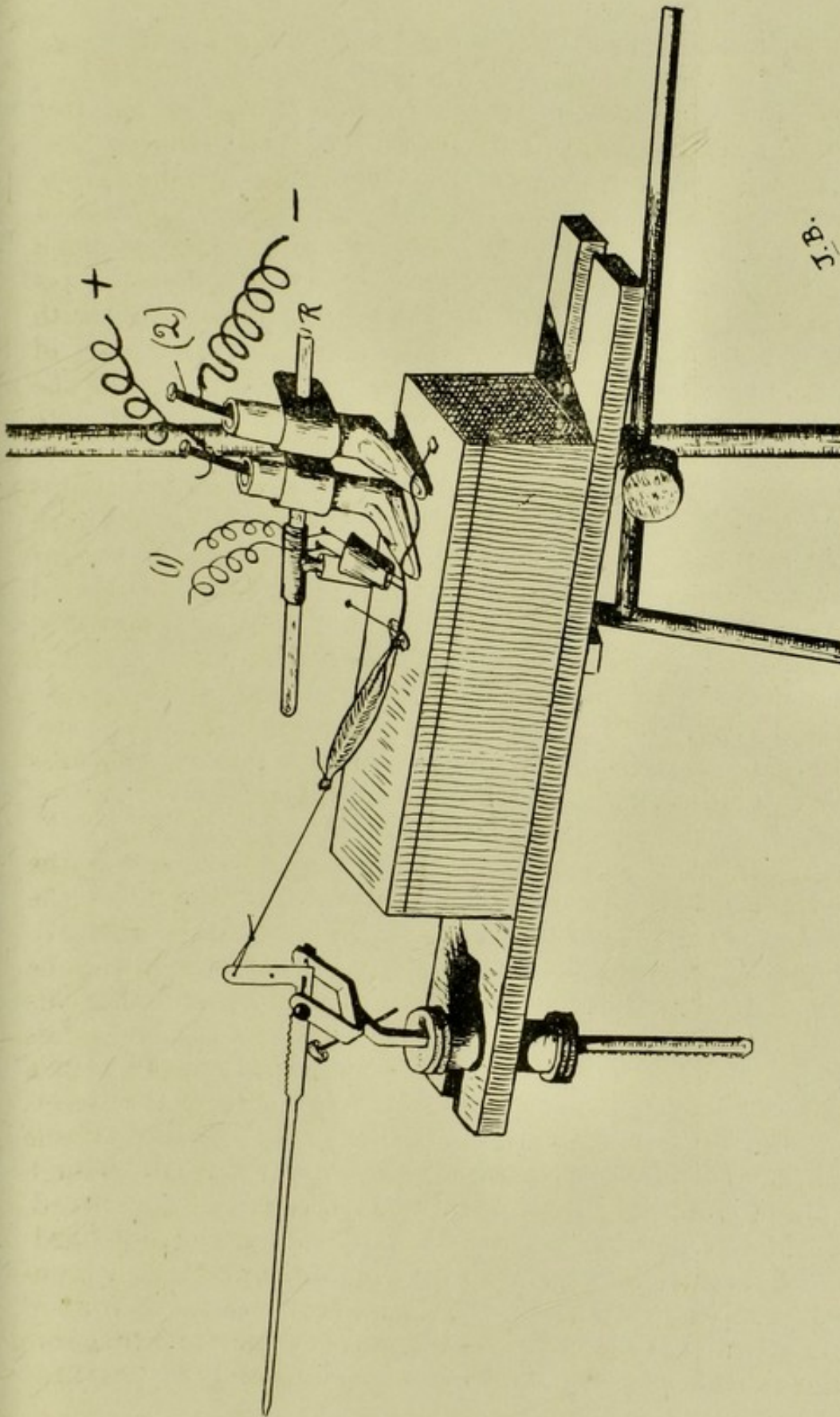


Fig. 12. (Exp. 130).

The pin electrodes (1) are connected to a simple stimulating arrangement (Fig. 7). The non-polarisable electrodes (2) form part of a galvanic circuit arranged as shown in Fig. 13.

R, a glass rod which carries the electrodes.

the other leading-off wire is to be fixed to the movable rider ; the other ends of these leading-off wires are fixed one to each of the lateral binding screws of the sliding commutator, or, if Pohl's commutator is used, one to each of the binding screws in line with the vulcanite top of the "cradle." The ends of the electrode wires are now to be connected to the commutator. If the Pohl's commutator is used, the cross wires are to be kept in and the electrodes are connected, one opposite to each cross wire and both at the same side. If the sliding commutator is used, each electrode wire is fixed diagonally from corner to corner of the sliding piece, and all four binding screws tightened on the wires. Care must be taken that the wires do not touch where they cross.

Now prepare the **non-polarisable electrodes** ; saturate the lower part of the boot with normal saline ; fill the small hollow on the top of the "foot" with saline ; fix the electrodes to the glass supporting rod and place them on the myograph stand so that the nerve can be laid across the point of each boot as shown in fig. 12. The electrodes should not touch the moist blotting paper on the myograph platform. Last of all insert the amalgamed zinc rods in the cavity of the "leg" part and nearly fill that space with saturated watery solution of zinc sulphate ; this must be done very carefully with a pipette, and the zinc sulphate must not come in contact with either muscle or nerve.

Now trace out the direction of the current from the positive back to the negative pole of the battery. Note how the position of the commutator changes its direction so that one or other of the non-polarisable electrodes can be made the anode or cathode.

Examine also the way in which the monocord can be used to obtain various strengths of current ; the two ends of the thin monocord wire have been connected to the battery by ordinary wires, and they therefore have a fixed difference of potential with a steady fall of potential all along the wire ; if the two leading-off wires are widely separated in their contacts with the monocord, that is, if the rider is placed close to the binding screw (*b*) (fig. 13) which carries only one ordinary wire, the current is taken off from two points at widely different potential and is therefore stronger than if the rider is placed close to the binding screw (*a*) which

carries the other leading-off wire. For this experiment, begin with the rider placed about the middle of the monocord wire, and arrange the commutator so that the electrode nearest to the muscle is the anode.

The object aimed at in the experiment, which can now be proceeded with, is to stimulate the nerve with induced electricity of a certain strength at a point which can be subjected at will to the influence of the positive or of the negative pole.

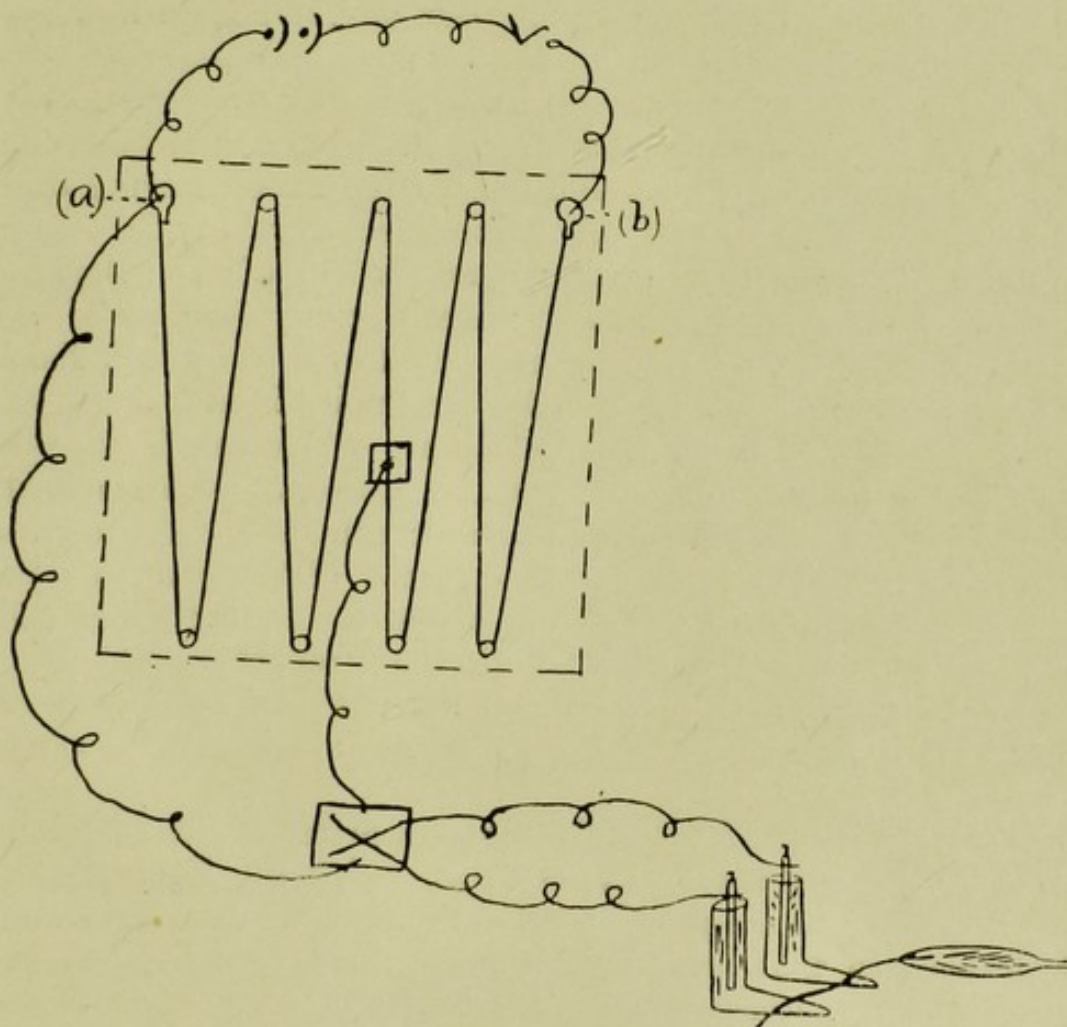


Fig. 13.

Apparatus for investigating the influence of a galvanic current on nerve.

Arrange the upper part of the nerve on the non-polarisable electrodes and place the ordinary pin electrodes from the induction coil on the nerve close to the lower of the two non-polarisable electrodes; with the key of the polarising (galvanic) circuit open, find the position of the secondary coil which gives a minimal stimulus on breaking the current in the primary circuit. Record the height of this contraction on a stationary drum; rotate the drum to the left for about half an inch; remove the writing lever from the blackened surface by means of the lever stop; close the key of the polarising stream and thus subject the stimulated part of the nerve to the influence of the anode; a contraction may follow on closing this key, but is to be disregarded; while the polarising current is flowing replace the lever against the drum and repeat the faradic stimulus used previously. If the experiment is successful there will be no contraction or a diminished height of contraction due to the anode having diminished the excitability of the stimulated part of the nerve. Remove the writing lever again; open the key of the polarising current; reverse the commutator; again close the key of the polarising current and again record the effect of the break shock from the induction coil. The stimulated point is now under the influence of the cathode, the excitability is increased, and the same stimulus is now more efficient and causes a higher contraction. If the muscle remains in tetanic contraction during the flow of the polarising current the strength of the current must be diminished by moving the rider nearer to binding screw (a); if the results are not obtained with the strength of polarising current at first employed, increase the current by moving the rider nearer to (b), (Fig. 13).

(131) **Influence of the galvanic stream on the conductivity of nerve.**—Use the same arrangements as in the foregoing experiment, but shift the pin electrodes to the other side of the non-polarisables, so that the nerve impulse must pass through the portion of nerve affected by the galvanic stream before it can reach the muscle. First test whether the impulse can pass when the polarising current is not flowing. If so, close the key of the polarising circuit and thus send a galvanic current along the nerve (contraction may occur); now, while this current is on, test whether the same faradic stimulus as was used before can

cause contraction of the muscle; usually it cannot. From the results arrived at in the experiment on excitability (130) it will be necessary to have the cathode next the stimulated point, otherwise the result might be due to the effect on excitability. The stimulated point should also be as far removed as practicable from the non-polarisable electrodes. By special methods it can be shown that the diminution of conductivity is due to the influence of the cathode.

(132) **Experiment to show that with galvanic stimulation the cathode stimulates on closing the circuit, the anode on opening, and that the former is the stronger stimulus.** Connect a Daniell cell to the monocord through a mercury key as in (98), fig. 5, using as electrodes two ordinary wires attached to the monocord and its rider. Pith a frog, and prepare the lower half of the body (87); lay it on the frog plate with the anterior aspect upwards; hook the bared ends of the electrode wire under the sciatic plexus, one on each side, and secure the wires in position by twisting each once round an ordinary pin and inserting the pin in the cork plate. Beginning with a weak current, try the effect of making and breaking the circuit. It will be found that contraction occurs at "make" in the limb affected by the wire from the zinc (cathode) and at "break" by the anode, and the former appears with a weaker current than the latter.

(With a certain strength of current both limbs contract at both "make" and "break." This is due to the formation of secondary anodes and cathodes where the current passes from nerve to the other tissues which form part of the circuit).

The results in this experiment are explained by the fact that any sudden (cf. the rheonome, exp. 99) increase in excitability acts as a stimulus; thus, when the current is suddenly set up by closing the mercury key the excitability of the tissue around the cathode is as suddenly raised and stimulation ensues; at the same time the excitability around the anode is depressed and remains so till the circuit is broken, when it suddenly returns to or even rises above what it was before, hence stimulation results here also, but it occurs only on opening the key and is not so easily set up.

(133) When a current of galvanic electricity is arranged to pass through a piece of living nerve certain results have just been seen to occur on opening and closing the circuit; these results vary according to the strength and direction of the stream, and the whole series of results has been named "**Pflüger's Law of Contraction.**" (Contraction, because it is usually performed on the muscle-nerve preparation).

The arrangement of the apparatus in this experiment is similar to that of (130) without the faradic stimulating part. Make a nerve-muscle preparation and arrange it on the myograph for recording the contractions; fit up a polarising current of two Daniell cells in series, a mercury key, commutator with crossed wires, monocord, and non-polarisable electrodes, across which the nerve is to be placed (fig. 13). Trace out the direction of the current through the commutator; when the anode is next the muscle the current is said to be "ascending," if reversed, it is "descending." Begin with a weak current, *i.e.*, place the monocord rider so that the leading-off wires are close together. Close the mercury key, keep it closed for several seconds, and then open it. If no contraction follows on closing, increase the strength of the current by moving the rider to a new position on the monocord wire, till with a certain strength of current contraction occurs on closing and not on opening the circuit; record these effects, reverse the commutator and repeat the closing and opening; mark on the tracing which direction of current was employed. When contraction occurs on closing and not on opening the effects are those of a "weak current." Increase the strength of the current still more, till contractions occur at both closing and opening with both ascending and descending currents; these are termed "medium" current effects. With still stronger currents contraction may occur on closing a descending current and not on opening, and on opening but not on closing an ascending current; these are termed "strong" current effects, and are usually more difficult to obtain in ordinary class work.

Note that the terms "weak," "medium," and "strong" do not refer to any definite strength of electric current (in voltage or ampères) but to groups of effects obtained as one gradually increases the strength of the current applied to a given nerve. Note also that the effects may be to some extent mixed, *e.g.*

the ascending current may give "weak" effects when the descending current of the same strength gives "medium," and instead of no contraction one may obtain a diminished contraction.

If a very strong descending current is used, *i.e.*, strong compared to the excitability of the nerve, the contraction on closing the key may be a prolonged spasm ("closing tetanus"), or this may appear on opening a strong ascending current.

(134) While a galvanic current continues to flow through a nerve trunk it produces electrical disturbances in the neighbourhood of the electrodes which may be investigated by the galvanometer or capillary electrometer. These changes in electrical potential may stimulate nerve fibres in the same trunk which are not in contact with the electrodes and this accounts for what is known as **paradoxical contraction**.

To demonstrate this, make a gastrocnemius-sciatic preparation; in dissecting out the nerve note that it divides into two branches; follow the external (or peroneal) branch as far as possible before cutting it across; then finish the dissection in the usual way. Lay the preparation on a glass slide with the two branches of the sciatic clear of each other; apply a galvanic current to the peroneal branch by means of ordinary pin electrodes connected through a simple key to a battery of two Daniell cells—the gastrocnemius will contract.

(135) **Electrical stimulation of nerves in situ.** Apply faradic and galvanic stimulation to the nerves of the arm. One electrode is large and is placed on some indifferent part of the body such as the abdomen or between the shoulders; the other is small so that it can be applied to those points where the motor nerves come closest to the surface. Both electrodes are covered with wash leather and are to be soaked in strong salt solution before use. Note the response obtained in stimulating the various nerves of the arm with faradic electricity. Galvanic stimulation is carried out with the same electrodes. Use a commutator, and make the small electrode first the cathode and then the anode; close and open the key and note the order in which contraction of the muscles supplied by the nerve appear as the strength of the current is gradually increased. The order in

which these contractions occur in the healthy subject are (1) cathodal closing contraction, (2) and (3), anodal closing contraction or anodal opening contraction; (4) cathodal opening contraction. In diseased conditions changes in the sequence of these results occur, and are of importance in diagnosis, *e.g.*, if anodal closing contraction appears first it is known as the "Reaction of Degeneration."

(136) **Resistance of nerve fibres to fatigue.** That nerve fibres cannot be fatigued easily is shown by stimulating a nerve for a long time with repeated induction shocks, at the same time preventing the impulses from reaching the muscle by lowering or abolishing the conductivity of the nerve at some point between the stimulating electrodes and the muscle. For this purpose an ascending galvanic stream may be employed. After a long period of stimulation, remove the "block" and it will be found that the muscle responds.

(137) **Double conduction in nerve fibres.** Isolate the gracilis muscle in the frog. It will be found on the inner and posterior aspect of the thigh, its tendon of insertion being close to that of the sartorius. Dissect the muscle from below upwards; note its nerve of supply on the surface of the muscle next the femur; cut the nerve as far from the muscle as possible and then remove the whole muscle, and lay it on a glass slide. It will be seen that the nerve branches into two halves, one of which supplies the upper and the other the lower half of the muscle. Cut the muscle across so as to separate the parts supplied by these two branches. Fit up an induction coil to give interrupted shocks. Stimulate one of the branches of the nerve—both halves of the muscle contract. The impulses which originated at the stimulated point must have passed in both directions. It has been found that each fibre in the main trunk of the nerve to the gracilis divides into two where the trunk branches.

The above experiment (136) is apt to be confused with the experiment to show Paradoxical Contraction (134).

(138) The **velocity of the nerve impulse** may be measured as follows:—Make a nerve-muscle preparation in the usual way; fit up the apparatus for taking a simple muscle twitch but insert

a switch-commutator without crossed wires in the secondary circuit and attach two pairs of pin electrodes (cf. 128). Place the electrodes on the nerve, one pair as near to the muscle as possible and the other pair at the spinal end. Take two superimposed muscle curves on the same abscissa using a different pair of electrodes each time. One muscle curve, viz., that obtained with the "spinal" pair of electrodes, will rise from the abscissa with a longer latent period than the other, and the space between the points where the curves rise indicates the time taken for the impulse to traverse the length of nerve between the two electrodes. Put on a time tracing (tuning fork) and find the time value of this interval: measure the length of nerve involved and calculate the rate of transmission. The experiment is best performed with the pendulum myograph.

The velocity of the impulse in human nerve *in situ* can also be measured by stimulating the median nerve near the elbow and at the clavicle, and taking a tracing of the contraction of the thumb muscles with a Marey's tambour or transmission myograph.

For experiments on *nerve cells* see Chapter XI. (central nervous system).

ELECTRO-PHYSIOLOGY.

(139) **Galvani's Experiment.**—Suspend the lower half of a frog from an iron tripod by a copper hook passed under the sciatic plexus of each side. Tilt the tripod so as to allow the leg of the frog to touch the iron, a twitch will result. If unsuccessful, see that the copper and iron make good electric contact, and hold some iron or steel instrument, *e.g.*, scissors, horizontally against the leg of the tripod, rub it to and fro so as to make good contact and then allow it to touch the frog over the sciatic nerve. The stimulation here is due to the electric current, produced by the contact of dissimilar metals, flowing through the tissues.

(140) **Galvani's "Contraction without Metals."** Make a nerve-muscle preparation and cut away the bit of vertebral column. Place the muscle on a glass slide, raise the nerve by means of a bent glass rod and allow it to fall on the gastrocnemius. Contraction may occur, and is due to the differences in electrical potential of the injured end of the nerve and the comparatively uninjured muscle.

(141) **Rheoscopic Preparation.** Prepare two nerve-muscle preparations. Arrange these on a glass slide so that the nerve of the one lies on the muscle of the other. Tetanise the latter through its nerve with an interrupted current. The muscle of the second preparation also contracts. The term "secondary contraction" is applied to this result. It is due to the currents of action in the first muscle and since the second nerve muscle shows the presence of these currents it is termed the "rheoscopic preparation."

(142) **Demonstration of the electrical changes in dying and in active tissue by the galvanometer and capillary electrometer.**

CHAPTER V.

THE BLOOD.

Histological Examination of Blood (*See Schäfer's "Essentials of Histology"*). Experiments on phagocytosis, diapedesis, and amœboid movement, also those on the effect of water, salt solutions, etc., on the corpuscles are now to be performed if not already done.

(143) **Enumeration of Red Corpuscles** with the Thoma-Zeiss or Thoma-Leitz apparatus.

Clean the special slide and coverglass so that they make proper contact with each other ; this is shown by the appearance of Newton's coloured rings reflected from the surfaces in contact when viewed almost horizontally.

After cleaning the part, prick the skin so as to obtain a large drop of blood without squeezing. Use the larger of the two pipettes, viz., that marked 101 at the top of the bulb. Draw blood up to 0.5 or to 1 and then diluting fluid (1 to 3% sodium chloride) up to top of bulb, mark 101. Close the point and the upper end of the pipette with the finger and thumb of one hand and shake so as to mix thoroughly. Allow the diluting fluid in the stem to run out, and then place a small drop of the mixed blood and saline on the centre of the round glass platform of the counting slide ; the drop should be of such a size that it almost covers the platform when the coverglass is applied, and it must not be so large that it overflows into the circular depression.

Apply the coverglass, lowering it by one edge immediately

after placing the drop. Newton's rings should appear and remain permanent; to bring them out a little pressure may be brought to bear on the glass. Allow the corpuscles time to settle (one to two minutes), and examine under the microscope. Focus the lines which limit the squares. These squares are each equal to $\frac{1}{400}$ th of a square millimetre and since the distance between the surface of the round glass platform and the under surface of the coverglass is one-tenth of a millimetre each square represents $\frac{1}{4000}$ th of a cubic millimetre. The squares are further ruled into sets of sixteen by lines which bisect each fifth row of squares in both directions; this is merely to facilitate the counting.

Count the number of corpuscles in a large number of squares, say 50, and find the average in one square. In counting, include the corpuscles lying on the lines only on two adjacent sides in each, thus one may count as belonging to the one square, those lying on the right hand line and the line next to the observer in each case.

Multiply the average number per square by 4,000 and by the dilution 200 or 100 according as to whether blood was drawn to .5 or to 1. The result is the total number of red corpuscles in one cubic millimetre of blood.

(144) **Enumeration of White Corpuscles.**—Prepare the slide, coverglass, and skin as before. Use the smaller pipette, viz., that marked 11 at the top of the bulb. Draw blood up to 0.5 or to 1, and mixing fluid up to 11, so diluting the blood 1 in 20, or 1 in 10 respectively. The mixing fluid used in this case must be one which will destroy the red corpuscles while it makes the white cells more prominent—1% acetic acid tinged with gentian-violet is suitable. Mix the contents of the bulb, empty the nozzle of diluting fluid and apply a drop of the mixture as before. Count all the white cells in all the squares (= 400) moving the slide from side to side, twice to and fro. Multiply the total number so obtained by 10 and by the dilution. The result is the total number of white corpuscles in a cubic millimetre of blood.

(145) **Differential count of number of varieties of Leucocytes**—Having estimated the total number of white cells per c.m.m.

make and stain several large blood films and count the relative numbers of neutro-philic, eosinophiles and lymphocytes present. From this the numbers of the varieties can be calculated.

(146) **Reaction.**—Apply a drop of freshly drawn blood to glazed litmus paper. Allow it to remain for about a minute and then wash off with tap or distilled water. A blue spot in the position occupied by the drop indicates the alkaline reaction of the blood to litmus.

(147) **Specific Gravity** (Hammerschlag's Method). — The method is based on the fact that when a drop of blood is placed in a fluid of the same specific gravity as itself, it remains suspended. A mixture of chloroform (Sp. Gr. 1.52) and benzol (Sp. Gr. 0.89) is made so that its specific gravity approximates that of blood (1060). The mixture is put into a narrow jar or wide test tube and a drop of blood is added to it from a fine pipette. If the drop sinks the mixture is of lower specific gravity than the blood and some chloroform must be cautiously added, till the drops remain suspended; mix thoroughly after each addition of chloroform. If the drop rises and floats on the surface, add benzol in a similar way. When the mixture has been correctly adjusted, take the specific gravity with an ordinary hydrometer or urinometer.

(148) **Laking of Blood.**—To some defibrinated blood in a test tube add once or twice its volume of water. It becomes dark and at the same time transparent, so that print can be read through a thin layer of it. If much water be added some clouding due to precipitation of the globulins in the serum may result.

To some blood in a test tube add a few drops of chloroform or ether or solution of bile salts—laking occurs in each case.

(149) **Crenation of Corpuscles.**—To some blood add an equal amount of strong solution of sodium chloride,—the mixture reflects more but transmits less light and therefore appears of a brighter red than normal blood, but it is more opaque.

Coagulation of Blood.—This is due to the transformation of the *fibrinogen* of the plasma into *fibrin* which forms a meshwork of fine threads entangling the corpuscles. After a time the threads

shrink and *serum* is expressed. The fibrin network has already been seen (see Essentials of Histology).

Coagulation may be prevented by the addition of magnesium sulphate, a soluble oxalate, or a soluble fluoride. Jars containing blood, to which these were added as the blood was being shed, are put out and the following facts may be demonstrated (150)-(152).

(a) (150) *Coagulation is not due to the Red Corpuscles but to something in the plasma.* Centrifugalise some of the MgSO_4 blood so as to separate the red corpuscles out of the plasma or pipette off some of the plasma if the corpuscles have settled in the jar. Dilute some of the plasma so obtained with five to ten times its volume of water; divide into two portions; to one add a few drops of serum and place both on the water bath at 40°C . A fine clear gelatinous coagulum will form in the one to which the serum has been added, and possibly in the other also; if no clot forms in this second tube add some serum which has been heated to boiling and then cooled. Replace it on the water bath—no coagulation will occur. The inferences here are—that MgSO_4 prevents coagulation only when in concentrated solution, that the corpuscles do not form the clot proper (although in ordinary circumstances they are entangled in the clot), and that the property of the serum which causes coagulation is destroyed by boiling.

(b) (151) *Coagulation is prevented by the removal of Calcium ions.*—To some oxalated and therefore lime-free blood add several drops (10 to 20) of 5% Calcium Chloride and place the tube in the water bath at 40°C . Coagulation occurs. (Citrate also prevent coagulation due to an action on the lime salts and may be similarly examined).

(c) (152) *Coagulation is aided and hastened by the presence of thrombo-kinase found in the watery extracts of all tissues.*—To some blood which has been mixed when shed with sodium fluoride add, in one test tube a few drops of a strong solution of calcium chloride, in another tube the same amount of the calcium salt and also some watery extract of a tissue such as muscle or liver. The second of these two tubes will show a clot much sooner than the first when both are placed at 40°C .

The **Composition of the Blood** may be studied with oxalated plasma freed from corpuscles by the centrifuge, or with serum. The main bulk of the proteins consist of *serum-albumin*, *serum-globulin*, and *fibrinogen*; the last is absent from serum.

(153) *Separation of the Proteins of Serum.*—To some blood serum add an equal bulk of saturated ammonium sulphate solution—a precipitate of *serum globulin* results. Filter and fully saturate the filtrate with ammonium sulphate—the *serum-albumin* is precipitated. Filter again and test the filtrate for protein (25) (biuret test with strong NaOH)—no peptone present.

(154) Allow some serum to fall drop by drop into a large amount of water slightly acidified with acetic acid or even into plain water—a cloud results due to precipitation of some of the globulin. (See (29), the solvent power of the NaCl is lost on dilution).

(155) Allow some serum to dialyse in a parchment tube surrounded by water for two days—note the precipitate of globulin (*eu-globulin*)—soluble in salt solution—and note also that the filtrate from this precipitate gives another precipitate on half-saturating with ammonium sulphate (*pseudo-globulin*).

(156) *Estimation of the amount of protein in serum.*—Dilute some serum to fifty times its original volume, using saline solution as the diluent. Fill an Esbach's Albuminometer up to mark U with this and add Esbach's Reagent up to mark R. This reagent consists of a mixture of picric and citric acids. Let the tube stand in a vertical position for twenty-four hours and then read the height of the precipitate; the figures on the tube refer to grammes per litre. This method gives only approximate results.

(157) *Heat* some diluted serum—the proteins are coagulated—test the filtrate for proteins—(17)—no result, so that albumoses and peptones are not present in demonstrable amount. If time permits, find the heat coagulation temperatures of the serum proteins (32).

(158) After coagulating the proteins of serum by heat as above, the filtrate may be used to test for the presence of *extractives* such as glucose, urea, etc., and inorganic salts, such as chlorides, phosphates, etc.

(159) The *Guaiaec Test* is one frequently employed in testing urine for blood. It is given by blood even when much diluted, but a similar colour is given by various fluids such as milk, saliva, etc., and it is therefore not an infallible guide in the examination of urine for the presence of blood. To some diluted blood add a few drops of tincture of guaiac—the yellowish white guaiac resin is precipitated on contact with the watery fluid. To this mixture add some ethereal solution of hydrogen peroxide (“ozonic ether”). A deep blue colour develops.

Haemoglobin. — Haemoglobin is the chief constituent of the red corpuscles. In some animals it is present as a less soluble variety than in others and can therefore be made to crystallize more readily (31).

(160) **Iron is present in Haemoglobin.**—To some dried haemoglobin in a test tube add about double its bulk of 1 in 4 nitric acid, and heat for some time; cool, filter and test the filtrate for iron by adding ammonium sulphocyanide (red colour) or freshly prepared potassium ferrocyanide (Prussian blue).

Solutions of haemoglobin, its compounds, and derivatives give characteristic absorption spectra. These may be conveniently examined with a small spectroscope—the prisms of which are so arranged that the whole spectrum is visible at one time. Examine the spectroscopes provided. Note that the width of the slit can be adjusted according to light requirements, and that the spectrum can be focussed by adjusting the eyepiece. Direct the slit to the sky and arrange it so that Fraunhofer’s lines are visible, then direct it towards a sodium flame and note the position of the D line. It is usual to hold the spectroscope so that the red end of the spectrum is on the observer’s left hand side. In all work on the spectra of pigments the degree of dilution of the substance examined is of importance and it is generally possible to add the diluting fluid in such a way that various strengths of solution can be seen in the one tube.

(161) **Oxyhaemoglobin.**—Take some defibrinated blood in a test tube and run in some water directly from the tap, holding the tube obliquely under the end of the pipe; allow the water to continue running after the tube is full. In this way one obtains a solution of the oxyhaemoglobin so diluted that the upper part of the tube contains almost pure water, the lower part blood, and the middle part all gradations between the two. The corpuscles are laked of course.

Adjust the spectroscope as described above, and hold it in the right hand: dry the outside of the test tube containing the diluted blood and place its upper end against the slit, holding the tube by the lower end with the left hand.

On looking through the spectroscope probably no bands will be seen. Gradually raise the tube so as to bring a stronger solution of oxyhaemoglobin in front of the slit. Two bands will appear, one narrower than the other and nearer the red end of the spectrum. In stronger and stronger solution these two bands fuse into one and broaden out so as to obscure the whole spectrum.

Using the strength which shows the two bands clearly direct the spectroscope towards the sodium flame (the sodium is applied to an ordinary gas jet so that the whole spectrum is visible with a bright line in the position of D.). It will be seen that both bands lie between D and the violet end of the spectrum close to D. The band next D is the narrower and is more sharply defined than the other.

(162) **Haemoglobin — (Reduced Haemoglobin)** — Prepare a solution of oxyhaemoglobin of such strength as to show the two bands, then add a reducing agent—ammonium sulphide is generally employed. Keep the mixture at body temperature to hasten reduction. The spectrum now shows a single broad band, as if the interval between the two bands of oxyhaemoglobin had been obscured. Examine the spectrum in various strengths of solution using cold boiled water or weak ammonium sulphide dilution to prevent re-oxidation (tap water contains dissolved oxygen).

Stokes' solution is a better reducing agent than ammonium sulphide. It consists of 2% ferrous sulphate in 2% tartaric acid solution, and just before use, sufficient ammonia is added to

make it slightly alkaline. When reduction is effected by ammonium sulphide, a narrow band may appear to the left of D, this does not occur when Stokes' reagent is used.

(163) **Carbonic oxide haemoglobin** (HbCO).—CO unites with haemoglobin in the same proportion as O_2 but forms a very much firmer compound. Take some blood in a test tube; add water to liberate the haemoglobin and so facilitate the test; allow some coal gas to run into the top of the tube; close the open end of the tube with the finger and shake; repeat the introduction of gas and shaking several times. The blood has now become very pink—easily seen in the froth. Examine with the spectroscope; diluting with water as in (161). Two bands will be seen apparently just similar to those of HbO_2 although the one next the violet end of the spectrum is slightly narrower than the corresponding one of HbO_2 .

The real distinction between the two pigments is that HbO_2 is easily reduced while HbCO is not. Add Stokes' Reagent or ammonium sulphide and keep at 40°C . No reduction occurs even after prolonged action.

Another good distinction between the two pigments is that when an HbCO solution is much diluted with water it remains pink while HbO_2 when diluted shows a yellow colour. This can be easily demonstrated by making a very dilute (yellow) solution of HbO_2 with blood and saturating a portion of it with coal gas.

(164) **Methaemoglobin**.—To some diluted blood add a few drops of potassium ferricyanide (other substances which may be used are amyl nitrite, potassium chlorate, permanganates, etc.). Keep at body temperature. This solution becomes brown and the spectrum now shows in strong solution a band between D and the red end of the spectrum. This is the characteristic band; others may be seen on dilution in the position of the two bands of oxyhaemoglobin and a fourth band nearer to the violet end of the spectrum. Add to the still warm solution a few drops of ammonium sulphide and watch the changes that occur. First the spectrum of oxyhaemoglobin appears and then that of reduced haemoglobin.

(165) **Acid Haematin.**—To some diluted blood add some acetic acid (about one-tenth its bulk of glacial acetic acid is sufficient). Keep on the water bath at 40° for five to ten minutes and then heat to nearly boiling. Too rapid heating precipitates the proteins and causes turbidity. Slow heating obviates this by allowing acid albumin to form. Examine the dark brown solution of acid haematin, diluting if necessary with acetic acid. A band in the red will be seen in much the same position as the chief band of methaemoglobin. A few drops of ammonium sulphide cause no change (compare to Met-Hb). Further addition of the reagent causes a precipitate which dissolves in KOH and the fluid may then show the spectrum of haemochromogen (alkaline haematin reduced by the sulphide).

(166) **Alkaline Haematin and Haemochromogen.**—To diluted blood add some KOH. Warm gently at first then heat to near boiling, cool, and shake with air to restore oxygen to the compound. A faint band of *alkaline haematin* will be seen to the red side of D, but is frequently indistinct. On adding a reducing agent, however, a very distinct spectrum appears, viz., that of *haemochromogen*, or reduced haematin. This consists of two bands between D and the violet end of the spectrum, but not close to D. This distinguishes it from HbCO and HbO₂, and, further, the band next D is much stronger than the other, and persists in weaker solutions, so that it may be the only one to appear.

This formation of haemochromogen is a very delicate test for blood.

(167) **Haematoporphyrin.**—Take about an inch depth of pure clear sulphuric acid in a test tube and allow one drop of defibrinated blood to fall into it. Spectroscopically it shows two bands, one on either side of the D line—that to the red side of it being the narrower of the two.

(168) **Haemin** is the hydrochloride of haematin. It forms characteristic dark brown rhomboidal crystals on heating dried blood with glacial acetic acid and a crystal of NaCl. (See Essentials of Histology).

(169) **Quantitative Estimation of Haemoglobin.**—In clinical work this is done by diluting the blood to a certain extent in a graduated tube and comparing the tint of the solution with the tint of a standard colour in a similar tube. The extent to which it is necessary to dilute the examined blood before the colours become equal in depth gives directly the percentage of haemoglobin. In Haldane's modification of Gowers' apparatus the standard consists of a 1% solution of blood saturated with CO and preserved in a sealed tube. The estimation is made as follows :—Blood is drawn up to the mark 20 c.m.m. on the pipette and blown out into a small amount of distilled water previously placed in the dilution tube. A stream of coal gas from a narrow glass tube connected to the gas tap is run into the dilution tube so as to replace the air by gas containing CO. Close the end of the dilution tube with the finger and invert several times to allow the haemoglobin to become saturated with CO ; then add distilled water drop by drop till the tint of the diluted blood appears to correspond exactly with that of the standard. In comparing the two, they ought to be viewed by transmitted light from some white surface such as bright clouds or the opal shade of a reading lamp, and the tubes should be frequently transposed. Read the percentage and then continue the dilution till the sample is just appreciably lighter than the standard, read again and take the average of the two figures. This represents percentage of haemoglobin.

The capacity of the dilution tube up to the 100 mark is 2 c.c., and since 20 c.m.m. of blood is taken, dilution up to 100 gives 1% solution. The standard tube contains a 1% solution of blood which had an "oxygen capacity" of 18.5%, which means that each 100 c.c. of this blood contains an amount of haemoglobin capable of carrying 18.5 c.c. of oxygen when fully saturated with air (or O₂). From this one can find the oxygen capacity of the sample of blood taken. If, for example, the blood shows 108% as compared with the standard, then its oxygen capacity is $\frac{108}{100} \times 18.5 = 20$ c.c. Further, the relative amount of haemoglobin per red corpuscle can be stated if a blood count is made at the same time. Thus, if the haemoglobin is 50% while the corpuscles number 80% of the usual, then the haemoglobin per corpuscle is reduced to five-eighths of the normal. In some forms of anæmia

the haemoglobin percentage may be below normal, but the haemoglobin value per corpuscle may be increased.

There are various other methods used clinically where the standard consists of a series of glass discs coloured to represent various percentages of haemoglobin (Oliver's tintometer) or a wedge of coloured glass similarly coloured (Fleischl's haemometer) or a drop of blood on white filter paper is compared with standard coloured papers (Tallquist's method).

The "oxygen capacity" of blood is an exact gauge of its functional capacity as an oxygen carrier. This can be determined by the blood gas pump, or by the ferricyanide method using Haldane's apparatus. (Demonstration).

CHAPTER VI.

CIRCULATION OF THE BLOOD.

(170) Study the gross anatomy of a mammalian heart (of sheep or ox). Note the following :—The oblique groove on the front of the heart filled with adipose tissue and extending from the base nearly to apex, it indicates the position of the inter-ventricular septum ; a similar groove on the posterior aspect of the heart ; the auriculo-ventricular groove at the base of the ventricles ; the right auricle and its appendix ; the right ventricle, thinner walled than the left ; the corresponding parts on the left side ; the openings of the superior and inferior cavæ into the right auricle ; the openings of the pulmonary veins into the left auricle ; the aorta and pulmonary artery. Slit open all the cavities and note the following :—The muscoli pectinati in the appendices ; the Eustachian valve or fold on inner surface of the right auricle ; the tricuspid valve in the right auriculo-ventricular opening ; the chordæ tendineæ, muscoli papillares and “ moderator band ” of the right ventricle ; the pulmonary valves with their sinuses ; the interior of the left auricle ; the mitral valve and the thick wall of the left ventricle ; the aortic valves, their sinuses and the origin of the coronary arteries.

Slides illustrating the structure of the heart and vessels should now be examined.

(171) **Action of the valves.**—In another heart open the auricles and clear away the tissue so as to expose the auriculo-ventricular openings. Allow a fine stream of water from the tap to fall into the centre of the opening of either side—the valve

flaps will be seen to float up so that the upper surfaces of the edges come into contact. Examine the semilunar valves in the same way.

Make a small opening in the apex of the left ventricle, plug it with one forefinger, fill the ventricle with water so as to float up the valves and suddenly withdraw the finger—the valve flaps will be seen to fall back against the sides of the ventricle.

(172) **The beat of the frog's heart.** Pith a frog. Make a longitudinal incision through the skin from the floor of the mouth to the pubis, and another transverse incision opposite the fore limbs. Dissect back the flaps of skin. Incise the abdominal wall to one side of the linea alba so as to avoid cutting the anterior abdominal vein. Continue the incision upwards on each side of the sternum, which can then be removed. Expose freely the beating heart. Note the delicate pericardium, and then dissect it off. The frog's heart differs from that of mammals in that it consists of a sinus venosus which receives the veins, a right and left auricle, one ventricle, a bulbus arteriosus and two aortic arches. The parts contract in the above named order, the two auricles contracting simultaneously. Try to follow the order of the movements with the eye as far as possible, then proceed to take a tracing (cardiogram). Arrange the elbow piece of the myograph stand at the broad end of the cork platform; fit it with a straight lever instead of the crank lever (fig. 14); let the short arm of the lever

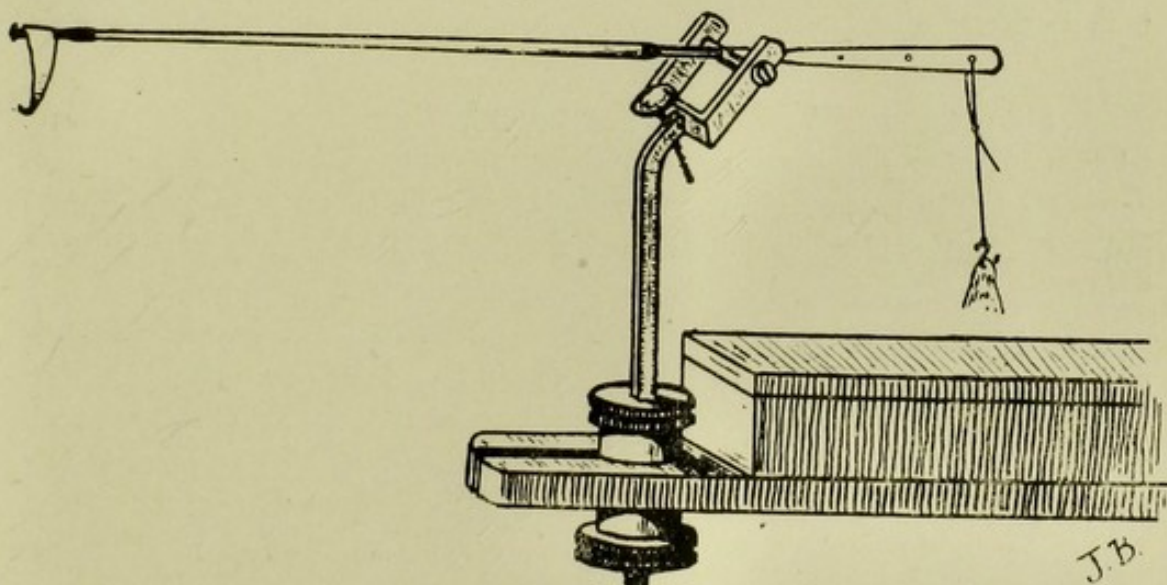


Fig. 14.

The crank myograph arranged for heart work.

project over the heart of the frog; insert a fine hook into the muscular tissue of the tip of the ventricle without grasping the ventricle or damaging it in any way; tie a short length of thread to the hook; make a running loop on the other end and pass it over the end of the short arm of the lever; adjust the parts so that the heart is stretched vertically upwards, the thread to the lever must be quite perpendicular; try various positions of the loop of thread along the lever till a point is found that gives a maximum amount of movement of the writing point. Before beginning to take the tracing look for the pericardial ligament—an extremely fine thread of tissue which connects the base of the ventricle to the pericardium—cut it through. Blacken a drum paper and arrange the driving cord for a slow rate of drum, one revolution in two minutes. Take a normal tracing of the beat, using the lever stop as in muscle work. Record also the effects of cooling the heart by applying ice to the sinus, and the effect of warming the heart by pouring over it some saline heated to 30° C. Put on a time tracing with the seconds clock, write the description on the paper, and varnish it as before.

(173) Listen with the simple wooden stethoscope, and the binaural stethoscope to the **heart sounds** of a fellow student. The first sound is most distinct where the cardiac impulse or apex beat is felt, the second sound is to be listened for at the second right costal cartilage, but both sounds can be heard in each of these two areas.

(174) **The cardiac impulse or apex beat.**—This is a movement of the chest wall over the apical part of the heart which can be seen and felt in the fifth intercostal space, a little to the inner side of a vertical line through the middle of the clavicle (mammary line). It is due chiefly to the hardening of the muscular fibres and the increased convexity of the front of the ventricle which occurs during systole. Palpate the area involved and take a tracing of the movement with a cardiograph. This consists of two tambours each covered on one surface with an indiarubber membrane; they are connected by rubber tubing; the one tambour is the recording one, and acts on a writing lever fixed

in a stand ; the other, the receiving tambour, is fixed in a hollow wooden shield so that when the edges of the shield are pressed against the fifth and sixth ribs the button attached to the rubber membrane comes in contact with the point where the movement is greatest. Instead of the latter a small glass funnel may be pressed over the part so as to make a small airtight chamber containing the beating area, the rubber tubing being slipped over the stem of the funnel.

The cardiograph should be held in position by elastic tapes passed round the body, the lever of the recording tambour should be horizontal, and the whole apparatus should be moderately distended with air before taking the tracing. Use a moderate rate of drum, about three revolutions per minute. The tracing will probably show large waves due to the respiratory movements and much smaller projections due to the heart beats. Record the time value with the seconds clock. The button of the receiving tambour must be very accurately placed ; if it is put in contact with the chest wall to one side of the apex beat a curve will be obtained which is almost the exact reverse of the normal, because the chest wall is drawn in near the apex beat while it protrudes at the centre of the area.

(175) **The Pulse.**—Feel the pulse in the radial artery at the wrist by compressing it against the bone. Count the number of beats per minute while the subject is standing, sitting and lying horizontally.

Sphygmograms.—Examine the mechanism of a Marey or a Dudgeon sphygmograph. In both there is a clockwork arrangement to give a moving surface which may have its time value determined by noting how many seconds it takes to run through a certain length of paper (usually six inches in fifteen seconds). The clockwork is set in motion by releasing a small lever on the top (Dudgeon) or at the back (Marey) of the instrument. The writing lever is acted upon by the pulse beat through a small spring armed with a button which presses on the artery. The amount of pressure exerted by the spring can be varied by means of an eccentric. In Marey's sphygmograph the lever is a long one and is acted upon directly by the spring. In Dudgeon there is a system of levers and it has the advantage that the writing

point moves at right angles to the direction of movement of the paper.

Use of Dudgeon's Instrument.—Before applying the instrument wind the clockwork ; see that the paper will run through easily when placed between the moving roller and the two small guiding wheels, and blacken one or two papers in the camphor flame. Rest the subject's arm in an easy position on your knee and feel for the position where the beat is most distinct ; the spot may be marked with a skin pencil or with ink. Then apply the button of the spring to the spot marked and secure the tape round the back of the wrist. Put a blackened paper in position and allow it to run in till it supports the writing point of the levers. Adjust the tightness of the tape and the pressure of the spring on the artery (by means of the eccentric) till a maximum amount of movement is obtained ; release the clockwork and allow the paper to run through to near the other end ; then stop the clockwork, remove the paper, label, and varnish it.

Use of Marey's Instrument.—The general directions are the same as above ; the arm should be bare to the elbow and supported on the special splint which accompanies the instrument ; the spring is applied to the pulse and the instrument placed with the writing lever pointing towards the elbow, and fixed by tapes passed round the arm. Adjust the pressure of the spring on the artery by means of the eccentric till a maximal amount of movement is obtained ; then adjust the level of the lever, take the tracing, label, and varnish it.

(176) **Blood Pressure.**—(Demonstration). A rabbit is anæsthetised with paraldehyde ; its carotid artery exposed, and a "wash out" canula inserted ; the canula is connected to a mercurial manometer or to a Hurthle's manometer, the connecting tube, which should be inelastic, being filled with a fluid capable of preventing coagulation, *e.g.*, 2% sodium citrate. The movements of the manometer are recorded on a revolving drum in the usual way and the effects of injection of "pressor" substances such as adrenalin or of "depressor" substances recorded ; also the effects of gravity on the pressure, of stimulation of the vagus, etc.

Venous pressure can be demonstrated in the same animal by means of a water manometer.

(177) **Capillary Pressure** is estimated indirectly as follows: (Von Kries' method). A small scale pan is suspended by threads carried from each end of a small rectangular piece of glass. The under surface of the glass has a piece of cover glass of known area fixed to it with Canada balsam; the whole is suspended from the finger in such a way that the small piece of glass presses on the delicate pink skin at the root of the nail, weights are then added to the scale pan till the skin area under the small piece of glass becomes pale. The pressure is calculated as follows:— Suppose the area of the skin blanched = 4 sq. m.m., and the weight of the apparatus plus the added weights amount to 1 grm. (=1000 c.m.m. of water); then the height of the column of water which would be required to overcome the lateral pressure in the capillaries of the area considered would be $\frac{1000}{4} = 250$ m.m., or 18.5 m.m. of mercury. The results obtained by this method are only approximately correct.

(178) **Arterial Blood Pressure in Man.**—The arterial blood pressure can be determined with a fair degree of accuracy by noting the constricting force necessary to compress the tissues of a limb to such extent that the pulse is obliterated (systolic pressure) and till maximal pulsation is obtained (diastolic pressure).

The constriction is produced by fastening a broad leather band round the limb, generally the upper arm. To the inner surface of this belt an elongated elastic bag is fixed, and this communicates with the pump and with a manometer which may be in the form of a hollow spring acting on an indicator (Hill and Barnard's sphygmometer), or a mercurial manometer. The bag is distended by pumping air into it and the pulse at the wrist is continuously observed. When the pulse can no longer be felt the systolic pressure has been reached. The air is then gradually released and the oscillations of the manometer noted; when these become maximal around a certain point on the scale, the diastolic pressure is indicated by that point.

The results are stated to be from 6 to 10 m.m. above the

true figures owing to the force required to compress other tissues in the limb besides the artery.

The difficulties in applying the method are chiefly in determining the "end points," *i.e.*, exactly when the pulse is completely lost and exactly at what stage the pulsation is maximal. These are to a great extent overcome in the instrument devised by Erlanger (in Howell's laboratory); in this apparatus a tracing is taken of the pulse beats during the observation and the end points are easily discerned.

The average pressures in the brachial artery are 110 m.m., systolic and 65 m.m. diastolic (Erlanger).

Various instruments have been devised for estimating blood pressure in the radial artery at the wrist or other accessible vessels by direct pressure over the vessel, but these are less reliable than those which depend on constriction of the whole limb.

(179) **Velocity of the Blood Stream.**—Demonstration of the use of the **stromuhr** in an artificial scheme of the circulation. The instrument consists of two glass bulbs fixed in a base plate which is double; the inlet and outlet tubes are connected with the central and peripheral ends of the cut tube (artery) and come opposite the openings of the bulbs, but the latter, along with the upper half of the base plate, can be rotated so that one or other of the bulbs comes into communication with the inlet tube. In actual practice one bulb is filled with olive oil, but in the artificial scheme air is used. When the bulbs are only half rotated the openings are not in apposition and the fluid goes by a small by-pass tube from the inlet to the outlet tube. Bring the empty bulb into communication with the inlet tube at an observed instant of time; when that bulb has filled and the air has been sent over into the other bulb rotate the bulbs rapidly so as to bring the air-containing one into communication with the inlet, and so on for a definite interval of time, say two minutes. Then calculate the velocity as follows:—Suppose the capacity of the bulb = 5 c.c., and the average time for one filling is 5 seconds, = 1 c.c. per second (= 1000 c.m.m.); measure the diameter of the artery (tubing), let it be say 3 m.m., then the sectional area of the tube is $(\pi r^2) = 3.15 \times (1.5)^2 = 7.08$ sq. m.m. The total

volume per second divided by this (1000 m.m. \div by 7.08) gives the length of the column of fluid which passes in one second=velocity, in this case about 140 m.m., or .14 metre. The velocity varies with each heart beat and by appropriate means a velocity pulse can be demonstrated.

(180) **Plethysmography.**—With each heart beat a wave of increased amount of blood is sent along the vessels simultaneously with the ordinary (pressure) pulse and the velocity pulse. This may be designated the “volume” pulse. It is demonstrated as follows:—The organ or limb is enclosed in a roomy vessel with rigid walls. The space between the walls and the organ communicates by a tube with the recording apparatus (Marey’s tambour, piston recorder or reservoir with float) and air or fluid transmission is used. Any change in volume of the enclosed organ affects the writing lever by increasing or diminishing the air or fluid in the recording part of the apparatus.

Application to Arm.—Enclose the arm in a Mosso’s plethysmograph and make the junction with the skin airtight. Connect the interior of the plethysmograph to a recording tambour, and to a burette containing water. The whole apparatus must be supported on a board suspended from a considerable height so that it is impossible for the subject to move the arm out of or into the plethysmograph. Clamp the tube leading to the burette, and record the pulsations of the tambour lever in the usual way. Ask the subject to take a deep breath, and note the effect on the volume of the arm as a whole apart from the fine pulsations due to the heart beat. For these large effects it is better to fill the interior of the plethysmograph with tepid water, clamp off the tambour tube, open the clamp on the burette tubing and note the actual amount of water transferred during the following—long deep respirations, clenching and relaxing the fist within the plethysmograph, moving one of the lower limbs vigorously, mental exertion, etc.

So far the circulation has been studied in its physical aspects—the experiments which follow refer to the vital properties of the heart and vessels.

(181) Expose the heart of a pithed frog and remove it entirely, cutting widely so as to take away all the sinus. Place

the heart in a watch glass previously moistened with normal saline and note that it continues to beat. Its action is therefore **automatic**, and **rhythmical**. It also possesses the power of **conducting** the impulse to beat because the contraction spreads from sinus to auricles and thence to the ventricle. Further it can **co-ordinate** these movements for the two auricles contract simultaneously. Cut through the ventricle midway between the base and apex, the apical portion comes to rest but can be made to contract rhythmically under the influence of a constant stimulus such as placing on it a crystal of NaCl.

(182) In another frog expose the heart and count or record the rate at which it is beating (172). By means of a glass tube drawn to a fine point direct a current of warm expired air against the ventricle and note whether the rate changes. Then direct the hot air against the auricles and lastly against the sinus. It will be found that the rate is increased only when the sinus is heated. One must be careful therefore not to allow spread of the heat to this part when testing the ventricle and auricles. The temperature of the breath may be sufficient in this experiment, or the tube may be gently heated in the flame before blowing through it.

These experiments show that the sinus is the most excitable part of the heart, and it has already been seen that the beat normally starts there.

(183) **Conduction**.—Gaskell's clamp (dem.).—The excised heart is supported in a special clamp, the jaws of which can be closed by means of a fine screw, and the auricles are attached to one lever, the ventricle to another. Both levers write on a smoked drum in the same vertical line.

While merely supported it will be seen that each auricular beat is followed by a ventricular contraction. Now approximate the jaws of the clamp so as to compress the tissues in the auriculo-ventricular groove. At a certain point there will be two or more auricular beats to each ventricular one.

(184) **Chemical conditions necessary for the Heart Beat** (dem.).—An excised heart is tied on to the end of a two-way

canula, *i.e.*, a double tube. A special normal saline (Locke's fluid) is allowed to flow into the heart from a small reservoir kept at a height of one to two feet, the fluid escapes during contraction by the other channel in the canula. The heart is then to be placed in a Schäfer's heart plethysmograph and the contractions recorded on a horizontal drum. In this way saline solutions of varying composition can be experimented with, and it is found that the best results are got with a fluid which contains besides the sodium chloride, potassium and calcium ions, and some dextrose.

(185) **Stimulation of the rhythmically beating heart.**—Take a normal cardiogram of a frog's heart (172), and while it is beating apply a single strong break shock (101) at various phases of the ventricular contraction. Apply the electrodes to the ventricle by fixing them in position with a pin; do not attempt to hold the electrodes against the heart. It will be found that if the stimulus reaches the ventricle between the beginning and the maximum of its contraction, no result follows. If the stimulus comes in during the relaxation an "extra systole" may result after which the heart makes a "compensatory pause."

(186) **Stannius' First Ligature.**—Pith a frog—expose the heart and arrange the apparatus as in (172) so as to obtain a normal cardiogram. Before taking a tracing pass a ligature under the two aortic arches and bring it round below the ventricle so that when tied it will constrict the heart at the junction of the sinus and auricles. Put a single knot on the thread and after taking a short length of normal tracing tighten the ligature in the above mentioned position. If correctly applied the auricles and ventricles come to a standstill, the sinus continues beating; if unsuccessful try a second ligature. The ventricular muscle can now be stimulated in the same way as striped muscle, as follows:—Arrange a simple stimulating apparatus consisting of a Daniell cell and key in the primary circuit of induction coil; short circuit key and pin electrodes in the secondary circuit. Fix the pin electrodes so as to touch the ventricle, magnify the amount of movement of the writing point by bringing the attachment of the thread as near the fulcrum of the lever as the stretching of the ventricle will permit, and use a frictionless

writing point and very lightly smoked paper. Try the effects of stimuli of different strengths and record the height of the contractions (102). Do not stimulate more frequently than at ten seconds intervals. It will be found that if the ventricle contracts at all it contracts to its maximal extent. It does not give minimal and sub-maximal effects.

(187) On the same preparation try the effect of an efficient stimulus repeated at intervals of five seconds or less, moving the drum a short distance after each stimulation. The first few contractions are successively higher and higher—this is known as the “**staircase**” effect.

(188) Put the drum in the primary circuit and take the curve of a simple cardiac contraction as in (105). Mark the point of stimulation and the lengths of the periods of contraction and relaxation. Take time tracing with tuning fork.

(189) Return to the arrangement used in (186), (187), but use the Neef's Hammer, and, while the drum is revolving and the lever is tracing an abscissa, stimulate. It will be found impossible to obtain more than a very incomplete tetanus of the muscle, due to its long “refractory” period.

(190) **Stannius' Second Ligature.**—Tie a ligature round the heart at the junction of the auricles and ventricle. It will be found that the ventricle resumes its rhythmic beating but soon comes to rest.

(191) **The Cardiac Nerves. Influence of the vagus on the heart of Mammals** (dem).—Several procedures may be adopted to show that stimulation of the vagus causes cardiac inhibition. (a) A fine needle on end of a straw lever may be pushed into the animal's heart through an intercostal space; (b) the blood pressure may be recorded; (c) the thorax may be opened and the heart exposed to view in an animal kept alive by artificial respiration from an air pump.

By any of these methods it may be shown that stimulation of the peripheral end of the cut vagus causes slowing, and then

cessation of the heart beat. On removing the stimulus the heart beats more vigorously than before.

In (c) where the thorax is opened the accelerator nerves may be stimulated where they surround the subclavian artery.

(192) **Cardio-inhibitory mechanism in the Frog.**—The heart of the frog may be inhibited by stimulation of (a) the origin of the vagus in the medulla oblongata; (b) the trunk of the vagus; (c) the sino-auricular junction (intracardiac inhibitory nerves).

Decapitate a frog by cutting transversely through the upper jaw and skull in line with the anterior margins of the tympanic membranes, then pith the spinal cord entering the pin about an eighth of an inch below the usual spot—junction of skull and vertebral column. The portion of the brain left between these two points includes the optic lobes and medulla oblongata from which latter the vagi arise.

Lay bare the heart and prepare to record the movements by the usual suspension method (172). Fit up a tetanising apparatus (Daniell cell, simple key, Neef's Hammer in primary circuit; short circuit key and pin electrodes in the secondary).

(a) Insert and fix the pin electrodes in the exposed cranial cavity so that one electrode lies on each side of the medulla. Then while a normal tracing is being recorded stimulate with the interrupted current at first with the coils at such a distance apart that the stimulus is just perceptible when applied to the tongue. If this fails to produce an effect, gradually increase the strength of the stimulation. Mark on the tracing the points where the stimulation was applied, and where it ceased.

(b) Isolate the vagus nerve. Three nerves emerge from the skull near the angle of the jaw in the frog—the glosso-pharyngeal, the vagus, and the hypoglossal. The glosso-pharyngeal and the hypoglossal are easily distinguished, both being comparatively superficial and both turn upwards along the floor of the mouth. It is advisable to dissect both these as far back as possible and then cut them away. The vagus will be found close to the carotid artery, crossing the base of the petrohyoid muscle. Its dissection is facilitated by placing a thick glass rod in the œsophagus. The nerve is accompanied

by its laryngeal branch which is relatively large and apt to be mistaken for the main trunk ; but the branch lies on the upper side of the carotid artery and can be traced towards the larynx, while the vagus itself is on the lower side of the artery. Pass a thread under the nerve, raise it gently, apply the electrodes and stimulate with the interrupted current (Neef's Hammer). There may result either acceleration or inhibition of the heart's action due to the fact that the vagus contains both cardio-accelerator (sympathetic) fibres, and cardio-inhibitory fibres. Weak stimulation has more effect on the inhibitory fibres, while strong stimulation effects the accelerators more.

(c) Fix the pin electrodes against the heart in such a position that the pin points touch the white crescentic line which marks the sino-auricular junction, and again record the effect of stimulation. The pin-points should be close together.

(193) **Effect of some Drugs.**—Paint the sinus with a dilute solution of *Pilocarpine*, or of *muscarine* using a camel hair brush. The heart stops beating in diastole. Now apply a solution of *atropine* in similar fashion, and the heart will resume beating. While the heart is under the influence of the atropine repeat (a) (b) or (c) of preceding experiment. No inhibition results because atropine paralyses the terminations of the vagus in the heart. Acceleration may be obtained if the trunk of the vagus is stimulated due to the uncomplicated effect of stimulation of the sympathetic fibres contained therein. On the same frog after thoroughly washing away the atropine, or on a fresh frog, the effect of *nicotine* may be tried. This drug paralyses nerve fibres where they end in connection with other nerve cells in ganglia. Suppose inhibition had been obtained by methods (a), (b), and (c), above before the application of nicotine, it will be obtained only by method (c) after painting nicotine on the heart, because in (c) the cardio-inhibitory fibres are stimulated after they have passed their cell stations.

(194) **Effect of Bile on the beating Heart.**—While a normal cardiogram is being taken in the usual way, dissect out the frog's own gall bladder without allowing the contents to escape. Hold it over the heart and cut it open so that the ventricle is anointed with bile. Note the slow weak beats which follow.

(195) **Innervation of the Vessels.**—Examine the vessels in the ears of a rabbit which has had its cervical sympathetic nerve divided on one side. Note the dilated condition of the vessels on that side and the absence of the slow rhythmical changes which occur normally. If the animal becomes angry, the vessels of the ears dilate, but those of the sound side become more dilated than those of the side operated on, due to the intact vaso-dilator fibres on that side. (Note also in this animal that the pupil on affected side is contracted, and the eye as a whole retracted).

(196) **Perfusion of Blood Vessels.**—Pith a frog; expose the heart and aorta; make a V-shaped incision in one of the aortic arches; insert and tie in a canula connected to a small reservoir of Ringer's saline solution; see that the canula is full to the point before inserting it. Tie the other aortic arch; suspend the frog vertically; cut the sinus open and bring the toes together so that the fluid which perfuses the whole circulatory system and which escapes at the sinus may drip from the toes. Count the number of drops per minute; add to the perfusion fluid some drug capable of affecting the calibre of the arteries, and again count the rate. Adrenalin is a good substance to use here.

CHAPTER VII.

RESPIRATION.

Examine microscopical preparations of trachea, bronchi, lungs, pleura, etc.

(197) **Graphic record of respiratory movements.**—The movements of the thoracic walls during inspiration and expiration are recorded by means of a *stethograph* consisting of a tambour and writing lever connected to a flexible indiarubber tube fitted to the inside of a leather belt. The belt is secured round the chest a little below the level of the nipple, and the movements of the lever are recorded on a slowly moving blackened drum.

Record also the effects of yawning, coughing, sneezing, and, if it can be obtained, hiccup.

(198) *Ratio of frequency of respiration to pulse rate.*—Determine this by counting each in the sitting and standing posture, and after running once or twice up and down stairs. The usual ratio is 1 to 4 or 5.

(199) **Respiratory Sounds.**—Listen with the stethoscope to the sounds heard during respiration when the stethoscope is placed over the trachea or large bronchi (between the scapulæ at the level of the fourth dorsal vertebra) — *bronchial* or tubular breathing, and in the axillary region, angle of scapula, or apex of lung — *vesicular* breathing. In bronchial breathing a sound is heard during both inspiration and expiration; the sounds are the same in quality of tone, are separated by a distinct silent period at the end of inspiration and beginning of expiration, and they

occupy practically the whole duration of each movement. Vesicular breathing, on the other hand, consists of two sounds which differ in character, the expiratory part being low in pitch while the inspiratory sound has a characteristic rustling sound; further, there is no distinct interval between the sounds, the inspiratory sound occupies practically the whole period of inspiration and is followed immediately by the expiratory unless the subject voluntarily holds his breath at the end of inspiration. Of great practical importance is the fact that in healthy vesicular breathing the expiratory sound is only about half as long as the inspiratory. In children, however, the sounds are equal in duration and have a harsher tone than in the adult. Note also the "vocal resonance." Ask the subject to repeat "one, one, one," or "ninety-nine," and note the intensity of the buzzing sound usually heard.

(200) **Spirometry.**—The spirometer is used to determine the volume of air that can be expired after the fullest possible inspiration = vital capacity. Two forms are in common use: Hutchinson's on the principle of a balanced gasometer, and various forms in which the current of expired air causes revolutions of vanes with which a series of indicators are connected as in the common household gas meter. Determine your vital capacity with each form of instrument.

(201) **Intrapulmonary Pressure.**—Connect a mercurial manometer to a mouthpiece by indiarubber tubing and determine the maximum expiratory pressure after a full inspiration has been taken. Estimate the inspiratory pressure in the same way, but be careful not to use suction.

(202) (**Dem.**) Effect of **division of the vagi** on the respiratory movements.

(203) (**Dem.**) Observe the symptoms produced in a small animal placed in an **air tight chamber**.

(204) **Chemical changes in respired air.**—Determine the amounts of CO_2 and of O_2 in expired air collected in a Hutchinson's spirometer. The CO_2 is absorbed by means of KOH , the O_2 by pyrogallate of potash in some form of gas analysis apparatus.

such as that devised by Haldane. The amount of CO_2 in expired air may also be determined approximately by the following method:—First find the capacity of the ungraduated lower end of a burette between the lowest mark (50) and the clip. This may be done by running in water from another burette. Now fill the burette with water, invert it and place it in the tall jar of water without allowing air to enter. Fill it about three-quarters full of expired air by means of the bent glass tube the lower end of which is to be inserted into the open end of the burette.

Read the volume of expired air which has been obtained being careful not to hold the burette in the hand; hold it by the indiarubber tubing or use a cloth between your hand and the glass. The water level inside the tube must be the same as that outside it when the reading is made. Now bring the open end of the burette near to the surface of the water and insert a small piece (say half to one inch long) of stick NaOH . Remove the burette from the jar after closing the lower end with a small cork. Shake to and fro so that the CO_2 may be absorbed by the solution of caustic soda. After about five to ten minutes replace the burette in the jar, uncork it, and again read the volume of the air with the water inside and outside the burette at the same level and calculate the percentage diminution of volume due to absorption of the CO_2 .

For a method (Haldane-Pembrey's) of estimating the respiratory interchange in small animals see exp. 305, H.

(205) **The gases of blood.** (Dem.) By means of a special form of mercurial pump (Leonard Hill's) the gases of a sample of venous or arterial blood are collected and analysed in a similar way.

Haldane and Barcroft's apparatus for estimating the O_2 and CO_2 in a small sample of blood by the ferricyanide method for O_2 and tartaric acid for CO_2 may also be demonstrated.

(206) **The oxydases or oxidizing ferments in the tissues.**—Add a drop of fresh blood from the finger to a few c.c.'s of ozonic ether or watery hydrogen peroxide in a test tube. An evolution of gas will be seen, due to the liberation of oxygen from the H_2O_2 by the oxydases present. The same ferments are said to be the cause of the Guaiac Reaction (159) of blood and extracts

of tissue, but the question is complicated by the presence of substances which act as catalytic agents and which withstand boiling, thus blood gives the guaiac reaction after boiling due it is believed to the stroma of the red corpuscles acting in this way.

(207) **Artificial respiration.**—Demonstration of Schäfer's Prone-pressure method, which consists in laying the subject in the prone position, preferably on the ground, with a thick folded garment under the chest and epigastrium. The operator puts himself athwart or at the side of the subject facing his head, and places his outspread hands on each side over the lower part of the chest below the scapulæ. He then slowly throws the weight of his body forward, so as to bear upon his own arms, and thus press upon the thorax of the subject and force air out of the lungs. This being affected, he gradually relaxes the pressure by bringing his own body up again to a more erect position without moving his hands. Repeat the movements twelve to fifteen times a minute

CHAPTER VIII.

ALIMENTARY SYSTEM.

Examine microscopical preparations of parts of the alimentary tract—palate, tongue, tooth, tonsil, gullet, stomach, intestines, and of the related glandular organs—salivary glands, pancreas and liver.

(208) (**Dem.**) Observe the **intestinal movements** in a rabbit, the abdomen of which has been opened in a bath of warm normal saline, also the means of recording the movements and the influence of section and stimulation of nerves.

(209) **The contraction of non-striped muscle.**—Cut a transverse or oblique ring of stomach wall of frog. Arrange it on the myograph stand using a straight lever with a frictionless point as in heart work (Fig. 14). Fix a pair of pin electrodes in position so as to touch the strip of tissue, connect these through a simple key to one or more Daniell cells, and take a tracing on the drum of the contraction wave of the non-striped muscle. The length of the latent period is appreciable, and can be measured by putting the drum in the circuit and so obtaining the point of stimulation. Galvanic electricity gives better results than faradic in this kind of tissue and the pins of the electrodes should be as widely separated as possible, or the current should be arranged to pass through the whole length of the strip. Spontaneous contractions may also occur in the strip.

CHEMISTRY OF THE DIGESTIVE SECRETIONS.

(A) **SALIVA**.—Collect some saliva and filter it. Note the reaction to litmus paper—alkaline.

(210) Add a few drops of acetic acid—precipitate of **mucin** in the form of a stringy coagulum results. The precipitate is insoluble in excess of acetic acid.

(211) Filter off the mucin and test the filtrate for protein; result—negative.

(212) Add some ammonium oxalate to some of the same filtrate—clouding, due to the presence of **calcium** appears.

(213) The saliva of many persons contains alkaline **sulphocyanides**. Test for this by adding a drop of HCl to some saliva, filter off the mucin, and to the filtrate add a small amount of dilute ferric chloride—a red colour appears if KCNS is present.

(214) **Ptyalin**—the digestive ferment of saliva is tested for by its action on boiled starch. Have ready some saliva, boiled starch mucilage, a water bath heated to 40°C., a series of small drops of iodine solution arranged regularly on a glazed white porcelain slab. Add to some starch mucilage in a test tube a small quantity of saliva, mix, and place the tube in the warm bath. As digestion proceeds test the contents of the tube from time to time by taking out a drop of the mixture on a glass rod, placing it alongside one of the iodine drops and allowing the two to mingle. The first result of ptyalin action is to change the starch mucilage into **soluble starch** or **amidulin**; this is indicated by a diminution in the opacity of the mucilage, the reaction with iodine being still a blue. Then later there appears **erythrodextrin** which gives a red colour with iodine; later still the mixture gives no colour with iodine but contains a dextrin which can be precipitated by alcohol or other means—**achroö-dextrin**. The final result is the reducing sugar, **maltose**; but some maltose is formed early in the digestion, and under artificial conditions where the products (dextrins and maltose) are allowed to accumulate there always remains some unchanged dextrin (achroö-dextrin). Test the digested

mixture for a reducing sugar by Trommer's and Barfoed's tests. To another portion add some alcohol and note whether a precipitate appears. (*Vide ante*—Polysaccharides). It will be noticed that the action of ptyalin is similar to the action of dilute HCl at boiling temperature, but the end product in the latter case is **glucose** due to the maltose being further hydrolysed.

(215) To some starch mucilage add some saliva which has been boiled and cooled. Keep at 40° C. No digestion occurs because all ferments are rendered inactive by boiling.

(B) **GASTRIC JUICE**.—This secretion contains free **mineral acid** (HCl) in a fairly definite proportion. A solution of HCl is put out similar in strength to that usually secreted. Test it as follows :—

(216) To ordinary blue litmus paper it reacts strongly acid. *Congo red* paper is turned blue, this indicates that the acidity is due to free acid, not to an acid salt such as sodium dihydrogen phosphate. Dry the piece of blued Congo paper, extract it in a dry test tube with ether—the blue colour remains (cf. 221).

(217) *Gunzburg's Test*.—(Reagent = 2 parts phloroglucinol, 1 part vanillin in 30 parts absolute alcohol). Add a few drops of this reagent to a similar amount of the acid solution in a porcelain basin and evaporate gently to dryness over a *small* flame. A carmine red colour indicates the presence of free mineral acid.

(218) *Boas' resorcin test*.—(Reagent = Resorcin 75 grains, white sugar 45 grains, dilute spirit 3½ ounces). Use in same way as for Gunzburg's test. Result—a purple colour if free mineral acid is present.

(219) *The Tropaeolin Test* (also known as Boas' test). Evaporate to dryness a mixture of equal parts of a saturated alcoholic solution of Tropaeolin OO and the suspected fluid—lilac to violet glancing colour appears if free HCl present.

(220) *Estimation of the strength of acid present.*—Measure out exactly, in a graduated pipette or burette, ten or twenty c.c. of the acid fluid into a small flask or beaker, dilute with some water, add a couple of drops of phenol-phthalein solution, run in some tenth-normal alkali from a burette noting the starting point and the end point; the latter is taken when the whole fluid assumes a very faint pink colour, remaining permanent for one minute or so; and then calculate the amount of acid present in 100 c.c.

Each c.c. of tenth-normal alkali exactly neutralises one c.c. of tenth-normal acid., so that each c.c. of $\frac{N}{10}$ soda used = 3.65 mlgm. HCl. (Normal HCl = 36.5 gm. per litre, tenth-normal = 3.65 gm. per litre = 3.65 mlgm. per c.c.) Suppose 8 c.c. of $\frac{N}{10}$ neutralises 10 c.c. of the acid fluid then the amount of HCl in the ten c.c. is 8×3.65 mlgm. = 29.2 mlgm. = .0292 gm. = 0.292 per cent.

The normal percentage of HCl present is 0.2%.

Abnormal acidity.—In some pathological states the stomach contains other acids than HCl, and it is of great practical importance to distinguish between these organic acids and the mineral acid (HCl). **Lactic Acid** is one of the commonest of these abnormal acids, but acetic and butyric acids also occur.

(221) Apply the following tests to a weak lactic acid solution :—

Litmus is affected in the usual way.

Congo-red Paper turns a bluish violet, and after drying, the colour can be extracted with ether, leaving the paper red.

Boas's Test (218) does not give a purple, neither does Gunzburg's give red.

Uffelmann's Reagent (120) gives a yellow colour, as with sarco-lactic acid. HCl completely discharges the colour of the reagent.

In many cases, however, both HCl and organic acids are present, and their separation must be affected. There are various good laboratory methods for estimating the relative amounts of

these, but the following simple procedures will be sufficient in most cases. Do these on a mixture of equal parts of the dilute HCl and lactic acid given you or on gastric contents from a suitable case :—

(222) The colour of Uffelmann's reagent will probably be completely discharged because of the action of the stronger inorganic acid. To about one-third full of a large test tube of the mixture add an equal amount of ether, cork and shake, holding the tube in a cloth while doing so. The ether extracts the lactic acid, and if the ethereal layer is carefully poured off into a porcelain basin containing a few c.c.'s of hot water the ether evaporates leaving the lactic acid. Evaporate off all the ether on a water bath and test the watery residue for lactic acid as in (221).

(223) **Pepsin.**—Prepare an artificial gastric juice from the mucous membrane of the pig's stomach by dissecting off the mucous membrane, mincing it, adding some 0.2% HCl and allowing it to digest for some hours. The fluid is then allowed to cool, and is filtered after standing for one to two days.

(224) To some of this artificial gastric juice add some raw meat fibre or washed fibrin, or a fine emulsion of partially coagulated egg white and note the changes which occur when the mixture is incubated at 40° C. The insoluble protein becomes swollen and gradually dissolves.

(225) Proceed to examine a *gastric digest*, either the one above mentioned or one similarly prepared and allowed to digest for several hours. First take the reaction to litmus, it is acid ; add diluted KOH till the solution is almost neutral, **acid albumin** will be precipitated, if the KOH has been added in too great amount the acid albumin will be redissolved (41), but can be again precipitated by adding acetic acid in slight excess ; filter off the precipitate of acid albumin if any is present and boil the filtrate to remove unaffected coagulable proteins ; filter again if necessary and cool the filtrate. (The bodies so far obtained, coagulable protein and acid albumin, might result from the action of the weak acid at 40° C. and do not necessarily indicate the presence of active pepsin). Half saturate one half of the last filtrate

with ammonium sulphate. A precipitate of **primary albumoses** may result; filter again, using a dry filter paper and funnel (keep the filter paper with this precipitate of primary albumoses); if primary albumose has been found, test the other half of the filtrate with nitric acid, a precipitate will appear which clears up on heating and reappears on cooling. The filtrate from the primary albumose precipitate is now to be tested for **secondary albumose** and **peptone** as follows:—Fully saturate the fluid with crystals of ammonium sulphate, shaking thoroughly and warming gently to secure complete saturation—a precipitate will indicate the presence of secondary albumose, filter and test the filtrate for the presence of protein by the biuret test (17); since peptone is the only protein which is not precipitated by full saturation with AmSO_4 a positive result with the biuret test indicates its presence in the filtrate, and the pink colour of the reaction is also characteristic; in doing the biuret test with a fluid containing AmSO_4 use very concentrated KOH, see (25). The precipitate of primary albumoses mentioned above is to be washed with half-saturated ammonium sulphate, then dissolved in water, and alcohol is to be added till the total volume is three times that of the watery solution; the concentration of alcohol will then be about 66%, and in this strength one of the varieties of primary albumose, viz., the *heteroalbumose*, is precipitated while the other *protoalbumose* remains in solution. These varieties of albumose can also be separated by dialysis, which causes precipitation of the hetero variety only.

The following scheme summarises the method of examining a digest (peptic or tryptic)

Neutralise———precipitate of albuminate (acid or alkali albumin.)

Filter if necessary and boil———coagulation of unaltered protein.

Again filter if necessary and half-saturate with AmSO_4 ——precipitate of primary albumoses.

Again filter if necessary and complete the saturation with AmSO_4 ——precipitate of secondary albumose.

Again filter and test with Biuret———pink colour indicates peptone.

(225a) In examining the contents of a stomach after a test meal both pepsin and rennin should be examined for. This is most readily done by neutralising the fluid with CaCO_3 ; filter; add some of the filtrate to fresh milk and keep at 40°C .—coagulation will result if rennin is present; the remainder of filtrate is to be mixed with finely emulsified egg white and HCl so that the percentage of the latter may be about 0.2 (7 c.c. of concent. HCl to the litre), the mixture is then incubated at 40°C . for some hours, neutralised with CaCO_3 filtered, and the filtrate may be completely saturated with AmSO_4 , filtered, and the new filtrate tested for peptone, or the neutralised filtrate may be boiled, filtered, and this filtrate tested for albumose by the nitric acid test. This test for albumoses is a most useful one, and is chiefly due to hetero albumose.

In examining vomited matter it is frequently important to ascertain if the fluid contains blood. If so, the haemoglobin has usually been converted into haematin, giving rise to the appearance of "coffee grounds"; obtain some of these by filtering or centrifuging, add KOH , heat gently, cool, add ammon. sulphide and examine spectroscopically for haemochromogen (166), or the test for haemin (168) may be applied to the brown substance.

(226) **Coagulation of milk by rennin** is another fermentative action produced by gastric juice. Add to a test-tubeful of milk a few drops of commercial rennet (which is usually an extract of the stomach of a calf), or the artificial gastric juice made from the stomach of the pig (223) may be used if neutralised, otherwise the HCl present will precipitate the caseinogen of the milk. Keep the test tube at body temperature, a coagulum forms which contracts and squeezes out whey. Test *boiled rennet* in the same way. *Calcium Salts are necessary*:—to another test tube of milk add a few drops of a soluble oxalate, mix, add rennet, incubate—no coagulation results; now add CaCl_2 in amount sufficient to combine with the oxalate and leave some calcium ions free, re-incubate—coagulation occurs.

(C) **PANCREATIC JUICE**.—As in the case of the gastric juice, a watery or glycerine extract of pancreas contains the chief ferments. They are four in number: trypsin, amylpsin, steapsin, and a milk-coagulating ferment.

(227) **Trypsin** is not present as such in the pancreatic juice but as the zymogen—*trypsinogen*—which is activated to trypsin by the enterokinase of the intestinal juice; a similar change

occurs when a pancreas is allowed to lie exposed to the air, so that extracts of the gland about a day after death contain active trypsin. If the gland is treated as described in (74) for preparation of its nucleo-protein, *i.e.*, extract for twelve hours with ammoniacal water, strain, precipitate the nucleo-protein by adding acetic acid cautiously, filter and dissolve the precipitate of nucleo-protein in half per cent. sodium carbonate—it will be found that this solution contains trypsin, possibly because it has adhered to the precipitate, for this is a characteristic of ferments that they are apt to cling to precipitates formed in their solutions.

Test the activity of a trypsin solution prepared in this way, or of a commercial extract of pancreas, on native protein by allowing it to digest for several hours at 40°. The action proceeds best in the presence of alkali up to the strength of 1% sodium carbonate; it ceases if free mineral acid is present. Examine the digest in the way described in (225) but for “acid” read “alkali” in relation to reaction and the form of meta-protein which results.

(228) Add some thymol to the remainder of the digest and allow it to incubate for several weeks to obtain the ultimate products (amido acids, etc.) which result from the tryptic digestion *plus the action of erepsin* derived from the tissue of the pancreas. The ultimate products most easily recognised are *leucin* and *tyrosin* which form characteristic crystals and *tryptophane*, which gives a violet colour on addition of water saturated with bromine, after acidifying.

(229) **Amylopsin.**—Test the action of a watery extract of fresh or of dried pancreas on starch mucilage in the same way as for Ptyalin (214); the products of digestion are the same.

(230) **Steapsin** — Make a concentrated watery extract of fresh pancreas; mix some of it with five to ten times its bulk of cream or milk; add a few drops of chloroform, a few drops of litmus solution, mix and if the reaction is acid (red colour) add a trace of sodium carbonate so as to have the mixture just alkaline and not excessively so. Divide this mixture into two; boil one portion; keep both at 40° C. till next day, and if the steapsin has acted, the unboiled fluid will show a red colour, or more red

as compared with the boiled portion, due to the formation of free fatty acid from splitting of the fat. It should be unnecessary to explain the reasons for the above procedure; the student ought to work out for himself the reasons for boiling one of the tubes, for adding chloroform, for using cream preferably to milk, for making the mixture before dividing into two, etc.

(231) Pancreatic juice and extracts cause coagulation of milk. Test this on fresh milk as in (226).

(D) **BILE**.—Bile may be included here among the digestive juices although it of itself has no marked digestive action on the food stuffs; it aids the action of pancreatic juice however.

(232) Test the *reaction* of ox bile to litmus paper; it is alkaline.

(233) **Mucin**.—Add a few drops of acetic acid—a precipitate of mucin is thrown down.

(234) **Bile Salts**—*Pettenkofer's Test*.—To some diluted bile or to the filtrate from the mucin precipitate (233) add a few crystals of cane sugar, and shake to accelerate solution and to cause a froth to appear; allow some concentrated sulphuric acid in excess to flow down the side of the tube—a purple colour develops where the acid comes in contact with the bile-and-sugar mixture; this colour is also well seen in the froth where it has been touched with the acid; now mix the contents of the tube and regulate the temperature of the mixture to about 70° C by cooling under the tap or heating over the bunsen—the purple colour ought now to become evident throughout the mixture and if cooled and diluted with alcohol a characteristic spectrum will be seen when examined with the spectroscope; it consists of one band between D and E, near E, and one near F. The rationale of the test is that the strong H_2SO_4 produces furfural from the sugar, and at the same time, cholalic acid from the bile salts, and the interaction of these two produces the purple colour. If too much sugar is added, the charring which results (51) obscures the result. Furfural solution (0.1%) may be used instead of sugar. This colour reaction is analogous to some protein colour reactions

(*e.g.*, glyoxylic (18) and Molisch's (20) where a colour results from the interaction of an aldehyde and a substance with an aromatic nucleus, in this case furfural (furfuraldehyde) and cholalic acid. The test is therefore unreliable in the presence of ordinary proteins.

(235) **Bile Pigments.**—*Gemlin's Test.*—To some diluted bile or bilious urine add some impure nitric acid so as to form a deep layer at the bottom of the test tube. At the plane of contact a series of colours appears, spreading up into the bile; (at the same time the mucin is precipitated if bile itself is used). The colours result from the oxidation of the **bilirubin** and **biliverdin** of the bile, and occur in the following order from above downwards, bilirubin (red), biliverdin (green), bilicyanin (blue), choletelin (yellow) or if concentrated (reddish). The reaction may also be done on a white porcelain plate by allowing a little bile to spread out on the plate in a thin layer and placing a drop of impure nitric acid in contact with it.

(236) *Sulphur Test for Bile.*—Bile salts can reduce the surface tension of a watery fluid so that fine grains of sulphur will sink through the fluid. Take two test tubes, one of water and one of diluted bile; add to each a few fine particles of powdered sulphur. The sulphur sinks readily through the bile but remains floating on the water.

(237) **Cholesterin**—previously considered as a constituent of protoplasin in general, occurs in relatively large amount in bile, and in some diseased conditions forms a gall-stone. Other forms of gall-stone consist chiefly of lime and pigments. Examine the appearances of some common gall-stones—note that the cholesterin ones are light and float in water. Extract some powdered gall-stone with alcohol on a water bath; allow a few drops of the extract to evaporate on a slide, and examine under the microscope for crystals of cholesterin. Repeat the microchemical test (75) with H_2SO_4 and iodine. If much cholesterin is present make a chloroform extract and repeat Salkowski's reaction (76) and (77).

(238) Bile has a slight power of *reducing* cupric hydrate solutions.

(239) One or other method of *isolating the bile salts* may be demonstrated while bile is being studied in the class.

(240) The *inorganic constituents* of bile consist of the usual chlorides, phosphates, sodium, potassium, calcium, etc. Iron occurs only in mere traces.

Action of bile in digestion.

(241) *On Proteins*.—Bile precipitates and is partially precipitated by the constituents of a gastric digest (chyme). Test this statement; the mixed precipitates consist chiefly of acid albumin, mucin and bile salts. Peptone is used clinically by some observers as a test for bile salts in urine.

(242) *On Carbohydrates*.—Bile aids amylase in the digestion of starch. Take some very thin starch mucilage, add some amylase, divide into two portions, to one add some bile, and place both on the water bath at 40° C. Test at intervals a drop from each tube against a drop of iodine on a porcelain slab, as in (214) and note the relative time taken by each to show the changes in the starch.

(243) *On Fats*.—Bile aids the emulsification and splitting of fat. Compare the permanency of the emulsion formed by shaking the following mixtures:—

- (a) Water and oil (*rancid* olive oil), (b) 1% sodium carbonate and oil, (c) 1% sodium carbonate and oil *plus* some bile. In (a) no real emulsion forms, in (b) a slight emulsion depending on the amount of soap formed if the oil is rancid, in (c) a good emulsion forms.

(E) **SUCCUS ENTERICUS** contains the ferments, Invertin, Maltase, Lactase, Erepsin, and Enterokinase.

(244) **Invertin**.—Test the effect of a watery extract of intestinal mucosa on the inversion of cane sugar, at 40°C. If inverted, the cane sugar yields glucose which will reduce Fehling.

(245) Test the effect of the same extract on a solution of maltose. If **Maltase**, the maltose-inverting ferment is present, Barfoed's reagent will be reduced by the products (44).

(246) Test the effect of the same extract on lactose. If **Lactase** is present the lactose is inverted, yields glucose which will give the fermentation test (50). Do control experiments with boiled extract of the mucosa in each case.

(247) **Erepsin**.—Add some of the watery extract of intestinal mucosa to a solution of albumoses and peptones so diluted that it gives a faint but distinct biuret reaction. Keep the mixture at 40°C and test from time to time. If the erepsin is present in small amount, the result (disappearance of the biuret reaction) may take some days to appear cf. (228); control as above.

CHAPTER IX.

THE URINE.

The **amount** of urine passed daily varies considerably, but is usually 40 to 50 fluid ounces (or about 1500 c.c.).

The **colour** varies from light straw to amber, according to the concentration. It is due to certain definite pigments—urochrome, urobilin, hæmatoporphyrin, etc.

(248) The **reaction** is generally acid to litmus paper due to acid salts (NaH_2PO_4) as shown by the fact that it does not affect congo red paper (c.f. (216) gastric juice). It may be amphoteric, neutral, or even alkaline during active digestion. Decomposing urine and normal herbivorous urine is alkaline.

(249) The **specific gravity** varies from 1015 to 1025, or less, or more according to the amount of water present. It is usually taken with a *urinometer*—a hydrometer specially graduated from 1000 to 1030. Remove any froth that may be present and allow the urinometer to float freely in the urine without touching the sides of the jar. Read the level at which the instrument floats. In light coloured urines this may be done conveniently by looking through the fluid, with the eye almost on a level with the surface; in this way the error arising from the meniscus which forms where the urine is in contact with the stem of the hydrometer may be avoided. The urinometer readings vary with temperature and are correct only at the usual room temperature (15°C.). For each 3° above or below that temperature add or subtract a unit to the last figure of the reading, *e.g.*, if the temperature of the urine is 18°C. and the apparent specific gravity by the urinometer 1016, the correct specific gravity is 1017.

CHEMICAL COMPOSITION.—The chief *organic* constituents of urine are—urea, uric acid and xanthin bases (alloxur bodies), creatinine, hippuric acid, ethereal sulphates, and pigments, with variable traces of other substances.

The *inorganic* constituents are sodium, potassium, ammonium, calcium, magnesium, partly free as ions and partly united to the acid radicles as chlorides, phosphates, sulphates, carbonates, etc.

The chief substances which appear *pathologically* in urine are albumin (and globulin), blood (corpuscles and pigments), bile (salts and pigments), sugar (glucose chiefly and the acetone bodies); and there may be an abnormal increase or diminution of the amount of water passed, or of a normal constituent, *e.g.*, increase of uric acid and urates, of ethereal sulphates, or of phosphates, etc.; or a diminution of urea, or of chlorides, etc.

(A) ORGANIC CONSTITUENTS.

Urea $\text{CO}(\text{NH}_2)_2$.—Urea is the chief solid found in urine.

(250) Examine the crystals of pure urea. Note the cooling sensation when they are applied to the tongue due to their rapid solution in water. Note the great *solubility* of urea in water, and in alcohol, and its insolubility in anhydrous ether and chloroform.

(251) In concentrated solution it forms additive *compounds with acids*. Test this by adding a drop of concentrated watery solution of urea to a drop of pure HNO_3 on a slide; examine the crystals of nitrate of urea which form. Proceed similarly with a concentrated solution of oxalic acid (oxalate of urea). The crystals take the form of rhombic plates in each case.

(252) *Heat* some urea crystals in a dry test tube. They melt and ammonia is evolved (test with moist red litmus paper). A sublimate forms on the colder upper part of the tube, consisting chiefly of ammonium carbonate. Heat till the contents of the tube become solid, then cool, add a few drops of water, a few drops of

KOH solution and a drop of watery CuSO_4 solution. A fine pink colour appears, due to the presence of *biuret*. (Proteins give a similar reaction on adding KOH and CuSO_4 , and the same name is generally applied to the reaction, but the atomic grouping which gives the colour is not quite the same in the two cases).

(253) Add some *KOH* to urea crystals and heat—ammonia is evolved (test with moist litmus) cf. action of alkali and heat on protein (34).

(254) Some *oxidising agents* such as sodium hypo-bromite (NaBrO), and nitrous acid decompose urea, liberating nitrogen and CO_2 . To some urea solution in a test tube add a few drops of sod. hypobrom.—A copious evolution of gas results. The reaction is expressed by the formula :—

$3 \text{ NaBrO} + \text{CO}(\text{NH}_2)_2 = 3 \text{ NaBr} + 2\text{H}_2\text{O} + \text{CO}_2 + \text{N}_2$
 since the solution of hypobromite is strongly alkaline, the CO_2 is absorbed, and only the N_2 is liberated. This forms the basis of the next exercise.

(255) *Quantitative estimation of Urea in Urine.*—The apparatus for this consists of a burette inverted in a tall jar filled with water and connected by tubing to a bottle containing a small glass tube.

Test whether the apparatus is airtight by inserting the stopper in the bottle and raising the burette; if there is no leakage the column of water in the burette remains standing at a higher level than the water in the jar.

Place 20 to 25 c.c. hypobromite solution in the bottle, and exactly 5 c.c. of urine in the small tube; place the tube inside the bottle without allowing the fluids to mix; insert the stopper securely; place the bottle in a jar or dish of water to cool; then read the level of the water in the burette with the water inside it at the level of the surface of the water in the jar. Now tilt the bottle so as to mix the urine and hypobromite solution and shake very gently; try to let some of the hypobromite get into the small tube to act on the urine that wets its inside; replace the bottle in the cold water; allow it to cool; then read the burette in the same way as before, *i.e.*, with the water inside

and outside the burette at the same level. Since the CO_2 is retained in the alkaline hypobromite, the increase in the volume of the gas contained in the apparatus (the difference between the readings of the burette) is due to the nitrogen evolved from the urea in 5 c.c. of urine. The calculation of the amount of urea present is made as follows:—From the formula given above (254) 1 gramme of pure urea should yield 372 c.c. of nitrogen gas at 0°C and 760 m.m. barometric pressure; but when the decomposition is done under the usual laboratory conditions, and with urine, which always contains other substances capable of yielding nitrogen with hypobromite, the amount of gas corresponding to 1 gram. urea is only 354.3 c.c., *i.e.*, 0.1 gram. would give 35.43 c.c.; suppose the burette readings before and after decomposition of the urea were respectively 48.7 and 21.3 = an increase of volume due to N_2 of 27.4 c.c., this would equal $(\frac{27.4}{35.43} \times 0.1)$ gram. urea in 5 c.c. = 0.077 gram., the percentage is therefore 1.54, and the amount in a total day's urine of 1500 c.c. = 23.1 gram., a somewhat low figure. Southall's and various other ureometers used clinically will be demonstrated.

Uric Acid.—Note the shape and colour of uric acid crystals under the microscope. The chief form is that of pointed oval plates arranged in stars, rosettes, and barrel shapes. They are almost always coloured with the urinary pigments. Uric acid occurs in urine united to bases and the compound frequently settles out on cooling.

(256) Examine such a deposit of *urates* and note that they dissolve on moderate heating. Crystals of uric acid may be obtained by strongly acidifying with HCl , such urine or even normal urine and allowing it to stand for a day or less.

(257) Note the *solubilities* of pure uric acid. It is almost insoluble in cold water (1 in 16,000), more soluble in hot (1 in 2000). It is insoluble in alcohol and ether. It dissolves in concentrated H_2SO_4 without decomposition, and is again precipitated on adding a drop of the solution to a test-tubeful of water. It dissolves in alkalis and alkaline carbonates forming urates.

(258) Make a small amount of a concentrated solution of urate of potash by dissolving uric acid in KOH with the aid of

gentle heat. Allow it to stand for forty-eight hours—the urate deposit which results then behaves like the urates found in urine *i.e.*, disappears on heating and reappears on cooling.

(259) Test a small amount of the concentrated urate of potash for *reduction* with Fehling's solution. A whitish precipitate of cuprous urate forms which on further heating gives some reduction. Reduction is also shown by *Schiff's Test*:—Dissolve some uric acid in sodium carbonate solution, moisten a filter-paper with it, apply a drop of silver nitrate, a black stain of reduced silver appears. In human urine the concentration of urates is insufficient to give reduction with Fehling's solution.

(260) Dissolve some uric acid in ammonia solution, add magnesia mixture and a few drops of silver nitrate solution. A gelatinous precipitate of *silver-magnesium-urate* forms.

(261) *Murexide Test*.—This is the most delicate test for uric acid, but it is given in modified form by the xanthin bases and a similar colour is produced with cholesterin (77).

To a few particles of uric acid or to several drops of urine add about five drops of nitric acid; evaporate gently on a small flame; when dry, cool and add ammonia at one point—a purple colour results. The colour is due to the formation of ammonium purpurate—purpuric acid having resulted from the oxidation of the uric acid. If KOH is added to the residue at another point—a bluish violet colour is obtained.

(262) **Xanthin Bases**.—These occur in small amounts along with uric acid. To about 20 c.c. urine add 5 c.c. magnesia mixture—a precipitate of phosphates results. Filter and add 2—4 c.c. silver nitrate solution to the filtrate. If the precipitate which results is white and curdy add more ammonia till the white (AgCl) disappears. Allow to stand for a little—a fine gelatinous precipitate settles out, consisting of a compound of silver and magnesium, with all the purin bodies. (There is a clinical method (Walker-Hall's) for estimating purin bodies, according to the amount of this precipitate, in the same way as albumin is estimated by Esbach's tube).

The term "alloxuric bodies" or "purin bodies" is applied to the group which includes uric acid and the nuclein or xanthin bases (xanthin, hypoxanthin, guanine, and adenin).

(263) **Creatinine.**—Repeat *Weyl's* and *Jaffe's* tests (125), (126), using normal urine instead of the muscle extract.

Creatinine in strong solution gives *reduction* tests, but it has also the power of holding reduced oxide of copper in solution, and so prevents the reduction from becoming apparent to the eye. It is therefore an important factor in obscuring the reduction test in urine where the amount of the glucose is very small (MacLean).

(264) **Hippuric Acid.** — (Always present in herbivorous urine—occurs in variable amount in man). Heat a small quantity of hippuric acid in a dry test tube. The crystals melt—become dark red—and give off an aromatic odour like that of oil of bitter almonds, due to phenyleyanide and hydrocyanic acid.

(265) **Organic or Ethereal Sulphates.** — These are bisulphates of potassium and sodium in which the hydrogen is replaced by indoxyl, skatoxyl, phenol, etc. The chief of these is *indican* or indoxyl-sulphate of potassium.

(a) To about ten c.c. of herbivorous urine add an equal amount of concentrated HCl, several drops of chloroform, and one drop of a 5% solution of commercial chloride of lime, or "bleaching powder;" shake; the *indoxyl* is set free by the HCl, oxidised to indigo-blue by the hypochlorite present in the bleaching powder, and this is dissolved and carried down by the chloroform which forms a blue-coloured layer at the bottom of the tube when the result is positive. A second drop or more of the bleaching powder solution may be added if the blue colour does not appear at first, but excess of this reagent must be avoided as it may oxidise the indigo-blue to a colourless compound. If human urine gives the test, the indication is that there is an abnormal amount of bacterial action going on in the alimentary tract.

(b) The *sulphate* part of the ethereal sulphate may be tested for as follows :—Add to the urine some acetic acid, and then barium chloride solution in excess ; keep the mixture on the water-bath till the precipitate settles ; filter or decant off the clear supernatant fluid ; divide into two parts ; to one add some HCl and boil ; compare it with the other for the presence of a precipitate (disregard any colour changes which may result on boiling with HCl). The rationale of the test is this :—The inorganic sulphates are precipitated by the barium chloride first added, the phosphates being held in solution by the acetic acid ; the filtrate contains the excess of barium chloride and the ethereal sulphates ; when this is boiled with HCl these are broken up, and yield inorganic sulphate, which unites with the barium present to give a further, though much smaller, precipitate of BaSO_4 .

(266) **Total Nitrogen.**—The nitrogen of the urine is chiefly contained in the foregoing substances—urea, uric acid, xanthin bases, creatinine, hippuric acid, and indican ; a variable amount also exists as ammonium salts and in the pigments, mucin, etc. The total amount may be estimated by Kjeldahl's method as follows :—Measure accurately 5 c.c. of urine into a round-bottomed Jena glass flask, specially used for these estimations ; add 10c.c. of pure H_2SO_4 , a crystal of CuSO_4 , and a few grammes (5-10) of potassium sulphate. Heat the flask over a bunsen flame on a tripod with wire gauze in a fume chamber ; the flask should be supported in an inclined position. Under the influence of the high temperature and acid the whole of the nitrogen becomes converted into ammonium sulphate, and the other elements are oxidised, *e.g.*, carbon forms CO_2 , hydrogen H_2O , etc. Allow the incineration to proceed for some time after the contents become clear, then cool and add water to dissolve the residue. The ammonia in this residue is then distilled over after adding strong NaOH, and estimated as described later (Exp. 305, C.3.).

(B) INORGANIC CONSTITUENTS.

Chlorides.—Test as in (2), acidify with nitric acid to prevent phosphates from being precipitated, and add silver nitrate.

(267) *Quantitative Estimation of Chlorides* (Volhard).—Measure accurately 10 c.c. urine into a 100 c.c. flask, add some distilled water, 4 c.c. of pure nitric acid, and 15 c.c. of standard silver nitrate solution measured from a burette, then fill up to the 100 c.c. mark with distilled water and shake. This causes precipitation of all the chlorides, and leaves some silver nitrate in solution, the amount of which is now to be estimated. Filter the contents of the flask through a dry funnel and paper into a dry 50 c.c. flask (or if the flask is wet it may be washed out with the portion of the filtrate which first runs through). Collect exactly 50 c.c. of the filtrate, empty it into a titration flask or beaker, and wash out the flask, and add the washings to the 50 c.c. Then add about 5 c.c. of ammonia iron alum solution to act as an indicator, and run in carefully from a burette sufficient standard ammonium sulpho-cyanide to cause a permanent red tinge in the fluid. Note the starting and ending points; calculation—Suppose 15 c.c. of AgNO_3 were taken (more may be required if the urine is rich in chlorides), and that 3 c.c. of NH_4CNS solution were sufficient to produce the end reaction with half the filtrate (*i.e.*, 50 c.c.), then 6 c.c. would have been required if the whole filtrate had been taken. Since 1 c.c. of sulpho-cyanide exactly combines with 1 c.c. of silver nitrate, the excess of silver nitrate in solution in the 100 c.c. flask after precipitating the chlorides is 6 c.c., and therefore 9 c.c. must have combined with the chlorides of the urine. Now 1 c.c. of the standard silver nitrate solution corresponds to 0.01 gm. of sodium chloride, and therefore the 10 c.c. of urine contains 0.09 gm. of chlorides reckoned as $\text{NaCl} = 0.9\%$ or 13.5 gm. in 1,500 c.c.

The strength of the standard silver nitrate is obtained from the combining weights of AgNO_3 and NaCl , thus NaCl (58.5 gm) \div AgNO_3 (170 gm) exactly satisfy each other, and therefore 29.06 gm. AgNO_3 in a litre of water would equal 10 gm. NaCl or 1 c.c. of the silver nitrate solution would equal 10 mlgm. of NaCl . The ammonia iron alum acts as an indicator in this way, that as soon as sulpho-cyanide has been added in amount sufficient to combine with the silver present, the next drop strikes a red colour with the iron salt; cf. (160) and (213).

(268) **Phosphates.**—Simply heating the urine causes precipitation of *earthy* (Ca and Mg) phosphates in some cases by

driving off the CO_2 ; the precipitate is soluble in acetic acid (distinction from albumin, *see* test (21)). Addition of alkalis or ammonia causes precipitation of the same varieties of phosphate; filter; the filtrate still gives the tests for phosphates (3) and (4) due to the presence of *alkaline* phosphates (K and Na). Traces of organic phosphates occur corresponding to the ethereal sulphates.

(269) *Estimation of Phosphates* (Uranium Nitrate Method). Measure accurately 50 c.c. urine into a small porcelain basin, add about 5 c.c. of sodium acetate solution and heat to boiling. Have ready a burette containing uranium nitrate, and a series of drops of potassium ferrocyanide arranged on a white porcelain slab. When the urine begins to boil, gradually run in some uranium nitrate solution from the burette, stir with a glass rod and test a small drop of the boiling mixture for free uranium nitrate by bringing it in contact with one of the drops of ferrocyanide. Cease adding the uranium nitrate when the mixture, tested in this way, shows a faint brown tinge, which indicates that all the phosphate in the urine has been precipitated as phosphate of uranium and that any further addition of uranium nitrate remains free in solution. Read the amount of uranium solution used to reach this end point; it is so made that 1 c.c. of it equals 5 mlgm. (.005 grm.) of P_2O_5 . From this it is easy to calculate the amount in percentage, or as total daily output if the amount of urine is given.

The reason for adding sodium acetate is that during the action between the acid phosphates of the urine and the uranium nitrate some free nitric acid is liberated; this would keep the uranium phosphate in solution if it were not for the sodium acetate which combines with the nitric acid and sets free acetic acid instead. Acetic acid is also added to the sodium acetate solution when it is made up; it keeps the earthy phosphates in solution till they combine with the uranium.

(270) **Sulphates** (inorganic) *see* (265 *b*) and (6); about one-tenth of the sulphate present in urine is in organic combination.

(271) The *bases* united to the foregoing acid radicles or free as kations, are sodium (7), potassium (8), calcium (9), magnesium

(10) and ammonia. The best way to demonstrate the presence of **calcium** and **magnesium** is to add to about 100 c.c. of urine sufficient ammonia to cause a copious precipitate of the earthy phosphates; filter; dissolve the precipitate from the filter paper with dilute acetic acid. This gives a concentration of the Ca and Mg salts, and on adding ammonium oxalate a large precipitate of calcium oxalate comes down; filter till clear, and add an excess of strong ammonia to the filtrate—the magnesium comes down as ammonio-magnesium phosphate.

Ammonia may be detected and estimated as follows:—(Schlössing's Method). A bell-jar fitting accurately on a glass plate is required; place 20 c.c. of $\frac{N}{10}$ acid in a small beaker or porcelain basin; over this, on a tripod, place another basin containing 20 c.c. of fresh urine with thymol added to prevent fermentation; add to this latter about the same amount of "milk of lime"; these are all arranged on the glass plate and then covered with the bell-jar, and the whole made air-tight by smearing the junction of the bell-jar and plate with vaseline. Let this stand for three days; then estimate the diminution of acidity of the $\frac{N}{10}$ acid by titrating with $\frac{N}{10}$ alkali. The ammonia has been gradually liberated by the lime and is then absorbed by the acid. On boiling some urine with KOH there is, of course, a copious evolution of ammonia derived from the urea, nitric acid, etc., but lime at room temperature does not attack these bodies.

(271a) **Deposits in Urine.**—The common forms of deposit in urine are:—(1) A pinkish coloured deposit of *urates* consisting of amorphous granules; it occurs in acid urine but persists if the urine becomes alkaline from decomposition. Urates dissolve on gently heating (256). (2) *Uric acid*—fine dark scattered grains like cayenne pepper—of characteristic form under the microscope. (3) *Calcium oxalate*, less easily distinguished macroscopically because always small in amount. The crystals are clear, colourless, octahedra as a rule, occasionally dumb-bell shaped. (4) A white deposit in an alkaline urine is generally *ammonio-magnesium phosphate*. The crystals have the form of "knife rests," or "feathery" phosphates. (5) "Stellar phosphates" or *phosphate of calcium* has a characteristic appearance under the microscope. (6) *Pus* can be distinguished by the

appearance of pus corpuscles when examined under the microscope; the deposit becomes glairy on addition of KOH, and forms "strings" on pouring it from one test tube to another; pus in urine gives the guaiac reaction. The rare forms of urinary deposits are, *leucin* usually associated with *tyrosin*, *cystin*, *hippuric acid*, etc.

If the suspended matter present in the urine is sufficient to form a distinct deposit, one can easily obtain some of it for microscopic examination, but when the urine is suspected of having material present which does not form a sediment one may use a small centrifuge and obtain a drop from the narrow end of the centrifuge tube for examination. In this way *blood cells*, *epithelium from the kidney and urinary passages*, *tube casts*, *bacteria*, etc., may be found. (Demonstration on the use of the small centrifuge).

Normal urine may frequently show a fine cloud of *mucus* as a deposit. It consists of mucin precipitated by the acidity of the urine. The mucin is not a true urinary constituent, but is added to it by the epithelium of the urinary passages.

(C) PATHOLOGICAL CONSTITUENTS OF URINE.

(272) **Albumin.**—This is serum albumin usually accompanied by serum globulin. The tests commonly employed in clinical work are (a) *coagulation on heating*, see test (21); (b) Heller's test, *nitric acid in the cold by the ring method* (22); (c) *acetic acid and potassium ferrocyanide* (23); (d) *Esbach's solution* used for estimating albumin may also be used qualitatively. In every case the urine ought to be clear before the tests are applied, therefore filter if necessary; and more than one test should be employed.

The *amount* of albumin may be approximately determined with Esbach's Albuminimeter as follows:—Clear the urine by filtering if necessary; take the specific gravity, and if it is over 1010 dilute the urine with a known amount of water till it is at or under that figure, *e.g.*, if the specific gravity is 1015 take 30 c.c. dilute up to 45 c.c. and mix thoroughly; fill the Esbach tube with

urine up to the mark U ; add the reagent up to mark R ; cork the tube, mix, let stand for one day, and read the height of the precipitate. In the above case the result would require to be increased in the ratio of 30 : 45 to give the true figure. (See also 156). The urine may require dilution not only on account of a high specific gravity but also because of an excessive amount of albumin. The urine in albuminuria may be pale and of low specific gravity, or concentrated and contain blood, or glucose or bile may also be present.

(273) **Blood.**—Red and white corpuscles may occur, or haemoglobin, or one of its derivatives such as methaemoglobin may be present without the corpuscular elements.

The urine may appear reddish, but if there is only a small quantity of blood it has a characteristic “ smoky ” appearance.

(a) If blood is present the *Guaiac Test* (159) should give a positive result, but this is not an infallible indication of its presence ; the reagents must be active—test with a few drops of blood from the finger mixed with water ; and some drugs when excreted in the urine give a similar reaction. Such drugs are rhubarb, senna, santonin and iodides. Oil of turpentine, which has been long exposed to the light and which then contains ozone may be used instead of ozonic ether.

(b) *Spectroscopic examination* of the urine (161) is the best. The spectrum of HbO_2 , or of one of its derivatives may be seen directly in the urine, or these pigments may be converted into haemochromogen (166) which in dilute solution has a more intense spectrum than any of the others. The blood pigment may be concentrated from a large bulk of urine, say 100 c.c., by forming a precipitate in it of the earthy phosphates with KOH, or of albumin coagulated by heat ; the haemoglobin or its derivative goes down along with the precipitate or coagulum, and can then be extracted with a small amount of dilute H_2SO_4 ; then render alkaline, heat, cool, add ammon. sulphide and examine for haemochromogen (166).

(c) *Microscopic examination* of the deposit from the urine, or of the result of centrifuging may show the presence of blood cells.

(d) *Heller's Test for Blood*.—Take about half full of a test tube of the urine, make it strongly alkaline with caustic alkali, boil; if a moderate amount of blood is present the deposit of earthy phosphates which is thrown down is coloured brownish-red by the haematin formed, while the supernatant fluid has a greenish tint.

(274) **Bile. Bile Pigment.** (a) Apply *Gmelin's Test* (235) directly to the urine. In the play of colour look especially for the green (biliverdin) because non-bilious urine may give some colour with the nitric acid due to indican and skatoxyl. (b) A more certain, though slower, method is to filter a large amount of the urine repeatedly through the same filter paper; the paper takes up the pigment; then spread the paper out flat on a porcelain slab and place a drop of the impure nitric acid on the centre; the rings of colour appear around the spot. (c) Huppert's modification of the test is:—add a small amount of milk of lime to about 50 c.c. of the urine; this carries down the pigment; filter off the sediment; wash with water on the filter paper; dissolve in acid alcohol (5 c.c. of HCl in 100 c.c. methylated spirit); heat the alcoholic solution—if bile pigment is present the solution becomes green or bluish green.

Bile Salts.—These occur less frequently in urine.

(a) Try *Pettenkofer's Test* with spectroscopic examination of the colour (234); (b) the *sulphur test* (236); (c) *Oliver's Test*—Acidify slightly with acetic acid and filter if the urine is alkaline or turbid, or both; reduce the specific gravity to 1008 if necessary, add to the urine three times its bulk of a peptone solution—a milky opalescence indicates bile salts.

The peptone solution consists of powdered peptone (Savory and Moore's is best), half a drachm; salicylic acid, 4 grains; acetic acid, half a drachm; distilled water, up to 8 ounces; filter till clear.

(275) **Sugar (Glucose).**—Diabetic urine in a case of even moderate severity is clear, and of high specific gravity (above 1030) and large amounts are passed per day.

- (a) It gives all the ordinary reduction tests.
 - (b) It gives the phenyl glucosazone crystals.
 - (c) It ferments easily.
 - (d) And rotates the plane of polarised light.
- Repeat tests (42) to (51).

(276) *Estimation of Glucose*.—Glucose may be estimated by the polarimeter (or saccharimeter) or by its power of reducing cupric salts.

(a) *The ordinary Fehling Method*.—Fehling's solution, when freshly made, is of such a strength that each c.c. is completely reduced by 5 mlgm. (.005 gram.) glucose. The urine generally requires dilution from 1 to 20 times with water. This must be done by measuring accurately 10 c.c. of urine from a graduated pipette or burette, and making it up to 100 c.c. with water. Place the diluted urine in a burette. Measure accurately 10 c.c. Fehling solution into a porcelain basin, add some water and boil. While the fluid is kept boiling run in the diluted urine, stirring all the time, till the last trace of the blue colour of the Fehling's solution has gone. The colour may reappear when the fluid is allowed to cool in air, but this is of no consequence. During the estimation the fluid must be kept boiling. The end reaction is somewhat difficult to preceive; a good plan is to continue the boiling for a little time till the deposit forms then tilt the basin slightly, and look through the edge of the hot fluid. Read the amount of diluted urine necessary to reduce the 10 c.c. Fehling = X c.c., then X c.c. contain .05 gram. glucose. If the dilution is 1 in 20, then $\frac{X}{20}$ of the original urine contains .05 gram. glucose, and $\frac{20 \times .05 \times 100}{X}$ = the percentage.

A rough indication of the amount of glucose present in urine may be done as follows: To freshly prepared Fehling in a test tube add an equal amount of the urine; boil for about a minute. If all the blue colour disappears the urine contains .05% or more glucose. If after boiling 1 volume of urine with 2 volumes of Fehling, the reduction is complete, then 1% or more present. In the same way complete reduction in a mixture of 1 of urine with 4 of Fehling indicates 2% or more.

(b) *Pavy's Modification of Fehling's Method* (Dem.) gives a sharper end reaction. The reduction is done in a flask closed except for a steam outlet and the nozzle of the burette, both of which pierce the stopper. The flask is suspended over the bunsen flame by means of the rubber tubing which connects the burette to its nozzle. The copper solution used contains an excess of NH_3 which holds the reduced copper oxide in solution (see 43), and so one can tell more readily when the solution is fully reduced, *i.e.*; when all the blue colour is gone. The procedure is as follows: 50 c.c. of Pavy-Fehling solution is placed in the flask. This is equivalent to .025 gm. glucose, for the Pavy-Fehling contains 10 times less copper than ordinary Fehling. Place the diluted urine in the burette, see that the nozzle is full to the point, and then insert the stopper into the flask, and proceed to boil the contents. The best dilution of urine to employ depends on the amount present. It should be such that the diluted solution has about 0.1% glucose (and this can be determined roughly in a preliminary test).

When steam begins to issue freely from the flask run in the diluted urine gradually, having first read the level of the fluid in the burette. When sufficient glucose has been run in, the blue colour is completely discharged, and this constitutes the end point. The calculation is easily understood. 50 c.c. Pavy-Fehling fully reduced means .025 gm. glucose in the amount of diluted urine run in; say X c.c., and suppose the original urine has been diluted 1 in M parts, then $\frac{X}{M}$ c.c. contains .025 gm. glucose, and 100 c.c.

will contain $\frac{100 \times M \times .025}{X} = \%$. A great deal of the accuracy

in this method depends on the rate of delivery of the urine from the burette. If too slow the NH_3 boils off before the end point is reached, and a deposit of cuprous oxide comes down which spoils the reaction. If the delivery is too rapid, one is apt to get beyond the end point because the reduction does not occur immediately. The entrance of air into the flask leads to re-oxidation of reduced copper, consequently the flask must not be allowed to cool, nor must one add too much cold diluted urine at a time.

(c) The saccharimeter may be used (51).

The reactions generally designated tests for "sugar" (Trommer, Fehling, Moore, etc) merely indicate the presence of a reducing substance in the urine. The results of these tests should be confirmed by the application of the Phenylhydrazin test, fermentation, or the polarimeter.

In the great majority of cases glucose is the reducing substance, but occasionally the following causes of reduction are met with (277) to (279).

(277) *Lactose* (See under Disaccharides c).—Lactose appears occasionally in the urine of nursing women. It gives the reduction tests except Barfoed, but this latter can hardly be depended upon as a crucial test. More important is the negative result with yeast fermentation within 12 to 24 hours. If left longer inversion of the lactose may occur, and then fermentation. The crystals of phenyl-lactosazone are somewhat characteristic in appearance—the clusters of needles being more ball-like than those of glucose.

(278) *Pentose*.—This monosaccharide ($C_5H_{10}O_5$) may occur in urine after eating certain fruits, such as pears, or as a rare disease condition (pentosuria); probably it frequently occurs undetected along with glucose in diabetic urine. It gives positive results with the reduction tests, yields an osazone with a low melting point ($158^\circ C.$), does not ferment with yeast, gives a red colour and a red precipitate when warmed with an equal amount of concentrated HCl and a few grains of dry *phloroglucin*; the *orcin* test is carried out in the same way with *orcin* instead of *phloroglucin*; if pentose is present the fluid becomes reddish-blue, then bluish-green, and shows a spectrum with an absorption band between C and D. Do these tests on the pentose solution provided.

(279) *Glycuronic Acid* occurs in traces in normal urine, and as a compound with such substances as morphine, chloral and chloroform when these are excreted in the urine. It may be present in sufficient amount to give reduction. It gives the *phloroglucin* but not the *orcin* test as applied above; it does not ferment; and it can be distinguished from other substances by a peculiarity in its optical activity—as it occurs in urine it is *laevo-rotatory*, but when boiled with acid and so dissociated from its combinations the free *glycuronic acid* is *dextro-rotatory*.

It is important to note that the reducing substance in urine which is of the greatest significance (glucose) is readily fermentable with yeast; and further, that urine contains a very small but definite amount of reducing substances the chief of which is glucose; probably owing to the creatinine, however, normal urine does not reduce Fehling, and gives no result on fermentation.

The Acetone Bodies (beta-hydroxybutyric acid, aceto-acetic acid, and acetone). These may occur in the urine in diabetes and to a less extent in some other conditions. Normal urine contains traces of acetone, however. They are closely related to each other and one may change into the other in the above order. There is no convenient clinical test for the first.

(280) *Aceto-Acetic Acid*.—Add to the freshly passed urine a few drops of ferric chloride—a precipitate of phosphate of iron will appear, continue adding the ferric chloride till no more precipitate comes down, filter and add a further small quantity of ferric chloride to the filtrate—a reddish claret colour indicates the presence of aceto-acetic acid. The claret colour may be sufficiently evident in the urine before filtering. Salicylates, if being taken by the patient, cause a similar reaction, but in such a case the result is still present after boiling the urine.

(281) *Acetone and Aceto-acetic acid together, or acetone alone* gives the following reactions:—(a) Iodoform test. Make the urine alkaline with KOH; add a few drops of watery solution of iodine in potassium iodide, a white turbidity and smell of iodoform indicates the presence of acetone.

(b) Legal's Test. Add to the urine a few drops of freshly prepared sodium nitroprusside solution and make alkaline with KOH. The urine becomes of a ruby-red colour; a similar colour appears in testing for creatinine (125) but a distinction can be made between these two, in this, that the addition of acetic acid in the case of creatinine causes the colour to change to yellow, green, and then blue, while with an acetone solution the colour becomes magenta on adding acetic acid. A recent modification of the test (Rothera's) is as follows:—Add a little solid ammonium

sulphate to 5-10 c.c. of the urine, then 2-3 drops of a fresh 5% solution of sodium nitroprusside, and 1-2 c.c. of strong ammonia, a characteristic "permanganate" colour develops. In the concentration in which it exists in urine creatinine gives no result with the nitroprusside test applied in this way, and it is moreover exceedingly delicate towards acetone.

Since the acetone bodies are volatile, they can be detected by their fruity odour, and can be distilled off from a large quantity of urine after acidifying with acetic acid; the distillate must be received into a flask kept as cool as possible, preferably with ice.

(282) **Secondary albumose in urine** ("Peptonuria"). Saturate the urine at boiling temperature with ammonium sulphate crystals filter, wash the precipitate with hot saturated ammonium sulphate solution, extract the filter paper and its contents with hot water, filter again, and do the biuret test (17). Urobilin may complicate the result in a highly coloured urine.

(For an account of the normal and pathological pigments of urine consult Milroy's Practical Physiological Chemistry).

(283) **Cryoscopy or Depression of the Freezing Point.** The apparatus required consists of a large jar to hold the freezing mixture which may consist of alternate layers of crushed ice and common salt; the lid of the jar is of wood and carries a large test tube within which, supported by a ring of cork is a smaller test tube to hold the fluid experimented on, (*e.g.*, urine); a special thermometer with a scale divided to read to hundredth's of a degree C. is also placed in the inner tube, along with a simple spiral wire of platinum or copper to act as a stirrer.

Arrange the apparatus as above, and watch the temperature as it slowly falls; when it reaches the freezing point of pure water, stir vigorously and keep stirring till the reaction is ended; the temperature sinks to a variable extent below the actual freezing point of the fluid, and when ice begins to form it suddenly rises and if left in contact with the freezing mixture it again slowly falls; the point to which it suddenly rises before beginning to

fall the second time is the freezing point of the fluid (urine). If the thermometer employed is graduated in the ordinary way with 0° as the freezing point, the depression of freezing point can be directly read, but where a Beckmann thermometer with an adjustable zero is used the freezing point of pure distilled water must be determined. The difference is then the depression of freezing point.

The average depression for urine is 1.85° C.

CHAPTER X.

METABOLISM AND DIETETICS.

(284) Storage of Glycogen in the Liver.

The presence of a large amount of glycogen can be demonstrated in the liver of an animal fed on carbohydrate-rich food some hours before being killed. Rapidly excise the liver just after death ; keep one small bit separate and rapidly cut up the remainder in boiling acidified water ; after the pieces have become coagulated remove them from the fluid, grind them up in a mortar with fine clean sand, and return the pulpy mass to the water ; continue the boiling for ten to fifteen minutes ; filter and distribute the filtrate to the class. Cut up the small separate piece of liver in a beaker of normal saline, place it in the incubator, and examine it next day for glycogen and glucose.

Further treatment of the filtrate.—First remove any protein that may be left by adding alternately a drop or two of HCl and of potassio-mercuric iodide (Brücke's Reagent) till no further precipitate of protein appears ; then filter ; measure the filtrate and add to it twice its volume of alcohol to precipitate the glycogen ; let the precipitate form and settle till next day then filter and wash with 66% alcohol ; then add some boiling water to the precipitate on the filter paper ; the glycogen goes into solution and comes through in the filtrate. Test this glycogen solution as in tests (67) to (71) inclusive. Test also the effect of saliva (ptyalin) on it (214).

In the separate piece of liver which was kept at 40° C. practically all the glycogen will be found to have been converted into glucose.

(285) **Phloridzin Glycosuria.**—This experiment is to be carried out by two members of the class chosen by lot. A rabbit is placed in a collecting cage, fed on the usual diet, and normal urine collected for a day. Then inject subcutaneously about quarter of a gramme of phloridzin dissolved in warm water. Continue to collect the urine in a fresh receptacle or catheterize the animal after several hours. Samples of the normal urine and of the phloridzin urine are to be tested for glucose by the class as a whole.

QUANTITATIVE ANALYSIS OF SOME FOOD STUFFS.

Some of these have been already examined, *e.g.*, meat (muscle), meat extract, gelatine, starch, cane sugar, and fats.

Milk.—The chief solid constituents found in all kinds of milk are proteins, fats, and carbohydrates, and salts in varying proportions to suit the environment and rate of growth of the young mammal.

The proteins are *caseinogen* (a phospho-protein), and small amounts of *lact-albumin* and *lacto-globulin*; the fats are *tripalmitin*, *tristearin*, *triolein* with glycerides of some of the lower and volatile fatty acids, *e.g.*, tributyrin; the carbohydrate is *lactose*; the salts are *potassium*, *calcium*, *sodium*, *magnesium* as *phosphates*, *chlorides* and *sulphates*. Cow's milk is put out for examination.

(286) The *reaction* to litmus paper is alkaline, or amphoteric (*i.e.* changing blue litmus red, and red litmus blue) in the fresh state, but it readily becomes acid on standing, due to the action of bacteria (*B. lactis*, etc.).

(287) The *specific gravity* varies from 1028 to 1034 and is higher when fat has been extracted, lower when water is added.

(288) *Microscopically* it shows fine fat globules of varying size.

(289) Milk gives the *Guaiac Reaction* (159).

(290) **Separation of the chief constituents of milk.** To about 10 c.c. of milk in a large test tube add four to five times its bulk of water and acidify very cautiously with acetic acid, mixing thoroughly after the addition of each drop (two drops may prove sufficient)—a flocculent precipitate of *caseinogen* and entangled fat appears. This precipitate readily dissolves in excess of the acid as can be shown by pouring some of the fluid into another test tube and adding more acetic acid. Filter, and keep the filtrate; wash the precipitate on the filter paper with water containing a trace of acetic acid (discard the washings); dissolve some of the precipitate in very dilute sodium carbonate solution and apply some of the protein colour reactions (15-20). Caseinogen is rich in aromatic substances (tyrosin, tryptophane, etc.) and therefore gives positive results with (15), (16), (18); as it contains no carbohydrate radicle tests (19) and (20) are negative. Allow the remainder of the precipitate of caseinogen + fat to dry in the oven till next day then extract some of the *fat* with ether, note the yellow colour of its ethereal solution and the greasy stain left on note paper where the ethereal solution has been allowed to dry (see 293); incinerate the remainder of the precipitate as in (74) and test for phosphate (3) (4).

The filtrate from the caseinogen-and-fat precipitate contains the other constituents. Test it for *lact-albumin* and *lacto-globulin* together by boiling after adding a little common salt (see 21.) Filter off the coagula of these and test the filtrate for *lactose* (see under Di-saccharides, C); test another portion of the filtrate for *calcium* (9) and for inorganic *phosphates* (3) (4).

(291) The action of *rennet* on milk can be again studied (226). Compare the amount of lime present in the whey with the amount present in a filtrate after precipitating the caseinogen as above, after diluting the whey to the same extent as the milk was diluted. The curd which results from rennet coagulation consists partly of the lime of the milk, and whey is therefore relatively poor in that element.

Cheese consists chiefly of casein and fat, part of which arises from the casein itself. The lactose originally present in the milk forms only a small percentage of the solids. Among the salts present are calcium and sodium, as phosphates and chlorides.

(292) Rub up some finely grated cheese with 2% sodium carbonate in a mortar and filter. The *casein* is to some extent dissolved and gives reactions very like those of caseinogen, *e.g.*, it is precipitated by acetic acid and the precipitate is soluble in excess. It gives the protein reactions, especially Millon's and xanthoproteic, (aromatic radicle).

(293) *Fat*.—Extract some grated cheese in a dry test tube with ether, pour the ethereal solution into a warm porcelain basin, and allow it to evaporate to small bulk, then place a drop of the fluid on clean white note paper, and allow the last of the ether to evaporate—a greasy translucent spot remains indicating the presence of fat.

The fat can also be detected by saponification, but should be first extracted in larger quantity by ether. The *salts* may be examined after incineration in the usual way.

Eggs (that of the hen is used for convenience). The **white** of egg consists chiefly of water with about 12% of proteins (egg-albumin, ovoglobulin, and ovomucoid). The **yolk** contains about 32% of fatty matter soluble in ether (true fat, lecithin, lutein, cholesterin, etc.); proteins constitute about 16%, the varieties being chiefly vitellin (a phospho-protein) and nucleo-protein; water and salts are the chief other constituents of the yolk. (Iron is present in egg-yolk in organic combination to the extent of .01%. It is relatively much richer in iron than milk is).

The following constituents of egg-yolk may be examined.

(294) *Fat and Lutein*.—Extract some fresh egg-yolk with ether by shaking in a test tube; pour off the upper ethereal layer and examine it as above described (293) for fat, and with the spectroscope for the two bands of lutein seen near the violet end of the spectrum; one of these bands is over F, and one lies between F and G. Repeat the extraction of the yolk with several fresh quantities of ether so as to free it from as much fatty matter as possible. Keep the ethereal extracts for examination of the lecithin and for recovery of the ether.

(295) *Vitellin*—The fresh egg-yolk used above, which has had its fatty constituents removed by ether, is now to be placed in a porcelain capsule on a warm bath for a short time to remove the ether; then place in a large test tube, add 10 % sodium chloride to nearly fill the tube, shake thoroughly and leave standing till next day. Then filter and pour the filtrate into a jar full of distilled water to which a few drops of acetic acid have been added; a precipitate of vitellin will settle out in fine flakes; after standing some hours pour off the supernatant fluid and dissolve the precipitate of vitellin in the least necessary amount of weak sodium carbonate solution. On this solution of relatively pure vitellin do the protein colour reactions (15–20) especially those for the aromatic radicle. Neutralise carefully the remainder of the alkaline solution of vitellin, add an equal amount of 0.4% HCl to make the percentage of the mixture 0.2, add an active pepsin preparation and allow the mixture to digest till next day; a sediment will then be found consisting chiefly of paranuclein derived from the vitellin. If filtered off, dried and incinerated with Na_2CO_3 this deposit will be found to be very rich in phosphorus. (Compare carefully the reactions of vitellin with those of caseinogen; both are phospho-proteins, rich in aromatic radicles and destined for use in building up the body of the young animal; they resemble nucleo-proteins in some respects, but that there is some great difference in the constitution of the two groups is shown by the fact that nucleo-proteins yield the xanthin bases on decomposition while phospho-proteins do not.)

(296) *The Mineral Constituents*. Incinerate a small piece of dried yolk as described in (1) and examine for the usual constituents of a tissue (2) to (10). Note the relative amounts of these as far as can be judged by qualitative tests; since the yolk contains practically all the constituents of the formed chick, the amounts of the mineral salts found represent an average in each case of all the tissues taken together. Make a final extraction of the incineration residue with 1 in 5 HCl and test for *iron* with a few drops of ammonium sulphocyanide (blood red colour) or with potassium ferrocyanide (Prussian Blue).

(297) *The Substances Soluble in Ether* have already been mentioned. After distilling off the ether and obtaining an ether-

free residue a series of operations may be carried out to separate the various substances. If time permits this may be carried out as given in Milroy's Practical Physiological Chemistry.

EXAMPLES OF SOME VEGETABLE FOODS.

These contain a preponderating amount of *carbohydrates* (chiefly starches) but *proteins* are also present, sometimes in large amount, and *fats* also occur, *e.g.*, olive oil.

(298) **Flour.** Add a small quantity of water to some flour in a porcelain basin, and knead it between the fingers till it forms a tough dough, this is due to the *gluten* and other proteins becoming viscid, and entangling the starch grains; wrap the dough in a piece of muslin and work it about in a quantity of water in the porcelain basin. The *starch* grains pass out into the water. Keep some of this fluid and test it later on for starch. Continue washing the dough till no more starch comes out, then open up the muslin and examine the gluten which remains. Suspend a little of it in water and apply the tests for protein; test the washings of the dough, after boiling and cooling, for starch (58-62), and for glucose (43). In good flour the latter should be absent.

(299) **Bread.**—In bread-making, a dough essentially similar to the above is first made, but yeast is added and fermentation is allowed to proceed; the development of the gas throughout the dough renders it spongy, and the digestive juices can readily penetrate. The baking is done at a high temperature, and a certain amount of dextrin is formed in the crust.

Test bread for protein by applying a few drops of Millon's reagent and allowing it to remain for a short time at room temperature—a deep red stain results. The xanthoproteic test may be similarly applied.

Make a watery extract of the "crumb" of bread, filter and test for glucose, starch and protein. Make a similar watery extract of the "crust", filter and test for dextrin; since the extract contains starch and amidulin, it is necessary to precipitate these by saturating with magnesium sulphate; filter and test the filtrate with iodine, *cf.* (70).

The following other food stuffs may be examined in a similar way if time permits.

(300) **Oatmeal** contains little or no gluten, and hence does not form a dough or bread in the same way as flour. It contains 10% of fatty matter. Examine dried oatmeal for fat, protein, carbohydrate and mineral matter including iron.

(301) **Pulses** form an important source of protein in vegetarian diets, the percentage being as high as that of meat (20%). Examine some pease-meal or lentil-meal in the same way.

(302) **Potatoes, Carrots, Parsnips, Onions, etc.,** consist chiefly of carbohydrates and water, with less than 2% of protein, and less than 1% of fat, and varying amounts of cellulose fibre, mineral matter, and extractives.

(303) In **Fruits**, such as grapes, pears, apples, etc., the chief constituent besides water is carbohydrate, chiefly as sugars (pentosanes in pears), along with varying amounts of organic acids such as citric, etc. The "jelly" of many fruits used in making jellies and preserves is a form of gum, not animal gelatine.

(304) **Nuts** contain a large amount of oil and protein, and lesser amounts of carbohydrate than the above, but they are less digestible than fruit or vegetables.

EXPERIMENT ON GENERAL METABOLISM.

(305) The following experiment illustrates the principles of dietetics as well as the method of studying general metabolism. The work is to be divided into separate parts, A, B, C, etc, one of which is to be done by one or more students according to the size of the practical class.

An omnivorous animal, such as a dog or rat is to be kept on a fixed diet of which the protein, carbohydrate and fat is to be estimated, and the intake and output of nitrogen, phosphorus, chlorine, calcium or other element determined in the food and excreta.

For class work rats are very convenient as they can be kept in a simple collecting cage such as that shown in fig. 15. The disadvantage of using a rat is that the faeces and urine are obtained together, and the percentages of food constituents absorbed cannot be stated, so that where a dog is available it should be used in preference to rats.

For either kind of animal a suitable diet consists in oatmeal and dried protein, and it can be prepared by adding a measured amount of boiling water to the weighed-out quantities of meal and protein.

Assuming that the experiment is continued for two to three days in a dog, or four days (two periods of two days each) in the case of the rats, the work involved will take the working time of one week, about eight hours, in a class of eighteen working in couples.

At the end of the experiment the lecturer will collect all the figures and draw up a statement of the results which will include—(1) the daily diet of the animal in terms of protein, carbohydrate and fat; (2) the energy value of the diet reckoned from the amounts of the substances and controlled by a direct combustion of the food in the bomb calorimeter; (3) where the urine and faeces are obtained separately the amount of each food stuff absorbed can be calculated as a percentage of what is eaten; (4) the balance of the various elements, *e.g.*, nitrogen, phosphorus, calcium, etc.; (5) the gain or loss of weight of the animal and the probable nature of the loss, thus a negative balance of 1 gm. nitrogen over the whole experiment might mean a loss of 6.25 gm. dried protein, or, since muscle contains 20% of protein, 31.25 gm. of “flesh.” (6) If the heat value of the excreta can be directly determined by the bomb calorimeter the “balance of energy” can be stated. Thus, knowing that a certain number of calories had been taken in as food, and that the caloric value of the excreta was so much, the difference represents what has been used by the body and either expended in work and heat or partly stored as the potential energy of glycogen and fat.

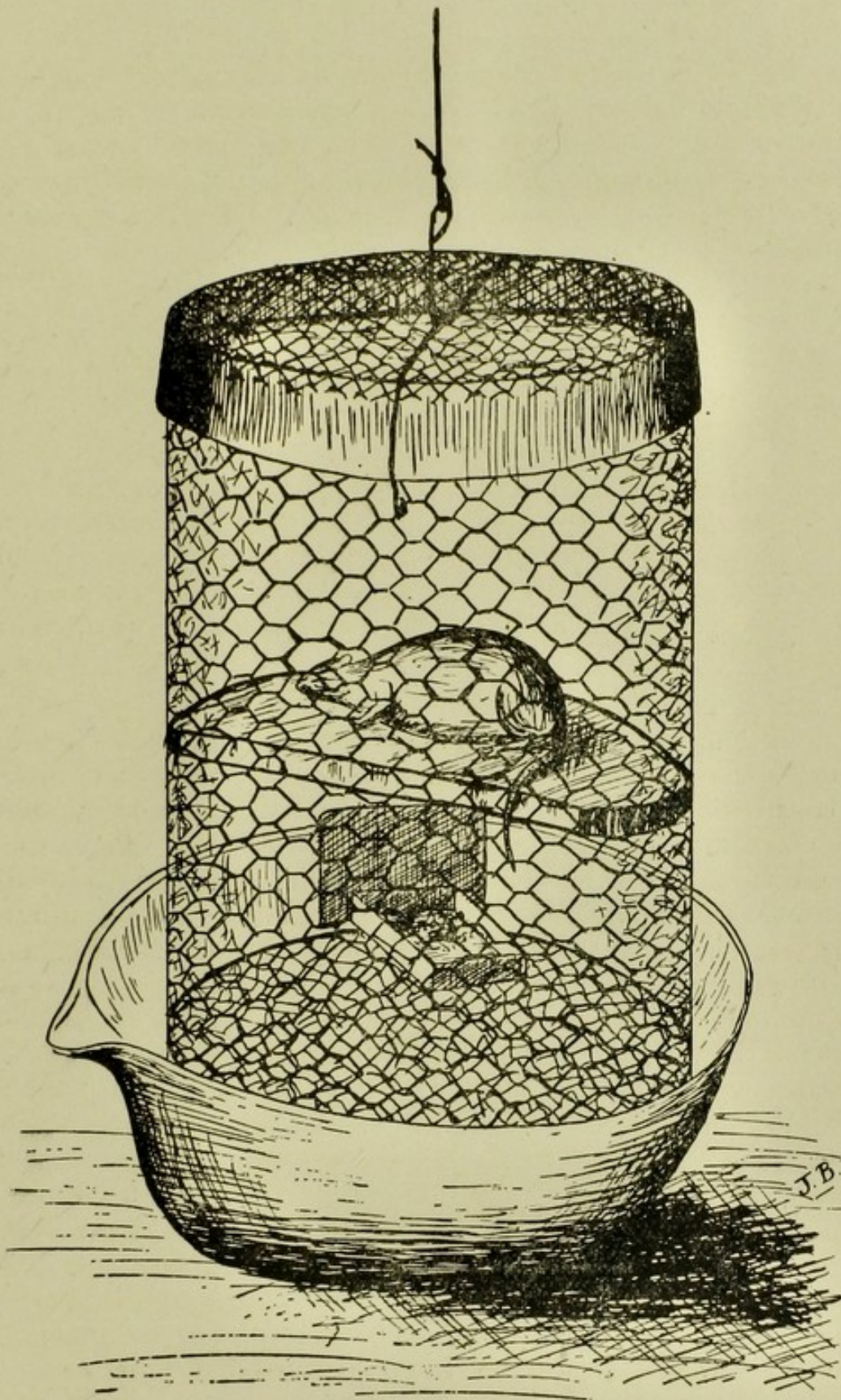


Fig. 15.
Rat's cage for metabolism experiments.

(A) THE WATER PERCENTAGE OF THE FOOD.

Apparently dry meal and dried protein, etc., contain a certain amount of adherent moisture, the percentage of which must be ascertained, as follows:—Procure a pair of well-fitting watch-glasses with a suitable brass clip for holding them in apposition. Clean, dry in the hot air oven, cool in the desiccator, and weigh the glasses and clip on the delicate analytical balance; weigh out about two grammes of the material on an ordinary balance, place this on one of the watch-glasses, cover it with the other, put on the clip and weigh on the delicate balance again. The difference in the exact weighings is the amount of meal or other undried material taken. Undo the clip, remove the one watch-glass and place all in the hot air oven at a temperature of 100° — 120°C . After an hour or more at this temperature, put on the upper watch-glass, clip both together, and allow the apparatus to cool in the desiccator. When cold, weigh again; this last weight may be that of the dried material, but in order to make sure that all the moisture has been driven off, the drying, cooling and weighing must be repeated until the whole attains a constant weight, or varies only by a milligramme or thereby. The water lost in the drying can easily be obtained by subtraction of the weight of the dried from that of the undried material, and the result expressed in percentage. The estimation must be done in duplicate, and if the results are divergent by 0.5% or more, another couple of estimations should be done. This method is not applicable to materials with constituents which are volatile.

(B) THE PERCENTAGE OF ASH.

This may be carried out on some of the same material as was used for A. Transfer the contents of two of the pairs of watch-glasses to a porcelain crucible which has previously been weighed (after careful cleansing, drying in the oven and cooling in the desiccator); see that every particle of the meal has been transferred to the crucible. Or, about four or five grammes of the undried material may be placed in the crucible, the weight of which has just been determined, and the whole re-weighed. Place the crucible on a clay triangle supported by a tripod as in (1), and incinerate, using a small flame at first. When the

mass is completely charred, cool, extract the mass several times with small quantities of hot distilled water, filter the extract each time through the same ash-free filter paper; allow the extracts to flow into a beaker and lay it aside at present. Then place the filter paper and its contents in the crucible, dry the whole in the oven and then continue the incineration till all the carbon is completely gone (Fig. 1). Cool the crucible, place it on the water bath and add to it some of the extract which was made early in the process. Keep adding some of the extract till it has all been evaporated to dryness in the crucible, including the washings of the beaker; then clean the outside of the crucible from any adherent matter, finish the drying in the hot air oven, cool in the desiccator and weigh. Subtract the weight of the crucible, the difference is the amount of ash in a certain amount of meal, etc. Express the result in percentage. The reason for the preliminary extraction with water is that the chlorides are to some extent driven off by the heat necessary to remove all the carbon. It also facilitates the complete reduction of the material to ash.

(C) THE PERCENTAGE OF PROTEIN.

Since it is difficult to separate the protein in a pure state from meal, etc., the method usually employed is to estimate the amount of nitrogen present, and multiply this by 6.25. This is done on the assumption that protein is the only nitrogenous substance present, and that the protein present is one that contains 16% of nitrogen; it is therefore only approximately correct, but is convenient for our purpose because we are about to determine the total amount of nitrogen ingested and excreted. The nitrogen is estimated by Kjeldahl method as follows:—

(1) *Weighing the material.*—About one gramme of oatmeal, or half a gramme of a dried protein, such as casein, fibrin, etc., is weighed on an ordinary balance and transferred to a small dry test tube. Prepare several of these and stack them together inside a small beaker. Weigh the whole accurately on the fine balance; transfer the bulk of the contents of one of these tubes to a hard glass flask (Kjeldahl flask), and replace the tube carefully beside the others, weigh again, the difference represents the amount of meal taken for nitrogen estimation. Prepare two such flasks.

(2) *The incineration.*—Take, in a dry cylinder measure, 10 c.c., of concentrated sulphuric acid and add it to the contents of the Kjeldahl flask. Before pouring it in, allow a small quantity of distilled water to run down the neck and inside walls of the flask, and then while pouring in the acid rotate the flask gently so as to spread the acid over the inside of the flask in a thin layer; this prevents the carbonaceous matter from sticking to the wall of the flask. Add also 10 grm. or thereby of potassium sulphate, and a small crystal of copper sulphate. Now place the flask on a tripod in an inclined position in the fume chamber and apply the flame, small at first and stronger later on when all bubbling has ceased. After some hours, or less, according to the ease of oxidation, the residue becomes clear. Continue the heating for some time after it has become clear, then allow the flask to cool on the tripod.

(3) *Distillation of the Ammonia.*—As already mentioned, the H_2SO_4 in the Kjeldahl method oxidises the elements present in organic substances and converts the nitrogen into ammonium sulphate. Our object now is to break up this compound with a fixed alkali, and measure the amount of ammonia evolved. When the flask is quite cold, add about a hundred c.c. distilled water (or less if the flask is small), and dissolve the crystalline residue which has formed in the flask. It may be necessary to warm the flask; if so, it may be cooled again, under the tap this time, when the residue is completely dissolved. Then prepare the condenser, see that the water can run freely through its jacket and that the tripod, bunsen burner, etc., are ready. Measure out very accurately from a burette a sufficient amount of tenth—or fifth normal acid (about 30 c.c. $\frac{N}{10}$ is enough for the amount of meal taken in this experiment). Run this amount directly into a very clean titration flask, add a drop of rosolic acid to act as indicator in case the amount of ammonia which comes over should be more than the acid taken can neutralise, and add some distilled water till the flask is about a quarter filled.

Measure out roughly in a cylinder 100 c.c. of very strong soda (23% or thereby), and have ready a long stemmed funnel. Now add some water to the incineration flask so as to fill it about one third full; add also a spoonful of talc to prevent bumping;

then place the funnel in the mouth of the flask—incline it to one side and pour in the 100 c.c. of strong soda so that it form a layer beneath the acid contents of the flask. Then without shaking more than you can help connect the flask to the condenser and apply the flame. The receiving end of the condenser should be fitted with a short length of tubing which at the beginning of the distillation should dip beneath the surface of the tenth-normal acid in the titration flask. Later on when the distillate comes over as condensed steam this may be taken out of the acid fluid but the opening of the tube should never be far from the acid, otherwise some ammonia may be lost. If the amount of $\frac{N}{10}$ acid taken at first has been insufficient, the distillate will become alkaline as shown by the pink colour due to the indicator; in such a case the estimation will still be good if an exactly measured amount of the $\frac{N}{10}$ acid is promptly added, sufficient to acidify the contents. The 100 c.c. of 23% soda is usually sufficient when 10 c.c., H_2SO_4 was taken to begin with, but of course more soda must be added if more H_2SO_4 was added to complete incineration. If the amount of soda added is sufficient, the contents of the distillation flask become deep blue when $CuSO_4$ is present.

Once the distillation has begun, the distilling flask must not be allowed to cool and must be kept connected to the condenser. After three-quarters to one hour's distilling, test with litmus a drop of the distillate as it leaves the tube—to see if ammonia is still coming over. When the distillate no longer gives the alkaline reaction, titrate the contents of the flask with $\frac{N}{10}$ or $\frac{N}{5}$ alkali. The difference between the amount of acid taken and the amount of alkali needed to reach the neutral point in the distillate is the datum for calculating the amount of ammonia which has been distilled off. Suppose this difference is equivalent to 20 c.c. of $\frac{N}{10}$ acid; obviously the amount of ammonia which this equals is 20 c.c. of $\frac{N}{10} NH_3 = 20 \times 1.7$ mlgm., or since it is nitrogen and not ammonia we want, 20×1.4 mlgm in the amount of substance taken, from which the percentage of nitrogen may be calculated, and this $\times 6.25 =$ percentage of proteid.

The nitrogen in the excreta is estimated in a similar way in samples of the whole urine and faeces separately in the dog. or of the mixed excreta of the rat.

(D) ESTIMATION OF FAT IN MEAL, &c.

First prepare a Soxhlet extraction apparatus fitted with a condenser (internal form) and flask, on a sand bath which is heated by a constant-level water bath.

Weigh an empty extraction thimble in a small beaker accurately on the analytical balance, then fill it nearly full of the meal and weigh again to obtain the quantity of meal taken. Dry the meal and thimble as thus prepared at a temperature of about 100°C . till next day. Place the loaded thimble in the extraction apparatus and pour over it sufficient ether to syphon over once and to come about half way up the syphon, then fit the condenser in, and start the bath; (keep the ether as far from the burners as possible).

Allow the extraction to proceed for two days, then remove the thimble and contents. Distill the bulk of the ether into the space previously occupied by the thimble, but before it syphons over into the flask disconnect it and syphon it over into the bottle of "used ether." Have ready a clean, dry, weighed fat flask, or other small glass vessel; filter the contents of the fat flask into this, washing out the flask and filter paper with several small quantities of ether. Drive off the ether from the clear filtrate on the water bath; dry in a water oven, cool, and weigh. The difference in the weight represents ethereal extract or "fat" in the quantity of meal taken. Express the result in percentage.

The method of estimating fat in milk will be demonstrated at this stage:—Ten c.c. of milk is allowed to soak into an Adam's fat-free paper which is then dried and extracted with ether in the same way.

(E) ESTIMATION OF CARBOHYDRATE.

Weigh accurately about two grammes of the meal, using a small tube as described in the method of estimating the protein (C,1). Put this into a flask capable of holding about five hundred c.c.; add about 200 c.c. of water and 7 c.c. of concentrated HCl (the percentage of HCl in the whole will then be about 1); arrange the flask on a sand-bath heated by a bunsen and fit a condenser in the mouth of the flask; allow the flask to remain

for one or two days on the hot sand-bath ; if the condenser is efficient there will be no danger of the contents becoming dry, but the apparatus should be examined from time to time to prevent such a result. The effect of this treatment is to hydrolyse all starches present into glucose, which can now be estimated by Fehling's method. Proceed as follows :—Cool the flask ; neutralise the contents by shaking with calcium carbonate ; filter ; wash the filter paper and any deposit which it may contain with water several times, adding the washings to the original fluid ; make the whole fluid up to 500 c.c. in a graduated flask ; mix thoroughly and estimate the glucose by Fehling's method (276*a*) without diluting further. The results are only approximate because reducing substances other than glucose are formed, and the cellulose is not attacked. State your result in terms of starch, *e.g.*, if 87% of glucose is obtained from the meal, the original starch would equal $\frac{C_6H_{10}O_5}{C_6H_{12}O_6} = \frac{162}{180} \times 87 = 78\%$.

The whole composition of the meal, etc., can now be considered ; add together the percentages of water, ash, protein, fat and carbohydrate, the result should be 100, for the amount of extractives is very small. In the case of oatmeal, the error is more likely to be in the carbohydrate estimation than in any other, owing to the cellulose.

(F) FEEDING OF ANIMAL, COLLECTION OF EXCRETA, ITS PREPARATION FOR ANALYSIS, &c.

The animal (or animals) must be weighed daily at the same hour, and then fed. Rats may be fed once a day, but larger animals such as the dog, twice.

The amount of food necessary requires some knowledge of its composition, and should be so arranged that the daily diet has (1) a sufficient amount of protein ; (2) a sufficient caloric value. For man this is accomplished by giving from 50 to 100 or more grammes protein daily, and a total caloric value of 40 Calories per kilogramme body weight, but for small animals the latter factor must be greatly increased because of the relatively larger surface in proportion to weight. The caloric value must also be increased if the external temperature is low, and if muscular

work is being performed by the individual. Generally speaking, about 3 or 4 grammes proteid daily, and 40 to 60 Calories is sufficient for a medium sized rat of 200 grammes.

A dog thrives better on a fairly high protein intake, so it is usual to allow them about 100 grammes protein per day, and 50 to 60 Calories per kilo.

The animal (or animals) must be kept at as equable a temperature as possible during the experiment, and dogs should be given a certain amount of exercise daily. The rats' cages should be placed within a wooden box over the laboratory ovens, as temperature affects them much more than large animals.

Having determined what the daily diet shall consist of, *i.e.*, so much oatmeal and so much dried protein such as dried casein, these amounts should be weighed out, mixed, and made up into packets, one for each day of the experiment.

When required, one such daily ration is placed in the feeding dish and about twice its weight of boiling water poured over it to make a soft pulpy mass. When the amount necessary for this has been determined, the same amount should be used each day so that the meal is cooked each day to the same extent. Any food left out over from one day should be separately analysed or else added to the *fæces* as if it had been eaten, but not absorbed. The total weight of the excreta should be taken where rats are used, or the volume of the urine and weight of the *fæces* when a dog is the subject of the experiment. To obtain the whole twenty-four hours' urine, dogs must be catheterised at a certain hour when the experiment starts; this urine is to be thrown away but all the urine obtained during the experiment is to be kept and the dog finally catheterised at the same hour when the experiment ends. If a suitable collecting cage is available, and if the animal is not too particular in its habits, these two catheterisations may be sufficient, otherwise it should be catheterised twice or three times daily. In rats this procedure is impracticable, and the usual method adopted is to collect the urine for four to six consecutive days, analysing every two days' excreta together.

Treatment of the excreta.—*In the dog*, the volume and specific gravity of the urine may be noted and the whole mixed

urine of one day made up to a definite quantity (500-1000 c.c.) with water; an aliquot portion of this is then taken for nitrogen, phosphorus, etc.; the size of this sample will depend on the element being estimated, and the amount of it expected to be present; thus a dog receiving 100 gm. protein daily, if on nitrogen equilibrium, excretes the nitrogen corresponding to that amount, say 16 gm.; suppose the urine to contain 15 gm. of this, then if it is diluted to 1000 c.c., 5 c.c. would contain $\frac{15}{200}$ gm. = .075 gm. nitrogen, and since 1 c.c. of $\frac{N}{10}$ acid equals 1.4 mlgrm. (.0014 gm.) nitrogen, one would require to use more than 53.6 c.c. of tenth-normal acid, or 26.8 c.c. of $\frac{N}{5}$ acid; such quantities are easily worked with. The 5 c.c. sample should be measured accurately in a pipette of the bulb form with only one mark, viz., that at 5 c.c., and the last drop may be procured by blowing through the pipette. It is inadvisable to wash out these pipettes; they are graduated to deliver 5 cc.

The fæces passed by a dog should be collected, mixed, and spread in thin layer on a sheet of glass of known weight, dried over a water bath or hot water pipes, weighed, and the dried fæces scraped off, powdered if necessary (a coffee mill does this very well), and sampled as described in the estimation of protein (C. 1).

The excreta of a Rat consists of mixed urine and fæces; empty the whole into a flask capable of holding 500 c.c. or more; wash the shelf of the cage, the wire netting of the bottom, and the collecting dish by directing a fine stream of distilled water from a wash-bottle against these parts, aiding this by rubbing any dirty places with a glass rod armed with india-rubber: When everything has been transferred to the flask, add several c.c. of sulphuric acid, mix and heat gently till all the pellets of faeces become broken up; this may be accelerated by vigorous shaking; when the fluid has acquired a uniform consistence, cool it, pour it into a 500 c.c. graduated flask, wash out the flask used for the mixing and add the washings to the main bulk, then add water up to 500 c.c., mix, and empty the whole into a clean dry bottle. This material can now be used for analysis, but before taking a sample for nitrogen, etc., the contents of the bottle must be thoroughly shaken and the flask or pipette filled before the heavier particles have time to settle. Knowing the amount of

protein given daily to the rat, calculate, in the way described above for dogs, the amount of this fluid which should be taken for nitrogen estimation so that the amount of $\frac{N}{10}$ acid shall not exceed 50 c.c. or that of $\frac{N}{5}$, 25 c.c.

(G) ESTIMATION OF PHOSPHORUS (as P_2O_5) IN THE FOOD AND EXCRETA.

Place a measured amount of excreta or quantity of dried substance weighed as directed in C,1 in a Kjeldahl flask; add 10 c.c. of a mixture of concentrated sulphuric and nitric acids, equal parts; and proceed to incinerate in the same way as in the Kjeldahl process. When the flame is applied or even in the cold, red fumes of NO_2 develop so that the flask must be placed in a fume-chamber with a good draught. Have ready a funnel provided with a glass stop cock and filled with the acid mixture; this should be supported in a stand so that the end of the funnel is over the mouth of the flask, the flask being inclined as in the Kjeldahl method. When the red fumes cease to come off, add some of the acid mixture drop by drop and continue heating and adding drops of acid till the contents of the flask become and remain clear; allow the clear residue to heat for twenty minutes to half an hour longer to ensure complete oxidation of the organic matter; then let the flask cool completely; add a little water and again heat to boiling to drive off the excess of nitrous acid. So far the process has been merely that of incineration (by Neumann's method); the older method of incineration was similar to that described in (13) except that a very large excess of sodium carbonate was added, the residue after incineration was cautiously acidified. The next step in the process consists in the precipitation of the phosphates by molybdate of ammonia. Supposing we have done the incineration by Neumann's method, make up the contents of the flask to 150 c.c. with water. This may be done in the same flask or, better, in a beaker marked at the 150 c.c. level; if the latter is used, the flask must be rinsed out several times into the beaker; add 50 c.c. of 50% ammonium nitrate solution, heat to 70° — 80° C., and then add 50 c.c. of a 10% ammonium molybdate solution; the result is a yellow precipitate of phospho-molybdate of ammonia which includes

all the phosphorus in the fluid. Neumann has described an accurate method of completing the estimation by titration, but the older method of converting the phospho-molybdate into triple phosphate ($\text{Mg}(\text{NH}_4)\text{PO}_4$) is more instructive for students' work.

After standing for some time (15 minutes or longer) filter off the precipitate, wash it several times with a weak nitric acid till the washings no longer give any precipitate on testing with ammonia. Then dissolve the yellow precipitate in ammonia (1 in 3), and add 20-30 c.c. magnesia mixture. A precipitate of ammonio-magnesium phosphate forms; allow to stand for 12-24 hours; then filter through an ash-free filter paper; wash chlorine-free with 1 in 3 ammonia; dry and incinerate in a platinum crucible. On incineration magnesium pyrophosphate results ($\text{Mg}_2\text{P}_2\text{O}_7$) from which the amount of phosphorus present in the original sample is estimated. The crucible is weighed with the ash and its own weight subtracted. In using a platinum crucible be careful not to allow the hot platinum to touch anything but platinum, therefore use a platinum wire triangle to support the crucible during incineration.

(H) THE CARBON BALANCE.

The carbon balance is more difficult to determine exactly than that of nitrogen, etc.

It requires an air-tight chamber into which the animal's cage can be put, some means of drawing air through this for ventilation purposes, and means of sampling the current of air and so estimating the amount of CO_2 produced. This gives the great bulk of the carbon output on a carbohydrate and fat diet; the amount in the urine can be estimated from the nitrogen as the carbon and nitrogen are present in fairly definite proportion to each other (this ratio would be quite definite if urea were the only nitrogenous organic constituent in urine).

The amount of carbon in the food may be calculated from the protein carbohydrate, and fat, or may be estimated by the bomb calorimeter, where combustion is very complete; the contents of the bomb being allowed to escape through soda lime and weighed.

(Dem.) (Fig. 16). The CO_2 for short periods, and small animals may be done by Haldane's method. The animal is placed in a small air-tight vessel which can be weighed with the animal inside. Air is drawn through this by means of a filter pump, and the current is made to enter (1) a soda-lime bottle to remove the CO_2 of the atmospheric air; (2) a drying bottle of CaCl_2 , or of sulphuric acid on pumice stone; (3) the space in which the animal is confined; (4) a bottle with H_2SO_4 distributed over pumice stone; (5) a soda-lime bottle, and (6) another H_2SO_4 bottle. The H_2SO_4 bottle (4) absorbs the moisture given off by the animal; the CO_2 is taken up by the soda lime, and the last H_2SO_4 bottle absorbs any moisture derived from the soda lime.

Before the actual experiment begins, the following weights are determined—(1) the weight of the animal in its air-tight cage, the connecting tubes (AA') being clipped at a given time; (2) the weight of the H_2SO_4 bottle No. 4; (3) the combined weight of the soda lime and H_2SO_4 bottles, Nos. 5 and 6. The bottles and animal chamber are then securely connected and the current of air drawn through for a fixed time reckoning from the time when the tubes of the animal chamber were clipped before weighing; this time may be half to one or more hours according to the capacity of the absorbing bottles. At the end of this period the three weighings mentioned above are repeated, and so one obtains the CO_2 and water output in grammes for a given time; the temperature of the animal chamber and the weight of the animal should also be taken, so that the results can be expressed per kilogram. body weight.

Another important datum can be obtained from the weights, viz., the "oxygen absorbed;" this is given by the difference between the loss of weight of the animal during the experiment and the combined gain of the absorbing bottles (due to CO_2 and H_2O). Some of the oxygen of the inspired air goes to oxidise elements other than carbon, such as sulphur, phosphorus, hydrogen, etc., and this oxygen is retained in the body of the animal, and hence its loss of weight and the gain in weight of the absorption bottles do not coincide.

If time permits, the experiment should be repeated at a different temperature, or under other conditions capable of affecting the CO_2 output.

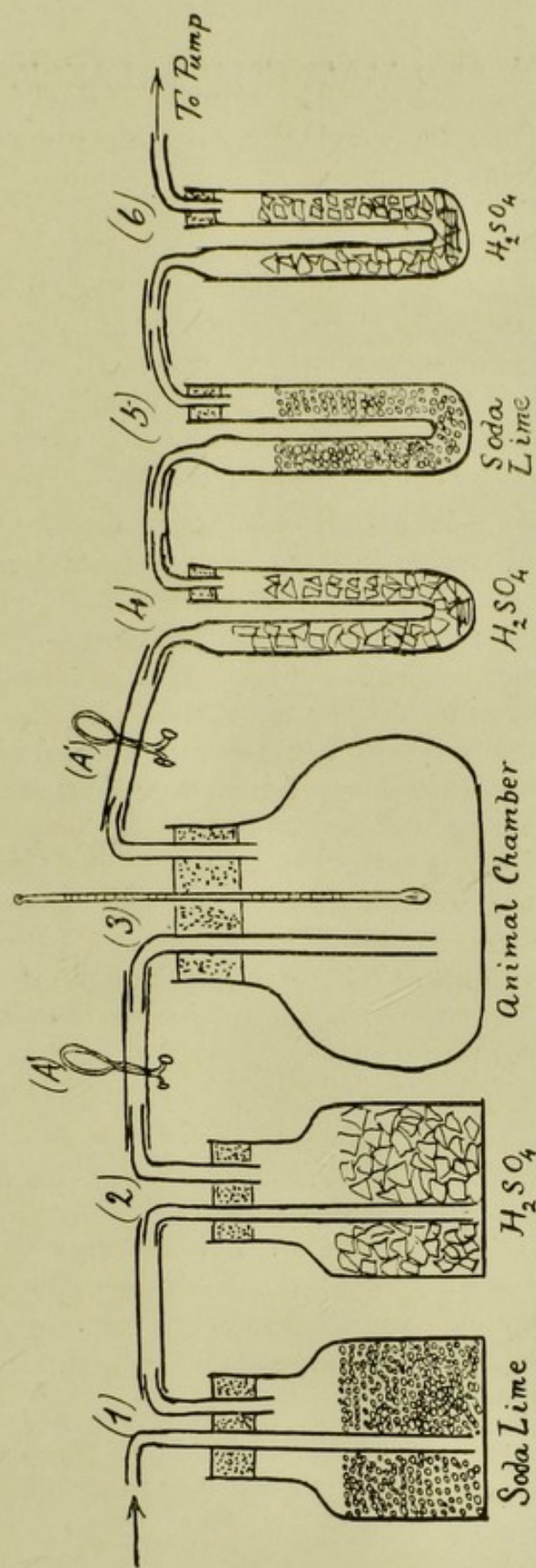


Fig. 16.

Apparatus for estimation of the respiratory interchange in small animals by the Haldane-Pembrey method; (3) the animal chamber, which may consist of a fat-flask when a mouse is used; (4), (5), and (6), thin glass U-tubes; these are light enough to be weighed on the analytical balance.

(I). THE LIME AND MAGNESIA BALANCE.

The **Calcium** intake and output may be obtained by estimating the amount present in food and excreta as follows :—

Incinerate a weighed amount, say 5 grm. meal in a porcelain crucible after adding some dry sodium carbonate ; the incineration may be hastened at the end by adding small pinches of potass. nitrate (as in 13) ; allow it cool ; dissolve the ash completely in dilute HCl, and filter. Add sufficient ammonia to make slightly alkaline, and then acetic acid till the fluid is distinctly acid. If a flocculent precipitate of phosphate of iron remains undissolved in the acid fluid it should be filtered off ; then add ammonium oxalate till no more precipitate appears. heat to near boiling and allow to stand 24 hours. Then filter through an ash-free filter paper, wash with water till the washings are chlorine- and phosphate-free, dry, incinerate, and weigh ; the result is CaO in the amount taken. Treat samples of the excreta similarly. In dealing with urine it is best to evaporate it down in a fairly large porcelain crucible and incinerate in the same way.

The **Magnesium** in food and excreta can be estimated in the filtrate from the calcium oxalate as above obtained. Concentrate the filtrate and washings ; filter if necessary, add some sodium phosphate and excess of ammonia. The result is a precipitate of triple phosphate which is treated in the same way as described under phosphate estimation, *i.e.*, the fluid is filtered after standing for twenty-four hours, the precipitate washed chlorine-free with 1 in 3 ammonia, dried and incinerated in a platinum crucible. The amount of magnesium is calculated from the weight of magnesium pyrophosphate ($\text{Mg}_2\text{P}_2\text{O}_7$) which results.

(J).

The **Chlorine** balance may also be estimated by incinerating the food and excreta with a large excess of sodium carbonate, dissolve the residue in dilute nitric acid and estimate the chlorine in the solution by Volhard's method (267).

INTERNAL SECRETIONS.

(306) Examine slides illustrative of the structure of the following glands :—thymus, spleen, suprarenal, thyroid, pituitary, pancreas, and sexual organs.

(307) Pith a frog; excise both eyeballs carefully; place one in each of two watch glasses filled with normal saline; note whether there is any difference in the size of the two pupils; add to one a few drops of dilute adrenalin chloride; continue to observe the size of the pupils; the one in the adrenalin will become larger (dilate); (stimulation of the sympathetic nerve supply to the iris causes a similar result, compare to exp. (195) where the sympathetic was paralysed by section).

(308) Take a muscle curve in the usual way from a gastrocnemius, sartorius, or hyoglossus, then inject adrenalin into the muscle and repeat the curve; increase in the height and duration of the contraction usually occurs.

(309) Addition of adrenalin to the saline perfused through the blood vessels of a frog causes a diminished flow due to vasoconstriction. Since the frog is pithed this must be a peripheral effect. Do this experiment as described in (196).

(310) Examine some dried thyroid substance for the presence of iodine as follows :—Add a little water to the dried substance in a porcelain crucible; then add about twice the bulk of the substance of pure solid NaOH; apply heat, gently at first to drive off the water, then more strongly till the whole mass is charred, then add cautiously a few particles of potassium nitrate to accelerate the oxidation, and continue heating and adding the nitrate till the mass just becomes white; avoid the use of more potassium nitrate than is necessary. Cool the residue; dissolve it in a little hot water; filter and cool the filtrate; then acidify with H_2SO_4 , keeping down the temperature by holding the tube under the running tap; add a few c.c. of chloroform and shake; if iodine is present in the substance originally taken, it is converted into an iodide by the oxidation in the presence of an alkali, and is then set free by the acid; the chloroform dissolves out the iodine and assumes a pink colour when the result is positive. Excess of potassium nitrate during the incineration may lead to formation of iodates, and so vitiate the result.

CHAPTER XI.

THE NERVOUS SYSTEM AND SPECIAL SENSES.

CENTRAL NERVOUS SYSTEM.

(311) Examine the slides illustrative of the general structure of the spinal cord and brain.

(312) **Reflex action in the Frog.**—Pith the brain of a frog in front of a line drawn between the anterior margins of the tympanic membranes, so as to leave the optic lobes and spinal cord intact; suspend the animal from the myograph stand by means of a pin passed through the snout so that the lower limbs hang free, and make the following observations:—(a) Note that pinching the toes on one side causes reflex withdrawal of that foot; (b) an electric shock or other form of stimulation, *e.g.*, thermal, has the same effect; if the stimulus is strong enough the excitement spreads to the other foot, fore limbs and whole body; (c) a “**purposive**” reflex movement may be elicited by applying to the skin of the abdomen a small piece of filter paper soaked in very dilute acid—the limbs are moved in such a way as to remove the irritant; wash away the acid from the skin of the frog; and (d) find the **reflex time** by Turck’s method, that is, allow one foot to dip into a beaker of very weak acid and count the number of seconds that elapse before it is withdrawn; repeat with a stronger acid after washing; the time varies with the strength of the stimulus, but only within certain limits. (e) **Inhibition of reflexes.**—Cut the head of the frog across so as to expose the optic lobes or medulla; take the reflex time by Turck’s method for a certain strength of acid; place a crystal

of common salt on the cut end of the medulla, or optic lobes ; after a minute or two find the reflex time again with the same acid ; it will be found to be much longer and sometimes the reflex is abolished ; (f) Another method of inhibiting reflex action may be tried on another frog—take the reflex time, tie a string tightly round one fore limb in order to cause a strong afferent impression, and again test the reflex.

(313) Prepare a frog as in (312), inject a few minims of a strychnine solution, and note that convulsive movements can now be set up by very slight stimulation of the skin, or even by tapping the table ; pith the cord—the reflexes and convulsions will disappear.

(314) Test your own **knee-jerk** by crossing one leg over the other so that the former swings freely from the knee downwards ; tap the patellar tendon smartly and note the involuntary forward movement of the leg and foot.

(315) **Simple Reaction Time** (dem.).—This may be measured with the pendulum myograph ; arrange the knock-over key as part of the primary circuit of an induction coil ; the electrodes of the secondary circuit may be placed on the skin (reaction time for pain), or connected with a telephone (for hearing), or the knock-over key may be made part of a galvanic circuit which operates an electro-magnet and pulls down a lever with a white disc attached (reaction time for sight) ; the point of time when the signal is given is shown on the moving surface by a dip of the writing lever due to the electro-magnet C (Fig. 17) being thrown into action ; the lever remains down till the response is given by opening key B. Take the time value of the moving surface with the tuning fork as usual.

SPECIAL SENSES.

(316) Examine slides illustrative of the structure of the special sense organs—eye, ear, olfactory mucosa, taste buds, nerve terminations in skin, muscles, etc. If opportunity offers dissect the eye of an ox, or other animal according to the directions given in Stewart's Physiology, or in manuals of Practical Anatomy.

(318) Observe, in a companion's eye, the **Purkinje-Sanson images** seen as reflections from the anterior surface of the cornea, anterior surface and posterior surface of the lens. Ask the subject to focus for a near object and then for a far one and note whether you can see any change in the size and position of the images. These observations can best be made with the phakoscope of von Helmholtz, the use of which will be demonstrated.

(319) Observe the **reflex contraction of the pupil** on stimulation by a bright light, and its dilation in a darkened room or on blindfolding; if one eye is blindfolded the other pupil dilates.

(320) (Dem.) **Kühne's Artificial eye** illustrates many important points in connection with the optics of the normal (emmetropic) eye, and abnormal conditions of refraction. It consists of a wooden trough which may be filled with water tinged with eosin to show up the path of the rays. At one end of the box is a curved glass window to represent the cornea; in some forms of the instrument this is covered with a water-tight cap and plain glass face so that when the cap is filled the cornea is thrown out of action; the retina is represented by a movable ground glass screen, and the lens and iris are also represented by movable parts; a ray of sunlight or other strong light is sent horizontally through the cornea and the following observations may be made. (a) The beam of light (parallel rays) is brought to a focus at one point behind the cornea and lens (= principal posterior focus); instead of using the whole beam of light a piece of thin metal with a stencil cut in it may be interposed, in such a case note that the image is reversed. Find the position of the screen which corresponds to the principal posterior focus.

(b) Remove the lens (cf. operation for cataract) and note that now the rays are no longer focussed on the retina; place the removed lens in front of the cornea, it overcompensates the defect and brings the rays to a focus in front of the retina, this is because it is now acting with air on each side of it instead of watery fluid; find a lens that will compensate the defect and bring parallel rays to a focus on the retina and then compare its strength (focal length) with that of the lens which was removed.

(c) Return the lens to its place and abolish the refractive power of the cornea by filling the front cap with water, and proceed similarly to find a lens which will compensate for the defect.

(d) Remove the iris—the image may be seen to be less distinct.

(e) Place the retinal screen further away from the cornea, after replacing all the working parts, a blurred image results comparable to *myopia* or short sightedness which is generally due to elongation of the eyeball in the antero-posterior direction. Correct the defect by a concave lens in front of the cornea.

(f) Proceed similarly to imitate *hypermetropia* (long-sightedness) by placing the retinal screen nearer to the cornea than normal—correct by using a convex lens.

(g) Place a cylindrical lens in front of the cornea and note the irregularity of the image due to increased refraction in one line. This simulates *astigmatism*.

(321) **Scheiner's Experiment.**—Make two pin holes in a piece of note paper at a less distance apart than the diameter of your pupil; stand with your back to the window and, holding the perforated paper close to one eye, look through the holes at a pin or needle held a foot or more away. At first you see a single image of the needle; then gradually bring the object nearer and the image will be found to become double. If now the right hand hole in the paper is blocked, the right hand image will disappear and similarly for the left. Now place the needle about fifteen inches from the eye and again look towards it through the two holes, but accommodate the eye for distance as if you were looking beyond the needle but yet paying attention to the image it produces:—A double image will again appear, and closing one of the holes, say the right, causes the other image to disappear, in this case the left hand one.

(322) Make three small pin holes arranged close together in triangular form in a piece of note paper, hold a pin quite close to one eye with the head up and hold the paper a short distance (1–2 inches) in front of the eye; at a certain adjustment of the objects each of the three holes in the paper will show the head of

the pin upside down. The pin in this case is held too close to the eye to be focussed but yet it lies in the path of the rays entering the pupil from the small holes; each beam from a hole casts a different shadow of the pin head on the retina in the erect position but the mind misinterprets this and reverses the image.

(323) **The Blind Spot: Mariotte's Experiment.** Make a dot and a cross about four inches apart on a piece of paper. Close one eye, say the left, gaze steadily at the left hand mark, gradually approximate the paper to the eye, at first both spots are seen but at a certain distance from the eye the right hand one disappears, to reappear again if the paper is brought still closer.

(324) **The Yellow Spot.**—Look at a bright white cloud through a solution of chrome alum—a pink area will be seen—due to the absorption of the blue-green rays of the solution by the pigment of the fovea centratis.

(325) **The Ophthalmoscope.** *Indirect Method.*—In an otherwise darkened room, place a shaded light near to the subject on the side of the eye to be examined; the subject is to be seated and the light placed opposite to or behind the ear so that the face is in shadow; seat yourself in front of the subject close to and facing him, during the examination your own eye should be about eighteen inches from the subject's eye; take the ophthalmoscope in the right hand, place the back of the larger mirror close to your eye and look through the central aperture; ask the subject to gaze steadily at a point in such a position that the eye is rotated slightly inwards and then direct a beam of reflected light into his eye through the pupil; a red appearance will be seen to occupy the pupil if everything is in line; when this is obtained, bring the convex lens into position about $2\frac{1}{2}$ in. in front of his eye, and in the path of the rays; move the lens till the optic disc is found.

The *direct method*, which is more difficult for students' work, will be explained.

Various other practical exercises on vision, and other special senses, have been omitted because the apparatus used can be easily demonstrated, and the experiments are fully described in the text books.

APPENDIX.

List of reagents kept on the shelves of the working bench The amount required for one session are also given. From left to right beginning with the upper shelf, the bottles are arranged as follows :—

Chemical Room.

Concentrated Pure Sulphuric Acid..	1½ oz.
Concentrated Pure Hydrochloric Acid	1 oz.
Fuming Nitric Acid	1½ oz.
Acetic Acid, 10%	3 oz.
Glacial Acetic Acid	1½ oz.
Oxalic Acid (crystals)	1 oz.
Alcohol, 96%	3 oz.
Alcoholic Potash	3 oz.
Ammon. Molybdate Solution	3 oz.
Ammon. Oxalate Solution	3 oz.
Ammon. Sulphate (crystals)	4 oz.
Ammon. Sulphide	3 oz.
Barfoed's Reagent	3 oz.
Barium Chloride, 10%	3 oz.
Calcium Chloride, 5%	3 oz.
Carbolic Acid, 5%	3 oz.
Chlorform
Copper Sulphate, 1 %	3 oz.
Ether
Fehling's Solution	3 oz.
Ferric Chloride, 2 %	3 oz.
Glyoxylic Acid	1 oz.
Guaiac Resin in Alcohol	1 oz.
Hydrogen Peroxide (watery)
Iodine 1% in 2% Potass. Iodide	3 oz.
Lead Acetate, neutral 10%	3 oz.
Lead Acetate, basic, sat. sol.	3 oz.
Litmus Solution	1 oz.
Magnesium Sulphate (crystals)	3 oz.
Magnesia Mixture	3 oz.
Mercuric Chloride, sat. watery soln...	3 oz.
Millon's Reagent	3 oz.
alpha-Napthol in Methyl Alcohol	1 oz.

Phenol-Phthalein in Alcohol	1 oz.
Picric Acid, sat. watery soln.	3 oz.
Potass. Ferricyanide, sat. watery soln.	3 oz.
Potass. Ferrocyanide, 5%..	3 oz.
Silver Nitrate, 1%	3 oz.
Sodium Acetate + Acetic Acid	3 oz.
Sodium Carbonate, 10%	3 oz.
Sodium Chloride (crystals)	2 oz.
Sodium Hydrate (solid)	1 oz.
Sodium Phosphate, 10%	3 oz.
Sodium Nitroprusside, 5%
Sodium Sulphate (dry)	1 oz.
Uranium Nitrate, standard soln.	3 oz.
Hydrochloric Acid, 1 in 5..	10 oz.
Sulphuric Acid, 1 in 5	10 oz.
Nitric Acid, 1 in 5	10 oz.
Ammonia, 1 in 5..	10 oz.
Saturated watery Ammon. Sulphate	10 oz.
Esbach's Reagent	10 oz.
Potassium Hydrate, 10%..	10 oz.
Sodium Hypobromite

Box of Litmus Papers ordinary and glazed.

[Where no quantity is mentioned the reagent is not to be put out or made up till required. Unless where specially mentioned, the Acids to be used are the diluted solutions in the larger bottles.]

Apparatus to be kept in the cupboard, or on the bench at each place :—

Iron Tripod with gauze top ; [the gauze to be heated to burn off the dressing before the class meets.]

Pipe-clay Triangle.

Bunsen Burner connected by tubing to gas tap, [the burner should have the gas exit narrowed or increased to give a flame two inches long, and the air inlet should be capable of adjustment]

Porcelain Crucible ($2\frac{1}{2}$ oz. capacity) and lid.

Crucible Tongs.

Porcelain Basin.

Mortar and Pestle.

Filter Funnel, filter paper (S. and S., 595, 11 c.m.).

Filter Stand with clamp for burettes.

Two Burettes, with nozzle, tubing and clip.

Graduated Pipette (25 c.c. in $\frac{1}{10}$ c.c.)

Measure Cylinder (100 c.c.).

Thermometer.

Water-bath with wire netting cover.

Glass Rod.

Slides and coverglass.

Clean duster.

Test Tube Stand with test tubes $\frac{3}{4}$ inch diameter and two large test tubes (6 x 1 inch).

The microscopes are kept in the adjoining room.

Experimental Room.

Apparatus required for muscle work in general :—

Recording Drum.
 Glazed Paper for drum.
 Camphor on porcelain slab and handles for rotating the cylinder in the flame, or luminous gas burner.
 Myograph Stand with crank lever.
 Frog, frog plate, dissecting instruments and dish for garbage.
 Normal Saline (0.75% NaCl in tap water)
 Thread, pins, blotting paper.
 Mounted Pin for pithing frog.
 Duster.
 Varnish (250 c.c. best white hard varnish + 1000 c.c. methylated spirit + 10 c.c. castor oil).

Where Electric Stimulation is to be used :—

Daniell Cell with amalgamed zinc plate, copper plate, porous pot, saturated solution of CuSO_4 , dilute (1 in 10) H_2SO_4 .
 Electric Wires, pin electrodes, emery cloth or sandpaper for cleaning ends of wires.
 Mercury Key.
 Induction Coil, Short-circuiting key.

Special apparatus, material and reagents required for some of the experiments. The material mentioned under one heading is frequently required for those which follow, and should therefore be left on the bench till no longer required, e.g., egg white mentioned under (15-20) is also required for tests (25), (30), (32).

CHAPTER I.

- (1-10). Minced tissue, e.g., meat, freed from fat as far as possible, and dried on the water-bath; ash-free filter papers (S. and S. 590, 11 c.m.); mounted platinum wire for flame test; saturated watery solution of sodium hydrogen tartrate.
- (11). Freshly made sat. watery ferrous sulphate.
- (13). Dry sod. carbonate and potass. nitrate.
- (15-20). Egg white pure and egg white diluted 1 in 5 with normal saline and strained; $\frac{1}{2}$ —1% gelatine; 2% Witte's Peptone in normal saline; caseinogen solution made from milk or solution of casein in 1% sod. carbonate; globulin solution (5% MgSO_4 extract of mince, strained); keratin shavings;
- (24). Tannic Acid 5%; salicyl-sulphonic acid, saturated watery solution; Potassio-mercuric iodide solution; trichloroacetic acid.
- (26). Mixture of primary and secondary albumose.
- (30). Parchment dialysis tube, 8 in. long; tall jars; blood serum.
- (31). Live rat or guinea pig.

- (32) Small test tube supported by a cork ring inside large test tube ; water bath.
- (33) 23% sodium hydrate ; undiluted egg white.
- (35) Sodium nitroprusside.
- (37-40) Water-bath.
- Blood serum diluted 1 in 5 with normal saline.
- (42) 2% Glucose.
- (45) Bismuth subnitrate ; sod. carbonate, dry ; Nylander's solution.
- (47) Saffranin solution ; indigo-carmin solution.
- (49) Phenyl-hydrazine, fluid ; 50% acetic acid saturated with sodium acetate.
- (50) Fresh brewer's yeast or active cake yeast ; fermentation tubes.
- (51) The spectro-polarimeter ; solution of glucose, 5 to 10%, cleared if necessary by adding lead acetate, shaking and filtering ; argand burner if sky is dull.
- (52-55) Laevulose, pure, or inverted cane sugar ; resorcin ; cane sugar 2% ; maltose 2% ; lactose 2%.
- (56-62) Starch powder, or raw potato, flour or other vegetable ; starch mucilage 2% ; tannic acid 5% ; dialysis tube.
- (63-66) Dextrin 2%.
- (67-71) Glycogen solution of sufficient strength to be distinctly opalescent.
- (72-73) Butter fat or suet ; Potass bisulphate crystals.

CHAPTER II.

- (74) Thymus gland or pancreas, minced and soaked overnight in ammoniacal water, and strained several times through flannel ; artificial gastric juice (0.2% HCL + pepsin) ; ash-free filter paper ; dry carbonate of soda.
- (75) Cholesterin crystals ; chloroform ; or solution of pure cholesterin in chloroform.
- (78) Keratin shavings.
- (80) Minced tendons of sheep's "trotters" extracted two or more days with lime water and strained.
- (81) Minced tendons ; (82) Gelatine $\frac{1}{2}$ to 1% ; (83) Dried and crushed bone ; 5% sulphurous acid.

CHAPTER III.

Frogs, frog plate etc., for exps. 85-113.

- (85-92) Apparatus for muscle work as already described ; scale pan and weights.
- (93-98) Apparatus for electric stimulation with the monocord instead of the induction coil ; thick wire for thermal stimulation ; crystals of NaCl ; (99) The Rheonome ; saturated solution of zinc sulphate ; (103-104) Induction coil and short-circuit key ; wires.

- (105-108) Drums must be carefully overhauled at this stage, driving cords adjusted, bearings oiled, stimulating pin and brass strip for contact attended to, and see that the friction pulley which transfers the motion to the base plate of the cylinder is close to the axis; tuning fork (simple or electrically driven); test tube; bunsen burner; thermometer; ice; extra stimulating pin for base plate.
- (109) Fit up Mosso's ergograph, use the 5 kilo. weight and pass the cord over the pulley in ceiling; arrange a Sherrington drum (slow speed) to take tracing. (110) Veratrin, saturated solution in saline; hypodermic syringe. (111) Usual apparatus for muscle work and faradic stimulation with extra stimulating pin. (112) Same with tetanus spring; seconds clock working electro-magnet with writing lever, all on a small tray for carrying round. (113) Glazed litmus paper and usual apparatus.
- (114-117) MgSO_4 extract of muscle (extract $\frac{1}{2}$ lb. minced meat overnight in 1000 c.c. of 5% MgSO_4 , strain several times); general chemical apparatus including water-bath, thermometer and test tubes for taking temperature of heat coagulation of protein; "extract of meat" on watch glasses.
- (118-123) Solution of meat extract, 5%; ether; (124-127) Creatinin solution prepared from meat extract as given in test (124); sodium nitroprusside, fresh, 5%.

CHAPTER IV.

- (128-129) Frogs; apparatus for muscle work and stimulation; small cork chambers for applying CO_2 to nerve; putty or soft sculptor's clay; bottle containing marble chip and fitted for the generation of CO_2 ; HCl ; switch commutator; extra pair of pin electrodes.
- (130-134) Two extra Daniell cells; two simple keys; monocard; commutator; non polarisable electrodes washed thoroughly and soaked overnight in normal saline; saturated solution of zinc sulphate; pipette; arrangement for supporting the non-polarisable electrodes. [This consists of a glass rod, R in fig. 12, clamped in the split horizontal limb of a small tubular T-piece, the vertical limb of which fit into the open upper end of the vertical limb of the large T-piece which carries the cork platform of the myograph; the electrodes are affixed to the glass rod by two bent strips of spring brass perforated by holes for the rod about $1\frac{1}{2}$ inches apart, see fig. 12].
- (135) Special electrodes covered with sponge or wash-leather; 5% salt solution, warmed.
- (136-137) Usual apparatus for muscle work. (138) Pendulum myograph and stimulation apparatus. (139-142) Tripods with copper hook attached; glass rod bent into shape of a hook; capillary electrometer and galvanometer.

CHAPTER V.

- (143-146) Haemocytometers (Gowers' and Thoma-Leitz); microscope; 3% sodium chloride solution; 1% acetic acid tinged with gentian violet; paper for making calculation on; glazed litmus paper.
- (147) Benzol and chloroform mixed to give a specific gravity about 1060; choloform bottle; benzol bottle; large test tube wide enough to admit hydrometer; hydrometer; pipette to deliver drops of blood.
- (148-149) Chloroform; ether; solution of bile salts; 3 -- 5% NaCl.
- (150-152) Jars containing blood which has been mixed when shed with the following:—27% MgSO_4 , 1 part — blood, 4 parts; 0.4% Potass. oxalate in normal saline, 1 part — blood, 4 parts; 3% sod. fluoride, 1 part — blood, 9 parts; these mixtures should be let stand 24 hours to obtain some plasma or the centrifuge may be employed for that purpose; blood serum; water-bath, thermometer and usual chemical reagents; watery extract of minced liver or other tissue freed from blood as far as possible. (153-160) Blood serum; dialysis apparatus; Esbach's albuminimeter and reagent; watery H_2O_2 or ethereal extract of same (= ozonic ether); ammon. sulphocyanide solution; dried blood.
- (161-168) Defibrinated blood; spectroscopes; luminous burners fitted for giving the sodium flame (cut some narrow strips of asbestos millboard, soak one end in strong NaCl solution, dry thoroughly, attach to the burner by a flexible lead wire so that the end of the asbestos strip just touches the flame); Stokes' solution (2% ferrous sulphate in 2% tartaric acid, ammonia to be added just before use till fluid is faintly alkaline). (169) Haldane's modification of Gowers' haemoglobinometer; distilled water; tube from gas tap connected to fine glass tube for delivery of CO; Haldane's apparatus for estimating blood gases and oxygen capacity.

CHAPTER VI.

- (170-171) Sheep's or ox's heart to each couple of students.
- (172) Frog, frog plate, etc; drum and apparatus for muscle work; heart-hook; ice; test tube and bunsen burner; seconds clock with writing lever; myograph fitted with straight lever and frictionless point, [the lever consists of a fine straw about seven inches long; about two inches from one end a piece of aluminium foil is wrapped closely round the straw and then transfixed by a piece of fine needle sharpened at each end, this forms the axis of the lever; the point is made as follows:—cut a small triangular piece of aluminium foil, $\frac{1}{2}$ inch wide at the base and $1\frac{1}{2}$ inches long; roll the base of this triangle two or three times tightly round

a needle or pin which is just a trifle thicker than the pin just to be mentioned; replace the needle by an ordinary pin $1\frac{1}{4}$ in. long; in this way a kind of "pennon" is formed with the pin as the staff round which the aluminium can revolve easily as on a hinge; coat the point of the pin with colophonium or other cement and push it while still warm into the open end of the straw lever till there is just sufficient of it left for the hinge to swing on; bend the aluminium into the arc of a circle; the point (apex of the original triangle) will then press against the surface of the drum paper by the action of gravity and can be made to press more or less heavily according as it is bent more or less acutely].

For experiments on heart work the shafting must revolve at a slow rate: let the cylinder revolve once in two minutes.

(173-175) Stethoscopes; cardio-graphs with recording tambours; sphygmographs (Dudgeon's and Marey's); papers for sphygmograph; camphor.

(176) Rabbit (weighed); paraldehyde; small burette for measuring dose; cylinder measure, 100 c.c.; distilled water; perforated mouth gag; stomach tube (flexible catheter and funnel); animal holder; dissecting instruments; intravenous injection syringe; manometer (mercurial or Hürthle's with connections to pressure bottle containing 2% sodium citrate, and to a suitable Franck's canula; ligatures; adrenalin.

(177) Capillary pressure apparatus.

(178) Martin's modification of the Riva-Rocci apparatus for estimating blood pressure in man connected by a side tube to a recording tambour writing on a slow drum; or Erlanger's apparatus for the same purpose.

(179) Fit up an artificial scheme of the circulation with an enema, syringe to represent the left ventricle and a long piece of tubing to represent a vessel; fix the bulb of the syringe between two boards hinged together and acted on by an eccentric worked from the shafting; various instruments, sphygmograph, cardiograph, manometers, stromuhr, etc., can be demonstrated on the scheme, but for the stromuhr the following arrangement is to be preferred:—Fix the jacket part of a condenser in a vertical position, insert a cork with a narrow tube in the wide lower end, leave the upper end open, connect the tube in the lower end to the water tap, connect the lower side tube of the jacket to the stromuhr and the upper side tube to an indiarubber overflow tube, let water run through the apparatus in amount sufficient to overflow even when the stromuhr is being operated; in this way the rate of flow is made constant and can be regulated by a screw clip on the tubing leading to the stromuhr.

(180) Mosso's Plethysmograph supported on a board swung from the easel or ceiling; burette connected to interior of plethysmograph; recording tambour and revolving drum; see that

the indiarubber collar is in efficient working order and have a large quantity of warm water (37° — 40° C) ready ; vaseline.

- (181) Frogs ; watch glasses. (182) Glass tube 8 in. long drawn to fine point.
- (183) Gaskell's clamp arranged between two levers, the upper of which is kept horizontal by a piece of very weak elastic ; arrange both levers to write on a drum in the same vertical line. (184) Schaffer's heart plethysmograph ; olive oil ; Locke's solution ; horizontal drum ; frog ; fresh defibrinated blood may be required.
- (185-192) Same apparatus as for (172) ; stimulation apparatus.
- (193) Pilocarpine nitrate 5-10%, muscarine 5-10%, atropine 5-10%, nicotine 5%, all made with normal saline ; camel hair brush for each drug. (196) Cylindrical tap funnel on stand connected by tubing to fine canula to fit frog's aorta ; adrenalin chloride (1 in 10,000) ; solution of amyl or other nitrite.

CHAPTER VII.

- (197-201) Stethographs, recording tambours or piston recorder ; stethoscopes ; spirometers of different kinds ; mercurial manometer with scale on one limb, and mouth-piece connected by tubing to other limb.
- (202-203) Rabbit and apparatus as for (176), but instead of manometer have a stethograph in the form of two cardiographs (with the wooden shield removed) mounted on a bent strip of lead so that the membranes of the tambours press on the two sides of the thorax ; these are to be connected by a Y-tube to the recording tambour.
- (204) Haldane's Gas Analysis apparatus in working order ; tall jar of water ; burette ; small cork for upper end of burette ; bent S-shaped tube ; extra burette in stand, nozzle, and clip ; solid NaOH ; duster.
- (205) Leonard Hill's Blood Gas Pump ; Haldane's apparatus for the same purpose ; defibrinated blood from the butcher, or animal to be killed ; mercury ; pyrogallate of potash ; strong KOH.

CHAPTER VIII.

- (208) Have a large water bath ready, heated to 40° C. from the night previous to experiment ; rabbit ; paraldehyde, etc., as for (176) ; dissecting instruments ; drum, tambour, and small balloon for recording movements. (209) Frog, frog plate, etc. ; myograph with straight lever as for (172) ; stimulation apparatus.
- (210-215) Starch mucilage 2% ; porcelain slab ; glass rod ; water bath, and usual chemical reagents.

- (216-220) 0.2% HCl (7 c.c. concent. HCl in 1 litre water); Congo red paper; Gunzburg's reagent; Boas' reagent; porcelain basin or broken piece of same; burettes; titration flask or beaker. capacity about 300 c.c. (221-222) 1% lactic acid solution; ether.
- (223-224) Pig's stomach, fresh; 0.2% HCl; raw meat or fish minced, or fibrin, or partially coagulated egg white.
- (225) Gastric digest (12 hours digestion of some of above material in 0.2% HCl *p'us* some active pepsin).
- (226) Fresh milk; rennet; water bath, etc.
- (227-228) Fresh pancreas minced, let lie one day and extract with 1% sodium carbonate or ammoniacal water as described in (227); protein for digestion as above (meat, fish, or egg); tryptic digest prepared in similar way to gastric but use 1% sod. carb. and active trypsin; Bromine water (later). (229) starch mucilage 2%. (230) Cream or neutral olive oil; fresh pancreas; (231-240) Ox bile; cane sugar, dry; sulphur, in powder; gall stones. (241-243) Gastric digest or 2% Witte's Peptone; starch mucilage; olive oil; 1% sodium carbonate. (244-247) Intestinal mucous membrane dissected off, minced, extracted 12 hours with water, and strained; cane sugar 2%; lactose 2%; maltose 2%; yeast; weak solution of Witte's Peptone.

CHAPTER IX.

Fresh normal human urine for tests (248-271) arrange for supply of pathological urines (248-254) Urinometers; urea crystals, dry; (272-280) fresh sodium hypobromite (dissolve 100 grms. NaOH in 250 c.c. water and add cautiously 25 c.c. bromine).

- (255) **Apparatus for quantitative estimation of urea in urine**, *viz.*, small wide-mouthed bottle with paraffined cork or rubber stopper perforated by a tube which is connected by rubber tubing to an inverted burette in a tall jar of water; small, short test tube to fit inside bottle and marked at 5 c.c. level; jar to hold water for cooling the bottle; cylinder measure.
- (256-262) Uric acid crystals; urine with urate deposit; broken porcelain for murexide test. (263-265) Fresh sodium nitroprusside; hippuric acid; herbivorous urine or human urine containing indican; 5% "chloride of lime."
- (266) **Apparatus for Nitrogen Estimation**, *viz.*, Kjeldahl flask; tripod, gauze, etc., in fume cupboard; H_2SO_4 ; K_2SO_4 ; CuSO_4 ; condenser; titration flask; fifth- or tenth-normal acid and alkali; two burettes; 23% soda; funnel; talc; rosolic acid indicator.
- (267) **Apparatus for Chloride Estimation**, *viz.*, 100 c.c. and 50 c.c. flasks; two burettes in stand; standard silver nitrate solution; standard ammon. sulphocyanide solution; ammonia iron alum; titration flask; and the usual chemical apparatus.

- (269) **Apparatus for Estimation of Phosphates in Urine, viz.,** Two burettes ; porcelain slab ; glass rod ; porcelain basin on tripod with gauze ; standard uranium nitrate and sodium acetate (kept on the shelves).
- (271) Bell jar to fit air tight on ground glass plate ; tripod to stand inside bell jar ; two small porcelain basins ; milk of lime ; tenth-normal alkali and acid in burettes ; vaseline.
- (271a) Urines with deposits, from hospital.
- (272) Albuminous urine ; Esbach's reagent and albuminimeter.
- (273) Urine containing blood ; spectroscopes ; burners to give sodium flame, etc., as for (161-168). (274) Bilious urine ; cane sugar ; flowers of sulphur ; Oliver's reagent (see text).
- (275) Diabetic urine ; phenyl-hydrazine and sod. acetate in acetic acid ; yeast ; polarimeter. (276a) **Apparatus for Estimation of Glucose (Fehling)**, viz., two burettes in stand ; tripod, gauze, and porcelain basin ; 25 c.c. graduated pipette and cylinder measure for diluting the urine ; glass rod.
- (276b) **Apparatus for Pavy-Fehling Method, viz.,** the same as the above but instead of the porcelain basin use a 300 c.c. flask with a rubber stopper, pierced by two holes, one for the nozzle of the burette, the other for a bent glass tube to act as an outlet for the steam ; put a screw clip on the tubing between the nozzle and the end of the burette.
- (277-279) Lactose solution 2% ; phenyl-hydrazine, etc. ; pure pentose solution or solution of gum arabic ; urine of rabbit or dog after a dose of chloral. (280-281) Diabetic urine containing aceto-acetic acid and acetone or an artificial solution taining acetone.
- (283) The cryoscope ; Beckmann's thermometer ; crushed ice ; coarse salt ; urine.

CHAPTER X.

- (284) Rabbit fed on carrots five hours before the class meets or fresh oysters ; scissors and forceps ; animal holder ; large porcelain dish with boiling water acidified with acetic acid ; large mortar and pestle ; piece of wire gauze for removing pieces of tissue from the boiling fluid ; clean sand ; large funnel and filter paper ; tall jar ; Brücke's reagent ; methylated spirit.
- (285) Rabbit in collecting cage ; phloridzin.
- (286-293) Fresh milk ; hydrometer ; ether ; rennet ; finely grated cheese ; mortar and pestle.
- (294-297) Hen's eggs ; spectroscope ; ether ; 10% NaCl ; 0.4% HCl ; pepsin ; dried yolk ; ammon. sulphocyanide. (298-304) Flour ; muslin ; loaf of bread and other materials as given in text.
- (305) Two rats or one small dog ; suitable cages or kennel ; catheter, if necessary, fitted up with wash bottle containing sterilised tap water ; several pounds of oatmeal ; or powdered dog biscuit ; dried and powdered protein, e.g., casein.

- A. Two pairs well-fitting watch glasses with clips; hot air oven; desiccator; analytical balance.
 - B. Clean porcelain or platinum crucible with clay or platinum triangle; ash-free filter paper; small beaker; constant level water bath.
 - C. Set of small, short test tubes, in light beaker, clean and dry; two Kjeldahl flasks; strong H_2SO_4 , etc., as for (266).
 - D. Soxhlet apparatus fitted with condenser and fat flask and placed on sand bath over a constant level water bath; extraction thimble; Adams' paper; fresh milk.
 - E. Flask to hold 500 c.c. or more fitted with condenser or long glass tube to act as such; sand bath; apparatus for glucose estimation (276a); CaCO_3 .
 - F. Ordinary balance for weighing up to several kilograms; wash bottle; glass rod with indiarubber on end; 1,000 c.c. or 500 c.c. measure flasks; several bottles to hold one or other of these amounts; sheet of glass 12 x 10 in. for drying dog's faeces.
 - G. Kjeldahl flask; cylinder funnel with glass tap supported in stand over flask; mixture of concent. H_2SO_4 and HNO_3 ; flask or beaker to hold 200-400 c.c.; ammon. nitrate 50%; ammon. molybdate 10%; platinum crucible and triangle; ash free filter paper; desiccator.
 - H. Mouse; apparatus for estimating CO_2 (Fig. 16); filter-pump fitted to tap to draw air through apparatus; place a gas-meter between the pump and the apparatus.
 - I. and J. Platinum crucible and apparatus for Volhard's estimation of chlorides (267).
- (306-310) Frog; watch glasses; adrenalin; apparatus for general muscle work and stimulation; hypodermic syringe in working order; thyroid gland substance; potass. nitrate; chloroform.

CHAPTER XI.

- (312-315) Frog; jars with 1 in 300 and 1 in 1000 sulphuric acid; acetic acid; electric stimulation apparatus; seconds clock fitted to bell; sodium chloride crystals; strychnine solution; hypodermic syringe; narrow tape; pendulum myograph fitted for estimating reaction time (Fig. 17); telephone receiver; two electro-magnets with levers.
- (316) Fresh ox eyes; dissecting dish and instruments.
- (317-324) Snellen's Types; phakoscope in dark room; Kuhne's artificial eye filled with water tinged with eosin; mirror on stand to reflect beam of sunlight; set of concave and convex lenses; cylindrical lens to show astigmatism; note paper and needles; solution of chrome alum in flat-sided bottles; ophthalmoscopes; have the room darkened and argand burners arranged for work with the ophthalmoscope.

Miscellaneous Instructions regarding the use of chemical apparatus.

Burners.—A bunsen burner gives most heat when it burns with a non-luminous flame and without noise; the hottest part is just above the inner mantle of the flame; if it is desired to diminish the flame, the air inlet should be diminished at the same time.

Bu ettes.—The correct way to read a burette is as follows:—Remove it from its clamp and suspend it from near the top by holding it between the adjacent sides of the forefinger and middle finger so that it swings freely and assumes a vertical position; fix some distant point of view on a level with the eye, such as the roof of a house and bring the burette in front of the eye so that the top of the column of fluid is in line with the point chosen, and then read the level at which the *bottom of the meniscus* stands; after running out the required amount of fluid allow a minute or so to elapse in order to allow the fluid which wets the inside to run down, and again read the level in the same way. Always see that the nozzle of the burette is full before you begin to run out the measured amount.

Pipettes.—Since a pipette is filled by suction, it should not be used for volatile or corrosive liquids such as ammonia, strong acids, or alkaline media such as Fehling's solution. When a sufficient amount of the fluid to be measured has been drawn up into the pipette, close the upper end with the tip of the forefinger which ought to be quite dry; read the level of the fluid as described for a burette, and allow the requisite amount to flow out by cautiously relaxing the pressure of the finger on the upper opening of the pipette. Burettes, pipettes, and measure flasks are used for exact work, measure cylinders on the other hand are less accurate and are used only for rough measurements.

Filtering.—In preparing a filter paper and funnel see that the paper is of a suitable size; when in position it should not project above the top of the funnel; fold the paper twice so as to produce the quadrant of a circle, but in making the second fold let one edge project a little beyond the other and then open out the wider of the two possible hollows, thus forming a cone the apex of which is an angle greater than a right angle; such a filter will cling to the funnel by its upper edge and leave a space between the lower part and the side of the funnel, through which the filtrate can run. The end of the funnel should not dip into the filtrate.

Before beginning the filtration, wet the paper thoroughly with fluid of the same nature as you are about to filter, except in some cases where a dry funnel and filter paper must be used (*e.g.*, in 267).

Test Tubes, Flasks, Beakers, etc.—When boiling a watery fluid in a test tube, keep shaking it gently, and, if only in the interest of the bystanders, never attempt to boil a long column of fluid

in a test tube by applying the heat to the bottom of the tube. If such a quantity must be boiled, use a large test tube or porcelain basin, or boil the fluid in the upper part of the tube only. Always proceed cautiously in neutralising strongly acid or alkaline fluids in a test tube, else the contents may be shot out by the heat evolved.

Bohemian glass beakers and flasks (flat bottomed) do not stand boiling well, unless the heat is gradually applied, the contents shaken occasionally, and the moisture which forms on the outside removed. Never heat a flask on a naked flame when it can be avoided; use a tripod covered with gauze to support the flask.

Jena glass beakers and flasks withstand heat better but the same precautions should be used. Flasks containing deposits and semi-fluid contents should be heated only on the sand bath or water bath. Measure flasks are on no account to be heated.

Thermometers.—Do not subject a thermometer to a temperature which may rise above that for which the instrument is graduated, *e.g.*, a hot air oven may reach a temperature of 120° C. and a thermometer graduated to 100° or 110° C. would be burst at that temperature.

Mixing and Transferring Fluids, &c.—To ensure the uniform mixing of fluids in a test tube, cylinder measure or flask, the mouth should be closed with the finger or ball of the thumb and the vessel inverted several times in succession. In the case of beakers and porcelain basins, use a glass rod to stir the fluids.

When the contents of one vessel are to be transferred completely to another, use several small quantities of fluid to wash out the first into the second instead of one large quantity.

Comparison of metrical with British measures.

1 gramme =	15.432 grains
1 cubic centimeter (c. c.) =	16.95 minims
0.065 gramme =	1 grain
0.06 c.c. =	1 minim
28.35 grammes =	1 ounce (avoir) = 437.5 grains
28.42 c.c. =	1 fluid ounce = 480 minims
1000 grammes (1 Kilogram) =	2.2 lbs. (avoir.)
1000 c.c. (1 litre) =	35.196 fluid ounces
4.546 litres =	1 gallon
1 metre =	39.37 inches
2½ centimeters approx. =	1 inch

To convert degrees Fahrenheit into degrees Centigrade or *vice versa*, use this formula.

$$\text{Degree F.} = \frac{9 \times \text{degree C}}{5} + 32.$$

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