Protoplasm : its definition, chemistry and stucture / by Gustav Mann.

PROTOPLASM

2

ITS DEFINITION, CHEMISTRY AND STRUCTURE

BY

GUSTAV MANN

M.D. EDINB., B.SC. OXON.

PHYSIOLOGICAL LABORATORY, OXFORD

DELEGATE OF THE OXFORD MEDICAL SOCIETY

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DEFINITION, CHEMISTRY,

AND

STRUCTURE OF PROTOPLASM.

(By Gustav Mann, M.D. Edinburgh, B.Sc. Oxon, Physiological Laboratory, Oxford.)

Hugo v. Mohl[1] described in 1844 the 'primordial utricle' as a membrane composed of a nitrogenous compound lying on the inner side of vegetable cell-membranes, and also stated that it occurred in the Confervae without a nucleus, and that it persisted in chlorophyll-containing cells after the nucleus had been absorbed. These observations no doubt led him to the theory expressed in 1846[2] that 'the semi-fluid, nitrogenous substance contained in the cell . . . is the precursor of the solid constituents found subsequently in a developing cell, and that it supplies the material for the formation of the nucleus and the primordial utricle.' This physiological consideration led him to introduce the word protoplasma.

Protoplasm is therefore, according to v. Mohl's definition, the mother-substance of the cell membranes and of the nucleus. That this conception is diametrically opposite to the one held by me will be shown later, and will, I hope,

[1] H. v. Mohl, *Botanische Zeitung*, 1844, pp. 273, 289, 305, 326, 337.
[2] Ibid., 1846, pp. 73, 89.

excuse me for having included the nucleo-proteids in my discussion.

Before attempting to give a definition of the word protoplasma, it is my intention to outline in the first instance our chemical and physico-chemical knowledge regarding the units out of which we believe protoplasm to be built up[1].

In protoplasm we have certain organic units which have received a great deal of attention, and also inorganic constituents which are greatly neglected. However much from a purely chemical point of view the isolation of the organic constituents is desirable, we should never forget, as I pointed out in 1902[2], 'that so-called pure ash-free albumins (proteids) are chemically inert, and, in the true sense of the word, dead bodies.'

For descriptive purposes Proteids [Protein-Substanzen; Substances albuminoides] may be divided into three groups.

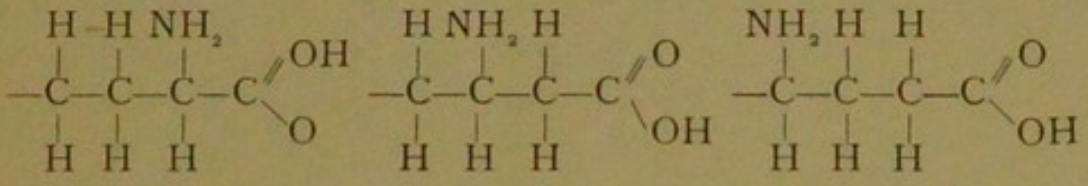
1. **Albumins** which occur in nature as 'native albumins.' They include the 'albuminoid' substances which form the supporting or connective tissues of the animal body.
2. **Proteids proper,** which are combinations of the native albumins with such other organic compounds as sugars or radicals containing phosphorus or iron.
3. **Derivatives** of the natural albumins and proteids, which retain in their chemical configuration the characteristics of albuminous substances, and are represented by the albumoses, peptones, peptids, and other compounds. These bodies are met with in nature as products of digestion and metabolism, but they may also be obtained artificially by hydrolysis of the more complex albuminous substances.

On subjecting these compounds to the action of acids or

[1] The author has given full references in his *Text-book on the Chemistry of Proteids*, Macmillan & Co., 1906, p. 606.

[2] Mann, *Physiological Histology*, 1902, pp. 2, 25, 224, 338, 345, 348.

alkalies, or to certain ferments acting preferably either in acid (pepsin) or alkaline (trypsin) solutions, they are broken up into smaller units called albumoses and peptones. On still further dissociating these latter we arrive at a number of substances known as amino-acids, characterized by the presence of one or more carboxyl-groups ($CO \cdot OH$) and one or more amino-groups (NH_2), attached to a carbon chain. According as to whether the NH_2-radical is attached to the first, second, third . . . carbon-atom next the carboxyl-group we speak of α, β, γ . . . amino-acids.



α-amino-acid. β-amino-acid. γ-amino-acid.

Now most amino-acids derived from protoplasm are α-amino-acids and are characterized by a sweet taste, a characteristic which led to the simplest of all amino-acids, namely amino-acetic acid, so abundant in gelatine or glue, receiving the name of sweet-glue or glycocoll. In addition to α-amino-acids there are also found certain bitter amino-acids which are β-compounds, such as the amino-valerianic acid formed during the autodigestion of the pancreas (Levene) and probably also tryptophane. There are no γ-acids present in protoplasm.

Enumeration of the Primary Dissociation-Products.

(The numbers marked with a * have their constitutional formula given hereafter.)

I. Albumins, as occurring in the cytoplasm.

A. Open-chain Amino-acids.

I. (*a*) mono-amino-mono-carboxylic acids.

*1. amino-acetic or Glycocoll $C_2H_5NO_2$.

2. amino-propionic $C_3H_7NO_2$.
   amino-butyric $C_4H_9NO_2$.
3. amino-valerianic $C_5H_{11}NO_2$.
4. amino-iso-butyl-acetic $C_6H_{13}NO_2$.

(*b*) mono-amino-mono-carboxylic-hydroxy acids.

*5. amino-hydroxy-propionic or Serin $C_3H_7NO_3$.
6. amino-tetra-hydroxy-caproic $C_6H_{13}NO_6$.

(*c*) mono-amino-di-carboxylic acids.

*7. amino-succinic or Asparctic acid $C_4H_7NO_4$.
8. amino-glutaminic $C_5H_9NO_4$.

(*d*) mono-amino-di-carboxylic-hydroxy acids.

*9. amino-hydroxy-succinic $C_4H_7NO_5$.
10. amino-hydroxy-suberic $C_8H_{15}NO_5$.

II. (*e*) diamino-mono-carboxylic acids.

*11. diamino-propionic $C_3H_8NO_2$.
*12. diamino-caproic or Lysin $C_6H_{14}N_2O_2$.
13. guanidin-amino-valerianic $C_6H_{14}N_4O_2$.

(*f*) diamino-mono-carboxylic-hydroxy acids.

14. diamino-trihydroxy-dodecanoic $C_{12}H_{26}N_2O_5$.

(*g*) diamino-di-carboxylic acids.

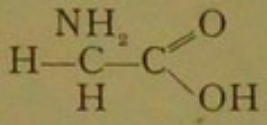
*15. diamino-glutaric $C_5H_{12}N_2O_4$.
16. diamino-adipic $C_6H_{14}N_2O_4$.

(*h*) diamino-di-carboxylic-hydroxy acids.

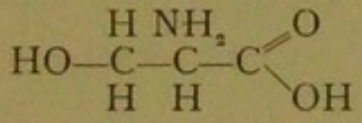
*17. diamino-di-hydroxy-suberic $C_8H_{16}N_2O_6$.
18. diamino-hydroxy-sebacic $C_{10}H_{20}N_2O_5$.
19. caseanic acid (?) $C_9H_{16}N_2O_6$.
20. caseinic acid (?) $C_{12}H_{16}N_2O_5$.

A. Open-Chain Compounds.

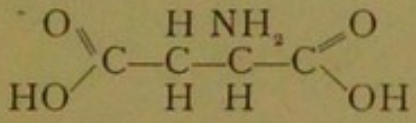
Glycocoll, or **Amino-Acetic Acid,** $C_2H_5NO_2$



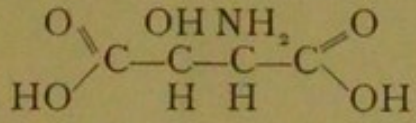
Serin, $C_3H_7NO_3$ is α-amino-β-hydroxy-propionic acid.



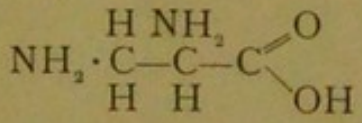
Aspartic Acid, $C_4H_7NO_4$



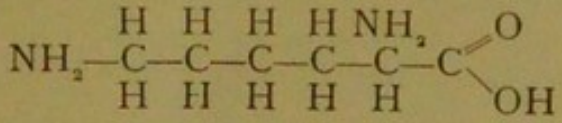
Amino-hydroxy-succinic Acid, $C_4H_7NO_5$



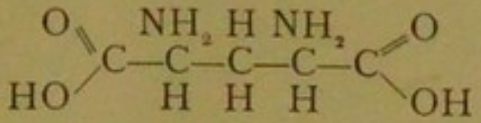
Diamino-propionic Acid, $C_3H_8NO_2$



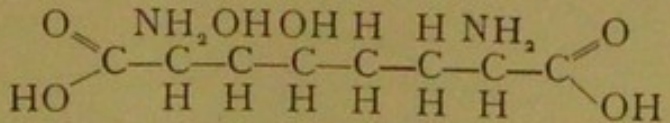
Lysin, $C_6H_{14}N_2O_2$



Diamino-glutaric Acid, $C_5H_{10}N_2O_4$



Diamino-dihydroxy-suberic Acid, $C_8H_{16}N_2O_6$



Suberic acid is $COOH(CH_2)_6 . COOH$.

B. Ring-compounds.

(*i*) pyrrolidin compounds.

*21.	α-pyrrolidin-carboxylic acid	$C_5H_9NO_2$.
22.	hydroxy-pyrrolidin-carboxylic acid	$C_5H_9NO_3$.

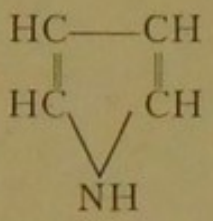
(*k*) imido-azol compound.

*23.	histidin	$C_6H_9N_3O_2$.

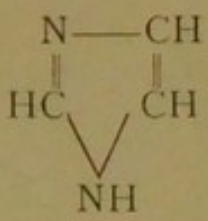
(*l*) aromatic amino-acids.

*24.	phenyl-amino-propionic or phenyl-alanin	$C_9H_{11}NO_2$.
*25.	phenyl-hydroxy-amino-propionic or tyrosin	$C_9H_{11}NO_3$.
*26.	indol-amino-propionic or tryptophane	$C_{11}H_{12}N_2O_2$.

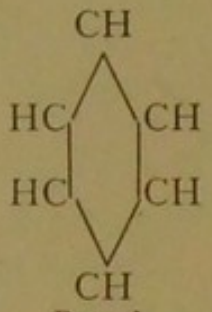
B. Ring-compounds.



Pyroll.

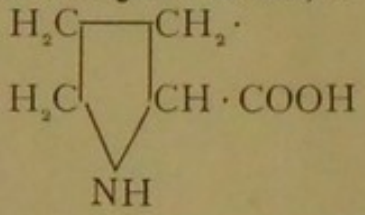


Imido-azol.

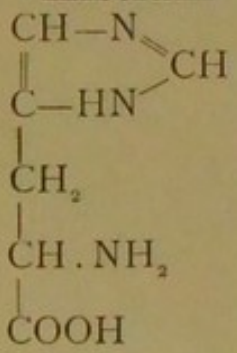


Benzol.

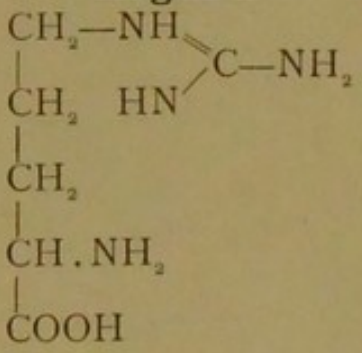
α-**Pyrrolidin-carboxylic Acid**, or **Prolin**, $C_5H_9NO_2$



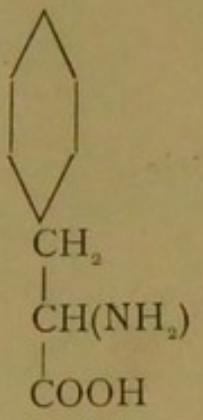
Histidin.



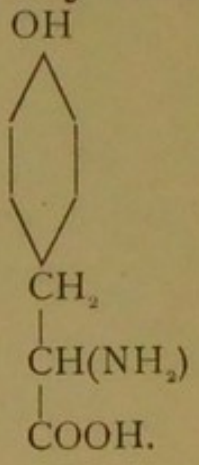
Arginin.



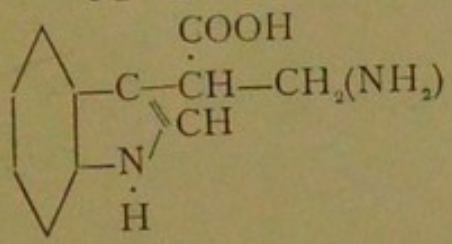
Phenyl-Alanin.



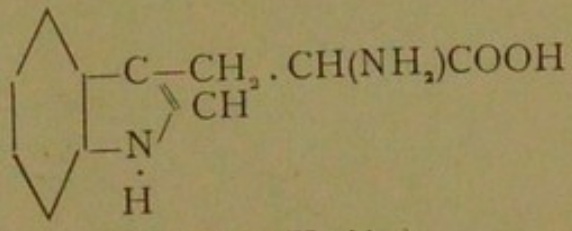
Tyrosin.



Tryptophane, or indol-amino-propionic acid $C_{11}H_{12}N_2O_2$



Tryptophane (Ellinger).



Tryptophane (Hopkins).

C. AMMONIA.

(*m*) ammonia.

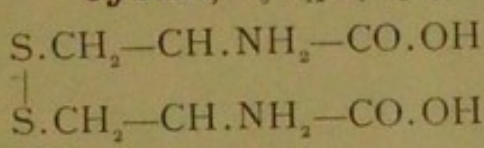
27. ammonia. NH_3.

D. THIO-AMINO-ACIDS.

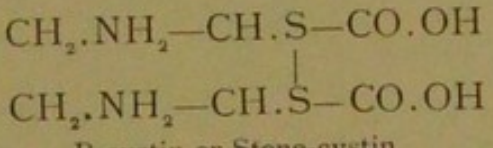
(*n*) diamino-di-thio-di-carboxylic acid.

*28. cystin $C_6H_{12}N_2O_4S_2$.

Cystin, $C_6H_{12}O_4N_2S_2$



A-cystin or Protein-cysten
(α-amino-β-thioglyceric acid
'disulphide.')



B-cystin or Stone-cystin
(α-thio-β-amino-glyceric acid
'disulphide.')

II. Nucleo proteids, as occurring in the nucleus.

*(*a*) pyrimidin-derivatives.

*1. 2·6 dioxy-pyrimidin or Uracil.

*2. 2-oxy-6-amino-pyrimidin or Cytosin.

*3. 2·6-dioxy-5-methyl-pyrimidin or Thymin.

(*b*) purin-derivatives.

*4. oxy-purin or Hypoxanthin $C_5H_4N_4O$.

*5. 2·6-dioxy-purin or Xanthin $C_5H_4N_4O_2$.

*6. 6-amino-purin or Adenin $C_5H_5N_5$.

*7. 2-amino-6-oxy-purin or Guanin $C_5H_5N_5O$.

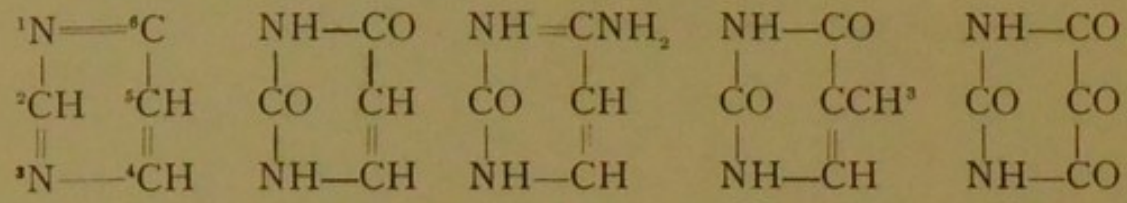
*8. guanylic acid $C_{52}H_{80}N_{20}O_{40}P_4$.

(*c*) laevulinic acid ($CH_3.CO.CH_2.CH_2.COOH$) $C_5H_8O_3$.

(*d*) metaphosphoric acid HPO_3.

(*e*) pentose $C_5H_{10}O_5$.

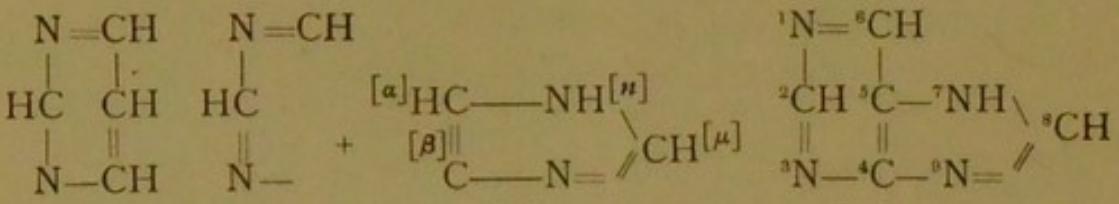
Pyrimidin and its derivatives.



Pyrimidin. Uracil. Cytosin. Thymin. Alloxan.

Purin and its derivatives.

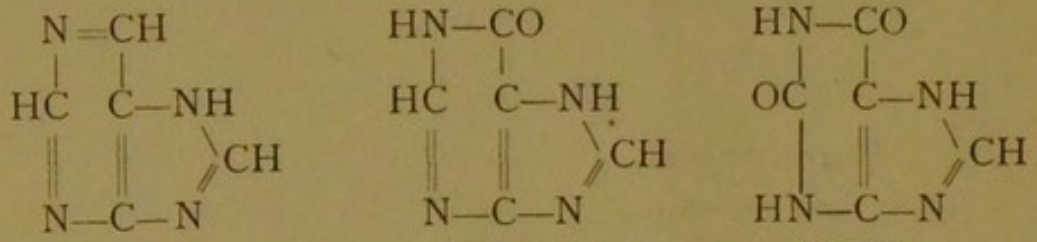
Purin, the mother-substance of these 'xanthin-bases,' contains a pyrimidin-remainder: the meta-di-azin and the imido-azol radical.



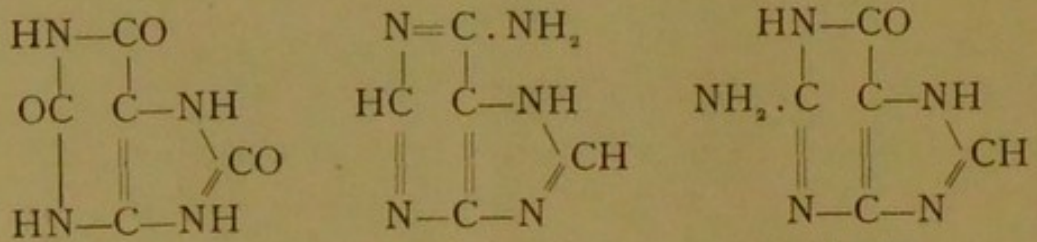
Pyrimidin. Meta-di-azin + Imido-azol (glyoxalin). Purin.

The following formulæ are arranged in this order:

purin→oxy-purins→amino-purin→amino-oxy-purin



Purin. Hypoxanthin (6 oxy-purin). Xanthin (2·6 oxy-purin).



Uric acid (2, 6, 8 trioxy-purin). Adenin (6-amino-purin). Guanin (2-amino, 6-oxy-purin).

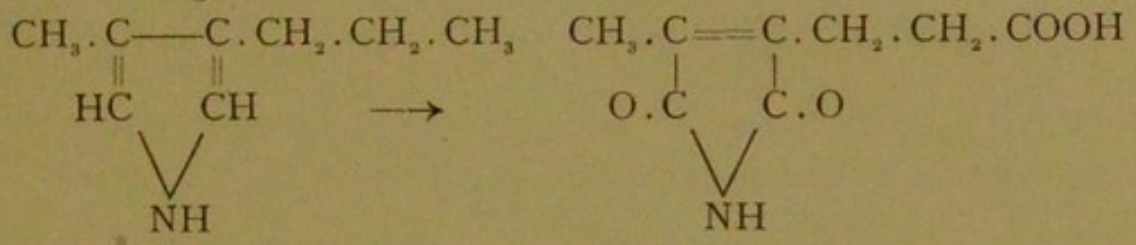
III. Haemo-proteids, as occurring in blood.

Haemoglobin $C_{785}H_{1203}N_{195}O_{218}FeS_3$.

*methyl-propylpyrrol or haemopyrrol $C_8H_{13}N$.

*haematinic acid (dibasic) $C_7H_9NO_4$.

Haemoglobin—derivatives.



Methylpropylpyrrol. Dibasic hæmatinic acid.

IV. Glyco-proteids, pronounced acids containing no phosphorus.

(*a*) Mucins: The arrangement of the carbohydrate radicals in the molecule is unknown. One of the secondary dissociation product is:

glucosamin $C_6H_{11}(NH_2)O_5$.

(*b*) Mucoids: The most characteristic substance is Chondroitin-sulphuric acid or 'Chondro-sulphuric acid' of unknown constitution, which contains sulphur in combination with an aminated polysaccharid.

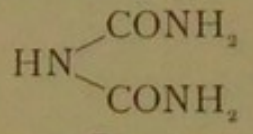
V. Phospho-glyco-proteids: contain phosphorus and a laevo-rotatory polysaccharid 'sinistrin' of unknown constitution.

VI. Albuminoids. The nature of these bodies makes their investigation exceedingly difficult, and they owe their characteristics either to a preponderance or absence of some of the amino-acids given above.

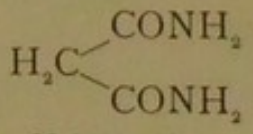
The Colour Tests.

None of the colour tests given by protoplasm are characteristic of it as such, as each test only indicates the presence of one or other of the radicals enumerated above. Thus the biuret-reaction, according to Schiff, is given by all

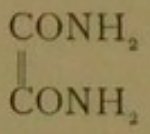
compounds in which two $CONH_2$-groups are linked either to a carbon-atom or to a nitrogen-atom or directly to one another, and which therefore correspond to one of the three following types:—



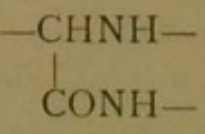
Biuret.



Malonamide.



Oxamide.



Grouping in albumins.

One of the $CONH_2$-groups may also be replaced by a CH_2NH_2 group or a $CSNH_2$-group.

Of these compounds oxamide may occur normally in gelatine (Kutscher and Schenck), while the grouping figured above is contained in all albumins. Millon's reaction shows the presence of tyrosin, as the latter is the only oxyphenyl-compound found in protoplasm. The xanthoproteic reaction indicates the presence of aromatic radicals, and is given especially well by tryptophane. The latter also gives the Adamkiewicz-Hopkins-Cole reaction with glyoxylic acid, and Rhode's reaction with aromatic aldehydes in the presence of sulphuric acid, while its derivatives give further the so-called pyrrol-reaction. Ehrlich's diazo-reaction indicates histidin, if tyrosin be absent; while the test of Molisch is an index to the carbohydrate radicals, as is also the glucosamin-test of Ehrlich. The black or brown colour obtained by boiling albuminous compounds with a lead salt and soda solution demonstrates the presence of sulphur.

Having described the dissociation-products it is possible to isolate from protoplasmic bodies, we have next to consider how they are linked up amongst themselves and also to such other radicals as iron and phosphorus.

The Linking of Protoplasmic Radicals.

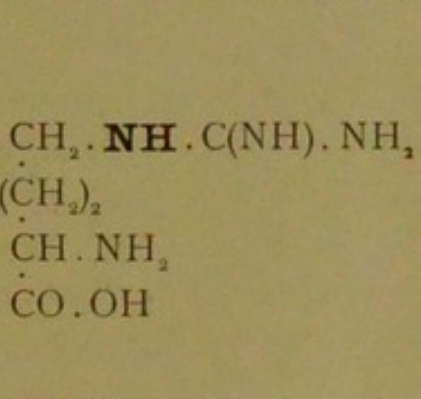
Schützenberger[1] advanced, in 1875, the view that albumins ought to be considered as derivatives of urea, $NH_2—CO—$

[1] Complete references are given in my *Text-book of the Chemistry of Proteids.*

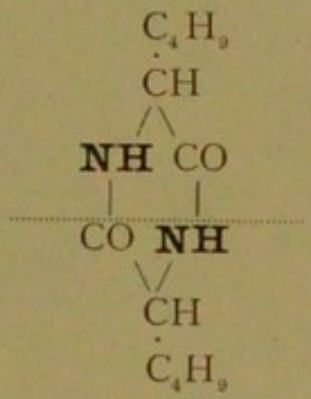
NH_2 and of oxamide, $NH_2—CO—CO—NH_2$, but this would only account for the guanidin-remainder, $—CNH \cdot NH_2$ occurring normally in arginin. The conception of Nasse, that albumins are built up as esters, containing the grouping: $\equiv C—O—C \equiv$ will also only account for a small percentage of the total amount of albumin, for radicals containing the alcohol-group OH, such as serin, tyrosin, oxyprolin, and the diamino-oxycarboxylic acids, are but few. For these reasons Hofmeister advanced in 1892 the theory that albumins are linked up according to the general formula:

$$—CH—\overset{|}{\underset{|}{}}—NH—CO \text{ or } —NH—CH—\overset{|}{\underset{|}{}}—CO—NH.$$

He based his view partly on the fact that this grouping occurs in arginin and in leucin-imide:



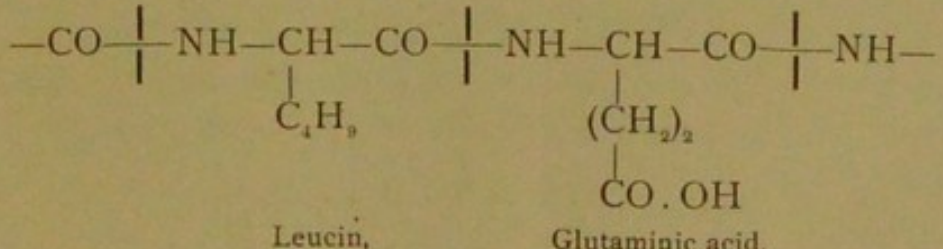
Arginin,



Leucinimide.

and partly on the fact that according to Löw and Schiff, relatively little NH_2 is present in albumins, judging by the amount of the nitrogen which is split off and the ease with which the biuret-reaction can be prevented on subjecting albumins to the action of nitrous acid.

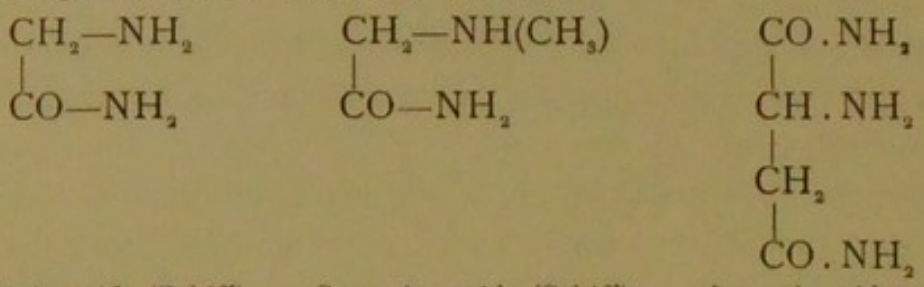
Hofmeister illustrated his theory by the following example in which leucin and glutaminic acid are linked together:



Leucin,

Glutaminic acid.

In this compound the radical —CH(CO—NH—)—NH— occurs, which is also met with in the following compounds giving the biuret reaction, namely,

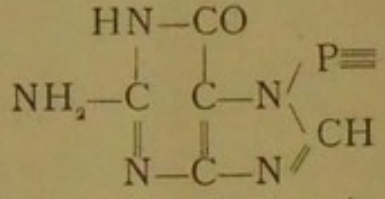


Glycinamide (Schiff), Sarcosin-amide (Schiff), Aspartic-acid-amide (Fischer).

Phosphorus which was just now alluded to in connection with plasminic acid, is one of the most characteristic constituents of nuclei as has been shown by Macallum's microchemical tests.

Burian[1] points out that purin-bases (see p. 9.) must be pre-formed in the nucleic acid molecule, as they are very readily separated from the nucleic acid remainder. They are liberated partially by heating nucleic acid to 60°, and completely by boiling the same for ten minutes in water or by dilute acids; this fact, along with the observation that nucleic acids do not give the diazo-reaction described above, led Burian to assume that the purin-bases are linked to the remainder of the nucleic acid molecule by the No. 7 nitrogen.

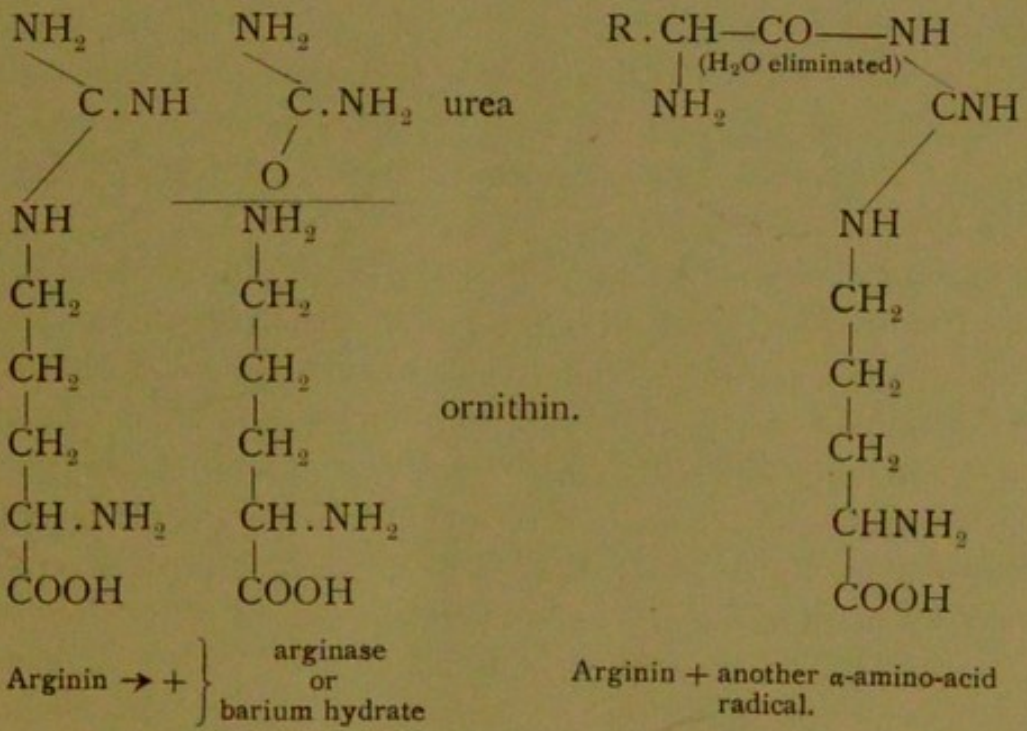
As nucleic acids are further very resistant to caustic potash, and in this they resemble other organic phosphoric-acid amides, there probably exists in nucleic acids a direct union between the phosphorus of the nucleic acid remainder and the No. 7 nitrogen of the purin-base. The union of guanin in nucleic acid would therefore be represented by the formula



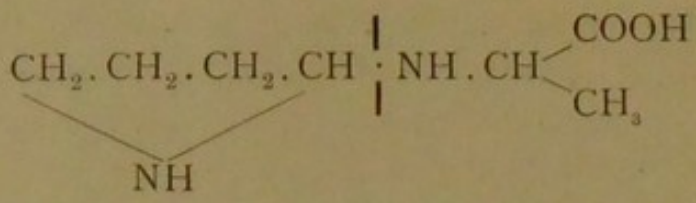
[1] R. Burian, *Ber. d. deutsch. chem. Ges.* **37.** 708 (1904).

Hofmeister's sound theory has been confirmed by the synthetic researches of E. Fischer aud Curtius, for there cannot be any doubt that ordinary amino-acids, when linked up, form neutral imino-compounds as far as the links are concerned; or, as Fischer puts it, amino-acids become 'amid-like anhydrides' or 'poly-peptids,' which, according to the number of amino-acids they contain, are called di-, tri-, tetra-peptids, and so on.

As direct oxidation of arginin does not yield oxaluric acid, but guanidin-butyric acid or guanidin + succinic acid (Kutscher) or urea and ornithin, when treated with barium hydrate (Schulze) or arginase (Kossel and Dakin), Seemann reasons that the arginin group must be attached at its guanidin-end to other amino-acids.

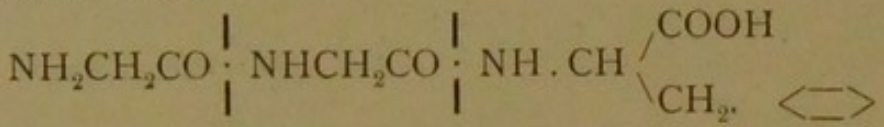


As examples of the way in which ring-compounds and sulphur is linked, we may take some of the polypeptids which Emil Fischer has obtained. Pyrrolidin carboxylic acid or Prolin (see p. 8) unites with alanin or amino-propionic acid (see p. 6) to form prolyl-alanin:

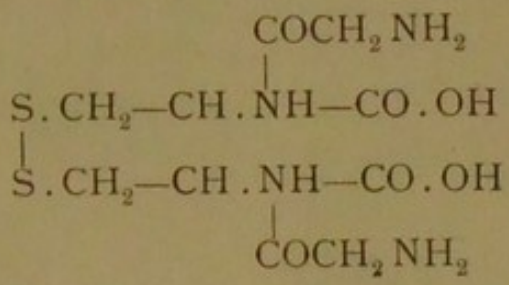


Prolin—remainder. Alanin—remainder.

and phenyl-alanin (see p. 8) may analogously be linked up with diverse amino-acids. Diglycyl-phenyl-alanin has *e.g.*, the constitution :



Cystin, the constitutional formula of which is given on p. 9, Fischer has linked up, amongst other amino-acids, with glycocoll. Thus diglycyl-cystin has the formula :

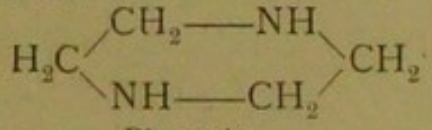


Iron is contained in most, if not in all, nucleo-proteids, and if we except the iron present in the haemoglobin, the main bulk of the remaining iron concerned in metabolism, is contained in the nucleo-proteids; nothing is known as to how the iron is linked up, but we know that it is present in a non-ionic or 'masked' state, and that therefore it cannot give directly the Prussian blue reaction, or the ammonium sulphide- or haematoxylin-tests.

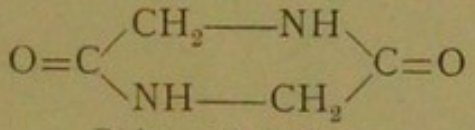
According to Ascoli iron does not fix on to the albumin at all, but to the nucleic acid or to the para- or pseudo-nuclein of the nucleo-albumin. Plasminic acid, which Kossel and Ascoli prepared from yeast, and which contains 27 per cent. of phosphorus, Ascoli believes to be a metaphosphoric acid, or the salt of such an acid with an organic base. Its most important property is that it renders iron 'masked.' If one add to a solution of metaphosphoric acid as much

ferric chloride as can be kept in solution by the excess of acid, and if one then add ammonia to neutralisation and precipitate with alcohol and ether, a substance is obtained which gives the following reactions. It is soluble in water, hydrochloric acid, and ammonia; its iron does not react to small amounts of ammonium sulphide, and not immediately to larger amounts, and it does not give up its iron to hydrochloric-acid-alcohol except under certain conditions. Plasmin behaves exactly as does this metaphosphoric acid: it too contains iron, and also in a non-ionic form, as its presence cannot be demonstrated by either the Prussian blue reaction or by other direct tests.

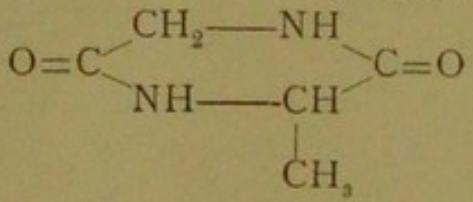
When examining the products of partially digested silk-fibrin, Fischer obtained a glycocoll-alanin compound, which arises by the union of one molecule of glycocoll with one molecule of alanin, there being given off two molecules of water. This glycocoll (or glycin)-alanin-anhydride, was the first di-keto-piperazin discovered in a derivative of proto-plasm.



Piperazin.

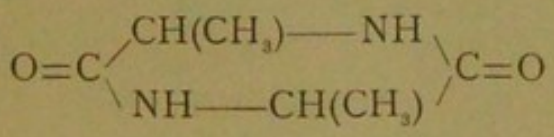


Di-keto-piperazin or
Di-glycocoll-anhydride.

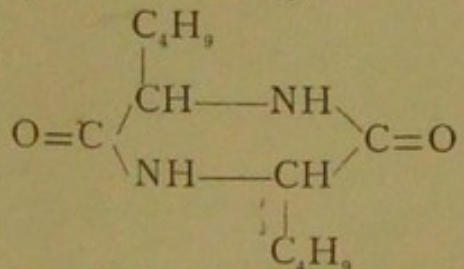


Glycin-alanin-anhydride.

Fischer has synthetized not only this glycocoll-alanin-anhydride, but two other di-keto-piperazins, namely



Di-alanin-anhydride.



Di-leucin-anhydride or
Leucin-amide.

The occurrence of anhydrides of amino-acids in protoplasm appears to be beyond doubt.

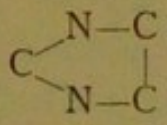
On passing to bigger complexes we meet at once with great difficulties. Kossel pointed out in 1901[1] that a systematic investigation into the quality and quantity of amino-acid constituents is the first essential, and to this we must all agree. He further proposed the theory that certain comparatively simple complexes, rich in diamino-acids, the so-called protamins obtained from ripe spermatozoa, might be considered as the nuclei round which all the others, chiefly mono-amino-acids are grouped. This view is, however, debatable. Emil Fischer[2] says 'Kossel's proposal to assume a "protamin-nucleus" in all albuminous compounds, and to make it the stepping-stone for a chemical system is going too far,' and in this I concur for physiological reasons, partly because of the very work which Kossel and his pupils have done. After Bang[3] had pointed out that in immature spermatozoa histone takes the place of protamin, Kossel[4] showed that in some fishes, for example the salmon, the body-muscle is converted into protamin, while in other fishes, for example the cod, the muscle becomes only changed into histone. It follows therefore that protamin is a derivative of albumin, and that it cannot be considered from the physiological point of view as a nucleus of the albumin molecule, except we assume with Kossel that the conversion of skeletal muscle into protamin is the equivalent of removing the mono-amino-acid 'impurities' by means of a physiological process taking place in the testicles.

I shall show later, on p. 57, that my researches into the functions of the nucleus allowed me in 1896 to draw another conclusion, namely that the nucleus is the agent by which mono-amino-acids are built up into bigger complexes. Kossel,

[1] A. Kossel, *Bericht. d. deutsch. chem. Ges.* **34**. 3214 (1901).
[2] Emil Fischer, *Ber. d. deutsch. chem. Ges.* **39**. 609 (1906).
[3] Baug, *Zeitsch. f. physiol. Chem.* **27**. 463 (1899).
[4] A. Kossel, *Biochemisches Centralblatt* **5**, reprint, p. 10.

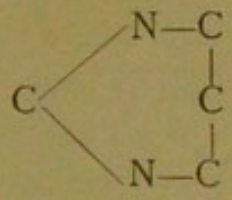
to a certain extent, has also arrived at this conception, for he pointed out in 1905[1] that the alloxur- or purin-bases, and further also a pyrimidin-derivative, which on hydrolysis gives rise to cytosin (or uracil) and thymin (see above) are not only rich in nitrogen, but also possess the C and N arranged alternately:

Imido-azol ring.



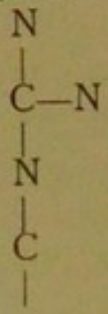
[In the alloxur bases; probably also in histidin.]

Pyrimidin ring.



[In the alloxur bases, in uracil, thymin, cytosin.]

Arginin.



and that this chemical peculiarity was characteristic of that part of protoplasm which is concerned with the processes of propagation and the formation of new substances.

The Protamins above referred to, as shown by the following table, are characterized by the presence of a high percentage of di-amino-acids, which renders them strongly basic:

TABLE SHOWING THE COMPOSITION OF THE PROTAMINS.

	Scombrin.	Salmin.	Clupein.	Sturin.	Cyclopterin.	α-Cyprinin.	β-Cyprinin.
Alanin (α-amino-propionic acid)	+	o	+	+	?	?	?
Serin (oxy-alanin)	o	+	+	o	?	?	?
Amino-valerianic acid	o	+	+	o	?	+	+
Leucin (iso-butyl-α-amino-acetic acid) ...	o	o	o	+	?	?	?
Diamino-valerianic acid (ornithin)	+	+	+	+	+	+	+
Diamino-caproic acid (lysin)	o	o	o	+	o	+	+
Histidin (imido-azol-alanin)	o	o	o	+	o	o	o
α-Pyrrolidin-carboxylic acid	+	+	+	o	?	?	?
Tyrosin (oxy-phenyl-alanin)	o	o	o	o	+	o	+
Urea	+	+	+	+	+	+	+
Tryptophane (indol-amino-propionic acid) ...	o	o	o	o	+	o	o
Ammonia	o	o	o	o	?	?	?

[1] A. Kossel, *Zeitsch. f. physiol. Chem.* **44**. 347 (1905).

The composition of protamins has been cleared up by Kossel's school in a very thorough manner. As will be seen from Kossel's figure on p. 21, it is possible to account for practically the whole of the nitrogen of the protamin salmin, while that of the albumin edestin of the Para-nut (Bertholletia) can only be accounted for to the extent of 58 per cent.

In this Figure the vertical line in the middle indicates in percentage figures the amount of nitrogen of those dissociation-products which could be isolated; the nitrogen-content of edestin was taken to be 18·64 per cent. (Abderhalden); the length of the horizontal lines indicates the ratio of the carbon to the nitrogen, except in the ornithin of the salmin and the leucin of the edestin, in which cases the lines have been shortened.

In connection with the grouping of various radicals in the protoplasmic molecule attention must be drawn to the anti- and the hemi-groups of Kühne and Pick.

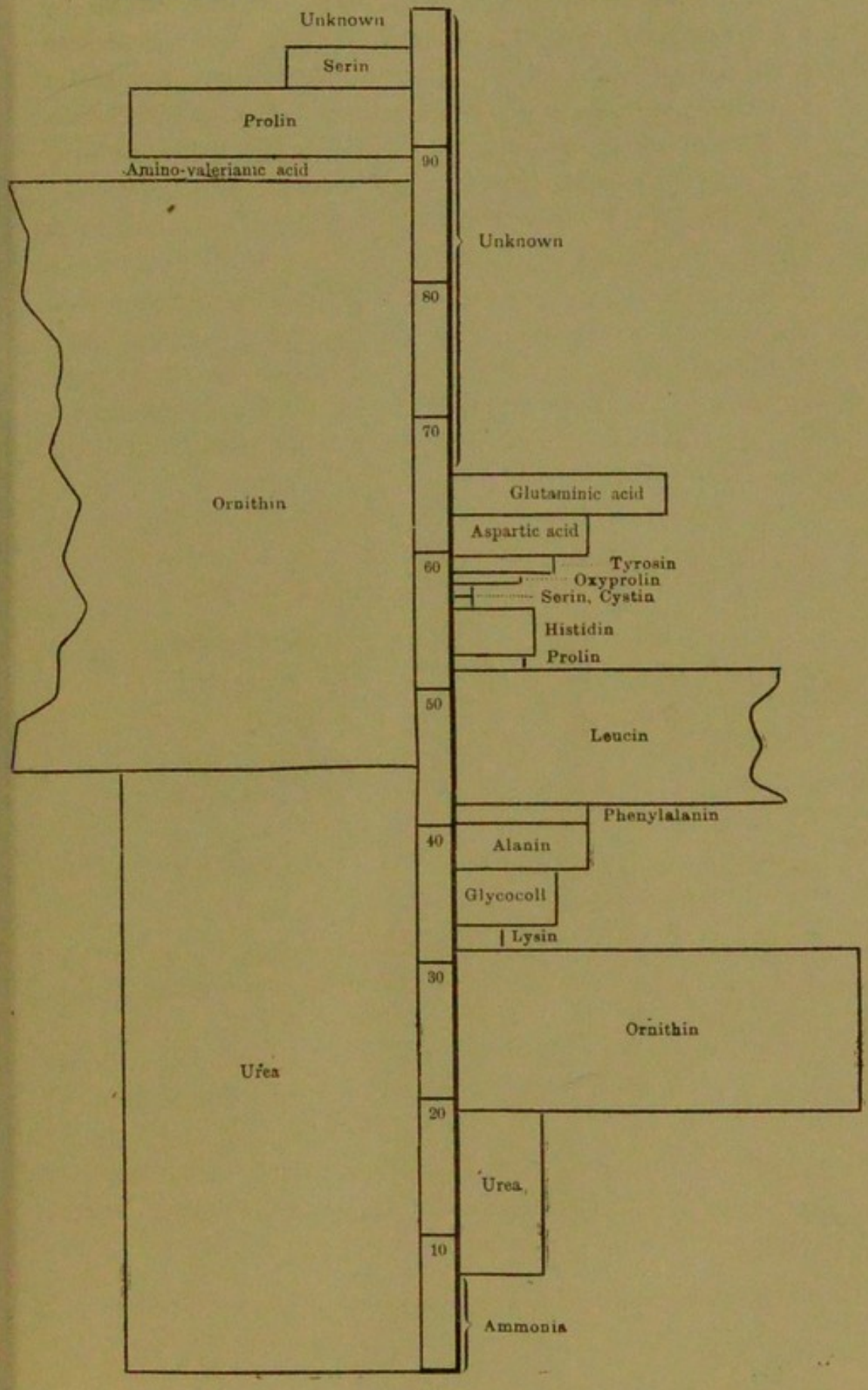
Anti-group.	*Hemi-group.*
Represented by gelatine	by casein
resists trypsin	is readily digested
not readily oxidized	readily oxidized
composed of hetero- and deutero-albumose	of prot-albumose
contains glycocoll, prolin and phenyl-alanin.	tryptophane and tyrosin.

So far I have given a very short review of the purely chemical aspect of protoplasm, and shall now proceed to the discussion of the physical and physico-chemical aspects. In my first book, in which the Theory of Histology has been treated, I advanced certain views which since that time have also been brought forward by pupils of Ostwald and Nernst. In the first instance it is absolutely necessary to have a clear conception of what we mean with the terms 'solution,' 'electrolyte,' 'hydrolyte' and 'colloid[1].'

[1] The following account is a reprint out of my *Chemistry of Proteids.*

Distribution of Nitrogen in

SALMIN **EDESTIN**



Solution.—A substance, on coming into contact with a fluid, is said to pass into solution when its molecules separate from one another and, diffusing into the fluid, mix with the molecules of the latter. The resulting mixture, consisting of the molecules of the solvent and the solute[1], may form so homogeneous a system as not to interfere in any way with the transmission of light, or, to use a technical term, the mixture may be 'optically void,' *i.e.*, contain no visible particles. On the other hand, the solute may consist of particles of such size as to interfere more or less with the transmission of light, when we speak of 'colloidal' solutions (see below) or of suspensions. All solutions are therefore mixtures of liquids or liquids and solids.

A substance in solution, as van 't Hoff has shown, is in every way comparable to a gas. There is, however, one difference, for in the case of an ordinary gas the amount contained in the fluid is proportional to the amount of the same gas outside the fluid, or, in other words, the gaseous tension in the fluid is proportional to the partial pressure exerted by the gas outside the fluid. In the case of dissolved solids, however, the solid cannot leave the fluid, because the very fact of a substance dissolving at all depends on definite electro-chemical interactions. Brühl[2] has shown that the power of acting as a solvent depends on the latter possessing some atom which is potentially polyvalent; for example, oxygen in water is divalent, but capable of becoming tetravalent; the nitrogen of ammonia is trivalent, but with a tendency to become pentavalent, and so on. To this must be added the conception that the body passing into solution may undergo an analogous change. In the light of Brühl's conception, and taking also into consideration that even pure water is partially dissociated, and pos-

[1] A solute in any substance which has passed into solution.

[2] J. W. Brühl, *Zeitsch. f. physik. Chem.* **10.** 1 (1899).

sesses a high dielectric constant[1], the following possibilities suggest themselves:—

1. The substance and the solvent, by mutually diffusing into one another, form mixtures without the solute undergoing electrical dissociation. This happens, for example, if sugar or mercuric cyanide dissolve in water, and also happens as the preliminary step in all cases where electrolytes dissolve; but in the case of electrolytes the primary 'passing into solution' is followed by a secondary chemical dissociation as described below.

I believe, when diffusion takes place, that the solvent has one electrical charge, while the solute has the opposite charge. The mixture being a binary system, it is impossible for an electrical current to pass through it, as this would mean moving both the solvent and the solute[2].

2. The substance undergoes in the solvent electrolytic dissociation, as in the case of electrolytes of salts containing radicals capable of giving rise to strong positive ions or kat-ions, and to strong negative ions or an-ions. Thus in the case of common salt, sodium becomes +, while chlorine becomes —:

$$NaCl + xH_2O \rightleftarrows Na^{\circ} + Cl' + H_2O.$$

In this case an electrical current passes through the mixture of solvent and solute, because we are dealing with a ternary system consisting of a medium, the solvent, in which both negative and positive ions, derived from the solute, are freely movable.

3. The substance, being composed of a potential, strong kat-ion and a potential, feeble an-ion, or *vice versa*, undergoes hydrolysis, which means that the weaker ion of the salt is replaced by a stronger ion derived from the solvent. If the solvent is water, and if the weaker ion of the solute is electro-

[1] A dielectricon is a substance without any electrical charge of its own, but capable of having an electrical charge induced in it.

[2] Mann, *Physiological Histology*, 1902, p. 45. See also in his *Chemistry of Proteids*, pp. 268 and 279, under Billitzer.

positive, it place is taken by the acid hydrogen-ion of water; if the weaker ion of the solute is electro-negative, then it is replaced by the alkaline hydroxyl-ion of water. Thus corrosive sublimate and water, or sodium carbonate and water, behave as follows:

$$NaCl + xH_2O \rightleftarrows [NaOH] + 2H^\circ + 2Cl'$$
$$Na_2CO_3 + xH_2O \rightleftarrows [H_2CO_3] + Na^\circ + OH'.$$

4. The substance, being composed of two feeble radicals, forms with the solvent a hydrate which is only capable of undergoing complete dissociation if along with this substance another salt is present, by the dissociation of which either acid hydrogen- or alkaline hydroxyl-ions are liberated. See below, and also footnote on p. 26.

Electrolyte.—An electrolyte is defined by Arrhenius[1] as a substance which imparts to water, which itself is a non-conductor, the power of allowing an electric current to pass through it, in virtue of the substance being in a state of electrical dissociation or ionisation, their being formed, while no current is passing, two sets of ions, the one having an electro-negative, the other an electro-positive charge.

Hydrolyte.—If only one of the components of a salt becomes an ion, while the other component transfers its positive charge to a hydrogen atom of the water, and thereby converts the later into the acid hydrogen-ion, H°, or its negative charge to the hydroxyl group, OH, of water, and thereby changes the latter into the alkaline hydroxyl-ion, OH', then the salt is said to undergo hydrolytic dissociation, and substances behaving in this manner may be termed hydrolytes. Examples of electrolytes and hydrolytes have been given under Nos. 2 and 3 in the previous paragraph on 'solution.'

Colloid.—This term was introduced by Thomas Graham[2] in 1861 for certain substances which differ from 'crystalloids' in diffusing very slowly in water, in being unable to pass through animal bladders and vegetable parchment, and in not

[1] S. Arrhenius, *Zeit. f. physik. Chem.* **1**. 631 (1889).
[2] Thomas Graham, *Phil. Trans.* **151**. 183 and 373 (1861).

crystallising readily. Graham states: crystalloids and colloids 'are like different worlds of matter,' while I hold that all colloids are electrolytes, as explained on p. 29.

It is necessary to distinguish between insoluble, semi-soluble, and soluble states of colloids. A soluble colloid is one in which all the component particles carry definite electro-positive or electro-negative charges, as will be shown later, while an insoluble 'colloid' is iso-electric, *i.e.* carries no electrical charges, and as long as a colloid remains in this insoluble state it exhibits none of the characteristics usually attributed to colloids and enumerated below. According to the nature of the particular colloid we are working with, the conversion of the insoluble into the soluble state is either comparatively easy or very difficult, and the more a colloid is rendered truly iso-electric, the more difficult is it, other things being equal, to re-convert it into the soluble form. This re-conversion in the case of albumins is often quite impossible, because when the iso-electric point is approached, the different groups of amino-acids in the albumin-molecule re-arrange themselves intra-molecularly to compensate for the removal of the electrically charged ions by means of which they were kept in solution. In addition to this change, amino-acids may also be converted from real acids and bases, into pseudo-acids and into pseudo-bases (see p. 35)[1].

Summing up our present knowledge, colloids, when 'in solution,' have the following characteristics:—

1. They polarise transmitted light.

2. Possessing a low osmotic pressure, they raise the boiling-point or affect the freezing-point of water only very slightly.

3. They are not coagulated irreversibly by a rise of temperature, provided electrolytes are absent and provided their chemical constitution does not become permanently altered.

[1] For a historical account of investigations into the nature of colloids up to the year 1902, see my *Physiological Histology*, Clarendon Press, 1902, pp. 28–70, while for the more recent work consult my *Chemistry of Proteids*.

4. They move either with or against an electrical stream which is being passed through them, and they are therefore either electro-positive or electro-negative, but they offer a great resistance to the flow of the electrical current, owing to their increased bulk and diminished surface.

5. They undergo hydrolytic dissociation, as in the case of arsenic trisulphide.

6. They are rendered more colloidal and are readily made insoluble by electrolytes, the potent ion of which[1] has an electrical sign the opposite to that carried by themselves, and they are made less colloidal by the addition of ions of the same sign.

7. Colloids of opposite electrical sign precipitate one another if they are in equivalent amounts, but if either of the two colloids is added in excess, then the colloidal precipitate, which was formed in the first instance, may re-dissolve.

8. One colloid in solution does not penetrate another colloid which forms a rigid system, or, in other words, colloids do not pass through animal or vegetable membranes.

9. As a rule they do not crystallise readily.

In support of my view that colloids are electrolytes the following facts may be mentioned.

Picton in 1892 divided arsenic-sulphide, As_2S_3, solutions, according to their physical state, into four classes, which he called α, β, γ, and δ. The α-solution is termed a pseudo-solution, because under a magnification of 1000 diameters the fluid is seen to contain crowds of minute suspended particles in rapid Brownian movement. The β-solution, forming the transition to the γ-variety, is composed of par-

[1] The potency of an ion is determined by the degree to which its electro-affinity is satisfied by the other ion with which it is linked together. If both ions have strong electro-affinities, as in the case of potassium chloride, then neither ion can exert its influence readily; but if one of the ions is weak, as, for example, the CO_3 radical in potassium carbonate, Na_2CO_3, and the Hg-radical in corrosive sublimate, $HgCl_2$, then the stronger ion causes the hydrolysis of water, or may act on other substances of the opposite electrical sign which are dissolved in the water along with itself.

ticles so small as to be microscopically invisible. The γ-solution differs from the α and β ones in diffusing and exerting osmotic pressure, but it cannot be filtered through a porcelain filter without the solid separating out, while the δ-solution contains sulphide particles of so small a size as to pass readily through the filter.

Now Picton's α-solution is comparable to what is ordinarily called a colloid, and his δ-solution to what is usually termed an electrolyte. The difference between a colloid and an electrolyte is, therefore, in one respect, purely one of size, or a quantitative one; the difference becomes qualitative only in respect to the unit of electrical charge carried by each individual particle. Polarisation-phenomena therefore do not allow us to distinguish between electrolytes and colloids.

The first observer to point out that 'inert' substances may decompose neutral salts in the presence of water, and that they may join either with the acid or basic radical set free, was v. Bemmelen, who investigated such porous substances as animal charcoal, silicic acid, and coagulated colloids. The fact that colloids may decompose such a 'neutral' salt as barium chloride and induce its hydrolysis shows that colloids must be chemically active, *i.e.* that they must be electrolytes or hydrolytes.

We further know that ions travel with or against an electrical current and so do colloids (see above p. 26) under the general characteristics of colloids. They further undergo hydrolysis and combine with different metals in equivalent amounts (Whitney and Ober).

1·9 per cent As_2S_3.	Chloride Solutions 25 ccm.	Amount of Salt added expressed in Grammes.	Free Chlorine remaining in Solution calculated as Acid.
100 ccm.	K	2·00	0·0038
100 ccm.	Ba	0·1394	0·0039
100 ccm.	Sr	0·1071	0·0042
100 ccm.	Ca	0·0706	0·0040

This table is exceedingly interesting, because it shows not

only a marked difference between the monovalent potassium and the divalent barium, strontium and calcium, confirming Hans Schultze's observation that the relative coagulative power of mono-, di-, and trivalent metals varies greatly, but also shows, according to my opinion, that within the divalent metals the power of precipitating colloids increases with the diminishing electro-affinity of the metals[1].

When a colloidal solution becomes semi-soluble, or, in other words, more colloidal, when, for example, Picton's δ-arsenic sulphide solution is changed into γ-, then β-, and ultimately into the α-variety, the following changes occur:— In a freshly prepared non-colloidal arsenic sulphide solution, As_2S_3 is dissociated into $[As_2O_3]^{\circ\circ\circ}$ and $3[H_2S]'$, and this dissociation is also met with in colloidal solutions, as has been shown by Freundlich. When the colloidal solution becomes less colloidal there occurs, according to my theory, a diminution in the amount of electrical dissociation; and this diminution is accompanied by a gradual increase in the size of colloidal particles. Picton and Linder were the first to notice that the size of the colloidal particles increases when the point of coagulation is neared, and that there is a reaction other than mechanical between solvent and solid, even in these cases of colloidal solution.

The change from an 'electrolytic' into a 'colloidal' solution I explain as follows:—'If to a solution containing a definite number of electro-positive (colloid + H)°-ions there is added an alkali containing the same number of electro-negative hydroxyl-ions, then the H° of the colloid

[1] Abegg and Herz, *Chemisches Practicum*, Vandenhoek and Ruprecht, Göttingen, 1900 (English edition, Macmillan), give the following table of electro-affinities:—

Kat-ions arranged in descending order of their electro-affinities—
K, Na, Li, Ba, Sr, Ca, Mg, Al, Mn, Zn, Cd, Fe, CO, Mi, Pb,
H, Cu, Ag, Hg, Pt, Au.

An-ions arranged in descending order of electro-affinities—
(F, NO_3, ClO_3), (Cl, So_4), Br, I, PO_4, CO_3, CrO_4, SiO_3, SH, H_2BO_3.
OH, CN, O, S.

and the OH′ of the alkali unite to form electrically neutral water, and the colloid, having lost its electrical charge, is precipitated; if however, not a sufficient number of OH′-ions are added to bind all the hydrogen-atoms, then the colloid-aggregates re-arrange themselves into larger aggregates.'

The observation that iso-electric, heat-coagulated albumin moves neither towards the anode nor towards the kathode, while after the addition of a trace of acid it moves towards the kathode, and after the addition of an alkali towards the anode, I explained in 1902 thus:—'As the proteid acquires the charge of the positive hydrogen-ion of acids, and the negative charge of the hydroxyl-ions of alkalies, we may assume the hydrogen- or hydroxyl-ions to unite with aggregates of proteid-molecules, and thus to form new ions consisting of the (colloid H +)° or (colloid + OH)′. The an-ion of the acid which was added (for example, the negative chlorine- or acet-ions) or the kat-ion of the alkali (for example, the positive sodium-ions) become the companion-ions to the (colloid + H)° or the (colloid + OH)′-ions.'

To the same conclusion as I expressed in 1902 in my *Physiological Histology*, namely, that colloids are electrolytes, have subsequently come Billitzer, working under Nernst, and Freundlich, working in Ostwald's laboratory.

Billitzer[1] arrived in 1903 at the conclusion that colloids may be regarded as ions, for he found it impossible to explain the movement of colloidal particles in an electrical field on v. Helmholtz's hypothesis[2] that a separation of the positive and the negative charge in electrolytes is brought about by the formation of a double electrical layer, which was so constituted that on two sides of a plane immeasurably thin[3] there were developed equivalent but opposite amounts of electricity.

Freundlich[4] explains the behaviour of colloids on the

[1] Jean Billitzer, *Ann. d. Physik.* **316.** 902 and 937 (1903).
[2] H. v. Helmholtz, *Pogg. Ann.* **165.** 228 (1853).
[3] See Lord Kelvin, *Nature*, 31st March and 19th May, 1870.
[4] Herbert Freundlich, *Zeit. f. physik. Chem.* **44.** 129 (1903).

assumption that the surfaces of colloidal particles are semi-permeable, which means that they allow of the ready passage of ions of the opposite electrical sign to that carried by themselves, *i.e.* of either kat-ions or of the an-ions, while the other ions which cannot enter the colloidal particles remain in the solvent. This explanation amounts to the same as that given by the author, namely, that the [colloid + the entered ion] is an ion. Very interesting in this connection is an observation made by A. Fischer[1], who noticed that 'the basic dyes are absorbed at once by the acid nucleo proteids, while with acid stains there is a delay, in about the proportion that methyl green will have stained already intensely, when acid fuchsin only just shows the faintest indication of staining. In the course of ten minutes, however, this difference disappears in material which was fixed in indifferent re-agents.' Reversely 'the acid dyes diffusing through the sections stain at first only the cytoplasm, and several seconds later the nuclei, which ultimately are also stained as intensely as the cytoplasm.' Fischer failed to understand the importance of his own observations, for he uses his facts to prove the absence of any real difference in the absorptive powers of nucleins with regard to acid and basic dyes; while to me[2], Fischer's observations have this significance: each particle, either kat-ion or an-ion, has an aversion for ions of its own kind or those of the same electrical sign; thus the positive kat-ion H° will not only repel other H° ions, but also, for example, those of potassium, K°. On the other hand, positive kat-ions will readily unite with negative an-ions.

The view that colloids are electrolytes is further supported by the fact that colloids of opposite electrical sign precipitate one another, as has long been known to histologists. Romanowsky[3] in 1891 combined equi-molecular proportions of the

[1] A. Fischer, *Fixirung, Färburg und Bau des Protoplasmas*, 1899, p. 94.

[2] Mann, *Physiological Histology*, 1902, p. 339.

[3] Romanowsky, *Zur. Frage d. Parasitol. u. d. Therap. d. Malaria*, St. Petersburg, 1891.

basic methylene-blue and the acid eosin, and thus obtained the water-insoluble eosinate of methylene-blue[1]. Quite recently the same phenomenon has been studied by Biltz[2], who calls these unions 'adsorption-compounds.'

After giving various further examples of the fact that hydrosols of opposite electrical sign mutually precipitate one another if they are mixed in equivalent amounts, he shows that mixtures of hydrosols possessing the same electrical sign—such as the purple of Cassius, which is a hydrosol of stannic acid and gold—are thrown down together by electrolytes having the opposite electrical load. He also found that the precipitating action of mixtures of electrolytes and colloids is an additive effect, and that in many cases an action which seems to be brought about by an electrolyte is caused at least partly by the presence of colloids. In this connection he draws attention to the work of Spring[3], who showed that solutions of the salts of plurivalent metals (such as aluminium chloride or ferric chloride) are not optically void, and therefore must contain colloidal hydroxide; and also to the work of Mylius[4], who accounts for metaphosphoric acid coagulating albumin, while orthophosphoric acid does not, by showing that metaphosphoric acid contains polymolecular particles, *i.e.* that it is in fact a 'colloidal' solution. Mylius further shows that all acids which precipitate ordinary white of egg, after it has been diluted, contain complex molecules.

Biltz objects to a chemical explanation of colloidal solutions, and considers Bredig's view to be correct, namely, that the cause of the relatively great stability of pure colloidal solutions is the electrical difference of potential between the colloid and the solvent. I have to point out that according to all laws of physical chemistry the first essential

[1] Other instances are given in my *Physiological Histology*, pp. 441–444.

[2] W. Biltz, 'Mutual Interactions of Colloidal Substances,' *Ber. d. deutsch. chem. Gesell.* **37.** 1095 (1904).

[3] Spring, *Bull. de l'Acad. Roy. de Belg.* 1900, p. 483.

[4] F. Mylius, *Ber. d. deutsch. chem. Ges.* **36.** 775 (1903). (The reader's attention is especially directed to this important paper).

for chemical interaction is the establishment of an electrical load, or, in other words, that only those substances which carry an electrical load are capable of acting upon one another 'chemically.' To say, as does Biltz, that we are dealing with adsorption-phenomena when a colloid is precipitated, is not giving an explanation at all, but amounts simply to stating the premise over again in a roundabout manner. If the cause of the adsorption is the ionic difference of potential between two substances, then adsorption means simply a chemical union.

The inter-relation of suspensions and colloids in viscid media; the behaviour of colloids upon one another, and how under certain circumstances one colloid may prevent the precipitation of a second colloid, is fully discussed by Arthur Müller[1].

In this connection the precipitation of colloidal solutions by the addition of 'neutral' salts must be mentioned. Even if we assume that the added neutral salt does not interfere in any chemical manner with the colloid we are experimenting with, it is evident, if my view is correct—namely, that electro-affinities in equivalent intensities but of opposite sign attached to two radicals lead to these two radicals forming insoluble compounds—that salts which are soluble and capable of electrical dissociation must for this very reason be composed of ions which differ from one another as regards their electro-affinities. If, however, either the kat-ion or the an-ion is stronger, then the unsatisfied balance of electro-affinity represents available energy which, when brought into contact with colloids, will lead to the precipitation of the latter, provided that the colloid has an electrical sign which is the opposite to that carried by the stronger ion of the 'neutral' salt.

If by adding water we dilute a solution of globulin made with 1 per cent. of sodium chloride, *i.e.* with a neutral salt, the globulin becomes precipitated, because the sodium of the

[1] Arthur Müller, *Ber. d. deutsch. chem. Ges.* **37.** 11 (1904).

NaCl has a greater dissociation-tension or electro-affinity than has the chlorine. As ions of opposite sign can only be present in equivalent amounts, it follows that the greater tendency of sodium to form ions in water is checked by the smaller dissociation-tension of the chlorine. It follows, therefore, that if we add a second radical, such as globulin, capable of becoming an electro-negative ion, that the sodium may develop its full dissociation-tension because of the formation of the compound:

$$[Na_2]^{\circ} \quad \begin{matrix} [Cl]' + \text{non-ionic Cl} \\ [\text{Globulin}]'. \end{matrix}$$

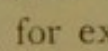
Whenever by dilution with water the globulin is removed mechanically from the sphere of action of the sodium, it ceases to be an electro-negative ion and will commence to separate out, the amount of separation depending on the amount of electrical change still carried by the larger aggregates.

Another explanation which is also possible, owing to the amphoteric nature of albuminous compounds, is that the globulin forms a complex ion with either the sodium kat-ion or with the chlorine an-ion, according to the formulae

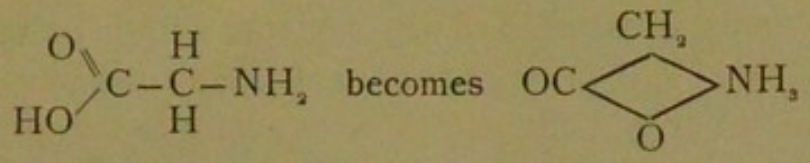
$$[Na]^{\circ} + [Cl + \text{globulin}]' \text{ or } [Na + \text{globulin}]^{\circ} + [Cl]'.$$

You may ask what right have we to consider protoplasm in the same light as a salt? It was pointed out above that we may isolate from protoplasm by its hydrolysis a number of fatty and aromatic amino-acids, and therefore it is necessary to study shortly what power these substances have of forming salt-like combinations.

Strecker seems to have been the first to advance the view that amino-acids fix metals by their CO.OH radical, while they bind acids by the NH_2-group, and the same conclusion has been arrived at by Bredig, Winkelblech, and Walker, who have studied amino-acids in the light of physical chemistry. Bredig uses the term 'amphoteric electro-

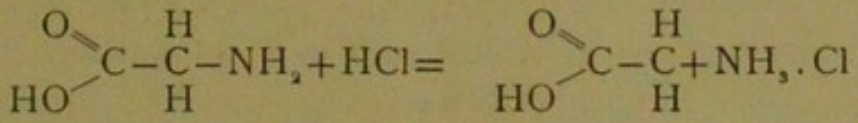
lyte' for any substance 'which may split off, or unite with, H° and OH' ions, or, in other words, any substance which can play the part of an acid towards a base or that of a base towards an acid. According to this definition, water is an amphoteric electrolyte because its hydrogen-atom H and its hydroxyl-radical OH may be converted into the chemically active ions H° and OH' whenever water comes into contact with certain salts, as will be shown more fully later. Alcohols $(C_nH_{2n+1})OH$ are also amphoteric electrolytes. Thus $(C_nH_{2n+1})OH$ can unite with sodium according to the equation $(C_nH_{2n+1}O)-H+Na^{\circ}=C_nH_{2n+1}ONa+H$, when the alcohol remainder $[C_nH_{2n+1}O]'$ plays the part of an acid, the feeble kat-ion H° being replaced by the strong kat-ion sodium, Na°. On the other hand, the hydroxyl-group OH may be replaced by a stronger an-ionic radical, such as a chlorine-ion; thus $[C_nH_{2n+1}]^{\circ}OH'+H^{\circ}Cl'=C_nH_{2n+1}Cl+H_2O$. This behaviour of alcohols depends on the presence of the OH radical, and it will readily be seen that other compounds which contain this OH radical will behave analogously. Such OH-compounds are, for example, serin and all phenols,  . Alcohols differ, however, from ordinary hydroxyl compounds, as they only form alcohol-salts in the absence of water. These alcohol-salts on coming into contact with water dissociate hydrolytically, because water is hydrolysed by alcohol-salts.

The simultaneous presence of acid and of basic radicals in one and the same molecule as occurring in all amino-acids must of necessity lead to a weakening of the acid or basic characters of the molecule towards other individual molecules, and must also set up within the amphoteric molecule a tendency towards 'internal salt-formation,' by which expression we mean that the acid and the basic radicals of an amphoteric electrolyte will tend to mutually satisfy one another. Whenever this tendency becomes an accomplished fact, then the previously open-chain compound is converted into a 'ring-compound.' Thus

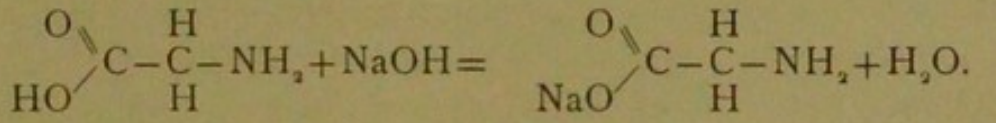


Chemically active glycocoll becomes chemically inactive glycocoll.

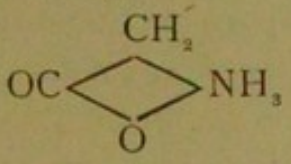
While glycocoll is in this inactive state it forms a true pseudo-acid-pseudo-basic compound; in other words, it cannot play the part of an-ion or that of a kat-ion till the ring-like compound is re-converted into an open-chain. This change can only be brought about by subjecting the pseudo-acid-pseudo-basic molecule to the influence of ions. If active or inactive glycocoll is brought into contact with a strong acid, such as hydrochloric acid, then glycocoll-hydrochloride is formed:



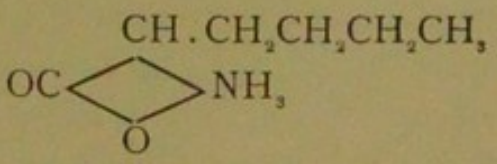
while with sodium hydrate it forms sodium glycocollate and water.



The proximity to or the remoteness from one another of the acid and basic radicals in the amphoteric amino-acid determines the ease with which an internal salt is made and unmade. As most of the normally occurring mono-amino-acids are α-compounds in which the basic NH_2-group is as close to the acid COOH-group as possible, it follows that the length of the primary chain does not much interfere with the internal salt-formation as long as only one NH_2 radical and one COOH radical is present:



Glycocoll.

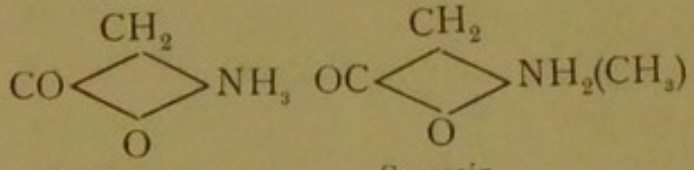


Leucin.

It is different, however, when two NH_2 and one COOH groups are present, or two COOH groups and one NH_2 as in

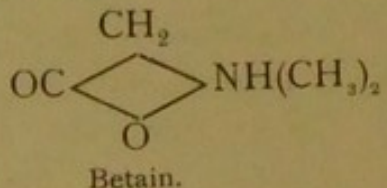
the case of the mono-amino-di-carboxylic and di-amino-mono-carboxylic acids.

The last point to be considered is the relative strength of the acid and the basic radicals in the amphoteric amino-acids. If the carboxyl-group COOH is replaced by the much more strongly acid sulphonic radical SO_3, then the basic character of the NH_2-group may be diminished to such an extent as practically not to make itself felt at all. This holds good, for example, in the case of sulphanilic acid, $C_6H_4(NH_3)(SO_2O)$. On the other hand, the basic character of the NH_2-group may be strengthened by the introduction of alkyl-radicals (methyl, CH_3; ethyl, C_2H_5) till it is 10 to 20 times stronger than ammonia (Winkelblech). The acid character of the COOH radical may hereby be overcome so completely as to prevent the latter from acting as an acid radical, at least at the ordinary room-temperature. Thus betain develops acid characters only at zero-temperature (Davidson). Glycocoll, sarcosin, and betain are in descending order less and less acid:



Glycocoll.

Sarcosin.



Betain.

The great inhibiting effect of the amino-group NH_2 on the carboxyl-group COOH is well seen by comparing acetic acid with amino-acetic acid or glycocoll. Thus $\frac{1}{3^1\frac{1}{2}}$ normal acetic acid, dissociating only to the extent of 2 per cent. is 500,000 times more strongly acid than is amino-acetic acid, while according to Winkelblech the ratios of the acidity to the basicity of certain amino-acids are as follows:

	Acidity to basicity.
Aspartic acid . .	53 millions to 1
o-amino-benzoic acid .	4 millions to 1
p- „ „ „ .	2·6 millions to 1
m- „ „ „ .	0·5 millions to 1

	Acidity to basicity
Asparagin	3000 : 1
Alanin .	250 : 1
Glycocoll.	120 : 1
Leucin .	115 : 1
Sarcosin .	72 : 1

The most remarkable property of amino-acids is their

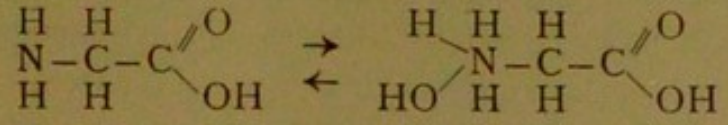
strong hydrolysis, which means that the salts which amino-acids form with other acids or bases are very readily broken up by the ions of water. If a strong base is linked to a strong acid—if, for example, equivalent amounts of hydrochloric acid and of caustic soda are dissolved in water—then the acid hydrogen-ion of the HCl unites with the alkaline hydroxyl-ion of the NaOH to form neutral water, while the sodium- and the chlorine-ions form a neutral salt. In this case the negative chlorine-ions and the positive sodium-ions possess a great electro-affinity for one another, and therefore they do not unite with either the feeble, acid hydrogen-ions or the feeble, alkaline hydroxyl-ions of the water. But if, instead of two such strong radicals as sodium and chlorine, there be present one feeble radical, *e.g.* an amino-acid, in combination with a strong acid or a strong base, then the strong radical will not join up with the amino-acid, which is a more feeble radical than are the ions of water, but will combine with one of the ions of the water.

Generally speaking, any amphoteric electrolyte H·R·OH will form, according to Walker, the ions H°, OH', HR°, and ROH', while the non-ionised portion must be either in the state of a hydrate H·R·OH or that of an anhydride R. Therefore an equilibrium in the solution depends on the factors

$$\overset{+}{H} \quad \overset{-}{OH} \quad \overset{-}{ROH} \quad \overset{+}{RH} \quad HROH \quad R$$

In working with amino-acids and with albumins it is necessary to constantly keep the salt-forming power of these substances before our mind's-eye. Glycocoll, being the simplest amino-acid, is therefore taken as a type.

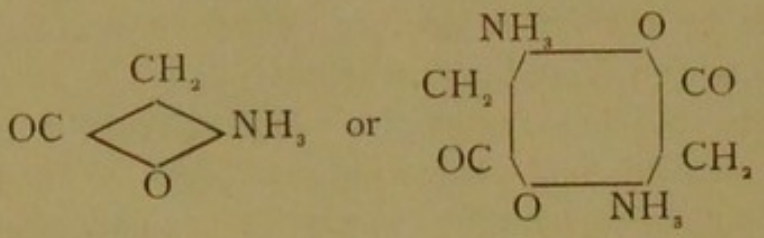
Normal Glycocoll, $NH_2.CH_2.COOH$.—Winkelblech assumes that a watery solution of glycocoll contains 99·967 per cent. of hydryated but non-dissociated molecules



Normal glycocoll. Hydrated glycocoll.

while the minimal remainder is made up of a few ions and of non-hydrated glycocoll molecules. He explains the neutral reaction of a watery solution of glycocoll as being due to the want of dissociation of the hydrated amino-acid. Although then the presence of glycocoll leads to a union of the water-ions with the amino-acid, there is no hydrolytic dissociation of the newly-formed compounds. A hydrated amino-acid does not dissociate, because both the acid and basic radicals are very feeble.

Walker and I believe that the glycocoll molecules in watery solutions either form internal salts or that they unite in pairs, in such a way that the acid radical of one molecule links on to the basic radical of a second molecule:



Whatever change an amino-acid undergoes, whether it form a ring-like compound on becoming an internal salt, or whether it form double molecules, or whether it become hydrated, or whether it unite with acids, the originally trivalent nitrogen always becomes pentavalent.

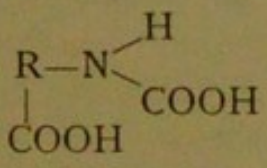
Glycocoll Hydrochloride, $ClH_3N.CH_2.COOH$, by hydrolysis sets free neutral glycocoll $H_2N.CH_2.COOH$ and hydrochloric acid, which then dissociates into the ions $H^\circ + Cl'$. The solution reacts strongly acid, owing to the hydrogen-ions, and it conducts the electric current mostly as hydrochloric acid, and to a very slight extent as the hydrochloride of glycocoll.

Glycocollate of Sodium, $H_2N.CH_2.COONa$; by hydrolysis neutral glycocoll and sodium hydrate are set free. The latter dissociates electrolytically into OH'- and Na°-ions. The solution has a strongly alkaline reaction owing to the hydroxyl-ions, and it conducts the current mostly as sodium hydrate, and to a very slight extent as sodium glycocollate.

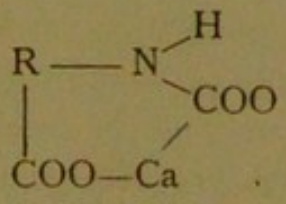
In connection with the union of amino-acids with carbon-dioxide Siegfried has made the following observation, which is of the greatest physiological importance :—

On saturating a mixture consisting of equal volumes of equinormal solutions of glycocoll and barium-hydroxide, an alkaline solution is obtained which remains clear when CO_2 is passed through it, and this continues to be the case till for each volume of glycocoll nearly two volumes of equivalent baryta water have been added. The solution so obtained gives off barium carbonate slowly on standing and quickly on boiling. Analogous results are obtained on substituting for glycocoll: i-alanin, l-leucin, sarcosin, phenyl-glycocoll, aspartic acid, glutaminic acid or asparagin, and on replacing barium-hydroxide by calcium or sodium hydroxide, and finally on substituting for CO_2 sodium carbonate.

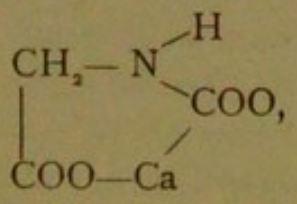
Analyses of the compounds which glycocoll, i-alanin, l-leucin, sarcosin, and phenyl-glycocoll form with calcium-hydroxide and CO_2 have shown that these amino-acids must contain the radical



Carbamino-acid radical.



limesalt of salt,

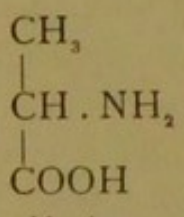


calcium carbamino-acetate or calcium glycocoll-carbonate,

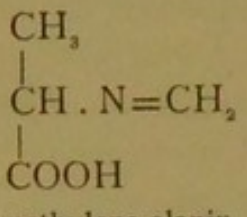
that is, the normal lime salts of the hitherto unknown dibasic carbamino acids of the glycocoll series. These compounds are therefore formed by the amphoteric amino-acids, simply adding CO_2, which thereby becomes de-ionised.

Quite analogous to the amino-acids behave the peptones, crystalline serum-albumin and dialysed horse serum. Siegfried points out that the union of CO_2 in the blood appears in a new light, expecially in connection with the hypothesis of Setschenow as to the conversion of serum albumin into carbo-albumin by the action of CO_2, and also in connection with the carbo-haemoglobin of Bohr.

The double nature of amino-acids, *i.e.* to act either as acids or as bases, is interfered with as soon as one of NH_2 or COOH radicals is bound up. Curtius and Göbel have shown that glycin-ester, $H_2N.CH_2.COOC_2H_5$, and E. Fischer that other amino-acid-esters are strong bases (as already mentioned in connection with sarcosin and betain; Schiff, on the other hand, found methylene-compounds to be acids, as the amino-radical is joined to formaldehyde; thus alanin is approximately neutral, while methylene-alanin is strongly acid :—



Alanin



methylene-alanin.

The presence of a second NH_2 or COOH group does not alter the general character of an amino-acid, but the basic character predominates in lysin, and the acid character in glutaminic acid, but notwithstanding this the latter can act as a base, for it forms chlorides.

Albumins behave in exactly the same way as do the amino-acids. According to Sjöqvist, Cohnheim, Cohnheim and Krieger, Erb, Bugarszky and Liebermann, and von Rhorer, albumins react as bases towards acids, being in some cases even more basic than the amino-acids. According to von Rhorer albumins are about 500 times more basic than is distilled water, and according to Sjöqvist about 74·2 more feeble than is anilin. With acids they form salts which undergo great hydrolysis.

Different albumins differ not only in their capacity for binding acids, but give different curves, when these are so constructed as to show that dissociation depends not only on the concentration but also on the excess of the acid. If a weaker acid be taken instead of hydrochloric acid, then the dissociation becomes even more marked.

Albumins behave quite analogously when they combine with bases; Bugarzsky and Liebermann and Spiro and Pemsel have shown that sodium albuminate exhibits marked and varying hydrolysis. One essential difference exists, however, between albumins and the simple amino-acids: the albumins are pluri-acid bases and pluri-basic acids.

The behaviour of albumins towards salts will have to engage our attention next. Spring was the first to point out the importance of the mobility of ions, for on comparing solutions having the same conductivity (chlorides of K, Na, Rb, Li, Ca, NH_4), he found that they produced flocculation in the order of the mobility of their ions, except in the case of lithium chloride, which takes much less time to coagulate than does the potash salt, because lithium chloride undergoes hydrolysis and thereby gives rise to the formation of hydrogen-ions, which possess the greatest mobility, and this has been confirmed by Posternak.

Other interesting points discovered by Posternak were that the same acid radical produces in different salts different effects:

HCl	NH_4Cl	KCl	NaCl
0·388	0·385	0·380	0·325

and that the same acid which in dilute strengths favours solution, causes precipitation when it is concentrated. This fact is attributed to a change in the electrical conductivity, thus

Strength of HCl.		Molecular concentration.		Conductivity.
1 : 1000	=	0·0273	=	0·95
1,415 : 1000	=	0·388	=	0·86

In the first case the quotient $\frac{\text{dissociated molecules}}{\text{non-dissociated molecules}} = 19$, while in the second case it is 6.

Ordinary albumins being electro-negative are coagulated,

according to Hofmeister and Pauli, by kat-ions in the following order:—

$$Li > Na > K > NH_4 > Mg$$

while the electro-negative an-ions tend to prevent coagulation in this order:

$$Fl > SO_4 > P_2O_5 > \text{citrate} > \text{acetate} > Cl > No_3 > Br > I > CNS.$$

Posternak has now observed the very interesting fact that if the reserve-material of the seeds of Picea dissolved in 1 : 1000 HCl be taken, that the order of the above salts is inverted, the electro-positive albumin is now precipitated by an-ions in this order:

$$CNS > I > Br > NO_3 > Cl > \text{acetate}$$

while the coagulation-inhibiting kations follow in this order:

$$Mg > NH_4 > K > Na.$$

This phenomenon is interesting in connection with the phenomena exhibited by heat-coagulated albumin, which may behave either as a kat-ion or as an an-ion (see p. 29). Pauli has also drawn attention to the fact that the above order, in which salts precipitate electro-negative albumins, is the same as that in which they prevent the inhibition of water by gelatine-plates and in which they increase the melting point of gelatine.

Pauli has carefully investigated the effect which is produced on egg-white by the addition of the neutral salts of the alkalies and of magnesium. In the first instance he confirms Schäfer's observation that two salts in combination will do what one salt by itself is unable to do, for if potassium or sodium chloride and sodium acetate be used in such strengths as not to cause coagulation, they will on being mixed give rise to coagulation, and $KCl + NaC_2H_3O_2$ will produce a greater effect than if $NaCl + NaC_2H_3O_2$ are used. In the former case the kation is different in the two salts while in the latter case they are the same. The dibasic magnesium sulphate + the monobasic sodium chloride also augment mutually their coagulating efficiency.

If the coagulating values of a series of kat-ions are indicated by $f, f', f'' \ldots$ and the inhibiting values of a number of an-ions by h, h', h'', then by combining electrolytes the three following states are possible :

$$\sum (f, f', f'' \ldots .) \gtreqless \sum (h, h', h'' \ldots .)$$

which means that it is possible to add to a solution of a coagulating electrolyte, other electrolytes which either increase or diminish or leave unaltered the coagulating power of the first electrolyte.

In the following table Pauli has arranged the kat-ions in ascending order from left to right, magnesium being the feeblest and lithium the strongest kat-ion, while the an-ions are so arranged that the one with the fullest inhibiting power, namely, fluorine, comes first, while the strongest inhibitor, namely, thiocyanate, comes last.

Kat-ions→ An-ions ↓	Mg	Ng_4	K	Na	Ll
Fluoride		+	+	+	
Sulphate	+	+	+	+	+
Phosphate		+	+	+	
Citrate		+	+	+	
Tartrate		+	+	+	
Acetate	−	−	+	+	
Chloride	−	−	+	+	+
Nitrate	−	−	−	+	+
Chlorate		−	−	+	
Bromide	−	−	−	−	+
Iodide		−	−	−	
Thiocyanate	−	−	−	−	

It will be seen from the table that the feeble precipitating power of magnesium and ammonium is already interfered with by the acetates and chlorides, while potassium is not affected by nitrates, and so on.

The criticism which I have to make to Pauli's very important investigations on the salting out of albumins, which

are fully detailed elsewhere[1], are that he has not taken into account that the addition of a solid soluble salt to the solution of a second salt must render the salt already in solution more concentrated, because it has to abstract water before it can pass into solution itself. Pauli has further assumed throughout that the salts only act upon one another, and not also on the albumin. If we consider what effects, especially the halogen salts, have in preventing, for example, the setting of gelatine, we must bear in mind than an analogous change may very well be produced in egg-white, and that for this reason in the above table the iodides and thiocyanates have apparently so strong an inhibiting action on all kat-ions. The formation of double salts has also not been taken into account, nor has sufficient attention been paid to the amphoteric character of the albumin. That, finally, so called 'neutral' salts are in reality not neutral, but are composed of ions in which either the negative or the positive electro-affinity preponderates, has already been explained on p. 33.

The Structure of Protoplasm.

Contemplating organised nature what strikes me most is its great stableness as compared with the unstableness of unorganised matter. This view may at first seem paradoxical, but is nevertheless true. If we bring NaCl, KNO_3 and H_2O together, even assuming that the water remains passive, we shall have NaCl, KNO_3, $NaNO_3$, KCl and the ions Na°, K°, Cl', NO_3', which means that many of the original NaCl and KNO_3 molecules have lost their individuality. It is different with protoplasm, for as long as it is living it possesses the power of attracting or shutting out other units according to the special characteristics which it has acquired by evolution. The fundamental characteristic of protoplasm is its colloidal nature, which means that it is composed of very large aggregates each of which has only a unit charge of either + or −

[1] Mann, *Chemistry of Proteids*, chap. 8.

electricity. Diagrammatically an ordinary electrolyte and a colloidal electrolyte (see also p. 28) may be represented in this way.

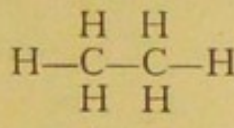
$[X_1]^{+ \text{ or } -}$ ordinary electrolyte $\qquad$ $[X_{100000}]^{+ \text{ or } -}$ colloidal electrolyte

What leads to the formation of colloidal matter ?

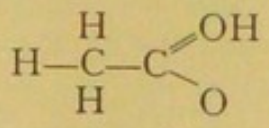
There is one factor which we may assume to be constant, namely, our solvent water. When water comes into contact with different salts, we find that the radicals which go to form the salt, possess the power of becoming ionised to different degrees.

The greater the power of a certain radical to become either a kat-ion or an an-ion the greater will also be its influence on other radicals, because its chemical power is in direct ratio to its capacity of becoming ionised. The less its power of forming ions the more will it tend to unite with other units similar to or identical with itself, because the least difference of potential must always be developed in a community of identical units.

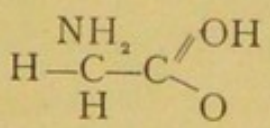
We thus have on the one hand such chemically exceedingly active substances as the halogen- and oxy-acid salts of the alkalies [*e.g.* KCl, KNO_3, K_2SO_4], and on the other hand the chemically inactive paraffins [CH_4, C_2H_6]. While the former because of their great ionic power in the presence of water will always interact with other active units and thus lose their individuality, paraffins on the other hand will under the same circumstances remain inactive. It is to me exceedingly interesting to see how our most primitive ancestors, the paraffins, by evolution gave rise to alcohols, aldehydes and acids, and thereby gradually acquired the power of interacting with the environment.



ethane.



acetic acid



glycocoll.

While an ordinary mineral acid such as hydrochloric acid

in semi-normal strengths is dissociated into ions to the extent of 100 per cent., acetic acid is dissociated 3 per cent., and amino-acetic acid or glycocoll forms only a few ions (see p. 37). Thus we have an inert compound, the paraffin, become a chemically active compound, namely, acetic acid, and the latter changed into a chemically less active substance, namely, glycocoll. There is, however, one great difference between acetic acid and glycocoll, for the former can only interact with kat-ions, such as sodium, while the latter interacts with both kat-ions and an-ions (see p. 38) being amphoteric in nature. It is this very property which also leads to the formation of internal salts, namely, to the pseudo-acid-pseudo-basic compounds (see p. 35). Internal salt formation satisfies the affinities of the compound in question for negative and positive radicals, and thereby safeguards it to a great extent against an inimical environment containing free acid or basic groups, as I pointed out in my *Physiological Histology* in 1902, p. 27. This view has been confirmed in a remarkable way by the researches of Wakelin Barratt[1], who found that Paramaecia may unite with small quantities of acid and larger quantities of alkalies without losing their neutral re-action or power of surviving.

Attention has already been drawn to the fact that a whole series of fatty and aromatic acids exists in protoplasm, and as each of these possesses only feeble acid or basic characters owing to its amphoteric nature, the acids readily unite with one another and form thereby large colloidal aggregates, which in a chemical sense are slow to act, and thereby preserve their identity.

It must not, however, be assumed because protoplasm appears in many instances in the living condition homogeneous that therefore it is structureless, nor should we mistake ap-

[1] J. O. Wakelin Barratt: *Die Wirkung v. Säuren u. Basen auf lebende Paramaecien; Zeitsch. f. allgem. Physiol.* **4**. 438 (1904), and ibid.: *Die Reaktion des Protopl. in ihrem Verhältnis zur Chemotaxis*, p. 87.

pearances which have been produced by re-agents for normal structures.

The chief conclusion I arrived at, in 1902, in my *Physiological Histology*, as far as fixing is concerned, was that histologists should beware of all fixing re-agents which are electrolytes, such as the salts of the heavy metals and acids, for all of these establish differences of potential and thereby lead to a more or less pronounced separation or dissolution of colloidal matter, according as to whether the more active ion has either the opposite or the same charge as that carried by the colloidal aggregates. I am convinced that many of the appearances which have been described as normal protoplasmic constituents are artefacts due to the action of electrolytes, but if we do not use electrolytes for fixing purposes, but employ non-electrolytes such as Osmium tetroxide (OsO_4) or formaldehyde (OCH_2) dissolved in isotonic 'normal' salt-solutions, we will still find definite differentiations of the protoplasm. As non-electrolytes act by forming additive compounds, without setting up differences of potential we have every right to suppose that structures which we may see after the use of aldehydes and osmium tetroxide have existed under living conditions.

Amongst the most readily recognized appearances in protoplasm, are the zymogen-granules of gland-cells. Granule-formation is in every respect identical with the formation of an emulsion; as in the case of milk, (till the specific gravity factor makes itself felt,) we have a balance between the size of the fat globules and the fatty acids and alkalies present, so in the cell-plasm. If we assume a cell-plasm to have a definite acid and basic capacity and the granules which are excreted by the nucleus to be either basic or acid in character (or by a subsequent change to become acid or basic), then the size of the granules and also their number will be determined by the amount of acidity or basicity of the cell-plasm. It has been pointed out (p. 28) that neutralisation of a number of charges on individual small units,

will bring these units together into bigger aggregates, each of which will have its own charge as long as an ion having the opposite charge is present in the solvent, and this also holds good for the zymogen granules. To me a discharge of zymogen-granules by the cell means simply that by a chemical stimulus the difference of potential between the cell-plasm and the cell-granules becomes disturbed, in consequence of which, the granule-phase becomes separated from the plasm-phase; as further all zymogen-granules in the cell-plasm are in an inactive state and in a rabbit remain so even after twenty-two days inanition, while the cell-phase is greatly reduced, we may say that the latter is the more changeable of the two phases, which again means that by an appropriate stimulus, such as the entrance of electrolytes into the cell, the cell-plasm will be made to become more colloidal, in consequence of which it will shrink and thereby force the granules to the surface of the cell.

The zymogen-granules I chose as a type of transient structure, for they are formed day after day only to be used up again in the general economy of the animal or plant, and under this heading come also reserve materials such as starch, glycogen, fatty compounds, &c.

As a type of a structure which is constant under certain definite conditions, we may take the fibrils of nerve-cells. We know that all nerve-phenomena are accompanied by definite changes in potential and we also know that such changes can only be due to chemical alteration in the plasma of which the various cell-processes are composed. It is exceedingly instructive that given one nerve-cell or a chain of nerve-cells that they remain fibrillar only as long as they are and can be made use of. As soon as afferent impressions or stimuli cease to act on a nerve-cell and its processes there occurs a disappearance of the fibrillar structure, showing that the fibrils are but the paths along which normally impulses are sent and also that stimulation is the cause of nerve-fibrils being formed. We have thus in the nerve-cell a mechanism so

constituted that irritation (stimulation) leads to coagulation along definite tracts, which allow a change set up at one end to travel till it reaches the other end.

Of recent years a great deal of attention has been paid to intra-cellular channels, as found in nerve-cells and various gland-cells (crescents of Gianuzzi, parietal-cells of the stomach, liver-cells, &c.). It may be that all these passages are excretory channels, or that some may serve for the absorption of food material. There is *a priori* nothing against the permanence of such intra-protoplasmic ducts, especially if we have to do with excretory tubes, for if we commence with a non-differentiated plasma, capable of reacting to electrolytes, it will follow, if the secreta be at all chemically active, that they must induce a coagulation of the plasma which surrounds them. Whether the cytoplasm so coagulated will remain coagulated, till the next time excreta are to be got rid of, or will become again homogeneous, is determined by the amount of coagulation the cell-plasm has undergone, and by the presence or absence of electrolytes capable of undoing the coagulation produced by the secreta or excreta. Very good examples of such intra-cellular tracts produced by nuclear substances diffusing into the cell-plasm will be found in the papers by Goldschmidt, who follows R. Hertwig in calling the structures in question, Chromidial-apparate [1]. It is quite impossible to enter into all the various differentiations which have been described in connection with protoplasm, and therefore I shall limit myself, as far as Bütschli's statements are concerned, in saying that in many instances there is undoubtedly a honey-comb-like structure, which seems to have been devised to bring about a ready diffusion of food material and of gases.

In this connection it may be pointed out that in all animals and in most plants the stability of the cytoplasm depends on the presence of an ample supply of oxygen. It is not neces-

[1] R. Goldschmidt, *Zoolog. Jahrbuch.* (Spengel) **21**, Heft. 1 (1904) and *Arch. f. Protistenkunde* **5**, 126 (1904).

sary to assume, as is generally done, that the oxygen serves only for purposes of oxidation, that it is always used up, for just as haemoglobin in the presence of oxygen takes up the latter and becomes oxyhaemoglobin, so do I believe that many compounds, especially in the nucleus, are kept constantly in an oxidised state, and that the oxygen-bond forms a link in the chain of chemical compounds to which I shall refer later.

Into the question of the centrosome and the part it plays during mitosis, it is necessary to go somewhat more fully. After Sachs had explained division of the cell as depending on centres of attraction dividing the nucleus between them, Fol in 1873[1] described the fibrils which pass outwards from what is now known as the centrosphere, and compared the appearances to the picture presented by iron-filings which arrange themselves round the two poles of a magnet. The same conception has been developed by Giard[2], who explains the formation of the spindle as due to physico-chemical phenomena and the establishment of electrical or electro-magnetical poles in the nucleus; by Ziegler, who made a magnetic model[3]; by Gallardo[4], who made an electro-static model by using a suspension of quinine-sulphate in oil of turpentine, and showed 'that the introduction of a third terminal put to earth produced a deviation of some of the fibres from the belly of the spindle to itself—the figure being, in fact, a "triaster," such as sometimes occurs in dividing-cells'[5]; by Hartog[7], who speaks of the cytoplasmic

[1] Fol, *Die erste Entwickelung des Geryonideneies: Jenaische Zeitschrift.*, vol. 7.

[2] Giard, *Bull. Sc.* **7**, 258 (1876).

[3] Ziegler, *Untersuchungen ü. d. Zelltheilung; Verh. Deutsch. Zool. Ges.* (1875).

[4] Gallardo, *Essai d'interpretation des figures karyokinétiques; Ann. Mus. Buenos Aires* (1896), and *Interprétation dinamica de la division celular* (1902).

[5] Quoted from Marcus Hartog's paper, *Proc. Roy. Soc.* **76**, 552 (1905).

[6] M. Hartog, *Compt. rend.*, June 10th, 1904, and *Proc. Roy. Soc.* **76**, 548 (1905).

figure of the dividing-cell being a strain-figure, under the action of a dual force, analogous to magnetism, and still more to statical electricity; he calls the force at play 'mitokinetic force.' Hartog, on the strength of his magnetic models [1], finds that the spindle-fibres, astral rays, the outer limiting membrane of the cytoplasm, the nuclear wall, and the free chromosomes along the cell-spindle, must all possess a high permeability to mitokinetism as compared with the other structures of the cell. 'A spindle figure can only be obtained in a field with the two unlike poles of a dual force . . . as the diffusion, osmosis, and surface-tension phenomenon are of similar character at the two poles of a cell, they cannot be the forces involved in the spindle.'

I shall revert to this later.

Darbishire [2] says, 'A consideration of the ontogeny and phylogeny of the centrosome seems to point to the conclusion that amphiasters, spindles, and fibres have no actual existence, but are the track of the influence which the centrosome or its homologue exercises on the chromosomes, owing to the arrangement of the constituents of protoplasm along the lines of that influence, like iron-filings in a magnetic field. . . . The word influence is used because it is sufficiently vague to prevent the reader from thinking that one had any conception of what it is.' Darbishire agrees with Boveri in the views expressed by the latter in his book 'On the Centrosome,' that 'the binary division of the cell is brought about by the binary division of the centrosome,' and also that the abnormal, multiple spindles seen in cases of polyspermy (Henneguy) are due to the abnormal number of centrosomes present. Hertwig's view, that the multipolarity of figures is caused by a superabundance of chromatic material; Rabl's conception that centrosomes are pulled out passively by a bipolarity of the cell, or Heidenhain's notion that centrosomes go passively

[1] Made with glycerine, gelatine or balsam (which two later are allowed to set) and with magnetic oxide of iron (Fe_3O_4).

[2] A. D. Darbishire, *The Centrosome, Trans. Oxf. Univ. Junior Scient. Club*, 1903.

to predestined places in the cell, are also not tenable. At the end of his paper Darbishire gives my views, which I shall state later.

Lillie[1] has likewise applied magnetic forces to explain the factors by which chromatic filaments and chromosomes become arranged in definite patterns during mitosis. By stringing small cubical pieces of cork at distances of about six millimetres along a delicate silk filament, and piercing the corks with small, similarly oriented, magnetised needles, floating this contrivance on water, and subjecting it to the influence of a magnet, he obtained the same appearances as are seen during the mono-spirem stages of karyokinesis, while the aster-stage could readily be demonstrated by stringing the magnets along a number of flexible wires capable of being bent into any desired shape.

During the last four years[2] I have taught that the centrosomes play the part of electrolytes by coagulating the cell-plasm or nuclear sap, as the case may be. As coagulation of a colloidal substance means that the colloidal particles aggregate to form bigger units (see p. 28), coagulation is equivalent to a contraction of the colloidal matter. If the colloidal particles possess adhesiveness, coagulation will produce an elastic system, arranged either in the form of a foam, or a net, or filaments, and the greater the amount of coagulation, the greater will also be the contraction of the coagulated material. When a change is produced in colloids by the action of electrolytes having an electrical load opposite to that carried by the colloid which we are enabled to see with the help of the microscope, we speak of structure; if the change is of such a nature as not to be resolved by the microscope we say the substance is homogeneous. By coagulating a colloid we may produce a system doing an enormous amount of work, provided there are fixed points to which the coagulating mass can

[1] Ralph S. Lillie, *Biological Bull.* **8**, 193 (1905).

[2] See end of Darbishire's paper.

attach itself. It is very instructive to take two similar vessels, containing an equal amount of globulin solution made by extracting ground lentils with 5 per cent NaCl, to add on equal amount of acetic acid sufficient to cause coagulation, and finally, to leave one vessel undisturbed while the other is shaken for one minute. In the former the coagulated material will separate out quickly, while in the second vessel, owing to the original system having been broken down there are no fixed points to serve as attachments of the coagulating material, and in consequence of this the coagulated globulin separates out very slowly owing to the original points from which the coagulation started having been disturbed; instead of having one contracting system there are now many systems.

In a cell about to divide the increased activity of the centrosome is an index of electrolytic changes taking place in it. Increased chemical activity always means that by the influence of some other chemically more active radical or ion there is induced in, or has been transferred to, the original inactive or sluggish matter the power of diffusion, which expresses itself in a lowering of the surface-tension. In consequence of the latter a centrosome will divide and may do so completely, or the two daughter-centrosomes may for a time be still adhering to one another by a delicate bridge. The increased activity, called forth by electrolytes, by imparting the same electrical charge to the two centrosomes will force them apart, and the centrosomes in their turn by liberating material which combines with the surrounding colloidal matter give rise to the formation of the spindle and the rays proceeding from the centrosphere. Continued action of the centrosomes on the cytoplasm or *vice versa* must lead to a contraction of the original fibrils formed in connection with the centrosome because of the increased coagulation, and must lead to a separation of the chromatin segments, and their migration towards the centrosomes if the latter are fixed points.

We have thus to explain the various changes set up in

cytoplasm during glandular secretion, during mitosis and so on, on purely physico-chemical grounds. The different means by which coagulation may be set up I have fully discussed in my books on the *Theory of Histology* and the *Chemistry of Proteids*, and it will suffice now if I enumerate the headings: (1) Setting of colloidal solutions by lowering of the temperature; (2) Conglutination or aggregation by mechanical means owing to dissolved colloid passing spontaneously out of solution and forming delicate surface pellicles exhibiting many of the characteristic properties of solid matter, as thereby the total energy of surface tension becomes diminished[1]; (3) Coagulation due to alterations in the electrical tension between the colloid and its solvent; (4) Salting out of albuminous substances owing to an increase in the concentration of salts (see p. 42); (5) Precipitation of colloids due to a withdrawal of hydrogen-radicals of the CO·O+H and the hydroxyl-radicals of the oxy-acids and phenol-compounds; (6) Precipitation due to the removal of salts as in the case of globulins (see p. 32); (7) The formation of irreversible salts owing to metals, such as calcium or mercury having low dissociation-energies; (8) The formation of additive-compounds: the amino-acids uniting for example with aldehydes; (9) The 'spontaneous' coagulation of albumins due to factors which are not yet fully recognised; (10) Coagulation by means of heat.

The osmotic properties of protoplasm have been so fully studied by Overton that his papers ought to be carefully read by everyone[2], and I have only to say that I fully agree with the conceptions put forward by him.

To me the protoplasmic structure means simply an equilibrium for the time being between colloidal aggregates which differ from one another in their constitution [and which are prevented from becoming inert or dead material by the pre-

[1] W. Ramsden, *Proc. Roy. Soc.* **72**, 156 (1905).

[2] E. Overton, *Uber d. allg. osmotischen Eigenschaften d. Zelle, &c.; Vierteljahrsehrift d. Naturf. Ges. Zürich*, **40.** (1895) and **44.** 88 (1899); and *Zeitsch. f. physik. Chem.* **22.** 189 (1897).

sence of inorganic and organic electrolytes]. Two important papers by Pauli[1] and Hofmeister[2] have also appeared dealing with protoplasm from the physico-chemical and the chemical points of view, and I have to content myself by drawing attention to them, with the exception of quoting one passage from Hofmeister: 'Even now, we may say, that the contemplation of the cell as a machine working with chemical and physico-chemical means leads nowhere to problems which could compel us to assume other than known forces, and, as far as we can see, there is no reason for that resignation, which either expresses itself in an 'ignorabimus' or in vitalistic deductions.'

Definition of Protoplasm.

At the beginning of this paper Hugo von Mohl's reason for speaking of protoplasm was given. He conceived it to be the mother substance of the nucleus and the cell-envelopes. This conception is, however, no longer tenable. In my paper 'What is Life?' I pointed out in 1898[3] that 'organic individuals possess the power of creating around themselves a new environment, the cytoplasm, which has the following functions: (1) To elaborate possible inorganic or organic food substances and thereby to make them directly assimilable by the nucleus (chloroplasts and zymogen-granules). (2) To protect the nucleus from deleterious influences outside the organic individual (as proved by the removal of the whole or greater part of the cytoplasm, invariably leading to the death of the nucleus). (3) To either attract food to the cell or to move the cell towards the food by means of the centrosomes which are to be regarded as special locomotor organs (*viz:* centrosomes in white blood corpuscles (M. Heidenhain),

[1] Pauli: *Der Kolloidale Zustand und die Vorgänge in der lebendigen Substanz; Braunschweig, Vieweg*, 1902.

[2] Hofmeister: *Die chemische Organisation der Zelle; Braunschweig, Vieweg*, 1901.

[3] Mann, *Trans. Oxford University Junior Scient. Club*, 1899.

the basal globules at the base of cilia) (v. Lenhossék). (4) To prevent all inter-communication with the outer world by the formation of cysts (amœba) or callus on sieve plates (plants), whenever deleterious agencies are at work.'

'Organic individuals, within physiological limits, are independent of chance, as the combination of compounds peculiar to each individual, leads to the formation of a new environment consisting of complex carbon compounds, which in their turn by acting on the world at large, so modify the latter as to make it directly assimilable by the nucleus. The nucleus in its turn forms its organic environment or cell-plasm by which it is kept in existence.'

This conception was based on the researches of my pupil Lily Huie, who for the first time showed by definite experiments[1] that the nucleus is the organ for forming cytoplasm.

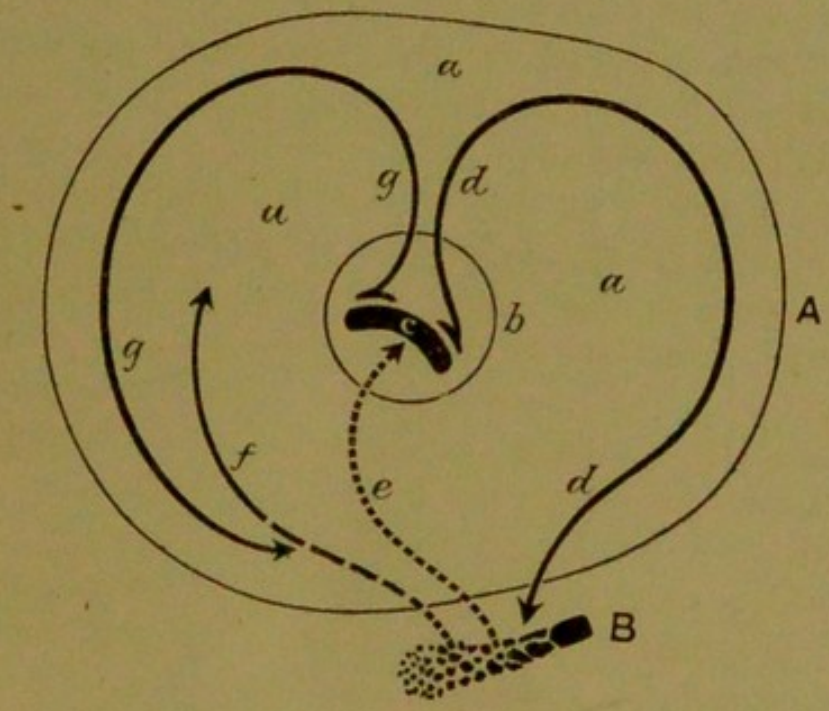
A typical gland-cell of the insectivorous plant Drosera rotundifolia contains in its resting condition a vacuolated cell-plasm with zymogen-granules, and a nucleus with large nucleolus and very scanty nuclear basophil chromation. A similiar cell, when examined 20 to 30 hours after feeding with egg-white, shows that the cell-plasm has mostly disappeared; that the nucleolus is also reduced to a mere shadow, while the basophil nuclear chromatin is enormously increased, having divided into 8 distinct chromatin segments. The same appearance may be obtained within one hour if the cell be fed on peptone. Two to three days after feeding the nucleus is engaged in re-building the cytoplasm, while seven days after feeding the cell-plasm has been completely reformed and the nucleus has assumed again the appearance it shows during the resting condition.

In conclusion let me give an abstract taken from my book on the *Chemistry of Proteids*.

'We have to distinguish between the origin of organic com-

[1] L. Huie, *Quart. Journ. Micr. Sc.* **39**. 387 (1896–97) and **42**. 203 (1899).

pounds and that of life. To be able to make marsh-gas, alcohols, aldehydes, acids, amino-acids, peptids, peptones, and albumin, however great an achievement in itself, is not the same as making life. To many people a living cell consists of "protoplasm" a substance they imagine to be one exceedingly complex body. They do not realise that in a cell we have a not very large number of comparatively simple compounds which only collectively form the protoplasm. What constitutes life, is the presence of a number of such "organic" compounds, capable of mutually re-acting upon one another, and thereby giving rise to new compounds, which cannot re-act chemically with the mother-substances from which they are derived, but which by inter-acting with new radicals give rise to a cycle of events.



'In the above diagram I have endeavoured to make my meaning clear. From the nucleus two arrows pass outwards: the one on the right represents the formation of "extra-cellular" zymogen granules, which have the function of ionising extraneous chemical compounds in such a way as to make them available to the cell-individual. These

enzymes change, for example, albumin into albumoses, peptones and amino-acids. The arrow on the left of the figure represents 'intra-cellular' zymogens, the function of which is a constructive or de-ionising one; they bring about an aggregation of those amino-acids and peptone-like bodies which have been liberated from proteid-food by the extra-cellular enzymes. The aggregates so formed constitute the main bulk of the cell-plasm, and they are subsequently partly transformed by the activity of the nucleus into the extra-cellular and intra-cellular zymogens already alluded to. The cycle of events just described is what we call life. Cessation of life, or death, will be produced either by the inability to procure food, which is necessary to counter-balance the wear and tear necessitated by the conversion of one chemical compound into another one, and this amounts to death by starvation, or secondly, by the inability of the nucleus to digest the food, and so make it available to the individual cell. In addition to these two kinds of physiological death, we have another form due to violence, as, for example, by the application of excessive heat or cold or inorganic (corrosive sublimate, &c.) or organic (bacteria) poisons.

'What must be the ultimate aim of chemical biology is to establish the sequence of events in the cycle from simple to more complex substances and the disintegration of the latter for the purposes of liberating energy and of so acting on other chemical compounds as to make these available to each individual cell.

'If we want to proceed systematically and not by guess-work, we have to pursue histological research based on a sound knowledge of chemistry and physics, and these we shall be able to understand and to modify the events in the life-cycle, for we will be able to accelerate and to slow down nuclear and cytoplasmic activities. The importance of such research in connection with cancer and all fevers cannot be over-estimated.'

As von Mohl's conception of the physiological function of the nucleus is no longer tenable, let us discontinue the use of the word 'proto-plasm' and substitute for it the term 'plasm.'