COLLOIDAL SOLUTION. THE GLOBULINS.

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(Ten Figures in Text.)

(*From the Physiological Laboratory, Cambridge.*)

CONTENTS.

COLLOIDAL solutions differ in their relation to small concentrations of salts, some, such as the hydrosols of metals, of silica and alumina, etc. are precipitated, others appear not to be changed, such as, for instance, hydrosols of albumen or gelatine, others again depend for stability upon the presence of salts. The chief and perhaps the only true example of the last class is the species of proteid known as globulins.

About five years ago I commenced the investigation of this last class with the object of gaining further insight into the mechanism of colloidal solution and the results were communicated to the Physiological Society in 1903, to the British Association in 1904, and a statement as complete as time would allow to the Royal Society as the Croonian Lecture of 1905.

In the communication to the Physiological Society I attempted to express the relation between globulins and salts, acids or alkalies in a

purely physical way, which may be briefly recapitulated as follows. The globulin is dispersed in the solvent as particles which are the colloid particles and which are so large as to form an internal phase. These particles at any one moment contain within themselves an excess of the most penetrating and fastest moving ion present, and they therefore have the electric charge of this ion. Therefore, in presence of acid they contain an excess of the hydrogen ion and they are charged positively, in presence of alkali they contain an excess of the hydroxyl ion and are therefore charged negatively, in presence of neutral salts the excess ionic concentration in the colloid particles will be so slight as to be unrecognisable and the particles therefore are uncharged: they are in point of fact related equally to both ions.

This purely physical hypothesis is attractive from its simplicity, it covers most of the facts, and it is identical with the theory of the colloidal state advanced by Perrin[1] as a result of certain most interesting investigations upon the origin of contact difference of potential. The hypothesis fails in two respects: (1) it does not adequately recognise the chemical phenomena involved, such for instance as influence of the selective affinity of metals for either hydrions or hydroxyl ions in the formation of metallic hydrosols[2], or the connection between the colloidal state and the hydrolytic reaction between water and soaps, or water and proteid-acid or proteid-alkali compounds. (2) It does not cover those cases of colloidal solution where there is no potential difference between the colloid particles and the fluid in which they are immersed. In globulin salt solutions and solutions of gelatine there is no trace of drift of the colloid in an electric field if the possibility of electrolytic decomposition of the organic colloid be excluded as in the method described later. Lastly the theory can be disproved by direct experimental evidence. If the relation between the colloid and the fluid depends solely upon the relative velocity of the ions present in the latter as I at first thought, and as Perrin thinks, then in a solution of globulin by a salt such as LiCl, or LiBr, in which the ionic velocities are in the ratio of about 1 to 2, the globulin should show a negative charge due to the faster anion. I failed to find indications of even the feeblest charge provided secondary electrolytic decomposition were excluded. This same experimental fact stands in the way of the acceptance of Perrin's theory of the relation of contact difference of potential to

[1] *Journal de Chimie Physique*, II. p. 61, 1904; III. p. 50, 1905.

[2] Linder and Picton, *Journ. Chem. Soc.* LXXI. p. 568, 1897; Burton, *Phil. Mag.* (not yet published).

ionic velocities since the ratio of the velocities of the ions in acids or alkalies is only of the order 1 to 4 or 1 to 5.

In the following paper I have tried to deal with the phenomena of colloidal solutions in the special case of proteids from a frankly chemical standpoint. The chief difficulty in the way of a chemical theory of colloidal solution is the apparent need for postulating the existence of continuously varying chemical compounds, or what van Bemmelen calls absorption compounds. The need to my mind is more apparent than real. The absence of transition points, the smoothing of the curves, is probably merely an expression of the inertia of the colloidal system due to the presence of electrified surfaces and to the large molecules involved. True equilibrium values for matter in the colloidal state, that is, values which have the same magnitude however the condition is approached, have not so far as I am aware yet been obtained in any one single instance. The form of the temperature curve for hydrosols of agar, or gelatine depends entirely on whether the particular temperature is approached by adding heat or by subtracting heat, and upon the rate of addition or subtraction of heat[1]. The reaction velocity of colloidal matter may be of the order of that of matter in the solid state so that, as van 't Hoff says of the hydrates of calcium sulphate, the time values are of the magnitude of geological epochs. Owing to the large size and low mobility of the molecules of colloids any surface once formed in a fluid plays to a great extent the part of a semi-permeable membrane, *e.g.* the surface of the colloid particles, and if that surface becomes strongly electrified it acquires a special stability. The system then as a whole is in equilibrium with *two* pressures[2]. In this way the instable state of spheroidal subdivision in which a new fluid phase first of all appears and which gives place rapidly to the crystalline state in crystalloid solutions[3] becomes subpermanent in colloidal solutions.

The globulin referred to except where express statement to the contrary is made is that precipitated from diluted ox serum by acetic acid. Four states can be recognised in both the solid condition and in solution, globulin itself, compounds with acid or with alkali, and compounds with neutral salts. Following old usage I propose to call the

[1] Hardy. *Proc. Roy. Soc.* LXVI. p. 95. 1900.

[2] Hardy. *Proc. Roy. Soc., loc. cit.*

[3] Lehmann, *Molecularphysik*, I. p. 730; Bancroft, *The Phase Rule*, p. 242, 1897, and especially Garnett, *Trans. Roy. Soc.* CCIII. A, p. 385, 1904.

compound with acid acid globulin, those with alkali alkali globulin, those with salts salt globulin.

Globulin itself is insoluble in water, though it forms filterable non-settling suspensions which are solutions of very low grade[1]. Acid globulin and alkali globulin can be separated in the solid state by dissolving globulin with minimal amount of acid or alkali and evaporating to dryness *in vacuo* over sulphuric acid and caustic potash.

When hydrochloric acid is used the dried HCl globulin is found on analysis by Carius' method to contain all the chlorine used to dissolve the globulin. HCl globulin therefore is stable *in vacuo* in presence of solid KHO and the acid may be regarded as being in true combination. Acid globulin dissolves in water.

Alkaline globulin can be separated in the solid state from, *e.g.* solution in ammonia by drying *in vacuo* over sulphuric acid. When redissolved the solid shows the same order of molecular conductivity as the solution from which it was dried out.

Both acid and alkali globulins ionise in solution. That is to say in solution the globulin is electrically charged and takes part in any electric transport, and the velocity can readily be measured by the boundary method. Therefore one must recognise "ionic" globulin as a species, but reasons will be given for regarding the ions as peculiar

[1] Defined exactly, a solution is a homogeneous mixture or a uniform phase of continuously varying composition. In this exact sense a hydrosol is not a solution but a mixture of matter in two states more or less clearly distinguishable since it consists of a fluid which has dispersed through it large particles, the colloid particles which are not of the same order of magnitude as the molecules of the fluid and which are separated from the fluid by a surface, the nature and properties of which may almost be said to define the colloidal state.

Colloidal solution therefore is a state of partial solution, and it passes, as Picton and Linder showed, by insensible gradation into true solution. Its exact position in the phenomena of the liquid state may be indicated as follows.

When a homogeneous liquid separates into two states, say a liquid and crystals, the new state appears first as minute droplets which represent an instable state and which changes to the stable crystalline state in a longer or shorter time according to the relative magnitude of the force of crystallisation and the surface tension (Garnett, *loc. cit.*).

Substances which do not readily crystallise, whose molecules therefore have feeble polarity, permit the instable emulsion state to become subpermanent, and this is the state known as colloidal solution. All cases of colloidal solution however are not necessarily states of incomplete crystallisation, though they are states of incomplete separation of a new phase.

To include these states Picton and Linder introduced the conception of grades of solution, the grade being high or low according to the degree of dispersion of the solvee in the solvent.

and special to the colloidal state. By reason of their ionisation solutions of acid and alkali globulin are good conductors.

Globulin therefore is an amphoteric substance and its acid function is much stronger than its basic function. As an acid it is strong enough to form salts readily with bases so weak as aniline[1], glycocoll, and urea, acting as a base it forms salts with weak acids, such as acetic and boracic acid, which are very unstable in presence of water.

The precipitation of globulin by salts. Globulin solutions can be precipitated by neutral salts, and in this respect they exhibit very characteristic relations. Each salt acts as a precipitant at low concentration, and at high concentration. Between these two it acts as a solvent. The first precipitation occurs only when acid or alkaline globulin is present, and it is similar to the precipitation of hydrosols by small concentrations of salt in that the colloid particles are electrically charged. The second precipitation is a separation of solid globulin from a solution of salt globulin, and it is the precipitation of the colloid from a hydrosol in which the colloid particles are completely uncharged.

Thus solutions of acid or alkali globulin are precipitated by the addition of neutral salts, further addition brings about re-solution always in the case of alkali globulin, sometimes in the case of acid globulin. Still more salt brings about reprecipitation. In the first solution the globulin is ionic, in the second solution it is not ionic.

The mechanism of these two precipitations in spite of views to the contrary[2] I take to be distinct.

The precipitation of colloidal solutions by salts has been explained in two ways as being due to (1) a condensation of the electrically charged colloid particles to larger and heavier masses owing to the nuclear action of those salt ions which carry a charge of the opposite sign to that on the colloid particles or (2) to a dehydrating action of the salts. In case one, as Picton and Linder first showed, the precipitate carries with it a fraction of the active ions. The solid phase, therefore, is a compound between the colloid and one of the salt ions, of the nature of an absorption compound, and the nuclear action of the salt ions is due to chemical reaction which is peculiar only in the chemically indefinite

[1] Of a weak base of limited solubility such as aniline it is almost more accurate to say that it is dissolved by globulin, than that the globulin is dissolved by the aniline. Aniline will not combine with anhydrous globulin.

[2] Pauli. *Beit. f. exp. Physiol. u. Pharmak.* VI. p. 233. 1905.

nature of the resulting compound. As Picton and Linder showed long since the compound formed between the colloid and the ion of the salt can be decomposed in quite an ordinary way, the salt ion being replaceable by an equivalent weight of another ion. It is wrong to speak of the action of salt on a colloid particle as "electrical combination" as though it were something quite apart. It is "electrical combination" only in the sense that reaction between electrolytes is electrical.

Now if the action between salt and colloid is chemical and identical for instance with the selective reaction in dyeing which discriminates between acid and basic dyes, it would be represented by a generalised equation of the form:—

$$C'H\cdot + B\cdot S' = CB + HS.$$

That is to say, the colloid functions as an acid and by the law of mass action, the compound $C'B\cdot$ is formed because of its insolubility.

Consider the hydrosol of a metal such as platinum. The colloid particles are negatively charged, they are anionic in character, and the charge is due to a reaction between the metal and water at the moment of formation of the hydrosol whereby the hydride PtH is formed which ionises in the sense

$$PtH \rightleftharpoons Pt' + H\cdot.$$

The chief number of the ions Pt' are in the form of masses so large as to have the properties of matter in mass, they are not of molecular dimensions and they form an internal phase[1]. Ionisation is a phenomenon of the surface of these masses only. It confers on the particle its electric charge, and it is in this case the "incomplete chemical combination" which Lord Rayleigh regards as the source of contact differences of potential to which I referred in an earlier paper[2]. In reactions therefore an electropositive colloid is a weak alkali, *e.g.* hydrosol of ferric hydroxide, and electronegative colloid a weak acid, *e.g.* silica.

This view of the source and nature of the charge on the particle of a hydrosol, as it seems to me, is proved by the researches of Burton[3], who finds clear evidence of selective chemical reaction when hydrosols of metals are formed in various media.

Though one may speak of the colloid particles as being ionic in nature they are sharply distinct from true ions in the fact that they are not of the same order of magnitude as are the molecules of the solvent,

[1] Hardy. *Proc. Roy. Soc.* xcv. p. 110. 1900.
[2] *Ibid.* p. 66.
[3] *Phil. Mag.* (not yet published).

the electric charge which they can carry is not a definite multiple of a fixed quantity, and one cannot ascribe to them a valency, and their electrical relations are those which underlie the phenomenon of electrical endosmose. To such ionic masses I would give the name "pseudo-ions," and I propose to treat globulin solution from the standpoint of a hypothesis of "pseudo-ions."

Amongst crystalloids there are substances which exhibit electrical phenomena in solution, the electrolytes, and substances which do not exhibit electrical phenomena, the non-electrolytes. Electrolytes are so called because the conditions of solution are such that the charged units of the solvee can carry equal amounts of + and − electricity and discharge on to the electrodes. It occurred to me that in non-electrolytic solution the particles of the solvee might be charged all of the same sign, and the condition such as to make discharge on to the electrode impossible. I was therefore led to look for instances of electrical convection in non-electrolytic solution and I satisfied myself that the solvee may be absolutely isoelectric with the solvent, but I failed to find a single such case when the solvent was water. Fields up to 500 volts per centimetre were used and in aqueous solutions such bodies as urea, glycerine, glycocol, cane-sugar, and iodoform moved. From their chemical constitution and relations the first five are weak bases or weak acids, and they move as such. Iodoform undergoes decomposition[1]. In a non-ionising solvent such as chloroform solutions of azobenzene showed absolutely no drift. They are completely isoelectric[2].

These facts are paralleled amongst colloids. One has hydrosols such as those of metals, of acid and alkali globulin, and of acid and alkali albumen, of silica, etc., in which the solvee is highly charged. One has relatively isoelectric hydrosols such as those of glycogen and starch, and hydrosols in which the colloid particles are completely uncharged such as solutions of salt globulin or gelatine[3].

On the theory already stated the charge on the colloid particle will be due to its acid or alkaline nature[4]. Thus cane-sugar acts in chemical combination as an acid, and in aqueous solution it moves as an anion. Similarly its chemical relatives, the colloids glycogen and starch, move in a field as though they were anionic.

[1] Hardy and Willcock, *Proc. Roy. Soc.* LXXII. p. 200. 1903.

[2] The experimental method is given in the appendix.

[3] The drift measured by Whitney and Blake (*Am. Chem. Soc.* XXVI. p. 1339, 1904) is due to partial electrolytic decomposition of the gelatine.

[4] Picton and Linder, *Journ. Chem. Soc.* LXXI. p. 568. 1897.

The theory of precipitation of colloidal solution by salts which has been considered so far, clearly is applicable only to cases when the colloid solution is electrically active. The precipitation of electrically inactive hydrosols by neutral salts needs some quite different explanation, and Billitzer[1], and Pauli[2] in a series of most interesting papers on the precipitation of proteids, are wrong in attempting to bring all cases of precipitation by electrolytes within the one theory.

The precipitation of electrically inactive hydrosols can be adequately explained on the lines suggested by Spiro[3] as a separation into two phases, one a solid phase rich in the colloid, poor in salt and water, the other a fluid phase, rich in water and salt, poor in the colloid, the action being exactly similar to the salting out of alcohol from a mixture of alcohol and water by the addition of magnesium carbonate.

Electrical precipitation is distinguished from salting out in the same way as is the precipitation of calcium from a solution of the hydroxide by potassium sulphate to the precipitation of potassium sulphate by calcium sulphate as the double salt $K_2SO_4CaSO_4 4H_2O$.[4] In the one case the precipitant is decomposed, in the other case it is not. The precipitation of electrically active hydrosols is distinguished also by the small concentration of electrolytes necessary to produce the change, whereas a high concentration is necessary to salt out.

According to Hofmeister[5] the precipitating action of salts is due to their "water depriving" action. Clearly this view is not applicable to the cases where salts act as precipitants at very low concentration. It is however a convenient way of considering salting out, the separation of the precipitate being due to the appearance of a lower hydrate of globulin just as the concentration of added salts determines by their dehydrating influence the particular hydrate of $MgSO_4$ which separates from a solution of the salt[6].

Cases of precipitation in which both actions concur obviously are possible, as when for instance an electrically active colloid by reaction with one of the ions of a salt is converted into an electrically inactive colloid which is separated as a solid phase only when the salt concentration

[1] *Wied. Ann.* II. p. 902. 1903; *Zeits. f. physik. Chem.* XLV. p. 307. 1903; LI. p. 129. 1905.

[2] *Beit. f. exp. Physiol. u. Pathol.* III. p. 225. 1901; V. p. 27. 1903; VI. p. 233. 1905.

[3] *Ibid.* IV. p. 300. 1902.

[4] Ditte. *Compt. Rend.*, p. 1266. 1904.

[5] *Arch. f. exp. Physiol. u. Pharmak.* XXIV. p. 246, 1888; XXV. p. 1, 1889.

[6] Van 't Hoff, Meyerhoffen, and Smith. *Preuss. Akad. Wiss. Berlin*, XLI. p 1034. 1901.

reaches a certain point. In this case, owing to the feeble chemical potential of the colloid, the first reaction by the law of mass action can take place only when the salt concentration reaches the point when the new compound is thrown out of solution. Precipitation of proteids by hydrolysed salts, such as ammonium sulphate, sodium acetate, and the salts of the heavy metals perhaps are examples.

The simple salting out of proteids so far as the imperfect knowledge which is available goes, is similar to the separation of a solid phase when ammonium sulphate is added continuously to a solution of potassium sulphate. The solid phase is a continuously varying solid solution of the two salts, therefore the composition of both fluid and solid phases alters continuously with addition of either component. There are no breaks in the curve. Both Pauli's papers and Osborne's[1] recent paper on globulins give indications however that when salts of the heavy metals are used the concentration curve shows breaks which would correspond to a change in the nature of the solid phase such as is seen for instance when lead iodide and potassium iodide in solution are in equilibrium with a solid phase. The latter according to the composition of the fluid phase is lead iodide, or potassium iodide, or a double salt[2].

In an earlier paper[3] I used the adjective "reversible" to distinguish certain colloidal systems such as gelatine-water from systems such as silica-water because in the former case gelation occurs with a fall of temperature, and is reversed by a rise of temperature. The word "reversible" was unfortunate in the first instance, since the last thing one would say of colloidal systems is that they undergo changes of state which are reversible in the strict physical sense of the word, but no other word equally convenient suggests itself. Pauli has applied the word in quite another way, and unless the point is cleared up confusion will arise.

When a new state of matter is formed in a system it may be said to be irreversible with respect to any one of the factors which produced it, which are the relative masses of the components, temperature, and pressure. A solid phase for instance may become again miscible with a fluid phase owing to a rise or fall of temperature, in which case the state is reversible with respect to temperature. I used the word reversible

[1] *American Journ. of Physiol.* 1905.

[2] Schreimenakers. *Zeits. f. physik. Chem.* x. p. 467. 1893.

[3] *Proc. R. Soc.* LXVI. p. 95. 1900.

originally only in this case. The solid phase may disappear when any one of the components is added, or removed, as when globulin is redissolved by the addition of water, or of salt. Pauli describes precipitates of proteids as being reversible or irreversible solely with reference to one component—water, though amongst the cases which he has studied are instances of irreversibility with respect to water, reversibility with respect to either of the other components salt and proteid: and the work of others from Alex Schmidt downwards shows that many of his cases of irreversible precipitates may be expected to be reversible with respect to temperature.

Preparation of the globulin. Except where it was otherwise stated globulin from ox serum prepared as follows was used.

The serum was centrifuged to free it from particles, diluted ten-fold, and acidulated with acetic acid. The copious precipitate was purified by being twice dissolved in alkali (usually ammonia) and precipitated by acid (usually acetic) or *vice versâ*. To free it from excess of adherent salts it was suspended in water centrifuged off, resuspended, collected on a hardened filter and washed with water. Sometimes prolonged dialysis was resorted to. Toluol and camphor were tried as disinfectants, and the dialysis was carried out in closed vessels. Water saturated with the former seems to be without action. Camphor acts on alkali globulin, producing an insoluble modification; it seems to be without action on acid globulin. Where it was specially important to follow the washing out of the salts, hydrochloric acid was used in the preparation in place of acetic as a precipitant, and the wash-water tested for chloride.

Acid and alkali globulin.

The point of solution by acids or alkalies is indeterminate. In the change from the mixture of globulin suspended in water to a brilliant transparent solution, or backwards from a brilliant solution to a suspension a continuous series of events occur which make it impossible to say where solution is complete.

The reverse change, clear solution towards suspension, may be produced by dialysis. When the acid or alkali is monovalent the solution gradually changes towards whiteness until it becomes opaque. At the same time no precipitate falls and, in the absence of any disturbing factor dialysis alone will not precipitate the globulin.

For example—some globulin was dissolved in acetic acid to a clear transparent solution; concentration of acid, 0·006 equivs. per litre. It was dialysed in a closed vessel against four successive lots of boiled distilled water which in each case were allowed to remain until equilibrium between dialysate and dialysee had been approximately reached.

	Acidity of dialysate gr. equivs. per litre	Condition of the dialysee
0.	0·006	Limpid transparent solution.
I.	0·0023	Transparent: light opalescence.
II.	0·00095	Translucent: white opalescence, smoky amber by transmitted light.
III.	0·00025	Opaque white: objects still visible through thin layers (0·5 cm.) if strongly illuminated.
IV.	0·00012	Completely opaque milky fluid, still no precipitation.

No. IV endured without precipitation for 20 days when the equilibrium was destroyed by moulds appearing and the globulin was thrown down *en masse*.

Similar phenomena are seen when strong acids such as HCl, or alkalies such as KOH, NaOH, or NH_4OH are used.

It follows from the above that the grade of solution which a given quantity of solvent will produce depends primarily upon the ratio mass of globulin to mass of acid or alkali. The effect of the degree of dilution, that is to say of the mass of the third component, water (freed from gases), on the grade of the solution is too slight to be detected when the solution is one of alkali globulin, and it is almost negligible for solutions of globulin in strong acids. Thus the continuous addition or subtraction of either component produces a continuous series of changes with no break corresponding to the separation of a second phase. As a special case take the removal of the component water by evaporation; at no point is there a separation into two phases, the components are miscible in all degrees. Dry globulin, dried *in vacuo* over sulphuric acid and caustic potash swells to a jelly in presence of exceedingly dilute alkali or strong acid, and the jelly is miscible with the fluid in all degrees.

As the ratio of the mass of acid or alkali to the globulin is reduced and the grade of the solution diminished its stability also is diminished, a smaller and smaller quantity of acid or alkali, as the case may be, or neutral salt being needed to destroy it.

The statement that no second phase separates needs qualification. The change from transparency to opacity means the appearance within the fluid of particles of the order of at least 10^{-6} cm. in size and which therefore are defined by a surface. They are not of molecular dimensions and they constitute a phase which separates in a special way in that it never aggregates to form a separate layer. It has long been known that in the separation of a new phase from a fluid the first stage is a labile state, in which the new phase appears as minute spheres condensed on to some nucleus; this is true even when each sphere will ultimately give rise to a crystal. The characteristic of the globulins, and probably of very many hydrosols, is that the labile stage of crystalloid solution

becomes subpermanent, the condition being in all probability one of false equilibrium.

Looked at in this way the change of state due to the continuous addition or subtraction of either acid or alkali or globulin is a continuous change in the composition of *two* phases, a fluid phase poor in globulin, and an internal fluid or solid phase rich in globulin. Both phases vary continuously, as do for instance the fluid and solid phases when either ammonium or potassium sulphate is added continuously to a system composed of both salts and water. The solid phase which separates is a continuously changing mixture of the two salts to which the name solid solution has been given.

Now it is possible to speak of the solution of acid in alkali globulin as being composed of two continuously varying solutions or of two continuously varying compounds, and the latter is the better because it takes account of a number of purely chemical phenomena such as reaction to indicators, hydrolytic reaction with the water, or electrolytic dissociation, which occur.

The system differs from a mixture of the gases ammonia and hydrochloric acid since though the latter in the solid state combine to form NH_3HCl the gas system composed of reacting weights (NH_3+HCl) does not contain the compound $NH_3.HCl$. Acid and alkaline globulins show by neutralisation and other phenomena that throughout there is true combination, though when the compound is between globulin and a weak acid the condition of free globulin and free acid, similar to $NH_{3_{gas}}+HCl_{gas}$ is realised at great dilution.

In the system two salts and water when a solid phase appears, the equilibrium over a certain range of concentration is usually between a fluid phase and a definite double salt (*e.g.* potassium and silver nitrates, calcium sulphate and potassium sulphate, silver chloride and sodium chloride), and the two phases do not vary continuously. When globulin is dissolved by the bibasic acid, sulphuric acid, a similar discontinuity is found. Removal of the acid by dialysis leads to the formation of a solid phase which separates completely as a precipitate and which appears to be a special insoluble compound of acid and globulin. After washing with water it is found to be insoluble by neutral salts, that is to say the special globulin feature is lost. It is, however, soluble by dilute alkali, and the globulin can then be recovered by neutralisation with its original properties unchanged.

The foregoing considerations makes it clear that to define the globulin system two curves are needed, one for the changes of com-

position of the external fluid, the other for the internal non-settling or settling phase, the two curves being those to which Roozeboom gave the names respectively of "liquidus" and "solidus[1]."

The solvent powers of various acids and alkalies. In order to measure the different solvent power of reagents two methods were employed, namely the addition of minimal amounts of acid or alkali to a suspension, which after standing for some time was centrifuged to get rid of the excess proteid, or the matching of solutions, taking care to allow for the depth of the layer, and to make the final comparison at the same concentration of the proteid.

In the second case one measures the amount of solvent needed to produce an arbitrary grade of solution at volume V. The first case is more complex, since it includes the distribution of acid and proteid between a fluid and a precipitate when the reacting masses are the same. The grade of solution in this case may differ very widely, being perhaps opaque white in the one case and transparent opalescent in the other.

By the second method some 250 measurements were made at temperatures from 2° to 30° and at concentrations from 0·28 to 4·18 dry globulin per 100 c.c. with 9 different specimens of globulin.

If the mean value for the amount of acid required to dissolve 1 gram of dry globulin is given in terms of HCl = 1 we have:

HCl	1·0	H_2SO_4.	1·91	Citric	3
HNO_3	0·995	Tartaric	1·994	H_3PO_4	2·9
$CHCl_2COOH$	1·0	Oxalic	1·9	H_3BoO_3	very great excess
CCl_3COOH	1·0				
$CH_2ClCOOH$	1·05				
HCOOH	1·25				
CH_3COOH	5·2				
CH_2CH_3COOH	7·56				

The values for strong monobasic acids are approximately the same within the limits of error. The first four acids are strong acids, and the value is that of HCl. Monochloracetic acid is rather less than half the strength of these, but the slightly diminished solvent action is within the limits of error. In the last three, which are less than a tenth the strength, the fall in solvent power is decided. As the dilutions employed were of the order of 0·005 normal all the acids are strong acids with the exception of the fatty acids.

[1] Cf. Heycock and Neville, "On the constitution of the copper-tin alloys," *Phil. Trans.* A. 202, R. 1. 1903.

	μ_v	0·006 N. μ_∞	t	$\frac{\mu}{\mu_\infty}$
HCl	3654	3672	18	
HNO_3	3636	3665	18	>0·99
CCl_3COOH	3570	3560	25	
$CHCl_2COOH$	3300	3584	25	0·92
$CH_2ClCOOH$	1550	3603	25	0·43
HCOOH	673	3740	25	0·180
CH_3COOH	207	3610	25	0·057
CH_2CH_3COOH	193	3560	25	0·054
H_2SO_4	3190	3552	18	0·898
Oxalic	1680	3500	18	0·480
Tartaric	850	3500	18	0·243

For the strong acids the solvent power is a molecular function,

$$HCl = H_2SO_4 = H_3PO_4;$$

and if solution takes place through salt formation the salt is $GHCl$, GH_2SO_4, or GH_3PO_4.

This result agrees with that of Bugarsky and Liebermann[1] for combination of egg albumen or albumose with HCl, and as they point out, it resembles the combination of NH_3 and HCl to form NH_4Cl.

This type of combination in which molecules combine together without replacement is characteristic of the amido acids which react with other acids as basic anhydrides,

$$CH_2\begin{cases}NH_2\\CO_2H\end{cases} + HCl \quad = \quad CH_2\begin{cases}NH_2HCl\\CO_2H\end{cases}.$$

The values for solution by alkaline bases show the same interesting relation. Taking the value for NaOH as unity we have

KOH	1
NaOH	1
NH_4OH	0·98
$Ba(OH)_2$	2·008

Aniline, Urea } dissolve; relation not measured quantitatively.

Here again the molecule of $Ba(OH)_2$ has the same solvent power as the molecule of KOH, NaOH, and NH_4OH. But the series differs from the acid series in the fact that the base NA_4OH which is about as weak as acetic acid has the same solvent power as the strong bases.

Aniline in aqueous solution readily dissolves globulin. Urea only with difficulty and at high concentration. It can be compared to

[1] *Pflüger's Arch.* LXXII. p. 51. 1898.

boracic acid, which also dissolves globulin but only in saturated or nearly saturated solutions. Picric acid is a good solvent of globulin, but a secondary change rapidly sets in which leads to precipitation, the precipitate being resoluble in excess alkali. Whatever the strength of acid or base (urea and picric acid alone were not tried) the effect of solution is that the globulin becomes electrically active and moves in a field as though true salt formation had occurred. That is to say, dissolved by acid it is positive ($GS \rightleftarrows G' + S'$), dissolved by alkali it is negative ($GB \rightleftarrows G' + B'$). It behaves like an amphoteric electrolyte.

Gravimetric determination of the solubility of globulin was made for HCl, H_2SO_4, and H_3PO_4 in order if possible to confirm the molecular relation described above, $G = HCl = H_2SO_4 = H_3PO_4$. The conditions are complex owing to the presence of the solid phase. The grade of solution is not the same, that of hydrochloric acid being much higher (more transparent, less opalescent) than the others. The mass of globulin retained in solution by equivalent weights of the acids depends not only upon the concentration of the acid but also upon the concentration of the globulin. It is not sufficient to define it as being in excess. There must be a partition of the acid between the globulin in solution and the globulin in the precipitate and one is measuring the distribution of the globulin between two solutions, the one poor, the other rich in proteid, which are separated under the stress of centrifugal force. The latter factor cannot be neglected, as van Calcar and Lobry de Bruyn[1] have shown that the composition of solutions of ordinary crystalline bodies such as sodium sulphate is altered by centrifuging. The four groups of measurements which were made agree in showing an approximation to the molecular solvent power already noticed.

Globulin 2·721 grs. per 100 c.c.

vol. in lit. of 1 equiv. of acid	HCl	H_2SO_4
1333	0·0084	—
1000	0·0186	0·0074
500	0·0775	0·0183
383	0·0939	—
328·6	0·0979	—
250	—	0·0479

The figures show the grammes of dry globulin in the solution per 1 c.c. $\frac{N}{100}$ acid added.

[1] *Rec. Trav. Chim. Leiden*, XXIII. p. 218. 1903.

A similar comparison of HCl and H_3PO_4 when equal amounts of $\frac{N}{100}$ HCl and $\frac{3N}{100}$ H_3PO_4 were run in gave:—

1 c.c. $\frac{N}{100}$ HCl = 0·0245 grs. 1 c.c. $\frac{3N}{100}$ H_3PO_4 = 0·0254 grs.

The differences in the solvent power of the various acids and alkalies can be explained by assuming that the proteid compounds undergo hydrolytic dissociation in the way so well discussed by Osborne[1] for caseinogen.

According to the well-known theory of hydrolysis the fraction hydrolysed will be greater the weaker the acid, and will be diminished by an excess of acid, the excess needed being smaller the stronger the acid. Therefore, in order to reduce opalescence to a minimum, the acid required is the amount necessary to combine with the globulin plus the excess necessary to depress hydrolytic dissociation. Call these amounts respectively m and n, then for a strong acid $m+n$ will not differ largely from m in value, for a weak acid $m+n$ will be much larger than m, and this is what the figures show. In the case of an acid so weak as boracic acid n is so large compared with m as to lower the solvent power to a very low level.

The effect of volume on the amount of acid necessary to dissolve, also can be explained by the theory of hydrolysis.

I take as the unit the gram equivalent $\times 10^{-5}$ and express in this unit the amount of acid necessary to dissolve 1 gram dry globulin.

Conc. proteid grs. per 100 cc.	$H\overline{A}$	HCl
0·28	183	23
1·46	56	15
4·18	40	13

On the theory of hydrolytic dissociation the fact that the weak alkali NH_4OH has the same solvent power as KOH, and NaOH can only mean either that the ammonium proteid compound is not ionised or that globulin is a much stronger acid than base. As we shall see, the electric conductivity shows that NH_4OH globulin conducts rather better than NaOH globulin, therefore the proteid must be a stronger acid than base, and this conclusion is supported by other observations.

In comparing the solvent power of acids and alkalies comparison is only possible for the strong acids.

Acids are always feebler solvents than alkalies.

[1] This *Journal*, xxvii. p. 398. 1901.

The actual figures are (as mean values) in the same units (equivs. $\times 10^{-5}$ to 1 gr. proteid).

NaOH, KOH NH_4OH $C_2H_3NH_3OH$	10	HCl, HNO_3 CCl_3COOH $CHCl_2COOH$ $CH_2ClCOOH$	18

There can be I think little doubt that the true ratio is 10/20, since the method of comparison being an optical one, the volume correction would increase the difference actually observed.

The degree of hydrolysis, that is the fraction of a salt split into free acid and free base by interaction with water, usually increases with rise of temperature. Osborne[1] used this temperature relation to support the view that the opalescence of certain compounds of caseinogen is due to liberation of the proteid by hydrolysis. The opalescence of caseinogen solutions increases with a rise of temperature, as it should do according to theory. But the opalescence of solutions of globulin diminishes with rise of temperature, even when the solvent is a weak acid. The amount of acid required to produce a given grade of solution therefore *falls* with a rise of temperature.

The change of state from 2° to 40° is not very great, and probably complex. If it were due to simple change in the degree of hydrolysis the opalescence should return on cooling. This it does either very slightly or not at all.

Globulin in combining with molecules and not equivalents of acids behaves like the amido acids; *i.e.* it acts like an anhydrous base. But judging from the solubility data it reacts also with the molecule of a base, *i.e.*

$$G = NaOH = Ba(OH)_2.$$

That is, it reacts as though it were an acid anhydride analogous to CO_2 in the hypothetical equation:

$$CO_2 + NaOH = NaHCO_3.$$

So far as I know no instances of such a reaction have ever been detected. The behaviour of globulins with indicators furnishes another suggestion.

Acid or alkali globulin and indicators. Freshly precipitated and washed globulin or globulin dried *in vacuo* in presence of H_2SO_4 and KOH reacts acid to thoroughly dialysed litmus, gives no colour with phenolphthalein, and only very slightly depresses the orange tint of

[1] *loc. cit.*

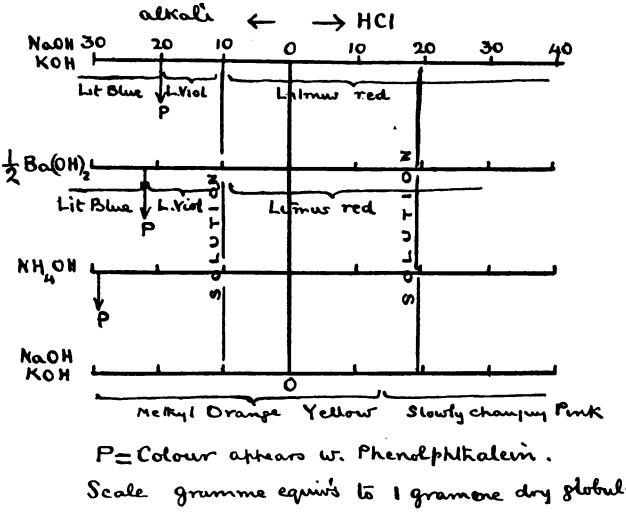
methyl-orange. The acid reaction with litmus might be due to CO_2, or to other acids held by the proteid.

To test this globulin, dissolved in NH_4OH and precipitated by HCl three times, was washed at 5° until the wash water was free from chlorine, and the proteid when dissolved in large excess of HNO_3 gave no clouding with silver nitrate. It was then dried and kept *in vacuo* over H_2SO_4 and KHO for some weeks. Distilled water free from CO_2 coloured with litmus was turned red on addition of the solid *in vacuo*. A solution of $Ba(OH)_2$ was made neutral to phenolphthalein by it *in vacuo*.

A strong alkali such as NaOH even in $\frac{N}{100}$ solution gives a fairly sharp neutral point with globulin, two drops excess giving decisive colour with phenolphthalein.

Methyl orange behaves in a specially interesting way. In presence of a suspension of globulin it is orange, on addition of $\frac{N}{100}$ HCl or H_2SO_4 drop by drop it turns at first bright canary, that is it sways over to the *alkaline* side, further addition of acid finally gives pink.

The relation of the point of maximum insolubility of the globulin to the reaction with indicators can be shown diagrammatically.



In the diagram, solution means the point at which opalescence is minimal. Now it is remarkable that in order just to neutralise globulin

to phenolphthalein with the strong bases KHO or NaOH the amount of alkali needed is approximately twice that required to dissolve the proteid. On the other hand, the point of solution and of neutrality to the same indicator coincide for $Ba(OH)_2$. Therefore for the monovalent alkalis there are two values, one the amount necessary for solution (of the order of 10×10^{-5} equivs. per 1 gram globulin), the other of the order 20×10^{-5} necessary to produce excess OH ions.

On the hypothesis of salt formation these facts suggest that globulin has at least two replaceable hydrogens, and that its acid salts of sodium or potassium are soluble, while its acid salt of barium is relatively insoluble, the neutral salt being soluble and neutral to litmus and phenolphthalein.

With a weak base, such as ammonia, the point of solution remains the same, but the point of neutrality to phenolphthalein is much more indeterminate but clearly beyond that for the strong bases.

The evidence which indicators furnish of the combination of globulin with alkalis being due to replaceable hydrogen is strengthened by other experiments.

In our ignorance of the effect of dilution upon solution of the colloid type one is chary of ascribing much weight to coincidences between values obtained for molecular conductivity and those given by ordinary solutions. If however colloidal solution be an emulsion, one is reassured by the remarkable fact that in partially miscible liquids near the critical point, though internal friction and some optical properties show abnormalities, electric conductivity data are normal[1]. Now alkali globulin compounds ionise freely, as is shown by the fact that the conductivity of the weak base NH_4OH is raised by the addition of globulin (Table, p. 276). Therefore it is a remarkable coincidence that the values for $\mu_{32 \times 32} - \mu_{32}$ for NaOH just neutralised with globulin gives Ostwald's characteristic value for a dibasic acid.

Two determinations at 18° gave respectively

$\mu_{32 \times 32}$	μ_{32}	diff.
55·3	34·4	20·9.

But, as Säckur[2] points out, with such low values for μ the simple difference cannot be used, but comparison must be made between the ratio of the differences to the value at ν_∞. By this method,

[1] Friedländer. *Zeits. f. physik. Chem.* XXXVIII. p. 385. 1901.

[2] *Zeits. f. physik. Chem.* XLI. p. 672. 1903.

where the values of $\frac{\mu_1 - \mu_2}{\mu_1}$ are compared, globulin comes out as five basic.

Whatever the significance of the molecular relation between globulins and alkalis it is fundamental so far as proteids are concerned, since it is exhibited also by acid and alkali albumen derived from egg-white and freed from electrolytes by prolonged dialysis, using the same units, equivalents $\times 10^{-5}$ needed for 1 gram dry proteid.

Acid albumen from egg-white (·475 grs. per 100 cc.).

	Solution	Faint pink with phenolphthalein
NaOH	27·3	57
NH_4OH	29	100
$Ba(OH)_2$	57	65

Alkali albumen from egg-white (·363 grs. per 100 cc.).

NaOH	36
NH_4OH	37
$Ba(OH)_2$	77

Measurements of the acid and basic function of globulin.

In order to get additional evidence as to whether globulin is a stronger acid than base I measured the degree of hydrolytic dissociation of the HCl and of the NaOH compound by the velocity of catalysis of cane sugar and of methyl acetate respectively.

Inversion of cane sugar. A suspension of ox-globulin containing 6·296 grs. dry globulin per 100 c.c. was used and the following solutions made:

(i) Globulin, HCl, cane sugar.

(ii) Water ,, ,, ,,

(iii) Water with globulin ash dissolved in it, HCl, cane sugar.

(iv) Globulin, HCl, and water in place of the cane sugar solution.

Acid and globulin were present in ratio 1 gr. globulin $= 9{\cdot}53 \times 10^{-5}$ equivs. acid.

Concentration of HCl 0·001837 equivs. per litre or $\frac{N}{544{\cdot}4}$,

,, ,, globulin 19·275 grs. per litre,

,, ,, sugar 8·2 %.

Solution (iii) was a control to determine the effect of the salts of the globulin on the velocity; 30 c.c. of the suspension was evaporated to dryness, carefully ashed and the ashes taken up with 30 c.c. water. This was used in making up solution (iii) in the same volume as the actual suspension in numbers (i) and (iv).

The four solutions were kept at 20 in closed flasks, the disinfectant being the hydrochloric acid present, and a trace of toluol.

At the outset the amount of NaOH $\frac{N}{100}$ necessary to produce maximal precipitation of

the proteid was determined, and the proteid was removed in this way from some of (i) and (iv) to get initial readings.

After 140 hours solution (iv) *i.e.* the control, was precipitated by $\frac{N}{100}$ NaOH, and the precipitated proteid filtered off. The filtrate gave practically no rotation.

The proteid therefore was removed from solution (i) in the same manner by the addition of $\frac{N}{100}$ NaOH: the same volume of NaOH solution being added to numbers (ii) and (iii).

It was found that the amount of alkali needed to precipitate the proteid increased remarkably in the flasks with sugar present (i) but remained unchanged in the flasks with no sugar (iv).

In the former it was abnormally *low* at first, complete precipitation being produced by alkali equal to one-third the acid added, but after 140 hours the amount of alkali necessary slightly exceeded the amount of acid originally added to dissolve.

	a_0	a_n	A	Constant
(i)	+11·039	+10·815	−3·753	$79{\cdot}4 \times 10^{-8}$
(ii)	,,	+10·215	,,	$2{\cdot}96 \times 10^{-8}$
(iii)	,,	+10·725	,,	$111{\cdot}7 \times 10^{-8}$

This gives two values for the amount of "free" acid according to whether comparison is made with HCl alone, or with HCl and globulin salt ash. The former gives 26·83 % of the HCl free, the latter 71·1 %. As the change of state of the proteid in presence of acid and sugar introduces an unknown error these figures can be taken only as indicating in a general way that the proteid is a weak base and that therefore a large fraction of the compounds with acids undergo hydrolytic dissociation in aqueous solution.

Catalysis of methyl acetate. Globulin purified by twice being dissolved in ammonia, and precipitated by HCl, then washed at 5° until filtrate gave no chloride with silver nitrate: dried *in vacuo* over H_2SO_4 and KHO.

Made into suspension with distilled water and kept as far as possible free from CO_2.

Three previous attempts to measure the constant showed that to obtain readable values and to take advantage of its antiseptic action it was necessary to have the methyl acetate present in a molecular concentration in excess of the NaOH.

The catalysis was carried out in a number of flasks at 20 and in order to obtain the constant for NaOH alone the same relative concentrations of alkali and ester were employed as in the globulin holding flasks.

Conc. globulin 1 litre = 25·76 grs.,
,, NaOH = ·0044 normal,
1 gr. globulin = $17{\cdot}127 \times 10^{-5}$ equivs. NaOH.

The NaOH used was freshly prepared from metallic sodium, and the globulin was dissolved in it to a point just short of giving colour with phenolphthalein, but in excess of the amount needed to dissolve (1 gr. = 10×10^{-5} equivs. NaOH). That is to say the point is as near as possible to the hypothetical neutral salt GNa_2.

Conc. methyl acetate 0·02395 gr. mols. per litre.

The fundamental equation for the velocity of the decomposition of the ester is

$$-dC = KC\,(C+s)\,dt^1,$$

where C is the concentration of the alkali and $(C+s)$ the concentration of the ester.

[1] Arrhenius. *Zeits. f. physik. Chem.* I. p. 115. 1887.

The solution of this equation given by Arrhenius and ordinarily used is an approximation which is valid only when S is very small compared to C. In my measurements S is large compared with C and therefore it was necessary to use the general logarithmic solution,

$$K=\frac{1}{s}\times\frac{1}{t_n-t_1}\log_e\left(\frac{C_n+s}{C_1+s}\times\frac{C_1}{C_n}\right).$$

I obtained a mean value for K for NaOH of 9·9 at 20°, Hantzsch[1] measured this constant at 25° and found 10·2.

The constant for the NaOH proteid was calculated by the equation given by Shield[2]:

$$K=\frac{\frac{c_2}{c-c_2}\log_e\frac{c_2-x_0}{c_2-x_1}-\frac{c}{c-c_2}\log_e\frac{c-x_0}{c-x_1}}{K(t_1-t_0)}.$$

K is the constant for NaOH; c conc. of ester, c_2 the conc. of the NaOH proteid is reckoned as the normality of the NaOH present, x is the amount of ester decomposed in time (t_1-t_0) reckoned in minutes. Concentrations are given in hundredths of a gram molecule per litre.

For the purpose of calculation the above equation is more useful in the form with Briggs' instead of natural logarithms:

$$K=\frac{c}{0\cdot4342945\times K\times(c_2-c)}\left[\frac{\frac{c_2}{c}\log_{10}\frac{c_2-x_1}{c_2-x_0}-\log_{10}\frac{c-x_1}{c-x_0}}{t_1-t_0}\right].$$

Titrations were carried out with 0·01 normal $Ba(OH)_2$ and phenolphthalein. That is to say one directly measured the amount of acetic acid produced by decomposition of the ester (methyl acetate + water = acetic acid + methyl alcohol) as the difference between titre 1 and the following titres in succession.

t	c_2-x	x	$c-x$	K
1490	·382	·058	2·337	388×10^{-9}
2665	·404	·036	2·359	333×10^{-9}
4024	·390	·050	2·345	399×10^{-9}
5644	·355	·085	2·310	$[853\times10^{-9}]$

The last value at $t=5644$ obvious bacterial decomposition had set in. The ester is a good antiseptic, and until it is largely decomposed bacteria do not seem to make headway. Considering the difficulty in judging the end point of the titration owing to the opalescence of the solution the agreement in the values for K are satisfactory.

From the mean value of K one finds that 0·288 % of the NaOH present is in the free form, that is to say, only 0·288 % of the globulin NaOH compound is decomposed by the water.

The amount of NaOH present was, as I have said, in excess of the amount necessary to dissolve the proteid and near to the hypothetical "neutral" salt, therefore it is interesting to see that the degree of hydrolysis agrees with that of Na_2HPO_3 when Shield's value for the latter is corrected for dilution by the effect of volume on the degree of hydrolysis in other salts.

If this value is correct therefore globulin acts as an acid of a strength not far inferior to phosphoric acid.

[1] *Ber. d. d. chem. Ges.* XXXVII. p. 3, 3066. 1899.
[2] *Phil. Mag.* (5), XXXV. p. 365. 1893.

These results show that acids react with globulins to form soluble compounds in molecular and not in equivalent ratios, but that the relation is obscured in the case of weak acids owing to the high degree of hydrolytic dissociation which occurs.

Alkalies also react to form soluble compounds in molecular and not in equivalent ratios, and owing to the stronger acid function of globulin the relation is less obscured by hydrolytic dissociation, so weak a base as ammonia not being displaced in the series.

The simplest interpretation of these relations would be that globulin compounds are:

$$G + HS = GHS \quad \text{or} \quad G + BOH = GBOH.$$

The first is similar to the manner in which acids combine with the amido-acids to form salts

$$RNH_2.COOH + HS = RNH_2COOH.HS.$$

No analogy can be found for the latter equation.

In this difficulty the reaction with indicators suggests that globulin has a series of replaceable hydrogens and that it can therefore form acid salts, the acid salt of barium being relatively insoluble. This would account for the facts and yet leave open the possibility of combination by replaceable hydrogen,

$$GH_n + NaOH = GH_{n-1}Na + HOH.$$

THE ELECTRIC CONDUCTIVITY OF SOLUTIONS OF ACID AND ALKALI GLOBULIN.

Measurements of conductivity were made, except where it is otherwise stated, at 18°. Platinum terminals coated with platinum black in the ordinary way are not suitable for work with proteids; the platinum itself, or substances absorbed by it, cause changes in the proteid such as to raise the conductivity. This source of error is avoided by heating the coated terminals to red heat as recommended by Whetham, the platinum black being thereby at once changed to a fine grey deposit.

The units employed in the following pages are ohms and reciprocal ohms, and specific molecular conductivity or μ, following the plan in the British Association Tables, is given in C.G.S. units $\times 10^{13}$. K is used to designate specific conductivity, R specific resistance.

A suspension of a globulin always has a conductivity which, though low, is large enough relative to the conductivity of solution in acids or alkali to form an important correction. As an instance of the relative values to be dealt with the following will suffice.

A suspension holding 7·737 grs. globulin per 100 c.c.

Part was dried and charred, the char extracted with water, the char ashed at low red heat and the ash taken up with water so that the final volume of electrolytes of char and ash was that of the original suspension.

18° $K_{\text{suspension}}$ 31×10^{-6} K_{ash} 20×10^{-6}

Various alkalies as 0·1 solution were added to dissolve the globulin,

1 gr. globulin = $10{\cdot}34 \times 10^{-5}$ equivs.,

and gave the following values corrected for the conductivity of the water used [3×10^{-6}].

K_{NaOH}	$288{\cdot}5 \times 10^{-6}$	μ 389	$\mu_{\text{corr.}}$ 352
$K_{\text{NH}_4\text{OH}}$	390 $\times 10^{-6}$	μ 526	$\mu_{\text{corr.}}$ 489
K_{HCl}	$409{\cdot}7 \times 10^{-6}$	μ 553	$\mu_{\text{corr.}}$ 515
$K_{\text{H}\bar{\text{A}}}$	$183{\cdot}4 \times 10^{-6}$	μ 283	$\mu_{\text{corr.}}$ 243

μ is the value obtained directly from the specific conductivity; it is $\frac{K}{c}$.

$\mu_{\text{corr.}}$ is the value corrected by subtracting $K_{\text{suspension}}$, or

$$\frac{K - K_{\text{suspension}}}{c}. \qquad c = 0{\cdot}00741.$$

The correction for the conductivity of the globulin alone cannot be neglected, it is doubtful how it should be employed. If $K_{\text{suspension}}$ represents undecomposed proteid compound either the observed conductivity or the sum $(K + K_{\text{susp.}})$ would be nearest to the true value; if it be due to absorbed salts then observed conductivity minus the conductivity of the suspension is probably nearest to the true value. I have chosen the latter alternative and the values for μ given are $\frac{K - K_{\text{suspension}}}{c}$ except when it is otherwise stated.

In determining the effect of volume upon conductivity, therefore, two sets of measurements are used, one of the globulin compound at certain degrees of dilution, the other of the suspension at the same dilutions. The corrected conductivity is the difference.

HCl globulin. 1 gr. globulin = $9{\cdot}318 \times 10^{-5}$ equivs. acid. 18°.

ν is the volume in litres of 1 gr. equivalent.

ν	107·2	[135]	216·9	433·8	867·6	1735	3470
μ	439	[515]	596	705·6	834	991	1167

NaOH globulin. 1 gr. globulin = $9{\cdot}75 \times 10^{-5}$ equivs. alkali. 18°.

ν	[32]	152·2	304·36	608·7	1217	2435	4869
μ	[320]	375·6	408·8	463	523·7	592	748

NaOH globulin. 1 gr. globulin = 18×10^{-5}.

ν	32	64	128	256	1024
μ	334	401	451	484	549

The values in brackets do not belong to the series, they were made at different dates and on different samples of globulin and in both the ratio globulin to acid was different:

$[\mu_{135}]$ HCl proteid. 1 gr. globulin = $10{\cdot}34 \times 10^{-5}$ equivs.

$[\mu_{32}]$ NaOH „ „ „ = 10 $\times 10^{-5}$ „

μ_{32} is difficult to obtain. The globulin solution at this concentration, and indeed up to and beyond ν_{107}, is as viscid as stiff treacle. Accurate measurements of volume therefore are practically impossible. A comparison of weights and volumes showed that the method adopted might give a maximal error of 1·8 %.

These values are plotted in curves (Fig. 1).

They are completely uncorrected for variations of viscosity—therefore it is remarkable that the curve of NaOH proteid should be of quite ordinary slope. The curves for the three phosphates of sodium are plotted alongside for comparison.

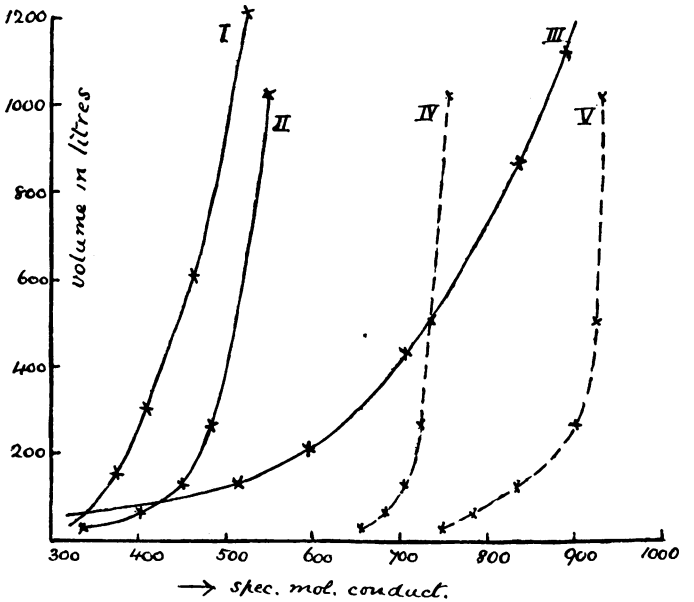


Fig. 1. Ordinates, the volume in litres occupied by 1 equivalent. Abscissæ, the specific molecular conductivity (μ) in c.g.s. units $\times 10^{13}$ at 18°. I. NaOH globulin. 1 gr. globulin $= 9{\cdot}75 \times 10^{-5}$ equivs. II. NaOH globulin. 1 gr. $= 18 \times 10^{-5}$ equivs. III. HCl globulin. 1 gr. $= 9{\cdot}32 \times 10^{-5}$ equivs. IV. NaH_2PO_4. V. Na_2HPO_4.

The table on p. 284 shows that in these solutions viscosity rises rapidly about $\nu = 110$. Botazzi[1] found that at a certain concentration of casein (8 %) and egg albumin (± 10 %) viscosity rises to an "enormous value." It is therefore remarkable that the curves should show so even a sweep. The fact is in agreement with the observation of Friedländer[2] already quoted that in partially miscible liquids near the critical point conductivity is normal, viscosity abnormal.

The chief point in a comparison of the curves for μ-volume is that

[1] *Arch. Ital. de Biol.* XXIX. p. 420. 1898.

[2] *Loc. cit.*

the molecular conductivity increases with increase of volume much more for HCl globulin than for NaOH globulin owing to the weaker basic function of the globulin. The molecular conductivity of a hydrolysed salt is given by the well-known equation

$$M_\nu = (1 - x)\,\mu_\nu + x\mu_{\text{HCl: NaOH}},$$

in which x is the fraction hydrolysed, μ_ν the molecular conductivity of the undissociated salt, and $\mu_{\text{HCl: NaOH}}$ the molecular conductivity of the free acid or base. Therefore as x increases with dilution and $\mu_{\text{HCl: NaOH}}$ is always greater than μ_ν owing to the high ionic velocity of $H^\cdot$ and OH, it follows that M_ν must increase with dilution the more rapidly the greater the degree of hydrolysis.

The slope of the curve for NaOH proteid is incompatible with any but a slight degree of hydrolysis: that for HCl proteid might signify a considerable degree of hydrolysis.

A comparison of the effect of globulin upon the conductivity of various acids and alkalies, in other words a comparison of the value for $\frac{K_{\text{salt}}}{K_{\text{acid}}}$ or $\frac{K_{\text{salt}}}{K_{\text{alkali}}}$, very clearly points to a stronger acid than basic function.

K_{salt} and K_{acid} are uncorrected for conductivity of the globulin suspension

	$\frac{K_{\text{sa.}}}{K_{\text{ac.}}}$ or $\frac{\mu_{\text{sa.}}}{\mu_{\text{ac.}}}$	1 equiv. = ν = litre	grs. prot. per litre	1 gr. = equiv. $\times 10^{-5}$
$\frac{H_3BoO_3}{3}$	0·9	0·72	4·78	29050·0*
C_2H_5COOH	0·84	151·7	4·54	145·2
$H\bar{A}$	0·8	225·6	16·72	23·49
$CH_2ClCOOH$	0·336	436·4	10·81	21·2
$CHCl_2COOH$	0·188	438·7	10·81	21·09
CCl_3COOH	0·191	442·7	10·81	20·9
HNO_3	0·24	453·4	10·81	20·41
HCl	0·24	454·7	10·81	20·3
H_2SO_4	0·18	273·2	8·96	40·8
H_3PO_4	0·22	289·7	12·05	28·63
	$\frac{K_{\text{sa.}}}{K_{\text{alk.}}}$ or $\frac{\mu_{\text{sa.}}}{\mu_{\text{alk.}}}$			
NH_4OH	2·3	1047	9·6	9·8
NaOH	0·29	1217·4	8·41	9·75
,,	0·21	304·36	33·67	9·75
,,	0·257	612·7	14·57	11·2

* This cannot be regarded as a measure of the solvent power of H_3BoO_3. The proteid was in large excess and the greater part precipitated on standing. A rough estimate would be 1 gr. proteid needs 100,000 equivs. to dissolve. The acid was twice purified by recrystallisation. The first column gives the relative specific conductivity of the solution of acid or alkali globulin (K_{salt}) to that of the same concentration of the acid or alkali alone ($K_{\text{ac.: alk.}}$).

The fundamental equation of hydrolysis is:

$$K = K_{\mathrm{HOH}} \frac{K_{\mathrm{salt}}}{K_{\mathrm{alk.}}\, K_{\mathrm{acid}}},$$

where K is the velocity constant of the reaction salt + water = alkali + acid and K_{salt}, $K_{\mathrm{alk.}}$, K_{acid}, are the respective ionisation constants in the Guldberg-Waage equation,

$$KC_{AB} = C_A \times C_B.$$

Therefore the degree of hydrolysis varies directly with the degree of ionisation of the salt (globulin HS or globulin BOH) and inversely with the degree of ionisation of the acid or alkali (HCl, NaOH, and globulin).

Assuming the salts globulin HS and globulin BOH to be ionised to about equal amounts in all cases, the degree of hydrolysis, that is to say the fraction x of HS and BOH in the free state, will be greater the weaker HS or BOH.

Since in the equation

$$\text{globulin HS} + \text{HOH} = \text{globulin HOH} + \text{HS},$$

the chief conducting species will be HS, and globulin HS, the molecular conductivity of the other two being negligible, it follows that the greater the degree of hydrolysis the more closely must the molecular conductivity approach that of the pure acid or pure base at the same concentration. Therefore the weaker the acid or base combined with the globulin the more closely should the value for μ_{salt} agree with $\mu_{\mathrm{acid:\,alkali}}$, and the table shows this to be the case throughout the acid series in which, owing to the globulin being a very weak base, the fraction x hydrolysed is always considerable, and with such weak acids as the first three must approach unity.

The features in the alkaline series again can only be explained by supposing that the globulin salt ionises freely and therefore is a good conductor, and secondly that it is not hydrolysed to any great extent even when combined with the weak base ammonium.

In the equation

$$M_\nu = (1 - x)\, \mu_{\mathrm{salt}} + x\mu_{\mathrm{acid:\,alkali}}$$

it is clear that if $\mu_{\mathrm{acid\ or\ alkali}}$ is less than μ_{salt} the value of M_ν will be reduced by hydrolytic splitting. This is the case with those salts of ammonia which ionise freely and hence are good conductors. $\mu_{\mathrm{NH_4OH}}$ is low at all dilutions, the base being a weak one.

Now the table shows that M_ν is considerably larger than μ_ν.

Therefore the ammonium globulin compound must ionise freely, and it must be hydrolysed to a relatively small extent, and this can be the case only if the globulin functions as an acid of considerable strength.

If NH_4OH globulin ionises freely and hydrolyses slightly, its molecular conductivity should exceed that of the NaOH globulin owing to the ionic velocity of $NH_4^{\cdot}$ being greater than that of $Na^{\cdot}$. A comparison made with the same globulin under identical conditions proved this to be the case.

18°, $\mu = \frac{K}{c}$, $\mu_{corr.} = \frac{K_{observed} - K_{suspension}}{c}$, c = gram equiv. NaOH or NH_4OH per litre.

	NaOH		NH_4OH		HCl	
v	μ	$\mu_{corr.}$	μ	$\mu_{corr.}$	μ	$\mu_{corr.}$
135	389	352	526	489	553	515
251	422	384	575	534	644	606
468	463	407	—	—	715	673

The most striking fact, however, is the difference in the values for $H\overline{A}$ globulin and NH_4OH globulin.

Acetic acid and ammonia have about the same molecular conduction. Therefore, if these globulin compounds behaved in the same way in solution, in the equation for hydrolysis

$$M_v = (1 - x)\,\mu_{salt} + \mu_{acid:\ alkali},$$

the relative values of μ_{salt} and $\mu_{acid:\ alkali}$ would be not far distant. The chief difference would be in μ_{salt} owing to the difference in the ionic velocities of acetion and ammonium ion, their respective values at infinite dilution at 18° being 36 and 67.

Later on we shall see reason to give a velocity of 15 to the globulin ion, therefore, assuming the degree of ionisation to be approximately the same, the ratio would be

$$\mu_{NH_4OH\ globulin} : \mu_{HA\ globulin} = 820 : 510 = 0{\cdot}63.$$

In both cases, owing to the small conductivity of NH_4OH and $H\overline{A}$, μ_{salt} must be greater than $\mu_{acid:\ alkali}$ if the salt ionises freely (as the figures for NH_4OH globulin show it must do) although the velocity of the globulin ion is somewhat low.

Therefore, the greater the degree of hydrolysis in HA globulin as compared with NH_4OH globulin, the smaller will be the relative value of the molecular conductivity of the former. For unhydrolysed salt the probable value of $\frac{\mu_{HA\ globulin}}{\mu_{NH_4OH\ globulin}}$ is as we have seen 0·64. The best values obtained give 0·3 for $\mu_{uncorrected}$ and 0·27 for $\mu_{corrected}$. And

whereas the addition of globulin raises the conductivity of solution of ammonia very markedly (*i.e.* M_v is $> \mu_{alkali}$), the conductivity of acetic acid is lowered ($M_v < \mu_{acid}$) except in certain cases due to impurities, when it is only very slightly raised. No fact which I have met with so impresses me as this high conductivity of NH_4OH globulin. The solution presumably is colloidal, it has a high viscosity as judged by the time of transpiration through a capillary tube, it is opalescent, and the globulin can be recovered from it by precipitation unchanged. Indeed the alkali I have most freely used in the purification of globulin has been ammonia. Yet the globulin ammonia compound has a high conductivity. How high and how close to the molecular conductivity of an ordinary ammonium salt can be seen from the following figures.

Ostwald[1] showed that ionic velocity falls with increase in the number of atoms in the ion. He also showed that as the ion increased in size the further addition of atoms produced less and less effect. The lowest value obtained by him was 29 for an ion of 28 atoms.

The globulin ion contains many hundreds of atoms, but direct measurement proves that its velocity is not excessively low, and in the ammonium compound it must lie between 10 and 20 at 18. Take it as 15. The velocity of NH_4 is 67, therefore, in our units,

$$\mu_\infty = v + v = 150 + 670 = 820.$$

Now the values actually obtained for NH_4OH globulin with different globulins and at different times were:

v	μ	$\mu_{corr.}$
135	526	489
251	575	534
1047	696	
1940	827	

At $v = 1940$ ionisation is usually nearly complete for any ordinary salt and the agreement with the calculated and observed value of μ is very striking.

It is too good—so good as to be suspicious—therefore an attempt was made to determine the magnitude of certain probable errors due to viscosity or electrolytic impurities.

As all the ordinary salts of ammonia conduct much better than the alkali itself, any impurity which combined with the ammonia to form a salt, or even was set free by it, would raise the apparent value of μ. In

[1] *Zeits. f. physik. Chem.* XI. p. 840. 1888.

passing I may notice that exactly the same holds for acetic acid whose conductivity is not raised by globulin. I therefore took a solution of starch of a conductivity higher than that of the suspension of globulin and compared its action on the conductivity of ammonia. I also determined the effect of the ash of globulin on the conductivity of ammonia.

In order to avoid loss by vaporisation of the salt the method adopted was as follows:—A given volume of suspension was dried down at 100, charred at a temperature considerably below red heat and the black carbon extracted with hot water, the carbon was then ashed completely at dull red heat, the process occupying 24 hours, and the ash taken up with water and added to the previous extract. The combined solution was brought to the same volume as the original solution. The final solution I call "ash."

The starch solution used was made from finest potato starch washed by decantation. It gave practically no proteid reactions. It was converted into a paste with boiling water, the "solution" being thick and opalescent.

Conductivity values are given $\times 10^6$.

Globulin suspended in water. 7·74 grs. dry globulin in 100 c.c.

K suspension 30·87. K water. 3.

Starch solution. 1·74 grs. solid in 100 c.c.

K starch 38·6.

K "ash." 20.

These solutions were titrated with 0·1 normal HCl, $H\bar{A}$, NH_4OH, and NaOH, the last being freshly made from metallic sodium, so that the final concentration was 0·0074 normal or $\nu = 135$, $c = 0{\cdot}0074$. At this stage the globulin was dissolved to a transparent opalescent solution, as viscid as a heavy oil, by the alkalies, the solutions in acid were however white and opaque.

1 gr. dry globulin $= 10{\cdot}34 \times 10^{-5}$ equivs.

Control solutions of the same concentration ($\nu = 135$) were made by adding the 0·1 normal solution to water.

Values of μ—those on the left—corrected only for the conductivity of the water, those on the right ($\mu_{corr.}$) corrected for the conductivity of the "ash," starch, or globulin respectively $\left(\frac{K_{obs.} - K_{susp.}}{c}\right)$.

	Water	Ash		Starch		Globulin	
	μ	μ	$\mu_{corr.}$	μ	$\mu_{corr.}$	μ	$\mu_{corr.}$
HA	172·8	191	164	178	131	247	210
HCl	3516	3554	3535	3301	3255	553	515
NH_4OH	122·7	155	123·5	158	111	526	489
NaOH	1961	1892	1865	1350	1301	389	352

How far are these numbers normal? Taking the globulin ion as having a velocity of 15, then we have:

$$NH_4OH \text{ globulin } \nu + \nu = 15 + 67 = 82,$$
$$NaOH \quad \text{„} \quad \text{„} \quad = 15 + 45 = 60,$$

$\frac{60}{82} = 0{\cdot}73$ and $489 \times 0{\cdot}73 = 357$, which is in close agreement with 352,

the value actually found. Similarly the ratio for $\frac{\text{NaOH globulin}}{\text{HCl globulin}}$ should be 0·7 and $515 \times \cdot 7 = 360$, which again does not depart widely from the number found. The HCl value is too high and that would be accounted for by the greater degree of hydrolysis, but the degree of hydrolysis is depressed here by the excess globulin present, beyond the amount dissolved by the acid. Calculated in the same way $\mu_{\text{HA globulin}}$ should be ± 300. The actual value is therefore low, and this is accounted for by the greater degree of hydrolysis replacing the globulin HA compound by badly conducting acetic acid.

The effect of the "ash" on conductivity is remarkably small, and practically nil in the case of NH_4OH. The effect of the starch solution also is surprisingly small. When the conductivity of the starch solution itself is allowed for the effect always is to depress conductivity. But the depression is small compared with the high viscosity and it varies in the different cases, which proves that it cannot be any simple function of the viscosity of the liquid.

Conductivity is depressed in the various cases as follows:

	$\frac{\mu\text{starch}}{\mu\text{water}}$	$100\,\alpha$
HA	·758	—
HCl	·926	4·3
NH_4OH	·904	—
NaOH	·678	19·3

I have calculated Arrhenius' α-function[1] for the extreme cases and the result appears in the last column. The values found by Arrhenius for non-colloid non-electrolytes, and various electrolytes range between 1·84 and 2·95. It is clear therefore that the high viscosity does diminish conductivity but its effect is surprisingly small seeing that the starch solution had a viscidity too high to be measured by any ordinary viscometer.

THE VISCOSITY OF SOLUTIONS OF ACID AND ALKALI GLOBULINS.

Sackür[2] refers the low conductivity of solutions of caseinogen compounds to their high viscosity. This explanation cannot be accepted without qualification for reasons already given and chiefly because the drift of the ionic proteid when directly observed is found to be remarkably rapid.

[1] *Zeits. f. physik. Chem.* IX. p. 487.
[2] *Ibid.* XLI. p. 672. 1903.

The observations already recorded on the effect of starch upon conductivity show that what is usually measured as the viscosity of a colloidal solution is not identical with or perhaps closely related to the internal molecular friction to which the ions are subject. I ventured to point out in 1899[1] that the high viscosity of colloidal solution is probably of complex origin and that its magnitude is very imperfectly analysed by ordinary methods of measurement. If colloidal solutions are heterogeneous states of matter the viscosity as measured by the flow in capillary tubes will be in part due to the influence of the friction of internal surfaces on the fluidity—a point which as I then drew attention to, was discussed as early as 1851 by Frankenheim[2]. Transpiration methods measure fluidity and this will only be simply related to the true molecular internal friction in homogeneous fluids.

Recently the point has been investigated by Garrett working under Prof. Quincke[3], by the two classical experimental methods in which the time of transpiration through a capillary tube, and the decrement in the period of oscillation of a disc immersed in the fluid are taken as measures of viscosity. In the case of colloidal solutions the two methods gave different values for the viscosity owing as Garrett points out to the different incidence in the two cases of the various factors which modify the fluidity.

The fluidity of a non-homogeneous liquid will depend upon (1) the internal friction of each of the several states, (2) the surface friction of the internal surfaces, and (3) the surface tension of the internal surfaces. To these I would add a fourth factor, the density of the electric charge if the internal surfaces be electrified. This will modify the surface tension and it will decrease the fluidity owing to the fact that electric work must be done in deforming the solution.

Electric conductivity will depend upon the internal friction of each of the several states, upon the distribution of the electrolytes between the different states, and upon the coefficient of ionisation in each of the states and in the surface film bounding the states[4]. Therefore change in gross fluidity, and that is what is measured, need not agree with changes in electric conductivity in magnitude though they usually will

[1] This *Journal*, XXIV. p. 180. 1899.

[2] *Journ. f. prak. Chem.* LIV. p. 433. 1851.

[3] *Ueber den Viskosität u. d. Zusammenhang einigen colloid. Lösungen.* Inaug. Dissert. Heidelburg. 1903.

[4] Molecular conductivity and chemical potential are greater in the surface layer of a solution. Reinold and Rücker, *Phil. Trans.* A, CLXXXIV. p. 505. 1893. W. Spring, *Zeits. f. physik. Chem.* IV. p. 658. 1889.

in direction. For instance the fluidity of gels is infinitely small, but Graham[1] and Voightländer[2] have shown that the velocity of diffusion of electrolytes in them is approximately the same as in water, and Whetham[3] has shown by direct measurement that the mobility of ions in agar agar jelly is very little less than in ordinary solutions.

The fluidity of a solution of gelatine decreases with lapse of time. But Garrett found that while the logarithmic decrement of a disc oscillating in the solution increased from 0·2502 to 0·4049 the electric conductivity remained constant.

For globulins I have found the following relations when the fluidity is measured by the transpiration method.

	Fluidity	Conductivity
HCl globulin	rises with time	remains constant
NaOH ,,	falls ,, ,,	falls with time

Time here means intervals up to 24 hours.

Bayliss[4] finds that in tryptic digestion electric conductivity does not change concurrently with change in fluidity, and instances might be multiplied further.

The absence of any simple relation between viscosity and electric conductivity was noticed by Reyher in the case of solutions of non-colloidal organic bodies, but there is a fair general agreement in the values[5].

Measurements of viscosity yield some unexpected evidence as to the relative acid and basic functions of globulin.

On the view that acid or alkali casein solutions are solutions of proteid salts there would be present if hydrolysis took place the following molecular species, expressed in ordinary notation:

$$BS,\quad B\cdot + S',\quad BOH,\quad B\cdot + OH',\quad HS,\quad H\cdot + S',$$

and Sackür[6] asks to which of these can the high viscosity of solutions of casein salts be attributed.

By a train of ingenious argument he fixes upon the proteid ion as the chief factor. As the casein acted always as acid in the cases considered by him, the high viscosity is due chiefly to S'.

This conclusion can be tested with extraordinary ease and certainty

[1] *Phil. Trans.* CLI. p. 183. 1861.
[2] *Zeits. f. physik. Chem.* III. p. 36. 1889.
[3] *Phil. Trans.* A, CLXXXVI. p. 507. 1895.
[4] Kinetics of Tryptic Action. *Arch. d. Sci. Biol.* XI. p. 261.
[5] *Zeits. f. physik. Chem.* II. p. 743. 1888.
[6] *Loc. cit.*

in the case of globulins since their solution in neutral salts contains no ionic, no electrically charged globulin, as is shown by the absence of all movement in an electric field, while solutions in dilute acids and alkalies always are "ionic." Therefore, if Sackür's view be correct, the former solutions should have relatively a quite low viscosity.

Some measurements of viscosity made to test this point gave striking results. The specific gravity of each solution was determined for Series III., and the values found to differ only slightly, therefore, as the formula is a linear one, viscosity may be taken to vary directly as the transpiration times.

Pressure 29 mm. mercury. Temp. 19.

Conc. of acid, alkali or salt		Gr. equivs. of acid, alkali or salt to 1 gr. globulin $\times 10^5$	Transpiration time for the same volume in seconds	State of the solution
		Series I. 0·62 grs. of globulin in 100 c.c.		
NH_3	0·0005 normal	8	57·1	Transparent whitey blue.
	0·0008	13	58·1	,, brilliant blue.
	0·002	32	58·7	,, ,, ,,
	0·01	161	57·5	,, ,, ,,
NaOH	0·002	32	58·7	,, ,, ,,
$H\bar{A}$	0·002	32	63·3	White opalescent.
	0·005	81	56·6	Transparent.
	0·015	242	58·1	,,
NaCl	0·15	2419	58	
		Series II. 2·13 grs. of globulin in 100 c.c.		
NH_3	0·006	28	234	Transparent.
	0·03	141	143·7	,,
$H\bar{A}$	0·012	56	130·5	Opaque.
	0·03	141	134·3	Whitish transparent.
NaCl	0·28	1315	107·2	Transparent.
		Series III. 7·59 grs. of globulin in 100 c.c.		
NaOH	0·0084	11	3292	(*a*) Transparent slight opalescence.
HCl	0·0084	11	753	White but non-settling.
$\frac{1}{2}MgSO_4$	0·32	423	226	Matches (*a*).
$\frac{1}{2}MgSO_4$	0·32	no globulin	60	
Water	... ...		48·5	

These figures show that the viscosity of solutions of alkali globulin and of acid globulin is much higher than that of solutions of salt globulin of the same strength and of the same grade. Therefore the presence of ionic globulin in solution raises the viscosity very much. In Series III. in which the proteid content is of the same order as the proteid content of blood plasma the difference is surprising.

Dilution diminishes the difference between the various solutions, as is seen on comparing Series I., II., and III.

When the opalesence of a solution is diminished by increasing the concentration of the solvent the viscosity is increased to a maximum; beyond this further addition of the solvent diminished the viscosity (Series I. and II. NH_3).

The viscosity of acid globulin is less than that of alkali globulin even when the grade of solution is approximately the same.

The relation to opalescence shows that viscosity is diminished as hydrolytic dissociation increases. Reyher[1] found a similar relation between hydrolysis and viscosity in the case of soaps, and Sackür in the case of caseinogen salts. Therefore one finds an interesting and somewhat unexpected confirmation of the conclusion already arrived at that the acid function of globulin is much stronger than its basic function in the fact that an equivalent quantity of alkali will produce a more viscid solution of globulin than an equivalent quantity of acid.

Excess of alkali diminishes the viscosity at both high and low concentrations, though the effect is decisive only for the latter. This is no doubt partly due to the free alkali or acid depressing the degree of ionisation of the globulin salt and so far it confirms Sackür's view that the high viscosity is due to the proteid ions, but, in view of the fact that ammonia globulin conducts better than ammonia itself, and that ammonia is a feeble base, the action is too marked to admit of this simple explanation. Taken in conjunction with the extraordinary rise in viscosity produced by increasing concentration it points to the presence in the solutions of complex ions which attain great size at high concentration and have then the properties of matter in mass. With their appearance the fluid ceases to be truly homogeneous and has the characteristic low fluidity of the heterogeneous state. Excess of alkali diminishes the size of these complex ionic states, and owing to their absence in dilute solution the proteid ion though present has no special influence on the viscosity (cf. Series I.).

Globulins as Pseudo-acids or Pseudo-bases.

Hantzsch[2] has drawn a distinction between true acids and true bases and pseudo-acids and pseudo-bases. Pseudo-acids do not contain

[1] *Zeits. f. physik. Chem.* II. p. 743. 1888.

[2] Hantzsch. *Bericht. d. deut. ch. Gesell.* XXII. pp. 306 and 575, 1899; XXXII. pp. 607, 3109 and 3066, 1899; XXXV. p. 210, 1902. Kauffmann. *Zeits. f. physik. Chem.* XLVII. p. 618. 1904.

a replaceable hydrogen but they can change to an isomeride of a salt-forming type, and pseudo-bases are substances which by an isomeric change can act as bases of the ammonium hydroxide type. In each case the substance itself is neutral in solution, but by a change in the molecular structure it may act as an acid or alkali as the case may be and form salts. The chief characteristic of these substances is that, owing to the change of molecular state, the process of salt formation is gradual, there is a measurable latent period. Cohnheim and Krieger[1] suggest that proteids may be pseudo-acids and pseudo-bases in virtue of a labile atomic grouping. The suggestion is tempting, but I can find no justification for it in the case of globulins. I failed completely to detect any latency of the kind described by Hantzsch in the reactions with acids or alkalies either with indicators, or by measurements of electrical conductivity. Amongst other indications of reactions with pseudo-acids or pseudo-bases is the existence of an abnormally large positive temperature coefficient of molecular conductivity. I measured the temperature coefficients for acetic acid globulin, and for NaOH globulin, and found them to be of normal magnitude

$$\text{H}\bar{\text{A}} \text{ globulin } 0{\cdot}5^\circ \text{ to } 23{\cdot}3^\circ \quad \frac{1}{K_{23\cdot3}}\left(\frac{\delta K_{23\cdot3}}{\delta t}\right) = {\cdot}0242,$$

$$\text{NaOH globulin } 0{\cdot}5^\circ \text{ to } 20{\cdot}5^\circ \quad \frac{1}{K_{20\cdot5}}\left(\frac{\delta K_{20\cdot5}}{\delta t}\right) = {\cdot}0214,$$

and neither system showed any appreciable lag.

One property which Hantzsch claims for pseudo-acids is manifested by globulins, namely in their inability to react with a dry base. Dry globulin is quite insoluble and unchanged by dry aniline.

Direct measurement of the specific velocity of ionic globulin.

In order to measure the movement of the globulin in an electric field I tried first the method already used by me[2] of analysing the contents of each limb of a U-tube through which a current had passed, but it was soon discarded in favour of the "boundary" method which was used by Whetham to measure the rate of movement of coloured ions[3].

Essentially the method consists in using two solutions, one of slightly greater specific gravity than the other, so that when carefully poured the

[1] *Zeits. f. Biol.* XL. p. 95. 1900.

[2] This *Journal*, XXIV. p. 288. 1899.

[3] *Phil. Trans.* CLXXXIV. A, p. 337. 1893.

one over the other a definite surface of separation is formed. One of the two solutions contains a salt with a coloured ion. By passing a current this ion can be made to move and its velocity and direction determined by the movement of the boundary of the coloured zone.

In observations on globulins the lower layer was the opalescent solution of the globulin in acid or alkali as the case might be, and the upper layer a clear solution of the same electrolyte, and the movement of the boundary between opalescent and clear regions is the measure of the movement of the globulin.

The theory of the boundary has been dealt with by Whetham (*loc. cit*) and by Orme Masson[1].

Very briefly stated it is as follows:

$$C = A\frac{n}{\eta}(U+V),$$

where C is the current, A the transverse sectional area of the tube, n the number of gramme equivalents of the electrolyte per litre, $\frac{1}{\eta}$ the charge carried by each monad ion, and U and V the average velocity of the cation and anion respectively.

Now the velocity of any ion varies directly with the fall of potential per unit of length (π), and the average velocity of the ions for a given value of π decreases as the concentration of the electrolyte expressed in gramme equivalents per litre increases. The factor which expresses this last relation has the same value as that which expresses the relation of specific molecular conductivity to concentration, and it is called the coefficient of ionisation (ϵ).

Therefore $$C = A\frac{n}{\eta}\pi\epsilon(u+v),$$

where $u+v$ are the actual velocities of the ions when free, and not the average velocities.

The principle of the method is due in the first instance to Lodge[2], who measured the rate of the hydrogen ion by means of a jelly containing phenolphthalein with enough alkali to give a colour. The tube connected two vessels containing sulphuric acid, so that when a current was passed the hydrogen ions entered the tube, and as they moved decolorised the phenolphthalein. Whetham[3] modified the method by

[1] *Phil. Trans.* CXCII. A, p. 331. 1899.
[2] *B. A. Report.* 1886.
[3] *Phil. Trans.* CLXXXIV. A, p. 343. 1893.

using a vertical tube containing two salt solutions of slightly different density, the lighter one floating on the heavier one. The heavier and lower solution contained a coloured ion and the rate of this ion was determined by measuring the rate of movement of the surface separating the coloured from the uncoloured solutions.

Picton and Linder[1] observed the movement of colloids in an electric field in a tube near the ends of which were two platinum electrodes. Whitney and Blake[2] have followed this method in their measurements of the velocity of colloids. The method is open to the objection that the colloid is concentrated by the field, it is driven into a smaller volume. This and secondary electrolytic decomposition I take it account for the complicated movements which these observers describe.

If the solution under observation is bounded by two fluid surfaces there is no compression of the colloid, and one reaps the further advantage of getting readings in pairs in which the most common source of error—namely electrification of the boundary surface—appears of opposite sign in the members of each pair. The chief advantage however lies in the fact that the sensitive colloid is removed from the electrodes by a column of fluid about 5 cm. long so that, provided each experiment be not too prolonged, the risk of secondary electrolytic decomposition is obviated.

The only method by which one can secure two fluid surfaces is by using a U-tube (Fig. 2), and having the liquid which contains the ion to be measured as the lower liquid. In order to avoid mixing, this liquid must be introduced from below and allowed to flow under the liquid which is to form the upper layers into which the electrodes will dip. The tube I had made is shown in the accompanying figure and I find that a similar form for use in the direct measurement of ionic velocities has been suggested by Nernst. On each limb of the U-tube a millimetre scale is etched.

The apparatus is used as follows. It is first washed out with the fluid which is to form the upper layers, drained, the tap turned and the U-tube filled with the fluid to a standard level. The second fluid—the solution of globulin—is then allowed to run slowly under the first fluid until the level in each tube stands at a standard mark—the 2 cm. mark in my experiments. The tube is then placed in an upright position in a bath at constant temperature, the electrodes lowered to a certain point in each limb and the apparatus is ready.

In order to avoid convection currents which would destroy the boundaries it is first necessary to bring the fluids, the tube and the bath to the same temperature.

Electrodes of very large surface were used, each consisting of a strip of platinum foil coated with grey platinum deposit 1 cm. broad and 4 cm. long rolled on itself.

The E.M.F. between the electrodes was measured by a Siemens-Halske millivoltmeter.

[1] *Jour. Chem. Soc.* LXXI. p. 568. 1897.
[2] *loc. cit.*

In order that a boundary shall remain sharp during the passage of a current it is necessary (1) that a specifically slower ion should follow one specifically faster, and (2) that the specific resistance of the fluid on each side the boundary should be the same. If these conditions are not fulfilled the boundary becomes indeterminate. The explanation was furnished by Whetham[1], and it may be illustrated by this simple example. Take the case of a difference in specific resistance, and let the current pass across the boundary from the liquid of lower to the liquid of higher resistance. By Ohm's law the potential slope will be greater on the distal (as defined by the direction of the current) than on the proximal side of the boundary. Any straggling ion which falls behind therefore will find itself in an area of lower potential gradient and it will be still further slowed; any ion which gets in advance of the boundary will find itself in a region of greater potential slope and it will be still more hurried forwards.

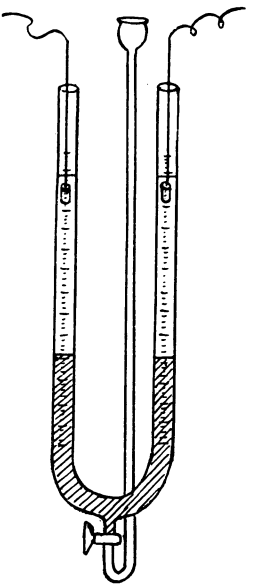


Fig. 2.

Lower layer methylene blue, 1 gramme-mol. in 144·93 litres. Temp. 13. The movement of the boundaries actually observed in 10 minutes is given in millimetres.

	−	+	Mean
·0476 *N*, $MgSO_4$ in both layers	4 ↑	4 ↓	4
·0244 *N*, ,, ,, ,,	4	4	4
·0012 *N*, ,, ,, ,,	6	2	4
·000 *N*, ,, ,, ,,	22	1	11·5
·0476 *N*, K_2SO_4 ,, ,,	4	4	4
·002 *N*, ,, upper layer only	20	3	11·5
·009 *N*, ,, ,, ,, ,,	4	2	3

The figures show the effect on the movement of the boundaries of diminishing the difference between the resistance of the layers by adding salt to the upper layer or to both layers. Mean values obviously are of use as giving an approximation only when the difference is small.

When there are two boundary layers, one preceding, one following,

[1] Whetham, *loc. cit.*

the theoretical conditions obviously are fulfilled when both boundaries remain sharp and move at the same rate. This is a very precise and accurate test and when it is satisfied also on reversal of the current the readings may be taken as thoroughly trustworthy.

The choice of an upper layer for globulins is very limited seeing that all electrolytes act energetically. It must always be an aqueous solution of the electrolyte used to dissolve the globulin. A second difficulty arises in the adjustment of the resistances. A solution of acetic acid has its conductivity altered only slightly by the presence in it of globulin, and an upper layer of the same concentration of the acid in water was found to give values for the boundaries which agreed within the limits of error of the readings. But solutions of strong acids and alkalies have their conductivity very much lowered by the addition of globulin. Adjustment of the aqueous solution of acid or alkali to the same resistance by dilution did not seem to satisfy the conditions, but the most perfect adjustment was obtained by dialysis.

For solutions in alkali toluol is the best disinfectant, for solutions in acid either toluol or camphor[1]. The dialysing membrane was ordinary parchment coated with formalised gelatine, and dialysis was carried out in glass vessels with the stoppers luted with soft paraffin.

The final equilibrium is a remarkable one. Both dialysee and dialysate stand at the same level—that is to say there is no difference in the osmotic pressure. This agrees with Weymouth Reid's observation[2], but with such prolonged dialysis I always found that the dialysate though perfectly limpid and free from opalescence gave a just detectable xanthoproteic reaction. The electric conductivity is identical. Therefore the product of the ionic concentration (supposing the ions to have the same valency in both fluids) into the mean ionic velocity must be the same for both dialysee and dialysate. It seems as though the proteid were of no account in the equilibrium. The globulin remains globulin throughout. That is to say on precipitation it is resoluble in a neutral salt. It is not changed to derived albumen The theory of this equilibrium is considered later.

The velocities are tabulated below:

[1] Camphor changes a solution of alkali-globulin into an insoluble gel.

[2] This *Journal*, xxxi. p. 438. 1904.

	Concentration	$\times 10^{-5}$ equivs. to 1 gr. globulin	Temp.	Specific velocity cm. sec. $\times 10^{-5}$
HA globulin	0·0004 *N* to 0·0100	17 to 80	13·2	19·78
			18	23
HA globulin dialysed in successive stages	character of the solution	transparent	18	22·5
		" highly opalescent	18	22·9
		white opaque	18	19·4
HCl globulin dialysed in successive stages	" "	transparent	18	11·5
		" highly opalescent	18	9·0
NaOH globulin dialysed	" "	transparent opalescent	18	7·66
	Not so good as the foregoing.			
H_2SO_4 *globulin*	0·00048 *N* to 0·0045 *N*		18	18·5
H_3PO_4 *globulin*	0·0092 *N*		18	23
For comparison with the above; measured in the same manner.				
$(AgCl)_x Cl_y$	...		18	57
Phase $\frac{\text{water}}{\text{phenol}}$:	the emulsion of phase $\frac{\text{water}}{\text{phenol}}$ in phase $\frac{\text{phenol}}{\text{water}}$, near critical temperature.		velocity of the former ±105 at 9·5	
[Ag·	$\nu = \infty$		18	58]

The accuracy of these values may be tested by calculating from those for the $H\bar{A}$ globulin the temperature coefficient. Expressed as a percentage of the velocity at 18° it is 2·6 % per degree. The coefficient for electric conductivity of $H\bar{A}$ globulin I find to be 2·69 % of the conductivity at 18°. These values are fairly close to Thorpe and Rodgers' determination of the change in the viscosity of water with temperature. Over the same range their figures give 2·58 % per degree of the value at 18°.

Two points are noticeable:

1. The specific velocity of the globulin in $H\bar{A}$ globulin is constant over a wide range of concentration both of the acid and the globulin. Over wide limits the ratio $\frac{\text{acid}}{\text{globulin}}$ does not affect it. As the ratio $\frac{\text{acid}}{\text{globulin}}$ rises the solution changes from milk-white or non-settling suspension stage, to transparency. Over the greater part of this range the velocity is constant while from the appearance of the fluid the colloid particles are altering in size. Therefore over this range the

velocity of the globulin particles must be independent of their size and the quantity of electricity which each carries cannot be constant. The particles, since they refract light, must be of much more than molecular dimensions. Each is bounded by a surface, and from Helmholtz' theory of electric endosmose, since the velocity is constant, the density of the charge per unit of surface is also constant. The total quantity of electricity on each must therefore be directly in proportion to the surface or

$$Q = \sigma 4\pi r^2.$$

The fact that all the colloid particles have the same specific velocity is remarkable since Zsigmondy and others have shown that the particles in colloidal solutions are not all of the same size. It agrees with Quincke's observation that the velocity of particles suspended in a fluid in a field is independent of their size, and it proves that the globulin particles cannot be simple ions, for the charge on an ion is a fixed quantity. Nor, as has been suggested, can the charge be due to a haphazard association of the colloid particles with ions which may be present. *It is due to a real electrolytic dissociation at the surface of the particles, the degree of dissociation depends upon the same factors which in the interaction between solvee and solvent determine the degree of dissociation of different electrolytes, and it fixes the density of the charge, while the amount of electricity carried per unit of weight is the product of the density and the area of the surface between the particles and the fluid in which they float.*

The stability of the system will vary with the density of the charge because amongst other things this measures the degree of interaction between solvent and solvee (the solvee here being taken to include the two other components, water and acid or alkali in the case of globulins), but it is not the only factor which operates since in solutions of salt globulins and salt alkali globulins the colloid is entirely uncharged.

Electrolytic dissociation is not the only thing which defines the degree of miscibility in ordinary salt solutions. K_2SO_4 and Na_2SO_4 do not differ in this respect, but the upper limit of miscibility with water is very much higher for the latter, and the reason is that the latter can form molecular compounds with the solvee while the former, for reasons which are entirely unknown, cannot.

Similarly one has electrolytic dissociation as the factor which chiefly defines miscibility in solutions of acid and alkali globulins, and in metallic hydrosols; while the capacity for forming molecular compounds

with the molecules of the solvee (salt and water) is the factor which determines miscibility in the case of the electrically inactive solutions of salt globulins, while both factors operate probably almost equally in the case of feebly electrical colloidal solutions such as those of starch and glycogen.

It is interesting to consider for a moment the possible relation between the density of the charge on the surface which separates the internal phase (colloid particles) from the external phase, if, as is not unlikely, the density of the charge defines the shape of the surface and fixes the limits within which the degree of curvature can change without destroying the stability of the system. Below a certain grade of solution which lies well within the region of "white opaque" solution the specific velocity falls. Therefore when the colloid particles become very large, that is when the curvature is diminished very much, the velocity and therefore the density of the charge is decreased, the limiting state being that of a plane uncharged surface. If this argument be correct the potential difference between the phases phenol/water and water/phenol is zero and the charge which can readily be demonstrated (cf. the table, p. 291) when the two phases have been shaken together to form an emulsion is due to the deformation of this surface which disturbs the equilibrium by placing the two phases under unequal pressures due to surface tension.

The second remarkable feature is the abnormally high value of the specific velocity of the globulin when combined with acetic acid or phosphoric acid, both as compared with known ionic velocities, and as compared with the value when combined with strong acids and bases. According to the table the specific velocity is greater in acid globulin than in alkaline globulin, and rises the weaker the acid. Therefore, on the theory of hydrolytic splitting of the globulin, the greater the degree of hydrolysis the higher the specific velocity of the globulin.

The other relation, namely that to known ionic velocities, must be considered with this.

The velocity of the globulin ion can be approximately calculated from the conductivity data by Ostwald's method[1]. For Na globulin the result is in round numbers 10 at $V = \infty$, 7·66 therefore is the order of magnitude one might expect with incomplete ionisation. Reckoned by the number of atoms the specific velocity of a proteid ion cannot be more than 10, and should be much less, and the values actually

[1] *Zeits. f. physik. Chem.* II. p. 840. 1886.

observed for HCl and NaOH globulin show that Ostwald's law of the decrease in ionic velocity with increase in the size of the ion ceases to hold beyond a certain size of ion. The values for HCl proteid and NaOH proteid therefore agree very well with the view that the globulin is in the ionic state, and they are low as compared with the velocity of movement when conditioned by electric endosmose.

The other values, however, are altogether outside the limits which could be reached by ions of such magnitude as proteid ions must be, and the theory of hydrolytic splitting furnishes an explanation.

According to it the globulin would be chiefly in the ionic state in NaOH globulin, chiefly in the state of anhydride or hydrate or of ionic complexes of compounds with lowered acid content (cf. p. 303) in HA globulin, while between these extremes would be the HCl globulin. Therefore the velocity observed in NaOH and HCl globulin, which it must be remembered is the mean velocity of all the globulin, no matter what its state may be, would be chiefly due to the globulin ion. It would be an ionic velocity and it actually is of the expected magnitude of an ionic velocity.

The velocity of the globulin in HĀ globulin will be mainly that of the free or partly free globulin to which the opalescence is due and which therefore is not in a state of molecular subdivision. It is present in masses so large as to be defined by a surface. Such masses, according to the view with which we started, would have a surface charge owing to ionisation at their surface, they are pseudions of the form $G_nG_x' + Na$. where n is large compared with x, and in their case the quantity of electricity would be a function of surface and not a fixed quantity.

Now the order of magnitude of the velocity of pseudions can be arrived at by measurements from known cases.

I. To 0·1 *N* NaCl I added a solution of silver nitrate until it became highly opalescent. This formed the lower layer in the cell, the upper layer being sodium chloride adjusted by trial and error to approximately the same conductivity.

Both boundaries moved at the same rate, and the specific velocity at 18° was found to be 57×10^{-5} cm. per second.

This value, by what is probably a coincidence, agrees almost exactly with the speed of $Ag^{\cdot}$ at infinite dilution; that is with the maximal velocity of a rapid ion, $Ag^{\cdot}$ having the value 58.

Clearly therefore the large pseudions $(AgCl)_x Cl_y'$ which are so large as to refract white light, do not conform to Ostwald's law of the decrease in specific velocity, will increase in the number of atoms.

II. A solution of pure sodium stearate from Kahlbaum was dialysed for 10 days against 5 changes of water. It became highly opalescent owing to the formation of acid salts. The dialysate formed the upper layers and the specific velocity of the boundaries, *i.e.* of the opalescence, was 50 at 18°. Again the value is much too high to be in agreement with Ostwald's values.

With these parallel cases as a guide we may conclude that the globulin which is turned out of combination by hydrolysis will show a higher specific velocity than "ionic" globulin in spite of the fact that it is very slightly dissociated. In other words the specific velocity is high, but the quantity of electricity carried per unit of mass is low, and this is the feature of a hydrosol.

The entire proteid moves with the same average velocity, which is therefore neither that of the fastest nor that of the slowest ions or pseudions. In ordinary solutions if U be the absolute velocity of an ion when entirely free, and α the ratio of the period in which it is in conjunction with the related ion and therefore not moving to the period during which it is free, then the observed velocity will be $\alpha(U)$, which is a measure of the mean free path of the ion.

In a globulin solution the globulin according to the above theory exists as free ions, as globulin combined in the molecular state, or as pseudions. Let the absolute velocity of the free ion be U, of the pseudion U_{coll}. If x be the fraction hydrolysed, then of the total mass x is the fraction in the state U_{coll}, therefore the observed velocity will be

$$\frac{xU_{\text{coll}}[\alpha(1-x)U]}{U_{\text{coll}} \times U}.$$

The equilibrium between dialysee and dialysate for globulin compounds with monovalent acids and alkalies is very remarkable. There is agreement within the limits of measurement in osmotic pressure[1], and therefore in the composition of the gas phase, and in specific electric conductivity.

Assuming that the molecules furnish the same number of ions, this means that in unit volume of dialysee and dialysate there are the same number of molecules which contribute to the osmotic pressure and the same number of molecules which are electrically active at any one moment. If one set of values cease to agree, so also do the other. Thus in some cases when owing to slight bacterial decomposition the osmotic pressure of the dialysee remains above that of the dialysate, the electric conductivity also remains different, but *below* that of the dialysate in the few cases which I have examined.

[1] Waymouth Reid, *loc. cit.*

These relations are explicable only on the view that the globulin solution is biphasic, the one phase being present as a number of separate spheres, and continuously varying with variations in the composition of the external phase.

In the equilibrium of dialysis there are four phases, one gas phase, internal and external phase of the dialysee, and the dialysate. If there be any tension on the surface of the internal phase there are two pressures, the lower one of the gas phase with which both the dialysate and the external phase of the dialysee are in equilibrium, and the higher one of the internal phase. The osmotic equilibrium is between the dialysate and the external phase of the dialysee, the agreement in electric conductivity is an equilibrium between the dialysate and both phases of the dialysee, since the internal phase carries a charge on its surface and therefore contributes to the electric transport. Considered in this way it seems at first sight that there must be a difference of pressure between dialysate and the external phase of the dialysee due to the globulin in the latter, and this may well be the case, the value being altogether too low for detection by experiment.

General theoretical consideration of the state of alkali and acid globulin in solution.

Attention was drawn to the possibility of direct chemical combination between proteids and acids or alkalies by the study of the action of the digestive juices.

Proteid was found to "mask" a portion of free acid or alkali. A few attempts have been made in recent years to investigate the nature and extent of the masking action by the newer methods of physical chemistry.

Sjöqvist[1], working in close touch with Arrhenius, and later Sackür[2], applied the method of electric conductivity, and Bugarsky and Liebermann[3] measured the electromotive force of concentration cells containing acid or alkali in presence of proteid, and from the values so obtained they calculated the concentration of the OH or H ions: they measured also the change in the freezing point due to the addition of various amounts of proteid to 0·05 N acid and alkali.

[1] *Skan. Arch f. Physiol.* v. p. 277. 1895.
[2] *Zeits. f. physik. Chem.* xli. p. 672. 1903.
[3] *Pflüger's Arch.* lxxii. p. 51. 1898.

Cohnheim[1] measured the free acid by the velocity of the catalysis of cane sugar. Paal[2], Spiro and Pemsel[3], Cohnheim and Krieger[4], Erb[5], and Osborne[6] used a more ordinary chemical method, namely the precipitation of the proteid-acid, or proteid-alkali compound and the determination of the quantity of acid held in the precipitate.

The methods used have been varied and all these workers, with the exception of Spiro and Pemsel, conclude that proteids combine to form salts with both acids and bases, they themselves being possessed of both basic and acid properties. Such bodies are known as amphoteric electrolytes, and from the nature of the case they must necessarily be weak acids and bases, since the presence in the same molecule of acid and basic groups diminishes the importance of both. Sjöqvist, who dealt with proteids as bases only, found them to be weak bases, and therefore, like all weak bases, their salts in aqueous solution are much hydrolysed. Bugarsky and Liebermann showed that proteids also are weak acids, the compounds with alkalies hydrolysing freely.

From the measurements of these workers it is possible to estimate the acid and basic functions. In the only native proteid used by them, egg albumen, they appear to be almost exactly balanced. Sjöqvist calculated the basic dissociation constant for egg albumen and found it to lie between the values for aspartic acid and urea. It is a base about 11 times weaker than asparagin, 6 times weaker than aspartic acid, but decidedly stronger than urea.

Proteid salts in aqueous solution are fairly good conductors, that is to say, they ionise. Bugarsky and Liebermann conclude that the degree of ionisation is not large, but they speak of true proteid ions. When reacting with acid, for instance HCl, the resulting salt has the form BHCl, and the ions are $BH \cdot Cl'$.

The proteid therefore combines like ammonia.

$$NH_4Cl \rightleftarrows NH_3H \cdot + Cl'$$
$$BHCl \rightleftarrows BH \cdot + Cl'.$$

All these workers are agreed that proteids form salts which behave quite normally. They ionise in aqueous solution, forming a true proteid

[1] *Zeits. f. Biol.* XXXIII. p. 489. 1896.
[2] *Ber. d. deut. chem. Gesell.* XXV. p. 1202. 1892.
[3] *Arch. f. physiol. Chem.* XXVI. p. 233. 1898–9.
[4] *Zeits. f. Biol.* XL. p. 95. 1900.
[5] *Ibid.* XLI. p. 309. 1901.
[6] This *Journal, loc. cit.*

ion. They react with water according to the ordinary equation of hydrolysis BS + HOH = BOH + HS. The only marked feature is the relatively low value for specific molecular conductivity, and this according to Sackür is due not to an abnormality in the salt itself but to the high viscosity of the solution.

If the standpoint of these various authors is accepted we are bound to consider the question whether proteids in aqueous solutions are colloids at all.

Since Graham's time true colloids as a class have been distinguished by the feebleness of their chemical reaction. But according to the authors quoted proteids react directly and fully, with acids and alkalies the reactions being sufficiently definite to admit the calculation of molecular and combining weights[1]. That there is however a theoretical difficulty not to be passed over so lightly has been recognised in the analogous case of soaps.

Krafft[2] urged the colloidal nature of these bodies on the ground that they gelatinise and do not lower the vapour pressure in concentrated solution.

Kahlenberg and Schreiner[3], however, using the method of electric conductivity, found soaps to be salts of ordinary type undergoing ionisation and hydrolysis in the ordinary way. They therefore refuse to regard soaps as colloids, on the ground that matter in the colloidal state does not react in this simple chemical way.

Smits[4], from direct determinations of vapour tension, proves the accuracy of Krafft's contention that the vapour pressure becomes that of pure water when the concentration of soap is sufficiently high; soap therefore, at these concentrations cannot be in true solution, it must be in colloidal solution. Kahlenberg and Schreiner, however, found even at concentrations when by the vapour pressure method the colloidal state must be fully established, soaps still are good conductors and therefore must be ionised. Smits regards this result as due to absorbed electrolytic impurities which act as the conductors, but this view is hardly tenable, for the impurities if sufficient to give normal values of molecular conductivity for the soap would also affect the vapour pressure.

Probably the controversy may be in part referred to our ignorance

[1] Sjöqvist, Bugarsky and Liebermann, Sackür, *loc. cit.*

[2] *Ber. d. deut. chem. Gesell.* XXIX. pp. 2, 1334. 1896, and earlier papers.

[3] *Zeits. f. physik. Chem.* XXVII. p. 552. 1898.

[4] *Ibid.* XLV. p. 608. 1903.

of the true interpretation of conductivity data derived from concentrated solution. A similar discrepancy between vapour pressure measurements and conductivity measurements appears in the work of Jones and Getman[1] on concentrated solution of chlorides, sulphates, and nitrates of various metals, and the explanation which they offer probably has a direct bearing on our subject.

The position with regard to the nature of soap solutions is exactly paralleled by the case of proteids. Reid[2] has shown that proteids in solution do not alter the vapour pressure, on the other hand in presence of acids or alkalies these solutions are good conductors, and as we have already seen the conduction cannot be referred either to the free acid or alkali, or to absorbed electrolytic impurities.

Further the proteid in presence of acid or alkali is electrically charged, and therefore so far is "ionic," and, as may be proved by indicators or by measurements of the rate of catalysis of methyl acetate or cane sugar, it neutralises the acid or alkali. These and other facts already discussed point to salt formation.

Proteids therefore show relatively vigorous chemical action, and as a class they gelatinise with difficulty[3]. In what respect then are they colloids?

The colloidal nature of their solutions appears mainly in the presence of a marked time element in the reaction. Kohlrausch found on dilution of sodium silicate, or on adding caustic soda to an already diluted solution, that the electric conductivity reaches a constant value only after a long time. Similarly van Bemmelen found that when $SnCl_4$ is added to water zinc oxide is liberated owing to hydrolytic decomposition and is dissolved in the HCl, but the relation between acid and oxide is not one of chemical equivalence. The zinc oxide slowly changes its state to one of true colloidal solution, the "colloid particles" retaining absorbed acid. Similarly with globulin solution I have found that viscosity, electrical conductivity, sometimes the one, sometimes the other, sometimes both continue to change for days. And the amount of globulin held in solution by a given quantity of salt is not constant for a given temperature and concentration but varies according to the previous history of the system; *e.g.* according to whether the particular volume relations have been reached by dilution of a more concentrated solution, or by direct solution of a suspension of globulin.

[1] *American Chem. Journal*, xxxi. p. 303. 1904.

[2] This *Journal*, *loc. cit.*

[3] Alkali globulin at high concentration sometimes gelatinises like a soap.

Enough has been said to show the need for caution in regarding the reaction between proteids and acids or bases as one of simple salt formation. The possibility of the phenomena belonging to the intricate borderland of absorption combination must not be lost sight of.

It must be borne in mind that the possibility and existence of definite chemical compounds between proteids and basic or acid radicles is not here called in question. The isolation of a score of such bodies would help little or nothing towards understanding the mechanism of solution of proteids. The existence of definite compounds of silica helps us not one whit to understand why silicic acid in aqueous solution has no definite heat of neutralisation, or why in presence of alkali it needs days to reach electrical equilibrium. And from the point of view of the dynamics of the body fluids what we need to know is the state of the proteid in aqueous solution.

The possibility of the relation between proteids and electrolytes in aqueous solution being that of "absorption" needs consideration, in view of the fact that the ratio of the reacting masses by weight is from 300 to 1000 to 1; and van Bemmelen[1] has shown that 1 molecule of colloidal Fe_2O_3 can absorb 0·31 molecules of, for instance, $Ba(OH)_2$.

Absorption compounds are marked by the indefiniteness of the reacting masses. The same indefiniteness is very clearly seen in certain relations of globulins. Dialysis of solutions of alkali, or acid globulin, proves that the quantity of acid or alkali necessary to dissolve unit weight of globulin is quite indeterminate, though the "grade" of solution, *i.e.* the degree of dispersion of the globulin does depend upon the mass ratio $\frac{\text{acid or alkali}}{\text{globulin}}$. Dialysis causes the solution to become more and more opalescent, but no precipitate settles out, and the entire mass of proteid retains its electric charge, the specific ionic velocity being almost unaltered.

Conversely indefinitely minute concentrations of acids or alkali will convert a globulin precipitate into a solution of low grade just as a minute amount of alkali will dissolve ("peptonise" as Graham called it) an indefinitely large quantity of silica, the solution being of proportionately low grade.

There is, therefore, no point in the solution of globulin by acids or alkalies at which it may be said to be complete. Continuous addition of reagents produces a continuous and parallel increase in the dispersion of the globulin, so that, starting from a solution which is opalescent and

[1] van Bemmelen. *Zeits. f. anorg. Chem.* xxxvi. p. 380. 1903.

opaque, that is to say which is optically heterogeneous, one ends with a solution which is transparent and is, relatively speaking, optically homogeneous. Similar variations in the grade of the solution were described by Picton and Linder[1], who were the first to draw attention to this important point, in hydrosols of sulphides and of silica and Siedentopf and Zsigmondy[2] detected them by the method of ultra-microscopical vision in metallic hydrosols.

If globulin is dissolved by acids and alkalies owing to chemical compounds of the nature of globulin salts, being formed it is clear from the facts of dialysis and of solution, and from measurements of the specific ionic velocity of the globulin, that the mass of alkali or acid needed to bring about solution of one gram of globulin, and to confer on it its maximal specific ionic velocity, is quite indeterminate, but the mass required to produce a given grade of solution, that is to produce a given degree of dispersion, is fixed and measurable.

If the globulin salt be $G_n(HS)_y$, and $G_n(BOH)_y$ respectively, the ratio $\frac{n}{y}$, the combining ratio that is, can vary continuously, and this I take it is the essential characteristic which van Bemmelen claims for absorption compounds.

A theory based on simple salt formation, therefore, will not cover the facts, it fails to explain what I take to be the central fact, namely the indefinite nature of the alkali or acid globulin compounds. It breaks down in another respect.

The molecular conductivity μ of a salt which behaves normally in solution is the sum of the mean velocity of the two ions,

$$\mu = U + V,$$

and the mean velocity is a value which can be directly measured by Lodge's[3] boundary method. It is low when the degree of ionisation is low.

Acetic acid ionises only slightly, its salts as a rule ionise well. Therefore the molecular conductivity of the salts stand as a rule much higher than that of the acid. But the molecular conductivity of acetic acid globulin is lower than that of the acid. Sackür and others explain these low values for the molecular conductivity of proteid compounds by supposing that the high viscosity of the solutions lowers

[1] *Journ. of Chem. Soc.* LXVII. p. 63. 1895.

[2] *Ann. d. Physik.* (4) X. p. 1. 1903.

[3] *Brit. Assoc. Report*, p. 389. 1886. "Mean velocity" is not the velocity of the free ion, but the average speed including periods of freedom and of conjunction.

the ionic velocity, and that the salts ionise freely. The explanation is contradicted by fact. Direct observation shows that the specific velocity of the proteid, the mean ratio of drift of the proteid in the electric convection, is not low but abnormally high. The low electrical capacity of acetic acid globulin in solution therefore cannot be explained by hydrolytic dissociation, low degree of ionisation, or high internal friction. The only other way which suggests itself is by the formation in solution of ionic complexes of large size and low electrical capacity per unit of weight.

The relation observed between concentration and viscosity, and the effect of excess alkali upon viscosity, also points to the existence of ionic complexes of a size so large as to form masses of matter separate from the general mass of the fluid.

Walker[1] found the molecular conductivity of solution of the acetates of asparagin, asparaginic acid and glycocol, to be lower at certain dilutions than that of acetic acid itself, and he suggested as the only explanation that molecular complexes are formed. Arrhenius[2] proposed another explanation based upon changes in the degree of ionisation, which would however directly conflict in the case of acetic globulin with the observed rate of drift of the proteid. Since these papers the formation of ionic complexes as a factor in reducing molecular conductivity has been freely recognised. Acid and alkali globulin in solution form such complexes, some of which are so large as to cease to have the properties of molecules. They form internal systems each separated from the general mass of fluid by a surface.

The acid or alkali must, I think, be held to be truly combined with the globulin, because it is wholly or partly neutralised, the proteid assumes an anionic, or cationic character as the case may be, and there are evidences of normal hydrolytic phenomena. The compounds may be called absorption compounds, but the further development of the theory shows that the distinction is one of name only.

Recent work on complex ions, chiefly by the method of concentration cells, has extended the possibility of reaction by chemical equivalents to a point where it seems to be undistinguishable from absorption combination. I propose to consider the nature of globulin compounds in the light of these recent investigations.

At the present moment the conception of the colloidal solution, which finds greatest favour, is that it differs from ordinary solution,

[1] *Zeits. f. physik. Chem.* IV. p. 319. 1889.
[2] *Ibid.* V. p. 16. 1890.

because the solution is not uniformly distributed throughout the solvent in a state of molecular dispersion, but is for the most part gathered into masses which may be either solid particles, as in metallic hydrosols, or fluid spheres, in which case the "colloid particles" are themselves a solution within a solution.

In an earlier paper[1] I ventured to describe colloid solution as being essentially biphasic, and to apply the terms internal phase and external phase to the general mass of the fluid and to the colloid particles respectively. In certain cases, as may easily be seen under the microscope, three phases may be present—external, internal$_1$ and internal$_2$, as when gelatine alcohol-water is over-cooled. Now the conception which I wish to introduce here is that the internal phase may form not necessarily because the solvent is supersaturated with respect to the solvee as a whole, but because it is supersaturated with respect only to one of the ions of the latter.

Consider the case of the double salts of silver or mercury and the halogens. The work of Bödlander and Fittig[2], of Sherrill[3], and of Bödlander and Eberlein[4] with others shows that when for instance AgI is dissolved by KI a double salt is formed, which ionises forming

$$[(AgI)_nK^{\cdot} + I'], \quad [Ag^{\cdot} + I'], \quad [K^{\cdot} + I'],$$

all these species being in equilibrium. An increase in the ratio $\frac{AgI}{KI}$ in the solution causes the value of n to rise and the complex ion to increase in size.

I have examined the system $(AgCl)_n(NaCl)_y$ which represents the class, and one cannot fail to be struck with the many analogies it offers to solutions of globulins. As the relative concentration of AgCl is increased the solution becomes gradually more opalescent, rising to complete opacity. Up to this point the solution is stable in clean vessels. The material which causes the opacity is ionic in character, that is to say it moves in a field with a normal ionic velocity, the value I found by actual measurement at 18° being 57×10^{-5} cm. per second. The constituent which causes the opalescence further is always positive in sign, clearly therefore it represents an overgrowth of the complex ion $(AgCl)_nNa^{\cdot}$ to dimensions so huge as to refract white light.

Now an ion of this size will very largely cease to have the property of matter in a molecular state, it is matter in mass defined by a surface.

[1] *Proc. Roy. Soc.* v. 66, p. 95. 1900.
[2] *loc. cit.*
[3] *Zeits. physik. Chem.* XLIII. p. 707. 1903; XLVII. p. 103. 1904.
[4] *Zeits. f. anorg. Chem.* XXXIX. 2, p. 197. 1904.

It moves in a field according to the laws of electrical endosmose—that is to say the total charge it carries is not fixed as it is for all true ions but the *density* of the charge is constant. This would account for the high specific velocity observed by me which is almost exactly that of Ag' at infinite dilution, whereas, as Ostwald showed, ionic velocity should decrease with increase in the size of the ion. A case such as this is, I think, best described by speaking of the fluid as being supersaturated with a complex ion, the instability of which decreases with increase in the ratio $\frac{n}{y}$.

Colloidal solutions, as we have seen, do not always contain colloid particles carrying a charge. There are, therefore, two types of hydrosol, electrically active and electrically inactive. On the view enunciated above in electrically active sols the colloid particles, *i.e.* the internal phase, is formed owing to supersaturation by an ionic species; in the latter it is due to supersaturation by an unionised species, that is by the molecule in the chemical sense. Therefore in those colloid solutions which are electrolytic the electric transport is due in part to the drift of charged particles so large as to be defined by a surface and to have the properties of matter in mass. These are the "pseudions" or "colloid ions." Each differs from a true ion in the fact that owing to its large size as compared with the molecules of the solvent it cannot partake of the molecular movement of the latter. It has *to a greater or less extent* the properties of matter in mass. It will contribute little or nothing to the osmotic pressure—nothing if it be so large as to cease completely to act as a "molecule" of the general fluid system. It will then have as little effect as have the particles of an emulsion.

Its relation to the true molecular species of the solvent will be peculiar in that, though the relation will be that of an average state, of a continuous interchange, it might be expressed statically by regarding the molecules of the surface layer of the pseudion as being in a state of incomplete ionisation, or orientation, so that the double electrical layer is formed which is postulated in the theory of electrical endosmose.

If a colloidal solution consists of two phases and comprises two components then for a given temperature the vapour pressure must be independent of the concentration. In the case of soaps this point is reached, according to Krafft and Smits, only when the concentration is above a certain minimum [at 0·18 mol. per litre], when it is constant and equal to that of pure water within the limits of accuracy of measurement. Below this concentration the vapour pressure rises with

increasing dilution (Smits). In this region of varying vapour pressure the solution cannot be biphasic since it is bivariant, and the components are two in number, water and soap. Are these dilute solutions not colloidal at all, and is the change to the colloidal state an abrupt one (as Krafft holds) due to the separation of a second fluid phase?

From the analogy of other cases an abrupt change to the colloidal state is not probable. In the many instances investigated by van Bemmelen changes of state are always gradual. On the other hand Smits, observations show that the pressure-concentration curve for soap must have the general form shown in *AB* (Fig. 3), and this is

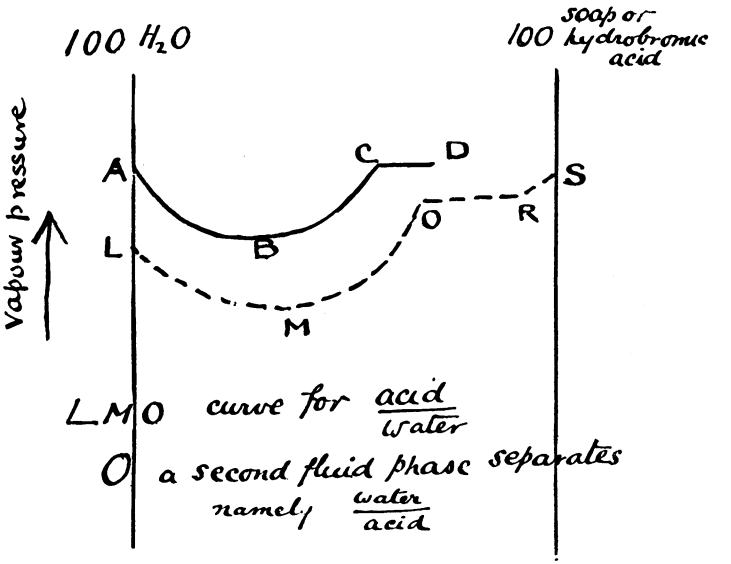


Fig. 3.

remarkably similar to the curve for hydrobromic acid and water[1]. *LMO* is the curve for the solution hydrobromic acid/water, at *O* a second fluid phase appears, and the system becomes monovariant, *OR* being the characteristic curve of constant pressure.

The difficulty may be avoided by the hypothesis that over the region of changing vapour pressure the internal phase is not fully separated. It is as it were growing by increase in the size of the particles, which, however, are still so small as to have to only a limited extent the properties of matter in mass. In other words, with increasing concentration the colloidal particles are completely separated only

[1] Bancroft, "The Phase Rule," p. 96. 1897.

gradually, just as van Bemmelen found them to appear gradually in the case of SnO or SnO_2[1] by mere lapse of time.

The conception of an imperfectly separated phase is not altogether visionary. Mixtures of isobutyric acid and water form two fluid phases. Friedländer[2] has studied these mixtures and he describes an appearance of opalescence which is not diminished by lapse of time over a range of 10° above the critical temperature for the complete separation of the phases. Konowalow[3] describes a similar stable opalescence in mixtures of pentane and dichloracetic acid. Over the range of temperature in which the opalescence is stable the vapour pressure does vary with the composition of the mixture, but the variation is much smaller than it would be if there were complete homogeneity. There is thus an incomplete separation of a second fluid phase, and the separation remains incomplete over a wide range of temperature (10°) and composition.

This conception of a heterogeneous fluid implies that in the external phase there will be some of the material in true molecular solution, which would pass through a porcelain filter while the internal phase would be arrested. A solution of pure sodium stearate strong enough to set to a jelly will yield a filtrate which contains a trace of soap, the filtrate from a clear filtered solution of NaOH globulin similarly contained 0·024 % of solids which gave the proteid and phosphorus reactions.

Salt globulins.

The special interest of these bodies lies in the fact that colloidal solutions as a class are precipitated, not preserved, by the addition of salts. Solutions of salt globulins, however, as their low viscosity and the decisive and clear way in which they can be precipitated shows, are much less colloidal than solutions of acid globulin or especially alkali globulin. Alkali globulins have a high viscosity and I have twice when endeavouring to measure electric conductivity at high concentration found the solution set to a firm jelly like a soap.

The solution of an insoluble body by an added neutral salt is not an exceptional phenomenon. Silver chloride is dissolved by sodium chloride, silver cyanide by potassium iodide, and calcium carbonate is dissolved by a wide range of salts[4]. Where the relations have been investigated

[1] *loc. cit.* [2] *loc. cit.*

[3] *Ann. d. Physik.* (5) pp. 12, 1160. 1903.

[4] Cameron and Seidell. *Journ. of Physical Chem.* VI. p. 50. 1902.

solution is found to be due to the formation of molecular compounds, and the quantitative and qualitative relations between globulin and neutral salts can be adequately and simply explained in the same way. Salt globulins therefore recall the double compounds which amido acids make with salts, while alkali and acid globulins recall the simple salt compounds which these bodies make when they react with free acid or free alkali.

The saturation of a salt solution with globulin alters the molecular conductivity of the former only slightly.

NaCl	·16 normal	conductivity depressed		2·4 %
	·11	,,	,,	2·3
	·09	,,	,,	2·1
$MgSO_4$	·3	,,	,,	1·4

These values are very small, and they need to be diminished still more for the increase in the internal friction produced by the globulin. Salt globulin however has a low viscosity, and in the light of the previous discussion this correction is probably negligible.

These low values show that the bulk of the salt is not attached to the globulin, 98 to 99 % being free.

Salt globulins in solution form no ionic globulin—or at any rate the concentration is such as to prevent its presence being recognised. Salt globulins therefore may be taken to be unstable in presence of water, stable only when their dissociation is completely suppressed by excess of salt. As only 1 or 2 % of the salt present is combined with the globulin, the solvent power of salts—the weight of globulin which one equivalent can keep in solution—will therefore be affected numerically only to an insignificant extent by variations in the combining weight of the salt and the globulin. What one actually measures is the concentration of salt necessary to prevent dissociation of the salt globulin. Owing to the extreme instability of the salt globulin this value is dependent upon a number of values which are obscure by reason of their small magnitude.

A comparison of the relative solvent powers of strong and medium alkalies, where by reason of the stability of the salt in aqueous solution we come nearest to the true combining ratios, and the solvent powers of salts, give us a measure of the depression of electric conductivity which would be produced by the presence of the globulin, if the combining ratios of salt and globulin, and alkali and globulin were the same. The salt globulin is neglected as it does not contribute to the electric transport.

To dissolve 1 gram of dry globulin it needs 10×10^{-5} equivalent of alkali, and from 1000 to 2000 of salt. The calculated lowering of conductivity in the latter case therefore would be between 0·5 and 1 %. The higher values observed are this theoretical value plus an increment due to increased viscosity.

The compound of silver chloride and ammonia which has been studied by Bödlander and Fittig[1] is analogous. The compound ionises in solution according to

$$AgCl2NH_3 \rightleftarrows Ag(NH_3)'_2 + Cl',$$

and also a minute fraction according to

$$AgCl2NH_3 \rightleftarrows Ag' + Cl' + 2NH_3.$$

The ions of the first equation are stable, of those in the second equation the ions Ag' and Cl' can coexist only at very low concentration. Therefore the system as a whole is stable only when NH_3 is present in excess sufficient to depress ionisation according to the second equation to the critical saturation point of $Ag' + Cl'$.

The stability of ionic globulin in presence of salt seems to be nil, and this is the critical value which defines the system salt + salt-globulin.

When a salt solution is saturated with globulin, there being no excess, the system therefore being monophasic, the solvent power is found to vary for different classes of salts but, on the whole, to rise with a rise in valency. This means that as a class the salt globulins are less easily decomposed by water the higher the valency of the salt.

The method adopted was either just to saturate a given volume of salt solution by running in a measured amount of a suspension of globulin in water, or the reverse. What one measures is the relative mass of salt and globulin at the concentration when the salt globulin compound just ceases to be stable.

	0·19	0·426	0·426	0·55	0·95	0·95	Globulin in 100 c.c. suspension in grs.
KCl	1	1	1	1	1	1	
NaCl	1	1	1	1	1	1	
KBr	1·4	1·37	—	—	1·2	—	
NaBr	1·5	1·37	—	—	1·3	—	
KNO_3	1·5	1·5	1·5	1·5	1·5	—	
$NaNO_3$	1·5	1·5	1·5	1·5	1·5	—	
$AmNO_3$	1·5	1·5	1·5	1·5	1·5	—	
$Ca(NO_3)_2$	1·8	—	1·6	—	1·5	—	
$Mg(NO_3)_2$	1·8	—	1·7	—	1·5	—	
$BaCl_2$	1·8	—	—	—	1·5	—	

[1] *Zeits. f. physik. Chem.* xxxix. p. 597. 1902.

	0·19	0·426	0·426	0·55	0·95	0·95	Globulin in 100 c.c. suspension in grs.
$MgCl_2$	1·85	—	1·6	—	1·5	1·52	
$CaCl_2$	1·8	—	1·6	—	1·5	1·52	
Na_2SO_4	1·99	2	1·99	—	1·86	1·89	
K_2SO_4	1·99	—	1·99	—	1·86	1·89	
$MgSO_4$	1·87	1·8	1·83	1·73	1·56	1·6	
Mg succinate	2·4	2	1·79	—	1·56	—	
$AmSO_4$	—	—	—	—	1·96	—	
K oxalate	—	—	—	—	2·1	—	
Am oxalate	—	—	—	—	1·96	—	
Na citrate	—	—	—	—	3·3	—	

The figures give the weight of dry globulin held in solution by 1 equivalent of salt in equilibrium with the CO_2 of the air, the values for KCl and NaCl being taken as unity.

The increase in solvent power with a rise in the valency is not peculiar to salt globulin solutions. It is seen in the solution of slightly soluble salts by other salts.

The following relative values were obtained from the series of papers by Cameron, Seidell, Atherton and Breazale[1]. The numbers give equivalents of $CaSO_4$, $CaCO_3$, or $MgCO_3$ dissolved by 1 equivalent of salt:

Concentration of solvent	Solvent	1 equivalent dissolves
1·5 n.	KNO_3, $NaNO_3$	0·7 $CaSO_4$
	$MgKO_3$	1·4 ,,
and chlorides "rather less than" KNO_3 or $NaNO_3$		
1·0	NaCl	0·006 $MgCO_3$
	Na_2SO_4	0·011 ,,
	Na_2CO_3	0·012 ,,
1·0	NaCl	0·0014 $CaCO_3$
	Na_2SO_4	0·0030 ,,

The valency rule is not absolute. It does not always hold for the fluid phase when a solid phase is present.

Normal salt was saturated with edestin crystals and filtered at 65°. Cooled slowly (some hours) to 22° and filtered from the crop of crystals at 22°. The edestin had been crystallised out by cold from a solution of NaCl edestin saturated at 70°; crystals washed with dilute alcohol.

Composition of the fluid in equilibrium with the crystals at 22°.

	Salt %	Equivs. per litre	Proteid %	1 gr. equiv. of salt = grs. proteid
NaCl	5·97 5·97	1·03	1·33 1·20	12·4
K_2SO_4	7·64 7·8	0·885	0·69 0·68	7·7

[1] *Journ. of Physic. Chem.* VI. p. 50, 1902; VII. p. 578, 1903, and VIII. pp. 335 and 493, 1904.

The table shows that solvent power is determined also by the nature of the anion, the order being $Cl < Br < NO_3$. This is explained by the difference in the capacity of ions for forming complexes such as those under consideration (*GBS*). Bödlander and Eberlein[1] find in respect to this property the order is:—$Cl < Br < CNS < I < CN$.

The concentration of globulin is determined not only by the concentration and nature of the salt but also by the initial concentration of globulin. The composition of the fluid phase is not defined by saying that the globulin is in excess since more than one solid phase may be present or separate out of the nature of various hydrates of globulin, or various molecular states of globulin, or of various compounds of globulin and salt. As in other three component systems it is not sufficient to say that the fluid is in equilibrium with a solid phase, it is necessary to define the particular solid phase. For instance in the case of calcium sulphate, which has been studied by van't Hoff[2], the solid phase in equilibrium with the fluid phase may be insoluble anhydrous calcium sulphate, soluble anhydrous calcium sulphate, or various hydrates such as $CaSO_4\frac{1}{2}H_2O$, and $CaSO_4.2H_2O$, and the composition of the fluid phase varies according to the particular solid phase with which it is in equilibrium, therefore, as van't Hoff points out, the system as a whole has the inertia of the solid state[3].

The effect of the concentration of Ox globulin upon the solvent power of the salt is manifested very readily when salt solution is saturated by running into it suspensions of various strengths of globulin in water.

Initial concentration of salt, in each case normal. Saturated with globulin by running in suspensions of different concentrations.

I. Concentration of suspension in grs. dry Ox globulin in 100 c.c.

II. Weight of proteid in grs. dissolved by 1 equiv. of salt.

NaCl	I.	0·19	·426	·55	·94	1·26
		0·34	·77	1	1·73	2·3
	II.	14·5	26	31	55	79
		0·47	·84	1	1·77	2·5
K_2SO_4	II.	22·3	42	55	92	99
		0·4	·77	1	1·7	1·8

[1] *loc. cit.* [2] *loc. cit.*

[3] See also a series of papers by van't Hoff on the formation of oceanic deposits. An interesting case also is the effect upon the curve of the solubility of calcium sulphate in solution of potassium sulphate of the nature of the solid phase when the latter is ordinary calcium sulphate or syngenite. Cameron and Breazale, *Journ. of Physic. Chem.* VIII. p. 334. 1904. See also Ditte, *Compt. Rend.* XCIV. p. 1266. At certain concentrations of KCl and $CaSO_4$ the composition of the fluid phase is determined by the solid phase which is the double salt $K_2SO_4CaSO_44H_2O$.

The lower row of figures gives the ratio for each case. It shows a close parallelism between the initial concentration of the globulin in the suspension and the concentration of globulin in the fluid phase. Over a certain range the numbers agree closely.

When I first detected the effect of the mass of the globulin upon the composition of the fluid phase the possibility of the presence of an unsuspected component became obvious. In order to eliminate this factor as far as possible I satisfied myself that the same relation occurs when pure crystals of edestin washed with 20 % alcohol are used.

An example from this globulin will illustrate the facts.

Crystals of edestin were suspended in water freed from carbonic acid. Two suspensions were made containing respectively 13·16 and 4·39 grs. of crystals per 100 c.c.

5 c.c. of each suspension was mixed with 10 c.c. of $\frac{1}{4}$ normal NaCl, in test tubes tightly closed with paraffined corks and set on a mechanical device which ran the fluid from one end of the tube to the other about 4 times a minute. This was done to secure complete equilibrium but later measurements of the rate of solution of edestin proved the precaution to be wholly unnecessary. After twenty hours the proteid in the upper layer of each tube was determined. The experiment was carried out in duplicate.

Salt $\frac{N}{4\cdot5}$. Proteid { I. 4·39 grs. per 100 c.c.
II. $\frac{1}{4} \times 4\cdot39$,, ,,

Fluid phase proteid { I. {0·45 grs. per 100 c.c.
{0·46 ,, ,, ,,
II. {0·16 ,, ,, ,,
{0·18 ,, ,, ,,

The undissolved residue in each tube was edestin, being entirely soluble in 10 % NaCl.

The composition of the fluid phase depends therefore upon each component, water, salt and globulin, and as the variation seems to be continuous the solid phase must be a continuously varying compound or solid solution containing all three.

The influence of the concentration of globulin in the system is probably in part related to the fact that a suspension of globulin in water differs from a suspension of, for instance, sand in this respect—that it is partly in solution and undergoes a true dissociation. The part dissolved may be of the nature of an impurity attached to the precipitate, but no matter how carefully prepared, Ox globulin suspended in water raises the conductivity of the water and the electrolyte, which is dissociated from the globulin, is so much a part of it as to be carried down again when the latter is centrifuged off. The globulin therefore is *ab initio* a solid solution or compound of low grade which dissociates in presence of water, the degree of dissociation increasing with dilution. Globulin reacts acid to litmus even when it has been dissolved and

precipitated ten times with volatile acid (HA or HCl) and alkali (NH_3), washed on a filter paper at nearly zero, and dried in vacuo over H_2SO_4 and KHO. The reaction of such a globulin in gas free water when tested *in vacuo* with litmus is decidedly acid.

The following table shows that the degree of dissociation of the globulin precipitate increases with dilution.

Specific Conductivity of a Suspension of Globulin.

V = vol. in c.c. of 1 gr. globulin.
C = grs. globulin per litre.
K = specific conduct. in recip. Ohms at 18°.

V	C	$K \times 10^{-6}$	$\frac{K}{C} \times 10^{-9}$
35·89	27·86	22·9	824
143·5	6·96	6·01	863
287·12	3·48	4·64	1333
574·24	1·74	3·25	1867

Salt globulins are so unstable and their solution so sensitive as to render the carbonic acid of the air an important component. This can be shown in many ways, but most simply by measuring the degree of dilution necessary to precipitate a solution of salt globulin with ordinary distilled water and with the same water freed from CO_2 by the passage of CO_2 free air, and from other gases by boiling.

$\frac{N}{6}$ NaCl was saturated with Ox globulin and centrifuged. Divided into two lots and diluted to $\frac{N}{15{\cdot}6}$ with (1) ordinary distilled water, and (ii) gas-free water respectively, and the precipitate centrifuged away as rapidly as possible. Proteid content of the upper layers:—

(i) 0·93 %, (ii) 0·76 %.

A portion of distilled water was freed from CO_2 by long continued bubbling through it of pure air, and from other gases by being boiled and rapidly cooled just before use.

Two burettes were filled, the one with gas-free water, the other with ordinary water, and determinations were made of the degree of dilution with the two samples of water necessary to precipitate salt globulin from clear saturated solutions. Ox globulin used 1 c.c. salt globulin solution:—

	c.c. CO_2 water	c.c. gas-free water
KNO_3 globulin	4 quite opaque	10 no change.
$MgNO_3$,,	4 opaque	4 very faint opalescence in 20′, deepening to partial opacity.
Na_2SO_4 ,,	2·4 faint opalescence, rapidly deepening to opacity	2·4 no change; 10′ later faint opalescence.
NaCl	1·6 opaque at once	1·6 remains clear for 10′, then becomes cloudy.
		15′, not yet opaque.

The slow clouding of solutions diluted with gas-free water is due to absorbed gas, and with care it may be seen to start from the free surface.

A thick suspension of Ox globulin precipitated from a solution in $MgSO_4$ by dialysis was diluted to thirteen volumes (1) with gas-free water, (2) with CO_2 water and run into 1 c.c. of a molar salt solution until a faint cloud appeared. Globulin in each suspension 0·426 grs. per 100 c.c.

Salt	c.c. suspension almost free from gases	c.c. suspension saturated with gases
NaCl	6·5	4·5
K_2SO_4	7·9	7·0
KNO_3	8·0	6·8

The NaCl globulin is the more easily decomposed by acid (H_3CO_4). Even more striking is the action of CO_2 in precipitating saturated solutions of salt edestin in gas-free water.

The solvent power of two salts acting simultaneously is the sum of their solvent powers when acting separately. The point is interesting, since, as Picton and Linder showed, when two salts together act as precipitants of colloids the precipitating power is the sum of their actions when measured separately only when the valency of the active ion is the same. When ions of different valency act simultaneously they inhibit one another. The solvent power of pairs of salts was found to be exactly the sum of the solvent power of each for the following pairs:—

KCl	KCl	KCl	NaCl	KB_r
NaCl	K_2SO_4	$MgSO_4$	K_2SO_4	Na_2SO_4

Inertia. Once a solid phase is formed it dominates the whole system by reason of the characteristic inertia of the solid state. I have often been struck with the fact that when silica is precipitated from solutions by salt the precipitate is at first resoluble and the change of state readily reversed. The precipitate, however, rapidly assumes its characteristic insoluble resistant nature. The gel. of silica in contact with water, as van Bemmelen showed, continues to change for years always in the direction of a more complete separation from the fluid. In these cases the equilibrium is labile and the composition of the fluid phase will alter continuously with the alteration in the solid phase. Van't Hoff points out the difficulties which arise in the study of the equilibrium states of calcium sulphate and water owing to the inertia of the solid phase, which may be one of various hydrates or one of two different molecular states of anhydrous calcium sulphate. The anhydrous form which separates first is a soluble calcium sulphate, and this slowly changes to insoluble calcium sulphate.

One can trace similar changes in globulin precipitates, which may be of the nature of differences in the degree of hydration of the globulin, of differences in the molecular state of the globulin similar to the distinction between soluble anhydrous and insoluble anhydrous calcium sulphate, or of differences in the amount of salt, acid or alkali combined with the globulin.

Increased stability of the solid phase of globulin due to contact with water. Globulin precipitated from a solution of acid or alkaline globulin by neutralisation and the precipitate is redissolved by salt. The amount of salt necessary to effect resolution rises and apparently reaches a maximum in 120 minutes, and the rate of solution slows with time. The increase in inertia is well seen in the following curve which represents solution of a globulin precipitate which had been kept in dialysing bags in contact with boiled water for three days.

Below the line where no solid phase can be separated by the centrifuge the curves were mapped by estimating the fraction of globulin in the total solid phase, above that line the change was followed by careful comparison of the change in the degree of opalescence. Curve *OABCD* represents in order solution in KOH, precipitation and resolution by HCl. Curve *OXYCZ* represents solution

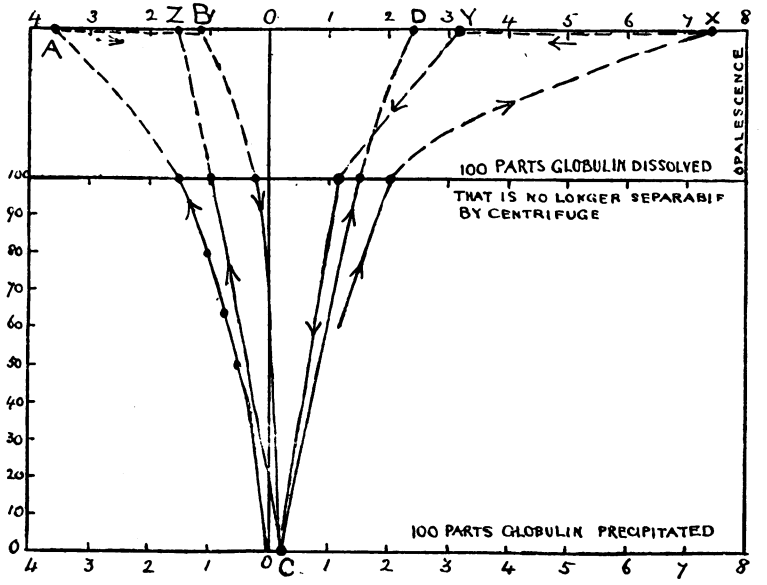


Fig. 4.

in HCl, precipitation and resolution by KOH. In both cases the curve starts from a suspension of ox-globulin containing in 100 c.c. 1·34 grs. dry globulin, and the figures to right and left of the line *OO* give respectively the number of c.c. of 0·01 normal KOH or HCl added to 4 c.c. of this suspension.

When globulin is dissolved by salt in presence of a small amount of acid (HCl) the end point depends upon the way in which the salt is added. If it be added slowly the first effect of the salt is to precipitate the globulin and this precipitate acts as a drag on the system so that final solution is only attained by excess of salt. If the salt solution be added rapidly with agitation the initial precipitate has not time to form and clear solution is obtained with less salt.

Two special instances of inertia are worth mention.

When the concentration of salt in the entire system is the same the concentration of globulin in the fluid phase is greater when the salt is added to a thick suspension of globulin than when the concentration of salt is reached by diluting a more concentrated solution of salt globulin free from solid.

Thus twice normal NaCl was added to a suspension of globulin so that the final concentration was 0·12 normal, and 0·09 normal respectively. And portions of 1·8 normal salt saturated with globulin were diluted with water to 0·12 normal and 0·09 normal with the result that precipitation of globulin took place. Therefore in the final state in each case excess solid globulin was present.

The various solutions were centrifuged and the globulin in solution determined with the following result:

Salt	Direct solution	Diluted from 1·8 normal
0·12 normal	4·46 %	2·16 %
0·09	2·776 %	0·35 %

The explanation is I think simple. If there were no inertia the state produced by solution and that produced by precipitation should coincide so long as excess globulin is present, and the state would be one in which salt, water, and globulin are distributed through both phases. But when solid globulin is dissolved the system has to come into equilibrium with residual solid globulin which will, owing to the inertia of the solid state, change to the salt globulin state slowly, while in precipitation on dilution the first-formed precipitate is a salt globulin in equilibrium with a certain relative mass of water. The lag in the one case is from one extreme condition of the solid phase, in the other it is from the other extreme. Similar cases are found in systems much less complex than colloidal systems, and they led van 't Hoff to a

generalisation which connects the degree of inertia with the valency of the ions[1].

An interesting example of inertia in respect to temperature is furnished by the compounds of edestin with potassium sulphate and sodium chloride.

When crystals of edestin are alternately melted by heat and separated by cold in sealed tubes under saturated solutions of K_2SO_4 edestin and NaCl edestin respectively, it was found that with a *rising* temperature the crystals in the NaCl tube melt slightly later than those in the K_2SO_4 tube, with a *falling* temperature they reappear very much earlier.

Warmed to 15°, the crystals in both tubes fuse, those in the NaCl tube lagging very slightly; cooled to 11°, the whole mass in the NaCl tube at once recrystallises, while that in the K_2SO_4 tube is still fluid ten minutes later.

The composition of the fluid phases from which crystals separated was :

	Proteid	Salt	Water
K_2SO_4	44·7 %	0·011 equiv. in 100 grs.	55 % by weight
NaCl	45·8 %	0·015 ,, ,, ,,	54·4 % ,,

From these two solutions it would be possible to construct a device which would resemble the thermal sense organs in indicating either a rising temperature or a falling temperature.

Looked at from the point of view of the Phase Rule most of the measurements made are of consolute points for the globulin rich and globulin poor phases, the consolute point being defined by opalescence due to an internal phase being minimal. Like ordinary three component systems which form phases of continuously varying composition the composition of the consolute depends upon the initial ratio of the components, *e.g.* water and globulin. In the phase diagram the curve for the conjugates is nowhere parallel to the base line globulin-water and therefore it is cut at different points according to the position on that base line of the initial system.

THE EQUILIBRIUM BETWEEN ACID, OR ALKALI, GLOBULIN AND SALTS.

It will be convenient to use BOH for alkali, HS for acid, and G for globulin, the latter symbol however is not taken to mean always the same reacting weight.

There are four components, BOH [or HS], G, BS, and HOH, and the equilibrium of these four component systems which are of practical interest are (1) the precipitation of solutions of alkali or acid globulin by low concentrations of salts, (2) the precipitation of those solutions on

[1] *Archiv. Néerlandaise*, VI. p. 471. 1901.

neutralisation, *e.g.* GHS + BOH = G + BS + HOH. In the second case the right-hand side of the equation which represents the condition when the solid phase forms contains the salt BS, and this is the agent which brings about the precipitation. Dialysis for instance in which salt is not formed does not lead to precipitation, at any rate from solutions where the acid or alkali combined with the globulin liberates only univalent ions.

Precipitation by neutralisation therefore is merely a special case of the precipitation of alkali or acid globulin by salts. The stability of the solution is diminished by reducing the ratio acid or alkali/globulin until the concentration of salt is sufficient to destroy it.

The solid phase which settles out may be alkali globulin or acid globulin of very low alkali or acid content, or globulin. This is the only possible explanation of the following facts. The solid phase is completely immiscible with the fluid phase, but it is usually only imperfectly separable from water. When the precipitate is dialysed or washed by decantation a stage is frequently reached at which solution of a low grade is reformed, the solution being filterable and stable on a centrifuge. The proteid has resumed its ionic character and the direction of movement shows that if it has been precipitated from alkali globulin it moves to the anode, if it has been precipitated from acid globulin it moves to the cathode. Between these two conditions of the precipitates there is a point where globulin is precipitated which does not resume its ionic character in presence of any volume of water.

Salts as precipitants. When salts at low dilution precipitate hydrosols a simple and remarkable relation exists. The precipitating action of the salt is due to one only of its ions, always that which carries an electric charge of a sign opposite to that of the colloid particle, and the power of the salt is much greater the higher the valency of the ion.

This valency rule can be detected in the precipitation of acid or alkali globulin by salts, but it is obscured to a greater or less extent by the fact that the salts themselves can form soluble compounds with globulins.

If transparent solutions of acid or alkali globulin are precipitated by salts it will easily be seen that the salts are arranged so far as precipitating power is concerned according to the ionic valency rule stated above, but it is equally obvious that some salts precipitate a larger

fraction of the globulin and give a heavier and coarser precipitate than others.

Putting this complication on one side attention was fixed solely upon the point of maximal precipitation for any particular salt, and four salts were examined in some detail, the acid and alkali being HCl and NaOH respectively.

The method used was to add a normal solution of the salt to the solution of globulin until the point of maximal precipitation was reached. The mass of globulin was constant throughout. The volume, that is the concentration, of the globulin varied. The following curves (Fig. 5) give the relative concentration of acid or alkali, and salt which correspond to the most complete separation of a solid phase. The curves

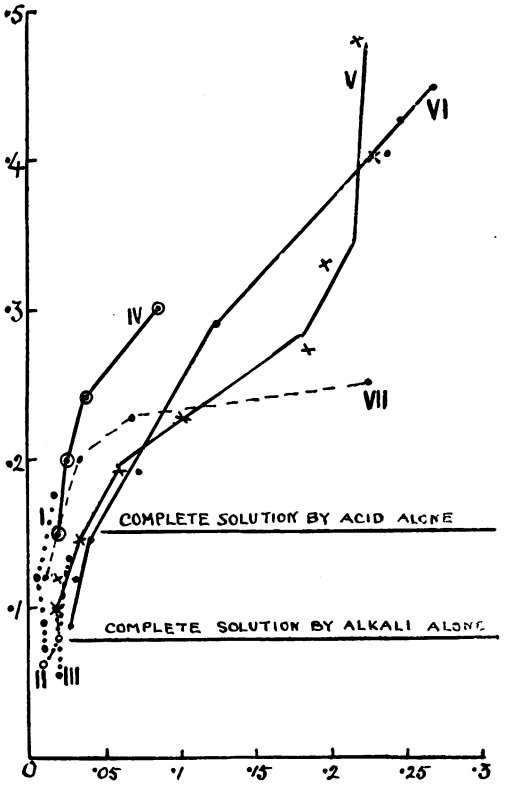


Fig. 5. Ordinates equivalents of HCl or NaOH × 100 per litre. Abscissæ equivalents of salt per litre. Dotted curves are salt alkali. I. $MgNO_3$: NaOH. II. $MgSO_4$: NaOH. III. K_2SO_4 : NaOH. IV. K_2SO_4 : HCl. V. $MgNO_3$: HCl. VI. KNO_3 : HCl. VII. $MgSO_4$: HCl.

for salt acid-globulin are much more extended than for salt alkali-globulin. The range of concentration of alkali over which a salt can precipitate is so small as to reduce the curves practically to short almost vertical lines, the slope of which is almost indeterminable.

The curves were followed downwards into the region of opaque opalescent solution as far as possible, as the horizontal lines which mark the level of clear solution show.

The curves for salt acid globulin are extensive enough to permit of some conclusion being drawn. At a concentration of acid when the globulin is completely dissolved the valency law holds, the electric sign of the proteid is + and the salts are:

$$K_2SO_4 = MgSO_4 > MgNO_3 \text{ and } KNO_3.$$

As the acid concentration rises an entirely fresh relationship is seen—that due to the solvent powers of the salts, those powerful solvents the sulphates ceasing to have any precipitating power, the nitrates continue to be good precipitants beyond the range followed.

At 0·002 to 0·003 HCl the concentration of K_2SO_4 or $MgSO_4$ needed to *precipitate* nearly reaches infinity, while at 0·23 $MgNO_3$ the concentration of acid needed to *dissolve* reaches infinity, and for 0·23 KNO_3 is rapidly rising.

The remarkable change of direction in the curve $MgNO_3$. HCl on the analogy of similar cases indicates a change in the constitution of the solid phase which is formed.

The curves for K_2SO_4.HCl and $MgSO_4$.HCl cannot be prolonged upwards, for beyond their upper ends a solid phase cannot exist. The curves for $MgNO_3$. HCl and KNO_3. HCl can be continued beyond the point to which they are actually mapped, that is to say these salts continue to precipitate with still higher concentration of acid.

Salts as solvents in presence of acid or alkali. I measured the converse relation for two salts, namely the effect of varying amounts of HCl and NaOH upon the amount of salt necessary to dissolve the same weight of globulin.

The salts were K_2SO_4 and $MgSO_4$ (Fig. 6). The ordinates give c.c. of normal salt, the abscissæ to the right of the line *OO* represent c.c. of 0·01 normal HCl, to the left c.c. of 0·01 normal NaOH. At 0·5 NaOH the mass of K_2SO_4 needed to dissolve the mass of globulin present (0·107 grs.) is zero. The ordinate *OO* measures the amount of salt needed to dissolve when neither acid nor alkali is added. The curve is the line which separates the system with no solid phase present from

the system with a solid phase present. The former lies above, the latter below the curve.

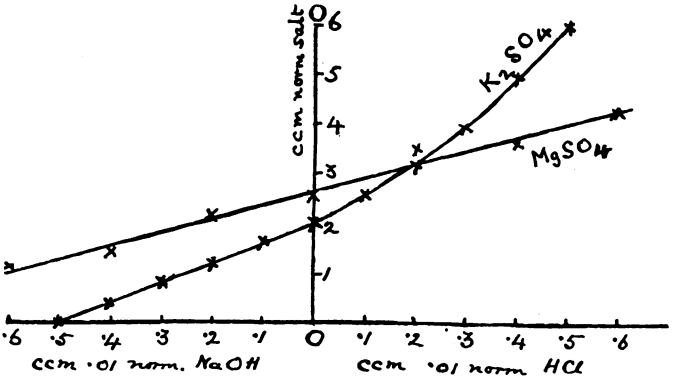


Fig. 6. Ordinates are c.c. normal salt, abscissæ to right, c.c. 0·01 normal HCl, to left 0·01 NaOH (for K_2SO_4 curve) or KHO (for $MgSO_4$ curve), in each case added to 5 c.c. of a globulin suspension. OO is line of salt alone.

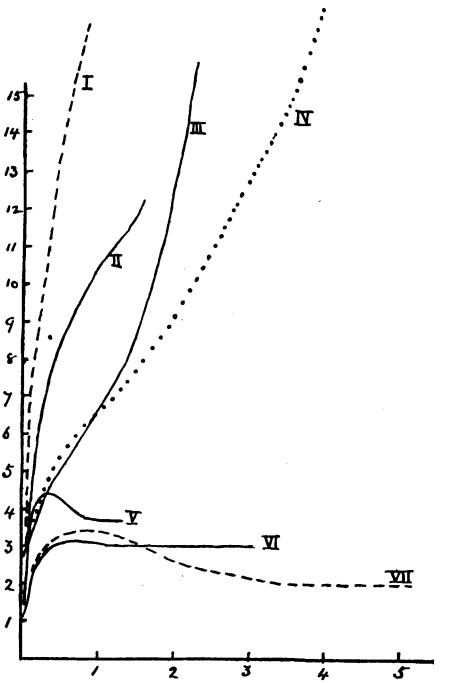


Fig. 7. The curves show the amount of acid (I, II, III, IV) or alkali (V, VI, VII) needed to dissolve to transparent solution 0·107 grs. dry globulin in presence of varying amounts of salt.

Acid or alkali as solvent in presence of salt. The difference in the relation of salt to acid globulin and to alkali globulin is shown in the following curves (Fig. 7), which indicate the quantity of hydrochloric acid or caustic soda needed to dissolve an arbitrary weight of globulin (0·107 grs.) to the same grade of solution—in presence of certain salts. The ordinates are c.c. of 0·01 normal HCl or NaOH, and the abscissæ are c.c. of normal salt to 10 c.c. of a suspension containing 0·107 grs. dry globulin.

The salt was added to the suspension first and it produced partial or complete solution. The acid or alkali was then run in, the globulin being reprecipitated by the first few drops. The curve for $Mg(NO_3)_2$. NaOH is too short to be plotted owing to the great solvent power of the salt in presence of alkali.

The curves show (1) that all the salts examined inhibit solution by the acid, and all the salts at first inhibit and then aid solution by the alkali; (2) that each salt has its own peculiar form of curve.

Acids and alkali as precipitants and solvents in presence of salts. In order that the precipitate should include a large fraction of the globulin the relative concentrations need to be adjusted with some accuracy. The distribution of globulin in the solid and fluid phases was determined by centrifuging and estimating the total precipitated. This gives the state of the system under the centrifugal stress, and as it has been shown that centrifugal force will concentrate the outer layers of an ordinary salt solution[1] this state must not be regarded as coincident with that produced by the much smaller force of gravity.

The following curves (Fig. 8) are drawn according to the recognised convention—the upper line represents the one phase system, all the

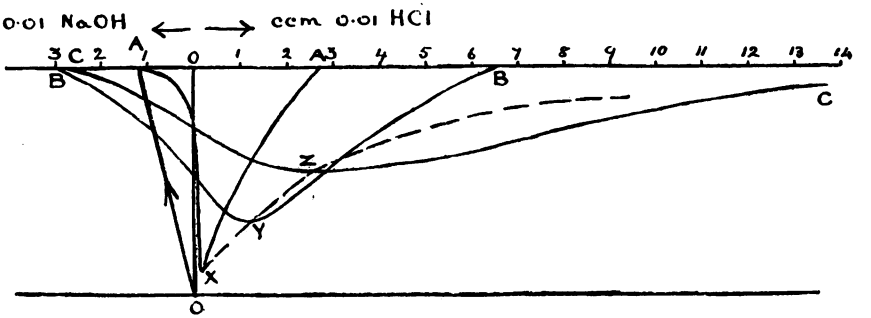


Fig. 8.

[1] van Calcar and Lobry de Bruyn. *Rec. Trav. Chim. Leiden*, XXVIII. p. 218. 1903.

globulin being dissolved, the lower line represents complete precipitation of the globulin. The apex of each curve is the point of maximal precipitation. To the left of the line *OO* the numbers give the amount of NaOH added in c.c. of 0·01 normal solution, to the right they give the number of c.c. of 0·01 HCl. Therefore the line *OO* is the line of neutrality so far as the NaOH and HCl alone are concerned. The lower *O* is a suspension of 0·1 gr. of dry globulin in 10 c.c. It was completely dissolved by alkali (*OA*), the curve being drawn as a simple straight line because the points intermediate between *O* and *A* were not mapped. The solution of NaOH globulin was precipitated by HCl 0·01 normal, and the curve *AX* does not reach the line of complete precipitation. The precipitate was redissolved by HCl (*XA*). One c.cm. of normal KNO_3 was added to 10 c.c. of the suspension and the curve *BYB* mapped, and 3·1 c.c. normal KNO_3 gave the curve *CZC*.

These curves show that the effect of increasing salt (KNO_3) concentration is to shift the point of maximal precipitation over to the acid side, and to render resolution by acid more and more difficult. At the same time the fraction of the globulin which can be precipitated becomes less and less, the trough of the curve gradually filling up, the apex moving along the dotted line.

The salt which is produced when a solution of acid or alkali globulin is precipitated by alkali or acid similarly shifts the point of maximal precipitation over to the acid side. In Fig. 9 two sets of

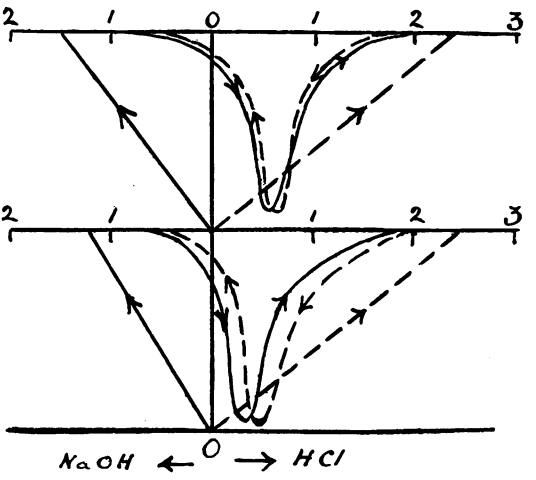


Fig. 9.

curves are given. In the lower, starting at *O*, the following events occur in order in the curves; solution by NaOH, precipitation and resolution by HCl, and, in the dotted curve, reprecipitation and resolution by NaOH. The second precipitation occurs further in the acid region. The other curve traces the same series of events with the same suspension after 20 c.c. 0·02 normal NaCl had been added to 100 c.c. of the suspension.

When an arbitrary and somewhat low salt concentration (0·13 normal) is chosen and the curves of solution and precipitation mapped, the effect of the valency law stated earlier is seen very prettily in the relative power which salts possess of inhibiting solutions of globulin by acid. In the following curves (Fig. 10) the point of maximal concentration is

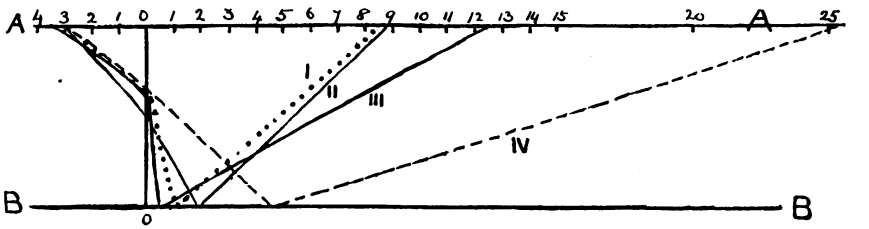


Fig. 10. *AA*, 100 per cent. globulin dissolved. *BB*, 100 per cent. precipitated. *OO*, no acid or alkali. At the beginning 1·5 c.c. normal salt added to 10 c.c. suspension. I. $Mg(NO_3)_2$. II. KNO_3. III. $MgSO_4$. IV. K_2SO_4.

placed on the line of complete precipitation as a convention since the fraction of globulin actually precipitated was not determined. The sulphates with bivalent negative ions inhibit solution by acids much more than the nitrates with monovalent negative ions, and also displace the point of maximal precipitation further to the acid side. This last feature is unexpected and interesting. It shows that the salt interferes with the action of the acid in decomposing alkali globulin, a relation readily understood on the hypothesis of the existence in solution of a salt alkali globulin compound.

These various cases render the complexity of the four component systems very obvious; there is however one feature of fundamental importance which is never obscured, and that is the antagonism between the solvent actions of salts and acids, and the additive nature of the combined solvent action of salts and alkali. This feature arises I believe from the fact that acid globulin is insoluble by salt. Salts will combine with globulin or with alkali globulin to form soluble compounds; they will not so combine with acid globulin.

If this be true, then, when acid globulin is precipitated and redissolved by salt, the acid must be displaced and the globulin redissolved, not as salt-acid globulin but as salt globulin. The displacement of the acid can readily be followed by methyl orange. When HCl globulin is precipitated by a salt this indicator shows that free acid is liberated[1]. But I failed completely to detect the liberation of free alkali when salt is added to a solution of alkali globulin. This suggests that precipitation of acid globulin is partly a definite chemical replacement of acid by salt,

$$GHS + BS = GBS + HS.$$

The fact that alkalies slightly assist solution in salts while acids very generally depress it suggests an interesting possibility, namely, that in the compounds GHS and GBS the acid HS and the salt BS are united to the molecule of G in the same way, so that they compete with one another, while in GB and GHS the base and salt are united to different parts of the molecule.

On this view the possible equations would be,

$$GB + BS \rightleftarrows GB.BS,$$

$$G + BS \rightleftarrows G.BS,$$

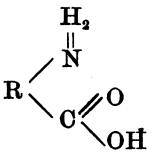
$$GHS + BS \rightleftarrows GBS + HS.$$

And the impossible equation is

$$GBS + HS \rightleftarrows GBS.HS.$$

Analogous case of amido acids. These equations can be developed further on the analogy of the amido-acids, if the globulin be taken to have a molecular structure in general agreement with that of Fischer's polypeptides.

The structural formula for an amido-acid is



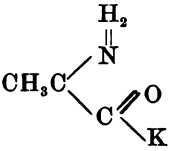
According to Bayer's oxonium theory the upper oxygen of the carboxyl group has two unsatisfied valencies, oxygen being tetravalent O≡. In the molecule it acts as O=. The nitrogen atom has two

[1] The following salts were tried and in each case free acid was detected, KNO_3, $CaCl_2$, and $MgSO_4$.

unsatisfied valencies. In the molecule it is N≡, while its upper limit of valency is N≣. Therefore there are two places where other atoms can be linked on without replacement.

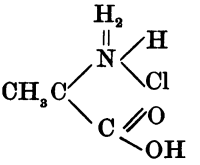
Consider the special case of amido-acetic acid. The following compounds are known.

(i) The salts with alkalies formed by replacement of the hydroxyl group:

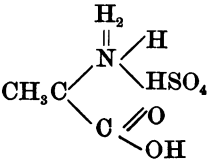


i.e. potassium acetate.

(ii) The salts with acids:

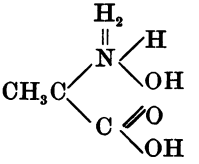


or



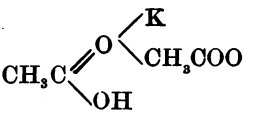
In this class the linkage is due to a change from N≡ to N=.

The hydrated form which theoretically exists in water would also probably have the formula



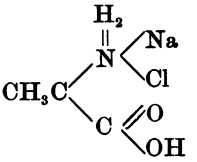
(iii) Double salts such as $CH_3C\begin{matrix}NH_2\\COOH\end{matrix}\cdot NaCl$.

Which of the two possible linkages holds the NaCl? Clearly it might be the bivalent O= on the analogy of the hyper-acid acetate of potassium.



But the probability is very much in favour of the linkage being by N≡, since N≣ is much more stable than O≣.

Therefore the probable formula is



That is to say the amido-acid can link on an acid or a neutral salt *but not both.*

Therefore the possible equations are:

$$R\begin{cases}NH_2\\COOH\end{cases} + HS \rightleftharpoons R\begin{cases}NH_2.HS\\COOH\end{cases}$$

$$R\begin{cases}NH_2\\COOH\end{cases} + BOH \rightleftharpoons R\begin{cases}NH_2\\COB\end{cases} + HOH$$

$$R\begin{cases}NH_2\\COOH\end{cases} + BS \rightleftharpoons R\begin{cases}NH_2BS\\COOH\end{cases}$$

$$R\begin{cases}NH_2HS\\COOH\end{cases} + BOH \rightleftharpoons R\begin{cases}NH_2HOH\\COOH\end{cases} + BS$$

$$R\begin{cases}NH_2BS\\COOH\end{cases} + HS \rightleftharpoons R\begin{cases}NH_2HS\\COOH\end{cases} + BS$$

$$R\begin{cases}NH_2\\COB\end{cases} + BS \rightleftharpoons R\begin{cases}NH_2BS\\COB\end{cases}$$

Appendix I.

The apparatus used to determine the absence of any drift in a field, and therefore of any charge on the molecules of the solvee, was as follows:

AB is a glass tube ground at the ends, on which is a shoulder at *E*, so that it can be lowered into the U-tube which it nearly fits. The lower part is graduated in millimetres, and filled with a gel of celloidin permeated only by pure chloroform. The gel was prepared by filling the tube to *C* with a solution of celloidin in ether and alcohol and setting it to a gel by immersion in chloroform. The ether and alcohol were removed by immersion in the dark for weeks in many changes of chloroform. The bend of the U-tube holds mercury, and the space *BC* a solution of azobenzene in chloroform. The space *AC* holds a similar

solution. Electrodes dip into AC and into the mercury and a field of 130 volts per cm. was maintained for six hours; after which the tube AC was removed and the azobenzene found to have diffused 9 mm. into the celloidin gel from each end. It has therefore moved at equal velocities in the field in a direction from anode to cathode, and from cathode to anode. There is no difference to indicate a drift of charged molecules.

In other experiments two plates of solid azobenzene each 1 mm. thick, were kept at differences of potential the gradient between them being 500 volts per cm. They were then immersed in chloroform and found to dissolve at the same rate.

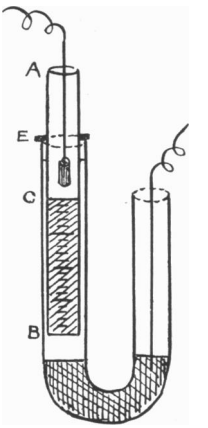


Fig. 11.

Appendix II.

The relation of the globulin to serum proteid.

Ionic globulin is absent from serum, therefore alkali globulin is absent. But salt-alkali globulin which does not liberate ionic globulin in solution may be present and stable, in spite of the low salt content, owing to the great stability of this substance in solution. Dialysis brings about partial precipitation by liberating alkali globulin, which in turn is precipitated by the free salt present, or by carbonic acid. Further decomposition and precipitation occurs when a stronger acid is added.

This hypothesis is simple, and can be extended very easily to embrace the methods of precipitation. It is also in agreement with general opinion, which regards serum as a mixture of certain proteids in solution.

It fails, however, to explain why serum is not precipitated by simple addition of acid, for acid globulin is extremely unstable in presence of salts, and on the whole the balance of probability is against it and in favour of there being in serum some (possibly one) complex proteid which breaks down readily into fractions whose composition and properties depend upon the degree of dilution and the reagents used.

The facts of the case are these. The proteids of serum are electrically inactive. Neither the whole nor any fraction moves in

a field. It is not possible to detect a trace of "ionic" proteid. Dialysis or dilution disturbs the equilibrium, and "ionic" globulin appears, and can be swept out of the general mass of proteid as an opalescent cloud before dialysis has been pushed to the point where precipitation occurs. The development of even minute quantities of "ionic" globulin can be detected in this way. The direction of the movement is towards the anode, therefore the globulin which appears is the anion of alkali globulin.

As dialysis or dilution proceeds the concentration of the liberated alkali globulin increases, the increase being shown by an increase in the density of the anionic globulin cloud which emerges from the serum in the electric field, and when the concentration reaches a certain point precipitation of globulin or of an alkaline globulin having a lowered alkali content occurs, due either to the action of carbonic acid or to the relative concentrations of alkali globulin and salt becoming such that the former is partly precipitated, just as a solution of pure alkali globulin can be more or less completely precipitated by the addition of small amounts of salt. What is known of globulins reduces the possibilities to these two, and as a matter of fact the action of carbonic acid seems to be the more potent. Dialysis in closed vessels against water freed from carbonic acid decidedly diminishes precipitation.

Be this as it may, when dialysis ceases to precipitate one is left with serum which has lost only a small portion of its proteids[1] and which still contains globulin in abundance. Further precipitation of this residual globulin is brought about by the addition of any acid, but even the most cautious acidulation fails to precipitate the whole, a second dialysis giving a still further yield.

Serum which has been dialysed against water with very low carbonic acid content until it ceases to give any precipitate, but which can still give with acid a large yield of globulin is in a most interesting condition. The whole remaining proteid is now ionic. It moves towards the anode quite uniformly, therefore it behaves as a whole as the proteid ion of an alkali proteid compound.

Now what does this mean? The presence of other proteids does not interfere with the movement of ionic proteid. This point cannot be too much insisted on. It lies at the root of the evidence. Therefore one starts with proteid which behaves uniformly and is electrically inactive,

[1] Hammarsten's values are not for serum, but for serum which has been first neutralised by acid.

one ends with proteid which behaves uniformly and is electrically active, and in the final stage there is no evidence of the presence of more than one kind of proteid ion. But this residue of uniform character still contains a globulin fraction. It can be split by saturation with a neutral salt or by acidulation into fractions differing in properties according to the mode of separation.

Serum proteid in uncharged serum is electrically inactive, but it is possible to make it as a whole electrically active.

When in the cell used for these experiments which is described on page 289 the upper layer of fluid is a solution of $\frac{1}{600}$ normal acetic acid and the lower layer is serum and a current is passed for 24 hours the serum proteid becomes slowly charged in such a way that it is repelled from both poles. Therefore in the anodic limb it becomes charged positively, in the cathodic limb negatively. The result of the double repulsion is that the proteid is condensed into a hard plate of rubber-like consistency just midway between the electrodes[1].

The phenomena would be explained on the theory which has been outlined in this way. In the anodic limb an excess of hydroxyl ions are liberated owing to the electric convection, and these, reacting with the serum proteid, convert it into cationic proteid. In the cathodic limb the converse reaction with hydrions occurs and is possible owing to the amphoteric nature of proteids. The entire mass of serum proteid is thus thrown into the ionic state and in this condition moves with uniform motion, the one half as cationic proteid, the other half as anionic proteid. The electric current, the most subtle of analysers, detects only one substance, and this substance owing to its amphoteric nature can exist in either the cationic, or the anionic state.

The concentrated hard rubber-like mass of proteid obtained in this way cannot be discriminated from serum proteid. It is easily soluble in distilled water, and in dilute salt solutions. From the solution in water a globulin fraction can be precipitated by acid, or by saturation with magnesium sulphate. From the solution in dilute salt solution a globulin fraction can be precipitated by saturation with salt (NaCl or $MgSO_4$). The filtrate after removal of the globulin by saturation contains a proteid which is precipitated by acetic acid. In this respect it is identical with serum albumen.

The plate of concentrated proteid contains the serum phosphorus but only a fraction of the serum pigment.

[1] Whitney and Blake, describe a similar double repulsion in the case of gelatine. *Am. Chem. Soc.* XXVI. p. 1339. 1904.

The position then is that in serum one has proteid which can be thrown into the ionic state and which then moves in a field as a single substance. From it an electrically active fraction, namely globulin, can be split off, and the proteid thereby becomes electrically heterogeneous. When the fraction is removed by dialysis and by acid the remaining fraction is again electrically homogeneous, but it is now ionic.

Now the globulin fraction has an abiding characteristic. In all its solutions its molecular state is so gross as to cause the molecules to be arrested by a porous pot. They will not pass such a filter, even under pressure. In this it is sharply distinct from the parent serum proteid, which is readily filterable. If globulin be present as such in serum it is not only non-ionic, but the agent which dissolves it must be something more than alkali and salt since either alone or together they will not produce so high a grade of solution.

The difference in the molecular state of globulin when once separated, and the electrical homogeneity of serum proteid and of the fraction (still capable of further subdivision by salting out) which remains after the alkaline globulin fraction which most readily appears has been removed suggests that serum proteid is a complex unit. If such a unit exist it is not saturated with globulin. Fresh ox serum has an extraordinary power of dissolving globulin, it will take up almost its own volume of the thick cake at the bottom of a centrifuge tube; and in ox serum so saturated there is not a trace of alkali globulin nor of any ionic proteid.

The question of the relationship of globulin and serum proteid can be approached from the chemical side.

Globulin contains phosphorus, two analyses by Carius' method giving 0·07 and 0·08 % by weight. In the analyses of blood globulins which I can find, this fact is nowhere mentioned, though Porges and Spiro[1] speak of calcium phosphate as a constituent of globulin precipitates, though the manner of its identification is not given. If calcium and phosphate were to be found in the ash in the requisite proportions it is certain that calcium phosphate does not occur in the intact globulin, for the phosphorus-holding portion is not dissolved by 48 hours' digestion at 40° with 50 % acetic acid.

Ox serum globulin was precipitated by ½ saturation with $AmSO_4$, and the precipitate purified by being dissolved and reprecipitated seven times. The preparation contains phosphorus.

Some of this preparation was dissolved and coagulated by boiling, and the fine

[1] *Beit. z. chem. Physiol. u. Path.* III. p. 277. 1902.

coagulum suspended in boiling 30 % acetic acid, and then digested for 48 hours with 50 % acetic at 40°. The proteid was filtered off and the filtrate found to give a good xanthoproteic reaction. The undissolved proteid was washed on a filter for two days with hot water. It still contained phosphorus, apparently in undiminished amount.

The phosphorus is not from entangled lecithin.

Ox globulin precipitated by acetic acid was coagulated by heat, digested for 48 hours at 40° with 50 % acetic, and washed on a filter with hot water until all the acid was removed. It now contained phosphorus. It was extracted with alcohol, dried and extracted with two changes of ether in a Sohxlet apparatus for five days, and it still contained phosphorus.

The property of serum globulins which first caught my attention in this investigation was their phosphorus content and the close association of the characteristic insolubility of the globulin with the presence of this element.

All the globulin fractions which can be separated from serum contain phosphorus in the first instance, most of them can be fractionated by secondary treatment into a phosphorus-holding proteid which is precipitable by dialysis and a phosphorus-free proteid which is soluble in water.

Consider first two fractions, the one separated from ox serum diluted to 10 to 15 volumes by acetic acid, the other separated from ox serum by saturation with magnesium sulphate. The first is purified by three times solution in ammonia, and precipitated by acetic acid. The second was purified by solution and precipitated by the salt until the whole of the proteid came down on saturation. The magnesium sulphate fraction, or shortly, the salt fraction, is, as Hammarsten showed, a much larger fraction of the whole serum proteid (63%), the acid fraction is only 17%.

The phosphorus content of these two fractions is very different, being for the acid fraction 0·07 to 0·08 % *P*, in the salt fraction 0·009 % *P*[1].

The acid fraction will not yield a fraction soluble in water or free from phosphorus by any simple treatment such as partial precipitation by acids or alkalies, by dialysis, or treatment with salts. I have only two sets of estimations of phosphorus bearing on this point, namely of fractions precipitated from solutions in very high dilute caustic by acetic acid. The first fraction to come down contained 0·068 % *P*, the second fraction 0·071 %, both fractions being prepared for analysis by washing with alcohol and ether.

[1] The analyses were carried out for me by Mr Hall, assistant at the University Chemical Laboratory, by the bomb method. The amount of phosphorus in the salt fraction is too small for accurate estimation. Mr Hall gives me the above figure as the best available approximation.

The salt fraction, as Burckhardt[1] first showed, readily splits on dialysis into a water soluble and a water insoluble portion. The former contains phosphorus, the latter is free from phosphorus, is soluble in water but is precipitated by saturation with neutral salt.

Consider another method of fractionating serum proteid. Ox serum is dialysed until precipitation ceases [P. i], the residue is precipitated by addition of acetic acid [P. ii], the filtrate is precipitated by saturation with magnesium sulphate [P. iii]. P. i, P. ii, and P. iii all contain phosphorus. Between P. ii and P. iii a fraction also can be interposed, namely by dialysis. I do not know whether this causes the final salted out fraction to be free from phosphorus.

P. i will not yield a phosphorus free or water soluble moiety, P. ii readily does so. Dialysis of solutions of the proteid in either dilute acetic acid, or sodium chloride, leads to the separation of a precipitate which contains all the phosphorus, the residue is a water soluble phosphorus-free proteid which is precipitated from its solutions by saturation with magnesium sulphate. From the former fraction I failed by subsequent treatment to separate water soluble proteid. The latter fraction is a proteid of somewhat singular properties. It is not precipitated by a stream of carbonic acid gas, nor by dilute acids. It is precipitated by very dilute alkali and the precipitate is resoluble on the addition of salts. It is entirely and completely precipitated by saturation with magnesium sulphate at 30°. It is not precipitated by 60% alcohol, though the slightest trace of salt at once and completely precipitates. It is coagulated by heat. It is very readily changed to derived albumen, as for instance by simple evaporation of the solution in air.

The two fractions into which P. ii splits differ not only in relation to phosphorus, but also in relation to the presence in the molecule of a sugar-yielding moiety. I succeeded in preparing osazone from the fraction which is insoluble in water and contains phosphorus. I failed in three attempts with the soluble phosphorus-free fraction.

P. iii gives a very poor yield at best of insoluble proteid. This examination of the various fractions is uncompleted and I am engaged in filling in the gaps, but the knowledge so far gained suggests that serum proteid will yield a phosphorus holding proteid which is insoluble in water and has all the properties associated since Schmidt's time

[1] *Arch. f. exp. Path. u. Pharmak.* XVI. p. 322, 1883.

with the word globulin. It corresponds to the englobulin of later writers[1].

It can be split off in association with varying amounts of a soluble proteid which corresponds probably to the pseudoglobulin of these writers.

When isolated it forms compounds with acids and alkalies which are remarkable for the indefiniteness of the combining weights, therefore englobulin itself may be merely a step in a series of proteid compounds, the magnesium sulphate fraction being at one extreme, and the so called nucleo-proteid of Pekelharing, which has all the characters of a globulin, at the other.

This is a very obvious possibility, it is therefore pertinent to ask why englobulin is dealt with in this paper as a chemical unit? Because in the first place it combines as a whole with bases so weak as aniline and urea, and with acids so weak as boracic, and in the second place when combined with any acid or any base the whole of the proteid becomes ionic, not merely a highly phosphated acid fraction, and the whole in its ionic state shares uniformly in the electric transport.

Summary.

This paper deals with the behaviour of globulins to acids, alkalies and salts, and the properties of the solutions considered as cases of colloidal solution.

In the three component systems water, globulin and monovalent acid or monovalent alkali, or neutral salt, when three phases are present they are vapour and two continuously varying states, one rich in globulin the other poor in globulin. The former may separate as a precipitate or it may remain dispersed throughout the latter as an internal phase which is not separable by gravity or centrifugal force, in which case it confers on the fluid a greater or less degree of opalescence. Both these phases vary continuously in composition with variation in the relative masses of globulin and acid, alkali, or salt. Such continuously varying states might be called solutions, solid or fluid, of the one component in the other, the precipitate for instance being a continuously varying solid solution of globulin, water, and salt, acid or alkali, like the continuously varying solid solution of ammonium and potassium sulphate which

[1] Marcus. *Zeit. f. physiol. Chem.* XXVIII. p. 559. 1899. Porges and Spiro. *Beit. z. chem. Physiol. u. Pathol.* III. p. 277. 1902; and others.

separates from a solution of both salts. The relations however are best described as continuously varying compounds of globulin, since there are signs in the behaviour with indicators, and in the electrical character of the solution, and in the replacement of acid by neutral salt, of undoubted chemical interaction.

Solution by acids or alkalies. (Electro-positive or electro-negative globulin.) The facts can best be explained by assuming that salt formation occurs. This agrees with the view of all previous workers on the subject. Globulins therefore have both an acid and a basic function—they are amphoteric electrolytes.

The globulin salts ionise in solution; therefore, in an electric field the entire mass of proteid moves. They also hydrolyse, but the hydrolysis offers special features resembling those which Jordis has pointed out in the case of sodium silicate, and Chevreul in the case of soaps. In both cases hyper-acid salts are formed. By dialysis the degree of hyper-acidity or hyper-basicity can be raised, but, as Jordis finds in the case of silica, with continuously increasing difficulty. With the rise in the degree of hyper-acidity or hyper-basicity, the "grade" of solution diminishes, but, in the case of globulins, a precipitation point is not reached with monovalent acids or alkalies. Electrically active solutions of globulin cannot be precipitated by dialysis, nor at any stage do they cease to be electrically active.

If G be used to denote globulin, the equation of hydrolysis or dialysis would be:

$$x\,\mathrm{GHS} + y\,\mathrm{HOH} = (\mathrm{GHOH})_y\,(\mathrm{GHS})_{x-y} + y\,\mathrm{HS},$$

$$\text{or } x\,\mathrm{GB} + y\,\mathrm{HOH}_x = (\mathrm{GH})_y\,(\mathrm{GB})_{x-y} + y\,\mathrm{BOH}.$$

In dialysis the ratio x/y varies continuously.

Clearly a relation of this kind agrees with van Bemmelen's definition of absorption compounds as chemical combination with variable composition.

The relative solvent power of various acids and alkalies has been measured. For strong and medium acids solvent power is measured by the number of gramme molecules present, not by the number of gramme equivalents:

$$\mathrm{HCl} = \mathrm{H_2SO_4} = \mathrm{H_3PO_4}.$$

Very weak acids have a lower solvent power $\mathrm{HCl} = 5\mathrm{HA} = \pm 30{,}000\mathrm{H_3BoO_3}$. Comparison was made between the amounts of acid necessary to reach the same grade of solution.

The relations are explained by the very weak basic function of globulin. Therefore, salts with weak acids are much hydrolysed, and to reach the same grade of solution an excess of acid is needed in order to lower the degree of hydrolysis. With alkalies the weak alkali NH_4OH dissolves as well as the strong alkalies, owing to the fact that globulin acts as an acid of considerable strength.

The acid and basic functions were measured by the well-known methods—the catalysis of cane sugar and of methyl acetate—and the acid function found to be much the greater.

Data derived from measurements of electric conductivity, and from the behaviour with indicators, support the view that salts are formed, and that the globulin acts much more strongly as an acid than as a base.

2. *Direct measurement of the specific velocity of globulin "ions"* was carried out by the boundary method.

As the basic function of a globulin is weaker than its acid function the salts GHS (globulin + acid) will be hydrolysed more than the salts GB (globulin + base). Therefore, in the equation of hydrolysis given above, y/x will be larger for the former than the latter. Similarly, comparing salts with $H\bar{A}$ and with HCl, y/x will be greater for the former.

With increase in the value of y/x the size of the molecule increases also. By dialysis, the size can be increased until it is large enough to diffract white light.

The large molecules are ionised, that is to say, they take part in the electric transport. And their specific velocity is exceptionally high. "Ions" of this order of magnitude have the properties of matter in mass. Each is defined by a surface and moves under the influence of a surface contact difference of potential. With their appearance the fluid would cease to be homogeneous. It would have internal surfaces. I propose to call such ions colloidal ions, or pseudo-ions. Their specific velocity, within wide limits, is independent of their size, and is controlled by the laws of electrical endosmose.

These conclusions are borne out by the boundary measurement and by the electrical conductivity of colloidal solutions.

For instance: theoretically the proportion of pseudo-ions in the following solutions of globulin should be:

$$\text{In } H\bar{A} > \text{in HCl}, > \text{in NaOH},$$

and the specific velocities at 18° were found to be 23×10^{-5}, $11{\cdot}5 \times 10^{-5}$, $7{\cdot}6 \times 10^{-5}$.

By Ostwald's law of the relation of specific ionic velocity to the number of atoms in an ion the value 23×10^{-5} is an utterly impossible one for an ordinary ion of the magnitude of the proteid molecule.

3. *Viscosity.* The viscosity of a globulin solution diminishes rapidly with the increase in the concentration of the ions, and more especially with the increase in the concentration of the true globulin ions as compared with the pseudo-ions.

4. *Solution by neutral salts.* Globulins are dissolved by neutral salts owing to the formation of molecular compounds (G . BS). These compounds are readily decomposed by water with liberation of the insoluble globulin (G . BS + HOH = G . HOH + BS). Therefore they are stable only in presence of a large excess of salt. Hence the solvent power of salts is from 200 to 500 times less than that of acids or alkalies.

A double salt of the form AB . CD ionises according to

$$\mathrm{AB.CD} \rightleftharpoons \mathrm{AB.C + D.}$$

I have never succeeded in detecting any trace of such ionisation in the case of globulin and neutral salts; possibly owing to the extreme instability of the ions.

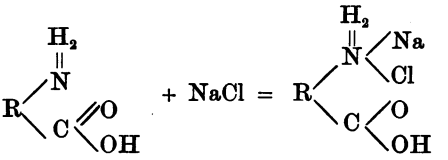
Owing to the instability of the dissociation products the globulin can be precipitated from its solution with neutral salts by simple dilution. No degree of dilution will precipitate globulin from solution with acids and alkalies.

The compounds of globulin and alkalies (GB) are more readily dissolved by neutral salts than is simple globulin. Compounds of globulins and acids are insoluble by neutral salts, being decomposed with liberation of the acid.

If on the analogy of an amido-acid a globulin combines owing to the presence of the NH_2 and the COOH group, then it forms salts with alkalies by replacement of hydrogen, and with acids by the change of the trivalent nitrogen of the amido group to the pentavalent form.

There are two possible places where the neutral salt might link on—the unsatisfied valencies of the nitrogen, or the unsatisfied valencies of

the upper O of the COOH group. According to the oxonium theory, O has a maximum valency O≡, but O≡ is much less stable than N≡; therefore, one would expect the linkage to be by the amido group—



And this would account for the fact that the acid can be turned out of the combination with globulin by an excess of neutral salt, but alkali cannot be.

The relation of globulin to the serum proteid or proteids is discussed and the complete absence of "ionic" globulin from serum is noted. The probability of globulin being formed owing to the decomposition of a complex proteid present in serum is urged.