THE EQUILIBRIUM BETWEEN VARYING CONCENTRATIONS OF ACIDS AND ALKALIES AND THE PROTEINS OF THE SERUM AND OTHER COLLOIDS, WITH A DISCUSSION OF THE NATURE OF COLLOIDAL REACTION OR ADSORPTION

By BENJAMIN MOORE, M.A., D.Sc., (Belfast), *Johnston Professor of Bio-Chemistry*; and A. DOUGLAS BIGLAND, B.A. (Cantab.), *University of Liverpool.*

From the Department of Bio-Chemistry, University of Liverpool

(*Received November 29th, 1909*)

It was shown by Moore and Wilson[1] in 1906 that the serum proteins possess a relatively enormous power of taking up acid or alkali within narrow ratios of hydrogen and hydroxyl ionic concentration. Thus serum titrated with alkali to the neutral point to phenol-phthalëin, and then to the neutral point to an indicator such as 'di-methyl' or methyl orange, shows a difference indicating no less a concentration than 0·14 to 0·16 Normal. Outside these two limits of neutrality to the two coloured indicators the hydrogen or hydroxyl ion concentration swings up very rapidly compared to the rate of change within the limits. We may hence speak of a 'neutral zone' for the protein of the serum. The traverse of this zone amounts to about 0·15 N, and within this zone the reaction is nearly neutral. Outside of it in either direction the acidity in the one case or alkalinity in the other mounts very rapidly. Moore and Wilson drew attention to the curious and interesting fact, that this concentration in regard to acid or alkali of the proteins amounts to about an isotonicity with the entire inorganic salts of the serum.

The tonicity for all mammalian sera is very nearly constant, amounting to about that of a 0·9 per cent. solution of sodium chloride, that is to say to about 0·15 N; this close coincidence can scarcely be fortuitous, and would support the view that the figure 0·15 N indicates an average reactivity of the serum to crystalloids, and shows that under normal conditions the amounts of crystalloids in the serum are regulated by the combining or adsorbing powers of the serum colloids.[2]

1. *Bio-Chemical Journal*, Vol. I, p. 311, 1906.

2. Forster, and also Sollman, on other and more purely physiological grounds, have suggested and discussed such a combination between proteins and salts in the plasma.

See J. Forster, *Zeitsch. f. Biologie*, Vol. IX, p. 297, 1873; Torold Sollman, *Amer. Journ. of Physiology*, Vol. VIII, p. 155, 1903.

When for any reason more crystalloids are present than can be held in this feeblest type of union by the proteins present, these free crystalloids become adsorbed (or united in some form) by the kidney cells and excreted; and, contrariwise, if salts are abstracted by the tissues, so that colloids are free, then crystalloids taken up from the alimentary canal are attached by the serum colloids to restore equilibrium in this direction.

The type of chemical or physical union here existing is one of the most delicate and labile with which we are acquainted; although its actual existence is demonstrated both by these titration figures, and by the delicate balancing of the tonicity of the serum by the excretory cells and tissue cells so as to preserve an almost absolute constancy in plasma tonicity. For example, this union between the crystalloids and proteins has no effect either upon freezing point or conductivity, the serum crystalloids behaving in these respects exactly as if they were entirely free. Also, the union cannot resist a gradient of osmotic pressure, the salts diffusing away from the proteins quite readily when placed in an osmometer with distilled water outside.

In addition to the evidence of such a feeble combination or union of some sort between crystalloids and proteins, arising from the titration figures to the two sides of the neutral zone, and of the minute physiological regulation of the tonicity of the serum or plasma, we know also from the direct measurements of osmotic pressure in serum by the osmomoter[1] that the state of solution of the proteins varies with the concentration and nature of the electrolytes present in common solution with them. Now, such variations can only possibly arise if there exists some variable or labile form of union between the proteins and the crystalloids.

In the series of experiments described in the present paper, we have attempted, by a special method, to follow up the conditions of equilibrium between inorganic acids and alkalies and the serum proteins, at intermediate positions within, and just without, the neutral zone, and not merely at the two ends, as is done when the serum is titrated in presence of the proteins to a neutral point to a coloured indicator.

There are immense difficulties in the way of determining by the electrode methods or other physico-chemical methods the concentration of hydrogen and hydroxyl ionic concentration in such fluids as bio-chemists have to deal with, and especially at the natural points of reaction of such fluids. To begin with, such reactions lie near the neutral point, as they

1. Moore and Parker, *Amer. Journ. of Physiology*, Vol. VII, p. 261, 1902; Moore and Roaf, *Bio-Chemical Journal*, Vol. II, p. 34, 1907; Adamson and Roaf, *ibid.*, Vol. III, p. 422, 1908; Lillie, *Amer. Journ. of Physiol.*, Vol. XX, p. 127, 1907.

do not depart widely from the reactions of most good tap waters. Within this region it is well known that the hydrogen electrode method gives experimental variations in the ratio of hydrogen and hydroxyl ions amounting to over one-hundred fold (10^2), and hence in this region, apart from the qualitative fact that the reaction is about what would be termed neutral in ordinary parlance, the electrode method can teach us nothing.

The second difficulty lying in the way, and most troublesome when coloured indicators are utilized, is that of the actual presence of the proteins, which by behaving like very weak acids or bases just like the indicators themselves, so dull off the end points that it is almost impossible to titrate with any degree of accuracy.

If the acid or alkali solution could be brought by some method into contact and equilibrium with the protein, and could then be removed without having its equilibrium disturbed or taking up from or giving out to the protein in the process, we would possess a fluid removed from most of these practical drawbacks, that is, one in which hydrogen or hydroxyl ionic concentrations could be directly determined, or since it is now only a dilute solution in water of acid or alkali, it can be titrated, and the concentrations referred to well known determinations of these in such purely crystalloidal solutions.

This is the basis of the experimental method we have utilized in the present research.

It was shown by Moore and Parker, for the purposes of their research into the osmotic properties of proteins by the direct method, that the usual crystalloids, such as sodium chloride, etc., of the plasma, rapidly pass through parchment paper and attain equilibrium on the two sides within twenty-four hours.

This forms the basis of a method introduced by B. Moore for the estimation of crystalloids when in common solution with colloids which render direct titration impossible, and it has been used in this laboratory already for many purposes, as in the determinations of sugar in blood by Edie and Spence,[1] and in the study of distribution of electrolytes in the blood corpuscles and serum by Moore and Roaf.[2]

The principle of the method is very simple. A known amount of the serum or other colloidal solution in which the determination is to be made is taken and placed inside or outside of a sausage dialysis tube, on the other side is placed a known volume of the dialysing fluid. The whole is

1. *Bio-Chemical Journal*, Vol. II, p. 103, 1907.
2. *Ibid.*, Vol. III, p. 55, 1907.

left so long as preliminary experiments have indicated to be necessary for equilibrium to be established. On the basis that when equilibrium is established the concentrations of crystalloids are equal on the two sides of the membrane, it is easy to determine any given crystalloid such as phosphates, chlorides, or dextrose in the outer protein-free fluid, and from this to calculate what it originally was in the serum or blood. For example, suppose 25 c.c. of serum are placed inside and 75 c.c. of distilled water outside, and at the expiration of twenty-four to forty-eight hours the outer fluid shows on analysis of 50 c.c. by some accurate method, such as the gravimetric method, that this contains 0·02 gm. of sugar, then, since this is contained in 50 c.c., the total amount in the 100 c.c. is 0·04 gm., and as 25 c.c. of serum were taken the 0·04 gm. belongs to 25 c.c., and accordingly in 100 c.c. there would be $0{\cdot}04 \times 4 = 0{\cdot}16$ per cent. of sugar.

This example shows how the method may be applied for determining the normal amount of any given crystalloidal constituent of the serum.

For the purpose of determining equilibria between serum proteins and acids or alkalies, the method of calculation and procedure varies somewhat. Here a known number of cubic centimetres of decinormal acid or alkali is added to a known volume of serum or to the known volume of dialysing water placed outside, and as before a definite volume of the outer fluid is measured off and titrated after a sufficient time-interval for equilibrium has been allowed to elapse.

In the case of any particular experiment with serum, a series of dialyses were always run simultaneously with gradually increasing amounts of added acid or alkali in each tube.

The arrangement of the experiment was that always 10 c.c. of serum *plus* the desired number of cubic centimetres of decinormal acid (or alkali) were mixed and introduced into a sausage-shaped dialysing tube. Outside there was placed a sufficient amount of distilled water to bring up the total amount of fluid within and without to 100 c.c. in every case. After allowing forty-eight hours to elapse, which, as we shall see, is a quite sufficient period for the establishment of equilibrium, 50 c.c. of the outer dialysate were removed and titrated with 'di-methyl' as indicator.

The calculation of amounts of free acid and of acid in union with protein is exceedingly simple. For the concentration of free acid within and without the dialyser is the same, when equilibrium is completely established. Accordingly, the total volume being 100 c.c. by doubling the titer of the 50 c.c taken from outside, we get the total amount of free acid, and simply subtracting this from the total number of cubic centi-

metres of decinormal acid originally added to the 10 c.c. of serum inside, we get the amount expressed also as cubic centimetres of decinormal acid in union with the proteins of the serum.

The ratio is thus directly obtained of free and of combined or adsorbed acid—or, perhaps, simply and without theorising, it might be called 'united' acid.

We shall see, as found by van Slyke and van Slyke[1] for insoluble casein and acid, that this 'united' acid increases in amount with increase in acid concentration, and does so in no simple linear relationship to the concentration, so that *in van Bemmelen's sense of the term the process is one of adsorption.*

We shall endeavour, however, to show in our theoretical discussion that, in our opinion, this does not suffice to determine whether the action is a purely physical, surface, or solid solution one, or is a chemical reaction.

The experiments are very conveniently carried out in wide mouthed glass stoppered bottles, capable of holding about 150 c.c., and wide enough to take the parchment sausage tube used without folding laterally upon itself. It is well to sterilize both bottles and parchment tubing before commencing, and the bottles must of course be dry.

The glass stoppers inserted afterwards so as to hold the doubled top of the parchment paper between bottle neck and stopper serve to hold the parchment tubes in position, and at the same time prevent the ingress of dust. Two drops of 'di-methyl' added to the outer fluid serve to show the progress of the dialysis, and also for titration afterwards. In working with alkali, the indicator may be changed to phenol-phthalëin. The method is less satisfactory for alkali than acid, especially for summer work, as the contents of the tubes often decompose within forty-eight hours. This is never seen with acid-containing tubes, even in hot weather. The acid we have used has been decinormal sulphuric acid, but similar experiments can be performed with other acids.

Before commencing the dialysis experiments, we performed a number of simple titrations in presence of the proteins to get the limits of distinct colour change to 'di-methyl' and to phenol-phthalëin in relationship to the breadth of the neutral zone as given by Moore and Wilson's figures, and to get the tonicity figure we determined the depression of the freezing point of each sample of serum used so as to get a figure for the total crystalloidal content to compare with the titration figure. In regard to

1. *Amer. Chem. Journ.*, Vol. XXXVIII, p. 383, 1907.

these figures, it must be admitted that they cannot be taken as accurate within closer limits than about 0·5 c.c. at each end. Making every allowance for this, however, there is a most remarkable coincidence between the acid-alkali value of the proteins and the total crystalloidal value as shown by the freezing point. Neither values show much variation in the sera we have tested.

We shall first give the record of these results, then that of the dialysis figures.

A. Total reactivity of Serum Proteins, compared with total Crystalloidal Content

When the difficulties of determining the freezing point with sufficient accuracy for these purposes on the one hand, and of titrating to a coloured indicator in presence of proteins on the other, are considered, the comparative closeness of the estimates of the 'Normality' of the serum by the two methods as shown in Table I, p. 38, appears to us to be more than a coincidence.

The results given by the dialysis method (*vide infra*, p. 41) show the interesting fact that at the place where the reaction in the protein-containing fluid was just on the turn in colour to the indicator, there was a good deal of free acid present. This appears to us to indicate that there is probably some union between the dye of the indicator and the protein whereby the indicator is protected from the action of the acid until practically all the protein power for union with acid has been used up.

When we look at the figures obtained by the dialysis method, we see that if concentrations are avoided at which injury and permanent change is caused to the protein, then there is a limit attained at which further increase in acid concentration does not practically increase the amount of 'united' acid, and only the amount of free acid concentration goes on increasing.

After this range of almost constant amount of the 'united' acid has been passed, there is again a sudden increase in amount of 'united' acid. This break we believe to be due to slow permanent alteration of the proteins by action of the acid or alkali. We have neglected such observations with higher amounts of acid or alkali, as they are, in our view, due to quite a different cause. Such injury is more readily caused by alkali than by acid.

If the real maximum point of complete union of protein and alkali or acid be taken, and the two titration values to acid and to alkali be added

TABLE I

	Type of serum	Total reactivity of the proteins of sera. 10 c.c. of serum taken for titration: Acidity to di-methyl in c.c. N/10 H_2SO_4	+	Alkalinity to phenophthalein in c.c. N/10 NaOH	=	Total reactivity in c.c. N/10 conc.	=	Therefore proteins correspond to a normal concentration of	Total crystalloidality of same sera: Depression of freezing point Δ	Corresponding conc. of sodium chloride solution in percentage	Normality
Experiment	I—Pig ...	14·9 c.c.	+	2·3 c.c.	=	17·2 c.c.	=	0·172 N	0·64	0·97	0·167 N
„	II—Pig ...	13·9 c.c.	+	3·1 c.c.	=	17·0 c.c.	=	0·170 N	0·64	0·97	0·167 N
„	III—Pig ...	12·6 c.c.	+	3·2 c.c.	=	15·8 c.c.	=	0·158 N	0·59	0·90	0·155 N
„	IV —Sheep ...	10·6 c.c.	+	2·1 c.c.	=	12·7 c.c.	=	0·127 N	0·65	0·99	0·170 N
„	V—Sheep ...	12·3 c.c.	+	2·9 c.c.	=	15·2 c.c.	=	0·152 N	0·64	0·97	0·167 N
„	VI—Ox ...	11·3 c.c.	+	2·1 c.c.	=	13·4 c.c.	=	0·134 N	0·62	0·95	0·164 N
„	VII—Ox ...	11·0 c.c.	+	2·1 c.c.	=	13·1 c.c.	=	0·131 N	0·62	0·95	0·164 N
„	VIII—Pig ...	10·9 c.c.	+	2·7 c.c.	=	13·6 c.c.	=	0·136 N	0·64	0·97	0·167 N

together, it is interesting to note that results very similar to those obtained by the direct titration method are obtained for the total 'binding'[1] power of the proteins. They correspond again quite closely to the mean 'total crystalloidality' or tonicity of the serum.

Taking the mean of the maximum figures for combination from the data of the following Section (Section B), we obtain the following results:—

FIGURES OBTAINED FROM 10 C.C. OF SERUM IN EACH CASE

Combined acid, N/10			Combined alkali, N/10		
1. Pig's serum (V)	=	11·8 c.c.	5. Pig's serum (VI)	=	5·5 c.c.
2. Sheep serum (III)	=	11·8 c.c.	6. Sheep serum (IV)	=	5·5 c.c.
3. Ox's serum (III)	=	10·5 c.c.			
4. Ox's serum (IV)	=	11·7 c.c.			
Average	=	11·4 c.c.	Average	=	5·5 c.c.

Total reactivity = 11·4 + 5·5 = 16·9 c.c. N/10.

Therefore total reactivity to acid and alkali of the proteins = 16·9 c.c. in 10 c.c. = 0·169 N. Reactivity.

The depressions of freezing point for these several sera were as follows:—

		Δ
1. Pig's serum (V)	=	0·629
2. Sheep's serum (III)	=	0·544
3. Ox's serum (III)	=	0·615
4. Ox's serum (IV)	=	0·590
5. Pig's serum (VI)	=	(lost)
6. Sheep's serum (IV)	=	0·617
Average Δ	=	0·599

Translating this Δ into terms of concentration of an isotonic solution of the most abundant crystalloid of the serum, viz., sodium chloride, we found as our mean measurement for a 0·9 per cent. solution of sodium chloride a Δ of 0·596; this is sufficiently close to the above average to be regarded as identical.

Hence the result is reached that the average tonicity of our sera is equivalent to that of a 0·9 per cent. sodium chloride. Now the molecular weight of sodium chloride (23 + 35) is 58, so that 0·9 per cent. corresponds to a $\frac{9}{58}$ Normal or to a 0·155 Normal. This figure is to be compared with 0·169 Normal obtained above for the total uniting power of the proteins.

1. We have made use of such words as 'union,' 'united,' and 'binding,' to prevent any theoretical conceptions of 'combination' versus 'adsorption' interfering with the statement of our experimental results.

B. Determinations by the Dialysis Method of the Equilibria between Serum Proteins and Acid (Sulphuric) and Alkali (Sodium Hydrate) at Different Concentrations

These experiments were carried out in the manner described above. To 10 c.c. of the serum the amount of acid or alkali specified in the first column was added, the two were thoroughly mixed and placed inside the sausage tube. Outside were placed 100 c.c. of distilled water *minus* the volume of serum and added alkali or acid placed within, so that there were altogether, inside and out, 100 c.c. For example, if inside were 10 c.c. serum + 12 c.c. N/10 acid = 22 c.c., then outside there would be 100 — 22 c.c. = 78 c.c. of distilled water.

To the outside fluid two drops of indicator, 'di-methyl' or phenolphthalëin were added, and the whole was left stoppered as described for forty-eight hours. Then 50 c.c. of the outer fluid were taken and titrated to neutrality, this titer doubled gave free acid (or free alkali, respectively), and the doubled figure subtracted from the total amount initially added gave 'united' acid or alkali, respectively.

Re-dialysis experiments were made by setting up the tubes after each experiment with the same volume of distilled water outside. These experiments of re-dialysis served to show that the acid or alkali is not permanently fixed to the protein, but dialyses out when the concentration is lowered in the solvent. Otherwise they were parallel to the original experiments, so the figures for them are not repeated.

EQUILIBRIUM TIME

Experiment IX.—To determine time necessary to ensure complete equilibrium. For this purpose a series of tubes were taken just as in the other experiments, but instead of varying the amount of acid in each, a constant volume of 14 c.c. of N/10 acid was added in all cases to 10 c.c. of serum and 76 c.c. of distilled water outside, and then the different tubes were titrated as to content of acid in their outer fluids as above described at the following varying time periods from the commencement of the experiment:—

	Time interval from start	Number of c.c. of free acid shown by doubling titre
Tube No. 1 ...	12 hours ...	3·2 c.c.
„ No. 2 ...	15 hours ...	3·6 c.c.
„ No. 4 ...	19 hours ...	3·4 c.c.
„ No. 5 ...	24 hours ...	3·8 c.c.
„ No. 6 ...	36 hours ...	4·0 c.c.
„ No. 7 ...	48 hours ...	4·0 c.c.
„ No. 8 ...	72 hours ...	4·0 c.c.

It is obvious from these figures that equilibrium was complete at the end of thirty-six hours, and as in our experiments under absolutely similar conditions and with the same paper, the time interval allowed was forty-eight hours, we believe that it may be taken that in all cases equilibrium was complete when titration was carried out.

In this test experiment with a concentration of acid in the solvent of 4 c.c. of N/10 per 100 c.c., i.e. of 0·004 N acid, the amount taken up was accordingly 10 c.c. of N/10 acid, which again corresponds fairly closely with the figures given below in the actual experiments.

A second experiment on the same lines gave similar results.

EQUILIBRIA WITH ACID

Experiment X.—Pig's serum (V), containing proteins 7·33 per cent. (by Kjeldahl), Δ = 0·629. Equilibria with acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
8 c.c.	...	2·1 c.c.	...	5·9 c.c.
10 c.c.	...	2·5 c.c.	...	7·5 c.c.
12 c.c.	...	4·0 c.c.	...	8·0 c.c.
14 c.c.	...	4·9 c.c.	...	9·1 c.c.
(16 c.c.	...	7·7 c.c.	...	8·3 c.c.) ?
18 c.c.	...	6·6 c.c.	...	11·4 c.c.
20 c.c.	...	8·2 c.c.	...	11·8 c.c.
24 c.c.	...	12·2 c.c.	...	11·8 c.c.

The figures are c.c. of N/10 H_2SO_4. It is seen that saturation of the proteins occurs with between 18 and 20 c.c. of added acid when concentration in the solution stands at about 7 c.c. of N/10 in 100 c.c or 0·007 N, and about 11·6 c.c. of N/10 acid have been taken up by the 0·733 gm. of protein in the 10 c.c. of serum. The titration at 16 c.c. is doubtful as to correctness, and is hence placed in brackets.

Experiment XI.—Sheep serum (III). Contained 6·85 per cent. of protein, Δ = 0·544. Equilibria with acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
8 c.c.	...	1·6 c.c.	...	6·4 c.c.
10 c.c.	...	2·2 c.c.	...	7·8 c.c.
(12 c.c.	...	2·6 c.c.	...	9·4 c.c.)
14 c.c.	...	4·4 c.c.	...	9·6 c.c.
16 c.c.	...	4·8 c.c.	...	11·2 c.c.
18 c.c.	...	6·2 c.c.	...	11·8 c.c.

Experiment XII.—Ox serum (III). Contained proteins, 7·49 per cent., Δ = 0·615. Equilibria to acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
8 c.c.	...	3·2 c.c.	...	4·8 c.c.
(10 c.c.	...	4·8 c.c.	...	5·2 c.c.)
12 c.c.	...	4·4 c.c.	...	7·6 c.c.
(14 c.c.	...	3·6 c.c.	...	10·4 c.c.) ?
16 c.c.	...	5·4 c.c.	...	10·6 c.c.
18 c.c.	...	7·6 c.c.	...	10·4 c.c.

In this experiment the results are somewhat irregular, but a maximum is reached here also at a low concentration.

Experiment XIII.—Ox serum (IV). Proteins 7·03 per cent., Δ = 0·590. Equilibria with acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
8 c.c.	...	1·8 c.c.	...	6·2 c.c.
10 c.c.	...	2·2 c.c.	...	7·8 c.c.
12 c.c.	...	2·8 c.c.	...	9·2 c.c.
14 c.c.	...	4·2 c.c.	...	9·8 c.c.
16 c.c.	...	5·0 c.c.	...	11·0 c.c.
18 c.c.	...	6·4 c.c.	...	11·6 c.c.
20 c.c.	...	8·2 c.c.	...	11·8 c.c.

Here also a maximum union is obtained at about 16 c.c. of added acid with about 11 c.c. in combination, concentration of acid for maximum union about 0·005 N.

Experiment XIV.—Sheep serum (VI).

Total acid added		Free acid		United acid
6 c.c.	...	1·4 c.c.	...	4·6 c.c.
8 c.c.	...	1·6 c.c.	...	6·4 c.c.
10 c.c.	...	2·4 c.c.	...	7·6 c.c.
12 c.c.	...	3·0 c.c.	...	9·0 c.c.
14 c.c.	...	4·4 c.c.	...	9·6 c.c.
16 c.c.	...	5·4 c.c.	...	10·6 c.c.
18 c.c.	...	7·4 c.c.	...	10·6 c.c.

Result much as in preceding experiments, complete uptake of between 10 and 11 c.c at about 5 c.c. of free N/10 acid in 100 c.c., i.e., at 0·005 N concentration.

EQUILIBRIA WITH ALKALI

Experiment XV.—Pig serum (VI). Proteins 6·60 per cent. Equilibria with alkali (N/10 NaOH).

Total alkali added		Free alkali		United alkali
2 c.c.	...	0 c.c.	...	2 c.c.
(3 c.c.	...	1 c.c. ?	...	2 c.c.)
4 c.c.	...	1·6 c.c.	...	2·4 c.c.
6 c.c.	...	3·4 c.c.	...	2·6 c.c.
8 c.c.	...	4·4 c.c.	...	3·6 c.c.
10 c.c.	...	5·6 c.c.	...	4·4 c.c.
12 c.c.	...	6·8 c.c.	...	5·2 c.c.
15 c.c.	...	9·2 c.c.	...	5·8 c.c.

It is to be observed that the uptake of alkali is less than that of acid, amounting when the full amount is reached to less than 6 c.c., and requiring a somewhat higher concentration of alkali (0·007 to 0·009 N) to hold it in union with the protein.

Experiment XVI.—Sheep serum (IV). Proteins 6·87 per cent., Δ = 0·617. Equilibria with alkali.

Total alkali added		Free alkali		United alkali
2 c.c.	...	1 c.c.	...	1 c.c.
4 c.c.	...	2·2 c.c.	...	1·8 c.c.
8 c.c.	...	4·6 c.c.	...	3·4 c.c.
12 c.c.	...	6·6 c.c.	...	5·4 c.c.
15 c.c.	...	9·4 c.c.	...	5·6 c.c.

Here again the important result is that there is full uptake at about 12 c.c., with about 5·5 c.c. taken up and 6 to 7 c.c free (i.e., in 0·006 to 0·007 N).

The main experimental results of this series of experiments may be summarised as follows:—

1. Serum proteins of the mammalia are capable of taking up a very considerable amount of alkali or acid when there is a certain concentration of acid or alkali kept up.

2. Even with the first addition of acid or alkali some free acid or alkali is left in solution; this is usually taken as a criterion of adsorption. In van Bemmelen's sense these are adsorptions, but in our opinion molecular chemical union occurs.

3. The amount taken up varies with the concentration of free acid or alkali in the solvent, but a maximum is reached in both cases above which practically no more is taken up with considerable increase in concentration, short of the amount necessary to injure the protein.

4. The acid or alkali concentration required to maintain almost complete union is a very low one, amounting to about 0·006 to 0·007 Normal.

5. The amount of acid taken up is about double that of alkali. Ten c.c. of serum take up about 11 c.c. of N/10 acid, and about 5·5 c.c. of N/10 alkali.

6. If the average amount of serum protein be taken in the experiments at 7 per cent., the above figures mean that 0·7 gm. of protein in the 10 c.c. take up 11 c.c. of N/10 acid or 5·5 of N/10 alkali respectively. The combining weight of the protein, supposing one of protein to unite with one only of acid or alkali, corresponds to that amount which neutralises 10,000 c.c of N/10 acid or alkali. This gives approximately 640 for acid and 1,280 for alkali. Now the osmotic equivalent of serum proteins, as determined by direct measurement in the osmometer, amounts to about 40,000. So that this indicates that each solution aggregate of protein

can unite with about sixty molecules of acid or thirty molecules of alkali.[1]

7. The interval between the limits of uniting power of protein for alkali and acid in the two directions of acidity and alkalinity correspond approximately with the *total* amounts of crystalloid contained in the plasma, indicating a labile union between crystalloids and serum proteins.

8. This interval may be defined as the 'neutral zone' of the plasma.

C. Equilibria of Casein Solutions with Acid and Alkali

The above method of measuring uniting power of serum proteins was also applied in a few experiments to casein suspensions. The 'adsorbing' power of casein for acids has been carefully determined by the van Slykes[2] in a method which in principle consists in leaving casein in contact with acids of the definite concentrations and then filtering off from the insoluble casein and taking the electrical conductivity of the filtrate. The filtrate contained small amounts of protein and could not be titrated directly with accuracy. In the present method this inconvenience is entirely obviated, for we found on careful testing that the dialysate was always protein free. Hence the dialysate could be titrated after reaching equilibrium in the ordinary way.

Experiment XVII.—Casein emulsion (I). Containing 9 per cent. of casein as precipitate. Equilibria to acid (N/10 H_2SO_4).

Total acid added		Free acid		United acid
3 c.c.	...	0·6 c.c.	...	2·4 c.c.
6 c.c.	...	1·8 c.c.	...	4·2 c.c.
9 c.c.	...	3·2 c.c.	...	5·8 c.c.
12 c.c.	...	5·8 c.c.	...	7·2 c.c.
(15 c.c.	...	8·2 c.c.	...	6·8 c.c)
18 c.c.	...	10·6 c.c.	...	7·4 c.c.
21 c.c.	...	13·6 c.c.	...	7·4 c.c.

It is seen here again that free acid is present from the beginning and that there is a limit of union at about 12 c.c. of added acid with a concentration of free acid of about 6 c.c. (i.e., a concentration of 0·006 N), and with about 7 c.c. of acid taken up by the casein.

Experiment XVIII.—Casein solution (II). Containing 9 per cent. of casein. Equilibria to alkali (N/10 NaOH).

Total alkali added		Free alkali		United alkali
3 c.c.	...	0 c.c.	...	3·0 c.c.
6 c.c.	...	0 c.c.	...	6·0 c.c.
9 c.c.	...	0·8 c.c.	...	8·2 c.c.
12 c.c.	...	2·0 c.c.	...	10·0 c.c.
15 c.c.	...	3·8 c.c.	...	11·2 c.c.
18 c.c.	...	5·0 c.c.	...	13·0 c.c.
21 c.c.	...	7·0 c.c.	...	14·0 c.c.

1. The importance of this point, that each solution aggregate of protein possesses at the saturation point the power of uniting with as many as 60 molecules of acid is pointed out later in discussing the affinity constant of the colloidal reaction.

2. *Loc. cit.*

A result somewhat different from that with acid is seen here, the casein taking up about 8 c.c. before there is an appreciable pressure of free alkali allowed to develop. This looks like a very stable union of casein and alkali as shown in other ways by many observers.

The curious result then follows that on adding more alkali there is a further uptake held in by alkali pressure and approaching a maximum just where the quantity in union has been doubled, i.e., where the amount in unstable union equals that more firmly held. This occurs with a pressure of alkali corresponding to 7 c.c. of N/10 in 100 or to 0·007 N concentration. These two results correspond probably with the two amounts of alkali taken up at the neutral point to litmus (neutral caseinate of Söldner) and that taken up at the neutral point to phenolphthalëin (basic caseinate of Söldner). The former is the amount taken up when dilute alkali and excess of casein are shaken up together and then separated by filter or centrifuge. The second is the body obtained when more alkali is added to the filtrate until it is just alkaline to phenolphthalëin. The second of these is not the double of the former, and our experiments show that it represents an artificial point, the true basic value being almost exactly double the neutral value. Since in shaking up with excess of casein all free alkali is taken up, it is clear that Söldner's neutral caseinate corresponds to our point of no free alkali. Moreover, Söldner found one gram of casein to take up 8 c.c. of N/10 alkali while we first get pressure of free alkali when 7·7 c.c. have been taken up.

D. Effects of Heat Coagulation upon the Equilibria of Serum Proteins with Acids or Alkalies

These experiments are interesting in view of the prevalent notion that such unstable unions are, by those who speak of physical adsorption, thought to depend on the amount of surface of the solid or colloidal adsorbing phase of the system.

If this were correct, then since boiling ought to diminish surface of colloid by causing coagulation, one might naturally expect less 'adsorption.' The experiment actually showed that more was taken up in the boiled condition, but shelter against this might be taken by those holding the physical side, admitting that, after boiling, a certain amount of the acid went into true chemical union, either with the protein or with some hydrolytic products split off from it.

In any case, here follow the experimental results, obtained by the usual method, as above.

Experiment XIX.—Equilibria with alkali.

	Pig Serum			
	Boiled		Unboiled (Same sample)	
Total added	United	Free	United	Free
3 c.c. ...	2·4 c.c. ...	0·6 c.c. ...	2·0 c.c. ...	1·0 c.c.
4 c.c. ...	3·0 c.c. ...	1·0 c.c. ...	2·4 c.c. ...	1·6 c.c.
6 c.c. ...	4·8 c.c. ...	1·2 c.c. ...	2·6 c.c. ...	3·4 c.c.
8 c.c. ...	6·0 c.c. ...	2·0 c.c. ...	3·6 c.c. ...	4·4 c.c.
12 c.c. ...	8·0 c.c. ...	4·0 c.c. ...	5·2 c.c. ...	6·8 c.c.
14 c.c. ...	9·4 c.c. ...	4·6 c.c.		

The boiled samples, it will be seen, not only throughout hold more in union at a lower pressure but a maximum is not even attained with 9·4 in union. This probably means considerable hydrolysis followed by stable chemical union.

Experiment XX.—Equilibria with acid.

	Pig Serum			
	Boiled		Unboiled (Same sample)	
Total acid added	United acid	Free acid	United acid	Free acid
10 c.c. ...	0·8 c.c. ...	2 c.c. ...	7·4 c.c. ...	2·6 c.c.
12 c.c. ...	— ...	(lost) ...	7·9 c.c. ...	4·1 c.c.
14 c.c. ...	— ...	(lost) ...	9·1 c.c. ...	4·9 c.c.
16 c.c. ...	9·8 c.c. ...	6·2 c.c. ...	8·3 c.c. ...	7·7 c.c.
18 c.c. ...	11·0 c.c. ...	7·0 c.c. ...	11·4 c.c. ...	6·6 c.c. ?
20 c.c. ...	11·2 c.c. ...	8·8 c.c. ...	11·8 c.c. ...	8·2 c.c.

Here the quantities free and united are about the same in the two cases, boiled and unboiled, and any slight difference there may be is in favour of the unboiled specimens.

E. Other Colloidal Solutions

In order to further test the question of amount of colloidal surface at an interface between colloidal aggregate and solvent, having an influence on this equilibria of acid or alkali with serum proteins, a few experiments were instituted with other suspensions and colloidal solutions.

The results which were small or negative showed conclusively that this uptake of acid or alkali is a specific thing, only occurring when there is something of the nature of affinity between colloid and acid or alkali.

For example, animal charcoal in suspension which possesses such a high power of taking up and binding organic coloured bodies possessed no attractions whatever for acid in solution, and certainly no acid was taken up.

The suspensions or solutions tested were as follows:—

1. Animal charcoal, 5 per cent. suspension in water. Reaction neutral.
2. Starch paste, 1 per cent. paste.
3. Gum acacia, 5 per cent. solution.
4. Colloidal platinum suspension (Bredig).

The experiments were carried out as above described, 10 c.c. of each of the above being treated with 10 c.c. of N/10 H_2SO_4 and dialysed against 80 c.c. of distilled water outside. It was found that the starch, charcoal, and colloidal platinum showed no appreciable uptake of acid. Gum acacia in two different experiments gave a union of 2·4 c.c. and 3·0 c.c. respectively.

A Short Review of Related Literature

The literature on the subject of crystallo-colloids stretches far into the past, for ever since proteins have been isolated and analysed it has been known that they contain in all cases crystalloids in some intimate relationship, so that they cannot be freed from these without undergoing important alterations in their physical, chemical and physiological properties. Much controversy has been waged as to whether this so-called ash of the protein is an impurity or an integral part of the molecule, and it is only recently that physical experiments in osmotic pressure variations associated and related to crystalloidal changes have brought what may perhaps be regarded as a final proof that the crystalloid forms not an impurity, but rather a central and essential part of the colloidal aggregate or protein molecule.

It is impossible to quote here the whole of this extensive literature, and we intend only to refer to a few of those papers which have a special bearing on the present experiments. Many papers we may exclude which refer only to insoluble forms of protein saturated with acid or alkali, or to protein salted out of solution. The main problem we are here dealing with is protein in equilibrium with acid and alkali at varying concentrations of these reagents; this we have been able to investigate by the dialysis method. So far as we are aware, dialysis has not been used before for this precise purpose of inducing an equilibrium at definite acid and alkaline concentrations, by other observers. Dialysis has been used by Hardy[1] in the case of globulin, to follow the 'grade' of solution

1. *Journ. of Physiology*, Vol. XXXIII, p. 251, 1905.

corresponding to different acid concentrations. This observer has also used the methyl acetate inversion method to study the degree of ionization of acid in presence of globulin, but so far as we know he has not calculated the amounts of free and united acid in equilibrium at varying concentration. Nor would the methyl acetate method lend itself so well as dialysis to such a purpose, since we possess no guarantee that the united acid or alkali would not aid the free acid or alkali in attack on the substrate.

Some of the earlier results on the composition of the crystalline proteins formed by combination between crystalloids and various proteins are very interesting, in view of the results we have above obtained for the ratios of crystalloid (acid or alkali) and protein at the saturation point. Thus, Schmiedeberg,[1] that great pioneer of protein chemistry, obtained in 1877, beautifully formed glistening polyhedral crystals of a compound of magnesium oxide with a globulin of vegetable origin from Brazil nuts (*Bertholletia excelsa*). This crystalline magnesium-globulin compound of Schmiedeberg was examined and analysed by Drechsel,[2] who found that the compound dried at 110° C. contained 1·40 per cent. of MgO, which leads to a molecular weight of 2817 on the assumption of one atom of magnesium in the molecule. Drechsel also improved the technique for obtaining this compound by introducing dialysis with alcohol, and now obtained a molecular weight of 2757. Also, Drechsel was able by dialysis with alcohol outside to obtain the corresponding sodium oxide compound, which was found to contain 3·98 per cent. of Na_2O, leading to a molecular weight of 1496.

This figure is worth comparing with the figure calculated on similar lines for the sodium hydrate compound with serum proteins, existing at saturation point, with a free concentration of alkali in the solvent of about 0·007 Normal NaOH. At about saturation in Experiment IV, 10 c.c. of a 6·6 per cent. protein solution holds in union 5·2 c.c. of N/10 sodium hydrate. That is to say, 0·66 gm. of protein corresponds to 5·2 c.c., therefore as a gram-molecule of sodium hydrate is contained in 10,000 c.c. of N/10 alkali solution, we have as the weight of protein combining with this $\frac{10{,}000}{5{\cdot}2 \times 0{\cdot}66} = 1{,}260$ to 1280. This figure, therefore, would represent the mean molecular weight of the serum protein, on the supposition that one molecule of protein combines with one molecule of sodium hydrate. As has been pointed out above, direct osmometric

1. *Zeitsch. f. physiol. Chemie*, Bd. I, S. 205, 1877.
2. *Journ. f. prakt. Chemie*, N.F., Bd. XIX, S. 331, 1879.

measurements show that each solution aggregate combines with a considerable number of sodium hydrate molecules.

It is remarkable how closely of the same order are these older figures with more modern ones, such as our own, and others with casein, which will be mentioned later. It is also to be noticed that the figures for the divalent magnesium are about double those for the monovalent sodium.

The low values for the crystalloidal portion in such analyses caused at the time the results to be distrusted, and researchers turned towards regarding the absence of crystalloid as a test of the purity of proteins. This led to Harnack's work and the production of the so-called ash-free protein.

This substance caused much debate in its time, and it was pointed out that it had lost many of the usual native protein properties.

We now know from direct osmometric work that an ash-free protein can exist, but that it is an inert protein which has lost its solution properties and no longer shows osmotic pressure.

The purified pressure-free globulin of Weymouth Reid[1] is a modern congener of Harnack's pure ash-free protein, but the question in all probability is not one of purity, but one rather of the presence of crystallo-colloidal union or its absence.

Harnack[2] also prepared two distinct copper proteins from egg albumen, one containing twice as much copper oxide as the other. On the supposition of one copper atom, his figures lead to simplest molecular weights of 4696 and 2348. Loew[3] prepared two similar silver-proteins with percentages of silver closely corresponding to those of copper in Harnack's substances, and showing probably thereby that stochiometric relationships do hold in such cases. These two distinct pairs of compounds may be correlated with the two casein compounds mentioned later, as given by the work of more modern authors, and with the two values given by our Experiment XVIII. In this experiment we get no appreciable free alkali at 6·0 c.c. of added N/10 alkali, and a concentration of only 0·8 c.c. of N/10 in 100 c.c. (i.e., 0·0008 N concentration) with a total of 8·2 c.c. united. We shall, therefore, not go far out in assuming that appreciable free alkali is first present when 7 c.c. of casein are bound. Now, as 10 c.c. of 9 per cent. casein solution were taken, it follows that 0·9 gm. of casein unites, without free alkali pressure developing, with 7 c.c. of

1. *Journ. of Physiology*, Vol. XXXI, p. 438, 1904.
2. *Zeitsch. f. physiol. Chemie*, Bd. V, S. 198, 1881.
3. *Arch. f. d. ges. Physiol.*, Bd. XXXI, S. 402, 1883.

N/10 sodium hydrate. This leads, on a similar calculation to that given above, to a molecular weight of 1587 for the casein, with one molecule of NaOH to one protein molecule. On adding more alkali further uptake occurs, until a total of about 14 c.c. of N/10 sodium hydrate have entered into union before saturation is reached. Now this figure is just double the first, leading to a molecular weight of approximately 793 for a single molecule of sodium hydrate. The acid-caseinate compound, on the other hand, only forms under acid pressure, and becomes completed at about a pressure of 0·006 N, when the uptake is about 7 c.c. by 0·9 gm. of casein, corresponding to the first of the two alkali-caseinates.

These figures may be compared with those quoted by Söldner and many other observers using precipitation methods for acid caseinates and saturated uptake by alkali for alkaline caseinates.

We need hardly point out that we do not consider these as true molecular weights, but only as expressing the simplest stochiometric relationships of crystalloid and colloid at certain well marked points.

Many approaches, both by ordinary chemical analysis and by physico-chemical methods, have been made to the study of the interactions of proteins with acid and alkali.

Precipitation methods, followed by analysis of the acid-protein or alkali protein, have been utilised by Söldner,[1] Courant,[2] Paal[3], Spiro and Pemsel,[4] Cohnheim and Krieger,[5] Erb,[6] Osborne.[7]

Physical methods, such as conductivity determinations before and after protein additions, have been employed by Sjöqvist[8] and by Säckur,[9] and after removal of the acid-saturated protein by Lucius L. and Donald D. van Slyke.[10]

The electromotive force of concentration cells and the depression of freezing point have been utilised as methods by Bugarski and Liebermann;[11] Cohnheim[12] and also Hardy[13] have experimented by the

1. *Landwirthsch. Versuchsstat*, XXXV, p. 351, 1888.
2. *Arch. f. d. ges. Physiol.*, Bd. L, S. 109, 1891.
3. *Ber. d. deut. chem. Gesell.*, XXV, S. 1,202, 1892.
4. *Arch. f. physiol. Chemie*, S. 233, 1898-9.
5. *Zeitsch. f. Biol.*, XL, S. 95, 1900.
6. *Ibid.*, XLI, S. 309, 1901.
7. *Journ. of Physiol.*, XXVII, S. 398, 1901.
8. *Skand. Arch. f. Physiol.*, Bd. V, S. 277, 1895.
9. *Zeitsch. f. physikal. Chem.*, Bd. XLI, S. 672, 1903.
10. *Amer. Chem. Journ.*, Vol. XXXVIII, p. 383, 1907.
11. *Arch. f. d. ges. Physiol.*, LXXII, S. 51, 1898.
12. *Zeitsch. f. Biol.*, XXXIII, S. 489, 1896.
13. *Loc. cit.*

catalysis method for cane sugar and for methyl acetate, as first suggested by Ostwald.

All the authors practically who have used the above physical methods are agreed that proteins or protein salts both ionize and hydrolyse in aqueous solution; and experiments have been made by Hardy and others to determine both ionization and hydrolysis quantitatively.

There is, however, one matter about which care must be taken in dealing with such purely physical methods for determining or proving chemical union (or colloidal reaction or adsorption) between colloid and crystalloid, and that is that assumptions must not be made which amount to begging the question in interpreting the results. The strongest possible evidence appears to be accumulating that crystalloids can be in union with colloid without materially altering their separate physical properties, and hence physical properties measured after preparing solutions of colloid with crystalloid, or by adding crystalloids to colloidal solutions, may give very nearly additive functions in an independent way, although the crystalloids have united in an adsorption or 'colloidal reaction' with the colloid.

This has been recently shown by Moore and Roaf[1] in work upon the crystalloids of the serum and its own red blood corpuscles. The degree of interference with the freedom and physical properties of the crystalloid on its union depends to a large extent upon the amount of true specific chemical affinity and union between the two.

For example, the electrical conductivity and depression of freezing point of the serum are practically identical with those of its electrolytes, but the phosphates bound in with the haemoglobin of the red blood corpuscles do not show their full conductivity till fully detached. Also, in the case of globulins, neutral salts preserve their full conductivity, while the conductivity of acid and alkali in presence of globulin are very appreciably reduced.

In making conductivity and cryoscopic determinations we must therefore be careful of the proof that the values are due to a crystallo-colloidal compound, and not merely to crystalloid nearly free as to these properties, although anchored by colloidal affinities in the neighbourhood of the colloidal aggregates.

For example, there is good evidence, as we have shown, that the neutral salts of the serum are united to serum proteins, but while so

1. *Bio-Chemical Journ.*, Vol. III, p. 55, 1907.

united they produce full effects upon freezing point and conductivity just as if they were completely free. It would be obviously false, therefore, to calculate a mean molecular weight for the serum proteins from a Δ of the serum. This would be tantamount to assuming that each serum solution aggregate had only one ion attached to it, whereas we know, from taking in conjunction the freezing point depression and directly measured osmotic pressure, that each solution aggregate has many such ions attached to it. The same is illustrated by the experiments of this paper and the simple molecular weights deduced above are merely sub-multiples of the true ones showing the relative weight of colloid to each molecule of crystalloid.

The difficulties of accepting an ordinary salt formation and ordinary ionization are apparent in the classical paper of Hardy[1] on the globulins, which contains a large amount of accurate physical measurements and more clear reasoning than most of the papers dealing with this intricate subject by physico-chemical methods.

The author, abandoning his earlier view of a physical explanation, attempts a treatment of 'the phenomena of colloidal solutions in the 'special case of proteids from a frankly chemical standpoint.' He states that 'the chief difficulty in the way of a chemical theory of colloidal 'solution is the apparent need of postulating the existence of continuously 'varying chemical compounds, or what van Bemmelen calls absorption 'compounds.'

We agree with this author that 'such a need is more 'apparent than real.' The wild maze into which such a postulation of continuously varying chemical compounds leads may be appreciated by anyone who cares to read the series of long papers on the so-called 'ion-protein compounds' by T. B. Robertson.[2]

This theory is by T. B. Robertson usually ascribed to J. Loeb, but the whole later development has been the product of the pen of the former writer. Loeb merely introduced the word ion-protein to explain his and other people's results on cell stimulation by certain electrolytic ions. Such results had long before been obtained by Sidney Ringer, Howell and Locke; similar effects were noticed by Loeb some years later in increase of the percentage of parthenogenetic division in one or two species of

1. *Journ. of Physiology*, Vol. XXXIII, p. 251, 1905.

2. *Arch. f. d. ges. Physiol.*, Bd. CX, S. 610, 1905; *Journ. of Biolog. Chemistry*, Vol. I, p. 279, 1905-6, and subsequent volumes.

echinodermata[1] (falsely called artificial fertilization), and in some experiments upon rhythmical contractions induced in skeletal muscle by certain ions and inhibited by others.[2] It was clearly obvious to Ringer, Locke and others that calcium, potassium and phosphatic salts produced their effects by some type of union with protoplasm. Meanwhile the ionic theory of solution developed; Loeb stated that the ion in question unites with protein in some obscure way to form something upon which he fastened the tag ion-protein; then T. B. Robertson developed the so-called 'ion-protein theory,' and the net result has been that the real pioneer of the whole subject, who first developed the physiological or functional side of the study of the relations of crystalloids and colloids in living cells, namely, Sidney Ringer, remains known, as far as we can judge from their writings, to most of the workers of the Loeb school only as the author of 'Ringer's Solution.'[3]

1. It is only in species where parthenogenesis naturally occurs that it can be increased in amount by changes in tonic environment. For example, no artificial cell division can be induced by the procedures recommended by Loeb in the unfertilised eggs of *Echinus esculentus*. An altogether fantastic view has been taken of the importance of these 'artificial reproduction' experiments.

2. The rhythmical contractions of skeletal muscle induced by sodium chloride were well known to Ringer and published by him several years before Loeb re-discovered them (see papers numbered XI and XII in the list given below). Ringer also states that they were known and described by Biedermann. The antagonistic action of calcium and phosphates in arresting these contractions which has been discussed in several papers from the Loeb school, was also described in detail and discussed by Ringer in these much earlier papers.

3. [Note by B. Moore]. At some distant epoch it may happen that some American researcher, endowed with an extra measure of that antiquarian zeal which characterises his countrymen may dig out from musty archives of forgotten lore the history of 'Ringer's Solution,' and then that pioneer may at last be awarded the honour he has so long deserved but not received. In order to assist this research I append a list of the more important papers by Sidney Ringer, M.D., Professor of Medicine, University College, London, dealing with this important subject of the action of inorganic ions on living tissues. They may be recommended as studies of careful, accurate and painstaking research in a difficult and intricate subject:—

I. Concerning the influence exerted by each constituent of the blood on the contraction of the ventricle. *Journal of Physiology*, Vol. III, p. 381, 1880-2.

II. A further contribution regarding the influence of the different constituents of the blood on the contraction of the heart. *Journ. of Physiology*, Vol. IV, p. 29, 1883-4.

III. A third contribution regarding the influence of the inorganic constituents of the blood on the ventricular contraction. *Journ. of Physiology*, Vol. IV, p. 222, 1883-4.

IV. The influence of saline media on fishes. *Proc. Physiol. Soc.*, December, 1883; *Journ. of Physiology*, Vol. IV, p. 6.

V. An investigation regarding the action of rubidium and calcium salts compared with the action of potassium salts on the ventricle of the frog's heart. *Journ. of Physiology*, Vol. IV, p. 371, 1883-4.

VI. Concerning the influence of saline media on fish, &c. *Journ. of Physiology*, Vol. V. p. 98, 1884-5.

VII. On the mutual antagonism between lime and potash salts in toxic doses. *Journ. of Physiology*, Vol. V, p. 247, 1884-5.

VIII. An experimental investigation showing that veratria is similar to lime salts in many

To return to the globulins, Hardy states that '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, the electric charge which 'they can carry is not a definite multiple of a fixed quantity, and one 'cannot ascribe to them a value, and their electrical relations are those 'which underlie the phenomenon of electrical endosmose. To such ionic 'masses I would give the name "pseudo-ions."'

It is obvious that Hardy's ion-proteins or 'pseudo-ions' are not the chemical complexes, 'ampholates' or 'ion-proteins' of T. Brailsford Robertson.

Hardy found that globulins unite in molecular proportions with both acids and bases, and finds some difficulty in explaining this. Combination of this type with acids could be explained, as he points out, on the analogy of the protein amino-acids to an ammonium base; but to explain combination with bases, the nearest analogy would have to be an acid anhydride such as CO_2 uniting with a molecule of sodium hydrate to form a bicarbonate. The behaviour with coloured indicators led Hardy to

respects as regards their action on the ventricle, also showing that veratria and lime salts are reciprocally antagonistic. *Journ. of Physiology*, Vol. V, p. 352, 1884-5.

IX. Regarding the influence of the organic constituents of the blood on the contractility of the ventricle. *Journ. of Physiology*, Vol. VI, p. 361, 1885.

X. A further contribution regarding the effect of minute quantities of inorganic salts on organised structures. *Journ. of Physiology*, Vol. VII, p. 118, 1886.

XI. Further experiments regarding the influence of small quantities of lime, potassium and other salts on muscular tissue. *Journ. of Physiology*, Vol. VII, p. 291, 1886.

XII. Regarding the action of lime, potassium and sodium salts on skeletal muscle. *Journ. of Physiology*, Vol. VIII, p. 20, 1887.

Note.—The rhythmic contractions of skeletal muscle re-discovered many years later by Loeb are all carefully described in these two papers in 1886 and 1887, as well as the effects of calcium and phosphatic ions in inhibiting them.

XIII. Concerning experiments to test the influence of lime, sodium and potassium salts on the development of ova and growth of tadpoles. *Journ. of Physiology*, Vol. XI, p. 79, 1890.

XIV. The influence of carbonic acid dissolved in saline solution on the ventricle of the frog's heart. *Journ. of Physiology*, Vol. XIV, p. 125, 1893.

XV. Further observations regarding the antagonism between calcium salts and sodium, potassium and ammonium salts. *Journ. of Physiology*, Vol. XVIII, p. 425, 1895.

XVI. The action of distilled water on Tubifex. *Proc. Physiol. Soc., Journ. of Physiology*, Vol. XXII, p. 14, 1897. See also Ringer and Buxton.

Concerning the action of small quantities of calcium, sodium and potassium salts upon the vitality and function of contractile tissue and the cuticular cells of fishes. *Journal of Physiology*, Vol. VI, p. 154, 1885.

Concerning the action of calcium, potassium, and sodium salts upon the eel's heart and upon the skeletal muscles of the frog. *Journ. of Physiology*, Vol. VIII, p. 15, 1887.

Upon the similarity and dissimilarity of the behaviour of cardiac and skeletal muscle when brought into relation with solutions containing sodium, calcium, and potassium salts. *Journal of Physiology*, Vol. VIII, p. 288, 1887.

See also Ringer and Phear. The influence of saline media on Tubifex Rivulorum. *Proc. Physiol. Soc.*, March, 1895, *Journal of Physiology*, Vol. XVII, p. 23.

The influence of saline media on the tadpole. *Journal of Physiology*, Vol. XVII, p. 423, 1894-5.

See also Ringer and Saintsbury, 'The Action of potassium, sodium, and calcium salts on Tubifex Rivulorum.' *Journ. of Physiology*, Vol. XVI, p. 1, 1894.

the view that the globulin may be regarded as having replaceable hydrogen atoms like phosphoric acid and the acid phosphates. If G. represents the globulin molecule, then the union of globulin with base, according to Hardy, might be expressed by:—

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

This leads to the great difficulty, in our mind, that only the sodium atom and not the hydroxyl ion unites with the globulin, and now on such a complex salt ionizing (since there might be 30 to 60 sodium atoms in each solution aggregate) the charge on the complex anion of colloid would be enormous. Also, the mechanism of union of acid on the one hand and base on the other would be quite different, a molecule of water being eliminated in the basic union, and a firm salt formed, while no elimination occurred in the acid union.

We can find no experimental evidence to justify the existence of such large ionic masses, as would require to be formed on Hardy's views, possessing surfaces much greater than the ordinary molecular dimensions. and so forming a comparatively large interface charged by the presence of a large number (30 to 60) of similar ions with similar charges.

The velocity of the complex globulin ion was worked out by Hardy, by the boundary method of Whetham, and found to be of the same order as that for simple ions of the crystalloids. On Ostwald's law, an ion with so many atoms as the complex globulin ion ought to have an exceedingly low velocity, and Hardy points out that this law cannot hold above certain numbers of atoms in the ion, citing also other instances of departure from it.

It appears to us, however, that the ion postulated by Hardy is scarcely an ion at all in the ordinary sense of the term, and that Ostwald's deductions made for *increasing atomic number with constant ionic charge* cannot possibly be applied. It is obvious that the ionic velocity must decrease as number of atoms increases *if the ionic valency remains constant.* But if the ' pseudo ion ' becomes a mass defined by surfaces of dimensions outside molecular limits, then it is quite obvious that a different condition altogether has to be dealt with, in which proportionately to the increase of size of the aggregate the number of ionic charges increases also. If salt formation occurs, for example, between 60 molecules of acid and one solution aggregate of globulin, and then there follows free ionization, the aggregate will then have 60 ionic charges to pull it along in the electric field. Also, all those charges with the same sign will be brought closer together than in the solvent and be out of the region of opposite charges.

The cross section of the mass to be urged through the solution will be much less than the united cross sections of 60 ions; accordingly, instead of moving much more slowly than an ordinary crystalloidal ion, the 'pseudo ion' should move many times more rapidly.

The above reasoning may be illustrated by the difference in rate of falling of fog particles and raindrops. Gravitation takes the place of electric potential, the globulin is the dust particle around which the condensation occurs, the charged ions are the minute water particles of the fog, the raindrop falling rapidly through the air is the globulin 'pseudo-ion.' But no such acceleration on the one hand, nor the delay on the other due to size on the supposition of an ordinary charged ion, occurs; instead, the rate of movement is an ordinary ionic rate.

We would venture to suggest a much simpler view regarding conductivity of colloidal solutions and movements of colloids in the electric field, which so far as we can see fits with all the experimental facts.

Our view does not regard the colloids as amphoteric electrolytes, nor indeed as electrolytes at all, nor as having electric charges intrinsically belonging to them as ions, nor as carrying electric currents. The current is carried, in our view, entirely by the electrolytes of inorganic character, and when there are no such electrolytes there is no current and no colloidal movement. The colloids move because they are united to the crystalloids and must follow their movements, and they are precipitated at either electrode because they are there detached from their electrolytes.

Each solution aggregate when united to a crystalloid holds in colloidal union both ions of the crystalloid, that is to say both anion and kation. For example, globulin in neutral salt solution is not united only to sodium ions or only to chlorine ions, but to an equal number of sodium ions and of chlorine ions, and this number is a large one, say, thirty to sixty of each ion. Similarly, acid globulin is not a salt compound, nor does it hold in union hydrogen ions alone or the corresponding anions alone, but an equal and large number of hydrogen ions and anions. So also for alkali-globulin, an equal and large number of hydroxyl and of corresponding kations are held in union.

These colloidally united positive and negative ions are in some respects free, so that they can alter the freezing point and conductivity. Secondary alterations in ionisation due to the union with the colloid altering dissociation, and concentration of the ions in the neighbourhood of the colloidal aggregate and other such causes may occur, so that

conductivity may be considerably altered from that of the free crystalloid because of the presence of the colloid.

The ionisation of crystalloid still, however, remains high: much higher than direct ionisation of such colloidal bodies would probably give on any ordinary hypothesis of direct ionisation of a colloidal salt. Also these unions are of molecular kind between colloid and crystalloid and not atomic,[1] they are only kept up as long as there is a certain concentration of the crystalloid maintained in the solution, and there is no true atomic linkage with displacement of the elements of a water molecule, as in salt formation. The attraction is specific and hence chemical, and at the saturation point there are definite stochiometric relationships; but it is a union such as can occur between two atomically saturated bodies (such, for example, as a salt and its water of crystallization), and not an atomic union.

It is obvious that in such a union intermediate bodies with varying percentage composition can be separated off such as the adsorption compounds of van Bemmelen, and the varying amounts of bound acid or alkali shown by our experiments indicate such bodies; but this does not rule out chemical union nor debar stochiometric relationships. The most saturated product, however, will always possess a definite composition, corresponding to all points of attachment on the colloidal aggregates being occupied by crystalloidal molecules, and at this point more simple stochiometric relationships may be expected, as in our experiments above recorded, and in the haemoglobin compounds.

Also, the electrical conductivities and movements of the colloids in the electric field are easily accounted for on this view. For, *the movements of the colloids simply correspond to the algebraic sum of the movements of the two ions in union with them.* When, for example, globulin is dissolved in neutral salts, it is just as much in union with them as it is with alkali or acid when it is in solution along with these—why, otherwise, should they dissolve it? But, here the sodium ion and the chlorine ion move at about equal rates, the algebraic sum is zero, and there is no drift of colloid towards either electrode. When globulin is in acid solution, say in hydrochloric acid, the two ions in union with it in equal number are hydrogen and chlorine ions, the hydrogen is by far the more rapid of these two ions, and is moving towards the kathode. *Accordingly the whole crystallo-colloidal aggregate must move towards the kathode,* just as the concentration of the hydrochloric acid does, as shown by Hittorf, when it

1. This at once explains why the unions of globulin with acid and alkali occur between molecules and not atoms as found by Hardy.

is there alone in solution in water. On arrival at the kathode, the hydrogen ions of the crystallo-colloidal aggregate are discharged as hydrogen atoms, the chlorine ions are set free from the globulin and set moving backward towards the anode, and the globulin having now lost the acid with which it was united colloidally, is precipitated at the kathode. Turning to the anode, the chlorine ions arriving here are set free as chlorine atoms, attack water molecules by secondary decomposition, oxygen is set free, and hydrochloric acid is formed; this unites with more globulin and, again on account of greater velocity of hydrogen ion, the whole moves toward the kathode. In this way all the acid-globulin is finally conveyed towards and thrown out of solution at the kathode. It, itself, has not been an ion, has carried no electricity, and borne no charge, but its colloidal attachment to the hydrochloric acid has induced its movement. The explanation is exactly the same for alkali globulin, except that here the hydroxyl is the faster moving ion towards the anode, carrying globulin therefore in that direction and precipitating it there.

All cases of colloids, both organic and inorganic, united with crystalloids can be explained on such a basis. The colloid invariably is found to move in the same direction as the faster of the ions of the crystalloid with which it is colloidally united, and it is usually precipitated at the corresponding electrode from loss of its crystalloid, unless it is still soluble in its absence.

We have also considered closely all the inorganic colloids and their direction of movement in the electric field, and have found no exception to this rule. Starch and similar colloids which do not unite in colloidal reaction (adsorption) with crystalloids do not move in the electric field.

An important paper by Mellanby[1] in the same issue of the 'Journal of Physiology' as that containing Hardy's paper, adds a good deal of precise information to our knowledge of the properties of the globulins. The paper deals very exhaustively with the problems of solution and precipitation of globulins by neutral salts, alkalies and acids, from both theoretical and experimental standpoints. The author draws the following conclusions from his work:—'Solution of globulin by a neutral salt is due to forces exerted by its free ions. Ions with equal valencies, whether positive or negative, are equally efficient, and the

1. *Journ. of Physiology*, Vol. XXXIII, p. 338, 1905.

efficiencies of ions of different valencies are directly proportional to the squares of their valencies.'

'The amount of globulin dissolved by a given percentage of neutral salt is directly proportional to the strength of the original globulin suspension.'

'Solution of globulin by acids or alkalies is of the nature of a chemical combination. The relative solvent efficiencies of strong acids and alkalies are of the same order as their chemical avidities.'

'The precipitation of globulin from solution in neutral salts by neutral salts depends upon a molecular combination between the salt and globulin, the compound so formed being stable only in excess of the combining salt. Precipitation by salts of the heavy metals depends upon the formation of a stable globulin compound.'

These four conclusions of Mellanby, supported as they are by most abundant experimental evidence, appear to us to be of fundamental importance in the study of crystallo-colloidal combinations. Our own experiments, carried out by a different method, entirely agree with the greater part of them and confirm them, and we could wish no better support for the views which we hold as to the relationship of crystalloids and colloids.

In the first place the experiments, like our own, would conform to the van Bemmelen conception of adsorption compounds, but the author, like ourselves and most other authors, arrives at the conclusion that the process is a chemical one. This is now further confirmed by our result of a maximum uptake when a certain low concentration of the crystalloid has been attained.

The second result of Mellanby, that the uptake of globulin (colloid) in presence of the same percentage of saline (crystalloid) increases with original colloid concentration is the complement of the result of the van Slykes (vide infra) and ourselves, that the uptake of acid or alkali (crystalloid) in presence of the same percentage of serum protein or casein (colloid) increases with the original crystalloid concentration. This result is of fundamental importance because it disproves the universally accepted dictum that the colloid being a solid or liquid phase its concentration can be taken as constant. This point we raise at length further on, as it is at present entirely unappreciated, and shows an intrinsic difference between colloid and crystalloid. *Because the colloid concentration is not constant throughout the reaction, but varies like that of a gas or dissolved substance, the equilibrium equation does not reduce to one of the first degree, representing*

a reaction of the first order and with only one variable, viz., the concentration of the crystalloid; but remains an equation of the second degree with two variables, viz., concentration of crystalloid and concentration of colloid (see p. 67). The plotted points of the equilibria of a 'colloidal reaction' are hence roughly on an hyperbola and not on a straight line. They are only roughly so because the 'affinity constant' diminishes as more and more colloidal molecular combination between colloid and crystalloid goes on.

Another important point in which Mellanby is in accord in his results with Hardy and many other observers is that there is a *molecular* combination between salts and globulin. That the combination between a colloid and a crystalloid is one between molecules and not atoms is, in our view, the fundamental and distinguishing characteristic of a colloidal combination. The question is discussed at length by one of us in a paper now in the press,[1] and taken in conjunction with two other experimental findings this goes a long way towards explaining some of the seeming anomalies of colloidal compounds. The first of these two points is that what might be termed 'molecular valencies' can have much higher relative values than atomic valencies. Most atomic combinations giving origin to crystalloids take place with atoms having valencies not above four (as in carbon) or five (as in nitrogen); but in molecular unions or combinations one molecule may have a valency towards another as high as sixty to one, as, for example, in the case of our proteins with acid. We see for example a valency ratio represented by 24 to 1, when the alums combine with water to form the crystalline alums. For such molecular unions characteristic especially of colloids, there need be no complete atomic anchoring. A tendency to basic linkage of a weak type in a protein molecule, such as a feeble amido group, with no free atomicity serves to hold in 'molecular swinging' in the neighbourhood an acid *molecule* (not an hydrogen ion). There is an off and on kind of attachment, a dynamic rather than a static equilibrium, ready to vary up and down with varying conditions, to join more strongly or let go as slight alterations occur in the environing system.

This is the essential vitally important characteristic of the biotic molecule; and the simpler crystallo-colloidal compound, of alkali or acid or neutral salt with protein, leads up towards it.

In thinking, therefore, of crystallo-colloidal compounds, and still more so of living chemical units, we must depart from the statical, fixed, dead idea of a stereotyped molecule, and replace it by the conception of a

1. The rôle played by molecular affinities in bio-chemical reactions. B. Moore, *Archivio de fisiologia*, Vol. VIII, dedicato a Guilio Fano.

dynamical, labile, living molecule—variable and unstable in its component parts, and so giving a mechanism for energy exchanges, but as a whole stable and unaltering throughout a long series of subordinate interchanges.

When the concentration of the crystalloid reaches or exceeds that maximum at which the colloid is completely saturated, as occurs at the top of our series in each experiment, then the crystallo-colloid once more becomes fixed and like a stable purely crystalloidal compound. In such a condition it is useless in bio-chemical operations in the body, its essential lability is gone, and until crystalloidal pressure falls again it cannot react in the living cell.—Excess of oxygen acting upon living cells illustrates this point, and is as fatal to life as dearth of oxygen.

The number of researches upon the nature and composition of the union between casein and alkalies, and especially of casein and calcium, is quite extraordinary. Nearly all these confirm the facts first elucidated by Söldner, namely, that there are two classes of compounds of casein with bases: one neutral to phenol-phthalëin, termed the basic caseinate, and containing, according to Söldner, 2·32 per cent. of calcium oxide; the other neutral to litmus and formed when excess of casein is shaken up with dilute lime water or other alkali, the lime salt of which contains 1·55 per cent. of calcium oxide. As we have seen, the latter corresponds to our condition where no free alkali is present; the second, we have reason to believe, does not correspond to our maximum uptake of alkali, which corresponds very closely to double the amount for the neutral compound. Söldner's two figures are roughly in the ration of 2 : 3. Figures of different observers vary, but on the whole are in fairly close agreement for the so-called basic caseinates.

The caseinates of many bases have been prepared and investigated by W. A. Osborne,[1] such as ammonium, potassium, sodium, lithium, magnesium, strontium, and calcium caseinates. Osborne shows that casein acts as a stronger acid than carbonic acid, forming these salts from free casein and the carbonates or bicarbonates with expulsion of carbon dioxide, he also found that casein united with alkaloids such as caffein and strychnin. The caseinates of the alkaline earths form Ramsden surface films like milk on heating, and Osborne also found in confirmation of a forgotten discovery of Ringer[2] that the calcium caseinate undergoes a kind of coagulation on heating, which is reversible and re-dissolves on cooling. Para-caseinates are formed on acting upon the caseinates with

1. *Journ. of Physiology*, Vol. XXVII, p. 398, 1901; *ibid.*, Vol. XXXIV, p. 84, 1906.
2. Ringer, *Journ. of Physiology*, Vol. XI, p. 464, 1890; *ibid.*, Vol. XII, p. 164, 1891.

rennin, but as Ringer first showed, and Osborne confirms, are not thrown out until excess of calcium salt is present. Ringer was the first to elucidate the true action of calcium in milk-curdling.

The behaviour of casein with acids has not been quite so frequently investigated as in the case of the bases, but has received also a good deal of attention, as apparently being such a clear case of 'adsorption' on account of the insolubility of the casein-acid adsorpate.

Any account of the literature would be very incomplete without some reference to the most admirable paper by Lucius L. van Slyke and Donald D. van Slyke,[1] of the New York Agricultural Experiment Station, on the action of dilute acids upon casein when no soluble compounds are formed. The authors allowed carefully prepared pure casein to remain in concentrations of acids of 0·008 N and lower until equilibrium was established, filtered clear, and determined concentrations of acid in filtrate, from which amounts taken out by the casein could then be calculated by difference. The acid concentrations were determined in the filtrates by electrical conductivity and by titration methods. Over 0·008 N acid could not be employed because casein dissolved and interfered with the determinations. Conductivity water was used throughout, and long series of experiments carried out with hydrochloric, sulphuric, lactic and acetic acids.

The concentrations used by these authors were lower than our own, for we were able by the dialysis method to work without protein disturbance interfering with our titrations. Our results abundantly confirm those obtained by the van Slykes.

We completely agree with them that the process is one of adsorption in their experiments, and it is so in ours also, giving as it does all the criteria of van Bemmelen; we prefer, however, in spite of all this, to regard it (and all such adsorptions) as peculiar types of chemical reaction, which we should like to see called 'colloidal reactions.'

In the next section, in which we discuss our results in detail, we assign reasons why all components of the reacting system save the acid cannot be taken as constants, so that the reaction on a chemical basis does not for equilibrium reduce to the linear function $C_A = Kt$, where C_A is acid concentration, K a constant, and t temperature.

We are well aware of Ostwald's dictum that 'only gaseous and dissolved substances have varying concentration',[2] and agree that this holds

1. *Amer. Chem. Journ.*, Vol. XXXVII.

2. Ostwald, 'Principles of Inorganic Chemistry,' translation by Findlay, p. 327, 1902. Quoted by the van Slykes.

for crystalloids, such as the system [$CaCO_3$, CaO, CO_2] and many others. Here the constancy of concentration of $CaCO_3$ and CaO, the solid constituents, is obvious so long as there is any of either of them present; but is it so with a colloid? The colloid, as we have seen, is capable of uniting with 30 to 60 molecules of acid or alkali. Suppose at one stage each colloidal molecule is united with five molecules of acid or alkali, and at another stage is united with ten molecules of acid or alkali, and further suppose, as our experiments seem to indicate, that its 'affinity constant' decreases as it approaches complete saturation—is it then still to be regarded as of constant concentration? We believe it is then of varying concentration like any soluble constituent, and it is here that the intrinsic nature of the 'colloidal reaction' or 'adsorption' comes in. In our discussion we deduce the result, on this basis, that the curve should roughly be a hyperbola, and according to Ostwald's second additional criterion of adsorption quoted by the van Slykes the curve should approximate to an hyperbola.

On the exponential or logarithmic formula $\frac{C_1^k}{C_2} = K$ these authors find that the hydrochloric acid, lactic acid and acetic acid k is nearly 1, for sulphuric acid k is about 1·95 or almost 2. *Both* these sets could approximately lie on an hyperbola, the latter lying on the more curved part, and the former on the flat part where the hyperbola would almost appear as a straight line.

Whatever may be the interpretation of the results, experimentally they are in complete agreement—our own and those of the van Slykes—and, whether we ultimately find the problem is one of surface condensation and therefore so-called 'physical,' or one of specific colloidal reaction and therefore so-called 'chemical,' the value of the work as experimental research remains unaltered. At present, on account of the maximum value we have always obtained and because the action is a specific one for acids and bases with the proteins, we lean towards the chemical explanation.

Theoretical Discussion of the Results

The discussion of the meaning of our own results, especially in regard to the controversy as to chemical reaction *versus* adsorption, has been purposely postponed until after the statement of the experimental findings themselves, because we are of opinion that such results and the dialysis method for obtaining them in such colloidal solutions, possess a permanent

value as experimental matters, apart from any theory which may be given to account for them.

In the preceding critical account of the literature it has been impossible to avoid touching and commenting upon certain aspects of the contending theories; we desire now to deal more in detail with the theoretical side of the subject.

In regard to the nature of the action taking place, while it shows certain of the characteristics supposed to exist in typical adsorptions, and would be classed at present as a typical adsorption, it is in our opinion due to chemical affinity between the acid or alkali and the protein, and we would venture to suggest for all such reactions the name of 'colloidal reaction' instead of 'adsorption.' The word 'adsorption' is an essential one to retain, but it ought not in our opinion to be used to cover sets of phenomena so different as, condensation of moisture on glass; absorption of gases by charcoal, kaolin, and other inert bodies; uptake of water by fibres; union of dyes with fibres and tissues; formation of surface films on liquid and solid interfaces of a two-phase or three-phase system; unions of proteins or other colloids with acid, alkalies, and other electrolytes; and formation by molecular union or complex formation of inorganic colloids, such as the sulphides, etc.

There exists no experimental evidence that all these divers types of interaction are identical in kind and in causation, and the use of the single word 'adsorption,' to signify them all, is highly unscientific and misleading, and calculated more to cloak ignorance than to lead to experimental investigation.

Some of the above phenomena are almost certainly purely physical and due to surface cohesion, where molecular affinities enter in, not at all, or in very diminished degree; others are partly physical and partly chemical,[1] that is to say, dependent on molecular relationships arising from chemical dissimilarity, and we feel certain that some of them, such as those we are dealing with here, are almost purely chemical. If the word adsorption could be restricted to signify the purely physical process or the physical portion of a mixed process, and the term 'colloidal reaction' be applied to the chemical process where a colloid takes part in a reaction, or to that portion of the mixed process (such as dyeing of fibres

1. We dislike to use the word 'chemical' in contradistinction to 'physical,' as in the last resort both chemical and physical phenomena are ultimately physical in the broad sense of the word. We use the two words in obedience to established usage, meaning by 'physical' energy forms not involving *specific* molecular energies, and by 'chemical' energy exchanges which occur specifically between two chemically dissimilar substances, causing them to become more firmly united as a result of their specific chemical dissimilarity.

probably is) which is chemical, we believe a distinct advance in terminology of colloidal unions would be made.

Before passing to the more detailed consideration of the interpretation of our own results, we would also enter a protest against the scientific absurdity of allowing one single characteristic and that often of a doubtful nature, to outweigh all other evidence, no matter how strong or based on a number of different well-established facts.

No result bearing upon these colloidal unions seems nowadays to be of any value unless it can be plotted in a curve. Men appear only to be able to think in curves, although figures are often quite as easily understood, and show results better and more truthfully to those who will take the pains to examine them. There is nothing so easy as by proper (or improper) use of suitable co-ordinates, and choice of different scales of magnification in plotting ordinates and abscissae, to gloss over an imperfectly carried out experiment, and by judicious use of French curves make the experiment illustrate either one formula or another quite different one. Certain parts of a hyperbola or parabola can be made to look very like a straight line, or by the judicious choice of (k) a logarithmic curve can have for a good way an indistinguishable look from a conic, and lie so close to it, that a set of experimental points might well belong to either curve. After the curve has been plotted, the next thing is to see whether a presentable number of the experimental points can be tortured into, or somewhere near to, a curve represented by the formula $\frac{C_1^k}{C_2} = K$, and if this can be done, no matter how near k may lie to the values of 1 or 2 then, there is no help for it, and the case is one of adsorption.

This is really no exaggeration of the view taken by some recent authors, who appear to close their eyes, under the hypnotic influence of this equation and its supposed curve, to all opposed experimental facts.

For example, the union of oxygen with haemoglobin, a subject of fundamental importance to be correctly understood in bio-chemistry, has for years been believed to be a dissociable chemical union. The evidence in regard to this comes from several discrete sources, and appears to be about as well an established fact as possible in chemical science.

For once, thanks to the iron which the haemoglobin molecule contains, its molecular weight, although it is colloidal, is well known; it can be obtained in pure crystalline form and analysed, and its molecular weight can also be determined by direct measurement of osmotic pressures. The amount of oxygen it takes up (although it varies with the oxygen

pressure at low pressures) at and above the partial pressure of oxygen in the earth's atmosphere, is practically constant. Hence the amount of oxygen capable of combining at saturation point with a known weight of haemoglobin is well and definitely known. Further, the stochiometric relationships, on comparing the two combining weights, are of the simplest description, viz., one molecule of haemoglobin with one molecule of oxygen.

This would appear to be one fairly strong piece of evidence that haemoglobin and oxygen enter into chemical union, and that the smaller amounts of oxygen at lower pressures are due to partial dissociation.

Further, haemoglobin unites with other gases, and its union with carbon monoxide has been particularly well worked out. Here the coefficient of the reaction is higher, and saturation occurs at a lower partial pressure with carbon-monoxide than in the case of oxygen. But, at the saturation point, carbon-monoxide replaces oxygen, volume for volume, and the stochiometric relationships are again one molecule of haemoglobin to one molecule of carbon-monoxide. This again is somewhat convincing as to the action being of a chemical nature.

Lastly, if we look at the spectrum of haemoglobin free of oxygen we see a quite characteristic definite spectrum, with one well-marked band; allow access of oxygen, and the spectrum at once changes to a quite different, but equally characteristic, two-banded one, which reverts again to the one-banded spectrum if the oxygen be pumped off. At the same time that these spectroscopic changes occur the solution markedly changes in colour, showing a dark purple when free of oxygen, and a bright scarlet when united to oxygen. These are the chief points of evidence in favour of the existence of a chemical union between haemoglobin and oxygen, and, to our minds, they are quite convincing.

But simply because the points showing amounts of oxygen taken up at various partial pressures *appear* to lie on an exponential curve, all this evidence has been neglected by a recent worker on colloids, and the phenomenon of oxygen and haemoglobin union described as an adsorption.

It seems to be pretty unanimously forgotten by workers on the subject that an exponential curve is the very prototype of an *empirical* curve, the formula as the exponential index is altered, giving a series of traces like a set of French curves, to one of which nearly any set of experimental points can be made to approximate with that degree of accuracy usually expected in such a case.

Another assumption, experimentally unwarrantable, which has been

made in many papers devoted to such discussions on adsorption or chemical reaction is, that, supposing the union to be a chemical one, the ratios of free and combined crystalloid must be very simple because the concentration of the colloid does not alter during the reaction. The reason assigned for the colloid concentration not altering is that it is not in true solution but exists as a solid or fluid phase, in a heterogeneous system with interfaces between its aggregate and the solvent, which contains the crystalloid in varying concentration. It is in fact exactly similar to a plate of a metal, such as zinc, being attacked by an acid, and as long as there remains excess of metal its concentration (which is merely represented by its surface) may be taken as constant and written down as such in the equilibrium equation.

Unfortunately for the study of colloidal changes with crystalloids, the above reasoning is entirely fallacious; the colloid cannot be regarded as a discrete phase of solid, and therefore possessing a constant concentration, until it is all used up. Its concentration varies throughout the reaction just as does that of the crystalloid uniting with it, and it is this fact which makes all the difference to the form of the equilibrium equation, on the provisional assumption of chemical combination. Even we assume that there are interfaces between the colloidal aggregates and the solvent, large compared to the molecular dimensions, so that the colloidal solution comes to resemble an ultra-microscopic emulsion. Still, we have no grounds for assuming that the concentration, being represented by united superficial area of the aggregates, is constant, and hence that crystalloidal pressure (acid or alkali concentration, for example, in our experiments) must remain nearly constant and at a low value until all this surface is covered with adsorbed material. For, as crystalloid is attracted to the colloidal surface, the attached crystalloidal molecules will all the time be packed closer and closer together, and this can only be accomplished by a higher and higher concentration of crystalloid in the solvent. Whether, therefore, we take a physical or a chemical basis to go upon, the concentration of colloid, as measured by its surface or otherwise, does not remain constant, but continuously diminishes just as if it entered into true chemical union. Accordingly we have no right on the basis of chemical union to expect that great simplification of formula and form of curve which would arise if the colloid concentration were constant. It is necessary to point this out at length, because this erroneous assumption has been made by previous authors and has led to the wrong criterion, that in case there is chemical combination, then the amount of crystalloid

taken up at varying concentrations of crystalloid must remain constant, which it does not do experimentally. Also, it has been supposed that with less crystalloid present than that required for union with the whole of the colloid, then all of the crystalloid should be taken up.

Again, in the case of union of a mono-molecular type, where one molecule of colloid unites with one molecule of crystalloid, as we have seen is the case with haemoglobin and oxygen, investigation of the equilibrium equation shows that instead of the relationship being a simple linear one between oxygen absorbed and partial oxygen pressure, as it would be if the concentration of the haemoglobin remained constant, it becomes a conic section oblique to the axes of co-ordinates. An investigation of the equilibrium equation is mathematically exceedingly simple, and as it well illustrates the point at issue, we venture to give it.

The identical reasoning employed will hold for any case in which one molecule of a colloid unites chemically with one molecule of a crystalloid,[1] but as the form of the oxygen absorption curve with varying oxygen partial pressures is familiar to all biological chemists, we shall describe the deduction of the formula in terms of oxygen and haemoglobin.

Let C_1 = concentration of oxy-haemoglobin.
,, C_2 = ,, of reduced haemoglobin.
,, C_3 = ,, of oxygen in oxy-haemoglobin (i.e. absorbed oxygen, usually plotted as one co-ordinate by Hüfner or Bohr).
,, C_4 = ,, of free oxygen (proportional to oxygen partial pressure and usually plotted as the other co-ordinate).

This means that C_1, C_2, C_3, and C_4 are the four concentrations, which exist together at an equilibrium point for any given partial pressure of free oxygen (C_4).

Then, we have first the following facts to simplify our equation:— Since the total amount of haemoglobin present either as oxy- or reduced haemoglobin is constant, it follows that oxy-haemoglobin *plus* reduced haemoglobin is a constant, say A, that is

$$C_1 + C_2 = A$$

Next, since we have as an experimental fact that one molecule of haemoglobin unites with one molecule of oxygen, it follows that the concentrations of oxy-haemoglobin and of absorbed oxygen in it are equal, or, in other words, $C_1 = C_3$.

1. The same equation as we shall show later also holds for a multimolecular reaction between a colloid and crystalloid, unlike the case for two crystalloids of different valency uniting.

Coming now to the equilbrium equation, the tendency to union of reduced haemoglobin and the free oxygen is proportional to the concentrations of these two substances, i.e., to $C_2\,C_4$, and the tendency to move in the other direction by dissociation of combined oxygen from combined haemoglobin is proportional to the concentration of these two, i.e., to C_1C_3, or since $C_1 = C_3$ to C_3^2. Accordingly at the equilibrium point, since these two tendencies balance, we can introduce a constant and equate them, giving us, as equilibrium equation:—

$$C_2\,C_4 = k\,C_3^2$$

To get an equation between the two amounts of free oxygen (C_4) and combined oxygen (C_3) we must eliminate C_2 (the concentration of reduced haemoglobin). This elimination, the equation $C_1 + C_2 = A$, enables us to perform for $C_1 = C_3$ $\therefore$ $C_3 + C_2 = A$ or $C_2 = A - C_3$. Substituting this value for C_2 we get finally for our equilibrium equation between C_3 and C_4:—

$$(A - C_3)\,C_4 = k\,C_3^2, \text{ this may be written}$$

$$k\,C_3^2 + C_3\,C_4 - A\,C_4 = 0, \text{ where } C_3 \text{ and } C_4 \text{ are two variables.}$$

This final form is a conic with its axes oblique to the axes of co-ordinates, and may be a parabola or hyperbola, according to the experimental value of k. Anyone familiar with the dissociation curves of Hüfner and Bohr will realise how closely they resemble hyperbolae or parabolae.

This is quite a different result from the straight line or linear relationship of direct proportionality between oxygen taken up and partial oxygen pressure, which follcws if oxy-haemoglobin concentration is supposed to remain constant, and so both theoretically and experimentally the assumption of constancy of concentration of colloid in such interactions is shown to be erroneous.

There is a most important and fundamental difference between the crystalloidal and colloidal chemical reaction, to which no attention, to our knowledge, has hitherto been given. It may be formally stated thus:— As the valency of one of the substances increases in a purely crystalloidal reaction, the degree of the equation of equilibrium goes up accordingly, and the conditions of equilibrium become excessively complicated; on the other hand, in an equilibrium where one of the constituents is a colloid, although the colloid molecule or solution aggregate may be capable of combining with a huge number of crystalloidal molecules (such as thirty to sixty, as in our experiments in this paper, see page 43), the degree of the equilibrium equation does not rise, but it always remains roughly expressed by an equation of the second degree.

In the reactions of serum proteins with alkali and acid, which we have been experimentally examining above, there is clear evidence that each solution aggregate of protein is capable at the saturation point of union with thirty to sixty molecules of alkali or acid. One almost shudders to think of the complexity of the equilibrium equation deduced on the basis of chemical combination on the usual lines for such a reaction; it would certainly be an equation of high degree, and require many experimental points for the solution of its constants.

Does this necessarily rule out chemical combination altogether? By no means; we have simply been translating our preconceived notions, founded on crystalloidal reactions to a region where they do not hold, and where the phenomenon though still chemical (or possibly chemical) is arranged differently.

The obvious difference which alters the whole situation is that intermediate compounds with variable numbers of attached crystalloids can exist in the case of the colloid and that these cannot exist in the case of the two crystalloids of different valency combining with each other to form a crystalloidal compound. Suppose A, for example, is a trivalent crystalloidal ion and B a monovalent ion, capable of uniting to form the crystalloidal compound AB_3, then for the formation of a molecule of AB_3, the simultaneous collision of three molecules of B upon A is required (using the kinetic theory to state our case). This means (if C_A and C_B express concentrations of A and B respectively) that the tendency towards union is measured by the product $C_A \cdot C_B^3$, and the opposite tendency to dissociation or ionization is expressed (C_{AB_3}, being the concentration of the compound AB_3) by C_{AB_3}. Accordingly, the equilibrium equation becomes $C_A \cdot C_B^3 = k \cdot C_{AB_3}$, which works out to an equation of the fourth degree. If there were any cases of very high valencies, such as thirty or sixty, amongst crystalloids we should have equilibrium equations of similarly high complexity.

Now, all this follows because such compounds as $=AB''$, or $-AB'_2$, cannot exist in the case of crystalloids. For if they could exist there would be no need for three ions of B to be within the sphere of influence of A at the same instant. The intermediate ion ($-AB''-$) could remain stable and in a certain concentration in solution, till it was joined by a second B, and became $-AB'_2$. Similarly, $-AB'_2$ could exist until it was joined by a third B′ and become AB_3, after which no more of B′ could be taken up. This last stage is precisely what has happened in our present experiments when the amount of 'united' acid has reached its fixed maximum point.

It is this difference in behaviour of colloidal reactions which distinguishes them from incomplete mono- or bi-molecular reactions of crystalloids. The laws governing an adsorption, or, as we prefer to call it, a 'colloidal reaction,' differ in certain fundamental respects from those governing an incompleted or balanced crystalloidal reaction, and hence, *even on the view that both are purely chemical*, the one is different from the other.

One fundamental difference we have just pointed out, namely, that no matter that even thirty or sixty crystalloidal molecules or ions are uniting with one solution aggeggate of colloid, the degree of the equation does not rise but is *roughly* represented by an equation of the second degree. Simply because any number from one to one hundred of the crystalloidal molecules for which attachments are possible may be present on an aggregate at any given time, or the attachment may be vacant. Another important difference is that the colloidal is a reaction between molecules, the incompleted crystalloidal reaction is one between atoms or unsaturated radicles.

We may now further enquire why the second degree equation represents equilibrium conditions in the colloidal reaction, *so much more roughly*, than it does the simple reaction between two crystalloidal equivalent ions or molecules.

To understand this we must return to the derivation of the equation of equilibrium and the assumptions tacitly made therein. The equation of equilibrium for two crystalloids tending to unite is derived by assuming that the tendency to union is determined *solely* by the concentrations at the moment in the solution of the two ions or molecules uniting. If the two concentrations are C_1 and C_2 and we introduce a constant k_1, we can express this by equating it to $k_1 C_1 C_2$. On the other side the tendency to disrupt is proportional to the concentration of the substance formed (C_3), and again introducing a constant this is measured by $k_2 C_3$. For equilibrium, the two tendencies balance, and we get the usual equilibrium equation $k_1C_1C_2 = k_2C_3$. Now, all this holds rigorously enough for the crystalloidal reaction where there are no intermediate stages or any gradual accumulation of the one combining substance on the surface of the other. Hence the constant is here a real fixed constant, the tendency to union being determined *solely* by solution concentrations. With the colloidal reaction, however, the case is different, and something more than concentrations comes in. For, as the concentration of crystalloid goes on

increasing, the colloidal aggregate becomes more and more charged up with crystalloidal ions or molecules already in place. The presence of such united ions must obviously have a *secondary* influence on uptake of further crystalloidal ions or molecules, *in addition simply to the increased number of free places*, the change in which number alone is expressed by the changing concentration. The action is somewhat similar to that seen in constructive synthesis in organic chemistry, where each successive similar group or radicle is only introduced into a given molecular conformation with increasing difficulty, although a similar place or linkage exists for it. This means that k will not remain constant, but, *for these secondary causes*, will vary as the concentration of the crystalloid undergoes considerable variation.

On exactly similar lines to those given previously for haemoglobin and oxygen (see p. 68) an equation can be deduced to represent the equilibrium between the serum proteins and acid or alkali concentrations of the form $k C_3^2 + C_1 C_2 - A C_2 = 0$, where C_1 and C_2 are concentrations of united and of free acid respectively, and A is the total available combining power of the protein. But, for the above reason of decreasing affinity between partially combined aggregates and further crystalloidal acid or alkali, it is obvious that k will continuously decrease as C_1 approaches A in value; that is to say, as complete saturation of the protein is approached. This is precisely what happens when such an equation derived on the assumption of a constant specific affinity (the chemical affinity is represented by the constant k) is applied to an experimental case, where the affinity is all the time diminishing as the concentration of the acid or alkali is forced up.

In the limiting case, where the concentration of the combined acid or alkali (C_1) becomes equal to A; that is to say, when complete saturation occurs, the second and third terms of the equation balance, and we get $k C_1^2 = 0$, and since C_1 is not zero k must now have reached a zero value. This means that at the saturation point, all chemical affinity for crystalloid has been satisfied.

Substitution of the values for C_1 and C_2 from our tables in the experimental part of the paper shows the same result that k is decreasing with each increase in C_1 and C_2, or, in other words, the affinity is growing less continuously.

By empirically employing an exponential (or logarithmic) equation of the form $C_1^k = KC_2$, values for k can be obtained which, instead of varying continuously in one direction, will wriggle round a mean point with irregular variations accompanying similar perturbations in the experimental technique. But it is absurd to attach any importance to a constant so obtained. We do not know what it means. It certainly is

not an affinity constant, nor one related to any known physical or chemical property, but purely and simply an empirical figure. By so doing we are merely grinding out of a mathematical equation an unknown result, which we have thrown in by making an unknown assumption. We should simply be satisfying our minds unwarrantably, by obtaining a constant of the meaning of which we possessed no knowledge.

The value that we get from the simply deduced equation from the mass action law is, first that it demonstrates to us that there is an upper limit of possible union of the acid or alkali with the protein or other colloid. This is also demonstrated by our experiments. Secondly, both equation and experiments show that free colloid and free acid or alkali exist together, and a certain value of concentration is necessary for a definite amount of combination. Thirdly, we see that the *affinity constant* diminishes with increasing saturation, and becomes zero at complete saturation. Inspection of the figures also shows this decreasing affinity. Lastly, considering the results in conjunction with the known osmotic pressures of the serum proteins, we see that a large number of acid or alkali molecules or ions unite with each solution aggregate of protein, and the form of the experimental results demonstrates that this occurs in such a way as to distinguish completely the colloidal reaction from the incomplete crystalloidal reaction. The difference lies in the colloidal reaction proceeding by stages, while in the crystalloidal reaction of higher degree this is impossible, the intermediate stages being incapable of existing.[1]

We venture, in conclusion, to make a final protest against a loose form of expression which has crept into the literature of colloids in describing adsorptions (or colloidal reactions), namely, that they do not obey the mass action law. This is used, quite wrongly in our opinion, as a great criterion of physical adsorption *v.* chemical combination. Narrowly examined it really is found to mean, that the phenomenon, *as postulated and understood by the authors*, does not obey the law of mass action.

The reason why it does not *appear* to obey the mass action law is that there are secondary and varying factors at work which have been summarily left out of the equation. We get out of mathematics what we have put in, ground round for us into another shape.[2] That is all.

1. This assumption is quite different from that of a long series of intermediate bodies described earlier in the paper; for there is but one body, the colloid, at various stages of saturation with a second body, the crystalloid; in the so-called ion-protein theory, there are a vast number of intermediate saturated complexes or 'ampholates,' each forming a chemical individual.

2. Mathematics may be compared to a mill of exquisite workmanship, which grinds you stuff in any degree of fineness, but, nevertheless, what you get out depends on what you put in, and as the grandest mill in the world will not extract wheat-flour from peascods, so pages of formula will not get a definite result out of loose data. (Huxley, 'Lay Sermons,' p. 216.)

Substances capable of reacting upon one another can no more disobey the mass law than an energy-change can disobey the fundamental laws of thermo-dynamics, or a falling body disobey the laws of gravitation.

A feather floating in the air *appears* to be disobeying the law of gravitation until we understand the phenomenon, and then the disobedience disappears.

In our view, then, such unions as we have been studying are chemical, in that they occur between bodies still of molecular dimensions; in that the attraction between such bodies is of a specific character and dependent on the molecular conformation of the two reacting bodies; in that (especially at the upper limit) definite mass relationships (or stochiometric relationships) exist; in that molecular valencies occur, although the figures representing ratios of valency are higher than in the case of atomic reactions.

But we are well aware that such unions, because they occur between molecules and not between atoms, are a step onwards towards the more general cohesions and surface reactions which may be called 'adsorptions,' and are generally regarded as purely physical in character.

There is no real demarcation between physical and chemical phenomena. In modern physico-chemical notions, the atom is losing its position as a fixed constant, just as is that of the cell in cytology and biochemistry.

Just as we have now to concede ultra-microscopic organisms and invisible germs, and have to take the enzyme as something endowed with energetic properties from the cell, a kind of bio-chemical unit of lower value than the cell; so in physics we are coming to regard the electron as a more minute form of matter than the atom, a something capable of carrying an electric charge, which, added to or taken away from the atom, changes its character. Proceeding in the opposite direction, when we pass one stage upward from atomic reaction we come to molecular reactions, or, as we have termed them, colloidal reactions; thence to cell formation. There is nowhere any hiatus but only a series of gradual transitions. It is at these transition points that interesting problems await investigation. It is also just at these interesting points that cause and effect are so difficult to correlate. For example, where two phases exist with a contact surface, it can be demonstrated thermodynamically that if the presence on the surface layer of any dissolved substance tends to lower surface-tension then that substance will increase in concentration on the surface. Is this a physical or a chemical

result, and which is cause and which effect? At first sight it looks as if it were a purely physical change and that there is no specific molecular affinity, or, in other words, no chemical reaction. But why does the dissolved substance lower surface tension? There must obviously be something specific in this because it does not occur markedly with every solvent and solute, and in cases chosen specifically for examination by experiment it is quite obvious that there is specific affinity between solvent and solute. Even in ionized aqueous saline solutions, the older simpler view of perfectly free ions in solution, having served its day is passing away on account of the many things it quite fails to render any account of, and it has to be recognised that there are complexes (or molecular unions) between ions and solvents. These complexes on the one hand are not the old stereotyped solution hydrates, but on the other they remove for ever the free unchained ion, and introduce the conception of a chemical union between ion and solvent, which union is initially responsible for the ionization.

So, too, in the case of surface tension and surface film formation, the physical changes are dependent upon chemical affinities. The surface tension is lowered because the dissolved colloid and its solute are tending to unite in molecular union; on or near the surface, the dissolved colloid is less under the disruptive influence of the solvent acting in mass and tending to send it into solution. A colloidal particle arriving on the surface has passed half way out of solution, and if there is anything on the surface with which it can pass into molecular union or adsorption it will remain bound there. If, however, there is nothing with which it can unite on the surface, it must raise the surface tension, because it remains there in true solution, and all molecules in true solution must raise surface tension, because surface tension is in two dimensions what osmotic pressure is in three dimensions. This is obvious, because if we dissolve a substance in true solution in a solvent the osmotic pressure shows the volume energy has increased. If now we take equal volumes of solution and of pure solvent and draw these out into two equal and similar surface films, so thin that the whole thickness of the layer of each is within surface action, then it follows that the difference in amount of work necessary to do this must exactly equal the work of solution, for now surface tension replaces the properties of volume, and all the previous volume energy must be located on the surface.

To return to surface film formation then, if the solute remains in true solution on reaching the surface it must raise surface tension, and

hence an equilibrium must soon be established. No surface film will be formed under such conditions, and there can be no adsorption. On the other hand, if the molecule reaching the surface enters into molecular union either with similar molecules or with dissimilar molecules for which it possesses molecular affinities lying on the surface or interface, then surface tension will not rise, and surface films will form or surface condensation occur.

Without some such *specific* factor—which we may term physical or chemical, as we choose—there is no obvious reason why adsorption co-efficients should vary in each individual case. Why, for example, palladium should not adsorb all the permanent gases equally, or haemoglobin take up nitrogen, oxygen, and carbon monoxide in equal amount at equal pressures.

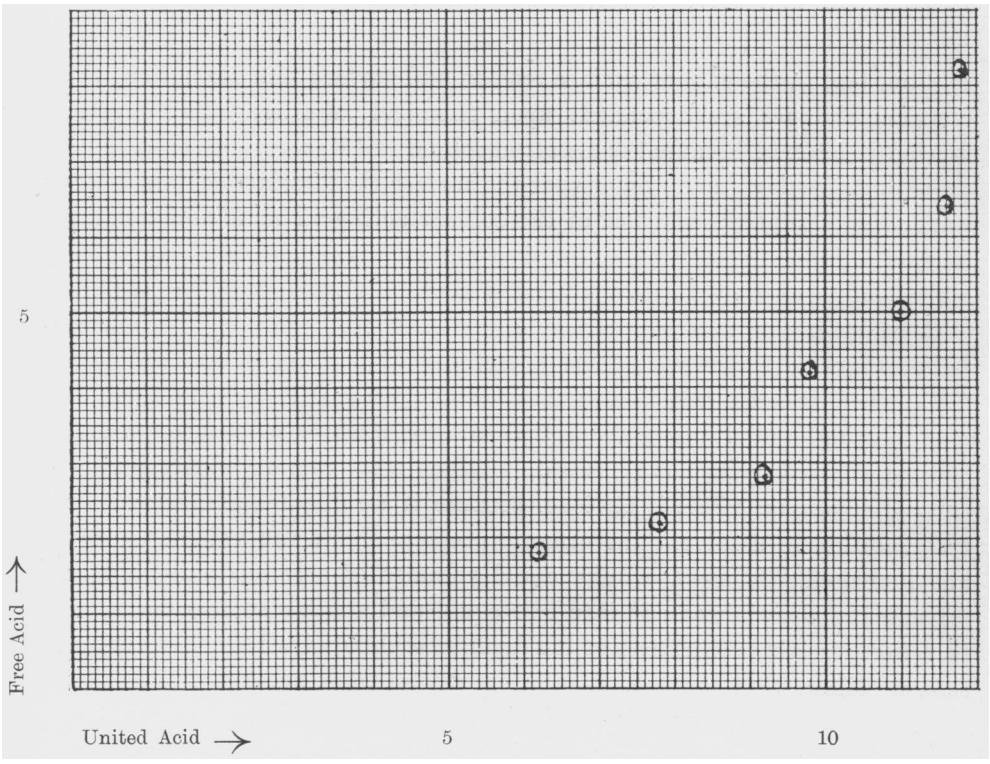

FIG. 1. *Expt. XIII.*—EQUILIBRIUM OF SERUM PROTEINS WITH SULPHURIC ACID.

Ordinates show free acid as 1 1000 N, and abscissae united acid in c.c. of N/10 to 0·703 grm. of serum protein in 7·03 per cent. solution.

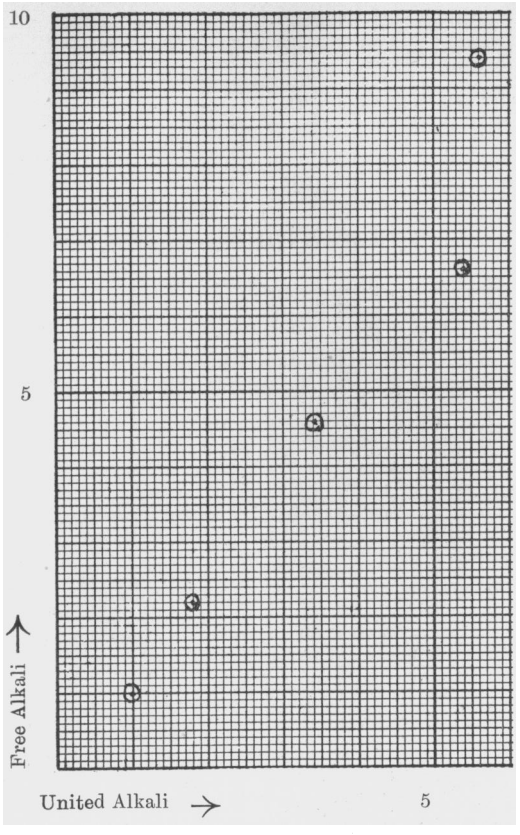

FIG. 2. *Expt. XVI.*—EQUILIBRIUM OF SERUM PROTEIN WITH ALKALI (NaOH)

Ordinates show concentration of free alkali in 1/1000 N, abscissae united alkali in c.c. of N/10 alkali uniting with 0·687 gramme of protein in 6·87 per cent. solution.

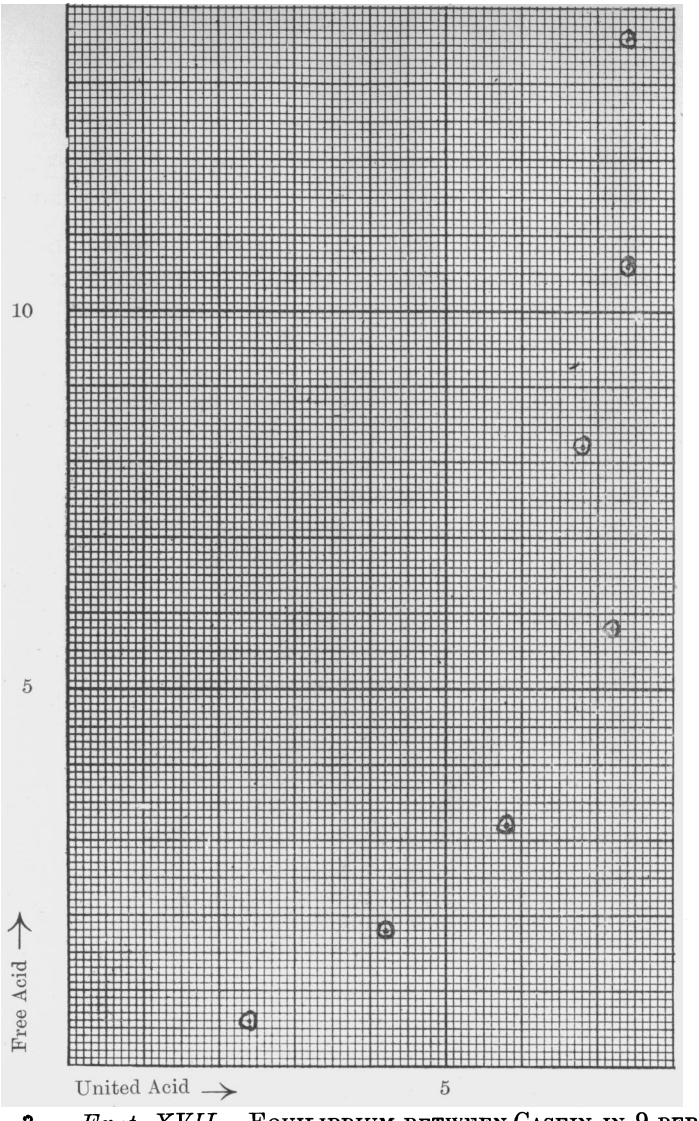

FIG. 3. *Expt. XVII.*—EQUILIBRIUM BETWEEN CASEIN IN 9 PER CENT. EMULSION AND SULPHURIC ACID

Ordinates show concentration of free acid in N/1000; abscissae amount in c.c. of N/10 acid taken up by 0·9 gramme of casein in 9 per cent. suspension.

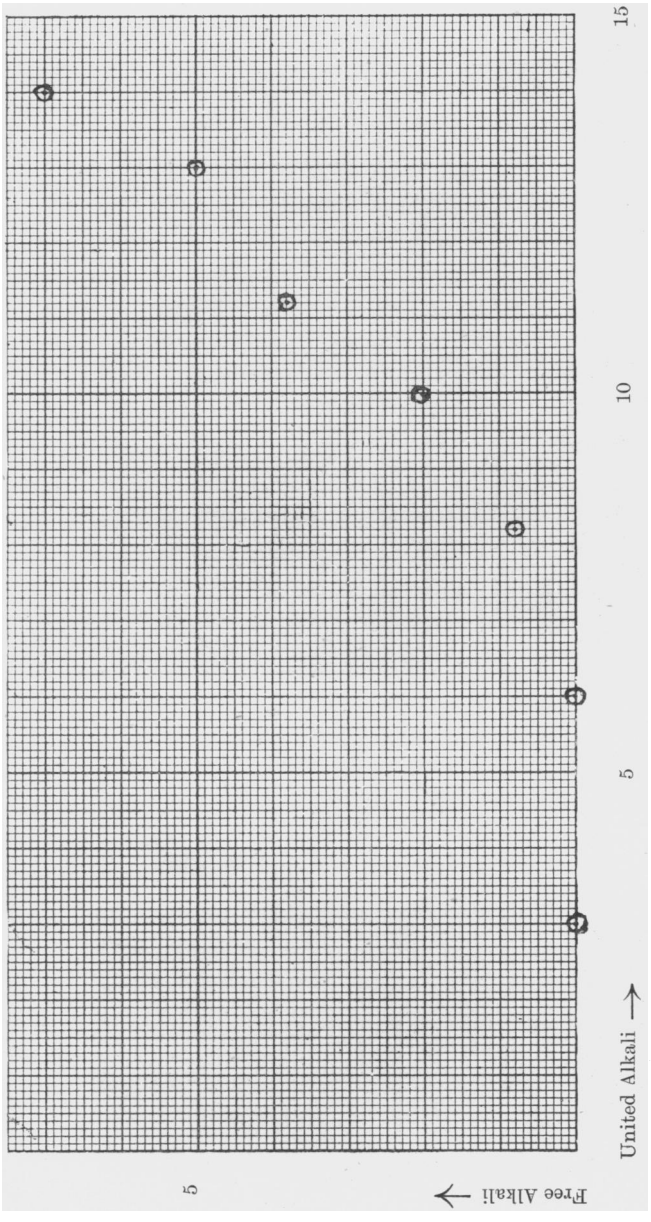

FIG. 4. *Expt. XVIII.*—EQUILIBRIUM BETWEEN CASEIN IN 9 PER CENT. CONCENTRATION AND ALKALI (NaOH).

Ordinates show free alkali in N/1000 concentrations, abscissae united alkali in c.c. of N/10 united with 0·9 gramme in 9 per cent. concentration.