ON SOME ASPECTS OF ADSORPTION PHENOMENA, WITH ESPECIAL REFERENCE TO THE ACTION OF ELECTROLYTES AND TO THE ASH-CONSTITUENTS OF PROTEINS

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I. THE GENERAL LAW OF ADSORPTION AND THE NATURE OF THE UNION BETWEEN PROTEIN AND ASH

The great difficulty of obtaining preparations of protein bodies free from ash-constituents is well-known to all observers who have had occasion to make the attempt to do so. It is, indeed, a matter of doubt whether a true protein absolutely free from inorganic electrolytes has hitherto been in the hands of investigators.¹

It is easy by dialysis, or other means, to remove a portion of these constituents, but beyond a certain limit it appears to be a matter of extremely prolonged labour to get rid of the remaining small amount.

1. See *Die Grösse des Eiweissmolekuls*, by Fr. N. Schulz, 1903, p. 9.

One is therefore justified in some degree in distinguishing with Schulz¹ between the 'essential' and 'accidental' ash-constituents of proteins. The discussion as to the state of combination of these constituents in the protein molecule concerns in fact only the former, that part namely which it appears difficult or impossible to remove without destroying the character of the protein itself.

Now of recent years a considerable amount of research has been undertaken with regard to the nature of the phenomena known by the name of 'absorption' or 'adsorption'.² Too much space would be taken up in giving a complete list of this work, and I must content myself with mentioning the names of Van Bemmelen, Biltz, Schmidt, Walker and Appleyard, and Craw.

The main result of these investigations, so far as concerns us for the present purpose, is the discovery of what we may call the 'Law of Adsorption.' Suppose we have a series of solutions of such a dye as Congo Red, for example, in progressively diminishing concentration, and in each of these we place the same amount of filter-paper, we find that a part of the dye is taken up by the paper *and in relatively larger proportion the more dilute the solution.* To take some examples from the paper by G. C. Schmidt.³

Picric acid and cellulose :

Concentration of picric acid		Amount adsorbed
0.889	...	0.111
0.340	...	0.095

Eosin and silk :

Concentration of eosin		Amount adsorbed
0.666	...	0.84
0.376	...	0.75

Iodine and carbon :

Concentration of iodine		Amount adsorbed
0.161	...	3.275
0.087	...	2.958

1. *Loc. cit.*, p. 11.

2. Notwithstanding the suggestion of Zsigmondy (*Zur Erkenntniss der Kolloide*, Jena, 1905, p. 61, footnote) to make use of the name 'absorption' in honor of Van Bemmelen, the chief worker on the question who uses this form, I think that, in English at all events, 'adsorption' is less likely to lead to confusion, and 'absorption' can be still used for phenomena like the solution of gases in water.

3. *Zeitsch. f. Physik. Chemie.*, XV, p. 60. 1894.

These numbers show that there is some kind of 'affinity' between the bodies adsorbed and those which take them up. Put in other words the law states that there is not a proportionality between the concentration of the solution or partial pressure of the solute and the amount adsorbed. Ostwald¹ indeed points out that there is no hard and fast distinction to be drawn between chemical affinity and adsorption which latter he sometimes speaks of as 'mechanical affinity.' He calls attention to the complete series of transitions between the two phenomena, and, in referring to Van Bemmelen's researches, he remarks that they frequently leave it a matter of uncertainty as to whether the facts brought forward should be regarded as of a chemical or physical nature. In any case all the evidence shows that electrolytes when adsorbed are non-ionized and no longer take part in the electrical conductivity of the solution. This circumstance renders the determination of conductivities a rapid and convenient method of investigating certain aspects of the problem as will be seen later.

In the course of a series of experiments on the changes of conductivity in protein solutions brought about by the action of enzymes I have had occasion to take into consideration the possibility of the splitting-off of inorganic electrolytes under these conditions. The results of some preliminary experiments suggested to me that the most satisfactory explanation of the nature of the 'combination' between protein and ash was that the constituents of the latter are present in adsorbed form rather than in true chemical union.

Before passing on to the description of the experiments made to throw light on this problem, I may, in order to make clear the point of view, give the results of a preliminary experiment on an undoubted case of adsorption, viz., congo-red and filter-paper.

Experiment: Seven flasks were taken, each containing 50 c.c. of a solution of congo-red in 10 per cent. alcohol. These solutions were of a regularly diminishing concentration from 0.014 to 0.002 per cent. Congo-red forming a colloidal solution is very readily precipitated from watery solution by traces of electrolytes, so that an alcoholic

1. *Lehrbuch d. Allgem. Chemie*, 2te. Aufl. Bd. I, p. 1084, et seqq.

solution is more easily worked with. For the same reasons, the filter-paper used was Schleicher and Schüll's analytical paper, and of this a disc 12.5 cm. diameter was added to each of the above flasks. After 24 hours standing the depth of colour in each solution was determined by Gallenkamp-Heele's colorimeter, the results being as follows :—

Concentration of solution	Proportion of dye in solution	Proportion of dye in paper
0.014	40 %	60 %
0.012	20 %	80 %
0.010	9.3 %	90.7 %
0.008	4 %	96 %
0.006	1.3 %	98.7 %
0.004	trace	practically all
0.002	"	"

The figures show at once that the amount of dye taken out of solution by the paper is not directly proportional to the concentration of the solution ; nor, on the other hand, is it independent of this concentration as would be the case if a true chemical compound were formed. In fact, from a 0.014 per cent. solution $\frac{40}{100} \times \frac{0.014}{2} = 0.0028$ gram is taken up by a certain amount of paper, whereas from a 0.008 per cent. solution only $\frac{4}{100} \times \frac{0.014}{2} = 0.00028$ gram, or one-tenth of the former, is taken up by the same amount of paper, although there was still a quantity of free congo-red in the solution. The peculiarity of these phenomena, which I have spoken of above as 'the law of adsorption,' is well shown by the series of numbers in the third column.

Now, if we put these data into the form of a curve as is done in Fig. 1, where the ordinates represent the percentage of dye left in solution and the abscissae the original concentration of the solution, we see that the curve forms part of a hyperbola, and only approaches the axis (*i.e.*, zero concentration) asymptotically.¹ In other words, however diluted the original solution may be, there will practically be always a certain amount of the dye left unadsorbed by the paper. It follows

1. This hyperbolic form of the curve is pointed out by Ostwald, *Allgemein. Chem.*, Bd. I, p. 1,096.

Walker and Appleyard (*Jour. Chem. Soc.*, 69, 1896), p. 1334, also find a logarithmic formula for the adsorption of picric acid by silk.

FIG. 1

also that if we attempt to wash out the dye by means of 10 per cent. alcohol it will require, theoretically, an infinite number of changes of the solvent to effect complete removal of the dye. I found, in fact, that if the piece of paper which was stained deep-red in the 0.002 per cent. solution was allowed to soak in 10 per cent. alcohol, a trace of dye only was removed each time a fresh supply of alcohol was added; the alcohol became very faintly pink, the colour only to be detected in a deep layer, while the paper did not perceptibly lose in depth of colour after several extractions.

II. WASHING-OUT OF ELECTROLYTES

It will be seen, on a little consideration, that the great difficulty of removing the last small part of the ash-constituents of proteins may be readily explained on the hypothesis that the case is one of adsorption. The hyperbolic form of the characteristic curve shows

that, as the asymptote is approached, a less and less percentage of these constituents will be taken away by repeated changes of the extracting solvent.

In order to test this hypothesis, I have taken commercial gelatin and extracted it with repeated changes of distilled water at room temperature. The comparative amounts of electrolyte removed by each change were estimated by taking the electrical conductivity. Gelatin at room temperature not being dissolved by water this latter can be poured off and replaced by fresh with ease. Of course, the water imbibed by the gelatin in swelling cannot be changed; this does not affect the result to any serious degree, merely making the process of extraction a more prolonged one. The experiment was performed as follows:—10 grams of Coignet's gold-label gelatin were allowed to soak in distilled water until swollen and sufficient water added to enable 100 c.c. to be poured off. About 180 c.c. were needed in all. A little toluol was added, and the mixture left to stand for twenty-four hours. The water was then poured off and its conductivity determined. A fresh 100 c.c. of water were then added, and the process repeated every twenty-four hours. After the first three or four extractions the conductivity of the extract was so low that I found, with the apparatus used, that more accurate readings were obtained by concentrating the fluid in a platinum capsule over the water-bath to a definite volume (12 c.c.) before measuring its conductivity. Even when their concentration is thus increased, the electrolytes may be looked upon as practically completely dissociated, so that no appreciable error can be put down to this circumstance. It might be objected that impurities in the distilled water would also be concentrated, but I found on testing this possibility that the conductivity of the distilled water used, which had originally a conductivity of 5 gemmhos (= reciprocal megohms) did not increase by concentrating 90 c.c. down to 12 c.c. Presumably, this is to be accounted for by the fact that the slight conductivity was almost entirely caused by carbon dioxide, which would be driven off on heating. The numbers obtained were as follows:—

No. of extract	Conductivity in gemmhos		No. of extract	Conductivity in gemmhos
1	1094	...	7	23.25
2	475.5	...	8	18.25
3	232.3	...	9	15.94
4	120.4	...	10	15.45
5	62.8	...	11	15.40
6	29.41			

We see at once that after a certain time each further change of water contracts only an infinitesimal amount of electrolytes. Since the conductivity of the distilled water was 5 gemmhos, this must be subtracted from the values given in the table, leaving only 10 gemmhos for the electrolytes washed out from the gelatin. These electrolytes consist in all probability chiefly of calcium sulphate, and a calcium sulphate solution of 0.25 per cent. has a conductivity of 2039 gemmhos, so that, neglecting differences of dissociation, a solution with a conductivity of 10 gemmhos will contain only $0.25 \times \frac{10}{2039} = 0.0013$ per cent. It may be said that at this stage the ash was nearly all washed out. In order to test this possibility, I determined the ash of the gelatin. The incineration was performed in a platinum capsule, care being taken not to let the temperature get above dull redness, and not to prolong the heating after all charred portions had lost their blackness. The original gelatin contained 0.55 per cent. of ash, and after extracting with water eleven times it still contained 0.036 per cent. Therefore, when the rate of extraction had already become almost negligible, there was still left in the gelatin a considerable amount of ash.

On the hypothesis that the electrolytes are merely mixed with the gelatin and washed out by diffusion, the rate of diminution of conductivity of the successive extracts would have been much greater. This can be seen by calculating on the basis of the amount washed out by the first and second extractions, what would have been, *e.g.*, the conductivity at the seventh extraction. The proportion removed at each change would, obviously, have been the same. If the above calculation be made it will be found that the conductivity of extract No. 7 would have been 7.4 gemmhos instead of 23.25 gemmhos as found,

Fig. 2 gives the curve of this experiment showing the same hyperbolic form as the adsorption curve of congo-red, Fig. 1.

FIG. 2

Since the last values are the most interesting, and if the whole had been plotted out, the final course of the curve would have been on a much smaller scale than in the figure, the first two values are omitted, the curve commencing at the third. The ordinates represent specific conductivities of the extract in gemmhos, the abscissae are at equal intervals. The value No. 6 is obviously an experimental error. In drawing the curve the conductivity of the distilled water used is taken as the axis of abscissae. The position of the other asymptote is not shown since only part of the hyperbola has been drawn.

Before leaving this experiment I may mention that an attempt was made to re-dissolve the ash and determine its conductivity. A quantity of the gelatin after the last extraction was dried in a toluol oven and weighed 0.75 grams. This was incinerated and the ash redissolved in the quantity of water corresponding to that in which the

gelatin had soaked, viz., 13.5 c.c. It did not, however, entirely go into solution; yet notwithstanding this, the conductivity was 29 gemmhos as compared with the 10 gemmhos of the water in which the gelatin had been soaked. The signification of this fact is that it confirms the deduction drawn from the previous experiment that electrolytes are held by the gelatin in such a manner that they can only be separated by an enormous number of changes of water.

If gelatin which has been washed free from the greater part of its electrolytes be placed in a dilute potassium chloride solution, it takes up a certain quantity of this electrolyte, which can be washed out by repeated changes of water, and the curve of the conductivities of the series of extracts is of exactly the same form as that of Fig. 2.

The fact mentioned in the last paragraph makes it of interest to see whether direct evidence can be obtained of the adsorption of electrolytes by gelatin. This can be done in the following way:—

Experiment: 30 c.c. of $\frac{N}{10}$ KCl were found to possess a conductivity of 16,600 gemmhos at 39.7° C. At room temperature 2.3 grams of 'washed' gelatin were placed therein and allowed to remain for 24 hours. The conductivity of the solution was now found to be diminished to 14,590 gemmhos at 39.7°. This means that at least 12 per cent. of the potassium chloride had been taken out of solution.

The fact can also be shown in other ways though in not so obvious a manner. If we take distilled water and add successively equal amounts of KCl, each increment produces a slightly less increase of conductivity than the previous one. The reason is that as the solution becomes more concentrated the KCl becomes less dissociated; to a less degree also increased ionic friction will contribute to the result. If, on the other hand, we add to the water first some 5 per cent. or so of gelatin and afterwards the successive doses of KCl the result is different. In a particular experiment 10 grams of gelatin were placed in 200 c.c. of distilled water, the gelatin having been previously washed fairly free from electrolytes. The conductivity of the water was raised to 26.9 gemmhos. When 2 c.c. $\frac{N}{10}$ KCl were added and allowed to stand for 24 hours the conductivity was increased by 200 gemmhos.

Another 2 c.c. were added, and after 24 hours the conductivity was found increased by 208 gemmhos. That is, as I interpret it, a part of the first dose of KCl was taken up by the gelatin. It is impossible to compare directly these figures with those obtained on a similar experiment with distilled water without gelatin, since the proportion of water taken up in the swelling of the gelatin is unknown.

Again, if gelatin has the power of taking up electrolytes, it is to be expected that commercial gelatin and 'washed' gelatin would show a difference in their power of taking up more, since commercial gelatin has already a larger percentage. The following experiment was made to find out if this is so :—

Experiment : 12 grams (air-dry) of commercial and 'washed' gelatin were soaked for 20 hours in distilled water. The conductivity of the water was then found to be :—

Commercial gelatin : 553 gemmhos.

Washed ,, : 77 gemmhos.

Two solutions (very dilute) of calcium sulphate and phosphate were made equal in conductivity to each of these extracts by adding, drop by drop, a saturated solution of these two substances in distilled water. Of these solutions a volume was taken equal to that of the water in two flasks in contact with the two kinds of gelatin, this was found to be (by weighing) 176 grams. Then to each of the four flasks 15 c.c. of the above-mentioned saturated solution of calcium sulphate and phosphate were added, and the increase of conductivity so produced was determined. If a part of the added electrolytes were taken up by the gelatin there should be a greater rise of conductivity in the cases of the watery solutions than in those where gelatin was present. The results actually obtained are rather difficult to interpret. To take first the 'washed' gelatin and the watery solution equal to it in conductivity. The rise of conductivity in the latter by the addition of 15 c.c. of the calcium sulphate solution was 163 gemmhos at 10°. Where the gelatin was present the first effect was a rise of 238 gemmhos, owing, no doubt, to the electrolytes not at once diffusing into the water contained in the swollen gelatin, this steadily diminished, and in about forty-five minutes had become 128 gemmhos. But then

the conductivity commenced to go up again, finally becoming 160 gemmhos. If, therefore, we take this last as the true value, the amount of electrolyte taken up by gelatin was very small, a difference of 3 gemmhos only between the two cases. If, on the other hand, we take the lowest value to which the conductivity fell, the difference is considerable, viz., 75 gemmhos.

In the case of the commercial gelatin and its equivalent calcium sulphate solution, the rise produced by the addition of 15 c.c. of saturated calcium sulphate and phosphate was 68 gemmhos in the watery solution and 56 gemmhos in that where the gelatin was present, a difference of 12 gemmhos. The addition of the electrolyte at first caused a rise of 106 gemmhos where the gelatin was present, it fell to 72 in one hour, but the final value was not reached for several hours, as opposed to the first case, where it was reached in two-and-a-half hours.

This experiment affords a certain amount of evidence that electrolytes are taken up by gelatin, but does not distinguish between adsorption and chemical combination. As we shall see later, the effect of heat shows it to be, in all probability, a case of the former.

III. THE KINETICS OF ADSORPTION

1. *Velocity of Reaction.*

Very little information is to be obtained as to the rate at which bodies are taken up by adsorbing substances. A few experiments that I have made on this question may therefore be of interest.

Experiment: 50 c.c. of 20 per cent. alcohol in test-tube lined with filter-paper. In thermostat at 39°. When warmed, 1 c.c. warmed 0.5 per cent. congo-red added, stirred, conductivity taken at intervals by pipette electrodes. The curve (Fig. 3) shows the time course of the diminution of conductivity. It will be noted that the curve is hyperbolic and that it arrives nearly at the asymptote in 10 minutes. At this temperature the velocity is considerable. The first part of the curve in all probability escaped observation, owing to the great initial velocity of the reaction as shown by the shape of the curve.

FIG. 3

In another experiment, in which the proportion of paper to dye was greater than in the previous one, equilibrium was reached in six minutes. So that the rate of change is proportional in the first place to the concentration of the adsorbing body. I have not enough data, however, to warrant any further discussion of this point. The reaction is one taking place in a heterogeneous system and its mathematical expression a complex one. It is probable that diffusion plays a considerable part as indicated by the results of the next experiment, which was made to obtain some idea of the temperature coefficient of the reaction-velocity. It was found that, for some reason or other—perhaps some action of the platinized electrodes on the dye—at low temperatures the electrical conductivity method did not give satisfactory results, so that the colorimetric method, though more laborious and less sensitive, was made use of.

Experiment: 50 c.c. of 0.005 per cent. congo-red in 5 per cent. alcohol in each of two flasks, a circle of filter-paper added to each, and

one kept at 50°C , the other at 10°C . After a certain time the solution was poured off and a new piece of paper and fresh dye solution, previously warmed, or cooled respectively, added and a different time of action allowed. The flasks were thoroughly shaken at frequent intervals to allow access of the solution to the whole surface of the paper. This shaking caused the separation of bits of paper which rendered it necessary to allow the solution to deposit before making colorimeter observations; neglect of the precaution caused the loss of several of the earlier observations. Filtration is naturally inadmissible. The results were as follows, the colorimeter readings expressing the relative percentage of dye left in solution, the original solution = 100.

Duration of action	Colorimeter readings	
	50°	10°
5 minutes	95	circ. 98
8 "	90	...
20 "	85	...
30 "	...	90
40 "	80	...
100 "	77	70
200 "	...	60
5 hours	75	...
7 "	...	55
14 "	75	48
24 "	...	45

FIG. 4

The curves (Fig. 4) reproduce these two series up to seven hours. What we notice is that at the higher temperature the velocity is greater until the equilibrium position at this temperature is approached. The question of equilibrium will be discussed in the next section. In estimating the value of the temperature coefficient of the velocity, some uncertainty exists, since it will differ according to what stage of the reaction we take. If we take as criterion the time taken to reduce the concentration of the solution to a given percentage, say 90 per cent. at 10° , this time is about 2.5 times that at 50° , if we take 80 per cent. it is about 1.3 times. In either case, however, it is extraordinarily low; taking the higher value at 10° it takes twenty-five minutes to become 90 per cent., and at 50° it takes ten minutes, a difference of fifteen minutes for 40° ; so that, assuming uniformity of increase for the total interval, the time taken at 40° would be only $\frac{15}{4} = 3.6$ minutes more than at 50° , or ten minutes and 13.6 minutes respectively. This gives a temperature coefficient of the extremely low value of $\frac{13.6}{10} = 1.36$. In the table given by Van t' Hoff¹ there are only two values below this, viz., the dissociation of PH_3 and AsH_3 probably depending on the high temperatures at which the observations were taken, since, as Van t' Hoff points out, the velocity-ratios for 10° usually diminish as the temperature rises. The value found in the present case indicates that the theory of Nernst² as to the part played by diffusion-processes in heterogeneous reactions applies here; the corresponding value found by Brunner³ for the dissolution of benzoic acid in water is, in fact, 1.5.

A few observations on the effect of alcohol and certain electrolytes on the velocity of reaction may be shortly referred to. Congo-red is the sodium salt of a substituted aromatic sulphonic acid, and is less soluble in alcohol than in water, so that it seems probable that more dye would be taken up from a solution containing alcohol than from a watery solution of the same concentration. This was found

1. Vorles. über Theor. und Physik. Chemie., 2te Aufl. p. 225.
2. Zeitsch. f. Physik. Chemie., XLVII, 1904, p. 52.
3. Zeitsch. f. Physik. Chemie., XLVII, 1904, p. 62.

to be the case. Two solutions containing 0.005 per cent. of congo-red were taken, one in 50 per cent. alcohol, the other in water. From the former a circle of filter paper adsorbed at 14° in seventy minutes 45 per cent. of its contents, and from the watery solution only 30 per cent.

The action of a neutral electrolyte, sodium chloride, was similar, but more pronounced. From a solution containing 0.0042 per cent. dye and 2 per cent. NaCl, 75 per cent. was taken up in fifty minutes, and from a solution of the same concentration in dye, but without NaCl, only some 25 per cent. was taken up at the same time.

The action of strong acids is, of course, to set free the sulphonic acid from its compound, the free acid while more soluble in alcohol is less so in water, so that again we have a favouring effect on adsorption as follows:—Two flasks of the usual 0.005 per cent. solution of dye with each one circle of paper to 50 c.c. solution were prepared, one remained neutral, the other was acidified by the addition of one drop of 5 per cent. HCl. Though the solution became blue no precipitation of the colour-acid occurred in this weak concentration. After two hours at 13° to 14°, 35 per cent. was taken up from the acid solution and 12 per cent. from the neutral one. Before taking the colorimeter reading the acid solution was neutralized by a drop of ammonia.

It appears, then, that bodies which tend to diminish the solubility of congo-red favour its adsorption by the substances immersed in its solution.

The experiment with gelatin and calcium sulphate, previously related, shows that the rate of adsorption in that case is considerably greater than that of congo-red by paper, as would be expected from the greater mobility of the ions concerned. At 11° equilibrium was attained in less than two-and-a-half hours, whereas at the same temperature at least twenty-four hours was required for congo-red and paper.

2. *Equilibrium*

The most interesting fact under this head is the influence of temperature. As the curve (Fig. 4) shows, at the higher temperature,

less of the dye is contained in the paper and more in the solution. The curve (Fig. 5) shows the results of a few observations at various temperatures, ordinates being percentages in paper and abscissae temperatures. It will be noted that a straight line is formed. The values at the lower temperature are not quite so accurate as at the higher,

FIG. 5

since the former were obtained by allowing the flasks to stand in the laboratory, the temperature of which was not constant within 2° or 3° during the time necessary for equilibrium to establish itself. The upper values are accurate, being taken in a thermostat.

Attention has already been directed to the length of time necessary at low temperatures for the establishment of equilibrium in these dilute solutions. Ostwald also notes this fact.¹

The dissociation of adsorption compounds by raising the temperature can also be shown in the case of gelatin and inorganic electrolytes, though complicated in this instance, by the passage from the state of hydrogel to that of hydrosol. There is, however, as I find, no sudden change in passing from the one state to the other as regards conductivity. If one warms a gelatin hydrogel from 15° to 40° determining

1, *Loc. cit.*, p. 1093.

conductivity at frequent intervals, the curve, which is in fact a straight line, shows no kink of any kind at the point where the hydrogel liquefies to a hydrosol.

The experiment previously described on the relations of gelatin to calcium sulphate was continued as follows :—The two flasks containing gelatin and calcium sulphate were warmed to 53° , the rise of conductivity in the case of the washed gelatin was 397.2 gemmhos, in the commercial gelatin 1317 gemmhos. Now the point of interest is, whether this rise is greater than would be the case if no separation of additional electrolyte had taken place under the influence of heat. To decide this, solution of calcium sulphate was prepared, having at 10° the same conductivity as the solutions in contact with the two kinds of gelatin. These were then warmed to the same temperature as the gelatin. The rise of conductivity in the more dilute amounted to 376 gemmhos, and in that of the stronger to 1446 gemmhos. There is therefore an increase in electrolytes in the case of the washed gelatin corresponding to a difference in conductivity of 21 gemmhos. It is to be remembered, moreover, that gelatin in the state of hydrosol has a slight effect similar to that of other non-electrolytes in diminishing the conductivity of solutions in which it is present. This is, no doubt, due to an increase of internal friction in some way or other, and amounts, according to determination I have made, to 3.4 per cent. diminution of conductivity for each 1 per cent. of the gelatin present. So that each of the values for the gelatin solutions should be increased by, approximately, 3.4×4.9 per cent., the gelatin solution being 4.9 per cent. in concentration. This increases the two values to 463 and 1536 gemmhos. The differences in favour of the gelatin come out now to be 87 gemmhos for the washed, and 90 gemmhos for the commercial sample. The closeness of these two numbers serves to strengthen the view that the electrolytes causing the increase were in some way separated off from the gelatin under the influence of heat.

It has been shown above that the conductivity of a solution of the ash from a given sample of gelatin is greater than that of the solution with which the gelatine is in equilibrium. I thought it interesting, therefore, to compare the ash of the particular 'washed'

gelatine of the above experiment, as regards its conductivity, when re-dissolved to original volume, with the conductivity of the gelatin solution at 10° and at 53° . The ash from 100 c.c. of the gelatin solution weighed 0.0262 gram, it was re-dissolved in 96.6 c.c. of distilled water (to allow for the volume of the gelatin), the whole of it not going into solution. Now the conductivity of the gelatin solution was found at 6.2°C. to be 141 gemmhos, that of the re-dissolved ash was 270 gemmhos. It is obvious that this is more than sufficient to account for the additional electrolytes split off on heating. It also confirms the view already expressed that inorganic electrolytes are held in some kind of combination by gelatin. Since they are also separated to a certain degree by warming, the combination in question is in all probability of the nature of adsorption.

The various substances which increase the velocity of adsorption also affect the final equilibrium position but in the opposite direction to that in which it is affected by heat. The action of neutral salts is the most interesting and of the greatest practical and theoretical importance. Sodium chloride has a very striking effect, even in very low concentration, as the following experiment shows :—

Solution of congo-red 0.004 per cent. containing various percentages of sodium chloride. After a circle of paper had lain in each for 2 hours the following colorimeter readings were obtained :

Percentage of NaCl	Percentage of dye left in solution	Percentage of NaCl	Percentage of dye left in solution
0	85	0.02	33
0.0005	77	0.1	25
0.001	71	0.2	16
0.005	50		

The effect of sodium chloride, then, appears to be proportional to its concentration but not in direct linear proportion. The fact that in so weak a concentration as 0.0005 per cent. an obvious effect is produced indicates that the action of inorganic electrolytes is rather to be brought into relation with the well-known effect of these bodies on typical colloids, rather than with the precipitating action of alcohol, which requires a much higher relative concentration in order to produce a comparable effect. In investigating this action of electrolytes

the phenomena met with, although of considerable interest, showed themselves to be of a very complex nature, so that it will be better to devote a special section to the discussion of them later on.

At first sight the action of heat in dissociating the adsorption compound, as above described, appears at variance with the common practice of histologists in staining their preparations. I find that the beneficial effect of heat in this case is probably due to the fact that a piece of paper, for example, dyed with congo-red and exposed to a temperature of 100°C . loses the power to a large extent of giving up the dye to water, in other words it is fixed more firmly than if merely stained at room temperature.

According to the well-known laws of Van't Hoff, since the compound of cellulose and congo-red is dissociated more and more as the temperature rises, the formation of the compound should be accompanied by evolution of heat. I have attempted in various ways to detect such a production of heat but have been unable to do so. The two methods used were : (1) Immersion of a Beckmann thermometer in the centre of a series of co-axial cylinders of filter paper in a large beaker filled with NaCl solution to facilitate adsorption. A test-tube of 1 per cent. solution of congo-red was also immersed in the solution, and when the whole was at the same temperature, the dye was mixed with the saline solution. No temperature change was to be observed, although by aid of a lens, the thermometer could be read to $\frac{1}{1000}$ th of a degree ; (2) A disc of paper moist with NaCl solution was allowed to rest on the face of an ordinary bismuth-antimony thermopile contained in a vacuum-jacketed vessel and connected to a low-resistance d'Arsonval galvanometer. The vessel also contained a small tube of concentrated congo-red solution, closed at the bottom by a glass rod with indiarubber tube around its lower end. When the galvanometer was steady the glass rod was raised and the dye allowed to flow over the paper. No deflection indicating the production of heat was observed. It is to be noted, however, that at the temperature of these experiments, the process would not be very rapid, and it is possible that a slight heat production would be conducted away as fast as it was formed.

3. *Reversibility*

The question as to how far the process is a reversible one is of some theoretical interest. It has been already mentioned that if the temperature is raised up to 100°C ., a part of the dye is fixed in the paper, so that under certain conditions, the reversibility is not complete. At ordinary temperature it appears complete. If a circle of paper be dyed with congo-red, rinsed with water and then placed in water along with another similar circle of paper, it will be found that after some time the two pieces of paper will be of the same depth of colour. At the same time, as already pointed out at the beginning of this paper, to completely extract all the dye from a piece of paper needs an enormous number of repeated changes of water.

As regards temperature reversibility is apparently complete.

Experiment: Two flasks containing congo-red and filter-paper were placed, one at 50° , the other at 11° ; next day both flasks were placed in a thermostat at 25° , when equilibrium was attained after about 30 hours, the colorimeter readings were:

From 50°	-	53 %
„ 11°	-	57 %

Again, if sufficient time be allowed for equilibrium to be reached, a preparation may be alternately warmed and cooled, and nearly the same readings obtained at the same temperature. It appears, however, that after several days contact the dye becomes partly fixed. For example, in one case the reading at 38° was 66 per cent., it was then cooled to 13° with a reading of 48 per cent.; on again warming to 38° , 50 per cent. only was reached, and on cooling again to 13° , after two days the value became 25 per cent.

Gelatin, also, shows fairly complete heat reversibility as regards its adsorbed electrolytes. At 11°C . a particular hydrogel had a specific conductivity of 162 gemmhos, after being heated to 53° and again cooled to 11°C . its conductivity was 157.8 gemmhos. Another preparation containing more electrolytes had an initial conductivity of 609 gemmhos, after heating to 55.5° and cooling again to 11° , it became 542 gemmhos, or less than its original conductivity. Perhaps in the first measurement equilibrium was not completely attained.

When adsorption has taken place under the action of electrolytes a very considerable degree of fixation occurs, so that the dye is not given up again to water, or only to a very slight degree.

Experiment: Two similar pieces of paper dyed to same depth of colour, one in solution of NaCl 0.02 per cent., the other in distilled water. Of course the latter solution was more concentrated as regards the dye. These pieces were rinsed in water, pressed between blotting-paper, and then placed in equal volumes of distilled water along with another piece of paper each. After 24 hours the one which had been dyed in distilled water had given up a considerable amount of pigment to the water, and the two pieces of paper were very nearly equal in depth of tint. The paper dyed in the presence of NaCl gave up no colour to be detected by the eye, although the second piece of paper was faintly pink, showing a slight extraction from the dyed piece.

IV. THE ACTION OF ELECTROLYTES

When we consider that a solution containing only 0.0005 per cent. of NaCl has a distinct effect in augmenting adsorption of congo-red by paper, viz., 23 per cent. taken up as against 16 per cent. from distilled water, it is plain that the phenomenon is not of the nature of a 'salting-out,' comparable to the precipitation say of egg-albumen by ammonium sulphate. The precipitation of such solutions as those of the colloidal metals and hydroxides is rather suggested. Now, there is considerable evidence that a large number of the anilin dyes exist in watery solutions in a colloidal form. Congo-red, having a molecular weight of nearly 700, would be expected to be one of these: Its solutions, in fact, do not diffuse through Schleicher and Schüll's parchment-paper thimbles, although they do so, very slowly, through ordinary parchment-paper. According to Michaelis¹ under the ultra-microscope they are heterogeneous, being resolvable into sub-microscopic particles. In the electric field the dye migrates to the anode, so that the particles are negatively charged. It is, however, somewhat difficult to make oneself certain of this fact on account of

1. *Deutsche Med. Wochenschrift*, 1904, No. 42.

the electrolytic decomposition. Colloids do behave as electrolytes, as is well-known¹, and I have made one rough determination, by Whetham's boundary method, of the velocity of the coloured ion in congo-red solution. At 13° the boundary, which was not very sharp, moved 11 mm. in one hour under a potential fall of 3 volts per cm. Hardy² finds for globulin about 7 mm. in the same time, and for methylene-blue considerably more, about 40 mm. The electrical conductivity of congo-red solutions is comparable with that of inorganic electrolytes, a $\frac{M}{100}$ solution at 40° having a specific conductivity of 5600 gemmhos. I do not lay any stress on the absolute value of this measurement since the preparation was not specially purified, and possibly contained a small amount of inorganic salt.

On the whole, then, we may regard congo-red as being a negatively charged colloid. As such it would be specially sensitive to di- and tri-valent kations, and this is in fact the case.

All the experiments to be described in this section, except when otherwise stated, were made in the same way, viz., to 50 c.c. of the solution of electrolyte 10 c.c. of $\frac{M}{1000}$ dye solution and one piece 12.5 cm. diam. of Schleicher and Schüll's extracted filter paper added. After about 24 hours the amount of dye taken up by the paper was estimated by taking the colorimetric value of the dye left in solution.

It is easily seen at once that calcium salts are much more active in promoting adsorption than those of the monovalent alkali metals, e.g., the amount taken up from a solution containing $\frac{M}{500}$ CaSO_4 was 85 per cent., from a $\frac{M}{200}$ KCl 67 per cent.

Considering this fact the effect of tap-water is not surprising, since New River water contains an equivalent in Ca^{++} ions of about equal amount to the content therein of $\frac{M}{300}$ CaSO_4 . From tap-water 85 per cent. was adsorbed in one experiment, and from distilled water only 27 per cent.³ Moreover, these facts show that in order to obtain any reliable data, special care must be taken as to the purity of the paper used. As an illustration, I give the following experiment :

1. Hardy, *Journal of Physiology*, XXXIII, 1905, p. 292.
2. *Loc. cit.*, p. 291 and p. 289.
3. The absolute amounts in different experiments can only be compared by taking their *ratio* to the amount taken up from distilled water in each case, since the temperature was not the same in all.

Equal weights of the following samples of filter-paper were placed in 50 c.c. distilled water with 10 c.c. $\frac{M}{1000}$ dye as usual. The amounts taken up by each are given :

1.	Dreverhof's ordinary, No. 333	...	...	95 %
2.	"	"	washed in distilled water	59 %
3.	Dreverhof's extracted paper	...	...	46 %
4.	"	"	washed	46 %
5.	Schleicher and Schull's extracted paper	...	...	46 %

I may mention here that Dreverhof's extracted paper turns blue when a drop of dilute congo-red is placed on it, this does not happen with Schleicher and Schüll's, nor with Swedish paper. As the above experiment shows this slight acidity does not seem to affect its adsorbing power, and as will be seen later H^+ ions have comparatively little favouring effect.

The result of this experiment seems to suggest the possibility, that if one could obtain a complete absence of electrolytes no adsorption would take place.

As regards trivalent kations I have only tested aluminium sulphate, but as even in a concentration of $\frac{M}{1000}$ precipitation took place the result was valueless.

It is scarcely necessary, perhaps, to remark that if the dye is precipitated by the electrolyte no adsorption by the paper takes place, the large particles merely float about in the liquid. What is needed is the local concentration of electrolyte on the surfaces of the adsorbing solids.¹

Although the greater favouring power of divalent as compared with monovalent kations is easy to demonstrate the order in each of these classes is difficult to decide since the action of each member is so nearly the same as that of the others. From a number of experiments the following seems to be the order :

1. Notwithstanding the fact that actual precipitation must not take place in these experiments the addition of electrolytes to congo-red, for example, causes an increase in the size of the colloidal particles even when no actual precipitation occurs ; so that the solution is on its way to precipitation even when this does not actually occur. The specimen of congo-red used in all my experiments showed the Tyndall phenomena very faintly in solution in distilled water ; but when NaCl was added the beam of light became much more distinct and the light reflected from it at right angles was polarized. When $CaCl_2$ was added in sufficient amount to slowly precipitate the dye (about $\frac{M}{100}$) the polarization ceased as the particles became larger than the mean wave-length of light.

This is very nearly the same as that found by Pauli¹ in the case of precipitation of proteins.

It is a matter of some interest to see whether in this case of adsorption the anions have a retarding action like that shown by Pauli in the above-mentioned experiments. That there is some action of this kind can I think be shown by consideration of the comparative action of chloride and sulphate of the same metal, say potassium. The difficulty lies in the fact of the great preponderance of the opposite action of the kation, probably on account of the negative charge of the congo-red. If we take equimolecular solutions of KCl and K_2SO_4 we find their effect almost exactly the same within limits of experimental error. Now there are, in the dilute solutions employed, very nearly twice the number of kations in the K_2SO_4 than in that of the KCl, necessarily, therefore, the SO_4 ion has a greater retarding power than the Cl ion. In determining the order of the anions in this respect the same difficulty presents itself, as in the case of the opposite effect of the kations, but in a more marked degree. The approximate order as regards power of inhibition is the following :—

The results of these experiments will be best shown in a table analogous to those given by Pauli. The numbers give the percentage amount of dye adsorbed at room temperature under the influence of the body formed by the combination of kation and anion shown vertically above and horizontally at the left-hand side of each number, and in $\frac{m}{200}$ concentration.

CONGO RED							
	H	Li	K	Na	NH_4	Mg	Ca
OH	26	58	...	68	$79 \left(\frac{m}{12}\right)$	...	89
CNS	...	...	...	...	64	86	...
Acetate	...	...	58	70	81	...	...
Cl	...	...	70	75	72	...	91
F	...	...	...	75	...	...	...
Oxalate	12	...	80	...	65	...	...
SO_4	4	71	74	78	78	91	> 100
PO_4	...	...	...	...	74	...	...

1. Hofmeister's Beiträge, III, p. 225 ; V, p. 27 ; VI, p. 233 ; and VII, p. 531.

There are some irregularities especially as regards NH_4 salts. The value put against CaSO_4 means that the concentration had to be taken at $\frac{m}{500}$ to avoid precipitation, and at this strength the value was 84 per cent.

The action of OH^- and H^+ ions needs a little further explanation since it seems to vary according to concentration in both cases. It is somewhat remarkable that in concentrations of $\frac{m}{100}$ NaOH and NaCl have almost exactly the same amount of favouring action; in $\frac{m}{1000}$ on the contrary, while NaCl has a distinct *favouring* action, viz., 58 per cent. adsorbed from NaCl as against 30 per cent. from water, NaOH has a very slight *inhibiting* action, viz., 25 per cent. against 26 per cent. from water.

In one experiment I took three solutions of equal content in OH^- ions, viz. :—

$\frac{m}{1000}$ NaOH	...	31 %
$\frac{m}{12}$ NH_4OH	...	79 %
$\frac{m}{100}$ Na_2CO_3	...	89 %

The amount adsorbed in presence of these is shown opposite each one.

I could not detect any influence of anilin-water on the process.

Sulphuric acid $\frac{m}{2000}$ has a favouring action, 33 per cent. against 21 per cent. from water, while in $\frac{m}{200}$ it has the opposite effect as the table shows. I am unable to suggest any explanation of these facts.

In connection with the well-known mutual precipitation of oppositely charged colloids² the behaviour of congo-red as a negative colloid is of interest. Colloidal platinum prepared by Bredig's method does not precipitate, but has a chemical action, turning the dye brown. This platinum sol is negatively charged so that precipitation would not be expected. On the other hand, according to Pauli¹ and other investigators, the precipitating action of salts of the heavy metals on egg-white is due to the presence in their solutions of metallic hydroxide in the colloidal form and presumably positively charged.

1. See Picton and Linder, *Journ. of Chem. Soc.*, 1892, page 148, etc., and W. Biltz, *Ber d. Deutsch. Chem. Ges.*, 1904, Bd. XXXVII, page 1111.

2. Hofmeister's *Beiträge*, VI, page 257, 1905.

This being so, the powerful action of such salts on adsorption is not to be wondered at. In fact, I find $\frac{m}{5000} \text{Zn}^{++}\text{O}_4$ to have an equal effect to that of $\frac{m}{200} \text{KCl}$.

This action of zinc sulphate gives the opportunity for testing by experiment whether in this case, as in Picton and Linder's cases of precipitation of colloids by electrolytes, the precipitating ion is carried down with the colloid to become attached to the paper. Since the 'precipitating' agent in the adsorption of a negative colloid is the positively-charged kation, and the most convenient method of detecting a disappearance of an ion, is the use of a concentration battery in the manner of Nernst, it is plain that we cannot conveniently determine the question where salts of alkali metals are concerned. A concentration battery in Zn^{++} ions is, on the contrary, easily arranged. In the present case I took two vessels, each containing an amalgamated zinc electrode immersed in $\frac{m}{500} \text{ZnSO}_4$ solution and connected together by an inverted U-tube filled with the ZnSO_4 solution. Such a battery being symmetrical has no E.M.F. A piece of filter paper was now placed in one of the vessels, the E.M.F. began to rise and attained a value nearly equal to what it would be by the Nernst formula, if all the Zn^{++} ions had disappeared from this vessel, viz., 0.0257 volt. This effect was, in all probability, due to adsorption by the paper. On now adding 5 c.c. of 1 per cent. congo-red in $\frac{m}{500} \text{ZnSO}_4$ the E.M.F. went down again, due to the addition of more Zn^{++} ions. Presently, as the dye became adsorbed, the E.M.F. commenced to rise again and when plotted on squared paper showed the usual form of the adsorption-curve. As far as it goes, then, the experiment shows a diminution in concentration of Zn^{++} ions. But there are two circumstances which deprive it of much value. In the first place I found that next day the E.M.F. of the battery had risen to 0.094 volt, a value above that possible by removal of all the Zn^{++} ions; there must have been, therefore, some secondary process set up by the congo-red. In the second place the possibility must be taken into account, that when congo-red and zinc-sulphate are mixed, a zinc-salt of the congo-red may be formed by double decomposition which might not be completely dissociated, this

salt might be the actual body adsorbed by the paper. Considering the great dilution of the solution, however, I do not think the latter objection is very serious.

Another property of colloids is that of being protected from precipitation of electrolytes when a small amount of a stable colloid, such as gelatin, is present. As pointed out by Zsigmondy,¹ the fact was known to Faraday, although its meaning was, of course, not understood at the time. The following experiment shows that gelatin also protects congo-red from the action of an electrolyte :—

- A. 50 c.c. H₂O + 1 c.c. 0.5 % dye
- B. 50 c.c. 0.05 % gelatin + 1 c.c. 0.5 % dye
- C. 50 c.c. 0.038 % NaCl + " "
- D. 50 c.c. 0.038 % NaCl containing 0.05 % gelatin + 1 c.c. 0.5 % dye

Adsorbed in three hours :

A. 30 %	...	C. 74 %
B. 31 %	...	D. 34 %

It will be seen that NaCl is practically without effect in presence of gelatin.

Egg-albumin has a similar effect, but less marked, probably on account of its content in electrolytes. I did not subject it to dialysis.

Gelatin is stated by Victor Henri² to be a negative colloid ; serum-albumin has recently been shown by Pauli³ to be positive in acid solution, and negative in alkaline solution. It was of interest therefore, to see whether any difference in the protective action of egg-albumin was to be noted in the two instances. The result showed that electro-positive albumin *increased* the action of CaSO₄, while electro-negative albumin *diminished* it :—

+ dye + paper as usual

Adsorbed :

- A. > 80 % (partially pptd.)
- B. 35 %

1. *Loc. cit.*, p. 65, footnote.

2. *Rev. Gen. des Sciences*, 1905, p. 641.

3. Hofmeister's *Beirrage*, VII, p. 535 and p. 536.

Adsorbed :

C. 75 %

D. 98 %

It is difficult to compare with these the action of the neutral albumin since acid and alkali have themselves an influence. But it appears that the sign of the charge on the stable colloid determines the nature of its action, although the particular albumin in this experiment had also a protective action in neutral reaction. I am unable to state whether it was naturally electronegative or otherwise.

Other electro-negative dyes, such as anilin-blue and nigrosin, under the action of electrolytes, give similar results to congo-red. The colloidal condition of the solution is, however, apparently a *sine qua non*. I could not detect any influence of electrolytes on picric acid, although it is difficult to observe a small difference, because of the comparatively feeble colouring power of this dye. I compared the depth of tint of the two solutions, one with electrolyte, the other without, in both of which the same amount of paper had been immersed, by placing them in two cells of equal thickness in front of a piece of plantinotype paper and exposed to light. No difference could be detected in the depth of tint on development of the paper, although, as is well known, this paper shows very small differences of tone.

Scharlach R. is interesting, because it is a so-called 'indifferent' dye, that is it does not form salts. It is insoluble in water; but as Michaelis¹ points out, when an alcoholic solution is mixed with five to six times its volume of water, it is not precipitated, but becomes a colloidal solution. In this state it behaves towards electrolytes like a negative colloid, and, in fact, in the electric field, as I find, it wanders to the anode.

Rosolic acid shows similar properties.

1. *Deutsche, Med. Wochens.*, 1904, No. 42.

In order to save space, I have put together in a table at the end of this paper a number of facts relating to various dyes in common use which have a bearing on the question under discussion.

The only other point I will refer to as regards the negative dyes is that eosin, although its colloidal properties are doubtful (see table), shows a much greater sensitiveness to the anion than the dyes hitherto mentioned, alkali in this case causes the taking up of *less* colour by paper than is taken up from water. We found in the previous cases that the action of the OH^- ion was overpowered by the opposite action of the kation (Na^+).

We turn now to the electro-positive dyes like toluidin-blue. It would be expected that in this instance the anions would have a favouring action, the kations the reverse. This is, indeed, the case, but like the negative dyes the effect of the kation is predominant, so that the result of adding any electrolytes, except alkalies, is to *lessen* adsorption. In the absence of electrolytes, as a rule, more dye is taken up by the paper than from the solution of an electro-negative dye. This would appear to be at variance with the general use of congo-red to dye cotton, but it is to be remembered that in practice there is always sufficient electrolyte present to reverse the relative behaviour of negative and positive dyes as shown when dissolved in pure distilled water.

For details as to various positive dyes see the table.

Methylene blue precipitates the electro-negative colloidal platinum.

I have not been able to detect any marked protective action of stable colloids in the case of electro-positive dyes.

In the circumstance that both the + and - dyes are more sensitive to kations than to anions they behave like albumin solutions to salts of the heavy metals as shown by Pauli.¹

On reference to the table at the end of this paper it will be noticed that in all the cases investigated where the dye is in the form of a salt with an inorganic base or acid, as the case may be, the sign of the charge of the colloid is determined by the organic constituent of

1. Hofmeister's *Beiträge*, VI, pp. 233 to 249.

the salt. In this respect they differ from the globulin of Hardy and the serum-albumin of Pauli, which in acid or alkaline solutions assume the sign of the charge of the H^+ or OH^- ions respectively. They are in fact more like certain complex ions, such as the ferrocyan ion, $Fe(CN)_6$, which, although containing an iron atom is, nevertheless, electro-negative.

I have been unable, for want of time, to test the behaviour of more than a few typical dyes of the two classes, but it appears that there is considerable difference of degree in the sensitiveness to electrolytes. This property indeed seems to depend on the degree of colloidalty of their solution and the amount of their electric charge. Each individual dye requires separate investigation.

As to the explanation of the action of electrolytes, I think the clue is given by the following considerations. V. Henri and Larguier des Bancel¹, in their work on colloids, observed that gelatin, as hydrogel, when immersed in a solution of anilin-blue or congo-red in distilled water took up little or none of the pigment, and give as the reason for this that both the bodies are electro-negative colloids and therefore mutually repel one another. If, on the contrary, a solution of a bivalent metal, such as barium nitrate, is added the gelatin becomes deeply stained. The negative charge of the anilin-blue is neutralized by the positive barium ions so that it can now freely attach itself to the gelatin. This case then resolves itself into one of the mutual precipitation of colloids, and the only doubtful point about the explanation is whether the negatively-charged particles of the gelatin hydrosol may be regarded as retaining their charge when in the form of gelatine. There seems no *à priori* reason why this should not be so, and there is also experimental evidence in favour of it. Picton and Linder² in referring to the adsorption affinity of the hydrogels of ferric hydroxide and arsenious sulphide for anilin dyes state that these hydrogels 'retain the same selective affinity for the dyes which will coagulate them' as they possessed as hydrosols, viz., 'the hydrogel of ferric hydroxide for anilin-blue, that of arsenious

1. C.R. *Soc. de Biologie*, LIX, p. 132, 1905.

2. *Journ. Chem. Soc.*, 88, 1905, p. 1934.

sulphide for methyl-violet. We regard this fact as evidence that the granular aggregates in these hydrogels still retain some charge. In other words, the difference of potential existing between the molecular aggregates and the field in the hydrosol state is not destroyed by coagulants, but only reduced to a point at which the forces of cohesion are just able to overpower the forces of repulsion brought into play by such difference of potential.'

It will readily be seen that if paper assumes a negative charge when immersed in water the phenomena I have described above fall into line with the behaviour of electrically charged colloids. We notice that of a negative colloid like congo-red the less is taken up by paper the more perfect the freedom of the water from electrolytes, there is mutual repulsion until the dye is discharged by a kation. On the other hand, the opposite holds as regards the positive dyes, there is attraction of the negative paper for the positive dye. The kation in this case probably acts by discharging the paper, being attracted thereto by its opposite charge.

Prof. Donnan, to whom I propounded the question, refers me to the work of Coehn¹, who found that when various non-conducting bodies are immersed in fluids of different dielectric-constants they assume a positive or negative charge according as their own dielectric-constants are higher or lower than the fluid with which they are in contact. For instance, glass (5.6) is negative in water (80) or alcohol (26), whereas in turpentine (2.2) it is positive. Now, paper according to Thwing² has a dielectric-constant of 2.82, and would therefore be electrically negative to water.

Moreover, Quincke³, in the course of his investigations on electrical endosmosis found that, as a rule, all non-conducting bodies in water took on a negative charge. Among the substances tested by him were cotton-wool and silk ; particles of these in water exposed to an electric field wandered to the anode and were therefore negatively charged.

1. *Wied. Ann.*, 64, page 217, 1898.

2. *Zeitsch. f. Physik. Chemie.*, XIV, page 292, 1894.

3. *Pogg. Ann.* 113, page 583, 1861.

The conclusion is, I think, justified that paper takes up very little electro-negative dye because it is itself negatively charged, and that when the dye is discharged by the addition of an electro positive colloid or a kation, there is no longer the same mutual repulsion between the dye and paper.

It would be of interest to test the behaviour of paper in turpentine, in which it would be electro-positive, if one could obtain an electro-negative colloidal dye in solution in turpentine.

This interpretation is confirmed by the results of experiments on silk :—

Experiment: From a watery solution of congo-red a piece of paper took up 26 per cent., a piece of silk (well washed), of the same weight took up only 5 per cent. From a $\frac{m}{200}$ $MgSO_4$ solution, paper took up 91 per cent. and silk 98 per cent. From toluidin-blue in water, paper took up 85 per cent. Silk rapidly took up the whole, so that I added another double amount of dye solution, the colorimeter reading thus showed that 67 per cent. of the whole, that is $67 \times 3 = 201$ as compared with 85 in the case of paper was taken up. The action of neutral-salts on the adsorption of positive dyes by silk was of the opposite sign to their effect on paper. For example :—

Paper : From water	...	...	85 % adsorbed
„ $\frac{m}{200}$ $MgSO_4$	...	...	51 % „
Silk : From water (concentration of dye three times that in case of paper)			67 % adsorbed
„ $\frac{m}{200}$ $MgSO_4$	...	...	91 % „

The results may be explained by the consideration that silk, owing to its lower dielectric constant than that of paper, would no doubt have a higher negative charge, which would make it less accessible to negatively-charged dyes, but more so to positively-charged dyes. I am unable to state why the action of electrolytes on the positive dye is opposite in the two cases of silk and paper, unless that for some reason silk is relatively more sensitive to the adjuvant action of the anion. If this were so, however, one would expect a more marked

inhibitory effect of the anion in the case of electro-negative dyes and silk, whereas we see that the action of $MgSO_4$ is rather greater in the case of silk than in that of paper.

The troublesome stain which appears on the surface of glass containing electro-positive dyes is no doubt due to the negative charge of the glass. This film is so adherent that water will not remove it, and I have found it necessary, when working with these dyes, to rinse all flasks used with concentrated nitric acid, in order to obtain concordant results.

Another fact receives its explanation from the negative charge of paper, viz., that from alcohol more negative dye is taken up than from water, in one experiment :

From 50 % alcohol	- 65 % taken up
„ water	- 53 % „

Since alcohol has a lower dielectric-constant than water it would naturally be expected, since the negative charge of paper is due to the difference between its dielectric-constant and that of the fluid in which it is immersed, that the negative charge would be greater the greater this difference is, and therefore in alcohol the charge would be less than in water and the attachment of a negative dye less difficult. On the other hand, *less* + dye is taken up in the presence of alcohol, thus :

From 50 % alcohol	- 5 % taken up
„ water	- 80 % „

Finally, I may refer to the action of acid in preventing the favouring action of electrolytes on adsorption of a negative dye, for example :

CONGO-RED				
From water	...	...	...	27 % adsorbed
„ $\frac{m}{200}$ NaCl	...	...	...	75 % „
„ $\frac{m}{200}$ NaCl + $\frac{m}{2000}$ H_2SO_4	...	...	...	31 % „

This result is probably to be explained by the fact that in the presence of sulphuric acid the colour-acid is set free from congo-red. This colour-acid is, no doubt, more strongly electro-negative

than the salt, so that the amount of kation which would suffice to discharge the salt would be insufficient to discharge the acid, it would therefore be comparatively ineffective in promoting adsorption.

In view of the theory set out in the preceding paragraphs it will be of interest to give two curves showing the form of the relation between the concentration of the kation and its effect on congo-red. The ordinates in Fig. 6 represent the percentages of congo-red taken up from a $\frac{m}{6000}$ solution containing electrolyte of the concentration given by the abscissae. The upper curve is that of CaSO_4 , the lower curve that of KCl .

FIG. 6

The commencement of the CaSO_4 curve is irregular, owing to partial precipitation of the dye having taken place. The inclination to an S-shape at the beginning of the KCl curve is, probably, also an indication of incipient precipitation. The extreme sensitiveness of

congo-red to electrolytes is also shown by the curves ; even at the low concentration of $\frac{m}{6000}$ of the electrolyte they do not fall to the level of the amount taken up from the particular sample of water used, which amount is represented by the horizontal line with an ordinate value of 20 per cent. It is noticeable, on the contrary, that the curves tend to become asymptotes. I am not prepared to give any interpretation of the form of these curves.

V. CONDITIONS OF DISSOCIATION OF ADSORPTION COMPOUNDS

Recent research has brought to light the important part played in vital phenomena by the mutual relations of colloids to electrolytes and to other colloids. Since these relations are apparently those of adsorption, it is of some importance to know what are the conditions under which this association is broken down.

1. *Temperature*

We have already seen that dissociation is favoured by rise of temperature, and that, on the other hand, if suddenly exposed to the temperature of boiling water, a dyed piece of paper becomes to a certain degree fixed in its condition, so that the dye can only with difficulty be extracted by water.

2. *Precipitation*

It was noticed by Cramer and Swale Vincent¹ that the inorganic constituents of various organic bodies are set free when these bodies are acted upon by precipitants of bases. I have observed the same fact in the case of the precipitation of gelatin by tannin.

Experiment: A 15 per cent. solution of gelatin had a specific conductivity of 1570 gemmhos at 38.5. 20 c.c. of this solution were mixed with 90 c.c. water and 10 c.c. of 10 per cent. tannin solution, filtered, and 100 c.c. filtrate concentrated to 20 c.c. The conductivity of this was now 2750 gemmhos. This 20 c.c. would represent $\frac{100}{120}$ of the first, so that the conductivity should be multiplied by $\frac{120}{100} = 3300$ gemmhos, that is, the conductivity was doubled by the action of

1. *Journ. of Physiology*, XXX, p. 150, 1903.

tannin. The amount of tannin added was intentionally less than that required for complete precipitation, so that the filtrate contained a little unaltered gelatin, but no tannin.

3. *Action of Chloroform*

Moore and Roaf¹ have shown that electrolytes are set free in blood by the action of chloroform.

4. *Excitation, Injury, and Death*

Macdonald² found that, as a result of injury, potassium salts are set free in the axis-cylinders of nerve fibres. Under certain conditions these salts may be taken up again by the colloids, and Macdonald suggests a theory of excitation and inhibition on this basis.

Hoeber³ finds that the excitability, the capacity of staining, and the colloidal consistency of nerve vary concurrently.

Wakelin Barratt⁴ finds that chlorine ions are set free when unicellular organisms are injured or killed.

Brailsford-Robertson⁵, as the result of his work on the phenomena of the heart-beat, comes to the conclusion that these phenomena are such as would be expected if there were a periodic driving out by anions of kations from combination with proteid.

Howell,⁶ having found that the vagus cannot exert its action on the heart-muscle in the absence of potassium ions, brings forward the hypothesis that the inhibition produced by the vagus is due to splitting off potassium from combination in the muscle.

In order to see whether there is any evidence of a sudden separation of electrolytes at the moment of death, I have made the following experiment :

The skins of two frogs were immersed in isotonic cane-sugar solution. The electrical resistance of the solution was measured, after soaking for some time, and found to be at 11° C., 6407 ohms. The solution containing the skins was then slowly warmed and the resistance determined at frequent intervals up to 56° C. The

1. *Proc. Roy. Soc.*, 73, p. 382, 1904, and 77 B, p. 98, 1906.

2. *Thompson-Yates Lab. Reports*, Vol. 4, p. 213, 1902.

3. *Centralb. f. Physiologie*, XIX, page 390, 1905.

4. *Zeitsch. f. Allgem. Physiologie*, V, page 33, 1905.

5. *Pflüger's Archiv*, 110, page 623, 1905.

6. *American Journ. of Physiology*, XV, page 291, 1905.

values when plotted on squared paper formed a regular curve, somewhat concave towards the axis of abscissae. There was no indication of any kink in the curve except for one determination at 16° , which was obviously an experimental error. That electrolytes were given off is shown by the fact that the diminution of resistance was considerably greater than would have been the case if the diminution depended only on the effect of the rise of temperature on electrolytes present at the beginning. The temperature-coefficient was, indeed, 4.14 per cent. per degree, instead of, at the most, 2.75 per cent., according to the determinations of Arrhenius. The separation of electrolytes also showed itself to be irreversible, since cooling down to 11° C., the resistance only increased to 4128 w. The shape of the curve shows that the rate of separation was greater at the lower than at the higher temperatures, but, as already remarked, there was no point on the curve to indicate that death occurred. It is obvious that the results may also be accounted for by gradual destruction of the impermeability of the living cell for most inorganic salts, but, if so, this process must also be a gradual one.

To compare with this experiment I made a similar one with egg-white, raising the temperature to 95° C. In this case the result was opposite to the above, viz., there was evidence of taking up of electrolytes. The physical state of the coagulated egg-white is, however, so different from that of the fresh substance that it is difficult to interpret the meaning of the observation.

5. *Coagulation of blood.*

It was found by E. G. Martin¹ that Ca^{++} ions have a marked stimulating influence in causing freshly isolated strips of the tortoise ventricle to commence beating spontaneously. This effect is shown by serum, but not by uncoagulated plasma. It appears, thus, that Ca^{+} ions are set free during the process of clotting, so that there should be an increase of electrical conductivity in the process. Robert T. Frank² was unable to detect any change of this kind. I have, on the contrary, succeeded in showing that there is such a change, not in

1. *Amer. Journ. of Physiology*, XI, p. 117, 1904.

2. *Amer. Journ. of Physiology*, XIV, p. 466, 1905.

the sense of an *increase* but a *decrease*, so that there is a disappearance of ions. This result is, I think, what would be expected from our knowledge of the relation of calcium to clotting, and the observations of E. G. Martin must be explained in another way. My experiment was made on fowl's blood. As shown by Delezenne, if kept from contact with the tissues this blood remains unclotted for a long time. It was collected from the external jugular vein and placed in a U tube furnished with an electrode in each limb and immersed in a water-bath at 10.2°C . When the temperature had become constant the specific conductivity of the blood was 5382 gemmhos. A small piece of muscle from the fowl was then dropped into one side of the tube. The conductivity steadily fell to 4360 gemmhos, which value was attained in 40 minutes; no doubt the process was completed earlier than this, but it was not wished to disturb the apparatus until it was to be expected that clotting had occurred. The diminution of conductivity therefore amounted to 18.5 per cent. of the original value.

6. *Action of enzymes*

I do not purpose here to enter into this subject in any detail, since it will be dealt with fully in a forthcoming paper. There is a certain amount of evidence that the splitting-off of inorganic constituents contributes to the rise of electrical conductivity observed in many cases, but the direct proof was found to be of considerable difficulty.

In the action of rennet on milk, I find a slight *increase* of conductivity. This is merely an additional fact showing the nature of this process to be quite different from that of clotting of blood. In all probability rennet-action is only an expression of pepsin-action in neutral or faintly alkaline medium.¹

VI. SPECIFIC ADSORPTION

The phenomena of adsorption show in this respect an approximation to true chemical combination.

It was shown by Schönbein² that when strips of filter-paper were immersed in solutions of various salts that the height to which the salts

1. Pawlow and Paraatschuk. *Hoppe-Seyler's Zeitsch.*, 42, p. 415, 1904.

2. Poggendorff, *Ann.*, 114, p. 275, 1861.

rose was less in the case of calcium and barium than in that of potassium or strontium, the calcium, etc., being more completely held by the paper. In the case of a solution of iodine in KI , while the latter rose nearly as far as water, the iodine was kept back in the lower third. These facts were explained by Schönbein as capillary attraction, but, as Ostwald remarks, there is no doubt that it is really an adsorption process that causes the separation of the bodies in question, capillarity only occasions the transport of the separated fractions.

These results of Schönbein have been extended and employed as a means of analysis by Goppelsroeder.¹

It is well known to histologists how certain tissues take up particular dyes in preference to others. In certain cases, as for example, the differences between paper and silk, as to their respective behaviour towards congo-red and methylene blue, this is probably to be accounted for by the fact of the electrical charge in the two dyes being opposite in sign combined with the negative charge of the substance to be stained, as already pointed out.

Acid fuchsin is used in Van Gieson's stain to show connective-tissue. In view of this, I thought it of interest to try the behaviour of gelatin in sheet towards this dye and toluidin blue respectively. The same amount of gelatin was found to take up 72 per cent. of the acid-fuchsin and only 32 per cent. of the blue. It is possible here that the electrolytes of the gelatin were responsible for the difference, since they would increase the adsorption of the acid dye and diminish that of the basic dye. This possibility is excluded when we compare the amounts of congo-red and acid fuchsin, both electro-negative dyes, taken up by gelatin or filter-paper. The following are the results of an experiment:

Equimolecular concentrations of dye.

Equal weights of gelatin and filter-paper.

Filter-paper from Congo-red	...	31 %
„ „ acid-fuchsin	...	32 %
Gelatin „ Congo-red	...	49 %
„ „ acid-fuchsin	...	95 %

1. *Mitt. d. techn. Gewerbe Museums, Wien*, 1899, and *Studien über die Anwendung d. Capillar analyse*, Basel, 1904.

Gelatin, then, has a specific adsorption affinity for acid fuchsin.

The preference exhibited by silk for methylene-blue over congo-red, previously described, may be explained in another way than by specific adsorption, viz., by the probable greater negative charge of silk.

That gelatin has a greater adsorption-affinity for calcium sulphate than it has for potassium chloride seems to be shown by the experiments on washing out electrolytes, given in the earlier part of this paper. It was found that KCl is more easily washed away than calcium sulphate.

VII. THE APPLICATION OF THE FACTS OF ADSORPTION-PROCESSES TO VARIOUS PHENOMENA

The theoretical interest of adsorption-processes has been pointed out by Ostwald,¹ who suggests the possibility that a mechanical theory of chemical affinity may be developed on the basis of a complete study of these phenomena.

From a more practical standpoint, it will be found that they play a very important part in numerous processes. I may refer briefly to a few of these.

1. *The Soil*

The power of the soil in holding back soluble salts and other bodies is, no doubt, due to adsorption. By this means these bodies are prevented from being readily washed away by the rain.

2. *Purification of sewage*

In the filter-process of nitrification, it appears that the complex organic substances, which are inimical to the nitrifying organisms, are kept back by adsorption in the upper layers of the filter.²

3. *The ash-constituents of organic compounds*

I have already given evidence that these are present in an adsorbed form, and shown that the difficulty of removing the last fractions of inorganic electrolytes is adequately explained on this basis.

1. *Op. cit.*, p. 1098.

2. See Dr. Harriette Chick, *Proc. Roy. Soc.*, 77B, p. 247, 1906

It is scarcely necessary to give instances of this difficulty, and I will merely refer to three instances. Macallum¹ in searching for the cause of the silver reaction of tissues met with considerable difficulty in completely removing chlorides. Mellanby² states that there is a molecular combination between neutral salts and globulin. Lange³ found that it was impossible to remove all ash-constituents from filter-paper by extraction with hydrochloric and hydrofluoric acids and washing.

4. *Dyeing*

On the whole, I think it must be taken as the true explanation of this process that it is, in the main, an adsorption. The very slight diffusibility of most dyes through parchment-paper seems to me a considerable objection to Witt's theory of solid-solution. If the dye is dissolved in the paper it should be readily given off again to water on the opposite side in the same way as hydrogen passes through palladium in the experiments of Ramsay.⁴ The theory to which my experiments lead is very much the same as that to which Picton and Linder have independently arrived.⁵ According to these observers, there are two stages to be distinguished.

Stage I. The 'coagulation stage' in which single ionic interchange takes place between the 'fibre substance' (colloid) and the dye, resulting in the separation of insoluble dye derivatives retaining a feeble charge.

Stage II. The 'colour absorption' stage, in which coagula produced in stage I attract and retain the oppositely-charged particles of the dye substance.

My experiments show that no actual precipitation of the dye must take place except in the substance to be stained, and I should be inclined to modify the above theory by omitting stage I, and reading instead of 'coagula produced in stage I' simply 'colloids of fibre-substance.' These bodies usually having a negative charge, it is

1. *Proc. Roy. Soc.* 76 B, p. 225, 1905.

2. *Journ. of Physiology*, 33, p. 359, 1905.

3. *Ber. Deutsche. Chem. Ges.*, 1878, p. 823.

4. *Phil. Mag.*, 38, pp. 206, 218, 1894.

5. *Journ. Chem. Soc.*, 88, p. 1,935, 1905.

necessary, in order for them to take up an electro-negative dye, that the latter shall be discharged by the presence of a kation. When the dye is electro-positive, on the other hand, neutral salts are unnecessary and usually deleterious, since both classes of dyes are most sensitive to the kation which would be inhibiting in the case of positive dyes. In certain cases alkali increases the adsorption of these latter, presumably owing to the powerful action of the OH^- ion more than counteracting the opposite effect of the kation. The common practice of using basic dyes in strong solution of borax and acid dyes in acid solution is in agreement with this view. It appears, that in the case of certain acid dyes, such as congo-red, the concentration of the electrolyte used in practice is unnecessarily high.

5. *The Staining of Histological Preparations*

Here again the balance of evidence is, as it seems to me, in favour of the adsorption theory.¹ It may be that there are exceptional cases where true chemical combination takes place, but they appear to be rare.

This being so, it is obvious that the part played by electrolytes must be taken into account. If electrolytes are split off from living cells when they die or are injured it is clear why these cells readily take up acid dyes under such conditions; moreover, since electrolytes are unnecessary when the substance to be stained is electro-negative, it is also clear why living cells can be stained with basic dyes, if, as seems probable, the structures in question have a negative charge. The reaction of the tissues is on the alkaline side of neutrality and, as Hardy has shown, globulin is electro-negative in alkaline solution, and Pauli has shown the same for albumin.

I am not forgetting the work of Overton on the solubility of basic dyes in the 'Plasmahaut,' and the insolubility of acid dyes therein. The action of electrolytes must also, nevertheless, make itself felt.

An interesting case, which at the same time presents considerable difficulty as to its explanation, is the staining of granules in the axis-cylinder of nerve-fibres. These granules are stained by neutral-red as

1. See Alfred Fischer, '*Fixierung, Färbung, etc. d., Protoplasmas.*' Jena, 1899.

shown by Macdonald¹, but only in the position and neighbourhood of an injured spot. They correspond to the distribution of potassium ions, and are interpreted by Macdonald as precipitates of the dye caused by potassium. He finds, in fact, that fairly strong solutions of potassium salts are capable of throwing down the dye. Since neutral-red is a basic dye these results seem at variance with the action of neutral salts on adsorption of these dyes as described in the preceding pages. I thought it of interest therefore to see whether neutral-red behaves differently to the basic dyes on which my experiments were chiefly performed. I found it, however, to behave quite in the same way. I did not, indeed, notice any tendency to precipitation even by 2.6 per cent. KCl. There are evidently, then, some other factors at work in the case of the staining by neutral-red of the granules produced by injury in the axis-cylinder of nerve-fibre.

Since congo-red showed itself to be particularly sensitive to electrolytes it would seem a suitable reagent for the detection of electrolytes, if split off from injured tissue. I have tested the behaviour of nerve-fibres to this dye. It is easily seen, under the microscope, that only the cut ends of the nerve are stained and not the uninjured parts of the fibre. I could not detect any appearance of granules, but did not look for them under a high power. It was to be noted also that connective-tissue fibres took the stain, as I imagine because they were non-living.

The fixation of adsorbed dye by heat has already been pointed out. This fact is of interest in connection with Altmann's method of using acid-fuchsin. The fixation of dye by electrolytes has also been mentioned.

Recently Emil Mayer² has shown that the affinity of the Nissl bodies of nerve-cells for basic dyes is abolished by previous treatment with neutral salts. This is in complete concordance with the results I have described.

It occurred to me that, considering the opposite action of neutral salts on electro-negative and electro-positive dyes it should be possible

1. *Proc. Roy. Soc.*, 76, page 325, 1905.

2. Hofmeister's *Beiträge*, 7, p. 560, 1906.

to vary the colour of paper stained in a mixture of two dyes of opposite character by the addition of electrolytes. This proved not to be so, the reason being the formation of dye-compounds, which appeared to be unaffected by electrolytes. One of these dye-compounds, that of eosin and methylene-blue, is well-known to histologists and is generally regarded as a salt of the methylene-blue base with the eosin acid. Similar compounds are formed by most pairs of acid and basic dyes. They are very insoluble in water though soluble in excess of either component. This latter fact suggests the possibility that they are not true compounds but adsorption-compounds of oppositely-charged colloids. As shown by Biltz mutual precipitation occurs in such cases and solution in excess of either colloid. On this account I have devoted some attention to these bodies. In the first place, if true chemical compounds are formed by a process of double decomposition the inorganic components should be found combined together in the solution. I tested this in the case of anilin-blue and methylene-blue by taking about 3 grams of the latter and the molecular equivalent of the anilin-blue. The precipitate was filtered off and the filtrate evaporated to dryness in order to estimate the chloride present. Unfortunately, by an omission to make a note of the amount of the filtrate from the silver chloride, the exact value was not arrived at. From the quantity of silver nitrate required to completely precipitate the chloride, it was obvious, notwithstanding, that practically the whole of the chlorine of the methylene-blue was contained in the filtrate. The precipitate was, therefore, a compound of the methylene-blue base with the anilin-blue acid, the sodium of the anilin-blue having at the same time combined with the chlorine of the methylene-blue. I should hesitate to state definitely whether this kind of combination was inconsistent with a process of adsorption, especially in the light of van Bemmelen's results ; on the face of it, at the same time, it appears more like a true chemical compound. Dr. J. H. Scott informs me that he has noticed that a new absorption-spectrum makes its appearance when solutions of eosin and methylene-blue are mixed. I have repeated this observation in the following way : A solution of methylene-blue was taken of such a strength as

to show distinctly the two dark bands on the red side of the D-line in a certain cell. An equivalent molecular solution of eosin was taken, so that when mixed in equal volume there should be two molecules of methylene-blue to one of eosin. This eosin solution was placed in a cell of equal depth to that containing the methylene-blue and the two cells then placed before the slit of the spectroscope. The combined spectrum of the two dyes was seen. Equal volumes of the two solutions were then mixed, a portion of the mixture at once placed in both cells and these then observed. By taking a double layer it would seem that there would be no change in the spectrum unless chemical changes had taken place. What one sees is that the band of methylene-blue next the D-line has almost completely disappeared, leaving a faint shade. No change is to be seen in the band in the red, nor in the eosin band at F. The same disappearance of the one band of methylene blue may be observed when any other acid-dye such as anilin-blue or congo-red is taken instead of eosin. On the other hand, if thionin or toluidin-blue is taken instead of methylene-blue, no obvious change is produced in the spectrum until precipitation occurs, when, of course the bands become much fainter. I believe the explanation of the disappearance of the methylene-blue band is to be found in the fact that it is the first to disappear on mere dilution. Now when an oppositely charged colloid is added to a solution of the blue the precipitate does not fall at once and it might be supposed there was no important change ; but, if the experiment is made in a vessel through which a beam of bright light is passing, the track of the beam is barely visible in the methylene blue, but almost immediately on adding the other dye the beam begins to appear, gradually becoming brighter as the particles increase in size. In point of fact then, methylene blue is really taken out of solution, and the first band to disappear would be the one next the D-line. The other thiazin-dyes, so far as investigated, do not show the same behaviour on dilution, the various bands all disappear or fade in nearly the same proportion.

These dye-compounds, although almost insoluble in water, are soluble in alcohol, so that it might be supposed that by taking a strong

alcoholic solution of a methylene-blue compound, one should see both the methylene blue bands. This is not so, and is due to the fact that in alcoholic solution of methylene-blue the band next the D-line is not to be seen at all distinctly. If the solution showing only the other band is gradually increased in depth or concentration, it is seen that this band broadens out until it reaches the D-line, but there is no indication of a gap between the bands. A fact which confirms the interpretation I have given of the change in the methylene-blue spectrum produced by combination with an acid-dye, is that scharlach R. in weak alcoholic solution produces the same change, and also a precipitate of a dye-compound. Now scharlach R. is an indifferent dye, not forming salts, but forms an electro-negative colloidal solution ; so that it would seem that here, at all events, the precipitate must be an adsorption compound. A similar state of affairs is to be observed in the case of rosolic acid. Here the possibility of double decomposition is also absent, except, of course, that by mass-action a small proportion of methylene blue rosolate might be formed. The only instance I have found where there is a real change in the spectrum is in the mixture of methylene-blue and picric acid ; picric acid is, however, a powerful reagent, and much weight cannot be laid on this fact as far as concerns the question before us. In mixing methyl-violet and an acid-dye no change is to be observed in the absorption spectrum.

The absence of change in the absorption-spectrum of these dye-compounds does not, of course, exclude the possibility of their being true chemical compounds.

With regard to the properties of these bodies, they are indiffusible, as such, through parchment-paper. In dilute watery solution they appear to be dissociated ; for, on dialyzing the eosin-methylene-blue compound, for example, it will be seen that after a time eosin begins to pass through, and is ultimately followed by the methylene-blue. Since this observation was made on a solution of the pure body, which had been thoroughly washed with cold water, the eosin and methylene-blue must have passed out as the free acid and base respectively. This dissociation is also shown by the fact that, at 100°, the solution is a fairly good conductor of the electrical current, and is electrolyzed by a constant current.

Although these compounds are, no doubt, colloids, they do not appear to carry a charge—or only a very small one. Electrolytes have a very slight effect in the sense of retarding adsorption. In the boundary apparatus the behaviour of anilin-blue-methylene-blue under electric stress is difficult to interpret, but since the phenomena are the same in neutral acid, or alkaline solution, it does not seem to be a question of electric charge. What happens is this : after the current has passed for some time it will be seen that electrolysis has occurred, so that on the anode side there is a layer of anilin-blue solution, and on the kathode side one of methylene-blue. The upper boundaries of these two are at the same level in both limbs, but the methylene-blue layer being deeper than the anilin-blue it makes the level of the unaltered compound on the kathode side lower than on the anode side, so that it appears as if negatively charged and moving to the anode. The behaviour to electrolytes, if any at all, is, on the contrary, in the sense of a positive charge ; so that I am inclined to think that the behaviour in the electric field is, in some way, due to different velocities of the two ions. In the case of the eosin-methylene-blue there was no similar difference in level of the unaltered dye, so that it may be that here the two ions are more nearly equal in velocity.

This absence of proof of any definite electric charge makes the compounds less interesting from the theoretical point of view in some respects. But it is, I suppose, what might be expected if these bodies are formed by the mutual neutralization of electro-positive and electro-negative colloids.

On the whole it seems impossible to give, in the present state of knowledge, a decided answer to the question as to the nature of these dye-compounds.

6. *Antitoxins*

It has been shown by Craw¹ that the combination between toxin and antitoxin follows more closely in its nature that of adsorption than that of chemical combination. The puzzling fact known as the 'Danysx-von-Dungern phenomenon' is, for example, satisfactorily

1. *Journ. of Hygiene*, 5, p. 115, 1905, and *Proc. Roy. Soc.*, 76 B, p. 179, 1905.

explained by what I have called in the earlier part of this paper the 'law of adsorption.' In illustration I may cite one experiment :

Two filter-papers of equal size (12 cm. diam.) were each cut into eight pieces. Two flasks, each containing 50 c.c. of a dilute congo-red solution were taken, and to one of these the whole of one paper was added at once, to the other piece by piece at intervals of about twelve hours. After all the paper had been added the amounts taken up from the solution were :

Added altogether	...	...	...	37 %
„ piece by piece at intervals	...	...	...	48 %

It is advisable in this experiment to wait a considerable time between the addition of each piece of paper on account of the slow attainment of equilibrium in the case of congo-red and paper.

7. *The part played in enzyme-action*

The interest of adsorption phenomena in this region is in connection with the combination between enzyme and substrate, which so much recent work indicates as an indispensable condition of attack. The fact that enzymes are carried down when a colloidal precipitate is produced in a solution containing them, has been long known and used as a means of preparation. But whether the union between a more or less specific enzyme and the body hydrolyzed under its influence is to be looked upon rather as a true chemical combination is a matter of dispute.

Certain experiments have been made by Dauwe¹ as to the taking up of enzymes by colloids. These were done chiefly on pepsin and various proteins, a few on the taking up of pepsin by agar and of emulsin by coagulated egg-white. Dauwe comes to the conclusion that the process is not one of adsorption, since the same weight of boiled egg-white takes up the same amount of pepsin whether the egg-white is in large pieces or fine powder. He considers that the hypothesis of solid solution applies to the case better. It seems to me that this conclusion is scarcely justified. Coagulated egg-white is not a homogeneous solid body, but porous, like charcoal, and therefore its active surface must not

1. Hofmeister's *Beitrage*, VI. p. 426, 1905.

be restricted to the external surface of the pieces of various size. Porous solids like charcoal, even in lumps, adsorb gases, and that not only on their surfaces but throughout their substance. Dauwe, indeed, appears to have thought of this possibility, but regards adsorption as the same thing as solid solution in such cases.¹ The distinction is, surely, analogous to that between true solution and colloidal solution. Although recent investigations teach that we must not draw a hard and fast line between these two kinds of solution, there is no doubt that the molecules or particles in suspension change their properties as their dimensions increase beyond what are ordinarily called molecular, and that they begin to have the properties, due to surface, of matter in mass. In the same way, as I think, we ought to keep the name 'solid solution' for such cases as alloys of metals, and give the name adsorption not only to the taking up of substances from their solutions by surfaces, in the usual sense of the word, but also by such surfaces as those forming the walls of pores. The criterion by which any given case is to be decided, is, of course, the way in which the relative amounts taken up vary as the concentration of the body taken up changes.

Dauwe rejects the hypothesis of chemical combination on the ground that the enzyme taken up can be extracted again by an appropriate solvent, for instance, pepsin from coagulated egg-white by a solution of egg-white. He regards the process as one of solid solution, or relative solubilities of the enzyme in the substrate and water, and brings forward in support of this view the experiments of Reichel and Spiro² on the apparent loss of rennet in the process of clotting of milk. These observers showed that the disappearance of the enzyme was to be accounted for by the taking up of it by the clot. Now, as I understand their results, they are rather in favour of adsorption. If it were a case of solid solution the percentage loss would be the same whatever the rennet concentration, but if one looks at the table on page 481 of the second paper referred to, it is seen that the percentage loss steadily *increases* as the concentration of the

1. *Loc cit.*, p. 443.

2. Hofmeister's *Beiträge*, VI, p. 68, 1905, and VII, p. 479, 1905.

enzyme falls ; in other words the more dilute the solution the more adsorption takes place. This is particularly well shown by the last experiment on the table. The mathematical expression given is also in accordance with this interpretation.

If the union of enzyme and substrate follows the law of adsorption it would be expected that there would be found some effect of this law on the relation of activity of enzyme to its concentration. In my paper on *The Kinetics of Tryptic Action*,¹ I showed that the form of the function in question varied according to the stage of the reaction, but if we take the early stages in which the action is most rapid there seems to be some effect suggestive of an adsorption process. The initial linear stage is very short, so that we may omit it, and, if we compare the relative times taken by concentrations of trypsin varying as 8, 4, 2, and 1 to produce an increase of conductivity of 1800 gemmhos in caseinogen solution, we find the values are :

Relative trypsin content		Time taken in minutes
8	...	54
4	...	79
2	..	126
1	...	233

so that the lower concentrations of trypsin are relatively more active. It is somewhat remarkable that the value of the exponent found in another experiment was 1.42 to 1.67² and this is the same as that given by Reichel and Spiro in their paper for the 'Teilungsfactor' of rennet, viz., 1.5 to 1.67. Whether this is merely an accidental coincidence I am not prepared as yet to state, the subject being still under investigation.

I have myself made one or two experiments similar to those of Dauwe, which are perhaps of sufficient interest to give here. It was shown by W. A. Osborne that calcium caseinogenate does not pass through a porous clay filter, trypsin, on the contrary, does do so. If then, we mix solutions of trypsin and calcium caseinogenate, the

1. *Archives des Sciences Biologiques*. Tome XI, Suppl. p. 261. St. Petersburg, 1904.

2. Page 26 of the reprint.

'compound' formed should prevent the appearance of trypsin in the filtrate. This I find to be the case. It may be said that what we have here is an instance of mutual action of colloids, but, as I think I have been able to show in the previous pages, there is no essential difference between this action and adsorption by solids from watery solution. On the other hand, it might be held by some, that the case is one of true chemical combination. To test this view I performed a similar experiment, taking caseinogen and malt amylase, and found that this enzyme is also held back by caseinogen, so that it appears to be merely adsorbed. This interpretation of the union of enzyme and substrate does not, of course, exclude a certain degree of specific relation between a particular enzyme and the substrate hydrolyzed by it, as we have seen there is considerable evidence of specific adsorption. It is probable, moreover, that it does not hold, in the same degree, for such enzymes as invertase, maltase, or lactase, when the substrate hydrolyzed is not colloidal, and where there seems to be a very close relationship between the chemical structure of the enzymes and the bodies split by them.

In another way the results described in the present paper are of interest in connection with enzymes. I refer to the action of electrolytes. From the work of Cole,¹ McGuigan,² and others, it follows that there is a certain opposition between the action of kations and anions. This indicates that enzymes are possibly electrically charged colloids. Victor Henri,³ in fact, speaks of trypsin as a negative colloid. I have tested the behaviour of a solution of Grüber's trypsin in the boundary apparatus, and found that it does indeed move to the anode, but how far this negative charge is due to the enzyme itself it is naturally impossible to decide until we have in our hands a pure preparation of the enzyme.

It is possible, however, to attack the problem in another way. Caseinogen in solution in alkali is electro-negative, if trypsin is also negative neutral salts, and especially those of bivalent kations, such

1. *Journal of Physiology*, XXX, pp. 202 and 281, 1904.

2. *American Journal of Physiology*, X, p. 444, 1904.

3. *C. R. Soc. di Biologie*, LIX, p. 132, 1905.

as calcium, should increase the adsorption. In some preliminary experiments I have made there seems to be some action of this kind, but it is difficult to obtain the correct relative concentrations so as to avoid precipitation. An experiment was also made on adsorption of trypsin by paper as follows :—

Four crystallizing dishes were taken, each containing a circle of filter paper, in two of them there was a watery solution of trypsin, in the other two a solution of trypsin of the same strength in $\frac{m}{350}$ CaSO_4 . After standing in a cool place for 24 hours, a paper from the watery solution was taken out, drained for 1 minute, placed in a flask, and heated to 100° in a steam sterilizer ; the same was done with a paper from the CaSO_4 solution. 160 c.c. of 5 per cent. caseinogen in ammonia were added to each, and they were then placed in the thermostat at 39° ; when warmed to the first was added a drained paper from the CaSO_4 trypsin, and to the second a similar one from the watery solution. The electrical conductivities were determined at intervals. There was not a very great difference between the two, but what there was showed that rather more trypsin had been taken up by paper under the action of CaSO_4 . The times taken to reach a change of 800 gemmhos were 165 minutes for the trypsin from CaSO_4 solution, and 192 minutes for that from watery solution. A similar experiment taking a $\frac{m}{350}$ solution of toluidini-blue instead of CaSO_4 was unsuccessful on account of mutual precipitation of the dye and trypsin. The precipitate, when filtered off and washed, showed itself to have considerable tryptic power. This subject requires further investigation.

The importance of the study of adsorption and surface-action in general in connection with the enzyme-action is emphasized by Bredig¹ in his article on 'Chemical Kinetics' in the *Ergebnisse* of Asher and Spiro.

8. *Oligo-dynamic action*

It has been pointed out by Pauli² that the puzzling phenomena called 'oligo-dynamic action' by v. Naegeli, and consisting in toxic

1. *Ergebnisse d. Physiologie*, I, p. 211, 1902.

2. Hofmeister's *Beiträge*, VI, p. 257 (footnote), 1905.

actions of distilled water which has been in contact with polished metal, are, in all probability, due to the presence of colloidal metallic hydroxides. Various solids, such as paper, are known to remove this toxic property. It occurred to me, therefore, that perhaps the adsorption of congo-red which occurs to some extent, even from distilled water, might be due to these hydroxides. I found that the ordinary laboratory distilled water caused considerably more adsorption of congo-red than that which I had prepared myself, using a tin condenser. From this latter, nevertheless, 22 per cent. was taken up. By allowing filter-paper to soak in this water previously the value was reduced to 18 per cent. It did not seem possible to get below this—no doubt the electrolytes dissolved from the glass and the traces remaining in the paper were sufficient to account for the effect.

VIII. SUMMARY OF RESULTS

1. The hyperbolic form of the curve of adsorption is confirmed.
2. The curve of electrical conductivity of successive distilled water extracts of gelatin has the same form.
3. It is impossible to wash out all the electrolytes from gelatin except by a practically infinite number of changes of water, each change removing a less percentage than the previous one.
4. The electrolytes are, therefore, neither chemically combined nor merely admixed, but in the intermediate form known as adsorption.
5. When gelatin has been washed nearly free from electrolytes it is capable of diminishing the conductivity of solutions of electrolytes in which it is placed. This it does by adsorbing them in a non-ionized condition.
6. The rate at which congo-red is taken up by paper is greatly accelerated by rise of temperature, but the total amount taken up when equilibrium is attained is less the higher the temperature.
7. The temperature-coefficient of the reaction-velocity is extremely low, so that the theory of Nernst as to the part taken by diffusion in heterogeneous reactions seems to apply to the case of adsorption.

8. At low temperatures equilibrium is attained very slowly, at room temperature at least 24 hours being required.

9. The adsorption-compound of gelatin and inorganic electrolytes is also dissociated as the temperature rises.

10. Raising the temperature rapidly to 100° tends to fix congo-red in paper so that it is afterwards extracted by water with considerable slowness.

11. No evidence was obtained of any production of heat in adsorption.

12. The reaction between congo-red and cellulose is reversible, as is also that between gelatin and electrolytes.

13. Dyes forming colloidal solutions are extremely sensitive to electrolytes in regard to their adsorption. The effect seems to be proportional to the degree of their colloidal nature or size of colloid particles.

14. The action of electrolytes may be expressed as follows : In the case of electro-negative dyes, like congo-red, kations facilitate adsorption, anions depress it. In the case of electro-positive dyes like toluidin-blue kations depress, anions facilitate. But in both cases the effect of anions is very small compared to that of kations.

15. The effect of bivalent kations is considerably greater than twice that of univalent kations.

16. Salts of the heavy metals which form positively charged colloidal hydroxides in solution have a very powerful effect in promoting adsorption of electro-negative dyes.

17. There is evidence of the carrying-down with the adsorbed dye of the facilitating ion.

18. The presence of a stable colloid, like gelatin, protects congo-red from the action of electrolytes. A negative charge seems necessary for this action, since egg-albumin, although exercising a similar action in alkaline solution, had the opposite effect in acid solution.

19. The explanation of the action of electrolytes, as well as that of other electrically-charged colloids is to be found in the negative charge of non-conductors, like paper, when immersed in water. (Quincke).

20. The different behaviour of silk and paper towards electro-negative and electro-positive dyes, as also the influence of alcohol on the process, is to be explained by the results of Coehn on the influence of the respective dielectric constants on the charge.

21. When gelatin is precipitated by tannin its adsorbed electrolytes are split off.

22. There is no evidence of a sudden separation of electrolytes at the moment of death. There is a gradual one when a living tissue is warmed from 11°C . to 56°C .

23. In the process of clotting of blood there is a *diminution* of electrical conductivity, so that ions (probably Ca^{++}) disappear from solution.

24. The adsorption affinity of gelatin for acid-fuchsin is greater than that for congo-red, while that of paper is the same for both.

25. A theory of dyeing is suggested on the basis of adsorption in relation to electrically-charged colloids.

26. An explanation, apart from different permeability of the cell, is suggested for the staining of living cells by basic dyes and the non-staining by acid dyes.

27. The nature of the compounds between acid and basic dyes is investigated. They appear to be uncharged colloids, but the evidence as to whether they are colloidal adsorption compounds or true chemical compounds is not decisive.

28. Evidence is brought forward to show that the union between enzyme and (colloidal) substrate is of the nature of adsorption. The adsorption of trypsin by paper is facilitated by calcium sulphate.

TABLE OF DYES

Name	Chemical nature	Molecular weight	Diffusion	Size of Particles	Electric migration	Electrical conductivity in gemmhos	State of solution	Action of							
								Kations			Anions			Negative colloid	Positive colloid
								H	K	Ca	OH	Cl	SO ₄		
Picric acid	Colour acid	229.2	Rapid	Partially sub-microscopic	...	...	?	...	o	...	...	...	...	...	...
Rosolic acid	Colour acid	304.2	Very slow	Marked Tyndall phenomenon	To anode in weak alcohol	...	Negative colloid in weak alcohol	...	+	+	...	...	(slight)	...	...
Methyl orange	Colour acid	327.37	Fairly rapid	...	?	...	?	...	...	...	...	...	...	...	...
Eosin	K salt of colour acid	647.9	Rapid	A-microscopic	?	2225 $\left(\frac{m}{100}\right)$	Negative colloid	+	...	+	-	...	...	...	...
Acid fuchsin	Na salt (acid) of colour acid	447.44	Slow	...	To anode	...	Negative colloid	...	...	+	...	...	...	...	...
Congo red	Na salt of colour acid	696.68	Very slow	Sub-microscopic	To anode	5660 $\left(\frac{m}{100}\right)$	Negative colloid	+	+	+	-	-	-	No ppt.	Negative protects, positive assists
Anilin blue	Na salt of colour acid	734.34	Very slow	Sub-microscopic	To anode	7380 $\left(\frac{m}{100}\right)$	Negative colloid	...	+	+	+	...	...	...	Ca
Nigrosin	Na salt of colour acid	About 722	Very slow	Sub-microscopic	...	...	...	...	+	+	+	...	...	...	...
Scharlach R	'Indifferent' dye	380.36	Nil into weak alcohol	Sub-microscopic	To anode in weak alcohol	...	Negative colloid	...	+	Ppt.	...	...	...	...	...
Methylene blue	Chloride of colour base	319.81	Slow	A-microscopic	To kathode ?	549 $\left(\frac{m}{100}\right)$	Positive colloid	-	-	-	+	...	+	Ppt.	No effect

TABLE OF DYES—Continued

Action of															
Name	Chemical nature	Molecular weight	Diffusion	Size of particles	Electric migration	Electrical conductivity in gemmhos	State of solution	Kations		Anions			Negative colloid	Positive colloid	Stable colloid
								H	K	Ca	OH	Cl			
Toluidin blue	Chloride of colour base	291·75	Very slow	A-microscopic	To kathode	...	Positive colloid	-	-	-	+	+	...	...	
Fuchsin	Chloride of colour base	337·8	Slow	Partially sub-microscopic	? Ppt. on kathode	...	?	...	o	...	...	...	...	...	
Fuchsin in anilin water	Do. containing free base	...	Very slow	Sub-microscopic	To kathode	...	Positive colloid	-	-	-	...	...	...	...	
Methyl violet	Chloride of colour base	(393·85)	Slow	Partially sub-microscopic	...	...	Positive colloid (V. Henri)	...	...	...	...	...	...	...	
Neutral red	Chloride of colour base	287·82	Very slow	Partially sub-microscopic	...	...	...	-	...	...	...	...	...	...	
Magdala red	Chloride of colour base	459·79	Very slow	...	...	...	Positive colloid (V. Henri)	-	...	...	+	...	...	...	
Safrania	Chloride of colour base	436·78	Very slow	...	To kathode	...	Positive colloid	...	o	o	o	...	...	...	
Eosin Methylene blue	Compound of + and - dyes	11138·32	Nil as such	...	o	82·5 $\left(\frac{m}{1000} \text{ at } 100^{\circ}\right)$	Colloid unchanged	...	o	o	o	...	...	...	
Anilin blue Methylene blue	Compound or + and - dyes	995·65	Nil as such	Tyndall phenomenon in weak alc. hol	o	...	Colloid unchanged	...	...	...	...	...	...	...	

NOTES TO TABLE OF DYES

The *molecular weight* of methyl-violet is given as that of the penta-methyl derivative.

Diffusion. The words 'very slow' mean that a certain amount had passed through parchment paper by the end of 24 hours.

The statements as to the ultra-microscopic behaviour of dyes in the column headed 'Size of particles' are taken from the work of Michaelis referred to in the text.

'Sub-microscopic' means that the solutions are resolvable into particles.

'A-microscopic' means that they are optically homogeneous.

Electric migration. It was difficult to be certain in the case of methylene blue since bleaching occurred by the electrolytic chlorine.

The measurements of *electrical conductivity* were taken at 39° C unless otherwise mentioned. They are not to be looked upon as absolutely correct, since the dyes were not specially purified, except the eosin methylene-blue compound.

State of solution. It is impossible to make definite statements as to certain dyes, *e.g.*, eosin, since they have some properties of colloids, but not all.

The columns headed 'Kations' and 'Anions' refer to the action of these bodies on adsorption by paper. It is to be understood that in all cases the action of Anions is very much less than that of Kations.

The columns 'Negative' and 'Positive colloid' refer to actual precipitation, not adsorption.

The column 'Stable colloid' refers to the effect of this on the facilitation or inhibition of adsorption by kations.