DIRECT MEASUREMENTS OF THE OSMOTIC PRESSURE OF SOLUTIONS OF CERTAIN COLLOIDS¹

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The statement is often to be found in recent contributions to the chemistry of colloidal solutions, that the colloidal solution is not a 'true solution,' but merely a suspension of colloidal particles in the solvent, possessing no osmotic properties such as are shown by true solutions of crystalloids.

This statement indicates that a hard and fast line can be drawn between colloids and crystalloids, and that the difference is not merely one of degree, arising from the great size of the suspension particle in the case of the colloid, as contrasted with the small size in the case of the molecule of the crystalloid.

There exists, however, at the present time not a particle of experimental evidence for the view that colloidal solutions are *qualitatively* different from crystalloidal solutions. Evidences of quantitative differences exist in abundance; between a typically colloidal and a typically crystalloidal solution the differences are many and striking, but between the two types there are many transitions without an obvious break anywhere, and as the character of the dissolved substance varies so do the osmotic and all the other physical characters of solution vary without a sudden change being detectable at any point.

To put an extreme case; there is no evidence that a visible suspended particle in an emulsion does not exert the same osmotic

1. The expenses of this research have been partially defrayed from a grant allotted to one of us by the Government Grant Committee, Royal Society.

effect as a molecule of a dissolved crystalloid, that the usual indirect methods do not show any osmotic pressure in an emulsion may simply depend upon the fact that the pressure on account of the low concentration of the emulsion particles is too small to be determined by such methods.

It will be shown in this paper, that when the more delicate method of direct measurement of osmotic pressure is used solutions of many colloids show a very distinct and definite pressure. At a certain size in the colloid molecule, or, as it might preferably be termed, 'solution aggregate,'¹ the direct method also ceases to give measurable readings of osmotic pressure, but in all probability this is only due to a certain size of solution aggregate being attained which transcends the delicacy of the direct method, and if we possessed a delicate enough method of detection osmotic properties might still be found in the suspended colloidal masses.

Thus, it has recently been shown by Ross² that particles of such a size as mammalian blood corpuscles can travel upwards through a solid agar-agar jelly, against the action of gravity, and permeate the whole jelly. In such a case there can be no convection currents, and the movement is probably due to diffusion of the corpuscles into the jelly. But true diffusion, apart from convection currents, or directive movement of charged particles in a field of varying electrical potential which is here obviously excluded, can only be due to osmosis.

If the diffusive movement of crystalloids be due, as is generally accepted, to osmotic pressure differences, why should not diffusive movement of colloids be due to osmotic pressure differences?

All staining acts due to the penetrating of colloidal dyes are also evidences of the diffusibility, and hence of the osmotic pressure, of colloids.

As the colloidal particles of the dye become adsorbed, or chemically combined with the tissue or fabric being dyed, fresh

1. This term was introduced by Moore and Parker (*vide infra*) to express the physical constant that in the case of the colloid corresponds to the molecular weight of the crystalloid; the solution aggregate may consist of many or few chemical molecules, but for purposes of producing osmotic pressure each solution aggregate has the same effect as a dissolved molecule or ion.

2. *Brit. Med. Journ.*, Vol. I, 1906, p. 1027.

particles pass in from the solution outside on account of the lowered concentration of the dye within the tissue ; but if the colloidal particles be supposed to exert no osmotic pressure there remains no reason why any particles should pass into the tissue, because there would exist no falling gradient of pressure urging them in that direction.

If a suspended or dissolved molecule or particle does not exert any osmotic pressure, there is no longer any reason why it should dissolve or be suspended (unless the specific gravity of the solvent is exactly the same as its own), nor any cause why the molecules or particles should distribute themselves uniformly throughout the solvent by a process of diffusion. By the formation of convection currents, or by mechanical shaking and admixture of solvent and particles, a *temporary* emulsion might be formed in absence of osmotic pressure, but the condition would be a temporary one only, the duration of which would depend upon the difference in specific gravity of particles and solvent, the size of the particles, the internal friction between particle and solvent, and other physical factors. The only active opposition to sedimentation is that arising from the osmotic pressure which produces diffusion from a region of high concentration to one of low concentration, and so equalises distribution of the dissolved (or suspended) substance throughout the whole of the volume of solvent.

While it thus necessarily follows that if osmotic pressure ceased to exist in the molecules or particles of a substance in solution or suspension, sedimentation must occur, the converse does not follow that because sedimentation does take place there exists no osmotic pressure. This condition may arise on account of the tendency of osmotic pressure to prevent sedimentation being overcome or outbalanced by the forces tending to produce sedimentation or separation into two phases.

At a certain concentration there is a balance or equilibrium between the two opposing forces, and at this point the solvent is just saturated with the solute or dissolved substance. If the concentration is less than that of the equilibrium point, then, if any undissolved

substance be present, the osmotic pressure tends to send it into solution until the equilibrium point is reached; if, on the other hand, the forces of cohesion and gravity outbalance the tendency to solution given by the osmotic pressure, then substance is thrown out of solution and sedimented until the equilibrium point is attained.

The fact that the osmotic pressure in the case of colloids or suspensions is low compared with that shown by crystalloids does not minimize the importance of its study, because that very circumstance, viz., large size of colloid molecule or solution aggregate, which renders the pressure low, provides the means of making the low pressure more effective by decreasing the permeability of any membrane containing the solution for the large molecule. So that osmotic pressure of colloids becomes apparent and is brought into action under conditions in which the greater osmotic pressure of crystalloids produces no effect. Further, the osmotic pressure of colloids is of especial importance in bio-chemistry because so much of the nutritive material of the living cell is presented to it in colloidal form, and so many of the normal and pathological products of cell activity are colloids. If such colloids possess no osmotic pressure then they can only be carried from one position to another by purely mechanical means, or by the activity of the cell, and the usual powerful aid to such processes given by the tendency to diffuse from a position of higher to one of lower concentration, will be entirely lacking.

It will, accordingly, be well to sift the supposed evidences against the possession of osmotic pressure by colloids before proceeding to a description of our experiments.

The first point is the large size of the colloid molecule, which is supposed by many to be so great and so nearly approaching to that of a mechanically suspended particle that the laws governing chemical molecules in solution cannot be expected to apply to it.

In reply to this objection it may be pointed out that the range of molecular weights in the case of typical crystalloids is much greater than that between the crystalloids with higher molecular weights and typical colloids. Thus the hydrogen ion, with a molecular weight of

1, and the cane sugar molecule, with a molecular weight of 342, have precisely the same effect in producing osmotic pressure, the great variation in the mass of the molecule causing not the slightest variation in the result. And bodies with even higher molecular weights, such as lecithin, with a molecular weight of 780, behave like true crystalloids in producing normal osmotic pressures. There is nothing inherently improbable, therefore, in a colloid, with a solution aggregate of 40,000 or 50,000, possessing a normal osmotic pressure corresponding to this higher value. The ratio between hydrogen ion and cane sugar molecule, where the law of osmotic pressure is admitted to hold rigorously, is 1 : 342, while that between cane sugar and serum albumen is 342 : 60,000 approximately, or 1 : 178; and for cane sugar to gelatine, which behaves as a typical colloid, it is 342 : 10,000, or 1 : 29. Accordingly, the range between typical colloid and typical crystalloid is shorter than that between different crystalloids.

A second argument against typical colloids possessing osmotic pressure is that the usual methods employed for estimating osmotic pressure, viz., lowering of freezing point or depression of vapour pressure (rise in boiling point), give negative results, or readings which lie within the limits of experimental error.

In order to make clear the relationship in the values to be expected in the case of determination of the solution aggregate of a colloid, (*a*) by an indirect method, such as determination of depression of freezing point, and (*b*) by a direct method, such as reading the osmotic pressure in millimetres of mercury, as shown by an osmometer like that described below, the following calculation may be set forth :—

Taking water as the solvent which interests us most, and supposing, for simplicity of calculation, we are dealing with a substance which is not ionized in solution, then the molecular depression of the freezing point is approximately 18.4°C. , which means that the molecular weight in grams of the substance dissolved in 100 c.c. would lower the freezing point 18.4°C. , or a normal solution containing the molecular weight in grams dissolved in a litre would give a lowering of freezing point of 1.84°C. Now when the molecular weight in grams occupies a volume of 22.33 litres the osmotic pressure

is one atmosphere, or 760 millimetres of mercury pressure. Hence, the normal solution containing the molecular weight in grams in 1 litre has an osmotic pressure of 22.33 atmospheres, or 22.33×760 millimetres of mercury, and this corresponds to a lowering of freezing point of 1.84°C. , the calculation of the osmotic pressure due to a lowering of freezing point of 0.001°C. is simple from this relationship, it is :—

$$\Delta \text{ of } 0.001^{\circ}\text{C.} = \frac{22.33 \times 760}{1.84 \times 1000} = 9 \text{ mm. of mercury}$$

When it is remembered that the smallest reading of difference of freezing point that can be measured is 0.005, that is half of one of the smallest divisions on the usual Beckmann's thermometer, it follows that an osmotic pressure of 45 millimetres of mercury would escape detection by the freezing point method.

A similar result follows for the boiling point method, and it is obvious that the only method known for estimating osmotic pressures of this order, is the direct method by a membrane impermeable to the colloid.

Accordingly, the fact that freezing and boiling point methods give no reliable readings does not prove that colloids possess no osmotic pressure.

A third argument usually urged against the possession of osmotic pressure by colloids is that the readings of osmotic pressure shown in the osmometer by the direct method are due to the osmotic pressure of crystalloids present as impurities along with the colloids. This argument is most difficult to discuss, both from the theoretical and experimental points of view, because there is no doubt that the presence of inorganic matter, which would undoubtedly have to be regarded as crystalloidal if present by itself, along with colloid in the solution is responsible in some fashion for the production of the osmotic pressure. It is precisely in the case of those colloids which are closely associated in solution with crystalloids, that positive results are obtained by the direct method.

No readings of osmotic pressure by the direct method have ever been obtained by any observer in a solution entirely free from

inorganic salts. In those cases where inorganic salts are not closely associated with the colloid, such as starch solution (*vide infra*) no osmotic pressure is shown, and further, when colloid, such as proteid, is dissociated from salts, the osmotic pressure is decreased. Hence, the osmotic pressure which can be directly demonstrated by the osmometer in the case of colloidal solutions is closely associated, in some manner, with the presence of crystalloidal substances also present in the solution.

This is far, however, from saying that the colloids are not responsible for the production of the osmotic pressure, or that the osmotic pressure observed is due to crystalloids present as impurities which would give the pressure apart from the presence of the colloids.

The position is one requiring careful analysis, and consideration of the various experimental results, in order to determine the share played by crystalloid and colloid in the production of the osmotic pressure.

In the first place, there is abundant proof in the experimental evidence recorded by all observers on direct measurement of osmotic pressure in colloidal solutions, that the osmotic pressure is not due to any substance, colloidal or crystalloidal, which can diffuse even slowly through the membrane.

This is shown by the fact that the manometer, attached to the osmometer, rises at first rapidly and then more slowly to a maximum value, *and then remains stationary for days at that maximum value.*

This would not be the condition of affairs were the pressure due to any substance capable of diffusing through the membrane; then there would be a rise to a maximum value, followed by a fall as the concentrations of the substance diffusing through tended to become more nearly equal on the two sides of the membrane. Such a condition is realised when a diffusible substance, such as crystalloid alone, is placed in solution on one side of the membrane and pure solvent on the other side. Thus, with a one per cent. sodium chloride solution on one side and water on the other side, although the difference in real osmotic pressure of the two fluids is initially enormous, there is only a rise of a few millimetres in the first

few hours, which rapidly subsides to zero, and then analysis for chlorides on the two sides shows practically equal amounts. A more slowly diffusing crystalloid shows a slower and smaller rise and a slower fall, but there is never a maximum which is followed by a constant summit or plateau. With a membrane of parchment paper, such as was employed in our experiments, the equalisation was so rapid that even with sugar solutions no appreciable rise of pressure was obtained, and analysis at the end showed zero pressure and equal concentration in sugar on the two sides of the membrane.

The osmotic pressure readings obtained with colloids could not therefore be due to crystalloids not associated with colloids, because such crystalloids must have diffused through the paper membrane unless prevented from doing so by some association with the colloid either by adsorption or chemical combination.

Under such conditions the crystalloid must be regarded not as an impurity but as an integral constituent of the colloid molecule or solution aggregate, which confers on the aggregate the property of giving osmotic pressure in measurable amount. In the absence of the crystalloid the colloid molecules, or aggregates, are not stable in the solution, but run together to form solution aggregates so large that the osmotic pressure becomes too low for measurement.

The change in value of the solution aggregate when the amount of crystalloid is altered has been drawn attention to previously by Moore and Parker.¹

These observers have shown that the osmotic pressure of the serum proteids changes, in the same sample of proteid, when the amount of diffusible salt present is changed, thus the same serum when in equilibrium against distilled water gave an osmotic pressure of 28.2 mm., as against 22.2 mm. with 0.7 per cent. sodium chloride, and 19.3 mm. with 1 per cent. sodium chloride, leading to values for the 'solution aggregate' of 40,383, 50,504, and 58,327 respectively. Still greater changes were observed when dilute alkali was allowed to act upon the serum, the osmotic pressure rising to about five times

1. *Amer. Journ. of Physiology*, 1902, Vol. VII, p. 261.

its value, and the solution aggregate falling approximately from 50,000 to 10,000.

These results show that the degree of association of the proteid to form 'solution aggregates' of varying size depends to a large extent upon the amount and nature of the crystalloids present, at the same time the colloid is absolutely necessary for the appearance of the osmotic pressure, the crystalloids alone upon one side as against pure solvent on the other producing no permanent pressure.

The permanent osmotic pressure readings obtainable, therefore, when colloids present in common solution with crystalloids are in equilibrium with crystalloids only in solution on the other side of the membrane of the osmometer, are due to colloid containing crystalloid in combination or adsorption. The function of the crystalloid is to keep the colloid in solution, and by its union with it prevent the formation of too large solution aggregates to give pressure readings, and the function of the colloid to render the crystalloid attached to it indiffusible so that it cannot pass the membrane, and hence gives rise to a pressure.

It does not follow that the presence of any particular crystalloid in co-solution with colloid, will necessarily give rise to an alteration in osmotic pressure. For this purpose it is necessary that the colloid and crystalloid must be capable of union in the form of combination or adsorption with one another. Thus it will be shown that starch, in distilled water, gives no measurable osmotic pressure, and that the addition of an equal concentration of sodium chloride on the two sides of the membrane has no effect in producing an osmotic pressure, but, on the other hand, as pointed out above, alteration of the concentration of sodium chloride present along with the serum proteid at once causes an alteration in the osmotic pressure.

It is clear, therefore, that in the case of those colloids which unite with crystalloids when in common solution that the crystalloids are not to be regarded as impurities, but as essential to the particular condition in solution of the colloid under which the osmotic properties are being measured, and that the osmotic pressure is due to the union

of crystalloid and colloid which preserves the solution aggregate of which the osmotic pressure is being determined.

The first definite measurements of osmotic pressure of colloids, by the osmometric method, were made by Starling,¹ who determined the osmotic pressure of serum, using a gelatine membrane, constructed between layers of peritoneal membrane.

Moore and Parker² published, in 1902, observations on the direct measurement of osmotic pressure with egg-albumen in native condition and after prolonged dialysis, serum-proteid and alkali-albumen formed from serum-proteid, and solutions of various soaps. These observers used parchment paper as a membrane, and a bell-shaped osmometer, and whilst criticising Starling's applications of the osmotic pressure of the serum proteids to the problems of lymph absorption and glomerular activity in the kidney, confirmed the experimental results obtained by Starling that the serum proteids possessed an osmotic pressure.

Moore and Parker introduced in their paper the term 'solution aggregate' for the aggregation of chemical molecules which gives rise to the unit of the colloid in solution corresponding to the chemical molecule of the crystalloid in solution. They showed that the osmotic pressure of a colloidal solution depended upon the value of this aggregate, and that in the case of dilute solutions of the sodium soaps, the value of the solution aggregate varied between thirty and sixty times the value of the molecular weight. As stated above, the value of the solution aggregate with the same colloid was shown to vary with the amount of crystalloid present. The effect of such variation in physical aggregation of molecules of proteid upon protoplasm formation, and in the building up of the minute structures of the cell, was considered, and it was pointed out that the activities of the colloid would vary with the degree of aggregation.

1. *Science Progress*, April, 1896; *Journ. of Physiology*, 1896, Vol. XIX, p. 312; 1899, Vol. XXIV, p. 317; Schäfer's, *Text-book of Physiology*, Vol. I, p. 307.

2. *Amer. Jour. of Physiology*, 1902, Vol. VII, p. 261.

E. Waymouth Reid¹, in a paper, published in 1904, on the osmotic pressure of solutions containing native proteids, arrived at the conclusion that the pressures observed by Starling, and by Moore and Parker, were not due to the proteids, but to certain other substances recoverable from the washings of the proteids after precipitation with neutral saline (Am_2SO_4).

The nature of these substances upon which, according to Reid, the osmotic pressure depends was not determined at the time, nor have been since, but it was shown that alone they did not produce pressure, but only when proteid was also present, or a membrane used which had been used previously with proteid.

Reid was led to the conclusion that the osmotic pressure shown by serum was not due to the proteids by the observation that the osmotic pressure was not exactly proportional to the amount of proteid

1. *Journ. of Physiology*, Vol. XXXI, 1904, p. 438.

[Note by B. Moore.—I desire to take this opportunity of replying briefly to certain criticisms made by Reid, in the above paper, upon the experimental methods used by Parker and myself in our former work, especially as some of them would also apply to the present experiments. Reid objects to the results on account of the osmometer used by us not having been stirred, but we expressly point out in our paper that the natural process of diffusion and differences in specific gravity in the bell osmometer acts as a most excellent stirrer, this being one of the chief advantages of that form of osmometer, as also of the one used in the present research, in this way we were able to dispense with the 80,000 daily mechanical stirrings used by Reid, and the analysis, mentioned on p. 276 of our paper, shows how complete the equilibrium was upon the two sides at the end of the experiment, the amounts of chloride being practically equal, so that it is difficult to see how stirring would have altered the results.

It is objected that our membrane being impermeable to soaps, the presence of soaps in the blood serum seriously invalidates the conclusion that the pressures observed are due to the proteids, but collateral evidence in our paper clearly shows that the osmotic pressure due to the traces of soaps present in blood serum could not appreciably affect the results, since it is stated by us that as much soap as one-half per cent. only causes a pressure of 6.5 mm. of mercury.

It is suggested by Reid that, as the duration of each experiment is not given, one cannot judge whether sufficient time was allowed for equalization of substances in solution (and to which the membrane was permeable) on the two sides of the membrane before the final reading of pressure was taken, and it is further suggested that the lower readings when sodic-chloride was used as outer fluid instead of distilled water, and where 1 % sodic-chloride replaced 0.7 % sodic-chloride solution, with one and the same sample, are internal evidence of the readings having been made before equilibrium had been attained. Considering the nature of the experiment which is being carried out, namely, that of recording the final value of a pressure which rises from zero, it would seem scarcely necessary to state that readings were continued until a steady pressure was attained; but, as it appears to be necessary, I may now state that the observations were continued until the pressure had been steady for two or three days. Further, in a note to our table, p. 276, we point out that with our osmometer a difference of 1 % of sodic-chloride on the two sides gave, at the maximum point of pressure in the process of equalization, only 4 mm. of mercury, and that the greatest difference of concentration of sodic-chloride on the two sides at the end of an experiment never exceeded one-twentieth of this amount, as determined by analysis, so that pressure due to difference in concentration of diffusible crystalloids might, we thought, be neglected. Regarding the differences in osmotic pressure when the proteid is in distilled water, 0.7 % saline and 1 % saline respectively, we expressly treat this at considerable length in our discussion of the results as an important point showing difference in aggregation of the proteid under these conditions.

It is this same alteration of state of aggregation of proteid due to lengthy manipulation, and removal of the natural alkali and salts of the serum, which appears to me to be the explanation of the loss of osmotic pressure observed by Professor Reid in his own experiments, and not the removal of hypothetical organic substances other than proteids from the serum.

present in the serum, and that after many washings of the precipitated proteid with neutral salts, such as ammonium sulphate, or by sodium chloride and acetic acid solution, and re-dissolving the proteid, a solution could be obtained which showed no osmotic pressure, and finally, that by adding the dialysed washings to such osmotic pressure free solutions, an osmotic pressure could be once more obtained.

In our opinion, these facts are capable of explanation otherwise than on the supposition that the serum proteids do not give an osmotic pressure.

In the first place, the fact that the osmotic pressure is not absolutely proportional to the amount of proteid present, is probably due to the fact already mentioned above, that the pressure depends not alone upon the amount of the colloid, but upon its degree of aggregation in solution, and this, in turn, upon the salts of the serum and its reaction, so that the proteid of the serum may be responsible for the pressure without there being an absolute linear proportionality in different samples of serum.

Secondly, in the prolonged process of precipitation and washing used by Reid, the serum proteid is removed from all the alkali and all the neutral salts with which it is combined or adsorbed in the natural condition, so that it is scarcely to be wondered at that its state of aggregation changes, and that it finally does not give a readable osmotic pressure.

Finally, with regard to the production of osmotic pressure once more on adding the dialysate of the first washing, it may be remarked that the pressures observed, of three to five mm. of mercury, are too small to inspire confidence in any deduction from them, and, secondly, that even then such readings are only obtained when the pressure free proteid is added to the dialysed washings, and that the true explanation of the small result obtained may be a partial return of the proteid to a less state of aggregation forming an approach to its natural condition in the serum.

More recently, Reid¹ has published experiments carried out

1. *Journ. of Physiology*, 1905, Vol. XXXIII, p. 12.

with haemoglobin solutions, and finds that this proteid does possess a genuine osmotic pressure, so that it is, according to him, in 'true solution,' while serum proteids are not. It would be interesting to test whether haemoglobin also loses its osmotic pressure after being precipitated and many times washed with ammonium sulphate solution, and if there was a partial restoration of osmotic pressure on dissolving the haemoglobin in the dialysed washings. Until this laborious process has been carried out, there is little logical basis for believing in a difference in character between the osmotic pressure of serum proteids and that of haemoglobin.

To sum up the experimental evidence hitherto published regarding osmotic pressure of colloids, osmotic pressure has only been recorded in solutions of colloids which are capable of adsorption with crystalloids, or of forming combinations with crystalloids. The pressure is not, however, due to crystalloids alone, for it is only found when colloid impermeable to the membrane is present, and the pressure remains permanent after all diffusible colloids have equalized on the two sides of the membrane. The pressure varies with the same amount of colloid, according to the state of aggregation of the colloid, and this can be altered according to the amount and character of the crystalloid present in the solution.

There is no evidence, save the failure of the direct osmometric method to give readings, that osmotic properties entirely cease whatever the size of the aggregate, and the failure of the method may be merely due to the low osmotic pressure given by such huge aggregates, for the fact of diffusion occurring is *prima facie* evidence in favour of the existence of an osmotic pressure if we possessed delicate enough methods to detect it.

EXPERIMENTAL METHODS

The osmometer¹ used for the experiments is illustrated by the accompanying sectional drawing (Fig. 1) and photograph (Fig. 2).

1. The construction of the osmometer, and preliminary results, were described in a short paper read at the Southport Meeting of the British Association, 1903. 'A new form of osmometer for direct determination of osmotic pressure of Colloids,' by B. Moore.

The drawing shows the osmometer alone in section, and the photograph the manner in which it is connected up and joined to the manometer by thick walled rubber tubing.

When it was desired to work at a constant temperature different from that of the laboratory, the osmometer was immersed in a water bath kept at the desired temperature by means of a thermo-regulator.

FIG. 1. Sectional view of Osmometer magnified $1\frac{1}{2}$ times.

The osmometer consisted of platinum linings, or capsules, supported and held in apposition by thick walled brass chambers.

Each half of the platinum lining was the same in construction, being a circular shallow capsule with a platinum tube leading away from its base and projecting beyond a similar, but larger, tube in the thick walled brass case. Around the rim of each platinum capsule was a flange, which faced over the similar flat flange shown on the outer brass case. A thick platinum grid came, when the apparatus was screwed together, between the mouths of the two capsules, and served to support the membrane of parchment paper and prevent it from sagging as it came under the osmotic pressure. As the drawing is placed, the upper chamber contained the colloidal solution and the lower the fluid used against the colloidal solution and containing no colloid.

Three circular rings of thin soft rubber were arranged, one between the lower platinum flange and the grid, one between the grid and the parchment paper membrane, and one between the membrane and the upper platinum flange. The platinum grid and upper case were prevented from turning round, as the apparatus was being screwed together, by means of two small brass pins, not shown in the section, which were let in at opposite ends of a diameter of the lower thick casing close to the screw thread; these projected and caught two recesses cut in the edge of the platinum grid, and also corresponding recesses in the upper case. This precaution is absolutely necessary, otherwise, the turning round which occurs on screwing up tears up the membrane and rubber rings, and invariably causes leakage.

The osmometer is screwed together by means of a male screw on the lower casing and a female screw on a circular joining piece of thick metal possessing a flange, which fits against a flange on the upper case as shown in the drawing. The thumb hold on the lower case enables the whole to be tightly screwed together.

The capacity of each chamber was about 20 c.c., the diameter 5 cm., and the depth 1 cm.; so that a large surface for diffusion and rapid equalization of crystalloids was offered, relatively, to the volumes of the chambers.

In fitting up the osmometer a circle of parchment paper, the

exact size of the platinum grid, is cut out from a sheet, thoroughly wetted, and applied to the grid, which is then placed in position and the apparatus screwed tight. Any leakage is then tested for by putting the osmometer in a vessel of water, blowing air in, and observing whether any air bubbles appear. In order to prevent blowing the

FIG. 2. Photograph of osmometer fitted up and joined to manometer.

membrane away from the platinum grid, the two outlet tubes of platinum may be joined, by rubber tubing, to a glass T piece, and blown up together, as any leakage is usually to the outside and not from one chamber to the other, good parchment paper in sheet being remarkably free from imperfections.

The next step is the filling of the osmometer. The chamber on the upper, or colloid, side is first filled with the solution, in which the pressure of colloid is to be measured, by means of a narrow tubed pipette, and the proximal limb of the mercurial manometer, which is of narrow bore, as also the connections and funnel on the manometer side (see Fig. 2) having been previously filled with the solution, or with oil, and clipped off at the end to be connected to the osmometer, the osmometer and tubing are joined. Care should be taken to avoid inclusion of air in making the connection, as far as possible, but a bubble or two of air only slightly delays the time of reaching a steady pressure reading. By care in manipulation, any air accidentally included can be got into the vertical tube, and so out by the funnel. The screw clip on the vertical tube is at first left open. The osmometer is then turned other side up, and the other chamber of the osmometer is similarly filled with the non-colloidal solution, which is to come into equilibrium with the colloidal solution, save for content in colloid. This chamber is, in turn, joined up to its tubing and funnel, which have been previously filled with the same fluid as the chamber, and the osmometer is turned so that this chamber lies lowest down. The two funnels are supported at the same level, and the level of the fluid in them is made the same. The clip is then left open on the colloid side for about half-an-hour to let the whole come, approximately, into equilibrium, then the screw clip is closed, shutting off communication with the funnel on the colloid side, but leaving open the communication with the manometer. A zero reading of pressure is now made on the manometer, and readings are continued at intervals, as seen in the tables of experiments, until a steady pressure is attained, as shown by constancy of reading.

It is important, as a saving of time, that the chamber on the colloid side be filled first, joined up as above described, and clipped on the rubber tubing just beyond the platinum tube, so as to get the pressure from osmometer to funnel upon the membrane before the other side is filled, for if the other side be filled first, the membrane is pressed away from the platinum grid, and it takes a considerable time before diffusion of water through the membrane increases the

volume in the upper chamber, and forces the membrane tight against the grid, until this takes place, the pressure does not begin to rise in the upper, or colloidal, chamber.

EXPERIMENTAL RESULTS

Experiments with Gelatine Solutions

A great advantage of the osmometer described above is that, on account of the unalterable nature of the parchment paper membrane, and of the platinum walls of the chamber, solutions of colloids which do not undergo heat coagulation on boiling, such as gelatine or starch, can be completely sterilized by raising the chamber and its contents to nearly 100°C , and keeping at that temperature for some minutes. The advantage of this is that an experiment, with such a substance as gelatine, can be continued for months if necessary, and the point in this way settled as to whether the observed osmotic pressure be due to some slowly diffusing crystalloid present as an impurity along with the colloid. If this were the case, equalization must, in the end, occur, and the pressure fall to zero.

In the experiment with gelatine recorded below, the same quantity of solution of gelatine was used throughout, during a period of two months and twenty-five days, or eighty-five days (Nov. 4th, 1902, till Jan. 28th, 1903). When taken out of the osmometer at the end of this long period it was, to all outward appearance, the same as when it was put in, forming a stiff, clear jelly, which melted at 24 to 25°C , began to re-set at 22.5°C , and was completely set at 21°C . The fluid on the other, or water, side of the membrane was a clear watery fluid, which gave a negative result on testing for protein by the biuret, acetic acid and ferrocyanide, and tri-chloro-acetic acid test. It contained 0.074 per cent. of organic matter, and 0.032 per cent. of ash.

The gelatine solution, which had been made up at the beginning of the experiment lasting nearly three months to contain 10 per cent.

of gelatine, contained, at the end, 9.56 per cent. of organic matter destroyed by incineration, and 0.17 per cent. of inorganic ash.¹

As soon after the commencement of the experiment as equilibrium had been reached, the gelatine solution showed an osmotic pressure of 74 mm. of mercury at 31° C. (Nov. 11th, 1902); near the end of the experiment, over two months later, during all of which time dialysis and equalization of any diffusible crystalloids present had been going on, it showed (on Jan. 27th, 1903) a pressure of 70 mm. at 26° C.

This constancy of the pressure, and its persistency after such a period, show that it is something existing when equilibrium has been attained, and not a passing phase due to slowly diffusing crystalloids, and since, in the absence of the gelatine, the soluble crystalloids would have equalized on the two sides in the course of a few days, we regard the experiment as a crucial one, showing that colloidal solutions can exhibit osmotic pressure.

The experiments with gelatine were also used to determine, as far as possible, the effect of temperature on osmotic pressure of colloids; gelatine, on account of its not coagulating by heat, being a suitable colloid for this purpose. The results show clearly that the osmotic pressure rises with the temperature. The rise is not proportional to the absolute temperature, occurring more rapidly, but this may arise from partial dissociation of the colloidal molecules, or solution aggregates, of the gelatine as the temperature rises.

When the temperature is lowered, so that it lies just above the point at which a jelly is formed, there is no sudden fall in pressure, but if it is kept for some days at this temperature a very marked continuous fall is obtained, showing that, in the neighbourhood of the temperature of formation of the hydrogel, a rapid aggregation to form much larger solution aggregates occurs. At temperatures just below that of formation of the hydrogel it

1. This amount of ash, calculated on the percentage of dried gelatine (organic matter), amounts to 1.74 per cent. of inorganic constituent. The long duration of dialysis in a chamber specially built for rapid equalization, and the difference in percentages of ash on the two sides, at the end, viz., 0.032 and 0.170 per cent. show that this inorganic matter is not to be regarded as an impurity in the gelatine, but as an intrinsic part in combination or adsorption with it.

is difficult, on account of some of the experimental difficulties introduced by the setting of the solution, to determine whether a low osmotic pressure exists or not, but in a portion of the experiment in which the upper surface of the gelatine was covered by oil, which also covered the connections to the manometer, it was found that a low pressure of about 10 mm. was still obtained at about 2° C. below the setting point of the solution.

A further fall in the temperature, to about 10° C. below the setting point of the solution, caused complete disappearance of all pressure.

After the gelatine solution has been kept for a short time at a higher temperature (70° to 80° C.) it not only shows a high pressure out of linear proportion to the absolute temperature, but, after the temperature is allowed to fall again, much higher readings than before are obtained at the lower temperatures, and this continues for some days *after which there appears to set in a recurrence to the former values.*

If the heating to the higher temperatures (80° to 90° C.) be continued for a longer period (see Experiment ii) a *permanent* condition of change with a higher osmotic pressure is produced, and with prolonged heating, as is well known, the gelatine is so permanently altered that it no longer sets on cooling, and, at the same time, protein derivatives are formed which pass through the membrane, and give a solution on the other side, showing the biuret and other protein tests.

(Commenced on November 4th, 1902, at 10.30 a.m.)

Experiment I.—Filled the osmometer with a ten per cent. solution of best French gelatine as above described on the upper side, the platinum tube leading from the platinum capsule and the connections to the osmometer were filled with pure neutral olive oil to prevent solidification of gelatine in these connections. In the under platinum capsule was distilled water.

		FIRST PERIOD					
		Time from commencement of experiment		Osmotic Pressure in mm. of mercury		Temperature in degrees Centigrade	
		Days	Hours	Mm.			
November 4th	...	0	0	...	0	...	15°
		1	8	...	57	...	23°
		2	0	...	64	...	25°
		3	0	...	82	...	29°
		4	0	...	90	...	40°
		8	0	...	80	...	32°
		9	0	...	85	...	36°
		10	0	...	88	...	40°
		14	8	...	77	...	30°
		15	0	...	78	...	27°
November 21st	...	17	0	...	64	...	22°

The temperature was allowed to fall, after keeping for some days at about 20° C., down to laboratory temperature (13° to 14° C.), the osmotic pressure falling as a result to zero. During the long intervals equalization of all diffusible crystalloid must have been going on.

On December 6th (i.e., 32 days after starting) the osmometer was disconnected and opened. On the water side a perfectly clear watery fluid was found, which showed no signs of any decomposition. Analysis showed that this contained 0.132 per cent. of organic matter and 0.065 per cent. of fixed ash.

The gelatine side contained a clear, transparent, colourless jelly which filled the whole of this capsule of the osmometer and the proximal half of the attached platinum tube, the india-rubber connecting tube being filled with oil. The jelly melted at about 25° C.

Since the jelly was so unchanged it was not immediately analysed, but used for a further continuation of the experiment.

SECOND PERIOD

The same identical quantity of gelatine was used again, being introduced into the upper capsule of the osmometer, covered with oil, and joined up as before with connections also filled with oil to the manometer; the lower capsule was filled with distilled water. Before introduction into the capsule of the osmometer the gelatine solution was heated for five minutes to 95° C. in order to sterilize. After the osmometer had been filled on both sides as above described and connected up, it was immersed in a large beaker in a constant level water bath, and the bath and osmometer raised to 85° C., and kept for 30 minutes at about that temperature, the clip on the T piece on the manometer side being left open. The temperature was then allowed to fall to 40° C., and regulated to about that temperature by a thermo-regulator. A zero reading of pressure was taken and the clip then closed on the T piece on the manometer side, so as to begin to allow osmotic pressure to develop.

	Time from commencement of this period		Osmotic Pressure in mm. of mercury	Temperature in degrees Centigrade
	Days	Hours		
December 8th, 1902,	0	0	...	37°
5 p.m., 32 days after	0	17	...	37°
first commencement	1	0	...	40°
of experiment	1	18	...	41°
	2	0	...	38°
	2	19	...	38°
	3	0	...	35°
	3	17	...	35°
	4	1	...	33°
	4	22	...	31°
	7	1	...	29°
	9	1	...	25°
	10	0	...	25°
	11	17	...	23°
	13	17	...	21°
49 days from first commencement	14	18	...	20°

The pressures recorded during this period are considerably higher than those obtained at corresponding temperatures during the first period. The only explanation we can suggest is that the high temperature of 85° C., to which the gelatine solution was subjected at the commencement of the period, caused a separation of the colloid aggregates to smaller dimensions, and that it takes a prolonged period for re-association to occur when the solution is allowed to cool but still kept considerably above the setting point. That the gelatine was not appreciably altered permanently is shown, first, by the fact that there was a reversion to the old values after the solution was allowed to set and then just raised to 40° C. (see Period III), and, secondly, because at the end of the entire experiment, about a month later, the gelatine was found still to form a clear stiff jelly, melting at almost the same temperature as at the commencement of the experiment.

On December 30th, 1902 (56 days from commencement of experiment), the pressure was allowed to drop to zero by opening the clip on the manometer side, and the temperature was kept 3° to 4° C. below the setting point of the jelly in order to see if an osmotic pressure manifested itself under these conditions. The pressure at first rose to about 20 mm., and then slowly fell to about 9 mm. The experiment, therefore, appeared to indicate a low pressure even in the hydrogel form, but it is difficult to be certain of pressure transmission in the hydrogel form of the gelatine, and hence this was pushed no further.

THIRD PERIOD

The osmometer which had been left at laboratory temperature (11° to 13° C.) from January 8th to 10th, 1903, showed zero pressure. The temperature was now raised and regulated at a level just more than sufficient to melt the hydrogel, and when the pressure had become approximately constant readings were taken.

	Time from commencement of this period			Osmotic Pressure in mm. of mercury		Temperature in degrees Centigrade
	Days	Hours				
January 15th	5	7	...	67	...	23°
Time from beginning,	6	0	...	72	...	25°
72 days	7	1	...	83	...	28°
	9	0	...	86	...	30°
	11	7	...	94	...	39°
	12	8	...	94	...	40°
	14	0	...	70	...	21°
84th day from beginning	17	0	...	70	...	26°

It may be pointed out that these pressures lie at the corresponding temperatures much closer to those of the first period than do the readings of the second period to either. This shows that the disintegrating effect produced upon the colloid aggregates by the heating to 85° C., and which remained in part for a long time after the temperature was reduced, had finally been recovered from either as a result of the hydrogel having been allowed to form or by the prolonged time which elapsed after the heating.

On January 28th, 1903 (85 days from beginning), the osmometer was finally opened for the second time, and the fluids on the two sides examined and analysed for total organic matter and ash.

On the water side there was found a clear, colourless, watery fluid, which gave a negative result with the biuret, acetic acid and ferrocyanide, and tri-chloroacetic acid tests for protein. Ten c.c. were evaporated down for analysis, and gave 0.074 per cent. of organic matter and 0.032 per cent. of ash.

On the gelatine side there was a stiff clear jelly, which became quite fluid at 24° to 25° C., and began to reset at 22.5° C., and was completely set at 21.5° C. A weighed amount of this jelly (22.8 grms.) was evaporated down to a constant weight on a steam bath, and yielded 2.1812 grms. of dried solid or 9.56 per cent. (the solution was made originally 10 per cent.), and after incineration the ash was found to be 0.169 per cent.

The points that may be specially noted in the experiment are the persistency and constancy of the osmotic pressure at the end of such a prolonged period. The greater rise with temperature of the osmotic pressure than is linearly proportional to the absolute

temperature showing a diminution in the value of the solution aggregate with rising temperature. The effect of a higher temperature in causing a sustained increase in the osmotic pressure, which only reverts approximately to its old level after many days, and the re-formation of the hydrogel condition. And, lastly, the important point, that even after such a long period for equalization of diffusible crystalloids the amount of ash is entirely different on the two sides, showing that a combination, or, if the term be preferable, adsorption, exists between gelatine and inorganic constituent. Hence the inorganic constituent is an essential part of the gelatine under the conditions in which it exists, and not an admixed impurity.

Experiment II.—In this experiment, which in point of time was carried out before the preceding one, the osmotic pressure was observed throughout a wider range of temperature, and a marked increase was found at temperatures above 70° C., but the prolonged heating at the higher temperatures permanently altered the gelatine so that at the end a thin fluid which did not gelatinize on cooling was found on the gelatine side. The fluids on both sides of the membrane at the end gave the biuret test with a colour between purple and pink, but the depth of tint was much greater in the case of the fluid on the gelatine side.

The experiment is, nevertheless, of interest in showing the great rise with temperature, and the persistence of a higher pressure on re-cooling similar to that shown in Experiment I.

	Time from commencement of experiment			Osmotic Pressure in mm. of mercury		Temperature in degrees Centigrade
	Days	Hours				
October 20th, 1902 ...	3	3	...	107	...	51°
	6	3	...	116	...	61°
	7	0	...	126	...	71°
	8	1	...	148	...	80°
	9	0	...	158	...	91°
	10	0	...	142	...	80°
	11	0	...	138	...	70°
	11	6	...	134	...	60°
	11	22	...	134	...	47°
	12	3	...	122	...	33°
	12	6	...	114	...	21°
	13	5	...	98	...	16°

In carrying out this experiment the pressure was allowed to become steady, and then, by altering the thermo-regulator, the temperature was raised approximately 10° C. at each interval in the first half of the experiment, and lowered 10° C. in the second half of

the experiment. The intervals of time are made shorter between the readings in the latter portion of the experiments, but the changes in pressure set down are not due to the instrument acting as a thermometer, but to actual variations in the osmotic pressure. This is shown by the fact that with rising temperatures the first initial change occurring within the first half hour of the alteration in temperature is larger than the true change in osmotic pressure, and is followed by a rapid fall to the correct osmotic pressure. Similarly with the temperature being lowered there is at first a sharp drop, due to the osmometer acting at first as a thermometer, followed rapidly by a rise as diffusion equalizes and shows the true osmotic pressure by a steady manometric reading.

For brevity these intermediate readings are not given, but the following may serve as a sample :—

Time				Osmotic Pressure	Temperature	
Days	Hours					
6	3	...		116	...	61°
Temperature raised to 70° C.						
6	6	(initial reading)		138	...	70°
7	0	(final reading)		126	...	71°
10	0	...		142	...	80°
Temperature allowed to fall to 70° C.						
10	1	(initial reading)		118	...	70°
11	0	(final reading)		138	...	70°

EXPERIMENTS WITH STARCH SOLUTIONS.

The experiments described above appear to us to prove conclusively that one colloid, gelatine, possesses in solution osmotic pressure, those now to be described show equally clearly that another colloid, starch, has no osmotic pressure within the limits measurable by the direct method, not even a single millimetre of constant pressure having been obtained throughout our work.

The starch used was potato starch, prepared in the laboratory, and made as pure as possible, by decantation from distilled water and thorough washing with alcohol and ether, from all soluble matter.

Experiment I.—A starch mucilage of approximately 1 per cent. concentration was prepared in the usual way by boiling with distilled water ; this was introduced into the upper chamber of the osmometer as already described, and the lower chamber was filled with distilled water.

	Time			Osmotic Pressure in mm. of mercury		Temperature in degrees Centigrade
	Days	Hours				
March 15th, 1904 ...	0	0	...	2	...	13°
	0	15	...	0	...	12°
	1	0	...	1	...	15°
	1	15	...	0	...	15°
	2	15	...	0	...	13°
	3	0	...	0	...	15°
	3	15	...	0	...	14
	5	15	...	0	...	14
	6	15	...	0	...	14
March 25th, 1904 ...	9	15	...	0	...	14

Examination of the fluid on the water side showed at the end of the experiment not a trace of starch. The mucilage on the other side was dried in a steam bath to a constant weight, and was found to contain 0.89 per cent. of starch.

Experiment II.—Since it is impossible to introduce a stronger starch mucilage than 1 per cent. through the narrow platinum tube of the osmometric chamber, the expedient was resorted to of making the stronger mucilages of starch within the chamber itself. For this purpose a certain weighed amount of the pure potato starch was introduced into the upper chamber in a dried condition, a sufficient amount of distilled water was introduced to fill up the chamber, the lower chamber was filled with distilled water, and the osmometer, after being attached to its connections, was heated up in a large beaker to a temperature sufficient to break up the starch granules and form a mucilage. Then, at the end, a weighed amount of the thick mucilage was taken, the water evaporated off in a steam bath, and the starch weighed when the weight had become constant. In this way the percentage of starch in the mucilage was estimated.

February 9th, 1903.—One gram of dry potato starch was weighed out and introduced into the pressure side of the osmometer, which was filled with distilled water. Distilled water was filled into the other side. The whole was heated up to 60° C. and left over the night.

February 10th, 10.30 a.m.—Pressure at zero, temperature 15.9° C., heated up to 90° C.; manometer at first rose from osmometer tending to act as thermometer to 12 mm., but fell back within an hour to zero.

	Time			Osmotic Pressure in mm. of mercury		Temperature in degrees Centigrade
	Days	Hours				
February 10th, 1903, at 6 p.m.	1	0	...	—0.2	...	60° C.
	1	16	...	0.0	...	16° C.
	1	18	...	+0.8	...	83° C.
	1	20	...	—0.2	...	82° C.
	2	0	...	—0.2	...	83° C.
	2	18	...	—0.2	...	15° C.

The experiment was then stopped, communicability of fluid pressure throughout the apparatus having been proved by the fact that a quick oscillation of the mercury in the manometer occurred when the rubber tubing on either side was compressed with the fingers. The osmometer was emptied and the contents on the two sides examined.

On the distilled water side there was found a colourless mobile fluid, as clear as water, which gave no colour reaction with iodine and no reduction with alkaline copper sulphate.

On the starch side there was found partially a thin translucent fluid and partially a stiff jelly, which retained the shape of the osmometer on removing, but was easily broken to pieces. The mixture was heated in a vessel, while it was stirred with a thermometer, it melted into a homogenous viscid mucilage containing no visible solid particles at 70°C. , and remained a viscous mass until 100°C. was reached. The percentage of starch determined as in the preceding experiment was 5.56 per cent.

Experiment III.—Starch mucilage in saline (1 per cent. sodium chloride solution) first against saline, secondly against distilled water.

Both chambers of the osmometer were well washed out several times with 1 per cent. sodium chloride solution after a new membrane had been fitted in.

One gram of dry starch was then introduced into the upper chamber, which was afterwards filled with the 1 per cent. solution of sodium chloride. The lower chamber was filled with the 1 per cent. sodium chloride solution only.

The whole was then heated to 85°C. , the screw clip to the funnel on the starch side screwed up, leaving the starch chamber connected to the mercurial manometer, and the experiment commenced.

	Time			Osmotic Pressure in mm. of mercury		Temperature in degrees Centigrade
	Days	Hours				
February 14th, 1903 ...	0	0	...	0	...	82°C.
	0	2	...	0	...	82°C.
	1	21	...	0	...	14°C.
	2	0	...	0.4	...	82°C.
	2	2	...	0.2	...	84°C.
	2	5	...	0.2	...	85°C.
	2	21	...	0.2	...	13°C.

The salt solution was now removed from the lower chamber, and after washing out several times it was filled with distilled water, and the osmometer set in action again.

	Time			Osmotic Pressure in mm. of mercury		Temperature in degrees Centigrade
	Days	Hours				
	3	0	...	0	...	13°C.
	3	5	...	0	...	84°C.
	3	22	...	0	...	13°C.
	4	0	...	0.2	...	84°C.
	4	4	...	0	...	84°C.

To make certain that the zero pressure obtained throughout¹ this experiment was really a true indication, and that the membrane was acting properly, the pressure in the starch-containing chamber was now artificially raised by the introduction of 1 per cent. saline under pressure from the funnel, and the clip screwed down. The results in the following table show that the pressure gradually fell to zero, indicating that this is the true equilibrium point, as found in the previous parts of the experiment :—

	Time		Pressure (artificially caused initially) in mm. of mercury	Temperature in degrees Centigrade
	Days	Hours		
February 18th, 1903,	0	0	...	43 ... 81°
4.15 p.m.	0	0½	...	35 ... 81°
	0	1½	...	20.2 ... 81°
	0	2	...	9 ... 81°
	0	18	...	0 ... 14°
	0	20	...	0 ... 81°

Examination of the fluids at the end gave similar results to preceding experiment. With the same membrane the 1 per cent. saline solution was introduced on one side, and distilled water on the other. During fourteen days there was no measurable rise in the osmometer, and at the end of the time the concentrations in sodium chloride were practically equal on the two sides. The membrane was examined carefully, and found quite sound and free from holes.

Experiment III.—One gram of dried starch placed in osmometer, March 24th, 1904, 10 a.m.; both sides filled with distilled water; kept at 100° C. for some time, then allowed to cool, and experiment started at laboratory temperatures. This experiment may be regarded as one with a mucilage saturated with starch at the temperatures stated, and at the end only the thinner portion was taken for determination of percentage of starch, and not the whole of the material in the osmometric chamber as in Experiment II of this section.

	Time			Pressure in mm. of mercury ²		Temperature in degrees Centigrade
	Days	Hours				
March 24th, 1904,	0	0	...	6	...	17°
6 a.m.	0	15	...	6	...	17°
	1	15	...	2	...	16°
	3	15	...	0	...	16°
	4	16	...	0	...	16°

The fluid in the water chamber of the osmometer was free from starch; that on the starch side contained a thick jelly and a thin, slightly translucent fluid. In this case

1. The small fraction of a millimetre obtained in some readings is probably a slight variation of the zero, and is much too small in value to be regarded as a true pressure.

2. The experiment was commenced with a small positive pressure artificially produced.

only the fluid portion was taken for determination of percentage of starch. The determination was carried out as before by drying to a constant weight, and gave 1·82 per cent. of starch.

Experiment IV.—This experiment was continued for eleven days, and showed zero osmotic pressure like all the preceding ones. Total percentage of starch, 6·02.

EXPERIMENTS ON THE HYDROLYTIC PRODUCTS FROM STARCH.

In these experiments, ptyalin was allowed to act upon starch for the varying intervals mentioned in each case. The results show that at a certain stage at which the blue reaction has almost disappeared a non-diffusible dextrin is present, giving a constant osmotic pressure, but further action makes the whole diffusible, and no constant osmotic pressure is obtained. It may also be noted that erythrodextrin, or some substance giving a red colouration with iodine, diffuses through the parchment paper.

Experiment I.—A starch mucilage acted upon by saliva (1 c.c. in 100 c.c.) at 40° C. for ten seconds, then heated to 75° C. and filtered. The mucilage became quite fluid in the process, showing that 'soluble starch' had been formed. No osmotic pressure was obtained throughout a period of eight days. The solution taken out at the end was more opalescent than at the beginning. The water side when tested gave a red colour with iodine and reduced Fehling's solution. It contained 0·09 per cent. of dried solids. The starch side contained 3·44 per cent. of dried solids, and 0·0012 per cent. of ash.

Experiment II.—In this experiment saliva was added, 1 c.c. in 100 of starch mucilage, and without destroying the ptyalin this was placed in the osmometer. No osmotic pressure was obtained throughout a period of eight days.

Experiment III.—One hundred c.c. of starch mucilage acted upon by 1 c.c. of saliva for 17 minutes at 40° C.; blue colour gone, red of erythrodextrin present. Ptyalin destroyed by boiling; filtered, and put into osmometer.

	Time from commencement		Osmotic Pressure in mm. of mercury	Temperature in degrees Centigrade
	Days	Hours		
October 24th, 1904 ...	0	0	...	0
	0	9	...	2
	1	0	...	18
	2	0	...	16
	3	6	...	26
	4	0	...	26
	5	0	...	26

There is accordingly in this stage a definite and steady pressure. The solution side contained 2.37 per cent. of dried solid at the end, about half of which was precipitable by alcohol; the water side contained 1.11 per cent. of total solid, of which only a trace was precipitable by alcohol.

EXPERIMENTS WITH SUGARS.

The experiments with the products of hydrolysis of starch, show that the sugars pass easily through parchment paper, and give no osmotic pressure effect in the osmometer. The matter was, in addition, tested directly by two experiments, one on maltose and the other on arabinose.

The experiments are of interest in showing how completely equality of concentration is attained on the two sides of the membrane, and suggested a method for the quantitative determination of sugar in blood and its derived fluids, which possesses many practical advantages.

Experiment I.—A solution of maltose made up in cold water was placed in osmometer, against distilled water on the other side. The experiment was allowed to run for six days, and no trace of osmotic pressure was obtained throughout, and on opening the clip at the end of the experiment not the slightest movement was seen in the mercury of the manometer. Analysis showed the same amount of maltose on the two sides within the limits of experimental error, viz., 0.35 per cent. on water side, and 0.38 on solution side.

Experiment II.—A similar experiment with arabinose; experiment kept up for five days; no pressure shown throughout; analysis showed 0.35 per cent. on water side, and 0.36 per cent. on solution side.

EXPERIMENTS WITH GUMS

Gum Acacia.—*Experiment I.*—A 6 per cent. solution of specially hand picked gum acacia was made up and placed in osmometer against distilled water. The experiment was continued for six days, for the last two of which the pressure was steady.

	Time from commencement			Osmotic Pressure in mm. of mercury		Temperature in degrees Centigrade
	Days	Hours				
September 15th, 1904, 9 a.m.	0	0	...	0	...	15°
	1	0	...	32	...	15°
	1	2	...	40	...	16°
	2	0	...	98	...	17°
	4	0	...	130	...	16°
	5	0	...	134	...	16°
	6	0	...	134	...	15°

Analyses of the two fluids at the end showed 0.006 per cent. of total solids on the water side, and 6.25 per cent. of total solids on the solution side of which 6.09 per cent. was organic, and 0.16 per cent. was inorganic ash.

In two other experiments similar results were obtained, a 6 per cent. solution giving an osmotic pressure of 142 mm. at 15° C., and a 10 per cent. solution an osmotic pressure of 276 at 13° C.

Gum Tragacanth.—This gum while it swells up enormously in water does not dissolve readily even in hot water, and the small amount used (0.72 per cent.), which is, however, sufficient to form a thick mucilage, was the highest concentration with which we were able to experiment. This solution showed a small pressure of 5 mm. of mercury at 17° C. The pressure was raised artificially to 20 mm., but slowly fell again. This shows that if gum tragacanth possesses an osmotic pressure at all in water it is scarcely measurable even by the direct method.

URIC ACID SOLUTION.

In certain respects, such as precipitation in gelatinous forms from solution, and salting out with crystalloids, uric acid and certain urates appear to behave as colloids. It was hence thought of interest to determine whether a saturated solution of uric acid in distilled water exhibited any osmotic pressure, and whether it passed through a parchment paper membrane.

The saturated solution was prepared by boiling up excess of uric acid with distilled water, and filtering hot from the chief excess, and again from re-precipitated uric acid after cooling.

The result showed no appearance of osmotic pressure throughout an experiment lasting for five days (at 15° to 16° C.), and at the end of that period, practically the same concentration in uric acid on the two sides of the membrane.

The determinations were made by titration with potassium permanganate in the usual way, with the following results:—

Ten c.c. of original solution placed in osmometer require 0.5 c.c. of KMnO_4 solution = 1.875 in 10,000.

Ten c.c. on uric acid side at end require 0.25 c.c. = 0.9375 in 10,000.

Ten c.c. on water side at end require 0.22 c.c. = 0.8250 in 10,000.

This shows that uric acid behaves in solution in water as a crystalloid, and readily diffuses through the membrane.

EXPERIMENTS ON BLOOD SERUM AND ITS CONSTITUENTS AND
EFFECTS OF REAGENTS.

Experiment I.—In this experiment it was sought to determine whether the presence of anaesthetics or organic solvents altered the osmotic pressure of the serum proteids. No change in osmotic pressure was obtained, and the experiment shows how closely readings come to the same point with the same sample of serum. Also, since the chloroform used acted as a preservative from any chance of bacterial growth, the results show that the osmotic pressure readings obtained were not due to any crystalloidal products of proteid decomposition. For brevity sake, only one daily reading is given, and the intermediate readings, which were consistent with these, are omitted.

The experiments were carried out in July, 1904.

No. 1.—*Serum (pig's) only, against 0.75 per cent. saline.*

Time d. h.		Osmotic Pressure mm.		Temperature
0	0	...	0	17° C.
1	0	...	12	17°
2	0	...	24	18°
3	0	...	32	20°
4	0	...	30	20°
5	5	...	31	19°
5	22	...	30.5	18°

No. 2.—*The same serum saturated with chloroform, against saline (0.75 per cent.) also saturated with chloroform.*

Time d. h.		Osmotic Pressure mm.		Temperature
0	0	...	0	19° C.
0	21	...	22	18°
1	5	...	28	18°
1	21	...	31	18°
2	0	...	31	18°

No. 3.—*The same serum saturated with benzol, against 0.75 per cent. saline, also saturated with benzol.*

Time d. h.		Osmotic Pressure mm.		Temperature
0	0	...	0	19° C.
1	0	...	34	18°
2	0	...	34	19°
2	19	...	34	19°

These experiments show that there is no appreciable change in the solution aggregate caused by the anaesthetic. It may be pointed

out that the amount of chloroform on the serum side, as shown by Moore and Roaf's¹ experiments, would, at the saturation point, be four or five times as great as on the saline side, and since this does not appreciably disturb the osmotic pressure, it must be in combination with the proteid.

Experiment II.—This experiment was on the effects of converting the serum proteids into alkali albumen by boiling with 1 per cent. caustic potash, and measuring osmotic pressure against saline (0.75 per cent.) to which an equal amount of caustic potash had been added. The result showed an immense rise in osmotic pressure, similar to that obtained by Moore and Parker (*loc. cit.*).

The experiments were carried out in December, 1904, and are at a lower temperature than the preceding one.

No. 1.—Serum, untreated, against saline (0.75 per cent.)

Time		Osmotic Pressure		Temperature
d.	h.	mm.		
0	0	...	0	12° C.
1	2	...	10	12°
2	0	...	14	12°
8	16	...	18	12°

Percentage of proteid = 7.97

No. 2. The same serum boiled with 1 per cent. of potassium hydrate against saline (0.75 per cent.) plus 1 per cent. of potassium hydrate.

Time		Osmotic Pressure		Temperature
d.	h.	mm.		
0	0	...	0	9° C.
0	5	...	12	10°
0	7	...	24	11°
0	11	...	40	11°
1	0	...	80	9°
1	9	...	94	11°
2	0	...	100	10°
6	0	...	103	7°
7	0	...	103	8°

Percentage of proteid 5.02.

The action of the caustic potash causes the osmotic pressure to rise to between five and six times its former value, and the increased pressure remains steady.

1. *Proc. Roy. Soc.*, Vol. LXXIII, 1904, p. 382. *Ibid.*, B. Vol. LXXVII, 1905, p. 86.
Thompson Yates & Johnston Lab. Reports, Vol. VI, Pt. I, 1905, p. 151.

Experiment II.—The same result was obtained in this experiment in which the globulin and albumin were separated, and their osmotic pressures taken before and after treatment by boiling with 1 per cent. of potassium hydrate. In the latter case, 1 per cent. of potassium hydrate was also added to the 0.75 per cent. saline on the other side of the membrane, as in the preceding experiment.

The globulin was obtained by half saturation with ammonium sulphate, and was twice washed and then made up to the volume of the original serum; the serum albumin was twice precipitated by saturation with ammonium sulphate, and then dialysed and made up to the volume of the original serum. The alkali-globulin or -albumin was made by adding 1 per cent. of potassium hydrate and raising the temperature to the boiling point.

The osmotic pressure rose, as in the previous experiments, to a constant maximum. To save space, only this maximum is given in each case.

Substance	Osmotic pressure	Temperature
Serum	18 mm. ...	12° C
Alkalized serum ...	103 „ ...	8°
Serum-globulin ...	7 „ ...	13°
Serum albumin ...	4 „ ...	12°
∴ Serum albumin + serum globulin ...	11 „ ...	—
Alkalized serum globulin	62 „ ...	10°
Alkalized serum albumin	44 „ ...	12°
∴ Alkalized serum-globulin + alkalized serum- albumin ...	106 „ ...	—

It is evident from these figures that the precipitation and washing of the serum proteids changes these so that the sum of the pressures is less than that of the total proteids of the serum in their natural condition; but that alkalization leads to osmotic pressures which give for their sum a pressure practically the same as that obtained by alkalization of the serum directly.

In all cases, the biuret test gave, at the end, a negative result in the fluids on the water side.

Experiment III.—On the effects of addition of considerable amounts of neutral salt on the osmotic pressure of the serum proteids. Since a large amount of neutral salt added to a proteid-containing solution has the effect of salting out the proteid in many cases, the present experiment was devised to determine whether, at a point short of precipitation, any increased aggregation occurred as shown by diminished osmotic pressure. Two experiments were carried out, in each of which a control of the same

serum, against 0.9 per cent. sodium chloride solution, was used. The second osmometer had 20 per cent. of crystallised magnesium sulphate ($\text{MgSO}_4 + 5 \text{H}_2\text{O}$) added on each side of the membrane, so that on one side there was serum + 20 per cent. crystallized magnesium sulphate, and on the other, 0.9 per cent. sodium chloride plus 20 per cent. magnesium sulphate. Since the two experiments gave similar results, details are only given in the case of one.

No. 1

Pig's serum against 0.9 per cent. saline.

No. 2

Pig's serum + 20 per cent. of cryst. mag. sulph. against 0.9 per cent. saline + 20 per cent. cryst. mag. sulph. after dialysing the two fluids against each other.

Time from commencement d. h.		Osmotic pressure in mm. of mercury		Time from commencement d. h.		Osmotic pressure in mm. of mercury	
0	0	...	0	...	0	0	...
1	3	...	14	...	1	2	...
2	3	...	21	...	2	2	...
2	18	...	24	...	2	17	...
3	18	...	26	...	3	17	...
4	18	...	27	...	4	17	...
5	20	...	27	...	5	19	...
6	18	...	27	...	6	17	...
7	18	...	27	...	7	17	...
8	18	...	27	...	8	17	...
10	18	...	27	...	10	17	...
12	19	...	27	...	12	18	...
14	18	...	27	...	14	17	...
16	18	...	27	...	16	17	...

The figures show that the maximum pressure in the salted serum rose to 31 mm. at the same period as the untreated serum stood at 27 mm.; that later, the pressure in the salted serum fell to 18 mm., while that in the unsalted remained steadily at 27 mm.

Analyses at the end showed, on the serum side, 85.62 per cent. of water, 5.93 per cent. of organic matter, and 8.45 per cent. of MgSO_4 ; on the saline side, 90.82 per cent. of water, and 9.18 per cent. of MgSO_4 , thus, the amount of saline is closely proportional to the percentage of water on each side.

The second experiment showed, in the salted serum, a maximum pressure of 32 mm. in two days, which fell to a constant 18 mm. in eight days; while the untreated serum rose to a maximum of 21 mm., and remained constant at that level until the experiment was terminated in ten days.

In our opinion, these changes are not due to diffusion of magnesium sulphate, for diffusion in our osmometer of such easily soluble crystalloids occurs very rapidly, while these changes in osmotic pressure occupied several days. They seem, therefore, to be due to some slow molecular or aggregation change induced by the excess of salt.

EXPERIMENTS WITH LECITHIN AND LANOLINE MEMBRANES.

Since it has been supposed that the peculiarities in the behaviour of cells to certain electrolytes and inorganic ions are due to a cell membrane which is permeable to some and impermeable to others, and that this membrane is composed of, or consists chiefly of, lecithins and other lipoids, which confer upon it these peculiar properties of selective permeability and impermeability, it was considered of some importance to prepare such membranes in the pores of parchment paper and coating its surfaces, and to test whether such a membrane showed similar permeabilities to those *supposed* by many authors to be shown by cells, and, if so, whether osmotic pressures would be developed when ions to which the membrane was not permeable were present in different concentrations on the two sides.

Both lecithin and lanoline membranes were prepared and tested, and it was found that these were readily permeable and that no trace of osmotic pressure was developed.

This demonstrates, in the first place, that a lipoid membrane does not furnish any explanation of the osmotic properties of living cells, and, secondly, that the presence or absence of an inorganic ion in a cell, or variations in concentration of such an ion inside and outside of the cell, are not to be ascribed to a barrier opposed by such a membrane, but that the explanation is rather to be sought in the

properties of the cell contents themselves, for combining with or adsorbing such constituents of its inorganic environment.

The lecithin was prepared by the method described by Roaf and Edie,¹ and dissolved in the smallest portion possible of ether. The discs of parchment paper, cut to the proper size to fit the osmometer, dried at 100° C. and cooled in a desiccator, were dipped in this strong lecithin solution and the ether allowed to evaporate off; they were then dipped a second time and allowed to dry again. In this manner, the parchment paper is thoroughly soaked with lecithin, becomes translucent, and is coated with a thin layer of lecithin on both surfaces.

Experiment I.—A membrane, impregnated with lecithin as above described, was fitted into the osmometer, which was filled with 1 per cent. sodium chloride solution on one side, and with 0.5 per cent. sodium chloride solution on the other. This arrangement was used in preference to distilled water on one side, in order to keep the lecithin from emulsifying in the water. The experiment was kept going for five days, during which time the pressure remained at zero, and afterwards a small positive pressure produced artificially slowly disappeared. The fluids on the two sides were titrated for chlorides, and the originally 1 per cent. solution was now found to be 0.89 per cent., and the originally 0.5 per cent. was now 0.52. From these figures it appears as if some of the salt had been adsorbed by the lecithin, but, in addition, the absence of pressure throughout, and the increase on the more dilute side, show that the membrane is permeable to chlorides. Also, the entire absence of pressure, in face of such a difference in concentration, clearly shows that the osmotic phenomena of the living cell, and the wide differences in the qualitative composition of the inorganic salts of cell and of plasma or lymph, cannot be due to the presence, surrounding the cell, of an impermeable membrane of lecithin which prevents ingress or egress of certain inorganic ions.

There is no experimental evidence of the existence of any impermeable membrane surrounding cells, and the differences of composition within and without are, in our opinion, more probably due to the special affinities of the cell protoplasm for different ions than to the action of any such hypothetical membrane.

Experiment II.—A similar experiment, but with a membrane of lanoline instead of lecithin, gave similar results—no osmotic pressure being obtained, and a rise in concentration in chlorides on the less concentrated, and a fall on the more concentrated side.

CONCLUSIONS

1. Evidence is given by direct measurement that certain colloids, which cannot be shown to definitely possess osmotic properties by the indirect methods, such as depression of freezing point or raising of boiling point, do show a very definite and easily measurable osmotic pressure when a membrane permeable to crystalloids is used in the osmometer.

2. As evidence that the pressure so obtained is due to colloid, and not to crystalloid, the following facts are brought forward :—

(a) Colloids diffuse in solution and hence must have a falling gradient of osmotic potential.

(b) The pressure reaches a maximum, and there remains constant ; if it were due to crystalloid not attached to colloid, this pressure would rise and then fall gradually to zero.

(c) The pressure is modified by varying amounts of easily diffusible crystalloids in solution, provided such crystalloids affect the condition of the colloids, although such crystalloids pass with ease and rapidity through the membrane, and accordingly themselves show no permanent osmotic pressure.

(d) Anything which is capable of altering the condition of the colloid, such as alkalization of the serum proteids, or hydrolysis of starch, causes corresponding changes in the osmotic pressure.

(e) Repeated precipitation of an alterable colloid, such as the serum proteids, causes a fall in osmotic pressure ; but this effect subsequently disappears on alkalization, the pressure for the fractions of alkaliized proteid adding up to the total for the fresh serum alkaliized as a whole.

3. Certain colloids, such as starch (potato) and probably gum tragacanth, have so high a state of aggregation that they show no osmotic pressure, even by the direct measurement ; those showing osmotic pressure clearly, which have been dealt with in this paper, are the serum proteids, gelatine and gum acacia.

4. At the stage of hydrolysis of starch at which the blue colour with iodine has just disappeared, the dextrans present give a permanent osmotic pressure.

5. In the case of gelatine the effect of variation of temperature upon the osmotic pressure has been observed. It has been found that the osmotic pressure rises with the temperature, and that the rise is more than proportional to the absolute temperature, indicating a dissociation and lowering of the solution aggregate as the temperature is raised. Prolonged heating to 80° C. or over causes a permanent change with higher osmotic pressure ; but heating for a short period gives a higher pressure, which persists for a few days, after which the osmotic pressure returns to its former value, and the physical properties of the gelatine solution are not permanently altered.

6. All the sugars experimented with behave as true crystalloids in passing readily through and equalizing on the two sides, and hence show no osmotic pressure ; uric acid solutions also behave as true crystalloids.

7. Serum globulin and serum albumin both possess osmotic pressure, but the process of separation has the effect of altering the state of aggregation so that the sum of the pressure shown by the two fractions is less than that of the serum from which they came ; but on alkalization the sum of the pressures equals that of the alkalized serum.

8. Addition of a considerable quantity of a neutral saline (magnesium sulphate) to serum, causes, initially, a rise in osmotic pressure, followed by a fall to a constant lower level, which is less than that given by the untreated serum.

9. The slow change in osmotic pressure observed in colloidal solutions, such as that back to normal conditions in gelatine after a short period at a higher temperature, and of the fall observed after addition of magnesium sulphate to serum, which requires some days to be completed, indicates a kind of hysteresis in such solutions, or a very slow return to equilibrium after the state of aggregation has been disturbed.

10. A lecithin or lanoline membrane is not impermeable to crystalloids, and hence shows no osmotic pressure when the concentration of crystalloids is different on its two sides.

Accordingly, the supposed presence of such a membrane will not explain the peculiar content of the cell in crystalloids, different from that of the bathing lymph around it. A more probable view is that the cell protoplasm has selective adsorptive powers for different ions, and that such ions exist in the cell in combination or adsorption with the cell substance.