ON THE EQUILIBRIUM BETWEEN THE CELL AND ITS ENVIRONMENT IN REGARD TO SOLUBLE CONSTITUENTS, WITH SPECIAL REFERENCE TO THE OSMOTIC EQUILIBRIUM OF THE RED BLOOD CORPUSCLE[1]

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Since the pioneer experiments of Sidney Ringer[2] first demonstrated the absolute necessity of certain saline constituents in the circulating fluid supplied to the isolated contracting heart, in order that the heart muscle should be enabled to carry on in a normal fashion its physiological functions, a host of observers have been fascinated by the study of the reaction of the living cell to changes in the soluble inorganic constituents of its environment.

Nor can any subject be regarded as of higher practical importance either in regard to the rational study of biological function, or of practical medicine which ought to form the applied science of such a study.

It is becoming ever more clearly recognised that the various drugs which show a specific action in different diseases, produce their results by the action of a specific inorganic or organic ion added to the medium which surrounds the cell, and possessing, by virtue of its constitution and the constitution of the cell affected, special affinity for that cell. So that one class of cell is most affected by one ion and another class by a different ion.

1. The expenses of this research have been chiefly defrayed from a grant made by the Government Grant Committee of the Royal Society.

2. *Journ. of Physiology*, Vols. III, IV, VI, etc. See Schäfer's *Text-book of Physiology*, Vol. II, p. 225.

It is on such a basis that physical chemistry has given a rational explanation of why, for example, all mercury salts produce the same specific action in syphilis, the result being due to the free mercury ion, and not being affected by the anion of the salt used except in so far as this quantitatively alters the degree of ionization and hence the concentration of mercury ion. Similarly, the ferric ion, in all iron salts, stimulates the production of erythrocytes in anaemia. So too, quinine, and the alkaloids generally, furnish a basic ion affecting specific cells of the organism, or of pathogenic foreign organisms present in it, for which at different stages they possess special affinities.

It is on this specific selective affinity of different cells of the organism or of the cells of parasites of the organism, for ions or molecules in solution in the medium bathing such cells, whereby these cells selectively adsorb or combine with such ions so changing the activities of the adsorbing cells, that the rational experimental science of therapeutics of the future must be based.

As an instance of how different organic ions attack and combine, or become adsorbed, by different cells, we may instance the observation of Whitley[1] in regard to the action of phenol-phthalëin and di-methyl-amido-azo-benzol on the eggs of *Echinus esculentus* (sea urchin) and *Pleuronectes platessa* (plaice) respectively. A trace of phenol-phthalëin rapidly killed the echinus eggs but had no injurious action on the plaice eggs; while, conversely, a trace of di-methyl-amido-azo-benzol rapidly killed plaice eggs, but had absolutely no injurious effect on echinus eggs.

If we suppose for a moment that the plaice eggs and echinus eggs represented two parasitic organisms infesting an animal which was to be treated, then di-methyl-amido-azo-benzol would have been a specific drug for the one, and phenol-phthalëin for the other, providing that these two bodies did not injuriously affect the higher animal acting as host.

It may be added that this holds not only for drugs, but for the toxins of disease which usually produce their effects on account of their specific adsorption by the body cells.

1. *Proc. Roy. Soc.*, B Vol. LXXVII, p. 137, 1906.

In view of the abundant evidence from physiology, pathology, and therapeutics of the ability of the cell to combine, or enter into adsorption with ions from its bathing fluid, and that the amount of adsorption or combination taking place varies in degree for each ion, and often in a highly specific manner from one type of cell to another ; it is somewhat surprising what a very passive rôle has been assigned to the cell in this process by most workers in biology and physiology in considering the normal adaptation of the cell to its environing medium.

It is one of the oldest and best established experimental findings of biology, that the inorganic constituents of the interior of the cell differ widely from those of the plasma or intercellular fluid by which the cell is bathed. The cell salts are rich in potassium and phosphates, while the plasma is exceedingly poor in concentration of these ions, and richer in sodium and chlorine ions. Since the cell receives all its nutrition, both inorganic and organic, from the outer fluid, the existence of such a difference points most powerfully to the cell contents possessing a special affinity, or adsorbing power, for the potassium and phosphates on account of which it takes them up from the intercellular fluid, although they are only present there at such low concentration ; and, on the other hand, to the fact that its constitution gives it no adsorbing or combining power for the sodium, so that although this ion is present in the intercellular fluid in many times higher concentration than the potassium ion it is not taken up by the cell, which has no attachment for it and no facility for holding it.[1]

This is not, however, the accepted view, according to which the cell is regarded as acting quite passively as to what it shall take up and what reject in the way of ions from its enveloping fluid. The whole exchange is supposed to be regulated for the cell by an inert membrane by which it is enclosed, and which, like a prison wall, keeps the potassium and phosphate ions within while it equally

1. See Moore, *Recent Advances in Bio-Chemistry*, edited by Leonard Hill, Arnold, London, 1906, p. 149 *et seq.*

prevents sodium ions from entering.[1] The membrane theory takes no trouble to explain how prisoners are introduced within the prison, yet introduced in some way they must be, for as growth proceeds, cells multiply, and there is no diminution in the number of imprisoned ions in each.

In the present paper we desire to suggest (i) that the *qualitative* differences between the electrolytes of the cell and the electrolytes of its environment, are due, not to the presence of an impermeable wall, but to the specific affinities of the cell protoplasm for certain electrolytes or ions, whereby it combines with or adsorbs them, and (ii) that in regard to *quantitative* relations there is maintained an *equilibrium* in regard to electrolytes between the cell and its environment, but this equilibrium does not necessarily consist in an equality or isotonism in regard to total osmotic pressure, and in practically all cases departs in some degree from equality.

Considering first the qualitative composition of the salts of cell and plasma, we may pass over the fact that the existence of a limiting membrane has not been proven in the vast majority of cases, and assuming the existence of such an impermeable membrane, discuss whether it is capable of explaining the profound differences in composition of cell salts and plasma salts which have been found by all previous observers, and are shown in our own determinations later in this paper.

Can the cell become vastly richer in potassium and phosphates and poorer in chlorides and sodium than its surrounding medium by the action of a membrane ?

The answer to this question is perfectly clear, the only membrane which could produce such an effect would be one which was permeable in one direction and not in the other, in other words, a Maxwell's demon would be required to open a trap-door in one direction and close it in the other.

Such a membrane, with pores or valves open in one direction

1. See Hamburger, *Osmotisches Druck und Ionenlehre in den medicinischen Wissenschaften*, Bände I u. III, where a full account is given of the literature of the subject and the experimental work by various authors, which is supposed to establish the membrane and permeability hypothesis.

and closed in the reverse direction, is unknown in inorganic nature, and has not been shown to exist in the case of living cells.[1]

On the other hand, it has been clearly established that colloids possess strongly marked affinities for combining with crystalloids, or for adsorbing them, and this condition is all that is requisite for explaining the difference in qualitative composition between cell salts and plasma salts.

Let us suppose that the cell starts with a complete equality, both qualitative and quantitative, as regards its crystalloids, with the surrounding fluid (plasma); then the known existing condition of richness in potassium and phosphates and poverty in sodium and chlorides obtaining within the cell can be realized by taking into account that the cell protoplasm combines or fixes in some chemical or physical way the potassium and phosphatic ions, while the plasma similarly holds the sodium and chlorine ions.

There are many examples known of such adsorption or combination between colloids and crystalloid substances when present together in common solution.

Perhaps the simplest and best known example which can be taken to illustrate such an action of adsorption in heaping up concentration in one portion of a heterogeneous system (such as cell and nutrient medium form) and depressing it in another portion of the system, is the taking up of oxygen by the haemoglobin of the red blood corpuscle.

Here, just as is the case with potassium and phosphatic ions, under normal conditions the concentration of oxygen in the red blood corpuscle is many times that which it has in the plasma. Circumstances of oxygen pressure can also be chosen which will *apparently* prove that the red blood corpuscle is impermeable to oxygen, notwithstanding this large amount of oxygen inside, on similar lines to those

1. It may be pointed out, since this point has escaped the attention of previous writers on the subject, that a passive valved membrane will not serve the purpose of obtaining an increased concentration or pressure on one side. The valves, to open, must either have energy supplied from outside, or must take momentum from the molecules passing through. In other words, the mere presence of valves permeable in one direction cannot explain increased concentration of the permeable substance on one side by passage from a more dilute solution. Any such concentration, in other words, requires a supply of energy.

which have wrongly been taken as proving that the red blood corpuscle is impermeable to potassium ions.

If, for example, pressures of oxygen be taken between 200 mm. and 1,000 mm., and the blood corpuscles and plasma be shaken up with the oxygen until equilibrium is attained, and then the concentration of oxygen in corpuscles and plasma determined. It will be found that while the amount of oxygen in the plasma is almost proportional to the pressures, the amount of oxygen in the corpuscles is very little different at 1,000 mm. to what it is at 200 mm. pressure.

The result, as we know well, is due to the fact that haemoglobin and oxygen combine or become adsorbed with each other and that the adsorption is almost complete at about 120 mm. pressure. At this point nearly all the large amount taken in by the haemoglobin in physical or chemical union is already within the corpuscle, and the further rise in pressure of oxygen only causes to be taken in the small amount capable of existing in uncombined or unadsorbed condition.

It is obvious, therefore, that the true explanation is adsorption and not impermeability of a membrane in the case of oxygen and haemoglobin.

Yet this is exactly the kind of experimental proof upon which the view is based, that although the red blood corpuscle is very rich in potassium yet it is impermeable to that ion. Because no amount of extra potassium, which can be shown by chemical analysis, enters the red blood corpuscle when it is washed with normal saline made with potassium chloride, or no *appreciable* amount of potassium passes out of it when it is contrariwise washed in a saline made of pure sodium chloride, the deduction is drawn that the red blood corpuscle is impermeable to potassium.

From what has been said above in the case of haemoglobin and oxygen, however, it follows clearly that such a deduction can only be drawn provided the concentration of potassium has been so reduced that the pressure of dissociation of the compound or adsorpate of potassium and cell constituents has been passed; for until this has been done the cell will not appreciably lose potassium, and at pressures above the dissociation value the cell will not appreciably take up

more than its normal amount. Further, if this dissociation pressure be a low one, the determination of small amounts of potassium leaving the cell will be a most difficult one, for the potassium will have to be determined in presence of large excesses of sodium chloride or other indifferent saline used to wash the corpuscles, which must be washed in isotonic solution or otherwise will break up. Also, it must be remembered that the amount of potassium coming out at each wash, with a saline of sodium chloride only, will be very small, since the potassium will only diffuse out until its concentration is at a level somewhat lower than the low level of its concentration in the natural serum, and at each successive wash the concentration in the sodium chloride saline will be lower than in the preceding wash.

A consideration of the low concentration of potassium ion in the normal plasma or serum shows at once that the dissociation pressure of potassium and cell contents must be very low (or in other words, that the combination between cell contents and potassium is a fairly stable one), because it is at this low potassium ion pressure existing in the plasma that the red blood corpuscle and the tissue cell become saturated with their potassium content, and accordingly the dissociation pressure must lie below this low value.

Accordingly it is clear that the exchanges of potassium between cell and plasma, especially when one remembers the poorness of quantitative methods for determination of potassium in presence of excess of sodium, may well be such as to fall within the limits of experimental error in direct chemical analysis.

Fortunately, there are more delicate physiological methods which clearly show that when the amount of potassium in the plasma is increased there is an uptake by the tissue cells, shewing that here there is no impermeability due to a membrane, and that the apparent impermeability is due to a protective action brought about by the cell contents possessing a specific adsorptive power.

Before passing to this physiological proof attention may, however, be drawn to the fact, which never seems to have been appreciated or have had attention drawn to it, of how low the concentration of oxygen is in the plasma when the red blood corpuscle begins to part

freely with its oxygen, and, what particularly concerns us here, of how impossible it would be to estimate this oxygen if we had to depend, as we have in the case of potassium, upon estimations of percentages in the plasma itself.

The partial pressure of oxygen at which appreciable dissociation of oxy-haemoglobin commences can safely be taken as lying below 150 mm., which is about the partial pressure in the atmosphere. Now the coefficient of solubility of oxygen in plasma is about the same as in water, and for the purposes of this calculation may be taken as 0·02. It hence follows that with a partial pressure in the air of 150 mm., the osmotic pressure of the dissolved oxygen in the plasma is only 150 × 0·02 mm. = 3 mm. This exceptionally low pressure, or rather about two-thirds of it, that is 2 mm. of mercury, is the *real* dissociation pressure of oxy-haemoglobin, and not the 150 mm. obtaining in the air mixture to which the blood is subjected. The name of *osmotic* dissociation pressure might perhaps be suggested for it, to prevent confusion with the partial dissociation pressure in the air to which it is of course proportional.

Now it is this osmotic dissociation pressure which we must take into account in considering the percentage by weight of oxygen in the plasma. When the calculation[1] is made it is found that with an oxygen partial pressure of 150 mm. in the air in contact with the blood, the percentage by weight of oxygen in the plasma is about 0·0005 per cent., that is about half a milligram of oxygen in 100 c.c. of plasma.

Now, if we take it that the potassium in the red blood corpuscle is no more firmly held than the oxygen, so that its appreciable dissociation commenced at about 3 mm. of osmotic pressure, this would correspond to a concentration of K_2O in the plasma of 0·5 mg. per 100 c.c., which is a quantity far outside the limits of chemical analysis in such a solution, and could only be determined if the potassium could be pumped off as a gas like the oxygen.

1. The calculation is as follows:—22,330 c.c. of oxygen, under normal conditions of temperature and pressure, weigh 32 grammes, what will 100 c.c., at 3 mm. pressure weigh $= \frac{32 \times 3 \times 100}{760 \times 22{,}330} = 0{\cdot}00057$. Corrections to body temperature which have not been introduced in the calculation, further reduce the amount to 0·00049, that is to very approximately half a milligram of oxygen in every 100 c.c. of plasma.

If we turn now to the physiological tests in the active tissues, we find that the permeability of the cells for oxygen and for potassium can be shown by exact parity of reasoning in the two cases.

Although the cells do not take up an additional amount of oxygen at two atmospheres of pure oxygen obvious to chemical analysis, as compared to what they take up under ordinary atmospheric conditions, of one-fifth of an atmosphere of partial pressure of oxygen, the physiological effects due to the increased oxygen pressure are profound. At about two atmospheres of oxygen pressure the nerve cells are upset in their oxygen metabolism and the animal dies in convulsions. The pressure of oxygen is so great that the combination between tissue cell and oxygen becomes a permanent fixed one, instead of an unstable one fluctuating in labile equilibrium and being made and unmade as the osmotic pressure of dissolved oxygen varies around the dissociation value of about 2 mm. The entire oxygen exchange so delicately balanced is upset and the cell is as much asphyxiated as if it were deprived of oxygen altogether, or were bound in fast combination with such a gas as carbon-monoxide. In fact, by another method, oxygen exchanges have been made impossible.

Exactly similar results are obtained in the case of the potassium ion which prove unmistakably that this ion passes into or out of the cell, causing increased or decreased potassium ion pressure in the cell when the concentration of that ion in the circulating fluid is raised or lowered.

This is most clearly shewn, perhaps, by the changes in beat of the isolated heart perfused by salines of different composition, as discovered by Ringer. When there is no potassium ion present, or too low a concentration, the automatic rhythmic beat soon stops in a characteristic way; when the low amount of 1 in 10,000 of potassium chloride is added then the beat becomes quite normal, and as the potassium concentration is increased beyond this optimum concentration the beat becomes characteristically irregular and abnormal, and at a slightly higher concentration the heart again ceases to functionate.

The behaviour is exactly as in deficiency or excess of oxygen,

and might be almost described as potassium asphyxiation, with diminution or excess.

Similar proof is given by administration of excess of potassium salts to the animal as a whole, as shewn by the profound depressant effect upon the nervous system of doses which would show to chemical analysis no appreciable increase in potassium ion concentration in either plasma or tissue cells.

In physiological action, then, we possess a test infinitely more delicate than chemical analysis, and the test proves conclusively the permeability of the cell for ions such as potassium and the phosphates. Chemical analyses of cell contents and nutrient fluid give also results which show specific adsorptive powers for the different inorganic constituents.

This specific power of adsorption, which is moreover not equal for different types of cell but varies in a characteristic manner, furnishes an explanation of many physiological and pharmacological phenomena, and does not occur merely in a few isolated instances but in the manifold reactions in which the living cell carries on its wide commerce of exchange in health and disease.

The point made out above, also, that exchange can only occur normally around about a certain optimum of concentration, cannot be too firmly grasped, for excess leads to stoppage of exchange equally with deficiency.

As some instances of this labile equilibrium between the cell and its nutritive constituents having for its chemical basis unstable chemical combinations or adsorpates possessing definite low limits at which they associate or dissociate, there may be mentioned, also, the organic foodstuffs, where there is evidence, always increasing, of combination in both blood and tissues between proteid on the one hand and carbohydrate or fat on the other. There exists at present little doubt as to a loose chemical combination in blood and tissue between proteid and carbohydrate. Further, in the case of the liver, it has been demonstrated that normal liver shewing little or no fat under the microscope may, on extraction, yield as much fat as a pathological liver microscopically shewn to be laden with fat globules. It is

obvious here that the fat in the normal liver must be in some combined form with the protoplasm which renders it homogeneous with the protoplasm and invisible under the microscope. Again, perfectly clear serum may easily contain over one per cent. of fatty extractives which, unless combined in some fashion, most probably with the serum proteids, would yield an obvious, opaque, milky emulsion quite different from the clear serum in which it is contained.

The same unstable, easily dissociated compounds are known to be formed in the case of constituents added from without, or formed by disease. Thus, for example, the stilling action of anaesthetics upon cell activity has been shewn to be due to the limitation of cell activity by the formation of such unstable combinations or adsorpates between cell proteid and anaesthetic, which persist only so long as the osmotic pressure is kept above a certain level, and dissociate and so leave the cell once more active when the pressure of anaesthetic falls again in the course of free respiration.[1]

Similar compounds or adsorpates have had to be assumed, and have now in many cases been proven, for toxins and similar bodies with cells, and for toxin and antitoxin with each other. Here the selective adsorptive power of special cells for special products above referred to, becomes peculiarly evident.

So also in the case of active selective drugs; the action is in each case due to a more or less unstable combination or adsorpate between drug and tissue cell or some special constituent of some definite type of cells. As examples may here be mentioned that of the bromine ion on the central nervous system, and of the nitrite ion, adrenalin, strychnine, or atropin on special nerve cells or nerve endings.

In blood corpuscles and in tissue cells we have, therefore, to deal, not with an impermeable membrane, but with a cell substance possessing specific adsorptive powers, and it is the low solubility in *free* solution in the cell contents as compared with the relatively larger power of taking up in adsorbed or combined condition which gives rise to an apparent impermeability, which is shown not to exist (i) by the presence in the cell of the apparently impermeable con-

1. Moore and Roaf, *Proc. Roy. Soc.*, Vol. LXXIII, p. 382, 1904; also *ibid.*, B, Vol. LXXVII, p. 86, 1905.

stituent in large quantity, and (ii) by the conspicuous physiological effects obtained by increasing or diminishing the osmotic pressure of the apparently impermeable constituent, and so limiting the play of dissociation and association on the part of the cell.

Turning now from qualitative considerations of the equilibrium between the cell and its external medium the plasma, to considerations of the *total* osmotic equilibrium of pressure between cell and plasma, as shewn by depression of freezing point in both cases, we find that the necessary condition is an *equilibrium* of total pressure between cell and outer fluid, and this equilibrium need not necessarily mean an *absolute equality* of osmotic pressure within and without.

It is quite clear in many cases that the cells can exist and functionate in a perfectly normal physiological manner when they are not in a condition of equality in regard to total osmotic pressure with the fluids which bathe them. Thus a salivary gland cell in secretion is in contact on the one side with plasma or lymph and on the other side with the secreted saliva possessing an osmotic pressure much lower than that of the lymph; it is, therefore, perfectly obvious that the osmotic pressure of the cell contents cannot be equal both to that of the lymph and that of the saliva, which are entirely different from each other in value. Similarly, in the case of the secreting cell of the uriniferous tubule, on the one side is lymph and on the other urine with a very varying osmotic pressure as shown by the depression of freezing point it possesses, yet the varying osmotic pressure of the urine never injures the tubule cell, although the osmotic pressure of urine and cell contents never can be in a state of equality. A third example is the columnar cell of the intestinal villus, which has most widely varying pressures on its intestinal side and manages to maintain in its living condition an equilibrium with these, and absorbs fluids of very varying osmotic concentration. In fact, it is the exception and not the rule that the living cell in the body must be in contact with fluids of equal osmotic concentration to its own cell contents. An equilibrium with regard to osmotic contents must exist between cell and bathing fluid, but the cell possesses an adaptability within certain limits, which vary with the type of cell, and the equilibrium

need not consist in an equality of osmotic pressure within and without the cell.

The usual inference that there is an equality of osmotic pressure is based on the plasmolysis experiments in plant cells, and on changes in volume of animal cells, especially the red blood corpuscles, on altering the osmotic concentration of artificial outside bathing fluids.

Now, such experiments are of value in shewing the osmotic pressure with which cells are in *equilibrium* at different volumes of the cell, but they do not demonstrate equality of pressure within and without, or prove that the cell, normally, has an *equality* of pressure with its environing medium under natural conditions; nor can the cell be regarded as a perfectly inert membrane quite free to move and accommodate itself without resistance to all changes in osmotic pressure in the outer medium.

It is further assumed in such experiments that the salts inside the cell and those placed in the medium without are *perfectly* impermeable to the cell, and as has been pointed out above, this is a most dangerous assumption.

It is well to analyse what is the fundamental thing being measured in such experiments on change of volume of cells placed in solutions of varying 'tonicity,' or osmotic pressure.

It is quite clear that the cell will not change in volume if the tendency to a flow of water is equal in the two directions. Hence, the two things pitted against each other in any such experiment, are really two rates of diffusion of water, one inwards and one outwards, and a constant volume, therefore, means an equality in these two rates of diffusion. This, however, may mean something quite different to equality of osmotic pressure within and without, and it is only in the limiting case of *complete* or perfect impermeability for all soluble constituents that equality of rates of diffusion, which the experiment shows, can be taken to mean equality of osmotic pressures. If a dissolved substance passes in and out without any resistance, then it cannot exert any pressure, and will lead to no diffusion of water and have no effect on cell volume. If it cannot pass at all, it will exert its full osmotic pressure when the cell has come to rest, and will exert

its full influence on the diffusion of water and on cell volume. Between these two extremes a partial influence will be exerted; and apart from adsorption, and allowing full time for equilibrium to be attained, then equilibrium will only be reached when the concentrations of free unadsorbed substance are equal on both sides of the membrane for each individual soluble component of the system of dissolved substances.

The plasmolytic method, as a machine or apparatus of physical chemistry for determining isotonicity of two given external fluids, each of which possesses an equally low permeability to the cell, is perfectly legitimate, so long as the assumptions made in the experiment are carefully borne in mind. These are that the permeability must be low for both substances and, theoretically, that the rate of permeability should either be equal in the two cases or so small as to be negligible during the period of the experiment, and secondly, if there is any permeability, as there nearly always must be, that the observations be taken at the same time interval from the commencement.

Given that these conditions are fairly well satisfied, then the plasmolysis method may be a good working method for determining approximate equality of osmotic pressure in different solutions.

It is quite another matter, however, to assume from such experiments that there is an *equality* of osmotic pressure between the cell contents and the environing medium of the cell.

In the case of the vegetable cell, turgidity of the cells and pressure of the contents against the cell wall would be impossible by osmotic action if the effective portion of the osmotic concentration of the outer sap were not less than the effective portion of the osmotic concentration of the cell sap within. When plasmolysis occurs, these two effective portions have become equal, leaving out of account any resilience of the cell contents.[1] But the experiments say nothing as to equality in osmotic concentration of sap within and without, and the effective factor in turgidity is the difference in the two. The effect of slow permeability must also be borne in mind, as well

1. See Drabble and Scott, *Bio-Chemical Journal*, Vol. II, p. 221, 1906.

as cell resilience, which is an important factor in many types even of vegetable cell.

In most types of animal cell the resilience becomes a most important factor, and enables the cell safely to bear great variations in the osmotic pressure of fluids in contact with it. Here the minute size of the cells requires careful consideration, since, given that the dimensions are sufficiently minute, a most delicate structure can support a very high pressure. For example, a very thin-walled capillary glass tube, if its bore be sufficiently small, can bear a pressure of several atmospheres without rupturing, which would at once burst even a thick-walled glass tube of wide diameter. Hence, in the case of a tissue cell of fixed dimensions a very considerable difference of osmotic pressure may be borne without any appreciable change in volume.[1]

Accordingly, we must be slow to argue from observations of changes in volume of cells subjected to treatment with salines of varying concentration, that there exists equality of osmotic pressure at these different volumes within and without the cell.

It seems somewhat strange that it has not occurred to any previous workers on the subject to determine freezing points of both serum and separated blood corpuscles and to contrast such determinations with each other; and further to allow corpuscles to come into equilibrium, as regards osmotic exchange, with salines of different concentration, then separate corpuscles from salines by centrifuging, and determine freezing points in both.

This method gives a direct experimental answer to the enquiry as to whether the osmotic pressures within and without are always equal, or whether there is an equilibrium at each pressure but not necessarily an equality or isotonicity.

In the present experiments we have carried out such measurements, amongst others, and have obtained interesting results which show that neither in the normal condition of natural serum and

1. This result is due to thickness of cell or tube wall, when very small dimensions are reached, being very great relatively to the thickness of wall in wider tubes.

untreated corpuscles, nor in the case of corpuscles brought into equilibrium with salines of different strength is there absolute equality, but rather an equilibrium which varies with the concentration.

It may be argued that the small but constant differences in depression of freezing point which we have observed between natural serum and corpuscles may be due to unequal changes in dissociation in the two cases between body temperature and the freezing point at which, of course, the freezing point determinations have to be made. This we freely admit, but at the same time the freezing point method is the only one available at present for determinations of osmotic pressure in serum and corpuscles, and is the one which has been hitherto relied upon for the supposed proof that there exists equality of pressure between intercellular fluid and cells or, in the present particular case, between blood serum and erythrocytes. We submit that if freezing point determinations are to be quoted in proof of isotonicity, then freezing point of serum and erythrocyte must be shewn to be the same within the limits of experimental error.

In the whole extensive literature of cryoscopic measurements on blood we have only been able to find one instance in which the freezing point of the separated corpuscles has been taken alongside of that of the serum. This measurement was made by Prof. G. N. Stewart[1]; the Δ found for whole blood was 0·628, that for the corpuscles was 0·597, and the author marks the latter with a note of interrogation, evidently taking the difference for an experimental error and pursuing the subject no farther.

We have found a constant difference in this direction throughout all our experiments, viz., that the depression of freezing point of the serum is always somewhat greater (0·02 to 0·03) than that of the erythrocytes, or in other words, that, at any rate at the freezing point, the osmotic concentration of the serum is greater than that within the red blood corpuscle.

When the corpuscles are shaken up with salines of different osmotic concentration, allowed to settle, centrifuged off, and then

1. *Journ. Physiol.*, Vol. XXIV, p. 129, 1899.

cryoscopic determinations made in (*a*) corpuscles and (*b*) supernatent saline, these differences become greatly exaggerated. The corpuscles do not come into equality of osmotic concentration with the outer saline, but preserve a varying pressure of their own, which stands in equilibrium with the outer pressure but is not equal to it.

We have also performed other series of experiments which bear in upon the question before us, of the relationship of inorganic constituents of cells and intercellular fluids, and how these salts are related by adsorption or otherwise to the organic constituents.

One method we have utilised is that of dialysis carried out in comparative experiments in serum and corpuscles. If the dissimilar constitution and composition of the electrolytes of (*a*) serum and (*b*) erythrocyte were due to an impermeable wall, then we should expect that when we disrupt that wall and allow cell contents to escape as, for example, by dialysis against distilled water, the salts previously held in prison by the wall would escape and pass readily into the dialysate, and then we should expect that determinations of osmotic concentrations in the two dialysates would give us at least approximately equal results. If, on the other hand, the electrolytes of the cell are held in adsorption, or some form of chemical combination by the organic cell contents, then we should expect that such adsorption might continue when both organic substance and electrolyte quit the cell as a result of dialysis; and as the organic substance cannot pass the dialysing membrane, neither can the electrolyte which is adsorbed with it. Hence, in this case, the Δ of the dialysate of the corpuscles should be much less than that of the serum. We are here assuming, of course, that the pressure of electrolyte does not fall sufficiently low in the process of dialysis to cause any considerable dissociation of electrolyte and organic cell constituent. This, however, is shown by theoretical considerations given earlier in the paper to be an improbable result, as the osmotic pressure of the electrolyte necessary to cause almost complete adsorption is very low, and hence, when dialysis occurs, osmotic pressure of sufficient value will be very soon restored by the diffusion of a very small amount from the adsorpate of organic constituent and electrolyte.

The results of our diffusion experiments very strongly support the view of an adsorpate between the organic constituents of the erythrocyte and the electrolytes contained therein, rather than the action of any membrane.

Similar results are obtained by repeated freezing and thawing, which lake the corpuscles, the freezing point remaining practically constant under such conditions, although the conductivity alters on account of removal of mechanical resistance.

In addition, we have conducted incineration of serum and corpuscles, and made chemical determinations of chlorine and phosphoric acid, in order, by our own experiments, to make certain of the very different composition of the two ashes shewn by all the older experiments. Further, we have made cryoscopic and conductivity experiments with ashes, dialysates, and original sera and corpuscles, which have clearly demonstrated to us that there is a labile osmotic equilibrium between serum and the cell, which is never characterised by absolute osmotic equality, but shows adsorption by the cell in all cases, and leads accordingly to different disposals of electrolytes and other soluble substances within and without the cell.

It is remarkable that in such an adsorbed form the electrolytes of the cell still have their due effect in producing osmotic pressure, as shewn by the cryoscopic method. Although attached in some form to the protein they still have a full effect in depressing the freezing point. There is, hence, a degree of freedom of the attached ions in adsorption which is lost in ordinary chemical combination, and this, to our minds, is one of the most fundamental differences between such adsorptions and chemical combination. For it is clear, when the relative percentages of salt and protein and relative molecular weights are considered, that there must be several ions in combination with each colloidal protein molecule, yet each of these has its full effect on freezing point as if it were free.

It is otherwise in regard to electrical conductivity, because here the ionic velocity is altered by the attached protein molecule which must be bodily moved with the ions in the electric field. Undoubtedly a part of the very high resistance of the corpuscles is

due, as shewn by Stewart and Oker Blom, to the mechanical effect of the want of homogenity of the fluid in the electric field[1]. This higher resistance, however, disappears only in part when the corpuscles are laked and the field made practically homogeneous save for the ghosts of the corpuscles, and the conductivity of the laked solution is still much lower than that of a dialysate reduced by evaporation to an equal volume, or of the ash after incineration and accompanying removal of haemoglobin.

Thus we have found, in confirmation of Stewart, that the conductivity of corpuscles is only one-fourteenth to one-nineteenth of that of serum; on laking by freezing and thawing alternately, the conductivity rises to about one-sixth of that of serum; dialysis brings conductivity up to one-half to one-third of the conductivity of the serum dialysate, and the ash of serum and of corpuscles have nearly the same conductivity.

This still high resistance after laking we attribute to the attachment of haemoglobin to the electrolytic ions, which is further confirmed by the fact that haemoglobin, as shewn by Gamgee, does move in the field during electrolysis.

This independence of ions as regards osmotic pressure (freezing point), and dependence on protein regarding ionic velocity (conductivity) is remarkable, and is probably connected with that extreme degree of labile equilibrium which is everywhere characteristic of the living cell and its energy exchanges.

The experiments on (A) fresh corpuscles and serum, (B) laking, (C) effects of foreign salines, (D) dialysis, (E) incineration, (F) estimation of chlorides and phosphates, and (G) conductivity, were usually made on the same samples of blood so as to admit of comparison with one another, but for clearness of description are placed below in different sections.

1. We have ourselves carried out conductivity experiments with sand and saline, and saline mixed with undissolved starch granules, which completely confirm Oker Blom's theoretical work on the subject.—*Arch. f. d. ges. Physiol.*, Vol. LXXXI, p. 167, 1900.

A.—On the Relative Freezing Points of (*a*) Corpuscles and (*b*) Serum in Fresh Untreated Blood

The blood was whipped as it came from the animal,[1] carefully collected and centrifuged as rapidly and completely as possible. Then serum and corpuscles were pipetted apart into dry vessels, and freezing points were determined as rapidly as possible. The same material was used for the conductivity experiments (Section G).

The value of Δ given is in each case the mean of at least three closely concordant freezing point readings.

No. of Experiment	Serum Δ	Corpuscles Δ
Sample 1	0·607° C.	0·577° C.
Sample 2	0·539° C.	0·519° C.
Sample 3	0·591° C.	0·573° C
Sample 4	0·586° C.	0·570° C.

Thus, the Δ of the corpuscles lies 0·02 to 0·03 lower than that of the serum, this would correspond to a difference of osmotic pressure of approximately 200 to 300 mm. of mercury pressure.

B.—On the Freezing Points (*a*) of Serum and (*b*) of Corpuscles after Laking the latter by Repeatedly Freezing Solid and Thawing

Here, no appreciable change in freezing point occurs, as might be expected were crystalloids set free by rupture of a membrane wall.

	Serum Δ		Corpuscles Δ	
	Freezing solid		Freezing solid	
	Before	After	Before	After
Sample 1	0·607	0·613	0·577	0·571
Sample 2	0·539	0·548	0·519	0·520

1. The blood of the pig was used for all the experiments given.

C.—On the Equilibrium Between Blood Corpuscles and Salines of Varying Concentration

In these experiments three strengths of saline were used, one of which was made very hypertonic, the second nearly isotonic, as shown by its freezing point, and the third hypertonic to the natural serum of the blood corpuscles used. The concentrations of the three solutions used were in the first experiment in the ratios of three, two and one, the more dilute being made from the strongest by diluting in this ratio with distilled water.

The corpuscles, after thorough centrifuging, were pipetted off and mixed with an equal volume, in each case, of the three strengths of the saline solution.

With the view of afterwards testing for potassium, and, if possible, estimating the relative amount of potassium and sodium coming out of the corpuscles by weighing mixed chlorides and determining percentage of chlorine, we used, as our saline medium, calcium chloride instead of sodium chloride. On account of other substances not chlorides, such as phosphates, dialysing out from the corpuscles, the percentages of sodium and potassium were so indeterminate that we could place no confidence in our actual figures by this indirect method of estimating potassium. We satisfied ourselves, however, by precipitation with platinic chloride and cobalt hexa-nitrite, that potassium was present in the calcium chloride saline after mixing with the calcium chloride and centrifuging.

When the centrifuged-off corpuscles had been placed in each of three centrifuge tubes with an equal volume of the three calcium chloride solutions of the three different strengths, they were thoroughly mixed, shaken up several times, and left for an interval of sixteen hours. If the small size of the red corpuscle is considered, it is evident that the process of equalization by diffusion must have been complete and equilibrium established by the end of this period.

The Δ for the serum (Sample 5) in the first of these experiments was 0·551° C., that of the strongest calcium chloride solution was 0·983, and, therefore, assuming no appreciable change in dissociation

from dilution, the calculated Δ of the other two solutions would be 0·647 and 0·323, so that No. 1 was strongly hypertonic, No. 2 slightly hypertonic, and No. 3 strongly hypotonic.

For the second experiment the blood of Sample 3, as used above, was taken, and the Δ determinations were made directly for each of the three calcium chloride solutions, which stood in the concentrations of approximately 2·03 per cent., 1·25 per cent., and 1 per cent.; the lowering of freezing point in each of these solutions before admixture with the corpuscles was found to be 0·919° C., 0·574° C., and 0·465° C., so that No. 1 was strongly hypertonic, No. 2 almost exactly isotonic, and No. 3 strongly hypotonic.

The solutions, after admixture with an equal volume of corpuscles, were left as before, being often shaken up, and then allowed to stand over night.

In the third experiment (Serum Sample 4), on exactly similar lines, three salines of barium chloride, showing Δ's of 0·605° C., 0·308° C., and 0·255° C., were used, instead of calcium chloride solutions. The weakest concentration gave rise to a considerable amount of laking, but the corpuscles still showed, even here, a lesser Δ. As in all the other experiments of the series, no laking was seen in No. 1 of this experiment, and only very little in No. 2.

Throughout the series of experiments it is seen that the osmotic pressure of corpuscles remains much below that of the saline, and this whether the saline is hyper- or hypotonic to the original natural serum of the corpuscles used for the experiments.

EXPERIMENT I ($CaCl_2$)

No. of solution	Original solution Δ	Saline after admixture and centrifuging Δ	Corpuscles after admixture and centrifuging Δ	Difference in Saline Δ and Corpuscle Δ
No. 1	0·983	0·847	0·788	0·059
No. 2	0·647 (calculated)	0·627	0·563	0·064
No. 3	0·323 (calculated)	0·409	0·362	0·047

EXPERIMENT II ($CaCl_2$)

No. 1	0·919	0·828	0·748	0·080
No. 2	0·574	0·598	0·561	0·037
No. 3	0·465	0·622	0·505	0·117

EXPERIMENT III ($BaCl_2$)

No. 1	0·605	0·615	0·581	0·034
No. 2	0·308	0·428	0·399	0·029
No. 3	0·255	0·396	0·375	0·021

D.—ON THE FREEZING POINTS OF (*a*) CORPUSCLES AND (*b*) SERUM AFTER DIALYSIS AGAINST DISTILLED WATER

The object of these experiments has been pointed out above.

In carrying out the experiments, a known volume of corpuscles or serum respectively, usually 50 c.c., was taken, placed in a wide piece of sausage-shaped dialysing tube of parchment paper; this was immersed in a wide-mouthed bottle containing a measured amount of distilled water, usually 450 c.c. All possible precautions were used by sterilizing bottle, dialysis tube, and water against putrefactive change, and dialysis was allowed to proceed for forty-eight hours, the dialysing tube being held in position by clipping it between bottle neck and bottle stopper when placing the glass stopper in the bottle.

After completion of the period of dialysis, the outer fluid was carefully collected in a graduated vessel, and measured. It was then evaporated down to such an aliquot part that it corresponded to the concentrations of the serum or corpuscles originally taken. Suppose, for example, the original amount of serum in the sausage tube was 50 c.c., and in the bottle outside there was 450 c.c. of distilled water, then it is obvious that the serum, when equilibrium was complete, would be diluted tenfold; hence, if at the end, 440 c.c. were found in the water bottle, this was evaporated carefully down to 44 c.c. before its freezing point was taken. Similar procedure exactly was used for the corpuscle dialysate to be compared alongside of the serum.

It was found that the corpuscle dialysate had a much less Δ than the serum dialysate, shewing the stronger adsorption between the organic matter of the corpuscle and the electrolytes of the corpuscle.

RESULTS OF DIALYSIS

	Serum Δ		Corpuscle Δ	
	Before dialysis	After dialysis	Before dialysis	After dialysis
Experiment I (Sample I)	0·607	0·562	0·577	0·232
Experiment II (Sample II)	0·539	0·568	0·519	0·262

E.—FREEZING POINT OF THE ASH AFTER INCINERATION OF (*a*) SERUM AND (*b*) RED BLOOD CORPUSCLES RESPECTIVELY

	Serum Δ		Corpuscle Δ	
	Incineration		Incineration	
	Before	After	Before	After
Sample I	0·607	0·450	0·577	0·357
Sample II	0·539	0·469	0·519	0·362

It is to be noticed that Δ is decreased after incineration for both serum and corpuscles; but that the two depressions are much closer than after dialysis where the adsorpate between protein and crystalloids in the case of the corpuscles keeps the crystalloids from passing the dialysing membrane.

F.—PERCENTAGE OF CHLORIDES AND PHOSPHATES IN (*a*) SERUM AND (*b*) CORPUSCLES RESPECTIVELY

The relative percentages of chlorides and of phosphates in serum and corpuscles were determined (*a*) after dialysis and (*b*) after incineration by the usual volumetric methods, with the following results.

	Serum percentages		Corpuscles percentages	
	Cl	P_2O_5	Cl	P_2O_5
Dialysis	0·3657	0·0197	0·1331	0·0329
Incineration	0·3373	0·0219	0·1704	0·1708

The results confirm the freezing point determinations, and show that in the case of the corpuscles the phosphates are held back from dialysis by some form of combination or adsorption with the protein;

the chlorides seem to be held in much looser combination, and in part dialyse through, but even here the figure is still much lower in the dialysate (0·1331 as against 0·1704) instead of being somewhat higher as in the serum due to slight volatilization during incineration with such a large excess of organic matter.

G.—ELECTROLYTIC CONDUCTIVITY OF SERUM AND OF CORPUSCLES RESPECTIVELY: (*a*) IN FRESH CONDITION, (*b*) AFTER LAKING BY FREEZING AND THAWING, (*c*) AFTER DIALYSIS AND REDUCTION TO ORIGINAL VOLUME, (*d*) AFTER INCINERATION AND MAKING UP TO ORIGINAL VOLUME

The conductivity was, in each case, determined in the usual manner at 40° C., by Kohlrausch's method, and the specific conductivity calculated.

The figures given, in order to save decimals, are sp. conductivity multiplied by 10^5.

	Treatment to which subjected	Sample I		Sample II	
		Serum	Corpuscles	Serum	Corpuscles
1.	Fresh... ...	1705	95	1519	109
2.	Frozen Solid (Corpuscles laked)	1602	310	1468	237
3.	Dialysed ...	1843	891	1623	754
4.	Incinerated ...	1608	1677	1697	1655

The figures support the conclusions of the other sections, and the changes in the conductivity of the corpuscles are most interesting. The first increase on laking is probably mechanical, due to removal of the resistance interposed by the want of homogenity caused by the presence of the whole corpuscles. The second increase with dialysis is due to the detachment from the protein of the greater part of the less firmly held chlorides, but the phosphates are here lost on account of their stronger attachment and to their being left behind in the dialysis tube.

Finally, in the incinerated corpuscles and serum both chlorides and phosphates are free, and the conductivity of the corpuscle crystalloids now becomes practically equal to that of the serum crystalloids.

Conclusions

1. The membrane theory fails to explain (*a*) the difference in composition of the electrolytes within and without the cell, (*b*) the physiological effects of perfusion or irrigation of cells by media defective or excessive in certain electrolytes normally present in the cell, (*c*) the selective uptake, accompanied by physiological effects, of certain soluble substances by different cells, such as food constituents, drugs, anaesthetics, and toxins.

2. These phenomena receive a simple explanation, on the basis of the formation of adsorpates or chemical combinations between cell protein (or protoplasm) and other constituents.

3. The cell when functionating normally exists in a labile or mobile equilibrium with such constituents, and is capable of undergoing reversible processes of association or dissociation with such constituents.

4. For each constituent there exists a certain range of osmotic pressure for that constituent, within which partial association and dissociation is possible, and it is within this range alone that labile exchanges are possible. Thus there is a minimal limit of starvation and a maximal limit of plethora for each indispensable constituent beyond which exchange becomes impossible in sufficient amount to meet the requirements of cellular life, and when these limits are passed in either direction, the chemical exchanges become embarrassed, and finally cell activities cease in a form of asphyxiation in a wide sense, showing similar effects at both ends of the range.

Anaesthetics, toxins, and drugs probably produce their effects by causing, when in sufficient concentration, adsorpates or compounds, which upset the cell's metabolism on account of their stability at the given osmotic pressures.

5. The osmotic concentration leading to formation of sufficiently stable adsorpates varies from substance to substance, and in many cases, as notably in that of oxygen, the osmotic concentration at the dissociation range is very low.

6. Cells normally exist and functionate in contact with fluids such as secretions, which are not isotonic with the cell contents.

7. There is not absolute isotonicity between the erythrocytes of blood and serum, the depression of freezing point for serum being always slightly greater than that for the corpuscles.

8. On laking the erythrocytes, the freezing point does not appreciably change.

9. The differences in freezing point are much exaggerated when the corpuscles are brought into equilibrium with either hypotonic or hypertonic saline solutions, and the rule holds that the osmotic concentration of the saline outside is always higher (as in the case of natural serum and corpuscles) than that of the corpuscles, whether the saline was originally hypotonic or hypertonic.

10. Dialysis also demonstrates an adsorpate between the protein and the electrolytes of the erythrocyte, since the depression of freezing point for corpuscular dialysate is much less than that for serum dialysate.

11. The results of dialysis and incineration, and determination of chlorides and phosphates, considered together, show that the adsorpate between protein and phosphates persists at a much lower osmotic concentration than that between protein and chlorides.

12. Determinations of electrical conductivity of corpuscles and of serum, of laked corpuscles and of serum, of dialysed corpuscles and dialysed serum, and of incinerations of corpuscles and of serum have been carried out which shew, (i) that in the natural condition of corpuscles and serum the conductivity of the former is only one-fourteenth to one-seventeenth of that of the latter, (ii) that this difference is partially mechanical, the ratio being reduced to one-fifth to one-sixth on mere laking (confirmation of Stewart's results), (iii) that the ratio is further reduced to about one-half on dialysis, due to setting free of crystalloids from colloids, although, in the case of the corpuscles, the greater part of the phosphates are still held back in adsorption or chemical combination with the protein, and (iv) that finally, on incineration, the conductivity of corpuscles and serum crystalloids becomes practically equal in value.