DIRECT MEASUREMENTS OF THE OSMOTIC PRESSURE
OF CASEIN IN ALKALINE SOLUTION. EXPERIMENTAL
PROOF THAT APPARENT IMPERMEABILITY
OF A MEMBRANE TO IONS IS NOT DUE TO THE
PROPERTIES OF THE MEMBRANE BUT TO THE
COLLOID CONTAINED WITHIN THE MEMBRANE

By BENJAMIN MOORE, M.A., D.Sc.; HERBERT E. ROAF, M.D.,
D.Sc.; AND ARTHUR WEBSTER, *University of Liverpool*.

*From the Laboratories of Bio-Chemistry and Physiology, University of
Liverpool*

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The most important experimental points settled by this research are the unequal distribution of alkali on the two sides of the membrane at the conclusion of each experiment, the high pressures observed, the flow of crystalloid *towards* the region of high pressure, and the proof that crystalloid, although associated with colloid, still exercises osmotic pressure.

This combination of observations appears to us to finally settle several outstanding questions as to osmotic pressure effects of colloids, and of the behaviour of living cells towards *apparently* impermeable ions which are, nevertheless, found in high concentration within the cells.

These experiments finally dispose of the older view, that osmotic pressures of colloids are not due to the colloids but to slowly diffusible crystalloids present as impurities (the ash of the colloid), which never get into equilibrium on account of the slowness of their diffusion through the membrane from the region of high pressure to that of low pressure.

For, the direction of movement of the crystalloid associating with the colloid, and causing the osmotic pressure as a crystallo-colloidal complex, is from the side of low pressure to the side of high pressure, and this to such a great extent as to leave no question about the matter. Until the colloid is almost saturated by crystalloid, practically no alkali remains on the non-colloid side of the membrane.

The alkali moves *against* osmotic pressure, into the colloid side, there to unite in some form with the colloid, and it is this union which causes the osmotic pressure. The purpose of the membrane is purely the mechanical one of holding together the colloidal aggregates, and its apparent impermeability to the ions is quite fictitious. Free ions pass through quite readily, ions anchored to colloid are retained on the colloid side and exercise pressure, and the membrane has no influence on permeability of free inorganic ions.

This observation throws a clear light on the behaviour of living cells towards ions: the varying concentrations of sodium, potassium, chlorine and phosphatic ions within and without the cell are an expression of specific affinities of the definite colloids of each particular cell-type for these ions, and do not mean that there is a membrane acting as a closed gate to these ions.

The osmometer containing the casein solution may be regarded as a cell, and the adsorbed or combined alkali is the analogue of the potassium ion of the living cell; the alkali has no appreciable concentration outside until the concentration within has become 0.03 N, and as alkali concentration is increased there persists always a higher concentration within and a lower without, just as the cell always has an almost negligible concentration of potassium ion without and a high concentration within the cell, and this concentration within the cell only slowly rises when the concentration outside is increased. On the contrary, if the crystalloid pressure outside is diminished, the specific affinity of colloid and crystalloid holds them together, so that there is only a very slow passage of crystalloid away from cell or osmometer. In this way a *fictitious* appearance of impermeability is produced.

There is no experimental evidence that the cell-membrane is impermeable to potassium, the actual presence of that ion within it shows that it must be permeable, and the analogous action of the osmometer membrane shows that the membrane is easily permeable to the free crystalloid, and that the high concentration of crystalloid within and low one without is due to a specific affinity of the colloid for the crystalloid.

In these experiments we can actually follow the process of the crystalloid being drawn through the membrane, *against* the gradient of osmotic pressure, the rise in osmotic pressure as a result of this, and the

establishment of a different concentration of the crystalloid on the two sides leading to the simulation of an impermeable membrane, such as is presented by the picture of the living cell.

Reasons have previously been assigned at greater length for the view that living cells are in reality permeable freely to ions which appear to pass with the greatest difficulty into or out of them.¹ In the first place, the heaped-up concentration of potassium and phosphate within the majority of living cells, as contrasted with the concentration of these ions in the outer plasma or other bathing fluids, is to us clear evidence that the cell, or cell membrane, is easily permeable to the potassium ion. Otherwise, in the process of reproduction of new cells as the organism grows, the initial concentration in potassium ion within must have rapidly fallen to zero, instead of remaining heaped-up high above the outer level, as one cell gave rise to many. This heaping-up of certain ions within the cell expresses for us a certain molecular affinity between the colloid contents of the cell and these ions, and it is that same affinity which prevents such ions being washed out when the cell is subjected to dialysis, and so causes the fictitious appearance of impermeability of a membrane upon which those observers still rely who believe in the existence of a cell-membrane impermeable, or only permeable with great difficulty, for these ions. Also, when the affinity of the cell colloids for an ion, such, say, as potassium, is satisfied, further uptake of potassium will be very slight and on a purely physical basis, so that when cells, such as red blood corpuscles, are bathed with salines rich in potassium, they take up but little more than their normal quantity. This, however, is no proof of impermeability, any more than is the fact that appreciable uptake of oxygen ceases when the haemoglobin is saturated with oxygen is a proof that the red blood corpuscle is impermeable or difficultly permeable to oxygen. All these experimental findings will present themselves in any living cell possessing a colloid with a molecular affinity for ions or other dissolved crystalloid substances, and such phenomena have nothing whatever to do with permeability, or impermeability, of a membrane to the ions or other crystalloids.

First, when the cell is washed with a saline solution free from the ion in question or containing that ion in very low concentration, the amount of the ion washed out of the cell may be practically inappreciable,

1. See Moore, 'Recent advances in Bio-Chemistry and Physiology,' edited by L. Hill, Arnold, London, 1907; Moore and Roaf, previous papers this Journal and elsewhere.

for it will only wash out till the concentration of free ion balances against the amount of adsorbed ion, and, depending on the molecular affinities, this concentration may be a very low one indeed, falling almost to zero in the case of the potassium ion. Thus, sodium ions can readily be washed out of cells because the cell proteins have no affinity for them, while potassium ions are retained to the last. This shows also how fresh-water organisms can saturate themselves with potassium and phosphatic ions from waters in which all tests for potassium would fail even after considerable concentration. A similar affinity of the colloidal constituents of the soil for potassium ions and phosphates retains these valuable constituents in the soil, while the sodium and chlorine ions are carried seawards by the rivers. No membrane retention hypothesis ever put forward can explain such facts as these.

Secondly, when a cell is washed with a saline containing a hyper-tonic quantity of one of these *apparently* impermeable ions, since the cell already contains, on account of its molecular affinities, a large amount of the particular ion, the further amount which it takes up physically is comparatively so small as to simulate impermeability and appear to lie within the limits of experimental error.

Physiological tests, which are always more delicately balanced than volumetric or gravimetric chemical tests with purely inorganic agents, clearly demonstrate, however, that the balance in the cell is being upset under such conditions. While the cell, to ordinary chemical tests, as pointed out above, appears to be at least difficultly permeable to potassium ions, physiological tests show that it is being profoundly affected. This is shown by the beautifully contrasted effects of perfusion with ordinary saline on the one hand, and with Ringer's solution on the other, in the case of the excised heart, as also in the effects of potassium starvation and potassium excess for practically all types of living cells. Witness also the profound effects of two or three grammes of potassium administered to a man of sixty to seventy kilograms, in depressing the whole nervous activities, and it must be admitted that the infinitesimal extra amount which has entered the cells has produced an enormous result. Such an effect cannot be explained by membranes. It is a result produced within the cell amongst the constituent cell-proteins affecting their aggregations, and fitness for chemical change and exhibition of chemical energy. Apart from living cells, we know experimentally that ions or crystalloids produce just such effects upon colloids in forming

their crystallo-colloidal aggregations. The solution-aggregate of the colloid varies in size with the nature and amount of crystalloids present, even far short of actual precipitation.¹ It is, therefore, easy to see that variations in amount and character of adsorbed crystalloids must lie at the very citadel of cell activities.

Now, what is claimed as the chief point of beauty and interest about the present series of experiments is that in an experimental and artificial system this march of events is clearly demonstrated.

At first there is the colloidal casein in the osmometer inert and showing no osmotic pressure, just as a living cell would be if deprived of all its potassium ion, then as alkali, which takes the place of the potassium ion of the living cell, is admitted slowly in the earlier experiments, and in increasing amounts in the later ones, the system, so to speak, becomes alive and active, and the osmotic pressure, which corresponds to the index of life, rises all the time till an optimum limit is reached, beyond which there is no further effect but rather a diminution in effect. It is not, of course, pretended that we have in this simple system of casein and alkali anything so labile and delicately balanced as a living cell. But in this simple type we see the same primary factors as in the cell, and the important point is that the chemical play and energy-discharge is within the osmometer as we hold it is within the cell. The function of the osmometric membrane is a mechanical one of holding crystalloid and colloid together, just as we hold that of the membrane of the living cell to be. The osmometer membrane is freely permeable to the alkali alone, it merely prevents back passage of the crystallo-colloidal mass, and this produces a fictitious appearance of impermeability, which we know, from having watched the experiment from the beginning, to be erroneous. We cannot watch the experiment from the start in the case of the cell, we take it up when the cell is already charged with potassium ion, and hence our first impression is that the cell possesses a membrane which is impermeable to the potassium ion. A moment of reflection convinces us, however, that the cell is now full of potassium, that it has arisen from a thousand previous generations of cells, all containing a like percentage of potassium to itself, and since in the thousand-fold repeated cell-division the volume has increased a thousand-fold of the entire mass of cells, it is irresistible

1. Moore and Roaf.

to conclude that potassium must have been going in all the time. Here the osmometric experiment shows us how the uptake or out-put of the crystalloid is regulated and how the fictitious and merely apparent impermeability is produced and maintained.

The thirst of the casein for alkali, demonstrated in the three experiments at the upper end of the table where the concentration of alkali is lower, illustrates the manner in which the colloids of living cells can extract and concentrate crystalloids for their purposes from infinitesimally low amounts in the fluids bathing them. Such, for example, as bone formation from the excessively low concentration of calcium ion in the blood, the formation of calcareous and siliceous shells in freshwater and marine organisms, and many similar cases. Such concentrations arise from affinities of a molecular type between colloids and crystalloids, which vary from time to time, so causing periods of uptake and deposition in a rhythmic manner.

The osmometer used was of metal lined by platinum and with a platinum grid supporting a parchment paper membrane. The two instruments used were those described by us in previous papers. The percentage of casein present was determined from the nitrogen of a Kjeldahl carried out in each case at the conclusion of the experiment, since the high pressure registered caused considerable dilution of the colloid solution during the experiment. Readings were recorded at intervals as shown in the protocols of experiments, and great care was always exercised that equilibrium was established before the experiment was concluded. Titration for free alkali was made, using phenol-phthalein as an indicator, when the end point is reached at the point where alkali and casein are combined. Of course no casein is present on the colloid-free side, and hence the titrations there are quite absolute. It is to be observed that in the beginning there is no free alkali on either side, and that the free alkali on the colloid-free side remains very low until about 0.07 to 0.08 N concentration has been reached, when the casein begins to be saturated at the levels used in the experiments.

It may be noted that, although the table seems but a short one involving little labour, that each line represents a prolonged experiment, with many observations and accompanying analyses.

Final concentration of Caseinogen	Observed Pressure	Pressure per one per cent. of Caseinogen	Initial concentration of Sodium Hydrate	Concentration of free Sodium Hydrate in Colloid chamber of Osmometer	Concentration of free Sodium Hydrate in Crystalloid chamber of Osmometer	Temperature at end of Experiment	Time in days Experiment lasted
<i>Expt. I</i> — 8.2 %	mm. 233	mm. 28	0.02 N	0.031 N Acid	Nil	11.5° C.	14
<i>Expt. II</i> — 5.4 %	310	57	0.033 N	0.02 N Acid	Nil	12.5° C.	10
<i>Expt. III</i> — 8.0 %	334	42	0.04 N	0.004 N	0.006 N	11.0° C.	14
<i>Expt. IV</i> — 5.6 %	460	82	0.06 N	0.025 N	0.013 N	15.0° C.	6
<i>Expt. V</i> — 5.7 %	488	85	0.065 N	0.024 N	0.018 N	19.0° C.	13
<i>Expt. VI</i> — 6.08 %	610	101	0.07 N	0.022 N	0.015 N	15.0° C.	10
<i>Expt. VII</i> — 5.37 %	686	129	0.08 N	0.047 N	0.025 N	15.0° C.	14
<i>Expt. VIII</i> — 4.7 %	588	125	0.09 N	0.048 N	0.034 N	13.0° C.	14
<i>Expt. IX</i> — 5.04 %	586	117	0.095 N	0.056 N	0.032 N	14.5° C.	14
<i>Expt. X</i> — 5.16 %	538	105	0.1 N	0.066 N	0.044 N	15.5° C.	9
<i>Expt. XI</i> — 5.1 %	508	100	0.1 N	0.06 N	0.042 N	13.0° C.	22

NOTE.—The concentration of alkali at the commencement was the same exactly on both sides of the membrane, and varied from one-fiftieth normal in the first to one-tenth normal in the last experiment. Observe the passage of alkali through the membrane to join casein, and the rising osmotic pressure demonstrating that the osmotic pressure is due to the combination of crystallo-colloid, and that the diffusion of crystalloid is against the gradient of osmotic pressure. Observe also that a maximum of osmotic pressure per one per cent. of colloid is reached at about 0.047 N of free alkali, and further increase of alkali lowers osmotic pressure.

Experiment I—Commenced December 6th, 1909. Ten per cent. caseinogen in 0.02 N sodium hydrate against 0.02 N sodium hydrate.

Time from commencement		Osmotic pressure in mm. of mercury	Temperature in °C.
Days	Hours		
0	0	0	14°
—	19	6	14
1	19	82	11.5
2	19	152	12.5
3	11	172	15.0
4	3	196	14.0
4	6	233	16.0
6	3	233	15.0
7	3	233	14.5
8	3	233	13.0

Analysis of contents of osmometer at end of experiment :—

Colloid Side : Clear solution, slightly opalescent. Titrated with N/10 NaOH and phenolphthalein, 5 c.c. required 1.55 cc. N/10 NaOH Nitrogen by Kjeldahl gave 1.33 per cent. nitrogen, equal to 8.2 per cent. caseinogen.

Crystalloid Side : Clear neutral solution.

Experiment II—Commenced February 1st, 1910. Ten per cent. caseinogen in 0.033 N sodium hydrate against 0.033 N sodium hydrate.

Time from commencement		Osmotic pressure in mm. of mercury	Temperature in °C.
Days	Hours		
—	18	116	13
1	2	166	12
1	17	216	14
2	17	256	11.5
3	17	288	13.5
4	20	306	16
6	1	310	15
7	0	310	16

Analysis of contents of osmometer at end of experiment:—

Colloid Side: Opalescent fluid. Titrated with N/10 NaOH and phenolphthalein, 5 c.c. required 1 c.c. N/10 NaOH. Nitrogen by Kjeldahl gave 0.86 per cent. N, equal to 5.4 per cent. caseinogen.

Crystalloid Side: Clear neutral fluid.

Experiment III—Commenced December 6th, 1909. Ten per cent. caseinogen in 0.04 N sodium hydrate against 0.04 sodium hydrate.

Time from commencement		Osmotic pressure in mm. of mercury	Temperature in °C.
Days	Hours		
1	0	226	11
2	0	242	11.5
3	0	348	12.5
3	8	348	15
3	23	338	14.5
5	2	336	16
6	23	332	15
7	23	332	14.5
9	23	332	13
10	23	334	12
12	23	334	11

Analysis of contents of osmometer at end of experiment:—

Colloid Side: Opalescent fluid, alkaline; 5 c.c. required 0.2 c.c. N/10 sulphuric acid. Nitrogen by Kjeldahl gave 1.28 per cent. N, equal to 8.0 per cent. caseinogen.

Crystalloid Side: Clear alkaline fluid, no protein present; 5 c.c. took 0.3 c.c. N/10 H₂SO₄.

Experiment IV—Commenced December 19th, 1909. Ten per cent. caseinogen in 0.06 N sodium hydrate against 0.06 N sodium hydrate.

Time from commencement		Osmotic pressure in mm. of mercury	Temperature in °C.
Days	Hours		
0	17	160	14.5
1	17	356	14
2	13	396	11
3	13	430	12.5
4	11	460	17.5
6	0	460	15.5

Analysis of contents of osmometer at end of experiment:—

Colloid Side: Pale yellow alkaline fluid; 5 c.c. took 1.45 c.c. N/10 sulphuric acid. Nitrogen by Kjeldahl gave 0.88 per cent. N, equal to 5.6 per cent. caseinogen.

Crystalloid Side: Clear alkaline fluid, free from protein; 5 c.c. took 0.65 c.c. N/10 sulphuric acid.

Experiment V—Commenced April 14th, 1910. Ten per cent. caseinogen in 0.065 N sodium hydrate against 0.065 per cent. sodium hydrate.

Time from commencement		Osmotic pressure in		Temperature	
Days	Hours	mm. of mercury		in °C.	
0	18	...	112	...	16
1	1	...	166	...	17.5
3	16	...	236	...	14.5
4	0	...	322	...	18
5	0	...	366	...	16
6	0	...	406	...	15.5
7	0	...	454	...	16
8	0	...	480	...	16
9	0	...	484	...	12
11	0	...	488	...	18.5
12	23	...	488	...	19.5

Analysis of contents of osmometer at end of experiment :—

Colloid Side : Opalescent alkaline fluid ; 5 c.c. required 1.2 c.c. N/10 H_2SO_4 . Nitrogen by Kjeldahl gave 0.91 per cent. N, equal to 5.7 per cent. caseinogen.

Crystalloid Side : Clear fluid, alkaline, no protein present ; 5 c.c. required 0.9 c.c. N/10 sulphuric acid.

Experiment VI—Commenced January 31st, 1910. Ten per cent. caseinogen in 0.07 N sodium hydrate against 0.07 sodium hydrate.

Time from commencement		Osmotic pressure in		Temperature	
Days	Hours	mm. of mercury		in °C.	
0	17	...	202	...	14
1	17	...	432	...	13.5
2	1	...	484	...	13.0
2	17	...	542	...	12.0
3	17	...	584	...	14.0
4	17	...	604	...	13.5
5	20	...	612	...	16.0
6	16	...	614	...	16.0
7	1	...	614	...	15.0
8	1	...	610	...	11.5
9	1	...	610	...	14.0
10	1	...	610	...	15.0

Analysis of contents of osmometer at end of experiment :—

Colloid Side : Clear alkaline fluid ; 5 c.c. required 1.1 c.c. N/10 sulphuric acid. Nitrogen by Kjeldahl gave 0.96 per cent. N, equal to 6.08 per cent. caseinogen.

Crystalloid Side : Clear alkaline fluid containing no protein ; 5 c.c. required 0.75 c.c. N/10 sulphuric acid.

Experiment VII—Commenced April 11th, 1910. Ten per cent. caseinogen in 0.08 N sodium hydrate against 0.08 N sodium hydrate.

Time from commencement		Osmotic pressure in mm. of mercury	Temperature in °C.
Days	Hours		
—	16	98	17
1	16	284	17
2	16	412	15
3	0	452	15
3	17	512	17.5
4	0	536	19
4	19	576	17
6	16	632	14.5
7	0	644	18
7	16	652	15.5
8	0	660	16.0
8	16	668	15.5
9	0	680	16.0
10	0	686	15

Analysis of contents of osmometer at end of experiment :—

Colloid Side : Clear alkaline fluid ; 5 c.c. required 2.35 c.c. N/10 sulphuric acid. Nitrogen by Kjeldahl gave 0.85 per cent. N, equal to 5.37 per cent. caseinogen.

Crystalloid Side : Clear alkaline fluid, free from protein ; 5 c.c. required 1.25 c.c. N/10 sulphuric acid.

Experiment VIII—Commenced February 26th, 1910. Ten per cent. caseinogen in 0.09 N sodium hydrate against 0.09 N sodium hydrate.

Time from commencement		Osmotic pressure in mm. of mercury	Temperature in °C.
Days	Hours		
1	22	332	12
2	6	392	14
2	22	458	10
3	6	490	15
4	0	520	14
5	0	542	13.5
6	0	558	13.5
6	8	564	16
7	0	568	13
8	3	576	15
9	5	580	16.5
9	22	580	14.0
10	22	582	14
11	22	584	13
12	6	588	16
13	6	588	16
14	6	588	13

Analysis of contents of osmometer at end of experiment :—

Colloid Side : Clear alkaline fluid ; 5 c.c. required 2.4 c.c. N/10 sulphuric acid. Nitrogen by Kjeldahl gave 0.76 per cent. N, equal to 4.7 per cent. caseinogen.

Crystalloid Side : Clear alkaline fluid free from protein ; 5 c.c. required 1.7 c.c. N/10 sulphuric acid.

Experiment IX—Commenced March 8th, 1910. Ten per cent. caseinogen in 0.095 N sodium hydrate against 0.095 N sodium hydrate.

Time from commencement		Osmotic pressure in mm. of mercury	Temperature in °C.
Days	Hours		
—	19	254	14
1	19	422	13
2	3	456	16
2	19	500	14
3	3	508	16
3	19	516	13
5	19	550	13
6	19	560	13
7	3	566	16
7	19	568	14
8	19	570	13
9	19	571	13
10	21	576	13
11	23	576	16.5
12	19	579	14.5
13	3	584	16.5
13	19	586	14.5
14	19	586	14

Analysis of contents of osmometer :—

Colloid Side : Clear yellow alkaline fluid ; 5 c.c. required 2.8 c.c. N/10 sulphuric acid. Nitrogen by Kjeldahl gave 0.8 per cent. N, equal to 5.04 per cent. caseinogen.

Crystalloid Side : Clear alkaline fluid, free from protein ; 5 c.c. required 1.6 c.c. N/10 sulphuric acid.

Experiment X—Commenced April 11th, 1910. Ten per cent. caseinogen in 0.1 N sodium hydrate against 0.1 N sodium hydrate.

Time from commencement		Osmotic pressure in mm. of mercury	Temperature in °C.
Days	Hours		
1	0	284	17
2	0	334	17
3	0	484	15
3	8	494	16
4	0	510	17.5
4	8	514	19
5	3	524	17
7	0	534	14.5
7	8	538	18
8	8	538	15.5
9	8	538	15.5

Analysis of contents of osmometer :—

Colloid Side : Clear alkaline fluid ; 5 c.c. required 3.3 c.c. N/10 sulphuric acid. Nitrogen by Kjeldahl gave 0.82 per cent. N, equal to 5.16 per cent. caseinogen.

Crystalloid Side : Clear alkaline fluid, free from protein ; 5 c.c. required 2.2 c.c. N/10 sulphuric acid.

Experiment XI—Commenced November 28th, 1910. Ten per cent. caseinogen in 0.1 N sodium hydrate against 0.1 N sodium hydrate.

Time from commencement		Osmotic pressure in mm. of mercury	Temperature in °C.
Days	Hours		
—	16	4	11
2	16	130	11.5
3	19	268	14.0
4	16	340	12.0
5	16	400	13
6	16	430	11
7	16	450	12.5
8	16	462	15
9	16	468	14
10	16	472	14.5
11	16	476	14
12	16	480	14
13	16	484	16
14	16	486	14
15	16	490	14
16	16	500	16
17	12	504	14
18	12	508	15
20	12	508	13
22	12	508	13

Analysis of contents of osmometer :—

Colloid Side : Clear yellow alkaline fluid ; 5 c.c. required 3 c.c. N/10 sulphuric acid. Nitrogen by Kjeldahl gave 0.81 per cent. N, equal to 5.1 per cent. caseinogen.

Crystalloid Side : Clear alkaline fluid, free from protein ; 5 c.c. required 2.1 c.c. N/10 sulphuric acid.