XXXIV. ON THE CHANGE OF THE OSMOTIC PRESSURE OF SOLUTIONS OF CERTAIN COLLOIDS UNDER THE INFLUENCE OF SALT SOLUTIONS.

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IN spite of certain controversies among investigators on the osmotic pressure exerted by colloidal solutions the positive proof that a definite, though small, osmotic pressure is set up was given by Starling [1896], Moore and Parker [1902], Lillie [1907], Moore and Roaf [1907] and others.

This osmotic pressure is affected by several factors, such as the mode of the formation of the colloidal solution [Lillie, 1907], the change of temperature [Moore and Roaf, 1907], the acidity or alkalinity of solution [Adamson and Roaf, 1908]. Further that the added salts also increase or diminish the size of the colloidal particles before any visible precipitation takes place, *i.e.* lower or raise the osmotic pressure, has been proved by Biltz and Vegesack [1909], Lillie [1907], Moore and Roaf [1907] and Loeb [1918].

The present research was started, by suggestion of Prof. Moore and under his direction, to prove by means of osmotic pressure measurements whether, first, salt solutions affect the "solution aggregate" of different colloidal solutions always in the same way, and, secondly, what is the influence, if any, of certain anaesthetics upon colloidal solution. While definite and regular results were obtained in the former experiments, those in the latter were not at all decisive.

METHODS.

As colloidal solutions gum arabic (1 %), egg albumin (5 %) and sheep serum (half diluted) were used. The membrane (dialyser) was of collodion and made on the outside of a large test tube. This was fixed to a well-fitting rubber cork with a strong string. The cork had two holes, one for a glass manometer tube and the other for an inlet and outlet tube. The length of the membrane was so adjusted as to limit its content to approximately 15 cc.

In the earlier part of the experiments the colloidal solution only was introduced into the dialyser, *i.e.* without mixing previously with any salt solution, thus letting the latter penetrate gradually from the outside. But

the procedure became more elaborate with the progress of the experiments, and the final method consisted of mixing the colloidal solution with the salt solution just before setting up the apparatus and then introducing into the membrane. The membrane was immersed up to the upper surface of the cork in a definite volume (120 cc. as a rule) of salt solution contained in a beaker. The manometer tube, together with a millimetre scale, was clamped in a vertical position during the experiments. The salts employed were copper sulphate, calcium chloride and alkaline sodium phosphate respectively.

The experiments were performed in series, *e.g.*, 1/10 *N*, 1/100 *N*, 1/1000 *N* solution and solvent (distilled water) only, the last as a control. The same salt solution of the same concentration was always placed inside and outside the membrane.

Usually the osmotic pressure was not very high, therefore the consideration of the distension of the membrane was of little importance.

As the salt solution could easily pass through the membrane the column of solution in the manometer increased in length, at first, very rapidly. After a certain time it began to fall, hardly staying at the highest point any measurable length of time. Then the column fell very slowly to a certain level at which it remained for a considerable time. When almost the same level was kept for at least two days, it was understood, in my experiments, that the expected equilibrium of osmotic pressure was attained. In most cases when this equilibrium was reached the outside liquids were replaced with distilled water, four or five times in succession with an interval of one day or more. The reversibility of pressure was fairly satisfactory.

RESULTS OF EXPERIMENTS.

The following are the examples of the abridged records of experiments carried out during five months. Similar results were always obtained when due precautions were taken.

Experiments with gum arabic solution. (See Fig. 1.)

Inside the membrane: 1 % gum arabic solution, 15 cc.

Outside the membrane: salt solutions of different concentrations, 120 cc.

Table I. SO_4Cu .

Day	Hour	Temp.	Osmotic pressure			
			1/10 <i>N</i> SO_4Cu	1/100 <i>N</i> SO_4Cu	1/1000 <i>N</i> SO_4Cu	distilled water
Sept. 1	afternoon (set up)					
" 2	4.00 p.m.	18.9° C.	2.5 mm.	13.2 mm.	48.0 mm.	163.3 mm.
" 3	2.30	16.9	7.5	14.0	45.1	150.5
" 5	4.00	18.9	8.5	12.8	42.0	135.5
" 6	3.20	18.3	9.0	13.0	42.2	130.0
p_H of the outside solution, about			9.0	7.2	6.5	6.5
The reversibility was tested:						
Sept. 13	10.00 a.m.	17.3	92.0	100.0	106.0	120.0

The curves in Fig. 1 indicate the rate at which equilibrium was reached both in direct and "reversed" experiments.

Table II. Cl_2Ca .

Day	Hour	Temp.	Osmotic pressure			
			$\frac{1}{10} N$ Cl_2Ca	$\frac{1}{100} N$ Cl_2Ca	$\frac{1}{1000} N$ Cl_2Ca	distilled water
Sept. 2	about noon (set up)					
" 6	3.30 p.m.	17.5° C.	12.3 mm.	17.0 mm.	50.0 mm.	108.5 mm.
p_H of the outside solution, about			6.0	6.5	6.0	6.5
The reversibility was tested:						
Sept. 13	10.00 a.m.	16.9	93.0	94.0	113.0	117.5

Fig. 1. Exp. with gum arabic solution. Effect of SO_4Cu . At the : line on the abscissa, the outside liquid was replaced by dist. water in succession.Table III. PO_4HNa_2 .

Day	Hour	Temp.	Osmotic pressure			
			$\frac{1}{10} N$ PO_4HNa_2	$\frac{1}{100} N$ PO_4HNa_2	$\frac{1}{1000} N$ PO_4HNa_2	distilled water
Sept. 3	morning (set up)					
" 7	12.00	17.2° C.	15.2 mm.	27.0 mm.	110.5 mm.	149.0 mm.
p_H of the outside solution, about			9.0	7.2	6.5	6.5
The reversibility was tested:						
Sept. 13	10.00 a.m.	17.2	251.0	220.0	129.0	130.0

Experiments with egg albumin.

Inside the membrane: 5 % egg albumin solution, 15 cc.

Outside the membrane: salt solutions of different concentrations, 120 cc.

Table IV. SO_4Cu .

Day	Hour	Temp.	Osmotic pressure			
			$\frac{1}{2000} N$ SO_4Cu	$\frac{1}{20,000} N$ SO_4Cu	$\frac{1}{200,000} N$ SO_4Cu	distilled water
Sept. 16	morning (set up)					
" 20	12.00	16.1° C.	12.0 mm.	53.9 mm.	55.1 mm.	52.2 mm.
p_H of the outside solution, about			6.0	6.0	6.0	6.0
The reversibility was tested:						
Sept. 27	4.00 p.m.	16.0	36.0	64.0	62.0	59.0

Table V. Cl_2Ca .

Day	Hour	Temp.	Osmotic pressure			
			$\frac{1}{20} N$ Cl_2Ca	$\frac{1}{200} N$ Cl_2Ca	$\frac{1}{2000} N$ Cl_2Ca	distilled water
Sept. 16	afternoon (set up)		14.0 mm.	17.0 mm.	36.0 mm.	50.0 mm.
" 20	12.00	15.7° C.	6.75	6.0	6.0	6.0
p_H of the outside solution, about						
The reversibility was tested:						
Sept. 27	4.00 p.m.	15.5	40.0	41.0	49.0	58.0

Table VI. PO_4HNa_2 .

Day	Hour	Temp.	Osmotic pressure			
			$\frac{1}{20} N$ PO_4HNa_2	$\frac{1}{200} N$ PO_4HNa_2	$\frac{1}{2000} N$ PO_4HNa_2	distilled water
Sept. 16	afternoon (set up)		22.6 mm.	30.0 mm.	44.0 mm.	47.0 mm.
" 21	2.00 p.m.	16.5° C.	9.0	7.2	6.75	6.5
p_H of the outside solution, about						
The reversibility was tested:						
Sept. 27	4.00 p.m.	15.9	40.0	48.0	51.0	59.0

Experiments with blood serum.

Inside the membrane: sheep serum half diluted, 15 cc.

Outside the membrane: salt solutions of different concentrations, 120 cc.

Table VII. SO_4Cu .

Day	Hour	Temp.	Osmotic pressure			
			$\frac{1}{2000} N$ SO_4Cu	$\frac{1}{20,000} N$ SO_4Cu	$\frac{1}{200,000} N$ SO_4Cu	distilled water
Sept. 30	afternoon (set up)		161.0 mm.	189.0 mm.	192.0 mm.	184.0 mm.
Oct. 5	12.00	16.6° C.	6.75	7.2	7.2	7.2
p_H of the outside solution, about						
Sulphate in 100 cc. of the outside						
solution as SO_3			5.36 mg.	2.86 mg.	0.06 mg.	0.00 mg.
The reversibility was tested:						
Oct. 17	10.00 a.m.	13.4	115.0	168.0	163.0	170.0

Table VIII. Cl_2Ca .

Day	Hour	Temp.	Osmotic pressure			
			$\frac{1}{20} N$ Cl_2Ca	$\frac{1}{200} N$ Cl_2Ca	$\frac{1}{2000} N$ Cl_2Ca	distilled water
Sept. 30	afternoon (set up)		105.0 mm.	120.0 mm.	169.0 mm.	176.0 mm.
Oct. 5	12.00	16.0° C.	7.2	7.2	7.2	7.2
p_H of the outside solution, about						
Chloride in 100 cc. of the outside						
solution as ClNa			37.7 mg.	11.5 mg.	9.25 mg.	8.0 mg.
The reversibility was tested:						
Oct. 17	10.00 a.m.	13.1° C.	178.0 mm.	168.5 mm.	155.0 mm.	163.0 mm.

Table IX. PO_4HNa_2 .

Day	Hour	Temp.	Osmotic pressure			
			$\frac{1}{20} N$ PO_4HNa_2	$\frac{1}{200} N$ PO_4HNa_2	$\frac{1}{2000} N$ PO_4HNa_2	distilled water
Oct. 1	morning (set up)		133.5 mm.	163.0 mm.	166.0 mm.	176.0 mm.
" 5	12.00	16.5° C.	9.0	7.2	6.75	6.75
p_H of the outside solution, about						
Phosphate in 100 cc. of the outside						
solution as P_2O_5			204.0 mg.	27.2 mg.	8.0 mg.	3.0 mg.
The reversibility was tested:						
Oct. 17	10.00 a.m.	13.2° C.	69.0 mm.	101.0 mm.	137.0 mm.	158.0 mm.

Unless specially mentioned the protein did not appear in the outside solution through the membrane (acetic acid and potassium ferrocyanide test).

As clearly seen in the above tables the salts used have all a more or less prominent influence upon the osmotic pressure exerted by the colloidal solutions such as gum arabic, egg albumin or blood serum. The salt solutions always reduced the osmotic pressure of the colloidal solution, and the more dilute the salt solution, the less decrease was brought about, thus gradually approaching the control with distilled water only. This fact suggests, at once, the important influence of electrolytes, because within the limits of concentration of the salt solutions employed here it may safely be concluded that the more concentrated the solution, the greater will be the number of electrically dissociated molecules. The trouble is the appearance of precipitation in the long run of experiments of this kind, and, therefore, all the statements here are based necessarily on the comparison with the control otherwise under the same conditions. Of copper sulphate an extremely dilute solution was made use of simply to avoid the unnecessary precipitation as far as possible.

The temperature, no doubt, affects the height of the osmotic pressure, but this effect is rather slight compared with that due to the electrolytes themselves. Notwithstanding the fluctuation of osmotic pressure parallel to that of temperature, the main course of change of osmotic pressure cannot be ignored.

The hydrogen-ion is neither the sole nor the principal cause in these cases, since the osmotic pressure does not always keep pace with its concentration. To quote a few examples, the hydrogen-ion concentration was about the same through the series in Tables II, IV, VII and VIII respectively. Nevertheless the pressure difference is very notable.

The more or less prominent reversibility of the pressure leads us to seek the probable cause in the physical reaction rather than the chemical change, as was propounded by several previous investigators. Mayer, Schaeffer and Terroine [1907] observed under the microscope that the submicroscopical granules of colloidal solutions (metal colloid, starch, albumin and others) disappeared on alkalisation and appeared on acidification. This is one of the convincing proofs that colloid aggregation can change at different reactions. It, moreover, strongly suggests that the decrease of the osmotic pressure of colloidal solutions under the influence of electrolytes may be most conveniently ascribed to a similar phenomenon.

So far as the results of our experiments were concerned the effects of three salts on the three colloidal solutions showed no qualitative difference among themselves.

Briefly speaking I am inclined to think, with previous investigators, that the diminished osmotic pressure under the influence of salt solutions depends upon an increased aggregation of colloidal particles.

The effects of anaesthetics (chloroform, ether, alcohol) were tested in the

same way. The only difference of the method lay in the use of a flask, instead of a beaker, to prevent the evaporation of these anaesthetics, connection with the manometer tube being made by a side tube. The solution inside the membrane was 15 cc., outside about 500 cc. The concentrations of anaesthetics were not always the same. Saturated solutions of chloroform in distilled water, 10 % ether, and 5 % alcohol were the most concentrated solutions in the series of experiments in each case. Roaf and Alderson [1907] found that anaesthetics such as chloroform, ether and carbon dioxide detached no salts from blood serum, which differed thus from other tissues (red blood corpuscles, brain, liver, muscle, kidney). Moore and Roaf [1907] observed that the presence of anaesthetics (chloroform) or organic solvents (benzene) did not alter the osmotic pressure of the serum protein (pig's serum). In their experiments the serum saturated with chloroform was placed against saline (0.175 %) also saturated with chloroform.

Despite the imperfections of the present osmotic pressure determinations the results of experiments with anaesthetics seem to favour somewhat positive effects though they are not very decisive.

SUMMARY.

1. Salt solutions (SO_4Cu , Cl_2Ca , PO_4HNa_2) added to colloidal solutions (gum arabic, egg albumin, blood serum) decreased the osmotic pressure exerted by the latter. No qualitative difference was confirmed among the salts or among the colloidal solutions within the concentrations here employed. The remarkable reversibility of the osmotic pressure suggests, at once, that it is a physical phenomenon, *i.e.* increased aggregation of colloidal particles by the electrolytes.

2. The effects of anaesthetics (chloroform, ether, alcohol) on the osmotic pressure of colloidal solutions were studied. The results, although not quite definite, seem to show that there is some effect.

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