XLIII. MEMBRANE EQUILIBRIA AND SELECTIVE ABSORPTION.

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Two general theories have been advanced to account for the unequal distribution of inorganic electrolytes on either side of a cell membrane.

According to one theory, the unequal distribution is due to the existence of a cell membrane, which not only prevents the passage of the colloid constituents of the cell, but also has the property of presenting varying resistance to the passage of different inorganic ions. According to the other theory, the cell membrane is capable of preventing the passage of the colloid constituents of the cell, but the unequal distribution of inorganic salts is due to the formation within the cell of relatively undissociated and non-diffusible compounds of the various ions with the cell constituents.

Direct experiments designed to test the former theory are beset with considerable difficulties, since the experiments have to be carried out on living tissues, and any considerable alterations in the surrounding medium are liable to alter the properties of the membrane. On the other hand, many artificial membranes exist which may be used in testing the latter theory, since the only properties which the membrane must have are those of preventing the passage of colloids and of allowing the free passage of inorganic salts.

In previous work [Wright, 1926] an attempt was made to ascertain how far the secretion of high concentrations of calcium in milk from the relatively low concentrations of this element in the blood-plasma could be accounted for on the basis of an unequal distribution caused by the presence in the milk cells of non-diffusible and relatively undissociated protein molecules. For this purpose, solutions of sodium caseinogenate were separated from solutions of calcium salts by a membrane permeable to inorganic ions but impermeable to the caseinogen molecules or ions. It was found that under these conditions the calcium tended to accumulate in high concentration on the protein side of the membrane, the distribution ratios¹ for the calcium varying from 2.2 to as high as 12.8. No attempt was made in the preliminary paper to examine in detail the physical chemistry of the systems under investigation.

¹ Throughout this paper, the term "distribution ratio" indicates, for cations, the ratio of the concentration on the protein side of the membrane to that on the non-protein side, and for anions, the ratio of the concentration on the non-protein side to that on the protein side.

The results appeared, however, to have such important applications in the more general study of the unequal distribution of salts in living tissues, that it was decided to carry out further, and more detailed, experiments. The results of these later experiments, which are described in this paper, show conclusively that (in the systems studied) the observed inequalities in the distribution ratios of the salts can be entirely explained if two factors—the degree of dissociation of the protein salts and the establishment of a Donnan equilibrium—are taken into account. ,

TECHNIQUE.

100 cc. of each of the two solutions under investigation were placed on either side of a dialysing vessel, the solutions were allowed to come into equilibrium by dialysing for at least 24 hours, and the contents were then removed and analysed.

The caseinogen used in the experiments was prepared by the method detailed by Van Slyke [1923] which gives a product of remarkably low ash content. The ash in the samples used was less than 0.1 %.

The dialysing vessels were of the type devised by Wright and Rule [1927], cellophane (0.0017 cm. thick) being employed as the membrane. This membrane is impermeable to caseinogen but freely permeable to inorganic salts.

With regard to analysis, calcium was estimated by McCrudden's [1909] method, and chloride by a macro-method based on that of Van Slyke [1924]. Sodium was determined throughout by difference¹. The hydrogen ion concentration of the solutions was not rigidly controlled, but the solutions were in all cases kept between p_H 6.7 and 7.0. It will be seen from the results that the small variations had no significant effect on the equilibria obtained. Toluene was added in order to prevent bacterial decomposition of the protein.

RESULTS.

Table I shows the results obtained with simple systems containing Na caseinogenate and NaCl. It will be seen that, at equilibrium, the distribution ratios of the sodium are in every case greater than the corresponding ratios of the chloride.

If Donnan's theory holds for such systems, this inequality in the distribution ratios can only be accounted for on the assumption that the Na caseinogenate is not completely dissociated. By calculation from the experimental data, it is found that a percentage dissociation of the caseinogenate of between 62 and 77 % (with a mean value of 68 %) is necessary in order to make the ionic ratios equal. Such a value agrees closely with that obtained from conductivity data by Pauli [1922].

¹ *I.e.* total anions – total calcium, assuming that 5 % caseinogen acts as a 0.025 *N* anion. All calculations in this paper are based on the analytical data, and not on the quantities of electrolytes noted as initially present. The latter are approximate values only.

Table I. *Equilibria in systems containing sodium caseinogenate and sodium chloride.*

Exp. No.	Approximate initial concentrations			Concentrations at equilibrium				Ratios at equilibrium		Percentage dissociation of caseinogenate
	Side I		Side II	Sodium		Chloride		$\frac{Na_I}{Na_{II}}$	$\frac{Cl_{II}}{Cl_I}$	
	Caseinogen %	NaOH <i>N</i>	NaCl <i>N</i>	I <i>N</i>	II <i>N</i>	I <i>N</i>	II <i>N</i>			
1	5.0	0.0250	0.1000	0.0688	0.0526	0.0438	0.0526	1.31	1.20	77
2	5.0	0.0250	0.0500	0.0443	0.0269	0.0193	0.0269	1.65	1.39	67
3	5.0	0.0250	0.0250	0.0337	0.0145	0.0087	0.0145	2.32	1.67	62
4	5.0	0.0250	0.0125	0.0288	0.0088	0.0038	0.0088	3.28	2.31	66
										Mean 68

Table II. *Equilibria in systems containing calcium caseinogenate and calcium chloride.*

Exp. No.	Approximate initial concentrations				Concentrations at equilibrium				Ratios at equilibrium			Per- centage dissocia- tion of caseino- genate
	Side I		Side II CaCl ₂ N	Calcium		Chloride		$\frac{Ca_I}{Ca_{II}}$	$\frac{Cl_{II}}{Cl_I}$	$\left(\frac{Cl_{II}}{Cl_I}\right)^2$		
	Caseinogen	Ca(OH) ₂ N		I N	II N	I N	II N					
	%											
5	2.0	0.0100	0.0250	0.0224	0.0132	0.0120	0.0129	1.70	1.07	1.14	29	
6	5.0	0.0250	0.0250	0.0362	0.0137	0.0115	0.0137	2.64	1.19	1.41	32	
7	5.0	0.0250	0.0125	0.0292	0.0073	0.0056	0.0072	4.00	1.28	1.64	27	
8	7.0	0.0350	0.0125	0.0396	0.0077	0.0053	0.0078	5.14	1.47	2.16	33	
											Mean 30	

Table II shows the results obtained with simple systems containing Ca caseinogenate and CaCl₂. The results are similar to those shown in Table I, but the distribution ratios of the calcium and the chloride (the ratio of the latter being squared¹) show a far greater divergence from one another than do the corresponding sodium and chloride ratios of the previous experiments.

If Donnan's theory holds for the calcium systems, the degree of dissociation of the Ca caseinogenate must be considerably lower than that of the Na caseinogenate: on calculation (by a similar method to that employed above) the percentage dissociation is in fact found to lie between 27 and 33, with a mean value of 30. Such a value agrees well with that obtained from conductivity data by Robertson [1920].

Table III. *Equilibria in simple and in mixed solutions.*

Exp. No.	Approximate initial concentrations					Concentrations at equilibrium						Ratios at equilibrium		
	Side I		Side II			Sodium		Calcium		Chloride		$\frac{Na_I}{Na_{II}}$	$\frac{Ca_I}{Ca_{II}}$	$\frac{Cl_{II}}{Cl_I}$
	Caseinogen %	NaOH <i>N</i>	Ca(OH) ₂ <i>N</i>	NaCl <i>N</i>	CaCl ₂ <i>N</i>	I <i>N</i>	II <i>N</i>	I <i>N</i>	II <i>N</i>	I <i>N</i>	II <i>N</i>			
3	5.0	0.025	—	0.025	—	0.0337	0.0145	—	—	0.0087	0.0145	2.32	—	1.67
6	5.0	—	0.025	—	0.025	—	—	0.0362	0.0137	0.0115	0.0137	—	2.64	1.19
9	5.0	0.025	—	—	0.025	0.0142	0.0100	0.0215	0.0035	0.0107	0.0135	1.42	6.15	1.26

In Table III typical results obtained in these simple systems are compared with the results obtained in a mixed system containing Na caseinogenate and CaCl₂. It is in this experiment, and in the succeeding experiments, that the very important influence of the degree of dissociation in systems of mixed

¹ To conform to Donnan's equilibrium equation.

electrolytes becomes apparent. It will be seen that the low degree of dissociation of the Ca caseinogenate has caused a preferential absorption of the calcium to the protein side of the membrane, with a consequent high distribution ratio of calcium of 6.15, and lowered distribution ratios of the sodium and chloride of 1.42 and 1.26 respectively.

Table IV. *Equilibria in systems containing sodium caseinogenate, sodium chloride and calcium chloride.*

Exp. No.	Approximate initial concentrations				Concentrations at equilibrium						Ratios at equilibrium		
	Side I		Side II		Sodium		Calcium		Chloride				
	Caseino-	NaOH	NaCl	CaCl ₂	I N	II N	I N	II N	I N	II N	Na _I	Ca _I	Cl _I
	gen %	N	N	N							Na _{II}	Ca _{II}	Cl _{II}
1	5.0	0.0250	0.1000	—	0.0688	0.0526	—	—	0.0438	0.0526	1.31	—	1.20
10	5.0	0.0250	0.0875	0.0125	0.0573	0.0487	0.0109	0.0026	0.0432	0.0513	1.17	4.17	1.17
11	5.0	0.0250	0.0750	0.0250	0.0501	0.0453	0.0197	0.0056	0.0448	0.0509	1.10	3.50	1.13
12	5.0	0.0250	0.0625	0.0375	0.0428	0.0403	0.0279	0.0103	0.0457	0.0506	1.06	2.71	1.11
13	5.0	0.0250	0.0500	0.0500	0.0367	0.0340	0.0347	0.0158	0.0464	0.0498	1.07	2.21	1.07
14	5.0	0.0250	0.0375	0.0625	0.0286	0.0276	0.0420	0.0218	0.0456	0.0494	1.03	1.93	1.07

A more extended study of mixed systems of this type is shown in Table IV. In this series, the concentration of the Na caseinogenate and the total concentration of chlorides have been kept constant, but the relative concentrations of NaCl and CaCl₂ have been varied. The following are the salient features of these results.

(a) As the total calcium in the system is decreased, an increasing proportion of calcium passes through the membrane. This is to be expected, since (owing to its low degree of dissociation) the smaller the ratio of calcium to caseinogen, the greater will be the relative amount of calcium bound by the caseinogen.

(b) As the total calcium in the system is increased, the distribution ratios of the sodium and chloride decrease, until, with high concentrations of calcium, the ratios approach unity. If the reversible reaction

is considered, it will be seen that the addition of CaCl₂ should tend to force the reaction from left to right, *i.e.* to form additional Ca caseinogenate and NaCl. Hence with increasing calcium in the system, the proportion of undissociated Ca caseinogenate and of freely diffusible NaCl should both increase, and (if Donnan's theory holds good) the distribution ratios of the sodium and the chloride should approach unity. The experimental results confirm this. Actually the distribution ratio of the sodium should always be slightly higher than that of the chloride, since there must always be a small quantity of undissociated Na caseinogenate in the system (the above equation being reversible). The method of analysis was not sufficiently accurate to reveal such a difference. The difference is, however, seen in the results of Exp. 9 of Table III.

Table V. *Effect of varying concentrations of sodium chloride on the distribution of calcium.*

Exp. No.	Approximate initial concentrations				Concentrations of calcium at equilibrium		Ratio at equilibrium $\frac{Ca_I}{Ca_{II}}$
	Side I		Side II		I	II	
	Caseinogen %	NaOH <i>N</i>	CaCl ₂ <i>N</i>	NaCl <i>N</i>	<i>N</i>	<i>N</i>	
9	5.0	0.025	0.025	—	0.0215	0.0035	6.15
11	5.0	0.025	0.025	0.075	0.0197	0.0056	3.50
15	5.0	0.025	0.025	0.75	0.0144	0.0101	1.42
16	5.0	0.025	0.025	2.5*	0.0152†	0.0118	1.29

* Contained appreciable quantities of calcium as an impurity; this accounts for the high analytical values obtained.

† Volume of solution reduced to 85 cc., *i.e.* it is actually a 5.9 % caseinogen solution.

It has been shown above that the presence of increasing concentrations of CaCl₂ causes a shift in the equilibrium in equation (1) from left to right. An attempt was made to cause the reverse reaction, *i.e.* to shift the equilibrium from right to left, by adding large quantities of NaCl to the system. Table V shows that this attempt was successful. As the NaCl in the system was increased (the quantities of CaCl₂ and of Na caseinogenate being kept constant), the proportion of calcium passing through to the protein side of the membrane decreased, until, with very high concentrations of NaCl, the distribution ratio of the calcium itself approached unity.

DISCUSSION.

The above results fully confirm the main conclusions of the previous paper [Wright, 1926]. Further, they demonstrate that the unequal distribution of inorganic salts on either side of the membrane can be fully explained if two factors—the degree of dissociation of the protein salt, and the establishment of a Donnan equilibrium—are taken into account.

It should be understood that the systems studied have not been selected for the purpose of illustrating striking distribution ratios: they have been selected primarily in order to elucidate the factors involved in the establishment of the unequal distribution. Nevertheless the results, together with those previously published, demonstrate the remarkable alterations in the distribution of salts on either side of an artificial membrane caused by the presence of a non-diffusible electrolyte (caseinogen). Further, they demonstrate the marked influence of the presence of one ion on the distribution ratios of other ions; for instance, in the influence of calcium on the sodium and chloride ratios in Exps. 9 to 14, and in the influence of sodium chloride on the calcium ratios in the experiments shown in Table V. It should be emphasised that such alterations in the distribution ratios are entirely independent of the nature of the membrane, provided that the latter fulfils the conditions necessary for the establishment of a Donnan equilibrium, *i.e.* impermeability to one ion of the system but complete permeability to all other ions.

In drawing attention to the more general applications of these results in

problems of cellular physiology, it is necessary to recognise that simple equilibria of the type discussed in this paper can only provide a partial explanation of the remarkable inequalities in the distribution of inorganic elements in living tissues. In the tissues, the various constituents are undergoing continuous transformations, which alter not only the internal phases of the cell but also the nature of the cell membrane itself. Recent papers on permeability have in fact tended to stress the importance of the constitution of the cell membrane in accounting for the accumulation of inorganic elements in living tissues; the membrane has been assumed to have the property of presenting varying resistance to the passage of different inorganic ions.

While such an assumption has in many cases received justification, the results detailed in this paper point to a further explanation, based on the degree of dissociation of the non-diffusible (protein) salts of the cell and on the establishment of a Donnan equilibrium, which may also be of considerable importance in accounting for the unequal distribution of inorganic elements in living tissues. Specific examples of the possible applications of these results may be cited (1) in the experiments of Stiles and Kidd [1919] on the absorption of salts by carrot tissues, (2) in the experiments of Hoagland [1923] on the influence of one salt on the distribution of another salt between the plant and its surrounding medium, and (3) in the experiments of Loeb and Nichols [1926, 1927] on the diffusibility of the calcium of blood-serum. The application of the results to the secretion of calcium in milk has been dealt with elsewhere [Wright, 1928].

SUMMARY.

1. The unequal distribution of salts on either side of an artificial membrane in systems containing caseinogen, NaCl and CaCl_2 , has been examined.
2. It is shown that the results may be quantitatively explained if two factors, the degree of dissociation of the protein salts and the establishment of a Donnan equilibrium, are taken into account.
3. The influence of one ion on the distribution ratios of other ions is demonstrated.
4. The applications of the results in the general study of the unequal distribution of salts in living tissues are briefly discussed.

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