XXVII. THE RÔLE OF THE PLASMA PROTEINS IN DIFFUSION.

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THERE is nothing more remarkable than the maintenance of a constant composition of the blood plasma under the most varied conditions. This is shown very clearly in the regulation of the reaction of the blood, and attention has now been directed by Haldane and Priestley [1916] to a similar control as regards the water and salts when large quantities of water or salt solutions are ingested or in the opposite condition of profuse sweating. The electrolyte concentration of the serum was shown by Priestley [1916] to be very slightly diminished when large quantities of water were taken and slightly increased when salts were added to the water. Again, after severe loss of blood, the volume of the circulating fluid is rapidly restored by the entrance of fluid from the tissue spaces and, as will be shown later, this fluid which enters must possess approximately the electrolyte concentration of the original blood plasma. Under these varied conditions there is one class of constituent of the plasma, as there is also one in the tissues, which can only with great difficulty leave its original situation and that is the colloidal element, the protein. Thus after the entrance of non-protein-holding fluids into the blood by ingestion or injection, the colloidal concentration is lowered and on removal of a similar fluid, as in sweating, it is raised. Again after haemorrhage the fluid which enters being either free from or poor in colloid leaves the colloidal concentration at a lower level than the normal. Variations in the colloidal concentration of the blood are therefore dependent upon the addition or withdrawal of non-colloidal solutions. Neither the proteins of the tissue fluids nor the proteins of the plasma can be readily transported, and the exchange of water and salts from one side to the other must be largely governed by the colloidal concentration on these two sides. To Starling and others we owe much of our knowledge of one way in which the protein concentration of a fluid may make itself felt, namely by affecting the osmotic pressure level and so controlling the filtration process through a membrane impermeable for the protein molecule. Before entering upon the consideration of the various ways in which the protein content of a fluid may affect saline diffusion, a brief reference will be made to certain changes which the blood plasma

undergoes after haemorrhage, in so far as these may contribute to our knowledge of the nature of the exchange between tissue fluids and blood. Many investigations have been carried out dealing with this subject and that there are certain well defined changes has been clearly established. After haemorrhage the blood shows a decrease in specific gravity, viscosity, and protein content. The chloride concentration is little if at all affected, while according to Taylor and Lewis [1915] the non-protein nitrogen and urea are apparently increased. Special reference however requires to be made to the degree of the alterations in specific gravity, viscosity, protein and electrolyte concentration with a view to the subsequent consideration of the part played by protein and salts in the exchange between blood and tissues. The effects of haemorrhage therefore were investigated in order to determine the nature and degree of the changes produced in the plasma.

EFFECTS OF HAEMORRHAGE.

The methods employed in the determination of these changes were as follows. The specific gravity was estimated in the usual way, a tube of the Sprengel type being employed and the tube filled with water and then with plasma at 18°.

Viscosity was determined by the Ostwald type of viscosimeter and determinations made either at 25° or 37°.

Conductivity was determined by the Kohlrausch method, the conductivity cell used being of the Washburn type and the observations were made in electrically regulated thermostats at 25° and 37°.

Nitrogen was estimated by Kjeldahl's method.

After loss of blood varying from 30—57 per cent. of its total volume, in both the cat and dog, the specific gravity, viscosity and percentage of nitrogen fall while the conductivity rises. The fall in specific gravity is very slight, the mean percentage fall in the cat being 0.2 and in the dog 0.13. The fall in viscosity is more distinct, ranging on an average from a 5 per cent. decrease in the viscosity coefficient in the dog to 10 per cent. in the cat. The nitrogen percentage shows a mean decrease of about 14.5 in the cat to 18 in the dog. The rise in conductivity, although always to be observed, is comparatively small, ranging from 1.92 mean percentage rise in the dog to 3.8 in the cat. These changes are given in detail in the following table (Table I).

The decrease in the nitrogen content of the blood after haemorrhage and the fall in viscosity are the most distinctive alterations, and the latter is the expression of the lowered protein content, as the non-protein nitrogen is evidently increased as a result of haemorrhage. The fluid which enters the blood after haemorrhage must have at least the electrolyte concentration of the normal plasma because even after the most severe loss of blood there is no evidence of a fall in conductivity. It is therefore evident that the blood plasma tends to maintain its normal electrolyte concentration not only after

Table I. *Effects of haemorrhage on blood plasma.*

	Sp. gr.	% fall	Viscosity	$K \times 10^4$ at 37°	% rise	Nitrogen %	% fall
Cat (2.23 kgs.) Before haemorrhage	1.0275	—	1.751 (η 25°)	—	—	—	—
	1.0249	0.25	1.545 "	11.7	3.1	—	—
Cat (2.6 kgs.) Before haemorrhage	1.0300	—	1.840 "	—	—	1.064	—
	1.0281	0.18	1.668 "	9.3	—	0.927	12.8
	1.0272	0.27	1.529 "	16.9	6.2	0.915	14.0
Cat (2.15 kgs.) Before haemorrhage	1.0265	—	1.604 (η 37°)	—	—	1.033	—
	1.0251	0.13	1.521 "	5.1	2.1	0.858	16.9
Dog (18 kgs.) Before haemorrhage	1.0249	—	1.593 (η 25°)	—	—	0.935	—
	1.0236	0.12	1.467 "	7.9	2.5	0.806	13.7
Dog (13 kgs.) Before haemorrhage	1.0250	—	1.409 (η 37°)	—	—	0.860	—
	1.0237	0.12	1.359 "	3.5	2.6	0.691	19.6
	1.0232	0.17	1.342 "	4.7	.66	0.662	23.0

the ingestion of fluids but also after severe loss of blood from the body. As to the nature of the salts entering the blood vascular system, from a small number of analyses it was evident that at least the chloride content of the plasma was not distinctly altered. From Luckhardt's [1910] observations the chloride content of the lymph and tissue fluids is rather higher than that of the blood serum in the same animal. The passage of such a fluid from tissue spaces into blood must affect the protein-salt equilibrium between tissue elements and the fluid which bathes them. It was therefore thought advisable to study by as simple methods as possible the influence of proteins on salt diffusion.

DIFFUSION OF NaCl FROM PROTEIN SOLUTIONS.

In order to study the rôle of proteins in saline diffusion attention was particularly directed to the velocity of diffusion under as nearly as possible constant conditions. The influence of proteins on the diffusion of salt from a protein salt solution into water was investigated in one or other of the following ways. The solutions were separated from one another either by means of a collodion membrane in tubular form, or as a flat diaphragm. The rate of change was followed by making conductivity determinations and also by analysing the saline content of the water at regular intervals. The water towards which the salt was diffusing was either kept running at a constant slow rate or it was changed at stated intervals in order that the concentration gradient should be fixed by the saline concentration in the protein salt solution and therefore be a function of the same. In order that the experimental conditions should be tested as regards their suitability for determining velocity coefficients, the rate of diffusion from a simple aqueous solution of the salt in the same concentration and under exactly the same conditions, as regards filtering membrane, rate of change of water, temperature, etc., was always determined either before or after, or both before and after, the corresponding experiment with the protein salt solution.

All experiments were carried out in one or other of two thermostats heated by electric radiators and with suitable toluene-mercury regulators.

One thermostat was kept at a constant temperature of 25°, the other at 37°, or in some cases slightly over that temperature.

The collodion membranes were made by a modification of Walpole's method [1915], the films being allowed to dry in a closed chamber saturated at 20° with alcohol-ether vapour instead of being freely exposed to air. By this method the passage of the protein through the membrane was diminished to a mere trace.

Conductivity determinations were made by the ordinary Kohlrausch method, the platinised plates being fixed in a definite position in the collodion tube. In the case of the diaphragm dialyser, two glass vessels of the small desiccator cover form (300 cc. capacity) with flanges were used, the collodion

diaphragm with a diffusing surface of 34 sq. cm. being clamped between the two vessels and the edges of the flanges carefully sealed with Chatterton's cement. Tests were always carried out before each experiment in order to exclude any possibility of leakage at the margin. The glass stopper bearing the platinised plates could be placed in either of the two chambers. The majority of the conductivity determinations were made in the fluid from which the salt was diffusing out. The chloride estimations were made by Volhard's method, and in cases where protein was present the modification of this method described by Rappleye [1918] was employed. In the first set of experiments, the diffusion rates of salt into conductivity water from (a) simple NaCl solution in water, (b) gum arabic solution with the same salt content, and (c) ox blood serum were investigated. The experiment was carried out in the diaphragm dialyser, the water being changed at the end of each three hour period and the chloride estimated. The conditions of the experiment in all three cases were identical. In each case the solutions were warmed to 37.5° before placing them in their respective chambers, and the conductivity water introduced at the end of each three hour period was also raised to the same temperature before replacing the preceding dialysate. The fall in conductivity in the three saline solutions was determined at frequent intervals and the one hour readings are given in Table II and are plotted out in Fig. 1.

Expt. 1 (Fig. 1). Diffusion from NaCl, NaCl-gum, and serum to water.

- Sol. A. 0.795 per cent. NaCl.
 „ B. 0.795 per cent. NaCl in 6 per cent. gum arabic.
 „ C. Ox blood serum (0.624 per cent. NaCl).
 Temperature 37.5°.

The dialysate was replaced by fresh conductivity water at the end of the third, sixth and ninth hours.

In Fig. 2 the results of the chemical analyses of the chloride content in terms of the percentage NaCl loss during diffusion are plotted; the experiments in each case were for a period of 12 hours.

The viscosity coefficients at 37.5° of the three solutions were

		$\eta_{37.5^\circ}$. (Water = 1.)
A		1.014
B	before diffusion	2.676
	after „	3.080
C		1.878

As may be readily observed on examination of the curves, the rate of passage out of the salt from the protein solution is evidently not a function of the viscosity, as the gum arabic solution is the more viscous fluid.

From about the fifth—sixth hour the velocity of diffusion from the serum becomes distinctly slower than in the case of the gum arabic or the simple salt solution. The diffusion from the gum arabic solution is somewhat slower and also more regular than in the case of the simple salt solution. If one regards the concentration gradient as determined by the salt concentration

in the solution from which the salt is diffusing and that therefore there is little back effect, then according to the law of mass action the rate of diffusion at the time t should be proportional to the concentration at that time. Taking x_0 as the original concentration in each case and x as the concentration at the stated periods, the velocity coefficients for the time unit one hour, determined from the equation

$$k = \frac{1}{t} \log_e \frac{x_0}{x},$$

are shown in Table II.

Fig. 1. Diffusion Curves. (Conductivity Measurements).

0.795 % NaCl against Water. $\odot$ ————— A.
 Gum Arabic solution in .795 % NaCl against Water. $\triangle$ - - - - B.
 Blood Serum against Water. $\odot$ ————— C.

The coefficients so determined are only approximate, but for comparative purposes between the three solutions they are valid.

Table II. *Velocity coefficients of diffusion in the three solutions.*

A.

Time in hours	NaCl present	$\frac{x_0}{x}$	$k = \frac{1}{t} \log_e \frac{x_0}{x}$
0	1.652 (x_0)	—	—
3	0.986	1.675	0.171
6	0.551	3.000	0.183
9	0.309	5.346	0.186
12	0.131	11.884	0.206

Viscosity $\eta_{37.5^\circ} = 1.0146$.

B.

0	1.669	—	—
3	1.018	1.639	0.164
6	0.618	2.700	0.165
9	0.378	4.415	0.166
12	0.228	7.320	0.165

Viscosity $\eta_{37.5^\circ} = 2.676$ before and 3.080 after diffusion.

C.

0	1.560	—	—
3	1.037	1.504	0.136
6	0.720	2.166	0.129
9	0.597	2.613	0.106
12	0.481	3.243	0.098

Viscosity $\eta_{37.5^\circ} = 1.878$.

In the case of blood serum there is a gradual fall in the coefficient, most distinct between the sixth and ninth hours, the gum arabic saline shows a very regular rate of diffusion proportional to the concentration while the simple watery solution of salt shows a rather variable rate under the experimental conditions due to back pressure effects, but the rate is faster at the lower concentrations.

As the gum arabic solution is the most viscous of the three it is evident as already mentioned that some factor other than that of viscosity is concerned in the diffusion of salt from serum. This of course in no way invalidates the experimental evidence in support of the advantage of the addition of gum to saline injections, the value of which is undoubted as shown by the researches of Bayliss [1918] and others. The blood serum differs from the gum arabic solution in possessing a constituent which requires a certain salt concentration for its solution, namely globulin, and it is during the later period of diffusion that this body separates out. The question whether the other protein in solution in the serum plays any part as a governing factor was examined in the case of dialysed horse plasma. The experiment was carried out in a collodion tube. In order that the effect of the globulin might be compared with the globulin-free (or globulin-poor) solution similar diffusion experiments were carried out with normal and dialysed (10 days) plasma.

The globulin suspension was removed from the dialysed plasma by centrifugalisation. The collodion tube was fitted with a rubber stopper through

which passed two glass tubes carrying platinum plates and also two tubes for filling purposes. The tube was then inserted in a glass jacket, the volume of the outer space being 200 cc. and that of the tube 90 cc. The apparatus was placed in a thermostat at 25°. The solution containing the salt, warmed to 25°, was placed in the inner tube and water, previously heated to the same temperature by passing through spiral glass tubing in the bath, was allowed to

Fig. 2. Diffusion Curves. (Percentage NaCl Loss.)

0.795 % NaCl. ○ ——— A.
 Gum Arabic containing 0.795 % NaCl. △ - - - B.
 Blood Serum. (0.624 % NaCl.) ● ——— C.

flow through the outer jacket at a rate of 250 cc. per hour. The outflow fluid was collected at two hour intervals and the NaCl estimated. The diffusion rates from the following three salt-containing solutions were then determined and are plotted in Fig. 3 as percentage NaCl loss against time.

Expt. 2. Diffusion from NaCl, normal horse plasma, and dialysed horse plasma (with salt).

Sol. A. 0.547 per cent. NaCl in water.
 „ B. Dialysed Plasma containing 0.574 per cent. NaCl.
 „ C. Normal Plasma 0.547 per cent. NaCl.

During the first six hours there is not much difference in the rates of diffusion of NaCl from normal plasma and from the simple watery solution of the salt, although the former shows the slower rate. Diffusion from the dialysed horse plasma is distinctly the slowest of the three. Thus 82.96 per cent. is lost from A, 75.57 per cent. from B, and 79.05 per cent. from C.

Fig. 3. Diffusion Curves. (Percentage NaCl Loss.)
 NaCl Solution $\circ$ ————— % NaCl=0.547; A
 Dialysed Plasma $\odot$ ————— „ =0.574; B.
 Normal Horse Plasma $\triangle$ - - - - - „ =0.547; C.

In the last six hours however there is a distinct difference between the globulin-free (or poor) solution and the globulin-containing one, the rate of diffusion being slower in the latter than in the former.

If one compares the velocity coefficients of the first four hours and the last eight hours in each of the three solutions, this becomes evident.

	A	B	C
k 1st 4 hours : k last 8 hours	1 : 1.08	1 : 0.92	1 : 0.78

Fig. 3 shows clearly that the curve of diffusion in the case of normal horse plasma begins to show the slowing in the diffusion rate about the fourth hour,

and as diffusion from the dialysed plasma is distinctly slower than from the normal in the preceding four hours the two curves converge from that point onwards.

It is evident therefore as in Experiment 1 that the globulin-holding solution disturbs the diffusion rate in the later stages and as the globulin-free solution in Experiment 2 does not show the same change, the protein other than globulin is not the main cause of the altered rate of diffusion observed in the later stage. As in the case of the blood serum or plasma the fall in diffusion rate is always accompanied by a separation of the globulin, it was thought advisable to investigate the salt diffusion under conditions where the globulin did not separate out on dialysis. The diffusion rates of salt from dialysed plasma and from globulin in acid solution (prepared from the globulin separated out from the normal plasma) were therefore investigated. During the period of diffusion the acid globulin only showed a faint trace of separation from the mixture, the solution becoming faintly opalescent. The globulin obtained by dialysis for a period of ten days from 200 cc. horse blood plasma, was removed by centrifugalisation, dispersed in 5 cc. 0.1N HCl, and the volume made up to 200 cc. with water. The mixture was allowed to stand for 24 hours at room temperature and the small residue of undissolved globulin was then removed. The solution was made up to 0.470 per cent. NaCl. The nitrogen value of the solution was 0.146 per cent. The rates of diffusion to water from 0.1 normal NaCl in water, 0.1 normal NaCl in dialysed plasma (nitrogen 0.796 per cent.), and 0.470 per cent. NaCl in the acid solution of globulin were determined. To compare with the acid solution of globulin an alkaline solution was prepared in the same way, 6 cc. 0.1N NaOH being added. The nitrogen value of this solution was 0.148 per cent. As the alkali globulin solution on dialysis showed a precipitate it could not be made use of for the purpose of this special experiment, in which the influence of globulin in solution throughout the diffusion process was being investigated. The salt-containing solutions were placed in the collodion tube of the same apparatus, water being allowed to pass through at a rate of 250 cc. per hour, and in addition at two hour intervals the outer fluid was removed and replaced by fresh conductivity water. The water which had run through during the two hour period was added to that drawn off at the end of the period and the chloride content estimated. The results are shown in Fig. 4.

Expt. 3. Diffusion from NaCl, dialysed horse plasma (0.1 normal NaCl), acid globulin (0.470 per cent. NaCl), alkali globulin (0.528 per cent. NaCl) to water.

- Sol. A. NaCl, 0.1 normal.
- „ B. Dialysed Horse Plasma.
- „ C. Acid Globulin.
- „ D. Alkali Globulin.

As is evident from the curves in Fig. 4, the diffusion rate from acid globulin is much slower throughout than from the other three solutions which show

diffusion curves of much the same form. The difference in the diffusion rate of salt from the acid globulin solution is possibly related to the influence of the acid on the hydrolysis of the globulin salt, retarding it to such an extent that the solution at the end was only faintly opalescent. Under those circumstances the globulin-salt compound had not evidently undergone dissociation to the same extent as in the alkaline medium. Hardy [1905], Pauli [1903],

Fig. 4. Diffusion Curves. (Percentage NaCl Loss.)

NaCl Solution	—————	⊙	% NaCl=0.585; A.
Serum Globulin (acid solution)	- - - - -	⊙	" =0.470; C.
" " (alkaline solution)	- - - - -	+	" =0.528; D.
Dialysed Plasma	—————	Δ	" =0.585; B.

Mellanby [1905] and others have studied the influence of salts on the solution or precipitation of the globulin and have recognised that the globulin forms salt compounds in equilibrium with the salt in solution. Pauli concludes that in the solution compounds of globulin with such a salt as NaCl are formed of the type Na-Globulin-Cl. It has been pointed out that, in cases where the effect of electrolytes in solution on living tissues is altered by the addition

of protein to the solution, the action is probably due to the removal of electrolytes from their sphere of action. All such experiments tend to show that certain proteins at least have a chemical (or physico-chemical) affinity for electrolytes.

In order to investigate the matter further certain experiments were carried out to determine the rate of passage of NaCl from an aqueous solution to one of egg white dialysed to such an extent that the globulin had just separated out. The rates at which the salt entered the solution containing a suspension of globulin were determined at first by following the conductivity changes in the protein mixture and also by estimating by chemical analyses the amount of the salt transported to such a solution compared to the rate of transport to water. As the conductivity changes were exactly of the same order as the chemical but were complicated owing to the suspension in the fluid, reference will only be made to the amounts of salt passing from the same volume of 0.03 normal NaCl in one case to the protein mixture and in the other to conductivity water.

Expt. 4. The experiment was carried out in the following way. Filtered egg white was taken, dialysed to the point of separation of the globulin, and 190 cc. of this mixture containing 2.731 per cent. protein were placed, after warming to 37°, in the upper chamber of the diaphragm dialyser. The lower chamber was filled with 300 cc. 0.03 normal NaCl. After a period of four hours had elapsed the fall in NaCl content in the lower fluid was determined.

The total chloride in the lower fluid at the outset was 0.5512 g. in 310 cc. and at the end of four hours, it had fallen to 0.4894, *i.e.* 61.8 mg. NaCl passed to the protein mixture in four hours or 15.45 mg. per hour. Using the same membrane and under the same conditions, but placing conductivity water in the upper chamber, the amount of chloride passing out towards water was found in the experiment to amount to 43.4 mg. in four hours or 10.85 mg. per hour.

The experiment was repeated with a membrane of slightly different permeability, all the other conditions remaining the same, when it was found that 76.27 mg. NaCl passed out to protein in four hours or 19.0 mg. per hour, while to water 52.65 mg. NaCl passed out in four hours or 13.16 mg. per hour. The rate therefore of saline diffusion in the first case to protein and to water respectively was as 15.45 : 10.85 or as 1.42 : 1, and in the second as 19.06 : 13.16 or as 1.44 : 1.

Expt. 5. Using the same membrane that was employed in the last case, the rate of passage of NaCl to dialysed egg white containing 6.08 per cent. protein was tested under exactly the same conditions. It was found that 107.6 mg. NaCl passed out in four hours or 26.9 mg. per hour. The rate therefore in this case with the higher protein content compared to the rate of passage into water was as 26.9 : 13.16 or 2.04 : 1.

Expt. 6. In the next experiment using a fresh diaphragm, the rate of passage of NaCl from a solution of very low concentration, 0.003 normal, to

an egg-globulin suspension also of low concentration (0.14 per cent. protein) was determined. In this case only 10 mg. NaCl passed through in four hours and in the comparable experiment with the same membrane but with conductivity water in the upper chamber 7 mg. NaCl passed through in the same period, the ratio of the rates therefore being 1.42 to 1.

In all the experiments sodium chloride passed more rapidly from the salt solution to the protein mixture (containing a globulin suspension) than to water. It is evident therefore that the decrease in the diffusion rate of sodium chloride from a salt protein solution such as blood plasma during the period of separation of the globulin is intimately related to the increased rate of passage of salt towards a globulin suspension which has as its accompaniment the solution of the globulin. The question as to whether "mechanical" or chemical affinity is the predominating factor in the fixation of NaCl by globulin (in suspension) is difficult to decide.

Expt. 7. With a view to determine whether the solution of the suspended globulin is the governing factor in the process, the proteins in a specimen of dialysed egg white were coagulated by raising the temperature to 80°. The mixture was then cooled to 37° and 230 cc. of the mixture, containing approximately 4.25 per cent. protein, were placed in the upper chamber of the diaphragm dialyser. The lower chamber was filled with 320 cc. 0.03 normal NaCl. The permeability of the collodion diaphragm was such that with the same strength of the salt solution in the lower chamber and conductivity water in the upper, the respective volumes being the same as in the experiment with the heat-coagulated protein mixture, the rate of passage of NaCl to water was 63.6 mg. in four hours or 15.9 mg. per hour.

In the case of the clotted egg white 64.4 mg. NaCl passed from the salt solution to the protein mixture in four hours or 16.1 mg. per hour. The diffusion rates therefore of salt from simple watery solution to water and to clotted egg white were approximately the same.

It appears therefore that the actual solution of the globulin is the determining factor in governing the rate. That the difference in velocity of diffusion is due to variations in the permeability of the membrane owing to blocking up of the pores with consequent diminution of the cross-sectional area of the diffusion path is unlikely, as in all cases when the globulin can undergo solution salt passes more rapidly to the mixture containing the globulin suspension than to water, and in the case of globulin in acid solution the diffusion of NaCl is delayed throughout the whole period although there is only a slight separation of the hydrolysed globulin. The quantitative relationships between the amounts of protein dissolved and the NaCl fixed, as also the influence of variations in reaction on the process, require further study. It is important however to bear in mind that a protein such as globulin forming a large percentage of the colloidal content of blood plasma, tissue fluids, and tissues possesses a certain salt-retaining power which must be of significance in the maintenance of the chloride content of the body.

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