CXVI. THE OSMOTIC PRESSURE OF CRYSTALLINE EGG-ALBUMIN.

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THIS work was begun with the intention of studying further the cause of the convexity of the osmotic pressure concentration curve of serum or plasma, which we have already discussed and ascribed to the Donnan effect [Marrack and Hewitt, 1927]; this effect should not make itself felt at the isoelectric point of protein. As the serum-proteins have different isoelectric points and the globulins probably change their degree of aggregation near their isoelectric point, as suggested by their viscosity [Chick and Lubrzynska, 1914], we decided to compare the behaviour of egg-albumin, the degree of aggregation of which seems to change little.

The work of Sørensen [1917] on the osmotic pressure of egg-albumin was done with $(\text{NH}_4)_2\text{SO}_4$ solutions as the external fluids. We have therefore been led to make fresh osmotic pressure measurements with other salts in the external fluid. We have in the course of this work investigated the following points.

- (1) Does egg-albumin give the same osmotic pressure in the presence of other salts as in the presence of moderate concentrations of $(\text{NH}_4)_2\text{SO}_4$?
- (2) Does the osmotic pressure fall with increasing concentration of sodium chloride as with increasing concentration of ammonium sulphate?
- (3) Is the minimum osmotic pressure found on the acid side of the isoelectric point with sodium chloride and acetate solutions, as with ammonium sulphate solutions?

We have also measured the osmotic pressure at hydrogen ion concentrations similar to those used for serum to see if we could find a similarly convex pressure-concentration curve.

Methods.

The egg-albumin was thrice crystallised by the method of Sørensen [1917]. Crystals were dissolved and subjected to a preliminary dialysis, to remove ammonium sulphate, against an appropriate buffer mixture.

The osmotic pressure was measured as described previously [Marrack and Hewitt, 1927], but the bottle was not rocked and the external fluid was stirred by a mechanical stirrer, with a mercury seal, passing through the cork.

After dialysis, the reaction of the external fluid was measured with a hydrogen or glass [Kerridge, 1925] electrode. Other analyses were made as in the previous paper.

As precipitation of protein occurred on keeping at 37° in the presence of high concentrations of sodium chloride, osmotic pressure readings were made only at 0°, in Exps. 62, 66 *a* and *b*, and 71 *a* and *b*, and the osmotic pressures at 37° calculated.

The partial specific volume of egg-albumin.

Knowing the number of grams of protein per 1000 cc. of solution, the specific gravity of the solution, the concentration of salt per 1000 g. of water, and the specific gravity of a salt solution of this concentration, we can calculate the "apparent specific volume" of the protein. This in all our experiments differed little from 0.749, the value found by Svedberg and Nichols [1926]. We may therefore regard the "partial specific volume" (and "partial molar volume") of the protein as approximately constant and equal to 0.749, and can calculate the volume of the salt solution in which a given weight of protein is dissolved by subtracting the calculated volume of protein from the total volume of the solution. Hence we can calculate the number of grams of protein per 1000 cc. of salt solution; all concentrations of protein are given in these terms.

The effect of change of temperature on the isoelectric point.

The change in the p_H of the isoelectric point of a simple ampholyte with a change of temperature from T_1 to T_2 is given [Stadie and Martin, 1924] by the formula

$$\Delta p_I = \frac{T_1 - T_2}{2.3 \times 2RT_1T_2} (q_a + q_w - q_b) \quad \dots\dots(1),$$

where q_a , q_b , q_w are the heats of ionisation of the acid group of the ampholyte, the basic group of the ampholyte and of water. It is therefore to be expected that the isoelectric point of an ampholyte will vary appreciably with the temperature, and this is actually found in the case of haemoglobin, the isoelectric point of which shifts by about 0.5 with a change of temperature of 20° [Warburg, 1922; Stadie *et al.*, 1925]. Such a shift would be of serious moment to us since we have worked at 37° and 0°.

We found that the p_H of a solution of egg-albumin (7 %) in 1 % sodium chloride, measured with the glass electrode, changed from 4.95 at 18° to 4.89 at 39°, and infer that the isoelectric point shifts by less than 0.1 with the changes of temperature on each side of 18° with which we deal.

In Exp. 44 in Table III with the high protein concentration 122 g. per 1000 cc. of salt solution, there is no evidence of excess of acid or base combined with protein at 37° and p_H 4.71; for if the activity coefficient of the acetate ion is affected by the protein to the same very slight degree that the activity coefficients of the chloride ion and the cations are affected, the concentration

of acetate ion inside will be 24.3 and the chloride + acetate will exactly balance the total base. The isoelectric point, therefore, must be close to p_H 4.71 at this temperature.

The effect of salt concentration on the osmotic pressure at the isoelectric point.

The osmotic pressures found with the ionic strength¹ of the external fluid ranging from about 0.024 to 0.12, that is salt concentration from about 0.19 to 1.1 % (Table I, Exps. 17 *a* and *b*, 31 *a* and *b*, 44, 29 *a* and *b*) lay on the

Fig. 1.

□ Sørensen's results calculated for 37°.

△ Dilute acetate + NaCl.

○ Acetate alone.

▽ Electrodialysed.

▷ p_H 6.75 to 8.0.

same pressure-concentration curve; they agreed well with those found by Sørensen with 4.35 g. $(NH_4)_2SO_4$ per 100 g. H_2O in the external fluid and varying protein concentration, and with his measurements with the protein concentration approximately constant and from 0.44 to 4.35 g. $(NH_4)_2SO_4$ per

¹ The ionic strength μ of a salt solution is given by the formula

$$\mu = \frac{v_a z_a + v_b z_b + v_c z_c + \dots}{2},$$

where $v_a, v_b, v_c, \dots$ are the number of gram-ions of the kinds *a, b, c, ...* per 1000 g. of H_2O , and $z_a, z_b, z_c, \dots$ their valencies.

100 g. H_2O . The results were the same whether the bulk of the salt present was sodium acetate (Exps. 17 *a* and *b*, 29 *a* and *b*) or NaCl (Exps. 31 *a* and *b*, and 44).

We can regard the osmotic pressure-concentration curve found in the presence of this wide range of different salts as a standard (Fig. 1).

With higher salt concentrations the osmotic pressure rose slightly above the standard (Exps. 81 *a* and *b*, 62, 66 *a* and *b*), but fell again to the standard at the highest concentrations ($\mu = 5.9$, *i.e.* NaCl 30 %, Exps. 71 *a* and *b*).

When the ionic strength of the external fluid was reduced to 0.006 with salt concentration of 0.05 % (Exps. 33 *a* and *b*, 38 *a* and *b*) the osmotic pressure at the lowest protein concentrations (33 *b*) agreed with the standard curve, but fell away to 15 % less than the standard at the highest protein concentration (Exp. 33 *a*).

Effect of protein concentration.

Up to a protein concentration of about 80 g. per 1000 cc. the pressure-concentration curve at the isoelectric point is a straight line passing through the origin, but above this concentration the pressure rises more rapidly than the concentration.

We may introduce a term in the pressure-concentration equation corresponding to "*b*" in Van der Waals's equation [Adair, 1925]; such a term would amount to about 1 cc. per g. protein.

It is not clear what meaning should be attached to this term. It may be the result of the carriage of a certain amount of water with the protein molecules; this water cannot be held as are water molecules round hydrated ions, for reasons which we have already discussed [1927], but it is possible that water and diffusible ions may be carried in the mesh of the loose structure of the protein molecule.

Molecular weight of egg-albumin.

It is natural to calculate the molecular weight from the slope of the tangent to the pressure-concentration curve at zero concentration; this is particularly obvious if we introduce a "*b*" term as suggested in the last section. Such a calculation gives a value about 43,000 which agrees with that calculated by Adair [1927] from Sørensen's [1917] osmotic pressure figures. It is not obvious how the latter obtained the figure 36,000 from his data; he gives this, however, merely as a tentative suggestion, though it has passed into the literature as though it were a fully established constant.

Svedberg and Nichols [1926], from the sedimentation equilibrium, concluded that egg-albumin prepared by simple dialysis contained a mixture of a substance of molecular weight 36,000 with sufficient substances of higher molecular weight to give a mean molecular weight of 43,000; but that electro-dialysed egg-albumin contained only a substance of molecular weight 36,000.

Samples of egg-albumin were electro dialysed for us by Mr Campbell Smith, with a current density of 1 milliamp. per cm.²; the conductivity of solutions 79 *a*, 79 *b*, and 80 were 1.0×10^{-5} , 1.3×10^{-5} , 1.26×10^{-5} mhos. at 17°. The osmotic pressures given by these solutions fell slightly below, rather than above, the standard curve corresponding to a molecular weight of 43,000.

It is possible that substances of higher molecular weight may be removed by electro dialysis, but form again on standing. It is difficult to understand how different workers could repeatedly get the same amount of impurity in their preparations of crystalline egg-albumin, although this impurity could be separated by the ultracentrifuge (*i.e.* was not adsorbed on the albumin molecules), unless the impurity was reformed in constant amount in solution on standing. The changes that occur in strong $(\text{NH}_4)_2\text{SO}_4$ solutions might be an exaggeration of this formation of substances of higher molecular weight.

The discrepancy between molecular weights obtained by Svedberg's methods and by osmotic pressure measurements in the case of egg-albumin is the more remarkable since these methods give the same result for haemoglobin [Svedberg and Nichols, 1927; Adair, 1927] and results in close agreement for serum-albumin [Svedberg and Sjögren, 1928; Adair, 1927]. The discrepancy mentioned by Svedberg and Sjögren between their molecular weight for serum-albumin and that given by Sørensen [1925] is most probably due to Sørensen's methods of calculation. The discrepancy between the molecular weights found for globulin by Svedberg and Sjögren, and Adair, is to be expected since globulins aggregate, particularly in the cold, as the concentration is increased; Adair must have used much stronger solutions than did Svedberg and Sjögren, and was working in the cold.

Effect of hydrogen ion concentration.

With medium salt concentrations (μ from 0.1 to 0.06) there was no appreciable change in the osmotic pressure when the p_{H} of the external fluid was reduced from 4.8 to 4.3 (Exps. 15 *a* and *b*, 41 *a* and *b*, 45 *a* and *b*). At p_{H} about 4.3 the level of the fluid in the capillary tube did not as usual reach a steady level in 24 hours, but continued to rise slowly; the slight discrepancy between the two pairs of readings with protein concentration 59.8 and 60.0 (Exps. 45 *b* and 41 *a*) was probably due to this. At p_{H} 3.98 the osmotic pressures were considerably above those found at p_{H} 4.7–4.8.

At these more acid reactions, particularly at p_{H} 3.98, a considerable precipitation of protein occurred; it is possible that the protein which remained in solution was, to some degree, denatured. However, at the lower temperatures no precipitates formed, but the osmotic pressures, allowing for the difference of temperature and the amount of protein which precipitated out, agreed with those found at 37°.

The rise of osmotic pressure at p_{H} 3.98 above that at p_{H} 4.7–4.8 is not more than the Donnan effect would account for.

With a low salt concentration ($\mu = 0.004$) the osmotic pressures at p_{H} 4.57

(Exps. 42 *a* and *b*) were above those found with similar salt concentration at p_H 4.81–4.83.

Thus with these low to medium concentrations of sodium acetate and sodium chloride there is no reduction of the osmotic pressure on the acid side of the isoelectric point, such as was found by Sørensen in the presence of higher concentrations of ammonium sulphate. We find, however, a tendency to some form of denaturation at these more acid reactions which tends to interfere with osmotic pressure measurements.

In pursuance of our original programme osmotic pressures were also measured at hydrogen ion concentrations below the isoelectric point (p_H 6.7–8.0); the external fluid in these experiments (1 *a*, *c* and *d*, 3 *a*, *b*, *c* and *d*, 9 *a* and *b*, 11 *c* and *d*) was a sodium chloride-bicarbonate mixture and the p_H was calculated from the Henderson-Hasselbalch equation as in our previous paper [1927].

The pressure-concentration curve found was similar to that found for serum at these hydrogen ion concentrations and its shape can be ascribed to the Donnan effect in the same way. The difference between the osmotic pressure at p_H 6.76 and that at the isoelectric point is nearly as great as the calculated Donnan effect for ideal solutions; but the increase of osmotic pressure when the p_H increased beyond 6.7 was less than that calculated. In agreement with this we find evidence, in the distribution of electrolytes (Table III), that the activity coefficients of the diffusible ions were more affected at the higher p_H . In this connection also an explanation may be obtained from the idea of the protein molecule as a sponge-like structure. The acid groups which first combine with base may be those outside the molecule, able to produce a Donnan effect in the solution, while the groups which combine with base only at higher p_H may be inside, and any Donnan effect that they produce may be, not between solution and the fluid outside the membrane, but between the free solvent and the fluid entangled in the molecule.

The effect of egg-albumin near the isoelectric point on the activity of the diffusible ions seems very slight (Exp. 44, Table III).

The effect of salt concentration on the activity coefficient of egg-albumin.

Scatchard [1927] has shown that the activity coefficient (f_2) of a non-electrolyte in an aqueous salt solution is given by the equation

$$\ln f_2 = \frac{N\epsilon^2\beta\mu}{RTD_0b} \quad \dots\dots(2),$$

where D_0 is the dielectric constant of water, D_2 that of the solution, and β a constant such that

$$\frac{1}{D_2} = \frac{1}{D_0} (1 + \beta m_2);$$

m_2 being the molar concentration of the non-electrolyte, b the mean radius

Table I.

Osmotic pressures of egg-albumin, near the isoelectric point, in presence of moderate concentrations of salt.

Exp. No.	Ionic strength of external solution	p_H of external solution	Protein concentration g. per 1000 cc. salt solution	Osmotic pressure at 37° cm. water	Osmotic pressure at lower temperature cm. water	
17 <i>a</i>	0.120	4.80	104.5	67.1	63.4 at 0°	} Acetate alone
17 <i>b</i>	0.120	4.80	63.0	38.6	34.3 at 0°	
31 <i>a</i>	0.127	4.81	77.6	49.6	46.8 at 11°	
31 <i>b</i>	0.127	4.81	44.4	27.45	24.3 at 11°	} Acetate 0.024 <i>N</i> . NaCl 0.103 <i>N</i>
44	0.127	4.71	122.0	81.0	—	
15 <i>a</i>	0.100	4.65	74.3	45.7	42.85 at 4°	} Acetate alone
15 <i>b</i>	0.100	4.65	38.9	23.7	21.6 at 4°	
29 <i>a</i>	0.024	4.90	99.7	62.6	57.4 at 3°	
29 <i>b</i>	0.024	4.90	55.0	32.9	29.0 at 3°	
79 <i>a</i>	0.120	4.80	47.6	28.45	25.55 at 0°	} Electrodialysed. Acetate alone
79 <i>b</i>	0.120	4.80	48.4	29.25	26.3 at 0°	
80	0.120	4.80	44.2	25.8	22.7 at 0°	

Table II.

Exp. No.	Ionic strength of external solution	p_H of external solution	Protein concentration g. per 1000 cc. of salt solution	Osmotic pressure at 37° cm. water	Osmotic pressure at lower temperature cm. water	Osmotic pressure calculated from standard curve 37°	
33 <i>a</i>	0.006	4.83	147.4	88.9	—	101.5	} Low ionic strength. Acetate alone
33 <i>b</i>	0.006	4.83	35.0	22.0	20.5 at 5°	22.0	
38 <i>a</i>	0.006	4.84	95.2	53.8	—	60.7	
38 <i>b</i>	0.006	4.84	90.5	50.5	—	57.5	
62	3.55	4.8	58.5	(39.5)	34.8 at 0°	36.5	} High ionic strength. Acetate (about 0.014 <i>M</i>) + NaCl
66 <i>a</i>	3.55	4.75	128.0	(96.0)	83.0 at 0°	85.0	
66 <i>b</i>	3.55	4.75	79.0	(54.0)	48.5 at 0°	49.5	
71 <i>a</i>	5.90	4.88	137.0	(94.0)	83.7 at 0°	92.5	
71 <i>b</i>	5.90	4.88	79.0	(50.8)	44.7 at 0°	49.0	
81 <i>a</i>	1.10	4.80	98.9	67.9	64.8 at 0°	63.0	
81 <i>b</i>	1.10	4.80	59.2	37.4	35.0 at 0°	36.7	} Low p_H . Acetate alone
41 <i>a</i>	0.060	4.31	60.0	37.0	34.5 at 5°	37.0	
41 <i>b</i>	0.060	4.31	33.0	18.4	15.9 at 5°	20.3	
45 <i>a</i>	0.060	4.27	102.0	65.8	62.3 at 12°	65.5	
45 <i>b</i>	0.060	4.27	59.8	38.0	34.5 at 12°	37.0	
46 <i>a</i>	0.036	3.98	74.1	57.4	56.8 at 1°	46.0	
46 <i>b</i>	0.036	3.98	72.1	56.9	55.9 at 1°	45.0	
42 <i>a</i>	0.004	4.57	142.0	90.5	—	96.7	
42 <i>b</i>	0.004	4.57	91.5	59.8	—	58.0	} High p_H . Bicarbonate + NaCl
11 <i>c</i>	0.156	6.75	94.1	91.6	—	—	
11 <i>d</i>	0.156	6.75	63.2	51.5	—	—	
3 <i>a</i>	0.157	7.0	71.5	61.0	56.0 at 1°	—	
3 <i>b</i>	0.157	7.0	43.8	32.8	29.45 at 1°	—	
1 <i>a</i>	0.158	7.0	69.8	58.95	53.95 at 1°	—	
9 <i>a</i>	0.158	7.66	54.8	44.9	39.9 at 1°	—	
9 <i>b</i>	0.158	7.66	34.0	24.35	20.7 at 1°	—	
3 <i>c</i>	0.157	7.68	71.5	61.3	—	—	
3 <i>d</i>	0.156	7.68	43.8	33.1	—	—	
1 <i>c</i>	0.156	8.0	69.8	60.5	—	—	
1 <i>d</i>	0.156	8.0	41.8	30.85	—	—	

Table III.

Partition of ions. Protein in g., other ions in mille-equivalents, per 1000 cc. of salt solution. Acetate concentration in internal fluid calculated. Base combined with protein as mille-equivalents per g.

Exp. No.	External fluid				Internal fluid				Base combined with protein	$\frac{A_e B_e}{A_p B_p} \times 100$
	p_H	Cl = A_e	HCO ₃ or acetate	Total base = B_e	Protein	Cl = A_p	HCO ₃ or acetate	Total base = B_p		
1 <i>c</i>	8.0	140.9	14.9	156.0	69.8	132.8	14.0	—	—	—
1 <i>d</i>	8.0	140.9	14.9	156.0	41.8	137.8	14.6	171.5	0.46	92.9
3 <i>c</i>	7.70	142.9	14.75	157.7	71.5	135.8	14.0	—	—	—
3 <i>d</i>	7.70	142.9	14.75	157.7	43.8	139.5	14.45	172.0	0.41	94.0
9 <i>a</i>	7.66	142.6	15.1	157.8	54.8	135.4	14.3	—	—	—
9 <i>b</i>	7.66	142.6	15.1	157.8	34.0	139.2	14.8	171.9	0.53	93.2
11 <i>c</i>	6.75	140.9	15.25	156.2	94.1	128.8	14.0	174.3	0.33	98.0
11 <i>d</i>	6.75	140.9	15.25	156.2	63.2	133.5	14.5	172.6	0.39	95.5
17 <i>a</i>	4.80	—	121.0	121.3	104.5	—	—	124.9	—	—
17 <i>b</i>	4.80	—	121.0	121.3	63.0	—	—	124.3	—	—
31 <i>a</i>	4.81	103.9	24.0	127.4	77.6	104.4	—	—	—	—
31 <i>b</i>	4.81	103.9	24.0	127.4	44.4	104.1	(24.0)	129.7	0.04	98.1
44	4.71	103.0	24.0	126.7	122.0	104.2	(24.3)	128.1	0.00	97.5

Bicarbonate
bufferAcetate
buffer

of the ions of the salt, μ the ionic strength of the solution and ϵ the unit of electric charge. According to this equation the activity coefficient is independent of the concentration of the non-electrolyte and is an exponential function of the concentration of the salt.

Equation (2) is strictly applicable only when the solution is very dilute, but Scatchard has shown that it can be applied to higher salt concentrations through the compensation of several factors, if there is no other cause for deviation from ideality than the electric charges of the ions; but it agrees extremely well with observations on the solubility of non-electrolytes including egg-albumin and serum-globulin in salt solution (the "salting out" effect) [Lindström-Lang, 1924; Randall and Farley, 1927]. We will therefore consider its relation to osmotic pressure.

We can deal with the relation of osmotic pressure to protein activity in a solution of three components, water, protein and salt, on the lines followed by Lewis and Randall [1923].

Suppose we vary the concentration of protein inside a collodion sac, which is immersed in a large volume of a solution of constant composition, and apply such a pressure P (the osmotic pressure) to the internal solution that equilibrium is attained and no further water or salt diffuses through the membrane.

Let n_s , n_w , n_p be the number of mols of salt, water and protein in a volume V ; $\bar{F}_s$, $\bar{F}_w$, $\bar{F}_p$ the partial molar free energies and $\bar{V}_s$, $\bar{V}_w$, $\bar{V}_p$ the partial molar volumes of salt, water and protein; a_s and f_p the activity and activity coefficient of protein.

Consider for convenience a volume V of the internal fluid in which the total number of molecules ($n_s + n_w + n_p$) is constant; let the molar free energy of this solution be F .

Since we may consider $\bar{F}_s$, $\bar{F}_w$ and $\bar{F}_p$ as functions of the variables n_s , n_w , n_p and P ,

$$d\bar{F}_s = \left(\frac{\partial \bar{F}_s}{\partial n_s}\right) dn_s + \left(\frac{\partial \bar{F}_s}{\partial n_w}\right) dn_w + \left(\frac{\partial \bar{F}_s}{\partial n_p}\right) dn_p + \left(\frac{\partial \bar{F}_s}{\partial P}\right) dP \quad \dots\dots(3),$$

$$d\bar{F}_w = \left(\frac{\partial \bar{F}_w}{\partial n_s}\right) dn_s + \left(\frac{\partial \bar{F}_w}{\partial n_w}\right) dn_w + \left(\frac{\partial \bar{F}_w}{\partial n_p}\right) dn_p + \left(\frac{\partial \bar{F}_w}{\partial P}\right) dP \quad \dots\dots(4),$$

$$\text{and} \quad d\bar{F}_p = \left(\frac{\partial \bar{F}_p}{\partial n_s}\right) dn_s + \left(\frac{\partial \bar{F}_p}{\partial n_w}\right) dn_w + \left(\frac{\partial \bar{F}_p}{\partial n_p}\right) dn_p + \left(\frac{\partial \bar{F}_p}{\partial P}\right) dP \quad \dots\dots(5).$$

The partial molar free energies of salt and water in the solution in the sac must be equal to their partial molar free energies in the external fluid; they are therefore constant, and $d\bar{F}_s$ and $d\bar{F}_w$ both = 0. Therefore multiplying (3) by n_s , (4) by n_w , and (5) by n_p , and adding,

$$\begin{aligned} & \left[n_s \left(\frac{\partial \bar{F}_s}{\partial n_s} \right) + n_w \left(\frac{\partial \bar{F}_w}{\partial n_s} \right) + n_p \left(\frac{\partial \bar{F}_p}{\partial n_s} \right) \right] dn_s + \left[n_s \left(\frac{\partial \bar{F}_s}{\partial n_w} \right) + n_w \left(\frac{\partial \bar{F}_w}{\partial n_w} \right) + n_p \left(\frac{\partial \bar{F}_p}{\partial n_w} \right) \right] dn_w \\ & + \left[n_s \left(\frac{\partial \bar{F}_s}{\partial n_p} \right) + n_w \left(\frac{\partial \bar{F}_w}{\partial n_p} \right) + n_p \left(\frac{\partial \bar{F}_p}{\partial n_p} \right) \right] dn_p + \left[n_s \left(\frac{\partial \bar{F}_s}{\partial P} \right) + n_w \left(\frac{\partial \bar{F}_w}{\partial P} \right) + n_p \left(\frac{\partial \bar{F}_p}{\partial P} \right) \right] dP \\ & = n_p d\bar{F}_p. \end{aligned}$$

Now the terms in the first three square brackets each equal zero [Lewis and Randall, 1923, ch. iv, equation (20)]; and

$$n_s \left(\frac{\partial \bar{F}_s}{\partial P} \right) + n_w \left(\frac{\partial \bar{F}_w}{\partial P} \right) + n_p \left(\frac{\partial \bar{F}_p}{\partial P} \right) = \left(\frac{\partial F}{\partial P} \right) = \frac{V}{RT}$$

[Lewis and Randall, 1923, ch. xiv, equation (9)].

$$\text{Therefore} \quad \frac{V}{RT} \cdot dP = n_p d\bar{F}_p \quad \dots\dots(6).$$

We may put $V = W + n_p \bar{V}_p$, since we find $\bar{V}_p$, the partial molar volume of egg-albumin, constant. W is then the volume of an amount of the protein solution, containing a constant total number of molecules, less the volume occupied by the protein; this, in practice, we find to remain constant when the composition of the external salt solution is held constant.

$$\text{Therefore} \quad \frac{W + n_p \bar{V}_p}{RT} \cdot dP = n_p d\bar{F}_p,$$

$$\text{or} \quad \frac{W}{RT} \cdot dP = \frac{n_p}{1 + \frac{n_p \bar{V}_p}{W}} \cdot d\bar{F}_p \quad \dots\dots(7).$$

$$\text{Now } \bar{F}_p = \ln \cdot a_p + \text{constant} = \ln \left(f_p \frac{n_p}{n_s + n_w + n_p} \right) + \text{constant}.$$

$$\begin{aligned} \text{Therefore} \quad \frac{W}{RT} \cdot dP &= \frac{n_p}{1 + \frac{n_p \bar{V}_p}{W}} \cdot d \ln \left(f_p \frac{n_p}{n_s + n_w + n_p} \right) \\ &= \frac{n_p}{1 + \frac{n_p \bar{V}_p}{W}} (d \ln \cdot n_p + d \ln \cdot f_p), \end{aligned}$$

since $n_s + n_w + n_p$ is constant

$$= \frac{1}{1 + \frac{n_p \bar{V}_p}{W}} (d n_p + n_p d \ln \cdot f_p) \quad \dots\dots(8).$$

Integrating

$$\frac{WP}{RT} = \frac{W}{\bar{V}_p} \ln \left(1 + \frac{n_p \bar{V}_p}{W} \right) + \int_0^{n_p} \frac{n_p}{1 + \frac{n_p \bar{V}_p}{W}} d \ln f_p \quad \text{.....(9)}$$

$$= n_p \left(1 - \frac{n_p \bar{V}_p}{2W} \right) + \int_0^{n_p} \frac{n_p}{1 + \frac{n_p \bar{V}_p}{W}} d \ln f_p \quad \text{.....(10)}$$

when $n_p \bar{V}_p$ is small compared with W .

If f_p has the value given by equation (2) it is independent of the protein concentration; so that f_p does not change over the range of integration, and $d \ln f_p = 0$.

$$\text{Consequently} \quad \frac{WP}{RT} = n_p \left(1 - \frac{n_p \bar{V}_p}{2W} \right) \quad \text{.....(11).}$$

This makes the osmotic pressure independent of the salt concentration. Equation (2) therefore agrees fairly well with what we have found in NaCl solution, but does not cover the effect of $(\text{NH}_4)_2\text{SO}_4$ in high concentrations. This also follows from the fact that $(\text{NH}_4)_2\text{SO}_4$ has a very different effect on osmotic pressure from that of NaCl, although according to equation (2) the effects should be similar, since the effect of NaCl on the solubility of non-electrolytes is similar and about equal to that of $(\text{NH}_4)_2\text{SO}_4$.

To give the osmotic pressures found with high concentrations of $(\text{NH}_4)_2\text{SO}_4$ we should have to introduce into equation (2) a negative term containing C_p and μ , which would signify that in strong $(\text{NH}_4)_2\text{SO}_4$ solutions the activity coefficient of egg-albumin falls with the albumin concentration and that this fall is greater the greater the $(\text{NH}_4)_2\text{SO}_4$ concentration. Such a term, although sufficient to account for the osmotic pressure effects, would not greatly effect the solubility relations because the term $\frac{Nc^2\beta\mu}{RT \cdot D_0b}$ becomes so great in high salt concentrations.

This fall of activity coefficient with increasing albumin concentration in high concentrations of $(\text{NH}_4)_2\text{SO}_4$ agrees with Sørensen's original suggestion that aggregation of the protein molecules takes place. This idea well suits the modern conception of long protein molecules which can form networks. Such linking undoubtedly occurs with proteins such as gelatin and globulins, and the striking thing is not that egg-albumin molecules aggregate under certain conditions, but that they should remain separate, behaving as separate molecules, over a wide range of conditions. The changes in the optical rotation of egg-albumin, with varying $(\text{NH}_4)_2\text{SO}_4$ and hydrogen ion concentration, found by Jennsen-Hansen [1927], suggest changes in the molecule similar to those which occur in gelatin [Kraemer and Fanselow, 1928], together with changes in the degree of aggregation.

Egg-albumin is a very large and complex molecule and it is enough to conclude that it forms simple solutions of separate molecules of constant weight in solutions of various salts of moderate concentration, without straining probabilities by trying to consider it as an inert mass even in the most extreme conditions.

SUMMARY.

1. In the presence of medium concentrations of sodium acetate and sodium chloride, isoelectric egg-albumin has the same osmotic pressure as in the presence of medium concentrations of ammonium sulphate.

2. Deviations occur from these values in the presence of high concentrations of sodium chloride and low concentrations of sodium acetate but less than those found in the presence of ammonium sulphate.

3. The molecular weight of egg-albumin calculated from our figures and from those of Sørensen is 43,000. Electrodialysed egg-albumin gave a slightly higher figure.

4. The fall of osmotic pressure on the acid side of the isoelectric point found by Sørensen in the presence of $(\text{NH}_4)_2\text{SO}_4$ was not found by us in the presence of sodium chloride and sodium acetate.

5. At p_{H} 6.7–8.0 the osmotic pressure-concentration curve of egg-albumin resembles that of serum, confirming that the shape of this curve is due to a Donnan effect.

6. Ammonium sulphate has a special effect on the activity of egg-albumin apart from the salting-out effect.

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REFERENCES.

- Adair (1925). *Proc. Roy. Soc. Lond. A*, **109**, 292.
— (1927). *J. Amer. Chem. Soc.* **49**, 2524.
Chick and Lubrzynska (1914). *Biochem. J.* **8**, 59.
Jennsen-Hansen (1927). *Compt. Rend. Lab. Carlsberg*, **16**, No. 10.
Kerridge (1925). *Biochem. J.* **19**, 611.
Kraemer and Fanselow (1928). *J. Physical Chem.* **32**, 894.
Lewis and Randall (1923). *Thermodynamics*, p. 236. (McGraw Hill Book Co.)
Lindstrøm-Lang (1924). *Compt. Rend. Lab. Carlsberg*, **15**, No. 4.
Marrack and Hewitt (1927). *Biochem. J.* **21**, 5.
Randall and Farley (1927). *Chem. Reviews*, **4**, 271.
Scatchard (1927). *Chem. Reviews*, **3**, 383.
Sørensen (1917). *Compt. Rend. Lab. Carlsberg*, **12**, 348.
— (1925). *Proteins*. (The Fleischmann Co., New York.)
Stadie, Austin and Robinson (1925). *J. Biol. Chem.* **66**, 901.
— and Martin (1924). *J. Biol. Chem.* **60**, 215.
Svedberg and Nichols (1926). *J. Amer. Chem. Soc.* **48**, 3081.
— (1927). *J. Amer. Chem. Soc.* **49**, 2920.
— and Sjögren (1928). *J. Amer. Chem. Soc.* **50**, 3318.
Warburg (1922). *Biochem. J.* **16**, 298.