THE OSMOTIC PRESSURE, AFTER RE-SOLUTION, OF SERUM DRIED FROM THE FROZEN STATE (F.D. SERUM)

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(With 2 Figures in the Text)

Introduction

At the present time, human serum is being dried from the frozen state on a large scale at the Pathological Laboratory, Cambridge, as a means of preserving it for subsequent transfusion into human beings. We will refer to this as F.D. serum.

If a perfectly clear sample of human serum be frozen it is generally opalescent after thawing, and human F.D. serum has invariably given an opalescent solution when redissolved.

For transfusion purposes it is desirable that the serum proteins should be unchanged, and recently the question has been raised as to whether the serum proteins undergo denaturation during the process of drying. Therefore we have thought that it would be of interest to publish results we obtained some years ago when testing the method for drying serum which has been described by Greaves & Adair (1936).

We determined the osmotic pressure of a specimen of human serum, some of the measurements being made on the fresh serum and some on material which had been dried from the frozen state. We made similar measurements on fresh and dried serum of normal rabbits and of rabbits which had been injected with crystalline egg albumin and which had produced antisera that reacted strongly with the antigen. The sera from the immunized rabbits contained a much larger proportion of globulin than those of the normal rabbits, so that the chances of detecting changes in the state of the globulins were improved.

We were unable to detect any appreciable difference between the osmotic pressures of the fresh and of the F.D. serum.

In addition to the determinations of osmotic pressures we measured the specific refraction increments and also the membrane potentials of dried and undried sera in equilibrium with a standard buffer solution. Previous work (Adair & Robinson, 1930*a*) had shown that the refraction increment of horse serum albumin was reduced if the serum were extracted with ether at room temperature before separation of the globulin and crystallization of the

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albumin. The serum lipids seemed therefore to influence the refraction of serum. We did not detect any significant change in the refraction increments of serum after drying, although the increased opalescence of the dried material after re-solution would seem to indicate that the state of combination of the serum lipids had undergone some alteration.

It has been shown in a former paper (Adair & Adair, 1934*b*) that the determination of the electric charge of haemoglobin, calculated from membrane potential measurements, gives a sensitive method for detecting small changes caused by manipulations in the preparation of the protein. We could detect no appreciable change in the charge of the serum proteins after drying and re-solution of the serum.

METHODS

The human serum was prepared from blood drawn by venepuncture and defibrinated under sterile conditions by the method which has recently been described by King (1939). Immediately after defibrination was completed, the blood was centrifugalized and the serum removed and preserved at $-10°$ C. until it was dialysed or dried. The rabbits were bled from the marginal ear vein and the blood was allowed to clot. After 16 hr. the serum was removed, centrifugalized and kept at $-10°$ C. until it was dialysed or dried.

All the dried sera used in the experiments recorded were dissolved in the phosphate buffer and were rapidly and completely soluble. After about 48 hr. dialysis at 0·5° C. both undried and previously dried specimens of sera deposited a small amount of precipitate at the bottom of the dialysing membrane.

Osmotic pressure measurements were made by the method of Adair (1925) in a room maintained at 0·5° C. An improved type of manometer was used, consisting of a glass tube engraved with a millimetre scale. For the more concentrated solutions the internal diameter of the manometers used was 1·5 mm., while for the solutions of a concentration less than 2%, which had low osmotic pressures, the internal diameter of the manometers was 3 mm. so that the capillarity correction was diminished.

In all the measurements recorded the dialysate was $M/15$ Sørensen phosphate buffer solution containing $M/30$ KH_2PO_4 and $M/30$ NaH_2PO_4, ionic strength 0·1333 with a pH of 6·91 measured at 0·5° C. or a pH of 6·81 at 20° C. Additional measurements were made with ultrafiltrates of serum as dialysates, but difficulties in maintaining a constant CO_2 tension caused fluctuations in the final pH values, and for the purpose of comparative measurements of dried and undried serum, it was considered preferable to use a standard dialysate of well-defined pH.

The fresh and dried sera were subjected to a preliminary dialysis for 24 hr. against the buffer and then put into collodion membranes of 70 mm. length and 11 mm. diameter, fitted with manometers and equilibrated with several changes of buffer in pyrex tubes 25 by 200 mm. Osmotic equilibrium was reached in 5–7 days. Osmotic pressures measured in mm. of solution were reduced to mm. of mercury at 0·5° C., using the following formula for ρ, the

density of the solution: $\rho = 1{\cdot}009 + 0{\cdot}00243\ C$, where C = grams of dry protein per 100 ml. solution.

Two methods were used for estimating the protein concentration:

(1) Determinations of the total nitrogen were carried out on samples taken from each osmometer at the close of the experiment, and the albumin-globulin ratios of the more concentrated protein solutions were estimated by the method described by Adair & Taylor (1936). The percentages of nitrogen in human and rabbit serum albumins and globulins are not known, but provisionally the values of 15·6% for albumin and 15·13% for globulin, determined for horse serum proteins (Adair & Robinson, 1930*a*), were employed. The values for C can then be calculated by formula (1):

$$C = \text{total nitrogen} \times [6{\cdot}41 + 0{\cdot}2 \times (\%\ \text{globulin nitrogen})]. \qquad (1)$$

(2) The protein concentrations were calculated from measurements of n', the refractive index of the protein solution and n'', the refractive index of the dialysate by formula (2):

$$n' - n'' = \alpha'\ C. \qquad (2)$$

α' = the refraction increment for 1% of protein. A mean value for α' was determined from all of the experiments in which C was estimated from formula (1). It was found that $\alpha' = 0{\cdot}00190$ for human serum and 0·00189 for rabbit serum and antiserum.

The membrane potentials were measured at 0·5° C. as described by Adair & Adair (1934*a*). The symbol E in Tables 1 and 2 represents the membrane potential expressed in millivolts. The protein solutions were negatively charged in all cases. Values for the ratio E/C were also calculated. This ratio appears to be independent of the protein concentration over the range investigated.

Table 1. *Osmotic pressures and membrane potentials of the serum of a man aged* 21 (*G. A. H. B.*)

Of the nitrogen 68·8% is albumin nitrogen and 31·2% globulin nitrogen.
Protein concentration = nitrogen × 6·535.
In Exps. 1–6 fresh serum, was used and in 1 D to 6 D F.D. serum.

No.	C from formula (1)	C from refraction	α' refraction increment	p osmotic pressure in mm. Hg	p/C	E membrane potential in mV.	E/C
1	6·92	6·84	0·001880	22·25	3·25	−1·67	−0·232
2	6·76	6·84	0·001925	22·80	3·34	−1·66	−0·231
3	4·63	4·63	0·001897	12·72	2·75	−1·13	−0·233
4	—	3·45	—	8·75	2·54	−0·90	−0·247
5	1·70	1·72	0·001917	3·98	2·31	—	—
6	0·79	0·81	0·001952	1·805	2·22	—	—
1 D	6·82	6·74	0·001880	21·8	3·24	−1·65	−0·233
2 D	6·99	6·92	0·001882	22·5	3·25	−1·63	−0·225
3 D	4·97	4·97	0·001900	14·0	2·82	−1·17	−0·224
4 D	3·36	3·40	0·001917	8·64	2·54	−0·88	−0·247
5 D	1·68	1·73	0·001950	3·81	2·20	—	—
6 D	0·90	0·90	0·001914	1·95	2·15	—	—

Table 2. *Osmotic pressures and membrane potentials of pooled sera of two normal rabbits*

Of the nitrogen 67·5 % is albumin nitrogen and 32·5 % globulin nitrogen.
Protein concentration = nitrogen × 6·475.
In Exps. 1–6 fresh serum was used and in 1 D to 6 D F.D. serum.

No.	C from formula (1)	C from refraction	α' refraction increment	p osmotic pressure in mm. Hg.	p/C	E membrane potential in mV.	E/C
1	5·36	5·36	0·001889	16·58	3·10	−1·42	−0·265
2	5·47	5·46	0·001884	16·92	3·10	−1·41	−0·258
3	4·10	4·14	0·001910	11·72	2·83	—	—
4	2·86	2·82	0·001870	7·30	2·59	−0·66	−0·234
5	1·35	1·34	0·001873	3·16	2·36	—	—
6	0·74	0·73	0·001856	1·69	2·28	—	—
1 D	5·45	5·48	0·001899	17·23	3·14	−1·32	−0·241
2 D	5·20	5·24	0·001903	15·92	3·04	−1·38	−0·263
3 D	4·03	3·98	0·001870	11·12	2·80	−1·03	−0·259
4 D	2·72	2·75	0·001908	6·97	2·54	−0·72	−0·262
5 D	1·22	1·25	0·001935	2·88	2·31	—	—
6 D	0·61	0·61	0·001890	1·34	2·20	—	—

Table 3. *Osmotic pressures of serum of rabbit* 2255 *which had been injected with crystalline egg albumin*

Of the nitrogen 34·1 % is albumin nitrogen and 65·9 % globulin nitrogen.
Protein concentration = nitrogen × 6·542.
In Exps. 1–4 fresh serum was used and in 1 D to 5 D F.D. serum.

No.	C from formula (1)	C from refraction	α' refraction increment	p osmotic pressure in mm. Hg	p/C
1	6·70	6·75	0·001904	15·22	2·28
2	3·36	3·39	0·001910	6·51	1·94
3	1·66	1·66	0·001889	3·01	1·82
4	1·24	1·24	0·001887	2·24	1·81
1 D	6·59	6·58	0·001889	14·71	2·23
2 D	3·35	3·36	0·001896	6·41	1·92
3 D	1·71	1·70	0·001876	3·09	1·81
4 D	0·78	0·78	0·001886	1·35	1·74
5 D	0·89	0·89	0·001879	1·58	1·77

The results of the experiments are given in Tables 1–3 and Figs. 1 and 2.

The measurements of the osmotic pressures of the fresh and F.D. sera and antiserum have been examined and compared by two methods. In the first place it was found that the results of the observations on fresh and dried human serum could be represented by a single curve, and that, at the concentration of normal serum, the alteration in osmotic pressure caused by drying seemed to be less than 0·3 mm. Hg or about 1 % of the colloid osmotic pressure. Such a close agreement was not obtained with the more dilute solutions, but the measurements of very small osmotic pressures tend to be less exact and it is probable that the deviations are not greater than the experimental errors. In the case of normal and immune rabbit sera, it was found that the osmotic

pressures of both fresh and dried serum agreed very closely, the differences being less than 0·05 mm. Hg.

In the second place, values of the ratio C/p were plotted against C, to determine the constants in the empirical formula 3, described by Adair & Robinson (1930*b*):

$$p = \pi_0 C/(1 - K_b C) = (10\ RT/M)\ C/(1 - K_b C) \qquad (3)$$

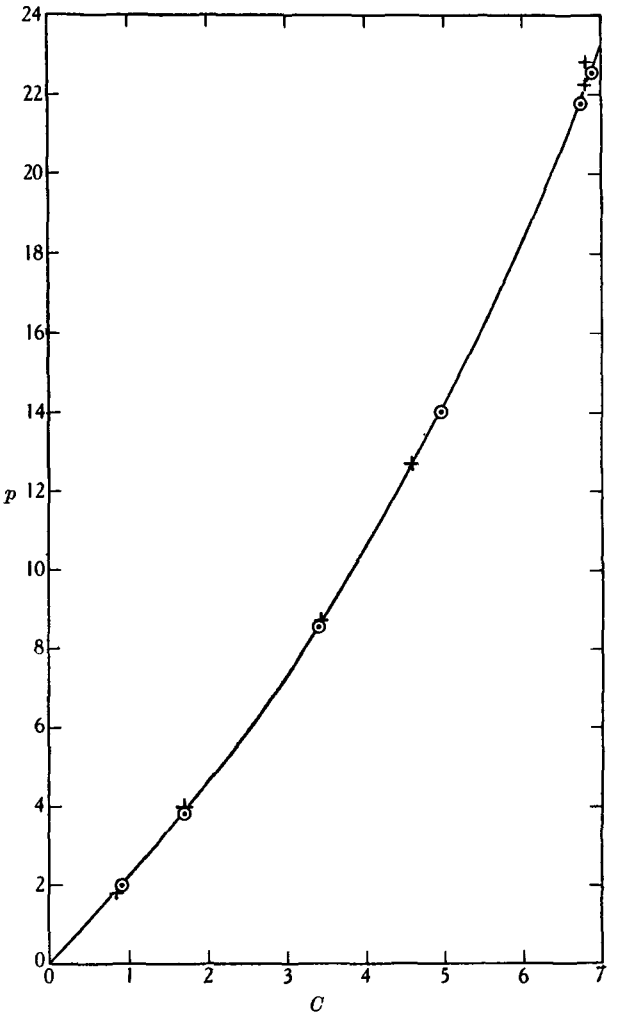

Fig. 1. p = osmotic pressures in mm. Hg of fresh and dried human serum at 0·5° C. in $M/15$ phosphate buffer pH 6·9. C = g. dry protein per 100 ml. solution. Curve calculated by formula $p = 2{\cdot}066C/1 - 0{\cdot}0542C$; crosses, fresh serum; circles, dried serum.

π_0 = constant, M = average molecular weight of serum proteins, K_b = a constant with a high value when the osmotic pressure increases more rapidly than the concentration.

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In the case of human serum, fresh and dried, it has been estimated that $M = 82{,}500$ and $K_b = 0{\cdot}054$. In the two most dilute solutions, measurements on fresh serum gave higher pressures and those with dried serum gave lower pressures than those calculated by formula (3), but the differences were probably due to experimental errors.

Fresh, pooled, normal rabbit serum gave values of 78,200 for M and 0·055 for K_b, whereas the F.D. serum gave 79,800 for M and 0·057 for K_b. It appears therefore that the constants agree within about 2%. In the case of the antiserum, the constants were the same for both the fresh and the F.D. serum,

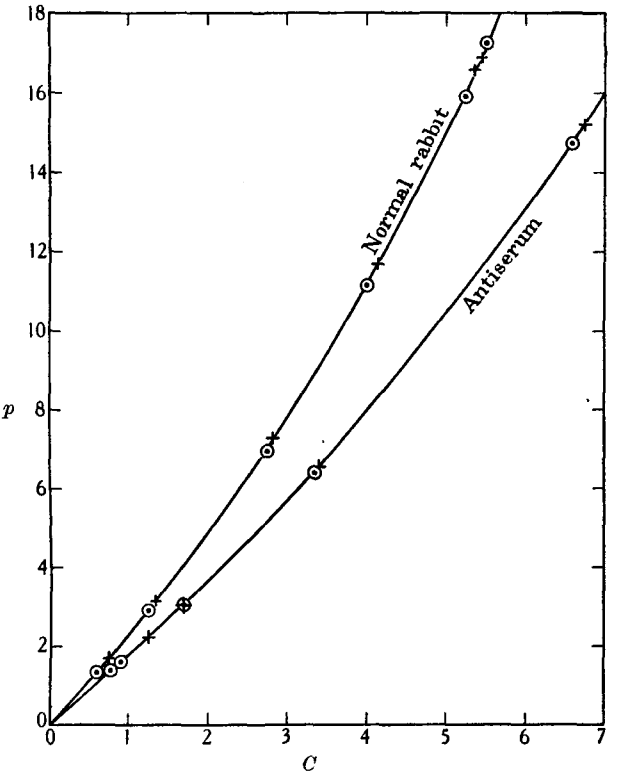

Fig. 2. p = osmotic pressure in mm. Hg. of rabbit serum at 0·5° C. C = g. dry protein per 100 ml. solution. Curve for normal serum calculated by formula $p = 2{\cdot}18C/1 - 0{\cdot}055C$: crosses, fresh serum; circles, dried serum. Curve for antiserum calculated by formula $p = 1.70C/1 - 0{\cdot}037C$: crosses, fresh serum; circles, dried serum.

namely 100,300 for M and 0·037 for K_b. The differences between the constants for normal serum and for antiserum are accounted for by the differences between the albumin globulin ratios, given in Tables 2 and 3.

The measurements of the membrane potentials, given in Tables 1 and 2, provide additional evidence in support of the view that the serum proteins are uninjured by drying, since values for the ratio E/C, the membrane potential per 1% of protein, for fresh and dried sera agree within the limits of error. It

may be noted that the values for E/C for human serum lie between values of 0·286 for horse serum albumin and 0·113 for horse serum globulin, equilibrated with the same buffer mixture, and it would seem that E/C is correlated with the albumin globulin ratio. The relationship between the membrane potentials and the electric charge of the serum proteins has been discussed by Adair & Robinson (1930*b*).

Conclusions

One of the main objects of giving a transfusion of serum, especially to a patient suffering from shock, is to assist in the maintenance of the colloid osmotic pressure of the circulating fluid. Our experiments indicate that the osmotic pressures of sera re-dissolved after drying from the frozen state are unchanged.

The osmotic pressure is mainly due to the serum albumin, because the molecular weight of this protein is smaller than that of the globulins, but, since serum may contain a number of globulin fractions, our measurements do not exclude the possibility of changes in the globulins which might affect the total colloid osmotic pressure by less than 1%.

Summary

Comparative measurements are recorded of the osmotic pressures of human and rabbit sera, both fresh and re-dissolved after drying from the frozen state (F.D. sera).

The process of drying did not alter the osmotic properties of the serum proteins.

The view that drying from the frozen state does not cause denaturation of the serum proteins is supported also by the facts that the refractive indices and the membrane potentials remained unchanged.

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