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The Osmotic Pressure of 'Defatted' Human Serum

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McCarthy showed [1938] that foetal sheep serum exerts a significantly higher osmotic pressure per gram of protein than does the maternal serum. This phenomenon could not be explained by the different albumin-globulin ratios of the two sera. Molecular weight determinations by McCarthy [1942] did not reveal any striking difference between foetal and maternal crystalline horse-serum albumins. Assuming that maternal and foetal serum albumins in the sheep are also identical, as is probable in the horse, we sought another explanation for the phenomenon observed in the foetal sheep serum. Since the lipid content of foetal blood is considerably less than that of the maternal blood [data for human: Slemmons & Stander, 1923], and since Macheboeuf [1929], Macheboeuf & Sandor [1931] have stated that the lipid constituents of serum protein exert a considerable influence on osmotic pressure, it seemed desirable to investigate further the effect of lipids on the osmotic pressure of the serum proteins. Experiments were therefore undertaken to compare the osmotic pressure of serum before and after lipid

extraction. The chief problem regarding the extraction was to find a suitable method which would remove the lipids without denaturing the serum proteins.

The method of McFarlane [1942] seemed the most convenient for our purpose, the method being also of especial interest since it is used in preparing pooled sera for transfusion. The method consists of shaking the serum with ether and freezing the mixture by cooling it to below -25° , then thawing it. The ethereal layer which separates during thawing contains a large proportion of serum lipids, and by repeated extractions approximately three-quarters of the lipids can be removed. McFarlane showed by electrophoretic measurements that the only change that takes place in the serum proteins is the diminution of the β -globulins.

METHODS

We extracted fresh pooled human serum by McFarlane's method. The serum was shaken with ether, frozen and thawed five times. Since the method employed by us may

differ slightly in detail from that of McFarlane, a summary of our procedure is desirable. The serum was frozen by surrounding the container with solid carbon dioxide ('Dricold', Imperial Chemical Industries Ltd.) and leaving it for several hours or overnight. The frozen serum was thawed at room temperature by inverting the vessel and letting the thawed liquid drip into another container. It was noticed that as the thawed serum collected, several layers separated out: usually the bottom part was crystal clear and the rest increasingly milky towards the top. After the yellow ethereal layer had been siphoned off and the extracted serum stirred up, the serum lost its opacity and became crystal clear throughout after each extraction, so that there was no need to centrifuge or filter it. While the frozen serum was thawing, a thick, creamy, yellowish material gradually separated on the surface of the ice. This remained in the first vessel; it had a low solubility in water and was not further analyzed. This material may be identical with the gelatinous deposit of low solubility in water, which McFarlane found to separate, after freezing and thawing the serum, subsequently to the last ether extraction, and which had to be filtered or centrifuged off to clear the serum. After the last ether extract had been siphoned off, the ether remaining dissolved in the serum was removed by passing nitrogen gas through the serum for approximately 1 hr. at 0°. After this, samples of the extracted serum and of the untreated serum, the latter having been kept frozen up to this time, were placed in collodion membranes and dialyzed for 48 hr. at 0° against M/15 phosphate buffer (M/30 $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ + M/30 KH_2PO_4) of pH 6.8. Ten osmometers were set up at 0° with various dilutions of each sample of serum according to the method of Adair [1925], with the same buffer for the outer fluid as in dialysis. The protein concentration of the serum in each osmometer was measured by a micro-Kjeldahl determination. Albumin N was determined on three of the more concentrated sera after precipitation of the globulins by saturation with MgSO_4 .

In calculation of protein concentration from N content, since the N contents of human-serum albumin and globulin are not known, the figures for horse-serum albumin (15.6% N) and horse-serum globulin (15.13% N) [Adair & Robinson, 1930a] were used. No allowance has been made for the presence of phospholipid N in the normal serum, nor for the loss of this after the extraction, since the corrections that might have been introduced are insignificant in comparison with the probable error in the determinations of total N. The serum was analyzed for its lipid content before and after the extraction. The distribution of lipids in the ether extract, and the N content of the latter, were also determined. Total fatty acids, fatty acids derived from neutral fats and cholesteryl esters, and total phospholipids were all determined by the methods of Bloor [1928, 1929]. Total free and ester cholesterol were estimated by the digitonin method as described by Popják [1943].

RESULTS

Table 1 shows the lipid content of the serum before and after extraction. It can be seen that practically all of the cholesterol, approximately two-thirds of the phospholipids and two-thirds of the neutral fats have been removed by the extraction. Table 2 shows the observed percentage distribution of lipids in the

Table 1. *Lipid content of serum before and after extraction*

	Before extraction (mg./100 ml.)	After extraction (mg./100 ml.)
Cholesterol: Free	44	—
Ester	148	—
Total	192	Traces
Total fatty acids	390	101
Fatty acids*	280	61
Neutral fats†	183	64
Total phospholipids	171	61
Total lipids	652	125

* Fatty acids derived from neutral fats and cholesteryl esters.

† Neutral fats = (fatty acids - ester cholesterol $\times 0.72$) $\times 1.05$.

Table 2. *Observed and theoretical percentage distribution of lipids in ether extract of serum*

	Lipid content of ether extract	
	Observed (%)	Theoretical (%)
Phospholipid	3.20	20.90
Neutral fat	4.20	22.60
Cholesterol: Free	2.00	8.35
Ester	56.00	28.05
Fatty acids of cholesteryl esters	36.00	20.10
Total	101.40	100.00

dry ether extract, together with the theoretical distribution calculated from the results of lipid analyses of the sera, assuming that all the lipids removed by the extraction were to be recovered in the ether extract. It is evident that the observed and theoretical lipid distributions differ considerably; the results appear to indicate that a large proportion of the phospholipids, neutral fats, and also of free cholesterol was lost with the material which separated on the surface of the ice during each thawing.

The N content of the ether extract was found to be 0.28 mg./100 mg. of dry extract; about 22% of this small amount of N is accounted for by the phospholipids. If the remaining fraction of N is protein-N, the amount of protein in the extract must have been negligibly small.

Total N determinations on the dialyzed normal and 'defatted' sera showed a discrepancy in total protein concentration of a magnitude difficult to reconcile with any observed volume changes due to dilution in the dialysis. The total protein concentration of the dialyzed normal serum was 6.82% and that of the dialyzed 'defatted' serum 5.49% (cf. Table 3, osmometers 1 and 11 respectively). It seems probable that N determination and lipid analysis on the material which separated out on the surface of the ice during thawing would have explained the discrepancies.

The percentage albumin and globulin contents of the dialyzed normal serum were 56.76 and 43.24% respectively; and those of the dialyzed extracted serum 61.64 and 38.36% respectively, indicating a diminution of globulin content. It is of interest to note that a loss of β -globulin following the ether extraction of the serum was reported by McFarlane on the basis of electrophoretic analysis, although no quantitative data are available in his paper.

Fig. 1. Relationship between protein concentration and osmotic pressure of normal and of 'defatted' serum. The data for the two curves have been calculated from the equation of Adair: $p = \pi_0 C / (1 - K_b C)$ [cf. Adair & Robinson, 1930b]. K_b (normal serum) = 0.0509; K_b ('defatted serum') = 0.0547. The circles and triangles represent observed values.

Fig. 1 illustrates the osmotic pressures, in mm. of Hg at 0°, of the normal and of the extracted sera; the detailed data are presented in Table 3. It would appear that the 'defatted' serum exerts slightly higher osmotic pressures for the same protein concentrations than the normal serum. The difference in higher concentrations amounts to 0.5–0.6 mm. of Hg, in lower concentrations to 0.2–0.3 mm. of Hg. Evidence on the question whether this difference is due to the removal of lipids, or to the higher percentage of albumin in the extracted serum, can be obtained by finding the value of π_0 for both sera, i.e. the limiting value of π , i.e. the ratio

$$\frac{p \text{ (osmotic pressure)}}{C \text{ (protein concentration)}}$$

Table 3. Data for osmotic pressures of normal and 'defatted' sera

Osmometer no.	Osmotic pressure (mm. Hg at 0°)	Protein concentration (g./100 ml. solution)	$1/\pi$
(a) Normal serum			
1	21.39	6.82	0.319
2	16.77	5.69	0.339
3	15.05	5.28	0.351
4	13.97	5.01	0.359
5	10.22	3.99	0.391
6	6.77	2.82	0.417
7	5.30	2.28	0.431
8	3.64	1.57	0.431
9	2.48	1.11	0.448
10	1.44	0.68	0.472
(b) 'Defatted' serum			
11	16.66	5.49	0.329
12	14.20	4.91	0.346
13	12.11	4.35	0.360
14	10.49	3.91	0.373
15	8.50	3.28	0.386
16	5.86	2.41	0.411
17	3.63	1.51	0.417
18	3.47	1.45	0.418
19	1.95	0.82	0.420
20	1.39	0.62	0.448

in an infinitely dilute solution, and then comparing the observed and theoretical values of π_0 . In the

Fig. 2. Relationship between protein concentration and $1/\pi (= C/p)$.

comparison of osmotic pressures, it should be noted that the unequal distribution of ions may affect

results for the more concentrated solutions. This complication is eliminated by calculation of the value of π_0 for infinite dilution [Adair & Robinson, 1930b]. In Fig. 2 observed values of $1/\pi$ are plotted against protein concentration. The straight lines have been drawn visually as the best fit for values of $1/\pi$ relating to protein concentrations above 2 g./100 ml. of solution, and the values of $1/\pi_0$ determined by extrapolation of these lines to the ordinate. The points relating to lower protein concentrations are neglected, as the unavoidable experimental errors at these concentrations produce significant deviations in the value of $1/\pi$. The values of $1/\pi_0$ for the normal and extracted sera thus determined by extrapolation are 0.4835 and 0.4720 respectively [cf. Adair & Robinson, 1930b], and hence π_0 for the normal serum is 2.07, and for the extracted serum 2.12. Adair & Robinson [1930b] showed that for an infinitely dilute solution of serum the osmotic pressure can be expressed by the formula

$$p = RT [10C_a]/M_a + RT [10C_g]/M_g,$$

where C_a and C_g denote the concentrations (g./100 ml. of solution) of albumin and globulin, and M_a and M_g the molecular weights of albumin and globulin. In calculating the theoretical π_0 , it is assumed that $M_a = 72,000$ and $M_g = 170,000$, values known for horse-serum albumin and globulin [Adair & Robinson, 1930b]. Then the value of theoretical π_0 is calculated by the formula

$$\pi_0 = 2.36X_a + 1.00(1 - X_a),$$

where X_a = g. of albumin/g. of total protein. For the normal serum $X_a = 0.568$ and for the extracted serum $X_a = 0.616$. Therefore the theoretical values of π_0 for the normal and extracted sera are 1.77 and 1.84 respectively. Thus there is an increase in the value of the theoretical π_0 of the same order as in the observed values, which is attributable to the

increased percentage of albumin in the extracted serum. The difference between the values of determined and theoretical π_0 for human serum proteins may be explained by the fact that constants for horse-serum proteins were used in the computation of theoretical π_0 .

Therefore we conclude from our experiment (a) that McFarlane's method for extracting lipids from sera removes also a part of the globulin complex without altering the colloid osmotic pressure properties of the remaining serum proteins, and (b) that the lipids extracted by the method do not influence the osmotic pressure of the serum proteins. The higher osmotic pressure effect of foetal serum remains still unexplained.

SUMMARY

1. Fresh pooled human serum was 'defatted' by McFarlane's method.
2. Lipid analysis of serum before and after extraction showed that practically all of the cholesterol, approximately two-thirds of the phospholipids, and two-thirds of the neutral fats, were removed by the extraction.
3. The extracted serum exerts slightly higher osmotic pressure per g. of protein than the normal serum. This difference, however, can be explained by the increased albumin-globulin ratio in the extracted serum.
4. The serum lipids, which can be removed by McFarlane's method, do not influence the osmotic pressure of the serum proteins.

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