THE DISPLACEMENT OF SERUM WATER BY THE LIPIDS OF HYPERLIPEMIC SERUM. A NEW METHOD FOR THE RAPID DETERMINATION OF SERUM WATER [1]

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In the course of studies on lactescence of serum, it was found that the insoluble lipids displaced serum water to a degree which was occasionally great enough to be of considerable practical importance. The immediate consequence of this displacement was the finding of spuriously low concentrations of water-soluble components of serum, although their concentrations were normal when repeated on sera from which the insoluble lipids had been removed by ultracentrifugation.

These observations clearly showed the need for a simple, rapid method for determination of serum water that would be applicable to sera with a high concentration of lipids. The method using Karl Fischer reagent (1) has been adapted for the rapid determination of serum water (2) but it was not known whether this would be applicable to highly lactescent sera. A new method based on freezing point depression meets the requirements stipulated and is reported herein. It is compared with the gravimetric method and the serum protein method of estimating serum water. The dilution effect of lipids on serum has been evaluated.[2]

METHODS

A. Chemical methods:

Lipids are reported as total fatty acids released by the hydrolysis of the lipid extract (4). Total proteins were determined by the Kjeldahl method when the serum was lactescent, and by the biuret method when it was clear. Chloride was determined by a micro modification of the Volhard method (5). A flame photometer was employed for the determination of sodium (6).

B. Ultracentrifugal studies:

Lactescent sera were centrifuged at 18,000 r.p.m. for one hour. The clear subnatant fluid was removed as previously described (7). The original and subnatant fluids were subjected to analysis.

C. Gravimetric estimation of serum water:

The water was evaporated and the weight loss used to calculate the serum water in grams per 100 ml. of serum (8).

D. Calculation of serum water from serum protein:

Serum water was calculated from the following formula, the derivation of which is explained elsewhere (8):

$$W_s = 98.5 - 0.745\ P_s$$

in which W_s = serum water in gm. per 100 ml. of serum, and P_s = serum protein in gm. per 100 ml.

E. Osmometric method for estimation of serum water:

Theory: The osmotic pressure of serum is determined before and after the addition of a known amount of dry sodium chloride designed approximately to double the osmolarity of serum. The only change taking place upon this addition is an increase in the molal concentration of sodium chloride. Since the osmotic pressure of a solution is determined by the molal concentration of solute particles, the exact molal concentration of the added sodium chloride can be calculated from the increase it causes in the osmotic pressure of serum. Knowing both the absolute amount and the molal concentration of the added salt, the volume of water in which it was dissolved can then be calculated.

A freezing point osmometer was used for the determination of osmotic pressure. Although osmotic pressure is not actually measured, advantage is taken of the proportionality between osmotic pressure and freezing point depression in such a way that the results are read directly as milliosmols per liter.

Reagents: 1. 0.161 molar sodium chloride solution. Dilute 9.404 gm. dry sodium chloride (reagent grade) to one liter.
2. Double distilled water.

Special equipment: A Fiske Associates freezing-point osmometer was used for the determinations of osmolarity.

Procedure: To prepare the dry sodium chloride exactly 2 ml. of the sodium chloride solution were pipetted into test tubes to be used later in the osmometer. These were brought to dryness in an oven at 98° C. and subsequently stored in a desiccator until ready for use. The addition of exactly 2 ml. of double-distilled water to the dry salt results in a 0.161 molal solution of sodium chlo-

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[2] Part of this study has been presented in abstract form (3).

TABLE I

Electrolytes of lactescent fluids before and after removal of insoluble lipids by ultracentrifugation

Patient* Age Sex	Date		Total fatty acids	Na	Cl	CO_2	Serum water	Comment
			mEq./L.	*mEq./L.*	*mEq./L.*	*mEq./L.*	*gm./100 ml.*	
B82609 18 M	8/17/53 2 PM	Original		102.5	74.5	13.2	72.7	Diabetic acidosis, mild
		Subnatant		135.0	102.0		90.2	
	9 PM	Original	526	103.0		16.3	72.5	
		Subnatant	33	134.5	100.0		91.3	
	8/18/53	Original	401	115.5	80.2	19.7	78.0	
		Subnatant	31	135.9	95.5			
39-38-38 3 M	8/21/53	Original	187	120.0	102.0	9.2	86.7	Nephrosis
		Subnatant	41	140.0	112.0			
Yan 45 M	11/11/53	Original	226	134.8			83.5	Essential hyperlipemia
		Subnatant	29	148.5			90.6	
B60586 31 F	11/17/53	Original	51	131.2			89.5	Diabetic acidosis
		Subnatant	30	142.4				
Chyle I	9/30/53	Original	102	143.0			91.9	
		Subnatant	10	149.0			94.7	
Chyle II	9/30/53	Original		132.0			83.3	
		Subnatant		149.0				
C63179 35 F	12/29/53	Original	46	135.0			89.3	Diabetic acidosis
		Subnatant	31	139.0			90.2	
B78936 50 F	1/8/54	Original	133				88.2	Essential hyperlipemia
		Subnatant	27				91.2	

* Entries are sera unless otherwise stated.

ride containing exactly 300 mOsm. per L. This may be used for the first standard required for the procedure below. (The addition of 2 ml. of water to salt prepared by evaporating 4 ml. of the saline solution results in a solution having an osmolarity of exactly 589.3 mOsm. per L. which may be used for the second standard.)

The osmometer must have been calibrated accurately initially. In addition it must be checked at the beginning and end of each run with two known standards, one having about the osmolarity of serum (300 mOsm. per L.), and the second about double this concentration. These may be prepared as indicated in the previous paragraph.

The osmotic pressure of native serum is first measured using 2 ml. of serum. Duplicate readings are taken. Readings usually check within 1 mOsm.

For the second measurement, exactly 2 ml. of the serum, which may include the thawed serum used in the first determination, are pipetted into a test tube containing the dry saline from 2 ml. of 0.161 molar sodium chloride solution. The tube is gently rotated to insure complete solution, and the osmolarity again measured. If necessary the first and second readings are corrected for any error noted in the readings of the first and second standards, respectively. The procedure is extremely simple, requiring about 8 minutes for the two sets of readings.

The serum water in ml. per 100 ml. serum is calculated from the following formula:

$$(R_2 - R_1)x = A$$

in which R_1 is the reading of the serum, R_2 is the reading of serum with added salt, x is the per cent water by volume, and A is the added sodium chloride expressed as net effective concentration in milliosmols per liter. When the dry sodium chloride is prepared as described above, $A = 289.3$.[3]

[3] If the activity coefficient of sodium chloride were 100 per cent at the concentration expected in this procedure, each millimol would produce 2 milliosmols. However, it is necessary to make a correction for the decreased ionic activity brought about by increasing concentration. At the osmolarity of serum, about 300 mOsm. per L., the activity coefficient of sodium chloride is 93.2 per cent, and at double the concentration it is 91.5 per cent, a decrease of 1.7 per cent (9). Moreover, the added salt also depresses the ionic activity of the original serum (assuming sodium chloride to be the most important determinant of osmotic pressure in serum) by the same amount, 1.7 per cent. Rather than correct the osmolarity of the original serum, a double correction is made for the added salt. The addition to serum of 161 millimols of sodium chloride is equivalent to $161 \times 2[93.2 - 2(1.7)]$, or 289.3 milliosmols.

Rather than test the method exclusively on normal sera in which little variation in serum water or lipids might be expected, miscellaneous sera from a variety of patients were selected to insure a wide range of water content and to exclude the possibility that various abnormalities of serum might interfere with the method.

RESULTS AND COMMENTS

A. Ultracentrifuge studies:

The most striking change in serum electrolytes following centrifugation occurred in the following case. A 16-year old diabetic boy was admitted in mild diabetic acidosis. For at least four months his diabetes had been poorly controlled and he had noticed a rash. He was slightly drowsy but otherwise he felt well. Examination revealed an extremely thin but healthy-looking boy who displayed the typical rash of xanthoma diabeticorum on the extensor surfaces of his arms and legs. The only other positive finding was lipemia retinalis. In view of the mildness of his illness it was startling to find that his serum sodium was only 102.5 mEq. per L., and the chloride 74.5 mEq. per L. The serum was markedly lactescent. After removal of the insoluble lipids by ultracentrifugation the electrolytes of the clear subnatant fluid were perfectly normal.

TABLE II

Serum water determined by three different methods: its relationship to concentration of proteins, lipids, and sodium in serum

Patient†	Date	Total fatty acids	Total proteins	Sodium		Osmotic pressure	Water			Specific gravity	Comment
				mEq./L. of serum	*mEq./L.* serum water		Grav.	Osm.	Protein		
		mEq./L.	*gm. %*			*mOsm./L.*	*gm. %*	*ml. %*	*gm. %*		
40-11-26	1/13/54	48.4	6.5	137	152	285	90.3	89.3	93.7	1.016	Obstructive jaundice
Serum A	2/19/54					314	92.7	92.5		1.028	Normal serum
Serum B	2/19/54					306	92.7	92.4		1.020	Normal serum
Serum C	2/19/54					315	92.4	91.5		1.019	Normal serum
Serum D	2/19/54					285	92.2	92.0		1.022	Normal serum
Ascitic fluid	1/26/54	1.5				285	98.7	99.7		1.005	
B82609*	8/17/53			102	141		72.7			0.999	Diabetic acidosis
	8/17/53	526		103	142		72.5			0.958	Lactescent, blood sugar 392
	8/18/53	401	5.8	115	147		78.0		94.2	1.002	Blood sugar 241
	8/19/53	231				297	78.5	78.0		0.976	Blood sugar 278
	8/21/53						86.3	85.6		0.951	
40-21-66	2/4/54	19	5.1	132	141	322	94.0	93.0	94.7	1.013	NPN 179 mg. %
39-38-38*	8/21/53	187	3.3	120	138		86.7		96.0	0.982	Lactescent
Chyle I*	9/30/53	102		143	156		91.9				
Chyle II*	9/30/53						83.8	84.0			
Yan*	11/11/53	226	8	135	162	287	83.5	83.8	92.5	1.010	Lactescent
B60586*	11/17/53	51		131	146		89.5			1.018	Lactescent, blood sugar 118
40-53-29	4/1/54	31	8				91.8		92.5	1.019	Renal disease
34629	3/19/54			129	139	311	92.8	92.5		1.019	Diabetic
28039	3/4/54					337	92.4	91.8		1.024	Blood sugar 341
Pooled serum	9/7/54	13.6		141	152	290	93.0	94.0		1.016	
Diluted serum	9/7/54						96.5	96.5			
A91724	9/28/54	9.8	7.2	145	157	284	92.5	93.0	93.1	1.015	Blood sugar 121
41-56-58	9/30/54	49	6.9			287	91.0		93.4	1.014	Icteric
39-35-61	9/30/54	15	8.1			297	91.3	91.8	92.4	1.017	Biliary cirrhosis
A58066	9/15/54	7.9	5.9	144	154	300	93.4	93.6	94.1	1.014	
A72286	10/8/54	11.5	6.2	141	152	287	92.9	93.2	93.9	1.011	Hyperthyroidism
41-33-53	10/8/54	30.2	5.6			320	91.7	92.4	94.3	1.001	NPN 91 mg. %, slightly lactescent
Kra	10/4/54	19.2	6.9			295	92.2	92.5	93.4		
A83882	11/12/54	13.2	7.0			295		93.0	93.3		Diabetic ketosis
41-04-06	11/10/54	8.8	2.9	132	138	267	95.8	96.8	96.3	1.006	Hypoproteinemia
	12/1/54		3.0	132	137	273	96.2	95.5	96.3	1.012	
41-69-79	10/29/54	32.9				306	90.4	91.6		1.034	Slightly lactescent
41-75-65	11/2/54		3.8	112	117	244	95.8	96.8	95.7	1.014	Hypoproteinemia
Pooled serum	11/29/54		5.9			285	93.7	93.5	94.1	1.017	
C63179	12/29/53	46.6		135	151	319	89.3			1.021	Diabetic acidosis, lactescent

* Ultracentrifugal studies shown in Table I.
† Entries are sera unless otherwise stated.

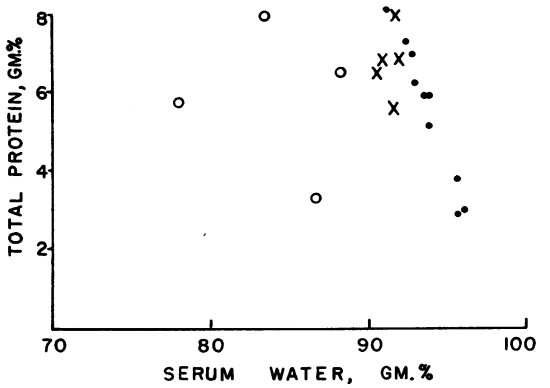

FIG. 1. RELATIONSHIP BETWEEN THE CONCENTRATION OF PROTEIN AND WATER IN SERA HAVING VARYING CONCENTRATIONS OF LIPIDS

● Serum clear, lipids normal or low. × Serum clear or slightly lactescent, lipids elevated. ○ Serum lactescent, lipids greatly elevated.

In Table I are listed the results of lipid, electrolyte, and gravimetric water determinations made on this and other lactescent sera or chyle, and on the clear subnatant fluids after centrifugation.[4] The discrepancy between the two sets of determinations was greatest in those sera having the highest lipids. While centrifugation removed most of the excess lipid, the concentration of soluble lipids in the subnatant layer was still greater than that of normal serum. This probably accounts for the finding of 91 per cent water in these subnatant layers in contrast to normal serum which contains about 93 per cent water (10).

B. Serum water determinations:

The serum water values estimated by the three methods listed above are given in Table II, together with the lipids, proteins, sodium, and specific gravity. The sodium is given both as concentration in serum and as concentration in serum water.

The serum water determined osmometrically agreed within 1 gm. per cent with that determined gravimetrically, covering a range of serum water from 78 to 99 gm. per cent. Duplicate analyses with the osmometer agreed within 0.5 ml. per cent. It should be pointed out that the gravimetric method would be expected to give results about 0.3 per cent lower than the osmometric method because the first is expressed as weight and the second as volume. The osmometric method was not influenced by hyperlipemia, icterus, or the presence of sugar or nonprotein nitrogen in excessive concentrations. Because of the congealing of lipids during freezing of extremely lactescent sera, the readings in such cases were facilitated if the serum was first diluted by an equal volume of the standard solution.

The relationship between serum water and protein concentration is shown in Figure 1. The anticipated linear relationship (8) was found when the lipids were normal, but when the fatty acids increased much above 30 mEq. per L. the relationship no longer held. The water estimated from the protein concentration then gave falsely high values (Table II) because this method does not take into account the variable contribution of lipids to total solids of serum.

The lipids may influence another commonly used relationship also based on the assumption that proteins are the only important solid of serum. It is seen in Figure 2 that the usually close relationship between serum protein and specific gravity determined gravimetrically does not hold for greatly lactescent sera.

C. Electrolyte studies:

The concentration of sodium in serum and in gravimetrically determined serum water is plotted

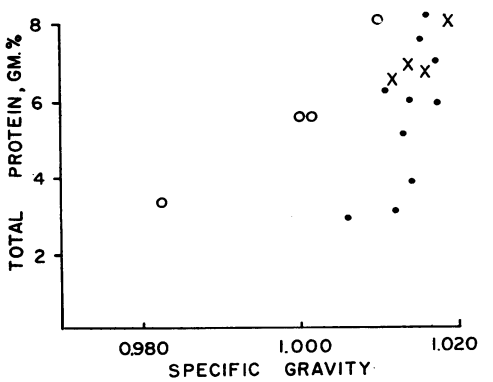

FIG. 2. RELATIONSHIP BETWEEN TOTAL PROTEINS AND SPECIFIC GRAVITY OF SERUM

● Serum clear, lipids normal or low. × Serum clear or slightly lactescent, lipids elevated. ○ Serum lactescent, lipids greatly elevated.

[4] The results of lipid analyses of these sera have been reported in detail (7).

against total fatty acids in Figure 3. If water constitutes 93 per cent of normal serum (10) and if the normal range of serum sodium is 131 to 144 mEq. per L., the normal range of sodium in serum water is 141 to 155 mEq. per L. As in the ultracentrifuge studies, the discrepancy became greater as lipids increased and some very low values of sodium in terms of serum were normal in terms of serum water (Figure 3 and Table II). One value (Yan, Table II) was normal in terms of serum but high in terms of serum water. This serves as a further indication of the displacement of serum water and its solutes by the lipids in lactescent states. A similar "volume effect" of lipids was noted by Popják and McCarthy in lipemic rabbit serum (11), although they did not encounter the extreme hyperlipemia found in some of the subjects of the present study.

An estimate of the water displaced by lipids of lactescent sera would be useful in interpreting electrolyte values in sera in which the concentration of lipids, but not of water, is known. Since protein is the only other solid which displaces water to a major extent, an estimation of this displacement was made by plotting the total fatty acids against the difference between the true serum water determined gravimetrically and that calculated from the protein. It may be seen in

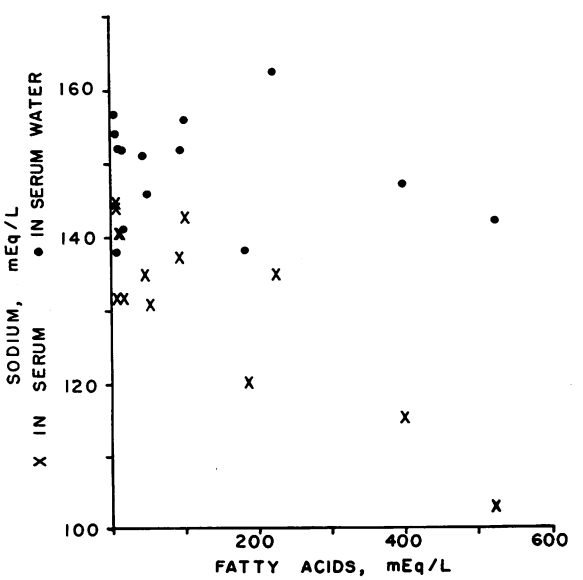

FIG. 3. RELATIONSHIP BETWEEN CONCENTRATION OF SODIUM IN SERUM AND IN SERUM WATER AND CONCENTRATION OF TOTAL SERUM FATTY ACIDS

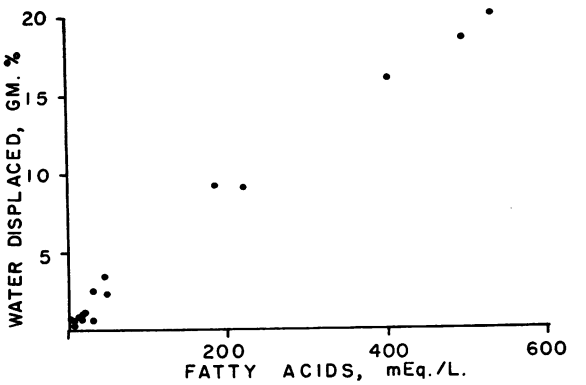

FIG. 4. WATER DISPLACED BY SERUM LIPIDS

The serum lipids are expressed as total fatty acids released by hydrolysis of the lipids. The water displaced is the difference between serum water estimated from protein concentration and that measured gravimetrically.

Figure 4 that the error in the protein method and hence the displacement of water by lipids increased linearly with increasing concentrations of fatty acid. The magnitude of the error in gm. of water per 100 ml. serum can be estimated roughly by multiplying the total fatty acids in mEq. per L. by the factor 0.04. In effect, this error represents the grams of water displaced by lipids in every 100 ml. of serum. Provided the total fatty acids are known, the degree to which water-soluble substances are displaced by lipids can be estimated. If total lipid weight is known, this estimation can of course be made more directly. While hyperlipemia sufficiently great to cause large displacement of serum water occurs only in greatly lactescent sera, the lipids in clear or only slightly lactescent sera can be high enough to displace as much as 3 gm. per cent.

This method of estimating the water displaced by serum lipids is only approximate, and likely to be of use only in retrospect. With an accurate rapid method for serum water estimation it is preferable to establish the precise concentration of electrolytes in serum water.

In a sense the osmotic pressure itself should give a measure of sodium in terms of its concentration in water, even in very lactescent sera, since the increased lipids in lactescent sera occur chiefly as a separate phase (7), and consequently can not influence the osmotic pressure. The latter conclusion was also reached by Popják and McCarthy (11). However, the true significance

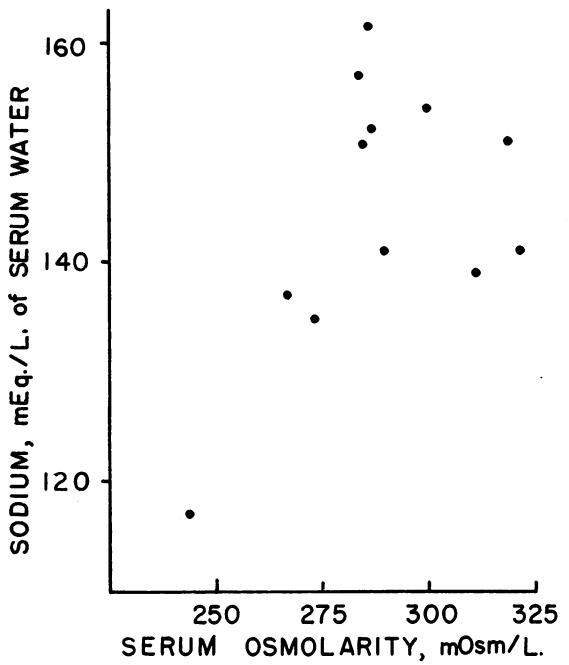

FIG. 5. RELATIONSHIP BETWEEN SERUM OSMOLARITY AND SERUM SODIUM EXPRESSED IN TERMS OF ITS CONCENTRATION IN SERUM WATER

of the osmolarity, shown in Table II, can not be ascertained without knowing the concentration of the solutes other than sodium chloride which influence it. In Figure 5, where the osmolarity is plotted against the concentration of sodium in serum water, it is obvious that the relationship is not a close one in pathological sera.

The fact that the osmometric method for determining serum water worked as well when the serum protein was 3 as it did when it was 8 gm. per cent suggests that the added sodium chloride was evenly dissolved in all the water of serum.

It is clear that for the accurate interpretation of serum sodium concentration, whether it be with regard to hypo- or hypernatremic states, the sodium must be expressed in terms of serum water. When the serum is markedly lactescent this becomes of added importance, the more so because abnormal sodium concentrations are often found in diabetic acidosis and nephrosis, diseases frequently associated with lactescence.

SUMMARY

1. Misleadingly low concentrations of electrolytes were found in the sera of patients with markedly lactescent sera. When the insoluble lipids were removed by ultracentrifugation, the concentrations of electrolytes in the clear subnatant fluids were normal or nearly so.

2. A new method for the simple, rapid determination of serum water is described. It is based on the depression of freezing point caused by adding a known amount of dry sodium chloride to serum. A freezing-point osmometer is employed for these determinations. This method checks within 1 gm. per cent with the gravimetric method.

3. The water in gm. per cent displaced by serum lipids can be roughly estimated as 0.04 time the total fatty acids in mEq. per L.

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