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Nature of Fatty Acids of Acetone Insoluble (Phospholipid) Fraction of Serum.*

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Although relatively little is known concerning the exact nature of the fatty acids present in the acetone insoluble (phospholipid) fraction of the serum lipids, practically all methods used for their determination consider these fatty acids to have a constant structure. Whether the method is based on oxidative, titrimetric or gasometric analysis or is dependent upon the determination of the lipid phosphorus, nitrogen, or choline, in the majority of the procedures it is assumed that the fatty acids in the phospholipid molecule contain 18 carbon atoms.

However, Wilson and Hansen[1] using a micrograviметric technic for the determination of the fatty acids of the serum found the average molecular weight of the total fatty acids in human subjects to be in the neighborhood of 290. Channon and Collinson[2] working with enormous quantities of ox blood also found the average molecular weight of the total fatty acids of the serum to be higher than that of an 18-carbon atom fatty acid, their values varying from 290 to 311. Further proof that long chain fatty acids are present in the blood is furnished by the studies of Brown and Hansen,[3] also, Brown, Hansen, Burr and McQuarrie[4] in human subjects, and of Tängl[5] in oxen, who found that arachidonic acid, a 20 carbon atom fatty acid, is present in appreciable quantities in the blood serum.

In an attempt to follow up a previous finding of a low degree of unsaturation of the total fatty acids of the serum in infants with eczema, the author[6] experienced difficulty in finding a satisfactory

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1 Wilson, Wm. R., and Hansen, Arild E., *J. Biol. Chem.*, 1935-6, **112**, 457.

2 Channon, H. J., and Collinson, G. A., *Biochem. J.*, 1929, **23**, 663.

3 Brown, Wm. R., and Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 113.

4 Brown, Wm. R., Hansen, Arild E., Burr, G. O., and McQuarrie, I., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 281; *J. Nutrition*, 1938, **6**, 511.

5 Tängl, H., *Biochem. Z.*, 1930, **226**, 180.

6 Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1198; *Am. J. Dis. Child.*, 1937, **53**, 933.

method for the determination of the phospholipid fatty acids of the serum. It was decided that the same fundamental principles used in the method of Wilson and Hansen[1] for determining the characteristics of the total fatty acids could be applied to the determination of the fatty acids in the phospholipid fraction. For this purpose the acetone insoluble fraction of the serum lipids was separated by precipitating with magnesium chloride according to Bloor's method.[7] The unsaponifiable portion of the phospholipid fraction was removed when the soaps were still dissolved in alcohol. After the fatty acids were liberated they were analyzed in essentially the same manner as described by Wilson and Hansen.[1] Although considerable difficulty was at first experienced in standardizing the method, subsequent findings have been consistent to the point where publication seems warranted. The details of the method will be described together with the findings in certain pathological conditions in a later publication. The data from 18 determinations on 14 essentially normal infants and children are presented in Table I. All specimens were obtained after a fast of 14 hours.

The acetone insoluble (phospholipid) fatty acids were found to comprise from 21 to 38% (average, 31%) of the total fatty acids of the serum when determined by this procedure. In each instance, excepting case 1, the iodine value of the phospholipid fatty acid was

TABLE I.

Values for the Acetone Insoluble (Phospholipid) Fatty Acids and Total Fatty Acids of the Serum with Their Respective Molecular Weights and Iodine Numbers as Determined on 14 Essentially Normal Infants and Children.

Case No.	Acetone Insoluble (Phospholipid) Fatty Acids			Total Fatty Acids		
	Mg%	M.W.	I.N.	Mg%	M.W.	I.N.
1	88	299	97	261	280	97
2	112	321	109	384	300	101
3	96	302	96	331	290	—
4	64	290	—	199	284	—
5	159	300	129	411	287	120
6	58	301	—	277	288	107
7	108	293	—	281	282	—
8	136	299	—	470	293	99
9	123	299	117	388	284	106
10	108	318	113	283	289	109
10	81	316	125	220	298	120
10	106	297	133	279	288	127
11	137	301	109	406	292	101
11	102	309	120	306	288	—
12	136	302	103	656	280	—
13	106	297	105	361	288	—
13	68	306	109	283	284	—
14	150	303	114	506	287	94

[7] Bloor, W. R., *J. Biol. Chem.*, 1929, **82**, 273.

higher than that of the corresponding total fatty acid. Of particular significance were the findings as regards the molecular weights of the phospholipid fatty acids, which were consistently higher than those of the total fatty acids. That the high molecular weights in the acetone insoluble fraction are not due to the presence of great quantities of arachidonic acid is indicated by the relatively low iodine numbers as compared to the great degree of unsaturation of arachidonic acid. If we can assume that the fatty acids in solution can be completely titrated and are stable, neither breaking down into shorter chains nor combining to form still longer chains, then it is necessary for us to look for other possible explanations for the high molecular weights encountered. The presence of such a substance as lignoceric acid, a 24 carbon atom saturated fatty acid, which is found in sphingomyelin extracted from certain tissues of the body, is a possibility worthy of consideration to account for the high molecular weights found in the fatty acids of the phospholipid fraction of the serum. The existence of these long chain fatty acids in the phospholipid fraction indicates the necessity of the recalculation of the factors employed for their determinaion in that these fatty acids are assumed to consist on the average of only 18 carbon atoms in most methods used.

Summary. This microgravimetric technic for the study of serum lipids in 14 normal infants and children disclosed that the acetone insoluble (phospholipid) fatty acids comprised about one-third of the total fatty acids. These fatty acids were more unsaturated and also had higher molecular weights than did the total fatty acids. Most methods used for the estimation of fatty acids of phospholipid fraction assume the presence of 18 carbon atoms. Our finding of high molecular weights indicates the necessity of revision of the calculations used in their determination.