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Comparison of Macro- and Micrograviметric Technic for Lipid Analysis of Serum.*

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Wilson and Hansen[1] using a micrograviметric technic for the determination of lipids found the average molecular weight of the serum fatty acids to be higher than that usually assumed. Later, it was observed that the fatty acids in the phospholipid (acetone insoluble) fraction had even higher average molecular weights.[2] In an attempt to determine whether these high values might be due to the small amount of material employed, a study was carried out wherein the results obtained by the use of this micrograviметric technic could be compared with those obtained by the use of similar procedures but with larger quantities of material. In addition, comparison was made of the iodine numbers when both small and large quantities of lipid material were used.

Blood was obtained from a 4-year-old child with a known lipemia (nephrosis). The lipids were extracted from 20 cc of serum with 400 cc of the usual Bloor mixture (alcohol-ether—3:1). After evaporation to dryness, the lipids were reëxtracted with petroleum ether and brought to a volume of 250 cc. Aliquots of 5 cc (containing 0.4 cc serum) were used for the microdetermination;[1] 100 cc aliquots (containing 8.0 cc serum) were used for the macro, the same steps being followed as in the micro method. The iodine absorption was estimated by Yasuda's micro technic[3] for the small sample and by the Hanus method for the large sample. The results obtained are presented in Table I.

Rather remarkable is the fact that the results obtained by both methods for the amount of saponifiable material recovered and for the molecular weights agree so closely. As seen in Table I the average of the total fatty acids recovered by the micro method was 1666 and by the macro method 1660 mg per 100 cc serum. The titration values for the serum fatty acids, and consequently the calculated values for the average molecular weights, were likewise

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

2 Hansen, Arild E., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 376.

3 Yasuda, M., *J. Biol. Chem.*, 1931-2, **94**, 401.

TABLE I.
Values of Total Fatty Acids (Saponifiable Fraction) of Serum Lipids as Obtained by Macro- and Microgravimetric Technics.

	Micro method	Macro method
Aliquots	0.40 cc serum	8.0 cc serum
Wt. of Sap. Fraction	6.64 mg	132.9 mg
	6.69	132.7
	6.66	—
	1659.3 mg/100 cc	1661.2 mg/100 cc
	1673.6	1658.8
	1664.0	—
Titration Value	2.28 cc .01N NaOH	4.55 cc .1N NaOH
	2.28	4.55
	2.28	—
M.W. (calc.)	291.4	292.1
	293.4	291.7
	292.1	—
I.N.	99.5*	104.9†
	99.8	105.6
	101.1	—

*Yasuda technic.
†Hanus method.

practically uniform by both methods. As regards the iodine numbers of the fatty acids, we found that the smaller quantity gave lower values than the larger quantity, the average iodine number being 100.1 by the Yasuda micro method and 105.2 by the Hanus method.

Because the iodine values obtained by the use of the pyridine sulfate dibromide reagent, which is employed in the Yasuda method, tend to be lower than those obtained by certain other methods and lower than those which would be theoretically expected,[3] the question arose as to whether more accurate results might be obtained by dilution of the Hanus reagent for use with micro quantities of fat. For this reason, determinations of iodine numbers of known solutions (mixtures of palmitic acid and linolic acid)† were made using: Yasuda's technic, the Hanus method, and the Hanus method with reagents diluted for adaptation to a micro technic. Representative data from the 10 experiments performed are shown in Table II. These reveal that the iodine numbers obtained by the usual Hanus method are practically identical with the theoretical numbers for these known substances. They also show that the modification of

† The linolic acid was obtained through the courtesy of Dr. J. P. Kass of the Department of Botany of the University of Minnesota. This fatty acid was chemically pure and was found to have an iodine number of 180 by the Wijs method and 178 by the Hanus method.

TABLE II.

Iodine Numbers of Mixtures of Palmitic Acid and Linolic Acid as Determined by the Hanus Method, Hanus Method Adapted to Micro Proportions, and the Yasuda Technic.

Mixture No.	Hanus	Micro Hanus	Yasuda	Theoretical
	87.7	82.3	79.1	
A-1	87.7	81.0	79.7	88.7
	87.6	—	79.7	
	115.6	105.3	105.3	
A-2	115.2	104.6	105.5	115.6
	115.3	105.6	105.5	
	89.0	83.5	83.2	
B-1	89.3	83.8	81.9	88.9
	—	—	83.4	
	117.7	108.8	107.0	
B-2	117.3	107.3	106.8	115.6
	—	—	106.3	

this technic to a micro method results in values very similar to those obtained by the use of the Yasuda method. Both the micro methods, however, give results from 6 to 10 points lower than those obtained by the macro method and those which would theoretically be expected.

Summary and Conclusions. Studies of the serum fatty acids from a child with a known lipemia (nephrosis) by means of the microgravimetric technic of Wilson and Hansen were made and the values thus obtained compared with those found by using 20 times the amount of fatty material. The results showed the amounts of saponifiable fraction recovered, the titration values, and the calculated molecular weights to be almost identical by both the micro and macro methods. Those obtained for the iodine numbers, however, by the use of the Yasuda micro method were consistently somewhat lower than those obtained by the use of the Hanus method and lower than would theoretically be expected. Dilution of the reagents used in the Hanus method for adaptation to a micro technic, resulted in iodine number values practically identical with those obtained by the Yasuda micro method.

From the data presented it seems warranted to conclude that the microgravimetric is a satisfactory technic for lipid analyses. Although the degree of unsaturation of the lipids tends to be somewhat lower when micro quantities are used, the pyridine sulfate dibromide reagent gives results comparable to the Hanus reagent diluted for adaptation to micro quantities.