suffer a 30% loss in this element as a result of the steroid injections. There was no significant alteration in the water content of the brain or other tissues examined. Whether or not the anti-epileptic effect of desoxycorticosterone acetate previously referred to is in any way related to the observed decrease in brain potassium cannot be determined on the basis of our present knowledge.

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Effect of Low Fat Diet on Lipids of Erythrocytes, Plasma, Serum, and Whole Blood of Dogs.*

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Although many reports have been made concerning the relative amounts of lipid in plasma and red blood corpuscles in both human beings and lower animals,[1-8] relatively little information is available concerning the qualitative characteristics of the fatty acids involved, especially as these may be affected by dietary conditions. Hansen and Wiese[9, 10] have reported that the fatty acids in the various lipid fractions of the serum were definitely less unsaturated in dogs maintained on diets extremely low in fat as compared with those found in control animals receiving lard as the source of dietary fat. The study here reported concerns the character of the fatty acids in the red blood cells in dogs and the effect of dietary fat on these fatty acids, as well as on those in the plasma, serum and whole blood.

Material and Methods. Six dogs, 3 on a low fat diet and 3 on the same diet except for the isocaloric substitution of 28% of lard for sucrose, were maintained under conditions described by Hansen and Wiese.[9] Blood was drawn from the jugular vein after a fast of 16-20 hours. The anticoagulant used was a saturated solution of ammonium oxalate, 0.024 cc per cc of blood. In the determination of the lipids of the erythrocytes, about 20 volumes of the usual 3:1 alcohol-ether mixture of Bloor were added rapidly to the red blood cells which had been laked with an equal volume of distilled water. The mixture was agitated, then heated in a boiling water bath. At times a solid gummy mass formed. This was ground with sea sand and returned to the extraction mixture. Simultaneous determinations indicated that the results were the same whether or not this mass formed. The extract was filtered through lipid-free filter paper into a volumetric flask, numerous additional extractions were made, the solution was cooled to room temperature and brought to volume. For the extraction of the lipids of whole blood, 3 cc of the oxalated blood were added slowly to the hot alcohol-

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1 Mayer, André, and Schaeffer, G., *J. de physiol. et de path. gén.*, 1913, **15**, 984.

2 Horiuchi, Y., *J. Biol. Chem.*, 1920, **44**, 345.

3 Sundstrom, E. S., and Bloor, W. R., *J. Biol. Chem.*, 1920, **45**, 153.

4 Iwatsura, R., *Arch. f. d. ges. Physiol.* (Pflüger's), 1924, **202**, 194.

5 Boyd, Eldon M., *Am. J. Dis. Child.*, 1936, **52**, 1319.

6 Bloor, W. R., *Biochemistry of the Fatty Acids and Their Compounds, the Lipids*, New York, 1943, Reinhold Publishing Corporation.

7 Erickson, B. N., Williams, H. H., Hummel, F. C., and Macy, I. G., *J. Biol. Chem.*, 1937, **118**, 15.

8 Bodansky, M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 630.

9 Hansen, Arild E., and Wiese, H. F., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 205.

10 Wiese, Hilda F., and Hansen, Arild E., *Fed. Proc.*, 1944, **3**, 97.

TABLE I.

Comparative Values for Total Fatty Acids Including Amount, Iodine Number and Average Molecular Weight Found in Erythrocytes, Plasma, Serum, and Whole Blood in 3 Dogs Reared on a Low Fat Diet and 3 Dogs on Control Diet with Lard as the Source of Fat.

Animal No.	Date	Hemato-crit	Erythrocytes			Plasma			Serum			Whole Blood		
			mg%	I.N.	M.W.	mg%	I.N.	M.W.	mg%	I.N.	M.W.	mg%	I.N.	M.W.
Animals on Low Fat Diet.														
19	10-13-42	51.6	235	100.1	—	490	95.4	—	440	94.4	—	334	99.2	—
20	10-20	47.0	295	105.8	285	447	96.5	281	402	96.3	290	324	100.0	284
19	11- 5	52.5	239	106.0	293	473	92.6	286	424	92.2	284	344	102.1	286
20	11-17	51.2	226	105.5	283	494	95.6	291	454	94.3	290	330	102.9	288
20	4- 6-43	52.3	290	98.6	297	*470	89.0	290	—	—	—	—	—	—
26	4-27	45.7	281	101.9	298	*416	91.3	291	—	—	—	—	—	—
Avg			261	103.0	291	465	93.4	288	430	94.3	288	333	101.1	286
Control Animals Receiving Lard in the Diet.														
17	10-13-42	53.8	219	117.5	—	428	110.6	—	385	110.9	—	312	113.3	—
15	10-20	52.5	242	109.8	284	505	115.4	285	461	115.3	284	358	114.9	286
17	10-29	55.7	235	111.6	286	582	106.9	286	514	107.1	287	364	112.0	279
15	11-10	57.2	247	114.4	—	640	116.9	287	559	117.0	—	390	116.1	281
17	4- 6-43	60.1	248	110.0	284	*414	105.7	285	—	—	—	—	—	—
24	4-27	47.1	213	120.5	288	*480	114.5	—	—	—	—	—	—	—
Avg			234	114.0	286	508	111.7	286	480	112.6	286	356	114.1	282

* Plasma removed after 10 minutes' centrifugation.

ether mixture. Plasma and serum extracts were prepared in the usual manner. Methods of analysis were those used by Hansen and Wiese,[9] except that alpha-naphtholphthalein was used in place of phenolphthalein in the molecular weight determinations. The results of the analyses are given in Table I.

Discussion. The majority of the authors reporting studies on lipid values for blood plasma and red blood cells[1-8] have found considerable variation in the quantity of the former, but a relative constancy in that of the latter. In our studies, the level of the blood fat in neither the erythrocytes, plasma, serum nor whole blood appeared to be greatly influenced by the amount of fat in the diet (Table I). As a matter of fact, there was more variation in this respect within each of the animal groups and in individual animals at different times than there was between the two groups. The quantity of total fatty acids per unit of erythrocytes was definitely lower than that per unit of plasma or serum. The unexpected and consistent variation between the plasma and serum fat levels was shown to be due to evaporation of water from the plasma during the relatively long process of centrifugation. The average molecular weight of the fatty acids of the cells was essentially the same as that of the other portions, indicating no difference in the length of their carbon chains.

As regards the effect of the diet on the qualitative characteristics of these fatty acids, our studies revealed a higher degree of unsaturation in all 4 of the portions of the blood of the animals receiving fat than in those on the low fat diet, but the difference was not as marked in the erythrocytes as in the serum and plasma. It should be noted that in the determination of iodine numbers of blood fats, if whole blood or cells are used for the analysis, differences in iodine numbers of the fatty acids may not appear significant. Epstein and Glick[11] were unable to note any difference in iodine numbers of blood lipids whether they used whole blood or serum, but our findings indicate that this is not true when abnormal iodine values are present. The finding that the characteristics of the fatty acids of the cells are not as greatly affected by the amount of fat in the diet as are those of the fatty acids of the serum and plasma is probably due in part to the fact that the cells contain relatively little of the cholesterol ester fatty acids, which in the serum have been shown to be the fraction most affected by

[11] Epstein, N. N., and Glick, D., *Arch. Dermat. and Syph.*, 1937, **35**, 427.

variation in the amount of fat in the diet.[9, 10] Even in the cells, however, this difference was notable, the iodine numbers in the low fat group ranging from 98-106 and those in the high fat group from 109.8-120.5, indicating that, although to not as great a degree as in the serum, the qualitative characteristics of the fatty acids of the erythrocytes can be affected by the diet.

In order to determine in which fraction of the lipids the greatest difference in the degree of unsaturation of the fatty acids existed, preliminary experiments were carried out with 4 animals (2 from each dietary group), wherein the fatty acids of the erythrocytes and plasma were separated into phospholipid, triglyceride and cholesterol ester fractions. Irrespective of the diet there was little variation noted in the degree of unsaturation of the fatty acids in the glyceride fraction of either the red cells or the plasma. On the other hand, the iodine values for the phospholipid and cholesterol ester fatty acids in both the red cells and plasma indicated that the character of these fatty acids was affected by the fat in the diet. The highest degree of unsaturation of the fatty acids in the erythrocytes was found to be in the phospholipid fraction, whereas in the plasma this was found in the cholesterol ester fraction.

Summary. From our studies of the fatty acids in the corpuscular and fluid portions of the blood, it appears that dogs on the low fat diet used in these experiments are able to synthesize sufficient fat to maintain a normal fat level in both the cells and the plasma but are unable to synthesize the highly unsaturated fatty acids necessary to maintain the degree of unsaturation normally found in both these portions. This confirms the previous conclusion of Hansen and Wiese[9, 10] from a study of the serum lipids.