Plasma Lipids in Fat Deficiency.* (21128)

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Essential fatty acid deficiency has been associated by different authors either with decreased lipid transport(1,2) or with increased lipid oxidation(3,4). In either case, the alteration should be reflected in the plasma lipids, a study of which should increase our knowledge of the fat deficiency disease. Surprisingly little information is available on the distribution of the plasma lipids in this disease. In most studies, the main subject of interest has been the changes occurring in the polyunsaturated fatty acids of the lipids. Some work has been done on the effect of low-fat diets fed for a limited time as distinct from fat-deficiency. Mellinkoff, Machella, and Reinhold(5) found that a low fat diet for 15 days was accompanied by a fall in serum cholesterol in 13 of 14 patients with lesions of the gastrointestinal tract. Recently, Wollaeger, Lundberg, Chipault, and Mason(6), in a study of 2 normal individuals, found that the total plasma fatty acids and cholesterol decreased somewhat during a period on a lipid-free diet. However, Brown, Hansen, McQuarrie, and Burr(7) found no change in the serum cholesterol or total fatty acids in one subject subsisting for 6 months on a diet extremely low in fat. Perhaps the only experiment in which the complete study of the effect of fat deficiency on the blood lipids was attempted is that of Hansen and Wiese(8). These authors studied at 3 months the serum lipids of dogs reared on a low fat diet from weaning. The results show slightly lowered total fatty acids, total cholesterol, and cholesterol ester (by difference) as compared with controls raised on the same diet plus lard.

Since a method recently developed in this laboratory makes possible the chromatographic separation of the plasma lipids(9), it was of interest to study their distribution in rats which had been on a fat-free diet from weaning and which showed definite signs of essential fatty acid deficiency. The results, which are described below, are compared with data secured similarly from fasted rats and from rats on a standard diet.

Experimental. Normal rats and rats which had been on a fat-free diet from weaning and had reached a growth plateau† were exsanguinated under ether anesthesia.‡ Extraction was carried out on plasma from individual animals or on pooled samples from several animals as described below. A total of 26 normal and 16 fat-deficient male Wistar rats was used in the experiment. Eighteen of the rats on the normal diet (Rockland Ray diet) were fasted 12-20 hours before being sacrificed. The conditions of extraction of the plasma lipids and the details of the chromatographic separations were as previously described(9) with 2 modifications.§

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† Diet and conditions of establishing the state of fat deficiency described previously(10). Rats in this experiment had been on fat-free diet for 22 weeks when sacrificed. Average weight 236 g as compared with 370 g for controls on a Rockland diet.

‡ Note that upon centrifugation of blood of fat-deficient animals, the red cells hemolyzed to such an extent that the plasma, which in the normal animals was clear with a light yellow tinge, was deep red and almost opaque.

§ Fifteen column volumes of 1% ether in petroleum ether sufficed to elute the sterol esters. The mixture used to elute the triglycerides was altered to 3% ether in petroleum ether for following reason: Fillerup and Mead(9) were in error in stating that fatty acids followed cholesterol into the eluate. Fatty acids freed of the products of autoxidation and other impurities have been found to follow directly after the triglyceride fraction. Adequate separation was not possible, when 4% ether in petroleum ether was used as the eluant. However, by using 3% ether mixture in a few experiments, separations of palmitic and oleic acids from triolein were accomplished with recoveries of each component amounting

TABLE I. Plasma Lipid Composition of Normal, Fat-Deficient and Fasted Rats.

No. of rats and condition	Total lipid, mg %	Composition of lipid (upper figure—mg % range; lower figure—mean % of total ± stand. dev.)			
		Sterol ester	Triglyceride	Free sterol	Phospholipid
Normal	355	60–95	57–157	46–74	61–157
2, 2, 2, 1, 1	280–512	22.3 ± 3.5	31.9 ± 3.8	15.5 ± 2.7	24.3 ± 3.8
Fat-deficient	227	49–75	34– 57	14–40	62–101
5, 5, 6	176–275	29.0 ± 1.4	20.9 ± 5.8	9.9 ± 2.5	36.9 ± .9
Fasted	330	65–94	15– 43	21–51	123–199
6, 6, 6	245–463	26.2 ± 4.2	13.1 ± 1.7	11.4 ± 1.8	46.3 ± 2.1

Results. The composition of the plasma lipids of normal fed and fasted rats is compared with that of fat-deficient rats in Table I. It is apparent that despite considerable variability in the values, the total plasma lipid of fat-deficient rats is lower than that of normal fed or fasted animals. This result is in essential agreement with those of Hansen and Wiese(8).

However, when the values for the individual lipids are compared, it can be seen that this decrease is a result of decreases in the sterol ester, sterol and triglyceride fractions, whereas the phospholipid fraction remains normal. When the values for the fat-deficient animals are tabulated as percentages of the total plasma lipid, it is of interest to note that with one exception they are not significantly different (at the 0.95 level) from similar values calculated for normal fed or fasted animals. In other words, in fat deficiency, the several classes of plasma lipids have been lowered to about the same extent with the exception of the phospholipid fraction, which seems to be relatively increased. It is possible that these data demonstrate that in the fat deficiency state, the rate of fat metabolism remains constant, since it has been shown by Entenman *et al.*(11) that the plasma phospholipids are formed and utilized in the liver and that conversion to phospholipid is probably preliminary to oxidation of fatty acids in this organ. This may seem a surprising conclusion in view of the fact that these animals are on a fat-free diet unless it be recalled that animals in such a condition may actually deposit increased amounts of fat(12). We may also compare these results with those from the fasted animals, which are metabolizing their depot fats and which show an increased percentage of phospholipid, in agreement with previously published results(13-15).

to about 90%. No free fatty acid has been found in plasma lipids which have been treated with due care during extraction and subsequent handling.

Summary. The composition of the plasma lipids of fat deficient rats had been compared with that of normal fed and fasted animals using a recently developed chromatographic method. Total plasma lipid was about 64% of normal but the percent distribution of the various classes at this lower level was not significantly different with the exception of phospholipid which was relatively higher.

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1. Schulitz, P. H., *Pharmazie,* 1952, v7, 9.

2. Alfin-Slater, R. B., Aftergood, L., Wells, A. F., and Deuel, H. J., Jr., *Fed. Proc.,* 1954, v13, 174.

3. Burr, G. O., and Beber, A. J., *J. Nutrition,* 1937, v14, 553.

4. Mead, J. F., Slaton, W. H., Jr., and Decker, A. B., Univ. Calif., AEP Rep. UCLA-283.

5. Mellinkoff, S. M., Machella, T. E., and Reinhold, J. G., *Am. J. Med. Sci.,* 1950, v220, 203.

6. Wollaeger, E. E., Lundberg, W. O., Chipault, J. R., and Mason, H. L., *Gastroenterol.,* 1953, v24, 422.

7. Brown, W. R., Hansen, A. E., McQuarrie, I., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1937, v36, 281.

8. Hansen, A. E., and Wiese, H. F., *ibid.,* 1943, v52, 205.

9. Fillerup, D. L., and Mead, J. F., *ibid.,* 1953, v83, 574.

10. Decker, A. B., Fillerup, D. L., and Mead, J. F., *J. Nutrition,* 1950, v41, 507.

11. Entenman, C., Chaikoff, I. L., and Zilversmit,

D. B., *J. Biol. Chem.*, 1946, v166, 15.
12. Smedley-MacLean, I., and Nunn, L. C. A., *Biochem. J.*, 1940, v34, 884.
13. MacLachlan, P. L., PROC. SOC. EXP. BIOL. AND MED., 1941, v48, 411.
14. Hodge, H. C., MacLachlan, P. L., Bloor, W. R., Welch, E. A., Kornberg, S. L., and Falkenheim, M., *J. Biol. Chem.*, 1947, v169, 707.
15. Petersen, V. P., *Acta Med. Scand.*, 1952, v143, 249.

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Physiological Action of 19-Norprogesterone in the Guinea Pig. (21129)

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Progesterone is the most potent antifibromatogen(1,2). However, there is no concomitant increase of the antifibromatogenic potency of testosterone when its progestational activity is increased by substitutions at C_{17} as with ethinyl-testosterone(3,4). Likewise, this progestational compound has no concomitant faculty to antagonize the hysterotrophic(3) or luteinizing action of estrogen (5).

It was of especial interest to examine whether the 3 mentioned actions (antifibromatogenic, antihysterotrophic, luteinizing) will increase with the progestational potency of derivatives of progesterone such as Δ^{11}-dehydroprogesterone(6) and 19-norprogesterone(7). The first of these compounds is 3 times, the second one 4 to 8 times as progestational as progesterone(6,8).

In a group of 30 animals we have found that Δ^{11}-dehydroprogesterone (Δ^{11}-dehydro-P) shares with progesterone (P) its antifibromatogenic and antihysterotrophic activity(9). But it is not certain whether antifibromatogenic potency of Δ^{11}-dehydro-P is greater than that of P; individual variations of the fibrous tumoral reaction in similar experiments and the error in calculation of quantities absorbed from subcutaneously implanted pellets especially when working with small quantities are considerable(10,11). The antihysterotrophic action of Δ^{11}-dehydro-P was certainly not greater than that of P. As we shall see the same result was obtained in new experiments. So far, we have not made a comparative study of the antiluteinizing potency of this compound.

Experiments. A group of castrated female guinea pigs were implanted subcutaneously with pellets of estradiol and Δ^{11}-dehydro-P, and another group with pellets of estradiol and 19-nor-P. In some experiments the pellets contained 40% of the specific steroid mixed with cholesterol, in others only 20%. This procedure, formerly used in experiments with P, assures absorption of very small amounts of the specific steroid. Classification of the fibrotumoral reaction was done as described previously(12).

Comparative results with P, Δ^{11}-dehydro-P, and 19-nor-P are given in Table I. This table shows that with Δ^{11}-dehydro-P the estrogen-induced fibrous effect is counteracted as with P. But, as found previously, with Δ^{11}-dehydro-P the antifibromatogenic activity was not strikingly superior to that of P (compare IV to II, and V to III); the difference was probably in the limit of the error (variation of the fibrous tumoral reaction; calculation of quantities absorbed). As to the antihysterotrophic activity Δ^{11}-dehydro-P was not superior to P.

With 19-nor-P both the antifibromatogenic and antihysterotrophic activity were considerably greater than those of P (compare VII to III).

The antiluteinizing activity of P can be expressed in the quantity necessary to counteract the luteinizing action of estrogen in intrasplenic grafts(5). With 16 to 23 μg per day absorbed from a 40% progesterone-cholesterol pellet frequency of corpora lutea is reduced

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