17302. Relation of Fat Deficiency Symptoms to the Polyunsaturated Fatty Acid Content of the Tissues of the Mature Rat.*

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In a previous study[1] on the appearance of essential fatty acid deficiency symptoms in the mature rat, only the external symptoms were considered. As one of the possible explanations for the ultimate disappearance of the scaly paws, it was suggested that synthesis of at least a limited amount of an essential fatty acid might take place within the animal itself. In order to check this possibility it was decided to examine the tissues for the amount of polyunsaturated fatty acids present at the various stages of development and disappearance of the symptoms.

Experimental and results. The kind of rats employed and the general procedures used in this experiment were similar to those described previously.[1] Fat-free diet A[1] was fed throughout the entire experiment. The fat soluble vitamins were suspended in propylene glycol and were fed once weekly. At the end of the depletion period, during which the intake of food was limited to approximately 5 g per rat per day, 9 out of 39 rats were killed and the entire body fat was extracted for analysis, with special care to prevent any change in the fat, *e.g.*, a low temperature (40-50°C) was maintained in the vacuum oven during the drying of tissues, and a nitrogen ebullition tube was used in all concentration operations.

The thirty remaining depleted rats were given food *ad libitum*, and 35 days later, when all showed symptoms of fatty acid deficiency, 10 rats were killed for analysis. After 71 days on the *ad libitum* regimen, at which time most of the symptoms had largely, though not completely, disappeared, 10 more rats were killed and their fat analyzed as representative of the third stage. Two groups of 5 rats each were killed on the 88th day (stage 3A); those in one group had been supplemented with 2 drops (approximately 100 mg) of ethyl linoleate daily during the last 23 days.

The polyunsaturated constituents of the body lipids were determined by the modified method of Brice *et al.*,[2,3] using alkali isomerization followed by ultraviolet absorption measurements with the Beckman spectrophotometer. It should be mentioned that Brice and coworkers estimated that the probable error of the results is roughly ± 10% of the quantity actually present when that quantity is about 10% of the total fat; and about ± 25% when unsaturated acid measured constitutes only 1% of the fat analyzed. The results of the analysis which represent the average of 4 separate determinations are presented in Table I.

On the basis of these data, certain trends may be observed. At the end of the depletion period (stage 1) the total amount of fat in the body is low, but the percentages of linoleic and arachidonic acid in the fat are very high; the amount of arachidonic acid per rat is also high.

In the second stage, when the deficiency symptoms appeared, a sharp reduction in the amount of linoleic acid and arachidonic acid was noted. The linolenic, which was not found in the first stage, appeared in a small amount at this stage. It is interesting to note that with the small reduction of arachidonic acid there was a steady increase in the

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1 Barki, V. H., Nath, H., Hart, E. B., and Elvehjem, C. A., Proc. Soc. Exp. Biol. and Med., 1947, **66**, 474.

2 Brice, B. A., and Swain, M. L., *J. Opt. Soc. Am.*, 1945, **35**, 532.

3 Brice, B. A., Swain, M. L., Schaeffer, B. B., and Ault, W. C., *Oil and Soap*, 1945, **22**, 219.

TABLE I.
Unsaturated Fatty Acids in the Bodies of Rats on Fat Deficient Diet and of Those Supplemented with Ethyl Linoleate.

		No. of rats	Avg wt., g	Avg lipid content/ rat, g	Linoleic*		Linolenic*		Arachidonic*	
					%	g/rat	%	g/rat	%	g/rat
Stage 1	Depletion	9	131	3.17	2.18	.068	0	0	5.76	.183
Stage 2	Appearance of symptoms	10	215	21.1	.38	.080	.37	.078	.686	.145
Stage 3	Recovery	10	276	38.4	.58	.222	.36	.138	.332	.127
Stage 3A	17 days later	5	282	40.4	.65	.252	.44	.178	.299	.121
Stage 3A +	linoleate	5	323	66.6	1.71	1.139	.21	.140	.388	.258

* As calculated by the method of Brice *et al.*[2,3]

amounts of linoleic and linolenic acids as the rats were maintained on the fat free diet beyond their recovery stage.

The supplementation of ethyl linoleate resulted in considerable increase in total fat and the levels of both linoleate and arachidonate present in the fat. At the same time the level of linoleneate declined.

Discussion. It is interesting that the appearance of the essential fatty acid deficiency symptoms coincides with a sharp reduction in the levels of linoleic and arachidonic acids. The accuracy of the zero value for the linolenic acid at the first stage is doubtful. The dependent nature of the simultaneous analysis for the three acids and the high level of arachidonic could have obscured the small amounts of linolenic which might have been present. The appearance of some linolenic in the second stage, when the levels of both arachidonic and linoleic declined sharply, seems to support this doubt.

The increase in the total amounts of linoleic and linolenic at the period when the deficiency symptoms had largely disappeared (3rd stage) is further indicated in the unsupplemented group of 5 rats which were killed 17 days later. Considering the fact that the diet of the rats was devoid of these acids, these findings support the explanation suggested in the previous paper,[1] namely that at least a limited amount of essential fatty acids is synthesized in the body of the mature rat. At these levels the increase of linoleic and linolenic acids only is observed. It is obvious, however, from the arachidonic acid level of the group supplemented with linoleate, that the level of arachidonate also increases in the body at least when the linoleate is administered orally or when it reaches a certain level in the tissues.

Sinclair[4,5] concluded that the essential fatty acids including arachidonic are probably synthesized by the rat on a fat free diet, but not to a sufficient extent to fulfill the requirements. Investigators at the Massachusetts Institute of Technology[6] fed rats a synthetic diet containing tri-di-deuterioiso-olein for 42 days. They analyzed the fatty acids from the carcasses and found that the arachidonic acid increased as the experiment continued, suggesting that arachidonic was synthesized by the rat. This was further supported by their findings that deuterium was present in the arachidonic acid molecule, for it indicated that the arachidonic was synthesized from the deuteriumated iso-oleic acid in the diet.

In the present study the supplemental feeding of linoleate, which raised the levels and amounts of linoleate and arachidonate in the bodies, appears to have lowered the levels of linoleneate. These findings support similar observations made by Rieckehoff *et al.*[7] They found that the trienoic acid content of heart fatty acids decreased upon supplementation with corn oil. They also found that when the fat deficient diet was supplemented with corn oil a considerable deposition of arachidonic or tetraenoic acid took place, indicating

4 Sinclair, R. G., *J. Biol. Chem.*, 1932, **96**, 103.

5 Sinclair, R. G., *J. Biol. Chem.*, 1936, **114**, xciv.

6 Anonymous, Report of Progress in Research III, Nutr. Biochem. Lab., Department of Food Technology, Massachusetts Institute of Technology, 1948, pp. 7-8.

7 Rieckehoff, I. G., Holman, R. T., and Burr, G. O., *Arch. Biochem.*, 1949, **20**, 331.

to them that this acid could be synthesized from linoleate. Nunn and Smedley-Maclean[8] also observed that supplementation with linoleic or with linolenic acids resulted in the production of arachidonate.

While this work was in progress, Rieckehoff, Holman and Burr[7] reported on the effect of dietary fat on polyethenoid fatty acids of rat tissues. One of their conclusions was that the deposition of polyunsaturated fatty acids takes place primarily in the phospholipid fraction with very little change in the neutral fat. They also found that the effect of the diet on the occurrence of the unsaturated fatty acids is considerably greater in some organs than in others, and that the polyunsaturated acids are particularly low in the skin and depot fat. Smedley-Maclean and Nunn[9] also found small amounts of arachidonate in the phospholipids of liver and muscle of fat deficient rats, but none in their neutral fat.

On the basis of these observations it becomes evident that the phospholipid fraction from the various organs (especially the heart, liver, brain and kidney) should be isolated and analyzed separately for better evaluation of the changes which take place in the essential fatty acid content of the rat. Otherwise, the small amounts of these acids are diluted in the relatively large quantities of fat extracted from the entire carcass, and the changes become less evident.

Summary. 1. Caloric restriction, causing severe emaciation, followed by *ad libitum* feeding on the same fat-deficient diet, precipitated symptoms of fat-deficiency in mature rats. When the rats, after depletion, were maintained on the fat-free diet *ad libitum* for sufficiently long periods, spontaneous recovery was observed.

2. The tissues of these rats were analyzed for linoleic, linolenic and arachidonic acids by the spectrophotometric method. The concentrations of linoleic and arachidonic acids in the body fat were found to vary with the appearance of the symptoms; it was high at the end of the depletion period, and low at the stage when fat deficiency symptoms were present. The higher levels of linoleic and linolenic acids at the time of recovery, after a long period on the fat-free diet, are considered as further evidence for the synthesis of essential fatty acids in the mature rat.

3. Supplementation of ethyl linoleate increased the level of arachidonate in the body fat.

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[8] Nunn, L. C. A., and Smedley-Maclean, I., *Biochem. J.*, 1938, **32**, 2178.

[9] Smedley-Maclean, I., and Nunn, L. C. A., *Biochem. J.*, 1940, **34**, 884.

17303. The Blocking Effect of Nembutal on the Ovulatory Discharge of Gonadotrophin in the Cyclic Rat.

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In a preceding investigation[1] it was indicated that in 4-day cyclic rats of the Vanderbilt strain, under our colony conditions, neurohumoral stimulation of the hypophysis occurs with great uniformity on the day of proestrus between 2 P.M. and 4 P.M., inciting ovulatory discharge of luteinizing hormone. Administration of intravenous dibenamine (N,N-dibenzyl-β-chloroethyl amine) or subcutaneous atropine at 2 P.M. or earlier characteristically blocks ovulation. Injection of either agent at 4 P.M. or later, however, rarely interferes. We have now found essentially identical time relationships with Nembutal (pentobarbital sodium).

Two dose levels of the barbiturate were

[1] Everett, J. W., Sawyer, C. H., and Markee, J. E., *Endocrinology*, 1949, **44**, 234.