Production and Counteraction of a Fatty Acid Deficiency in the Guinea Pig. (21208)

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Most mammals are known to require a dietary source of unsaturated essential fatty acids. With fat, as such, also supplied, growth in some animals may be somewhat accelerated. In the guinea pig, however, neither a requirement for unsaturated fatty acids nor for fat has been previously demonstrated. The present investigation is concerned with a study of these problems.

Methods. Three experiments have been conducted, each with 16 animals of the Hartley strain, 2 to 4 days of age, with an average weight per group of 101 or 102 g and with an equal number of males and females. Half of the animals were placed on the basal semi-synthetic diet previously described(1) containing 30% vitamin-free casein, 4 types of carbohydrates, 6% salts, 7.3% fat (corn oil), and liberal amounts of the known vitamins. The other half received the diet with fat omitted and cerelose substituted. The fat-soluble vitamins were added in alcohol solutions.

Results. After subsisting on the diet 25 to 30 days, the guinea pigs on the fat-free diet began to show a retardation in growth, with the differences in weight as compared to the control animals becoming greater with increasing length of time on the experiment. Table I shows the results of the 3 experiments.

After a period of 6 to 8 weeks (room atmosphere had low average humidity) on the fat-free diet many of the guinea pigs developed dry scaly dermatitis on the ears and feet which increased in severity with time. All showed severe drying of the inside of the ears. Marked scaliness was later observed over the entire body and superficial ulcers developed on some of the animals, apparently as a result of scratching. Sensitivity to touch and restlessness were notable. Some of the animals showed loss of hair from the abdomen and inner surface of the legs. The feet of some of the animals appeared to be swollen and bluish, and in some cases a cyanotic condition developed. Marked daily fluctuations in weight of the deficient animals were frequently observed. It seems unlikely that pyridoxine deficiency was involved in the production of the syndrome since the diet contained 16 mg pyridoxine per kg. The control animals showed none of the above symptoms.

TABLE I.
Effect of Dietary Fat (Corn Oil) on Growth.

Exp.	Time on diet (days)	% fat in diet	Wt gain (g)	Survivors*
I	72	0	277 ± 25	7
		7.3	405 ± 26	8
II	125	0	377 ± 45	6
		7.3	573 ± 43	8
III	161	0	427 ± 39	6
		7.3	690 ± 42	6

* 8 animals started per group.

Therapeutic trials were made with one series of animals. One of the guinea pigs, in a critical state as a result of the deficiency, was placed on the complete diet containing corn oil. Within 2 days improvement was observed and eventually there was full recovery. Three of the other severely deficient animals were treated for a period of one week with daily oral doses of linoleic acid (10, 30, and 60 mg doses, respectively). The feet of the animals at the 2 higher levels of dosage lost the bluish tinge and acquired a pink color, the dermatitis disappeared, and growth was somewhat accelerated. Toward the end of the one-week period of dosage even the animals on the 10-mg level showed improvement, particularly with respect to the dermatitis. Table II summarizes the changes in weight during a 4-week period following the treatment with linoleic acid.

At the start of the treatment the deficiency of the guinea pig receiving the 30 mg supplement was somewhat more advanced than that in the animals receiving the 10 and 60 mg doses, respectively. There was no doubt, however, about the beneficial effect of linoleic

TABLE II. Effect of Linoleic Acid on Growth of Fat-Deficient Guinea Pigs.

Supplement (mg daily for 1 wk)	Wt (g) 1 week before treatment	Wt (g) Time of starting treatment	Total gain (g) Wks after starting treatment 1	2	3	4
10	462	477	3	23	31	30
30	538	534	12	18	25	40
60	486	486	14	51	69	80
0	624	655	0	0	−12	4
0	576	587	8	12	15	6

acid in all 3 of the animals. Improvement in the animal which received the 10 mg dosage appeared to have subsided by the end of the third week. Eventually deficiency symptoms reappeared in all of the supplemented animals but latest in the one treated with the highest dosage of linoleic acid.

Summary. These experiments have shown that omission of fat from the diet of the guinea pig results in a syndrome characterized by: retardation of growth, dermatitis, marked drying of the inside of the ears and, in some animals, ulcers, loss of fur, and a tendency to a swollen, somewhat cyanotic condition of the feet. All of these symptoms were corrected by the oral administration of linoleic acid. These results afford the first demonstration of the necessity for the guinea pig of a dietary source of linoleic acid or, possibly, of some other fatty acid present in corn oil.

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1. Reid, M. E., and Briggs, G. M., *J. Nutrition,* 1953, v51, 341.

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Lipemia Clearing by Hyaluronidase, Hyaluronate, and Desoxycorticosterone, and its Inhibition by Cortisone, Stress, and Nephrosis. (21209)

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The lipemia clearing action of heparin(1) has been amply confirmed. A similar action has been reported for other compounds(2,3) but has been established with certainty only for sulfated polysaccharide anticoagulants (2,4,5). The apparent specificity of the type of compound that caused lipemia clearing and the anticlearing action of protamine and antiheparin dyes have suggested to some observers that heparin has a physiologic role in the transport and metabolism of lipids(5-9).

We have reported that hyaluronidase (H) has antihypercholesterolemic action(10). It was of interest therefore to determine whether it would also clear lipemia. Since in our studies of the ground substance(11-13) partially depolymerized hyaluronic acid (PDHA) and desoxycorticosterone (DCA) had effects resembling those of H while cortisone and stress were opposite in effect, it was of further interest to ascertain whether the same relationship prevailed with respect to lipemia clearing.

*Materials and methods.** Purified hyaluronic acid (HA) and PDHA were the same as that used in our previous studies(14). The HA had 3.4% N, less than 0.01% carbohydrate by the anthrone method(15,16) and no P, S, or halogen. The bovine testicular H (6000 TRU/mg N) used in all tests had 1.9 U proteolytic activity/mg by the method of Hadidian(17). Higher potency H (28000 TRU/mg N) was used to inactivate the lipemia clearing factor (CF) *in vitro* in order to eliminate significant proteolytic activity (less than 0.005 U/test). Sodium alginate was prepared by Dr. Harvey E. Alburn and was fractionated to yield a product with a molecular weight of 13,000-20,000 to approximate that of heparin. Plasma was decanted from citrated blood (0.1 ml M/10 Na citrate/ml) centrifuged at 2000 RPM until separation oc-

* All compounds were dissolved in 0.9% NaCl except DCA which was in oil (Cortate, Schering) and cortisone which was a 0.5% suspension of crystalline acetate in 0.5% acacia.