Effects of Chorionic Gonadotropin on Sex Organs of Male Rats Deficient in Essential Fatty Acids.* (19137)

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Considerable data are available indicating that testicular function is impaired in rats deficient in the essential fatty acids. Burr and Burr(1) observed that male rats on fat-free diets were unable to sire normal young, that they did not usually copulate and that the occasional copulation that did take place was infertile. Similar findings have been reported by Evans *et al.*(2), Quackenbush *et al.*(3), and others. Histologically the testes of rats fed a fat-free diet for approximately 5 months revealed complete absence of sperm and a marked degeneration of the seminiferous epithelium(2). Testicular dysfunction as judged by failure of sperm formation or atrophy of the secondary sex glands has also been observed in rats deprived of other nutrients including thiamine, riboflavin, pyridoxine, pantothenic acid, biotin, protein and others, as well as the fat-soluble vit. A and E. It would appear that in the case of at least some of these nutrients testicular dysfunction was due to an inadequate secretion of pituitary gonadotropin (resulting either from the nutritional deficiency *per se* or the attendant reduction in caloric intake). This is indicated by the fact that injections of chorionic gonadotropin restored normal structure and function in the testes of rats deprived of thiamine (4,5), pyridoxine(5), pantothenic acid(5), or protein(5). Gonadotropin stimulation, however, failed to promote spermatogenesis in rats depleted of vit. A(6) or vit. E(7-9), although it did correct the atrophic changes in the prostate and seminal vesicles of A-depleted rats(6,10,11). In the present communication data are presented on the effects of chorionic gonadotropin on the testes and secondary sex glands of male rats maintained for a prolonged period of time on a diet deficient in essential fatty acids.

Procedure and results. The basal ration employed in the present experiment consisted of sucrose, 72%; casein,† 20%; salt mixture,‡ 4%; cellulose,§ 4%; and the following synthetic vitamins per kg of diet: Thiamine hydrochloride, 72 mg; riboflavin, 72 mg; pyridoxine hydrochloride, 27 mg; calcium pantothenate, 67 mg; nicotinic acid, 10 mg; i-inositol, 500 mg; para-aminobenzoic acid, 200 mg; folic acid, 10 mg; 2-methyl-naphthoquinone, 5 mg; d-biotin, 1 mg; and choline chloride, 1.2 g. Each rat also received twice weekly oral supplements of the following fat soluble vitamins dissolved in propylene glycol (0.1 ml): 158 U.S.P. units of vitamin A,‖ 15.8 U.S.P. units of vitamin D,‖ and 2.8 mg of

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† Vitamin Test Casein, General Biochemicals, Chagrin Falls, O.

‡ Osborne-Mendel Salt Mixture.

§ Celluflour, Chicago Dietetic Supply House, Chicago, Ill.

‖ Nopco Fish Oil Concentrate, assaying 800000 U.S.P. units of vit. A and 80000 U.S.P. units of vit. D per g.

alpha-tocopherol. Twenty-four male rats of the University of Southern California strain were selected at 21 to 23 days of age for the present experiment. Animals were kept in metal cages with raised screen bottoms to prevent access to feces and were fed the above diet *ad lib.* for a period of 12 weeks. At this time evidence of essential fatty acid deficiency was observed in all rats as judged by failure to gain weight for a period of at least 3 weeks and dermatitis of the paws and tail. Rats were then divided into 3 groups, all of which were continued on the basal ration, but which received, in addition, the following supplements administered orally by graduated syringe 4 times weekly: Group I, 175 mg methyl linoleate¶ in 0.3 cc ethyl laurate (12 rats); group II, 8.75 mg methyl linoleate¶ in 0.1 cc ethyl laurate (6 rats); and group III, 0.1 cc ethyl laurate alone (6 rats). After 8 weeks of supplementation the body weight of rats in group I averaged 260.3 g (range 193 to 339 g) and all manifestations of essential fatty acid deficiency had been ameliorated. Groups II and III average 182.7 and 176.0 g respectively (range 111 to 225 g) and dermatological symptoms characteristic of essential fatty acid deficiency were marked in all rats. The level of essential fatty acids fed to group II was extremely low. Previous investigations from this laboratory(12,13) indicate that the optimum dosage of methyl linoleate for growth under conditions similar to those employed in the present experiment is from 20 to 40 times greater than the level fed to group II. Since no significant difference was observed either in the weight or gross appearance of rats in groups II and III, and since no difference was observed in the response of these groups in the present experiment, these groups will henceforth be reported and discussed as a single (negative) group in the present report. After 8 weeks of supplementation each group was divided into 2 parts. Half the animals in each group received 7 daily subcutaneous injections of 20 International Units of chorionic gonadotropin.** The other half were similarly treated except that saline solution was employed. The supplements fed for the preceding 8 weeks were continued for the duration of the experiment. All rats were autopsied 24 hours after the last injection. Testes, prostate and seminal vesicle weights were determined; testes were fixed in Bouin's solution, and sections were prepared and stained with hematoxylin and eosin.

Findings are summarized in Table I. No significant difference in testes weight was observed in the various groups regardless of whether chorionic gonadotropin or saline solution was administered. Gonadal weight was somewhat larger for rats in group I but when expressed as per cent of total body weight comparable values were obtained for all groups. Furthermore, no significant difference was observed histologically between the testes of rats fed a diet deficient in essential fatty acids and that of animals in group I or of rats of similar weight which had been fed a natural food ration. Mature sperm were present in the tubules of all rats and no evidence of seminal epithelial degeneration was observed in any of the rats fed a diet deficient in essential fatty acids. These findings are in contrast to those of Evans *et al.*(2), who reported complete absence of sperm in the testes of rats deficient in essential fatty acids but are consistent with the observation of Mackenzie *et al.*(14) that motile sperm were present in the epididymides of rats after 44 weeks on a low fat dietary regime. Both the prostate and seminal vesicle weights of deficient rats injected with saline were significantly less than that of comparable animals in group I. Administration of chorionic gonadotropin restored the weight of these secondary sex glands to normal values. Chorionic gonadotropin also increased the prostate and seminal vesicle weights of rats in group I, but to a smaller degree than in the deficient rats.

¶ Hormel Foundation, Austin, Minn.

** Antuitrin S, Parke, Davis & Co., Detroit, Mich. This material was diluted with saline solution to a concentration of 20 International Units per 0.1 cc.

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TABLE I. Effects of Chorionic Gonadotropin on Testes and Secondary Sex Glands of Male Rats Deficient in Essential Fatty Acids. Six animals per group.

Dietary group	Final body wt, g	Testes wt, g	Prostate wt, mg avg	Prostate wt, mg range	Seminal vesicle wt, mg avg	Seminal vesicle wt, mg range
		Saline series				
Deficient rats	198.8	2.222	165	(122-215)	510	(256-873)
Linoleic supplemented controls	268.5	2.679	249	(208-294)	1154	(979-1419)
		Chorionic series				
Deficient rats	182.8	1.843	353	(243-466)	959	(452-1903)
Linoleic supplemented controls	273.2	2.573	378	(315-428)	1390	(1090-1869)

Results of the present experiment suggest that the reduction in prostate and seminal vesicle weights of rats fed a diet deficient in essential fatty acids was due to insufficiency of pituitary gonadotropin (interstitial cell stimulating hormone) secretion. This is indicated by the fact that gonadotropin stimulation restored the weight of these organs to normal. Available data, however, do not indicate whether this insufficiency was absolute or relative and to what extent, if any, the sensitivity of the interstitial cells of the testes or the target organs of its secretion may have been altered as a consequence of essential fatty acid deficiency. Caloric restriction *per se* does not appear to be the cause of the deficient gonadotropin secretion inasmuch as the food intake of rats fed the deficient diet was not less than that of animals in group I. It is possible, however, that the reduced efficiency of food utilization in the deficient rats resulted in an inanition effect despite the apparent adequacy of calories consumed.

Summary. Rats deficient in essential fatty acids exhibited a marked reduction in prostate and seminal vesicle weight. Administration of either methyl linoleate or chorionic gonadotropin restored these organs to normal weight.

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Aureomycin and Terramycin in Human Saliva.* (19138)

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Many pathogens multiply in the oral cavity before spreading further. Antibiotics present in saliva might conceivably be effective in preventing the spread of microorganisms. However, little is known concerning the mean concentrations of antibiotics excreted in saliva of human subjects, and the changes in levels with time. Abraham *et al.*(1), Struble and Bellows(2), and Cutting *et al.*(3) detected penicillin in the stimulated saliva of medicated animals. Long(4) observed changes in the bacterial flora of the mouth and inferred from this that penicillin was excreted into human saliva. Direct measurements of such excretion have not been reported.

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