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Effect of β,β-Diethylalanine on Composition of Rat Livers. (21033)

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The effects of oral and intraperitoneal administration of β,β-diethylalanine (DEA) on growth and liver composition in rats has been studied with the view that it might be an antagonist of valine, isoleucine, or leucine. An antagonism involving one of these amino acids would be particularly advantageous for studying protein synthesis because, unlike methionine and tryosine, these amino acids are not known to be concerned with anabolic reactions aside from those occurring in the synthesis of protein molecules.

Materials and methods. Synthesis of DL-DEA. A modified malonic ester synthesis has previously been employed for the preparation of DL-DEA(1). The material for these experiments, however, was synthesized by HCl hydrolysis of the corresponding hydantoin (m.p. 182°) prepared by the general hydantoin synthesis of Bucherer *et al.*(2,3). The HCl was removed on an amberlite IR4-B ion exchange column. An overall yield of 41% was obtained. The product was identified by synthesis of the chloracetyl derivative(1) which gave the theoretical neutralization equivalent [m.p. 130°; DuVigneaud *et al.*(1) 127-128°]. On paper chromatograms the free amino acid gave a single ninhydrin positive spot. *Feeding of DL-DEA.* The control diet A contained vitamin-free casein (Labco), 10;

sucrose, 30; corn starch, 34.9; salts(4), 2.5; corn oil (Mazola), 15; cod liver oil, 3; Ruffex, 2; vitamin mix(5), 1; choline chloride, 0.1; L-methionine, 0.4; and L-glutamic acid, 1.10%. In diet B, 1.09% DL-DEA replaced 0.55% L-glutamic acid and 0.54% corn starch of the control diet. In diet C, 2.17% DL-DEA replaced 1.10% L-glutamic acid and 1.07% corn starch of the control diet. Twenty-four male white rats,* housed in individual cages, were placed on the control diet (diet A) for 4 days preceding the growth studies. After this period of adjustment to the synthetic diet, they were divided into 3 groups of 8 rats (groups A-C). Each group is designated by the letter of the diet on which it was maintained. *Ad libitum* food consumption was recorded every 2 days and body weights every 4 days. After 28 days on the experimental diets, the rats were sacrificed and the livers analyzed for lipide, water, and protein content (TCA insoluble material) as previously described(5). *Injection of DL-DEA.* In those experiments in which DEA was administered in large doses, 160-200 g rats were fasted for 12 hours prior to the first injection. Four intraperitoneal injections of 100 mg of DL-DEA in 1.0 ml of 0.9% saline were given at 2.5 hour intervals. The fasting was continued for 36 hours after the first injection, at the end of which time the rats were sacrificed and the livers analyzed as in the feeding experiments. Four female rats, 2 from each of 2 litters, received DEA injections while 4 female rats, 2 from each of the same 2 litters, served as controls. The controls were treated in a similar manner but received only saline solutions. The experiments were repeated on a similar grouping of 8 male rats. The rats were allowed fresh water throughout the experiment.

Results. Preliminary feeding experiments had indicated that DEA stimulated growth to a slight extent when substituted for corn starch in a basal diet. Diets A, B, and C were subsequently chosen so that each would contain the same amount of available nitrogen on the assumption that one-half of the DL-DEA nitrogen was utilizable. On these diets

* Holtzman Rat Co., Madison, Wis.

TABLE I. Effects of Feeding DL-β, β-Diethylalanine on Growth of Male Rats.

Group	Avg initial wt, g	Avg wt at 8 days, g	Avg wt at 28 days, g	Gain in wt/g food consumed (g) 1-8 days	9-28 days
A	74	107	176	.39	.32
B	73	84	156	.19	.32
C	71	85	154	.24	.31

the growth of groups B and C (Table I) was nearly identical for the 28-day period, but both groups showed initial growth lags when compared with group A, due at least in part to lowered food consumption. While it is possible that in both groups B and C the lower lipide content (Table II) is a reflection of the poorer growth of these groups during the first 8 days on the new diet, it seems likely that the last 20 days of parallel growth and nearly identical growth response per gram of food consumption of all groups would have overcome any such initial effect. It is, perhaps, more reasonable to suppose that DEA acts specifically to decrease the lipide content but that it has reached almost its maximum effectiveness at the lower level (diet B). The effect of feeding DEA upon the protein content (Table II) of the livers cannot be explained satisfactorily by the initial growth lag shown by groups B and C since the protein content was significantly higher only in group C on the higher level of DEA.

The injections of DL-DEA produced no significant differences in liver lipide, protein, or water content as compared with controls. The average liver lipide contents expressed as per cent of body weight for the male and female experimental and control groups ranged from 0.22 to 0.24. The average water content of the livers ranged from 70.1 to

TABLE II. Effect of Feeding DL-β, β-Diethylalanine on Liver Lipide, Water, and Protein.

Group	Total liver lipide of body wt, %	Water content of liver, %	Liver protein of body wt, %
A	.51 ± .05*	66.5 ± .5	.610 ± .010
B	.34 ± .04	68.3 ± .6	.622 ± .010
C	.31 ± .02	68.8 ± .2	.657 ± .008†

* Stand. error of mean.
† One sample lost.

71.6% for the same groups. Expressed as per cent of body weight, the liver protein contents of the female DEA injected rats and female control rats were 0.74 and 0.71 respectively, while the corresponding values for the male DEA injected rats and male controls were 0.80 and 0.81 respectively.

Although DEA was not a growth inhibitor on the levels at which it was administered, the differences in liver composition of DEA fed animals and controls are of interest, particularly in view of similar experiments with ethionine(5). The fact that feeding either DEA or ethionine can result in higher water and protein content of livers as compared with controls suggests that these effects are due to their properties as amino acid analogues *per se*, and not to interference with specific functions of the amino acids involved (*e.g.* transmethylation). Since the effect of DEA is to lower and the effect of ethionine is to raise the level of liver lipide(5), it appears likely that the mechanisms of action of these compounds on lipide metabolism are different. The scope of these experiments is not sufficient to suggest the mechanism by which the DEA diet lowers the liver lipide content.

Summary. The effect of the administration of β,β-diethylalanine (DEA) upon the composition of rat livers has been studied. Feeding DEA to male rats resulted, at the higher of 2 levels, in lower liver lipide and higher liver water and protein content than in controls. Injections of massive doses of DEA were without significant effect. The implications of these results with respect to similar studies with ethionine are discussed.

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Effect of Aldosterone and Desoxycorticosterone on Adrenalectomized Dogs.* (21034)

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Since 1950, data have rapidly accumulated concerning the presence in adrenal cortical extracts and in adrenal vein blood, of a highly active Na-retaining substance(1-6). This work has culminated in recent announcements from 3 laboratories of the isolation in crystalline form of a new mineralcorticoid(3,7,8), provisionally designated by Simpson *et al.*(7) as electrocortin. Chemical degradation of the new crystalline adrenal steroid by Simpson, Tait, Wettstein, Neher, Euw, Schindler and Reichstein(9) shows that the compound is 11β-21-dihydroxy-3,20-diketo-4-pregnene-18-al. According to these investigators, this reacts mainly as the 11-hemiacetal when in solution; they suggested aldosterone as a definitive name for the compound. Owing to scarcity of material, few detailed physiological studies on the pure substance have appeared (10-12).

Through the courtesy of Professor T. Reichstein, the writers secured enough crystalline material for detailed tests on 4 adrenalectomized dogs. The present study is a comparison of the life maintenance and Na-retaining activity of pure crystalline aldosterone with that of the free alcohol of desoxycorticosterone (DOC).

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