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Effects of B Vitamins, Yeast and Liver on Ovaries of Immature Rats Fed Massive Doses of Alpha-Estradiol.

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It is well established that massive doses of estrogens retard growth and inhibit gonadal development in the immature rat. Available data indicate that these effects may be counteracted, at least in part, by dietary means. Ershoff and Deuel[1] observed that the gonadal weight of immature rats fed massive doses of alpha-estradiol was significantly greater on diets containing yeast than on rations containing the B vitamins in synthetic form. In the present communication further data are presented on the effects of diet on ovarian development in the alpha-estradiol-fed rat.

Procedure and Results. Four basal rations were employed in the present experiment: diets A, B, C, and D. Diets A and B were purified rations containing the B-complex factors in synthetic form only. Diets C and D were similar in composition but contained yeast or desiccated whole liver in addition to the synthetic vitamins. All 4 rations were supplemented with 0.0 and 10.0 mg of alpha-estradiol per kg of diet.* Sixty-four female rats of the Long-Evans strain were selected at 21 to 23 days of age and an average weight of 42.0 g for the present experiment. Animals were kept in metal cages with raised screen bottoms to prevent access to feces, and were fed *ad lib.* the diets listed in Table I. Feeding was continued for 8 weeks. Animals were autopsied on the 56th day of feeding; ovaries were weighed and fixed in 10% formol, and sections prepared and stained with hematoxylin and eosin.

Results are summarized in Table II. In agreement with earlier findings[1] the effects of alpha-estradiol feeding in the immature rat were dependent on the diet employed. On the synthetic ration (diet A_2) ovaries appeared infantile both in weight and microscopic appearance. Similar results were obtained in alpha-estradiol-fed rats receiving additional B vitamins or yeast (diets B_2 and C_2). In the whole liver series (diet D_2), however, ovarian weights averaged approximately twice the values obtained on other alpha-estradiol-containing rations; and histologically ovaries appeared normal in 8 of the 10 rats in this series. Growth was markedly reduced in all rats fed alpha-estradiol-containing rations. Gain in body weight was somewhat greater in the liver series (diet D_2) than on other rations employed, but with the possible exception of the A_2 series these differences were not significant. On alpha-estradiol-free rations body and ovarian weights did not differ significantly on any of the diets employed, and histologically ovaries appeared normal in all groups.

Discussion. It is becoming increasingly apparent that the effects obtained in an experimental animal following administration of drugs or related products are dependent on the nutritional state and the diets employed. In acute deficiencies of essential nutrients it is readily recognized that resulting abnormalities in cellular metabolism may profoundly affect response to certain drugs. What is less well recognized, however, is that these drugs themselves may precipitate a deficiency state. Thyroid administration, for example, may induce a deficiency of thiamine, pyridoxine, and pantothenic acid,[3] while dicumarol, salicylates, and other drugs may precipi-

[1] Ershoff, B. H., and Deuel, H. J., Jr., *Am. J. Physiol.*, 1946, **145**, 465.

* The alpha-estradiol for these experiments was obtained from the Schering Corporation, Bloomfield, N.J. One mg alpha-estradiol = 12,000 R.U. or 120,000 I.U. estrone.

[2] Sure, B., *J. Nutrition*, 1941, **22**, 499.

[3] Drill, V. A., and Overman, R., *Am. J. Physiol.*, 1942, **135**, 474.

[4] Shapiro, S., Redish, M. H., and Campbell, H. A., Proc. Soc. Exp. Biol. and Med., 1943, **52**, 12.

TABLE I.
Composition of Experimental Diets.

Dietary component	Diet A_1 and A_2	Diet B_1 and B_2	Diet C_1 and C_2	Diet D_1 and D_2
Yeast*	0.0	0.0	10.0	0.0
Whole liver powder†	0.0	0.0	0.0	10.0
Casein‡	22.0	22.0	22.0	22.0
Salt mixture§	4.5	4.5	4.5	4.5
Sucrose	73.5	73.5	63.5	63.5

Alpha-estradiol was incorporated in diets A_2, B_2, C_2, and D_2 at a level of 10.0 mg per kg of diet.

To each kg of diet A, C, and D were added the following synthetic vitamins: thiamine hydrochloride 72 mg, riboflavin 9 mg, pyridoxine hydrochloride 15 mg, calcium pantothenate 67.2 mg, nicotinic acid 60 mg, 2-methyl-naphthoquinone 5 mg, and choline chloride 1.2 g.

To each kg of diet B were added: thiamine hydrochloride 144 mg, riboflavin 18 mg, pyridoxine hydrochloride 30 mg, calcium pantothenate 134.4 mg, nicotinic acid 120 mg, inositol 1.2 g, *p*-aminobenzoic acid 600 mg, folic acid 10 mg, biotin 1 mg, 2-methyl-naphthoquinone 10 mg and choline chloride 1.2 g.

Each rat also received 3 times weekly the following supplement: cottonseed oil (Wesson) 500 mg, alpha-tocopherol 1 mg, and a vitamin A-D concentrate‖ containing 50 U.S.P. units of vitamin A and 5 U.S.P. units of vitamin D.

* Brewer's Type Yeast No. 200, Anheuser-Busch, Inc., St. Louis, Mo.
† Whole Dried Liver Powder, Armour and Co., Chicago, Ill.
‡ Vitamin Test Casein, General Biochemicals, Inc., Chagrin Falls, Ohio.
§ Sure's Salt Mixture No. 1.[2]
‖ Nopco Fish Oil Concentrate, assaying 800,000 U.S.P. units of vitamin A and 80,000 U.S.P. units of vitamin D per gram.

TABLE II.
Effects of Alpha-estradiol on Body and Ovarian Weight in the Immature Rat.

Dietary Group	No. of animals	Initial body wt, g	Gain in body wt 8-wk period,* g	Avg ovarian wt,* mg
		Alpha-estradiol Series.		
A_2	10	42.8	97.9 ± 3.7	13.7 ± 1.1
B_2	10	42.1	105.1 ± 6.6	16.1 ± 1.3
C_2	10	41.9	103.4 ± 5.5	18.9 ± 1.4
D_2	10	42.2	117.9 ± 3.6	37.2 ± 2.7
		Control Series.		
A_1	6	41.7	146.4 ± 9.0	47.0 ± 2.4
B_1	6	42.0	152.3 ± 7.8	49.1 ± 3.0
C_1	6	41.5	146.8 ± 6.9	43.2 ± 3.2
D_1	6	41.7	169.7 ± 9.3	44.8 ± 2.1

* Including standard error of the mean calculated as follows: $\sqrt{\frac{\Sigma d^2}{n}} / \sqrt{n}$ where "d" is the deviation from the mean and "n" is the number of observations.

tate a deficiency of vitamin K.[4-6] In addition to the known nutrients, however, requirements for various unknown factors may also be increased following the administration of certain drugs. These "minor vitamins" are apparently dispensable under normal conditions or their requirements are so small they may readily be met by amounts present in the diet or through the synthetic activity of the intestinal flora or the animals' own tissues. Certain drugs or other "stress factors" may, however, increase requirements for these substances to the extent that deficiencies occur, manifest by retarded growth or tissue pathology, and preventable by the administration in appropriatee amounts of the missing nutrient.[7] Whole liver and yeast are potent sources of such unknown nutrients. The beneficial effects of these latter substances in animals inhaling carbon tetrachloride or fed toxic

5 Shapiro, S., *J. A. M. A.*, 1944, **125**, 546.
6 Collins, E. N., and Hoffman, A. D., *Cleveland Clin. Quart.*, 1943, **10**, 105.
7 Ershoff, B. H., *Physiol. Rev.*, 1948, **28**, 107.

doses of strychnine, promin, dinitrophenol, sulfanilamide, atabrine, and other drugs have been recognized for years.[8-11] Similar results have been observed following toxic doses of diethylstilbestrol[8] and desiccated thyroid.[12-14] In the present experiment toxic effects of alpha-estradiol were similarly modified by dietary means.

Findings indicate that the effects of alpha-estradiol feeding in the immature rat were dependent on the diet employed. On the synthetic ration (diet A_2) ovaries appeared infantile both in weight and microscopic appearance. Similar results were obtained with animals fed additional B vitamins or yeast (diets B_2 and C_2). Oral administration of desiccated whole liver, however, resulted in a significant increase in ovarian weight with ovaries resembling histologically those of the normal rat. On alpha-estradiol-free rations ovaries did not differ significantly in weight or microscopic appearance on any of the diets employed. Available data indicate that the beneficial effects of liver on ovarian development in the immature alpha-estradiol-fed rat were not due to any of the known B vitamins. This is indicated by the fact that ovaries remained infantile on diet B_2 although this ration contained all known members of the vitamin B complex in amounts exceeding their presence in the liver-containing diet D_2. It is suggested, therefore, that desiccated whole liver contains some factor(s) other than the known B vitamins whose requirement is increased following prolonged feeding of alpha-estradiol.† In previous work with smaller doses of alpha-estradiol yeast was also found to promote ovarian development in the alpha-estradiol-fed rat.[1] Such was not the case, however, under conditions of the present experiment.

Summary. Desiccated whole liver counteracted the inhibition of ovarian development observed in immature rats fed massive doses of alpha-estradiol. The protective factor in liver was distinct from any of the known members of the vitamin B complex. It was not present in significant amounts in yeast.

8 Chamelin, I. M., and Funk, C., *Arch. Biochem.*, 1943, **2**, 9.

9 Higgins, G. M., *Am. J. Clin. Path.*, 1944, **14**, 278.

10 Battelli, G., *Boll. soc. ital. biol. sper.*, 1940, **15**, 687.

11 Ershoff, B. H., *J. Nutrition*, 1948, **35**, 269.

12 Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 500.

13 Ershoff, B. H., *Arch. Biochem.*, 1947, **15**, 365.

14 Betheil, J. J., Wiebelhaus, V. D., and Lardy, H. A., *J. Nutrition*, 1947, **34**, 431.

† The unknown nutrient may be part of an enzyme system concerned with estrogen inactivation. It is possible that prolonged feeding of alpha-estradiol increased requirements for this factor on diets A_2, B_2, and C_2 to the point that a deficiency occurred, interfering with estrogen-inactivation and resulting in impaired secretion of pituitary gonadotropins.[15]

15 Zondek, B., *Clinical and Experimental Investigations on the Genital Functions and Their Hormonal Regulation*, Williams & Wilkins, Baltimore, 1941.

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The Ability of the Cat to Withstand Repeated Electrically Induced Convulsions.

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In carrying out electro-shock therapy on psychotic patients it is frequently observed that some fail to immediately respond to the electrical stimulus with a major convulsive seizure. At times, especially in attempting to establish the convulsive threshold for a particular patient, it becomes necessary to induce 3 or 4 minor responses before the