ple segment of aorta leading from the left atrium into the cavity of the left ventricle has been demonstrated. Orthograde flow from a reservoir of saline through the aortic tube into the left ventricle has been shown to occur in the living animal. No regurgitation has been observed. This observation has been documented by means of pressure determinations obtained within the portion of aorta lying in the left ventricle and that in the left atrium. An average systolic pressure of 104 mm Hg was observed in the ventricular portion and an average mean pressure of 12 mm Hg in the atrial portion. This demonstration of orthograde flow during diastole without retrograde flow during ventricular systole is felt to constitute evidence of an effective valve-like action.

The authors wish to express their gratitude to Mr. Paul Geller, Dr. Ruth Kaslow, and Mrs. Selma Rachlin for their assistance.

Received April 16, 1953. P.S.E.B.M., 1953, v83.

Influence of Low Protein Diet on Distribution of D-Amino Acid Oxidases in Rats.* (20372)

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In connection with a series of experiments on the influence of nutrition on the development of hepatic tumors, the effect of nutritional factors on the amino acid oxidases was studied. A decrease of amino acid oxidases was found previously in hepatic tumors(1-3) as well as with the livers of rats bearing transplanted liver tumors.

The present investigation attempted to answer the following questions: a) do nutritional factors influence the levels of amino acid oxidases, b) is such an influence general or restricted to any one organ, and c) does the administration of an estrogen together with a low protein diet produce any changes in the level of amino acid oxidase. The impairment of the detoxication of estrogens on low protein diet has been described(4). Experiments on the possibility of the production of hepatic tumors by estrogens added to a low protein diet are being carried out in this laboratory. In the rat, amino acid oxidases are present in appreciable quantities only in the liver and in the kidneys(5). In both organs a general D-amino acid oxidase has been demonstrated. In addition, a specific D-aspartic acid oxidase has been found in pig kidneys(6). In the present experiment the amino acid oxidases were studied both in the liver and in the kidney. Although next to D-L-alanine, D-aspartic acid was used as substrate, no attempt was made to separate the activity of the general D-amino acid oxidase from the specific D-aspartic acid oxidase.

Materials and methods. Young Wistar rats of both sexes, weighing 70-90 g at the beginning of the experiment were divided into 3 groups; one was kept on a full diet, one on a low protein diet, and a third group on a low protein diet with the addition of alpha estradiol. Rats fed on rat biscuits served as control on full diet. The low protein diet consisted of: Protein (vitamin-free casein) 8.5%, carbohydrate (sugar and cornstarch) 70%, fat (lard and vegetable oil) 17%, salt mixture 3.5%, and cod liver oil 1%. Vit. B and E were given to 1000 g of diet in the following amounts: thiamin hydrochloride 6 mg, pyridoxin 20 mg, inositol 15 mg, choline dihydrogenphosphate 100 mg, calcium pantothenate 20 mg, and alpha tocopherol 30 mg. Food and water were given *ad lib.* Alpha estradiol was fed to half the number of the rats on the experimental diet. Each of these rats received orally by eye dropper 5 μg estradiol dissolved in 0.1 ml olive oil daily

* This investigation was supported by a research grant from the National Cancer Institute, National Institutes of Health, Public Health Service.

with the exception of Sundays. Rats were killed at intervals, after having been on the experimental diet, with and without addition of estradiol for at least 3 months. The D-amino-acid oxidase was determined by measuring the oxygen uptake of liver and kidney homogenates in the presence of substrate by the Warburg technic. Each vessel contained 200 mg of homogenate, 0.3 ml of a 0.3% D-L-alanine or D-aspartic acid and M/15 phosphate buffer of pH 8.4. The central cup contained 0.2 ml of 20% potassium hydroxide. The total fluid volume was 3 ml. The experiments were carried out in duplicate at 38° C. Readings were taken every 10 minutes through 1-3 hours. The activity of D-amino-acid oxidase was calculated as milliliters of oxygen taken up by one g of wet tissue during one hour.

Results and discussion. All rats on low protein diet showed fatty livers; the male rats receiving estradiol showed pronounced cirrhosis and 4 out of 28 had cholangiomas. (This part of the experiment is now being repeated and will be reported at a later date.) The weights of the livers of rats on low protein diet were increased compared with the liver weights of rats on the normal full control diet. The other organs, with the exception of the genital organs of rats receiving estradiol, did not show significant differences between the rats on the experimental and on the control diet.

Since the effect of feeding the low protein diet on the D-amino acid oxidase was the same whether the rat received this food for 3 or for 9 months, and since it was found to be the same in the organs of male and female rats the results are discussed together. In every experiment the oxygen uptake of liver and of kidney was the same with alanine or with aspartic acid as substrate. Table I shows that the level of D-amino acid oxidases of the kidneys are the same in the control rats on full diet and in both groups of experimental animals on the low protein diet. The level of D-amino acid oxidases in the livers of rats on low protein diet dropped to about half of the values found in the rats receiving a normal full diet. No difference was found in the activity of D-amino acid oxidase in the liver of the rats on low protein diet on addition of

TABLE I. D-Amino Acid Oxidases in Livers and Kidneys of Rats on Low Protein Diet.

Treatment	No. of rats	Body wt Range	Body wt Mean	Liver wt, % of body wt Range	Liver wt, % of body wt Mean	Liver oxidase, ml O_2/g tissue/hr D-L-alanine Range	Liver oxidase, ml O_2/g tissue/hr D-L-alanine Mean	Liver oxidase, ml O_2/g tissue/hr D-aspartic acid Range	Liver oxidase, ml O_2/g tissue/hr D-aspartic acid Mean	Kidney wt, % of body wt Range	Kidney wt, % of body wt Mean	Kidney oxidase, ml O_2/g tissue/hr D-L-alanine Range	Kidney oxidase, ml O_2/g tissue/hr D-L-alanine Mean	Kidney oxidase, ml O_2/g tissue/hr D-aspartic acid Range	Kidney oxidase, ml O_2/g tissue/hr D-aspartic acid Mean
Control, full diet	10	123 217	174	3.4 5.1	4.10	365 610	449	325 535	434	.70 1.18	.86	240 480	342	220 475	338
Low protein diet	8	140 250	172	3.4 7.9	5.77	159 240	211	139 274	211	.64 1.17	.83	197 540	300	170 590	301
Low protein diet with estradiol	13	107 212	163	3.7 7.2	5.00	148 330	248	126 283	244	.51 1.38	.70	190 480	318	190 460	292

estradiol, though cholangio-fibrosis, and cirrhosis of the liver was more marked in the latter groups. The increase in the weight in the liver of rats on low protein diet is proportionally much smaller than the decrease in the level of the amino acid oxidases, accordingly a possible dilution of the enzyme in the liver cannot be taken as an explanation. In previous experiments(7) both components of the D-amino acid oxidase, the co-ferment riboflavin and the protein was estimated separately in liver of rats on protein free diet. The decrease in the amino acid oxidase in the livers could not be accounted for by the decrease of the riboflavin in the livers, and was thought to be due to a decrease of the specific enzyme protein. The amino acid oxidases in other organs were not studied. In view of the active participation of the liver in the protein metabolism, the finding in the present experiment that on reduction of dietary proteins a decrease of the amino acid oxidase occurred in livers only, might be of physiological importance.

Summary. 1. D-amino acid oxidases in livers and kidneys of rats on a low protein diet with or without addition of alpha estradiol have been compared with those of rats on a full diet. 2. The levels of D-amino acid oxidases in the kidneys was the same in all 3 groups. 3. In livers of rats on low protein diet, irrespective of feeding of alpha estradiol, the level of D-amino acid oxidases dropped to about half of that found in rats on full diet.

The author wishes to thank the Schering Corp., Bloomfield, N. J., for donating the alpha estradiol used in this experiment.

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Received May 26, 1953. P.S.E.B.M., 1953, v83.

Histochemical Studies on Glycogen Deposition in Uterus of the Rat* III. Effect of Starvation. (20373)

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It has been shown recently that glycogen is deposited in the smooth muscle of the uterus of the rat under the influence of estrogen, whereas progesterone, androgen and adrenal cortical extract each fail to produce this effect (1-3). This action of estrogen on the uterus is independent of both the adrenal cortex and hypophysis(2,3), but is, in a sense, analogous to the role played by the hormones of the latter organs in the deposition of glycogen in the liver and skeletal muscle. The glycogen content of the liver and skeletal muscle remains within normal limits in adrenalectomized(4) and hypophysectomized(5) animals provided a good nutritional state is maintained. Relatively short periods of fasting, however, result in a sharp reduction of liver glycogen in adrenalectomized animals and of both liver and skeletal muscle glycogen in hypophysectomized animals, although normal levels are promptly restored following treatment with the appropriate adrenal cortical or hypophyseal hormones(6,7).

The glycogen content of the uterus of intact and estrogen-treated castrate rats is not reduced by fasting(3). Whether estrogen can induce glycogenesis in the uterus after depletion of carbohydrate reserves, however, is not

* This investigation has been aided by a grant from the Montgomery County Society for Cancer Control, Dayton, O.

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