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Reproduction in the Rat on Purified Diets Containing Succinylsulfathiazole.*

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Nelson and Evans[1] have shown that placing normal adult female rats on purified diets deficient in pantothenic acid 2-3 weeks before mating or even as late as the day of mating resulted in impaired reproduction, *i.e.* failure of implantation, resorptions, or defective litters. The normality of the paired-fed controls receiving supplements of pantothenic acid eliminated the factors of inanition and of other possible specific dietary deficiencies as causes for these marked upsets in reproduction. One of the possible dietary deficiencies thus eliminated under the experimental conditions was that of pteroylglutamic acid, which has since been shown to be necessary for optimal lactation in the rat maintained on the above-mentioned control diet.[2] In the present communication we wish to report the reproductive behavior of adult females placed on a succinylsulfathiazole - containing diet (SST-diet) deficient in pteroylglutamic acid (PGA) for various periods prior to mating.

Experimental. Normal female rats (Long-Evans strain), 3 to 4 months of age were bred with normal males and placed on the deficient or control diet the day of breeding. Other groups of rats were maintained on the deficient diet for one, two, or three months and then bred as rapidly as possible. Vaginal smears were examined daily during gestation for the presence of erythrocytes, the sign that implantation has occurred; all rats were weighed at regular intervals.

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1 Nelson, M. M., and Evans, H. M., *J. Nutrition*, 1946, **31**, 497.

2 Nelson, M. M., and Evans, H. M., *Arch. Biochem.*, **13**, 265.

TABLE I.
Reproduction of Rats Maintained on Purified SST-Diets.

Time exp. diets fed	No. of rats bred	Failed implantation	Resorptions of implantations	Litters of implantations	Total No. young	Young born dead	Avg size of litter	Avg wt young
		%	%	%		%		g
		PGA—Supplemented.						
Day of Mating	10	0	0	100	87	0	8.7 (3-11)	5.9
		PGA—Deficient.						
,, ,,	12	0	0	100	112	0	9.3 (6-12)	5.5
35 Days before Mating	10	0	10	90	64	9	7.1 (2-10)	4.7
64 ,, ,, ,,	9	0	44	56	29	3	5.8 (3-9)	5.8
92 ,, ,, ,,	10	10	33	67	32	6	5.3 (2-9)	5.5

The PGA-deficient diet was composed of 24% alcohol-extracted casein, 63% sucrose, 8% hydrogenated cottonseed oil (Crisco), 4% salts,[3] and 1% succinylsulfathiazole. Crystalline vitamins per kilogram diet were: 300 μg d-biotin, 5 mg 2-methyl-1,4-naphthoquinone, 5 mg thiamine HCl, 5 mg pyridoxine HCl, 10 mg riboflavin, 10 mg p-aminobenzoic acid, 20 mg nicotinic acid, 50 mg calcium pantothenate 400 mg inositol and 1.0 g choline chloride. Control rats received the identical diet supplemented with 5.5 mg synthetic pteroylglutamic acid per kilogram diet. Each rat received weekly a fat-soluble vitamin mixture containing 800 U.S.P. units vitamin A, 115 Chick Units vitamin D, 6 mg synthetic alpha-tocopherol, and 650 mg corn oil (Mazola).

Results. Table I shows that the omission of pteroylglutamic acid from the SST-purified diet on the day of breeding did not interfere with reproduction. When the deficient diet was started one month before breeding, reproduction was slightly impaired as shown by the occurrence of resorption and of young born dead together with the slight decrease in the number of young per litter and their weight at birth. Extending the deficiency to two months before breeding resulted in marked upsets in reproductive behavior. Almost half of the animals resorbed instead of littering and the average size of the litter was markedly decreased. A further extension of the deficiency period to 3 months before breeding did not result in any greater impairment of reproduction. Additional groups of animals placed on the deficient diet at an earlier age have shown essentially the same picture.

It may be noted that no interference with implantation occurred in these PGA-deficient animals as was the case with pantothenic acid deficient animals during reproduction. A considerably longer deficiency period (1-3 months) was required to produce reproductive upsets with the SST-diet than was necessary for the pantothenic acid deficient diets (0-3 weeks). The resistance of our rats to a "folic acid" deficiency instituted after weaning has been previously pointed out.[2] Studies on the curative effects of pteroylglutamic

[3] Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 459.

acid after one to three months of the deficiency and on the relation of calorie restriction to these reproductive upsets on the SST-diet are in progress.

Summary. Adult rats maintained on a purified diet containing succinylsulfathiazole and supplemented with all the known vitamins except pteroylglutamic acid from one to 3 months before breeding showed impaired reproduction.

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Effect of Estrogens on Malic Dehydrogenase of Rat Liver.*

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The natural and synthetic estrogens are important in controlling the function of reproductive tissues, and they are also known to affect the metabolism and function of other tissues not directly concerned with reproduction. It is probable that these changes depend basically on the part played by the estrogens in certain of the enzymatic processes concerned with cellular function. As a means of obtaining information on these basic cellular processes McShan and Meyer[1] studied the effect of the estrogens on the succinoxidase system of liver and pituitary tissues. It was found that both natural and synthetic estrogens were effective inhibitors of this system, and that the inhibition was mediated through the action of the estrogens with the cytochrome oxidase of the system. This work has been extended to the study of the effect of the estrogens on the malic dehydrogenase system using the method reported by Potter[2] for the determination of this dehydrogenase. In addition results are given on the activity of malic dehydrogenase when the cytochrome c of the system is replaced by brilliant cresyl blue.

Experimental. Male and female rats of the Sprague-Dawley strain which were three to four months old were used in these experiments.

The rats were killed by decapitation and the tissues were removed immediately, weighed and placed in sharp-pointed homogenizing tubes containing 0.1 ml of glass-distilled water. These homogenizing tubes containing the tissues were kept in an ice bath during homogenization and until the tissues were placed in the Warburg flasks.

The method of Potter[2] for the determination of malic dehydrogenase was followed in these experiments. Two and one-half per cent water homogenates of the liver tissue were used throughout. Conventional Warburg flasks without side arms were employed. The main compartment of the flasks contained, in addition to the desired amount of liver homogenate, 0.3 ml of 0.5 M l-malic acid, 0.6 ml of 0.5 M glutamic acid, 0.6 ml of 0.1 M nicotinamide, 0.3 ml of a 0.5% (5 mg per ml) solution of coenzyme I (diphosphopyridine nucleotide), 0.3 ml of 4 x 10^{-4} M cytochrome c, 0.4 ml of 0.2 M phosphate buffer pH 7.4, and enough water to make a final reaction volume of 3.0 ml. The center wells of the flasks contained 0.1 ml of 2N NaOH. All solutions were made with glass-distilled water.

The cytochrome c was made from beef heart muscle according to the method of Keilin and Hartree[3] except that the final

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1 McShan, W. H., and Meyer, R. K., *Arch. Biochem.*, 1946, **9**, 165.

2 Potter, V. R., *J. Biol. Chem.*, 1946, **165**, 311.

3 Keilin, D., and Hartree, E. F., *Proc. Roy. Soc. (London)*, 1937, **122**B, 298.