Effect of 36-Hour Period of Pteroylglutamic Acid Deficiency on Fetal Development in the Rat.* (22541)

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Nelson *et al.*(1) have shown that a transitory period of pteroylglutamic acid (PGA) deficiency of only 48 or 72 hours during the second week of pregnancy markedly affected fetal development in the rat and invariably resulted in a high incidence of abnormal young or fetal death. The earlier phases of embryonic development were more severely affected than the later phases by either period of deficiency. In contrast, a 24-hour period of the PGA-deficient diet during the same week of pregnancy had little or no effect. In order to determine more accurately the length of time required for the PGA-deficient diet to affect fetal development and also to find the period of greatest sensitivity, the effects of a 36-hour period of deficiency have been studied.

Methods. The experimental procedures and diets were the same as those used previously to study the effects of transitory PGA-deficiency(1). Stock female rats of the Long-Evans strain were bred with normal males and given the PGA-deficient diet† for 36 hours, beginning with the 7th, 8th, 9th, and 10th days of pregnancy. Following the period of transitory deficiency, the animals were given the PGA-supplemented diet† for the remainder of the gestation period. Twenty to 25 rats were used for each experimental group. Additional groups of animals were subjected to 24- and 48-hour periods of deficiency instituted on the same days in order to confirm the results previously obtained with smaller groups for some of these periods.‡ All young were removed by cesarian section on the 21st day of gestation and examined macroscopically for abnormalities; the uterus was checked for the presence of resorbing sites.

Results. Table I summarizes the effects of 24, 36, and 48-hour periods of PGA-deficiency. A 48-hour period of deficiency started on either the 7th or 8th days of pregnancy was equally deleterious to fetal development as 97 to 100% of the embryos resorbed in both groups. When the same period of deficiency was started on the 9th or 10th days fewer embryos resorbed, 61% and 19%, respectively. Abnormal young and even a few normal young were found, showing that fetal sensitivity to PGA-deficiency decreased with increasing fetal age and differentiation. A 24-hour period of deficiency during the same days of pregnancy was, however, virtually ineffective. Only 10 to 11% of the embryos were affected, the percentage of fetal death being slightly higher than that observed in more than 40 control litters. Half of the control pregnancies had received the PGA-supplemented diet and the remainder a stock diet of natural foodstuffs. No abnormal young were present in the control litters whereas a few congenital anomalies were ob-

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† The PGA-deficient diet containing 1% succinylsulfathiazole and 0.5% x-methyl-PGA was the same as that used previously (Nelson *et al.* 1955). The PGA-supplemented diet contained the identical constituents, including the x-methyl-PGA for more than half the animals, but in addition, 50.5 mg synthetic PGA per kilo of diet. Lot N-125 of x-methyl-PGA was used throughout this study.

‡ No additional animals were subjected to the 48-hour deficiency period from days 9 to 11 or days 10 to 12. The data for these periods in Table I are taken from Nelson *et al.*(1).

TABLE I. Effect of Transitory PGA Deficiency on Fetal Development in the Rat (24, 36, and 48 Hr Periods).

PGA-deficient diet		No. rats bred	Wt change during gestation, g	Total No.	Embryos			Total affected, %
Hr	Days				Normal, %	Abnormal, %	Resorbed, %	
48	7- 9	22	+ 58	197	1	2	97	99
	8-10	23	+ 56	237	0	0	100	100
	9-11	34	+ 77	328	0	39	61	100
	10-12	21	+ 90	197	19	62	19	81
36	7- 8½	22	+ 92	206	67	6	27	33
	8- 9½	26	+ 79	256	20	15	65	80
	9-10½	24	+ 78	232	69	10	21	31
	10-11½	24	+101	240	92	0	8	8
24	7- 8	23	+109	217	90	0	10	10
	8- 9	23	+115	239	89	5	6	11
	9-10	21	+102	202	90	3	7	10
	10-11	23	+108	235	90	0	10	10
	None	42	+114	410	0	0	5	5

served in two groups subjected to 24-hour periods of deficiency. In contrast to the 24-hour periods of deficiency, the 36-hour periods markedly affected fetal development when instituted on the 7th, 8th, or 9th days. The absence of effects when the deficiency was started on the 10th day again demonstrated the rapidly decreasing sensitivity to this vitamin deficiency with increasing fetal age. The effects of the 36-hour period were greatest when the PGA-deficient diet was started on the 8th day, 80% of the embryos being affected. Approximately 30% of the embryos were dead or abnormal when the deficient diet was started one day earlier or later.

The types of abnormalities observed in the young resulting from 36-hour periods of deficiency instituted on the 7th or 9th days were similar to those previously observed for the corresponding 48-hour periods of deficiency(1). Cerebral and eye defects, cardiovascular anomalies, and gastroschisis were observed in the young from the day 7 to 8½ group; whereas cleft palate, harelip, syndactylism, clubfoot, cardiovascular and eye defects were observed in the young from the day 9 to 10½ group. In the abnormal young from the day 8 to 9½ group cardiovascular defects predominated; hydrocephalus, diaphragmatic defects, and gastroschisis were also found.

Discussion. The data presented demonstrate that fetal development in the rat can be severely injured by as brief a period as 36 hours of the PGA-deficient diet. The proportion of embryos affected varied with the time of instituting the deficient diet and was highest when the diet was started on the 8th day. The deleterious effects of the PGA-deficient diet given from days 8 to 9½ were presumably exerted during the 9th day (inasmuch as the first 24 hours were ineffective and we must assume that a few hours would be required for vitamin supplementation to counteract the antimetabolite, x-methyl PGA). Similarly, the PGA-deficient diet given from days 7 to 8½ presumably exerted its effects during the 8th day. Thus, the 8-day rat embryo appeared to be less sensitive to this vitamin deficiency than the 9-day rat embryo.

A similar difference in apparent sensitivity of the rat embryo to low doses of irradiation has been reported by Wilson(2) and by Hicks(3). It has been suggested by Russell and Russell(4) that the cells of the 8-day rat embryo may be just as sensitive to irradiation but have a greater regulatory power. The same explanation may be valid for sensitivity to PGA-deficiency. It is also possible that the cells of the earlier embryo may have fewer biochemical reactions for which the vitamin is necessary than cells of the 3 germ layers in the 9-day rat embryo. Bieber *et al.*(5) found a similar ineffectiveness of PGA antimetabolites during the early stages of development in *Rana pipiens*. Hitchings(6) has hypothe-

sized that early embryogenesis in *Rana pipiens* might occur at the expense of preformed purine-containing nucleic acid fragments stored in the ovum and that nucleic acid synthesis from smaller molecules involving PGA might begin only at neurulation, the earliest stage that PGA antimetabolites affect development in this species.

Summary. Fetal development in the rat was severely affected by a 36-hour period of pteroylglutamic acid deficiency when instituted early in the second week of pregnancy. The incidence of fetal death or abnormality was higher when the vitamin-deficient diet was started on the 8th day of pregnancy than on the 7th day but decreased rapidly thereafter with increasing fetal age.

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Vitamin E Deficiency and Biosynthesis of Glycine, Serine, and Methionine in Rabbits.* (22542)

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While studying the effect of vit. E deficiency on the incorporation of formate-C^{14} and glycine-1-C^{14} into the nucleic acids of various rabbit tissues, it was found that the deficiency also altered the level of isotope incorporation into the proteins of those tissues. The tissue protein exhibiting the greatest difference was that of skeletal muscle(1). This paper reports the results of the isolation of glycine, serine, and methionine from skeletal muscle of normal and vit. E-deficient rabbits following injections of either sodium formate-C^{14} or glycine-1-C^{14}.

Methods. New Zealand rabbits of both sexes were placed on a purified diet deficient in vit. E(2). Controls were given 4 mg of α-tocopherol acetate per kilo of body weight twice weekly. When symptoms appeared in 3-4 weeks, one deficient and one control rabbit were injected with either sodium formate-C^{14} (specific activity, 2.85 μc/mMole) or glycine-1-C^{14} (specific activity, 0.58 μc/mMole) at a level of 10 μc per 100 g of body weight. After 4 hours the animals were killed and the skeletal muscles of the hind legs were removed.

This tissue was homogenized in an equal volume of water and extracted with cold 10% trichloroacetic acid, alcohol, alcohol-ether, and hot 10% sodium chloride. The protein residue was then dried. The specific activities of these protein samples were determined by counting small aliquots in an end-window Geiger-Muller tube with window thickness of 2 mg per sq. cm. All values were corrected to infinite thinness. Approximately 10 g of the protein was hydrolyzed according to the method of Stein and Moore(3). The amino acids were separated with an ion exchange column, using Dowex 50-X4, using the method of Moore and Stein(4) with modifications. The hydrolysate was placed on the column in a citrate buffer at pH 3.1. The effluent was collected in 4 ml fractions at a rate of 50 ml per hour at 32°C. Each fraction was assayed for C^{14} content by counting an aliquot which had been evaporated to dryness.

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