same human donors over a period of 40 weeks has been found to be feasible and safe. The possibilities for obtaining large amounts of concentrated human antibodies and for stockpiling sterile human plasmas are discussed.

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Received March 15, 1956. P.S.E.B.M., 1956, v91.

Failure of Ovarian Hormones to Maintain Pregnancy in Rats Deficient in Pantothenic or Pteroylglutamic Acid.* (22348)

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Recent studies have shown that reproductive failure, *i.e.*, fetal death and resorption, can be prevented and pregnancy maintained by daily injections of estrone and progesterone in rats subjected to 4 different dietary deficiencies, those of thiamine(1), pyridoxine (2), protein(3,4) or potassium(5). The daily dosage levels of the hormones were the same as those found to be effective in maintaining pregnancy in rats hypophysectomized and oophorectomized after breeding(6). The present communication reports the failure of these hormones to prevent fetal death and resorption in rats deficient in either pantothenic acid(7) or pteroylglutamic acid(8,9).

Methods. The procedures for testing the efficacy of the hormones were the same as those used previously to maintain pregnancy in other dietary deficiencies(1-5). The experimental conditions necessary for each vitamin deficiency to result in fetal death and resorption in approximately 90% of the animals were determined, if not already known. The synthetic hormones were dissolved in sesame oil and given daily, either separately or in combination, by subcutaneous injection from the 3rd or 5th days of gestation period to the day before autopsy. Levels of estrone varying from 0.5 to 2.0 μg† daily were given separately and levels from 0.5 to 1.0 μg were given in combination with 4 mg of progesterone. Five to ten rats (Long-Evans strain) were used for each experimental group. Vaginal smears were examined daily for the presence of erythrocytes, the sign of implantation. The rats were autopsied near the end of the gestation period, from the 15th to the 21st day. The basal (control) purified diet was composed of 24% alcohol-extracted casein, 64% sucrose, 8% hydrogenated vegetable oil (Crisco or Primex), and 4% salts no. 4(10). Crystalline vitamins per kilo of diet were: 300 μg d-biotin, 5 mg 2-methyl-1,4-naphthoquinone, 5 mg thiamine HCl, 5 mg pyridoxine HCl, 5.5 mg pteroylglutamic acid, 10 mg riboflavin, 10 mg p-aminobenzoic acid, 20 mg

* Aided by grants from Roche Anniversary Foundation and the U. S. Public Health Service A-470 and A-841. We are indebted to Dr. Daniel A. McGinty of Parke, Davis and Co., Detroit, Mich. for generous supplies of estrone and progesterone; to Dr. T. H. Jukes, Lederle Laboratories, Pearl River, N. Y. for pteroylglutamic acid; to Dr. Elmer L. Severinghaus of Hoffmann-LaRoche, Nutley, N. J. for d-biotin, d-calcium pantothenate and dl-alpha-tocopherol; to Dr. Richard H. Barnes of Sharp and Dohme, Glenolden, Pa., for succinylsulfathiazole (SST); and to Dr. Randolph Major, Merck and Co., Rahway, N. J. for crystalline B vitamins and ?-methyl-1,4-naphthoquinone.

† Maintenance of pregnancy has been shown to vary with level of estrone given to protein-deficient rats(3).

TABLE I. Failure to Maintain Pregnancy by Injections of Estrone and Progesterone in Pantothenic Acid-Deficient Rats.*

Daily hormone dosage	Rats bred (No.)	Wt change during gestation (g)	Onset vaginal RBC (day)	Resorptions (%)	Litters (%)
Pantothenic acid-deficient diet					
None	15	+ 5	12.4	79	21
Estrone 1 μg	6	+14	12.2	83	17
Progesterone 4 mg	10	+ 3	12.2	80	20
Estrone .5 μg + Progesterone 4 mg	10	- 3	12.1	80	20
Pantothenic acid-supplemented diet					
None	10	+115	13.1	0	100

* All groups averaged 25-30 days of deficiency prior to breeding. Control group received pantothenic acid-supplemented diet only from day of breeding to autopsy (day 17-18). Hormone injections were given daily from day 5 to autopsy.

niacin, 50 mg d-calcium pantothenate, 400 mg inositol, and 1.0 g choline chloride. All rats received weekly a fat-soluble vitamin mixture containing 800 U.S.P. units vit. A, 115 chick units vit. D, 6 mg dl-alpha-tocopherol, and 650 mg corn oil (Mazola). Omission of calcium pantothenate from this diet resulted in a pantothenic acid-deficient diet similar to that used previously(7) but improved in vitamin supplementation. In the PGA-deficient diet PGA was omitted and 1% succinylsulfathiazole and 0.5% x-methyl PGA incorporated in the diet. Fifty mg PGA per kilo of diet (10 times the usual level) was added to this diet for control animals. The PGA-deficient and supplemented diets are identical with those used previously(8,9).

Results. Pantothenic acid deficiency. Normal female rats from the stock colony were placed on the pantothenic acid-deficient diet at 90 to 100 days of age and bred after 25 to 30 days of deficiency. Hormone injections were given from the 5th day of gestation to the 17th or 18th days, when all animals were autopsied. Table I demonstrates the failure to maintain pregnancy by estrone and

TABLE II. Failure to Maintain Pregnancy by Injections of Estrone and Progesterone in Rats Subjected to Pteroylglutamic Acid (PGA) Deficiency.*

Daily hormone dosage	Rats bred (No.)	Wt change during gestation (g)	Onset vaginal RBC (day)	Resorptions (%)	Litters (%)
PGA-deficient diet instituted day of breeding					
None	10	+15	12.5	100	0
Estrone 1 μg	5	+31	13.0	100	0
Progesterone 4 mg	5	+14	12.8	100	0
Estrone 1 μg + Progesterone 4 mg	5	+32	12.8	100	0
PGA-deficient diet instituted day 9					
None	22	+62	12.8	100	0
Estrone 2 μg	10	+62	12.6	100	0
Estrone 1 μg + Progesterone 4 mg	10	+71	12.6	90	10
PGA-supplemented diet instituted day of breeding					
None	5	+ 58	12.0	0	100
	21	+112	12.8	0	100

* Hormone injections were given daily from day 3 to autopsy when PGA-deficient diet was instituted on day of breeding but were not given until day 9 in 2nd group when PGA-deficient diet started day 9.

progesterone in these deficient rats. Resorption occurred in approximately 80% of the animals, whether uninjected or given the ovarian hormones, either separately or combined. In all groups vaginal erythrocytes (RBC) appeared normally on the 12th to 13th days. The animals showed no signs of the deficiency with the exception of a failure to gain weight during the gestation period.

Pteroylglutamic acid deficiency. Stock female rats were bred at 90 to 100 days of age and placed immediately on the PGA-deficient diet. Injections of the hormones were started 3 days after breeding and all rats were autopsied on the 15th to 16th day of gestation. Table II shows the ineffectiveness of the ovarian hormones in maintaining pregnancy. Resorption occurred in all animals, regardless of the presence or absence of injected hormones. A less severe PGA-deficiency was then produced by instituting the PGA-deficient diet 9 days after breeding, a procedure previously reported to result in 100% resorptions(9). Hormone therapy was instituted at the same time as the PGA-deficient diet. Again the hormones were ineffective as fetal death and resorption occurred in 90-100% of the animals in all groups.

In all PGA-deficient rats vaginal erythrocytes occurred normally on the 12th to the 13th days and the animals exhibited no external signs of the deficiency, although the maternal weight gain during the gestation period was less than that of vitamin-supplemented controls.

Discussion. The failure to maintain pregnancy by injection of ovarian hormones in this study was in marked contrast to the successful maintenance of pregnancy with the hormones in deficiencies of thiamine, pyridoxine, protein or potassium. Under the experimental conditions reproductive failure resulting from pantothenic acid or PGA deficiency did not appear to be due to lack of production or secretion of the maternal sex hormones as has been indicated for the other four dietary deficiencies. The signs of hormonal inadequacies observed in rats subjected to the other four dietary deficiencies or in rats hypophysectomized and oophorectomized after breeding, namely early appearance of vaginal erythrocytes and extremely rapid resorption of implantation sites, were not observed in rats deficient in pantothenic acid or in PGA. There were no disturbances in the estrous cycle in unbred rats given the pantothenic acid or PGA-deficient diets for the same length of time. In this study the maternal organism was only slightly affected, few or no external signs of either deficiency syndrome being observed, even though fetal death resulted. The deleterious effects of both pantothenic acid and PGA deficiencies on embryonic development are well established as multiple congenital abnormalities have been observed in the offspring when a less severe deficiency was employed(9,11-13). In contrast, congenital abnormalities have not been reported for those deficiencies in which pregnancy can be maintained by injections of the ovarian hormones.

Summary. Daily injections of estrone and progesterone, given separately or combined, failed to prevent fetal death and resorption in rats deficient in pantothenic acid or in pteroylglutamic acid. Dosage levels of the hormones were the same as those used successfully to maintain pregnancy in rats hypophysectomized and oophorectomized after breeding as well as in rats deficient in thiamine, pyridoxine, protein or potassium. Under the experimental conditions, reproductive failure in rats subjected to pantothenic acid or PGA-deficiency did not appear to be due to lack of the maternal sex hormones.

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Received March 13, 1956. P.S.E.B.M., 1956, v91.

Improved Urinary Excretion Test for Assay of Intrinsic Factor. II. Sensitive Counting Technic.* (22349)

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The measurement of urinary excretion of vit. $B_{12}Co^{60}$ ($B_{12}Co^{60}$) following oral administration is used in the diagnosis of pernicious anemia(1,2) and as an assay for intrinsic factor(3,4). The use of human beings as experimental subjects necessitates the use of $B_{12}Co^{60}$ of low activity.† Since Co^{60} is a gamma emitter, the instrument of choice for its measurement is the scintillation counter. Most present well-type scintillation counters however, cannot count more than a 10 ml sample and therefore the low activity present in a 24 hour urine sample presents a counting problem which can only be solved by excessively long counting or by concentration of the urine samples. Flat scintillation crystals, upon which a beaker of liquid can be placed, do not offer significantly greater sensitivity than the well-type scintillation counter. Weiner and Peterson(5) designed a G-M well counter for measuring the radioactivity in liquid samples contained in 3 liter bottles, whereas Baskin *et al.*(6) constructed a well-shaped scintillation counter with a 35 ml capacity.

Using an available small well-type scintillation counter, a beaker with a capacity of one liter was designed fitting into the well and surrounding the top and sides of the crystal. This paper reports on the results obtained with the use of this beaker.

Methods. The design of the scintillation counter‡ is shown on the left side of Fig. 1. The counter consists of an RCA 5819 phototube, a thallium activated sodium iodide crystal, 1¾ in. diameter and 1 in. thick with a hole ¾ in. in diameter and 1 in. deep, and a cathode follower preamplifier. A 4 ml sample can be counted in a screw-cap glass vial and the vial is then placed in the well separated by a brass sleeve to prevent contamination of the crystal housing. The beaker in counting position is shown on the right side of Fig. 1. It was constructed§ starting with a two liter beaker from which the bottom was removed. The new bottom enclosed the crystal on top and sides with a projection fitting downward into the well. The counter and beaker were enclosed in a lead shield 3 in. thick. The background was about 110 cpm.

Results. 0.5 μc of vit. $B_{12}Co^{60}$ was diluted to 1000 ml and increasing amounts of this solution were placed in the beaker and counted. The values reported in Table I are corrected for background. As is evident from Table I, the use of the beaker gives an increase in counting rate of approximately 40 times that obtained using the standard 4 ml vial. As one would expect, the efficiency of

* See Ref. 4 for Paper I in this series.

† The maximum permissible dose has been set at 3 μc by the Subcommittee on Permissible Internal Doses of the National Committee on Radiation Protection.

‡ Model S_1—Purchased from H. O. Anger, Berkeley, Calif.

§ Constructed by L. & K. Glass Co., Lyndhurst, N. J.