Incidence of Teratogeny Induced by Vitamin E Deficiency in the Rat. (30378)

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Thomas and Cheng(1) first reported that a maternal deficiency of vitamin E can induce a considerable variety of teratogenic changes in developing rat embryos. Subsequently these investigators noted(2) that when α-tocopherol was administered to E-deficient pregnant rats at any time up through the 8th day of gestation no abnormally developed young were found when the animals were sacrificed and the uteri opened and examined at term. When, however, tocopherol administration was delayed until gestational day 9, 10, or 11 almost half of the observed surviving fetuses (57 of 118) manifested gross congenital abnormalities.

More recently Cheng, Bairnson, Rao and Subbammal(3) reported on the influence of various tocopherol-deficient diets on the incidence of teratogeny. When female rats raised on various E-deficient rations were subsequently bred and administered 2 or 4 mg doses of tocopherol on the 10th day of gestation, the incidence of malformations in the surviving 21-day-old embryos ranged from 12% to 64%. Similar findings have very recently been reported(4) for delayed supplementation with single doses of tocopherol ranging from 1 to 8 mg.

Earlier unpublished observations in our laboratory(5) on the effects of delayed supplementations of tocopherol to pregnant, deficient rats had indicated a much lower incidence of teratogeny than that noted by Cheng and co-workers. The present study was thus undertaken to check these findings and to see whether strain differences in rats might play a role in teratogeny.

Materials and methods. Two strains of albino rats were used in this study, one obtained from the Holtzman Co. in Wisconsin, the other a Sherman strain which had been inbred in our laboratory for many years. At 4 weeks of age, 50 to 60 females of each strain were placed on tocopherol-deficient or tocopherol-supplemented purified rations. The basal (deficient) diet consisted of 69.8% sucrose, 20% vitamin-free casein, 5% U.S.P. XIV salt mixture, 5% U.S.P. cod liver oil (containing 1700 I.U. vit. A and 170 I.U. vitamin D per gram), and 0.2% choline chloride. Vitamins were added in the following amounts, expressed as mg/kg diet: *p*-aminobenzoic acid, 25; inositol, 25; niacin 5; Ca pantothenate, 5; riboflavin, 2; folacin, 1.5; thiamine·HCl, 1; menadione, 1; biotin, 0.1. The tocopherol-supplemented diets contained DL α-tocopherol acetate at levels of 5, 10, or 20 mg per kilogram of diet. A few animals of each strain were maintained on a commercial pelleted chow.

After 3 months, when random animals on the deficient (basal) diet were bred and invariably displayed fetal resorption, all females were mated with males which had been maintained on the pelleted chow. Vaginal smears were obtained from each female daily while in the breeding cages, the appearance of sperm being taken as the criterion for the beginning of pregnancy.

When gestation had proceeded for 9 to 11 days, certain of the deficient females were given tocopherol orally in the form of a 10% aqueous solution of water-soluble d-α-tocopheryl polyethylene glycol 1000-succinate (TPGS).* The supplement, equivalent to approximately 10 mg of α-tocopherol, was administered by stomach tube.

On the 21st day of gestation the animals were sacrificed, the uteri removed and opened, the number of implantation sites noted, and the developed fetuses examined carefully for gross abnormalities.

Results and discussion. No significant differences were noted between the 2 rat strains in their susceptibility to teratogeny from vit. E deficiency; of the 11 abnormal fetuses found, 6 were of the Holtzman strain and 5

* Generously provided by Dr. P. L. Harris, formerly of Distillation Products, Inc., Rochester, N. Y.

TABLE I. Gestational Performance of Rats on Tocopherol-Free and Various Tocopherol-Supplemented Rations.

Diet	Gestational day of tocopherol supplement*	No. of rats bred	No. of resorbed fetuses	No. of normal fetuses	No. of abnormal fetuses
Basal	—	14	124	0	0
"	9	23	128	60	1
"	10	23	173	18	2
"	11	22	186	10	1
Suppl., .5 mg†	—	13	31	71	4
" 1 "	—	11	3	75	3
" 2 "	—	6	0	52	0
Pellet	—	7	0	59	0

* Equivalent to 10 mg of α-tocopherol, by stomach tube.
† mg α-tocopherol acetate per 100 g of diet.

of the Sherman strain. Accordingly the results are grouped together in Table I.

Our data differ strikingly from those of Cheng *et al*(2,3) in the incidence of fetal malformations. Of the 92 fetuses which developed in our deficient rats receiving tocopherol supplements on the 9th, 10th or 11th day of gestation, only 4 (or less than 5%) showed gross abnormalities. In the present study delayed supplementation was carried out using a larger dose of tocopherol (10 mg) than was employed by Cheng and her co-workers (1.2 to 4 mg), but their data conflict with respect to the protective effects of different tocopherol levels. On the one hand they report(6) a markedly higher incidence of abnormalities with a 4 mg level of supplementation than with a 2 mg level, yet in another paper(3) they note that raising the level of tocopherol administered to deficient rats on the 10th gestational day from 2 mg to 4 mg reduced the incidence of malformed young by from 8 to 49%, depending on the particular ration used.

In any event, Cheng's data(3) show that teratogeny was less influenced by tocopherol levels than by subtle and seemingly unrelated changes in diet. Thus the substitution in one diet of vitamin-free casein for crude casein reportedly reduced fetal malformations by 50% or more, and addition of 2% of cod liver oil to another diet caused a similar, but even more striking, reduction in abnormalities. Also, Cheng has reported(6) the ability of progesterone or estrone to completely abolish the malformations normally observed with the delayed administration of vit. E. Such drastic, yet inexplicable, fluctuations in the incidence of teratogeny in the offspring of rats receiving the same tocopherol supplementation make one wonder to what extent the reported malformations are, in fact, caused by tocopherol deprivation. In any event, it seems to us unlikely that a higher level of tocopherol dosage can account for the markedly lower incidence of malformations observed in our animals in comparison to theirs.

Our findings are not inconsistent, however, with the thesis that some degree of congenital abnormalities can result from vit. E restriction. Furthermore, our data suggest that the incidence of malformations in fetuses of rats reared on tocopherol-insufficient diets is as great or greater than that of rats raised on tocopherol-free diets but subsequently provided with ample tocopherol on the 9th, 10th, or 11th day of gestation. Whereas a dietary level of 2 mg of tocopherol per 100 g of diet was adequate for preventing fetal resorptions and abnormalities in our rats, when the level was lowered to 1 mg% or 0.5 mg% abnormalities were noted in 4% and 5% respectively, of the surviving 21-day-old fetuses.

Summary. Holtzman and Sherman strain female albino rats were raised on tocopherol-free or tocopherol-supplemented diets for 3 months, then bred to normal males. On the 9th to 11th day of gestation most of the depleted females received a tocopherol supplement by stomach tube. All animals were sacrificed on the 21st day of gestation and the uteri and fetuses examined for resorptions

and congenital malformations. Of the 92 developed fetuses produced by those rats receiving the delayed supplements, only 4—or less than 5% of the total—showed any gross abnormalities. This finding contrasts sharply with earlier reports of others, wherein almost half of the surviving fetuses were found to be defective. The reasons for these differences are not clear. The reproductive performance of rats raised and bred on a diet containing 2 mg of tocopherol per 100 of diet was normal, but when the tocopherol level was lowered to 1 mg or 0.5 mg the incidence of fetal abnormalities became 4 and 5%, respectively, of the developed young. No differences were observed between the two rat strains.

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Blood Gas Transport in Chinchillas.* (30379)

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The family of rodents knows as the chinchillidae have only a few species extant. These are known as the viscachas and chinchillas and they occur naturally in the mountains of South America. According to Walker (1) there are only 4 species of viscachas and a single species of chinchillas *(Chinchilla laniger brevicaudata)*. Chiodi(2) has reported experiments with this species. We have studied 2 species of chinchillidae(3). Two viscachas were caught in their natural habitat in Northern Chile at an altitude of 15,000 feet. Blood studies on these specimens were not very complete because of field laboratory limitations. A colony of chinchillas *(Chinchilla laniger brevicaudata)* has been maintained in our laboratory at Duke University where these animals have thrived in good condition. Several have been used in numerous respiratory studies with uniform results. While chinchillas have a superficial resemblance to hares and rabbits (Lagomorpha), they are true rodents (Rodentia).

Studies were made of the respiratory function of 8 male chinchillas. These involved measurements of oxygen capacity, hemoglobin concentration, hematocrit for relative volume of erythrocytes, number of erythrocytes per cubic millimeter, the position of the oxygen-dissociation curve, effect of changes in pH on the oxygen dissociation curve (Bohr effect) and the effect of oxygenation of blood on the carbon dioxide dissociation curve (Haldane effect).

Methods. Blood samples were drawn into disposable plastic syringes by direct cardiac puncture. About 8-10 ml of arterial blood was obtained. Animals were tranquilized by an intraperitoneal injection of a small amount of Nembutal. Minimal amounts of Heparin (Organon) were used as an anticoagulant. Blood samples were kept cold until used for tonometric equilibration. All analyses were completed in approximately 2 hours from time of blood withdrawal. Analytical methods and equilibration procedures have been described fully in a previous study of mammalian blood(4). They are not repeated here because identically similar methods and procedures were followed in the present studies.

Results of studies of chinchilla blood are shown in Fig. 1 and Table I. Fig. 1 shows the position of the oxygen dissociation curves of whole blood at 37°C. No attempt was

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