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Fatal Syndrome Associated with Vitamin E Status of Pregnant Rats.* (28646)

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The syndrome traditionally associated with Vit. E deficiency in the female rat is fetal resorption. Other reproductive disorders apparently related to Vit. E status include mortality of pregnant rats at parturition(1,2,3, 4). Stamler(1) noted that rats fed diets containing peroxidized fat from the 13th day of gestation seemed healthy until near the end of pregnancy, when they exhibited restlessness, pallor, labored breathing, bloody nasal exudate, and sometimes convulsions, followed by death. Hemorrhagic edema, massive pleural effusion, renal or adrenal damage, and varying amounts of intrauterine bleeding, together with well-developed fetuses, were common findings at death. Oral supplements of Vit. E begun as late as the 19th day of gestation or injection of tocopherol by the evening of the 21st day prevented death.

Armstrong and Swanson(2) reported fatal toxemia of pregnancy in rats fed a diet containing 25% dried autoclaved pork. The syndrome, characterized by lethargy, paleness, water retention, hepatic damage, and convulsions, could be prevented with wheat germ oil (unpublished data).

The effect of varying doses of Vit. E early in pregnancy on the outcome of gestation in Vit. E-deficient rats has been investigated.

Methods. Four groups of weanling female Wistar rats were depleted of Vit. E by a diet containing 66.5% cornstarch, 16.5% vitamin test casein,‡ 4% Hawk and Oser salt mix,‡ 1% NaCl, 2% non-nutritive fiber,‡ 5% Torula yeast,§ and 5% cod liver oil. Another group was maintained on a stock ration which had supported reproduction in the stock colony for many generations. Daily vaginal smears were begun when the animals were 9 weeks old, and the rats were mated to littermate males. One group (CClo+0) received no tocopherol. Other depleted animals were given, orally, a total of 2, 6, or 20 mg dl-α-tocopherol‡ in cottonseed oil on the 8th and 9th days after confirmation of positive mating. Vaginal smears were examined daily after the 10th day for appearance of blood as a sign of implantation. Hemoglobin concentration and hemolysis by dialuric acid were measured on the 21st day of gestation. After delivery or resorption, animals were anesthetized with sodium pentobarbital. Blood was removed from the vena cava and refrigerated immediately. The liver, kidneys, adrenals, and any remaining fetuses were removed and weighed, and a weighed portion of liver was frozen for analysis of fat and nitrogen. Uterus, lungs, and other organs were examined.

Hemolysis by a solution of dialuric acid (40 mg/100 ml) was determined by the method of Friedman *et al.*(5). Hemoglobin was measured as oxyhemoglobin in a spectrophotometer calibrated against blood of known oxygen capacity. The ferric chloride-α,α'-dipyridyl microdetermination of Quaife *et al.*(6) was used for estimation of serum tocopherol. The correction for absorption due to carotene was omitted because this substance is absent from rat blood. Non-protein nitrogen in a tungstate protein-free filtrate of serum, digested with sulfuric acid and hydro-

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TABLE I. Comparison of Reproductive Performance of Rats Fed Stock Ration with Those Fed a Casein-Cod Liver Oil Diet and Given 0, 2, 6 or 20 mg Tocopherol During Pregnancy.

Group (diet + mg tocopherol)	Outcome of gestation (No. of animals) Complete resorption	Live young	Dead young	Died at parturition	Length of gestation (days)	No. per litter	Living young (%)	Mean fetal wt (g)	Implantation index*
By treatment:									
CClo†+0	10	0	0	0	—‡	—	—	—	0
CClo+2	0	1	6	8	26.3 ± .4§‖	5.0 ± .8‖	6	4.0 ± .3‖	64 ± 10
CClo+6	0	2	9	4	26.2 ± .3‖	5.5 ± .7‖	20	4.3 ± .4	68 ± 8
CClo+20	0	2	9	1	26.9 ± .5‖	5.3 ± .8‖	15	4.2 ± .5	73 ± 6
Stock	0	10	0	0	22.9 ± .3	9.1 ± 1.2	97	4.9 ± .1	88 ± 4
By response:									
CClo+2,6 or 20	—	—	—	13	25.9 ± .3¶	6.6 ± .5¶	0	4.6 ± .3	88 ± 5
CClo+2,6 or 20	—	—	24	—	27.2 ± .2¶	3.9 ± .5¶	0	3.6 ± .4	52 ± 6¶
CClo+2,6 or 20	—	5	—	—	24.2 ± .4¶	8.6 ± .2¶	58	4.2 ± .2	94 ± 4

* No. fetuses/No. implanted × 100.
† Casein-cod liver oil.
‡ Maximum wt was attained on 20th day.
§ Mean ± standard error.
‖ Significantly different from stock controls ($P < .05$).
¶ Significantly different from others grouped by response ($P < .05$).

gen peroxide, was measured with modified Nessler's reagent. Homogenates of liver were analyzed for nitrogen by macro-Kjeldahl procedure and for total fat by 2 extractions with alcohol, hexane, and ether(7). Means were compared by the *t* test.

Results. Vit. E deficiency in animals fed the casein-cod liver oil diet led to fetal resorption. The effects on reproductive performance of giving tocopherol on the 8th and 9th days of gestation are shown in Table I. As little as 2 mg tocopherol permitted fetal growth to a mean weight of 4.0 g. However, in this group, CClo+2, only one animal delivered living young, and maternal mortality was 53%. Incidence of maternal deaths decreased as the amount of tocopherol was increased. The percentage of living young was slightly greater with 6 and 20 mg tocopherol than with 2 mg. Number of young, average weight of young, and implantation index were not affected by the amount of tocopherol. Stock animals delivered more and healthier offspring than rats fed the casein-cod liver oil diet. Degree of hemorrhage at implantation was often greater in depleted rats than in stock controls. Gestation was prolonged in Vitamin E-deficient animals which were given tocopherol on the 8th and 9th days of pregnancy.

Animals that died at parturition appeared normal until about the 21st day. Paleness and lethargy warned of illness. Nine rats died before, 2 during, and 2 shortly after parturition. When renal failure was indicated by anuria or hematuria for at least a day, anorexia, lethargy, and sometimes vaginal hemorrhage also preceded death. Convulsive attacks often followed periods of dyspnea in animals with accumulations of pleural fluid at time of death. Death was associated with slightly higher intake of water (77 ml/100 g body weight) between the 16th and 24th days than preceded resorption (64 ml) or delivery by animals fed CClo (72 ml) or stock ration (63 ml).

Living young were never delivered after the 25th day of gestation. Only animals with large litters delivered soon enough that some of the young were still alive at birth. The implantation index was least for rats which delivered small autolyzing fetuses about the 27th day after mating. Such dead fetuses did not appear to have been grossly abnormal during development.

It was not possible to relate hemoglobin concentration or dialuric acid hemolysis on the 21st day of pregnancy (Table II) with the amount of tocopherol given or with outcome of gestation, but all groups which received tocopherol showed less hemolysis than the group which received none. Blood cells of stock controls were not lysed by dialuric acid. Concentration of hemoglobin in Group CClo+0 was significantly higher than in groups given 2 or 6 mg tocopherol. Weight

TABLE II. Components of Blood on 21st Day of Gestation and at Autopsy; Weights of Spleen, Kidneys, and Adrenals, and Weights and Gross Composition of Liver.

	21st day		Autopsy								
Group	Dialuric acid hemolysis (%)	Hemoglobin (g/100 ml)	(Serum tocopherol) (mg/100 ml)	Serum non-protein nitrogen (mg/100 ml)	Final body wt (g)	Spleen (mg/100 g body wt)	Kidneys (g/100 g body wt)	Adrenals (mg/100 g body wt)	Liver wt (g)	Liver fat (%)	N in fat-free liver (%)
By treatment:											
CClo + 0	73 ±10*	10.1 ±.4	.02 ±.01§	46 ± 2	148	225 ± 11§	1.03 ±.03§	33.5 ± .6§	6.67 ±.26§	6.1 ± .8	3.47 ±.04§
CClo + 2	43 ± 9 (14)†	8.7 ±.3	.10 ±.02§ (12)	77 ± 3‡ (6)	143	222 ± 16	1.26 ±.05§	44.3 ± 3.6§	7.35 ±.32§	11.6 ± 1.8§	3.29 ±.04§
CClo + 6	25 ± 8 (13)	8.9 ±.3 (14)	.07 ±.01§ (13)	73 ± 8 (10)	145	236 ± 14	1.14 ±.05§	34.2 ± 2.4	7.30 ±.34§	12.4 ± 1.0§	3.31 ±.06§
CClo + 20	28 ±10	9.5 ±.4	.08 ±.01§ (11)	77 ± 7 (10)	141	244 ± 17	1.11 ±.05§	35.2 ± 2.3	7.00 ±.39§	12.6 ± 1.8§	3.33 ±.06§
Stock	.4± .4 (9)	9.5 ±.5	.60 ±.14	68 ± 3 (8)	196	216 ± 17	.75 ±.02	30.5 ± .6	8.27 ±.26	5.2 ± .2	3.61 ±.03
By response:											
Died (CClo + 2, 6 or 20)	41 ± 9	8.6 ±.3	.15 ±.01‖ (7)	339 ± 53‖ (3)	140	178 ± 11‖	1.38 ±.04‖	50.2 ± 3.1‖	7.21 ±.37	11.3 ± 1.2	3.19 ±.05‖
Delivered dead young (CClo + 2, 6 or 20)	30 ± 7 (21)	9.3 ±.2 (23)	.07 ±.01	77 ± 4 (22)	146	265 ± 9	1.08 ±.06	32.2 ± 1.4	7.08 ±.24	11.3 ± 1.2	3.36 ±.04‖
Delivered live young (CClo + 2, 6 or 20)	20 ±13	8.5 ±.6	.06 ±.01	68 ± 8 (4)	139	225 ± 5	1.08 ±.04	35.5 ± 1.3	8.01 ±.59	18.8 ± 2.1‖	3.33 ±.08

* Mean ± standard error.
† No. of animals, if different from Table I.
‡ Excluding animals that died at parturition.
§ Different from stock controls ($P < .05$).
‖ Different from others grouped by response, or from each other ($P < .05$).

had begun to decrease by the 21st day in resorbing animals, so the hemoglobin measurements may reflect disappearance of increased blood volume associated with pregnancy. Hemoglobin concentrations of pregnant animals fed CClo were not different from those of pregnant stock controls on the 21st day.

Concentrations of serum tocopherol at time of autopsy were very low in all animals fed CClo, compared with stock animals. Groups given 2, 6, or 20 mg tocopherol had significantly higher concentrations than those which received none. Animals in Group CClo+0 had the lowest concentrations of serum non-protein nitrogen. At the time of death, blood was taken from 3 eclamptic animals fed CClo+2; massive renal hemorrhage was observed, and concentration of non-protein nitrogen in the blood was very high.

A striking consequence of maintaining pregnancy through dosage with tocopherol was the accumulation of fat in the liver (Table II). Abnormally high concentrations of hepatic fat were not observed in resorbing animals or in pregnant stock controls. Livers of stock animals were smallest in relation to body size and had the highest concentrations of nitrogen in fat-free tissue. Total hepatic nitrogen per 100 g body weight was similar for all groups, ranging from 144 to 149 mg. These data suggest that livers of animals which had received tocopherol, particularly those of toxic rats, had abnormally high content of water as well as fat. Frequency of inanition in prolonged gestation makes it unlikely that increases in non-lipid, non-protein components of liver were attributable to accumulations of glycogen.

Spleens of eclamptic rats were smaller and their kidneys and adrenals larger than those of animals which survived pregnancy while consuming the casein-cod liver oil diet. However, all animals fed the casein-cod liver oil diet had larger kidneys and adrenals, relative to body size, than rats fed stock ration.

Discussion. The direct effect of tocopherol on the outcome of gestation in Vit. E-deficient rats confirms suggestions that eclamptic syndromes of pregnant rats may be related to Vit. E status(1,3).

Prolongation of gestation with delivery of dead fetuses has been associated with Vit. E deficiency(8). Similar delay in onset of labor, with excessive hemorrhage and death of young, has resulted from essential fatty acid deficiency(9). To minimize oxidative changes of dietary fat in the present experiment, diets were prepared frequently and stored under refrigeration; leftover food was discarded at least every other day. However, some peroxidation of cod liver oil, and of its essential fatty acids, may have occurred. Indeed, the apparent water retention, changes in membrane permeability (shown in pleural effusion), and renal damage are reminiscent of essential fatty acid deficiency. If this syndrome is related to essential fatty acids, it must result from oxidative or other changes which are inhibited in the presence of tocopherol. Data from our laboratory have shown that, when the casein-cod liver oil diet was supplemented with tocopherol 3 times weekly throughout pregnancy or with 1 mg tocopherol daily after the 8th day of gestation, delivery was delayed until the 24th to 26th day, about half the young were stillborn, and livers were fatty, but parturition itself proceeded normally. These observations confirm those of Stamler(1) previously cited.

Feeding diets containing erucic acid for 16 weeks or longer resulted in interference with parturition and occasional death of the mother in labor(10). The syndrome was originally attributed to possible interference with metabolism of essential fatty acids; later it was observed that Vit. E, but not sources of essential fatty acids, prevented testicular atrophy in male rats and improved fertility and lactation in females fed erucic acid(11). Therefore, Noble and Carroll suggested that tocopherol deficiency might have been induced(11). Stamler's(1) inability to produce eclampsia with a diet containing oleic acid may have been due to the relatively short period during which the diet was fed. Depletion of Vit. E would occur less rapidly with monounsaturated fats than with polyunsaturated fatty acids, provided by corn oil or cod liver oil.

Although hemoglobin concentrations of eclamptic animals on the 21st day were not

different from those of other animals fed CClo, anemia apparently developed after that time. Eclamptic rats were dyspneic, pale, and weak, and had small spleens. Anemia attributable to Vit. E deficiency has been observed in monkeys and in human infants (12).

Excessive uterine hemorrhage was observed in eclamptic rats. Large amounts of Vit. A, and particularly Vit. A acid, can reduce prothrombin levels(13). Ten g of the casein-cod liver oil diet daily would provide almost 1000 I.U. of Vit. A per day, and oxidation of the vitamin might occur in a diet containing cod liver oil without added antioxidants. However, Vit. E-responsive toxemia has been produced by diets in which the amounts of Vit. A have not been excessively high(1,2).

Occurrences of the toxic syndrome and of fatty liver in pregnant rats, and enlargement of adrenal glands in eclampsia point to possible roles of hormones. Non-gravid animals did not show gross symptoms associated with the fatal syndrome, nor did the CClo diet *per se* induce fatty liver. Relationship of reproductive performance in Vit. E-deficient rats to altered function of the pituitary has been postulated(8,11).

The eclamptic syndrome appears to require a particular degree of Vit. E depletion at a specific stage in fetal development. This can be achieved by very rapid depletion with an "anti-Vit. E stress diet" in the last half of pregnancy(1), by slow depletion of Vit. E stores by a diet containing pork fat(2) or erucic acid(10), or by giving tocopherol to chronically depleted rats at a critical time in fetal development, as reported here.

Summary. Female albino rats, depleted of Vit. E for at least 5 weeks before mating, were given total oral supplements of 0, 2, 6, or 20 mg dl-α-tocopherol on the 8th and 9th days of gestation. Their reproductive performance was compared with controls fed stock ration. The casein-cod liver oil diet (CClo) caused fetal resorption, lowered concentrations of tocopherol in serum, and induced susceptibility to hemolysis in the presence of dialuric acid. Oral supplements of tocopherol on the 8th and 9th days of gestation permitted fetal development to continue, but did not insure normal birth. Gestation was prolonged and most of the young were stillborn. Maternal mortality at parturition was 53%, 27%, and 8% for groups given 2, 6, and 20 mg tocopherol. Death followed lethargy, pallor, dyspnea, and vaginal hemorrhage. Small spleen, large hemorrhagic kidneys, and large adrenals (relative to body weight), accumulation of pleural fluid, and high serum concentration of non-protein nitrogen were characteristic of animals which died at parturition. Hepatic fat of animals fed CClo and given 2 doses of tocopherol to maintain pregnancy was higher than of stock controls or of resorbing rats fed CClo.

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