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Relationship of Time of Therapy to Teratogeny in Maternal Avitaminosis E[1]

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Introduction

The literature concerning teratogeny that is attributable to faulty nutrition of laboratory induced origin was reviewed in a previous publication (Thomas and Cheng: 1952). Any review of this subject leads to the conclusion that there are numerous conditions of nutritional insufficiency which produce congenital abnormalities. Until reported recently (Thomas and Cheng, 1952) none had been ascribed to avitaminosis E. The usual abnormality in reproduction which prevails among pregnant rats in an advanced state of avitaminosis E is fetal resorption.

Adequate vitamin E therapy administered promiscuously is not sufficient to circumvent reproductive difficulties. Timing of therapy is an exceedingly important limiting condition. Therapy that is adequate when given during the first week of gestation in the rat frequently will fail if delayed until the second and third weeks of gestation. When given during the second week uterine responses will be variable. Thus, in a single uterus some feti will be resorbed, others will have developed congenital abnormalities, and still others will appear externally morphologically normal. When therapy is given during the third week the embryological contents of the uterus will be partially or completely resorbed.

The purpose of this report is to show more specifically than was possible at the time of our original announcement (Thomas and Cheng, 1952) the relationship of time of therapy to incidence of teratogeny in nutritional avitaminosis E. Also, data are presented summarizing the incidence of the different types of abnormalities thus far observed which are a part of this syndrome.

Material and Methods

That part of this study dealing with nutrition has been limited to certain phases of the reproductive performance of female rats markedly depleted of their reserves of vitamin E. Advanced depletion was attained by restricting young stock colony females of Sprague-Dawley origin from weaning time (45 grams and approximately 21

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days of age) to maturity (200 grams) to a vitamin E-depleting ration. This basal ration consisted of dextrinized starch, 49; lard, 22; casein, 18; dried brewer's yeast, 5; salt mixture (U.S.P. No. XIV), 4; and cod liver oil, 2 per cent. The ration was made granular by sieving after formulating and mixing. It was aged in the granular state for two weeks prior to feeding to enhance inactivation of any residual vitamin E present. An amount of ration was made each time to last only two weeks. Restricting females from weaning time to maturity to aged ration having this composition has always produced in previous experiments 100 per cent fetal resorptions during first pregnancies.

When each female weighed approximately 200 grams, she was transferred to a special cage to be mated to proven males maintained in service in the stock colony. All females placed in mating cages were "vaginal-smeared" daily until mated. When sperms were observed in the smear the female was allotted randomly to one of the 11 groups comprising this study. The day on which the sperms were noted has been arbitrarily considered the zero day of gestation which is in agreement with the reference point from which Nelson et al. (1952) started to count the days of gestation in rats used in similar studies. Embryonic implantation was assured by the appearance of red blood cells in vaginal smears made daily from the 11th to 15th days of each gestation.

The bred females used in this investigation were allotted randomly to 11 groups. Group 1 received only the aforementioned basal ration at all times and served as the negative control. Groups 2 through 10 were fed likewise, except that each female received via stomach tube a single dose of 1.2 mg. of d,l-alpha-tocopherol acetate, diluted to 0.6 ml. with Mazola, the 4, 5, 6, 7, 8, 9, 10, 11, or 12th day of gestation. Group 11 served as the positive reference group and received only stock colony ration at all times.

All females were housed in metal cages designed to minimize coprophagy and facilitate ease of cleaning, feeding, watering and weighing. Rations and water were offered ad lib. at all times. All females were weighed weekly from the time of weaning until they weighed 200 grams. Also, they were weighed the day of positive mating and on the 7, 14, and 21st days of gestation.

When each female reached the 21st day of gestation she was laparotomized and the uterus removed completely, weighed, incised longitudinally along the antimesometrial pole and a record made of the number and extent of resorptions; and number, condition and weight of dead and live feti. All fetal specimens pertinent to

this problem were preserved or appropriately processed for future examination macroscopically or microscopically.

Many feti illustrating typical abnormalities were "cleared". In cleared feti the soft tissues were rendered essentially transparent by aging them in an aqueous solution of one per cent KOH and subsequently preserving them in glycerine. The skeletal tissues were stained simultaneously with alizarin red S, thereby making them less difficult to examine macroscopically.

Results and Discussion

The usual abnormality in reproduction associated with female rats in an advanced state of avitaminosis E is fetal resorption. This disturbance is fairly easily overcome by appropriate therapy. However, when treatment is not properly timed during gestation other complications frequently occur. Some of these are in the nature of congenital abnormalities. The relationship of their incidence to time of therapy and the general nature of these congenital malformations will be described below.

The most important point to be noted from the data summarized in Table I is the occurrence of abnormal feti at term in the uteri of females given a single dose of 1.2 mg. d,l-alpha-tocopherol acetate on the 9, 10, 11, or 12th day of gestation, and the absence of abnormalities in feti when identical therapy was administered on

Table I.

Reproductive performance of avitaminosis E female rats given a single dose of 1.2 mg. d,l-alpha-tocopherol acetate on different days during gestation.

Group no.	Day of gestation tocopherol given	Conceptions	*Females having abnormal feti	*Females having no abnormal feti	Average wt. increase per female during gestation
	day	no.	no.	no.	Gm.
1	**Neg.	21	0	0	26
2	4	21	0	21	49
3	5	23	0	23	46
4	6	22	0	22	48
5	7	19	0	19	51
6	8	21	0	21	55
7	9	32	9	23	44
8	10	27	7	20	41
9	11	28	7	21	51
10	12	20	2	18	44
11	**Pos.	35	0	35	105

*Normality or abnormality based on macroscopic morphological appearance

**Groups 1 through 10 received basal E-depleting ration. Group 1 did not receive tocopherol supplement—other groups did. Group 11 received stock colony ration.

the 4, 5, 6, 7, or 8th day of gestation. These are not chance observations since they are based on a fairly sizeable number of conceptions. In addition, Table I shows that positively mated females increased weight rather poorly when limited to the basal ration only. This was due to several circumstances of which concurrent fetal resorption would appear to exert the greatest influence. The increase in weight of females was enhanced greatly when they received tocopherol therapy during pregnancy. Nevertheless, this greater increase was only about half that made by the positive control females which received stock ration Whether these differences in gain in weight are due primarily to differences in number of feti, better nutrition, or both is not known definitely. Information bearing on this is forthcoming.

The data in Table II summarize the outcome of fertilized embryos implanted in females given vitamin E therapy on certain days during gestation. When a single dose of 1.2 mg. of the vitamin was given on the 4, 5, 6, 7, or 8th day of gestation a small number of feti were dead and morphologically normal at term, a much larger number were live and normal, and a very much larger number were completely or partially resorbed. Important in this study is the observation that in none of these females were congenital abnormalities observable. On the other hand when identical supple-

Table II.

Condition of feti at term in avitaminosis E rats given a single dose of 1.2 mg. d,l-alpha-tocopherol acetate on different days during gestation.

Group	Day of gestation tocopherol given	Condition of feti				Average wt. of live feti	
		Dead		Live			
		Resorbed	Not resorbed	*Normal	*Abnormal	*Normal	*Abnormal
no.	day	no.	no.	no.	no.	Gm.	Gm.
1	**Neg.	208	0	0	0	....	....
2	4	196	8	27	0	4.4	....
3	5	208	4	37	0	3.9	....
4	6	208	8	38	0	4.6	....
5	7	147	4	43	0	4.1	....
6	8	150	6	59	0	4.7	....
7	9	244	6	45	15	4.2	3.9
8	10	231	4	2	23	3.5	2.8
9	11	232	3	14	19	3.6	3.2
10	12	211	0	1	2	3.1	3.3
11	**Pos.	24	0	347	0	5.2	....
Total		2059	43	613	59		

*Normality or abnormality based on macroscopic morphological appearance.
**Negative and postive control females.

mentation was administered on the 9, 10, 11, or 12th day of gestation an appreciable number of feti having single or multiple abnormalities occurred. Simultaneously, the number of normal and dead feti decreased while that of resorptions remained essentially unchanged, though surprisingly large.

The reproductive performance of the females in the negative and positive control groups was as anticipated; namely, complete fetal resorption and nearly 100 per cent normal feti, respectively. Also, the data in Table II show that the average weight of the feti in the positive control group was larger than that for live full-term morphologically normal or abnormal feti from females which had received the basal ration and supplementation. The data in both Tables I and II stress the importance of time of supplementation during gestation to incidence of teratogeny in maternal avitaminosis E.

In Table III data summarizing the incidence of several types of external abnormalities are presented. In the 59 abnormal feti produced there were 108 gross abnormalities observed. Most of them appeared in feti from dams that had received vitamin E supplementation on the 10th or 11th day of gestation, while approximately one third and one fifteenth as many appeared in feti from mothers given their supplement on the 9th or 12th day of gestation.

Table III.

Relationship of time of d,l-alpha-tocopherol therapy during gestation in rats to incidence of congenital abnormalities in maternal avitaminosis E.

Type of abnormality	*Day of gestation therapy administered and number of abnormal feti					Total
	**0-8(0)	9(15)	10(23)	11(19)	12(2)	59
Unbilical hernia	0	1	17	9	1	28
Hydrocephalus	0	11	2	4	1	18
Exencephalus	0	2	5	10	1	18
Rigid ankle	0	0	6	4	0	10
Receding maxilla	0	0	4	5	0	9
Hare lip	0	1	4	1	0	6
Edema	0	0	2	3	0	5
Cleft palate	0	0	3	0	0	3
Ectocardia	0	0	0	3	0	3
Kinked tail	0	0	0	3	0	3
Syndactylism	0	0	3	0	0	3
Receding mandible	0	0	1	1	0	2
Total	0	15	47	43	3	108

*Therapy consisted of a single dose of 1.2 mg. d,l-alpha-tocopherol acetate per female.

**Figures in parentheses denote number of abnormal feti.

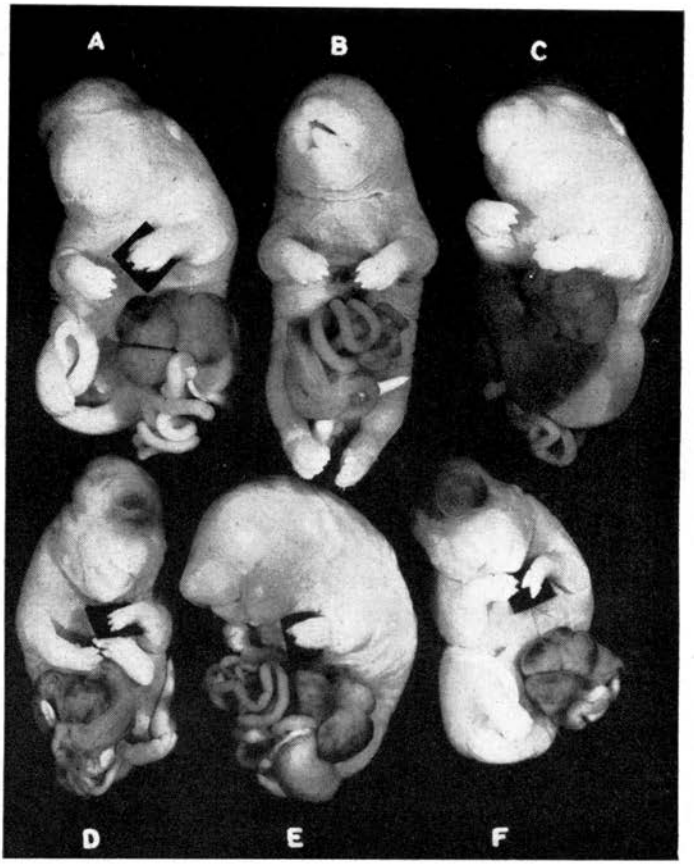

Fig. 1. Representative specimens of 21-day old abnormal rat feti from vitamin E deficient dams fed 1.2 mg. of d,l-alpha-tocopherol acetate on the 9, 10, 11, or 12th day of gestation; illustrating
A, kinked tail.
B, unilateral harelip.
C, ectocardia.
D, E, F, syndactylism.
A, C, D, F, exencephalus.
A thru F, unbilical hernia.

The principal types of gross fetal abnormalities which occurred are listed in Table III. Several of these are illustrated in Fig. 1. Those which occurred most often are umbilical hernia, 28; hydrocephalus, 18; exencephalus, 18; rigid ankle, 10; and receding maxilla, 9 times. Abnormalities which occurred less frequently are receding mandible, 2; syndactylism, kinked tail, ectocardia, cleft palate, 3 times each; edema, 5; and harelip, 6 times. Whether or not one type of abnormality is produced more frequently on the 9, 10, or 11th day of supplementation remain undetermined for the present. Later, when many more cases have been obtained a more

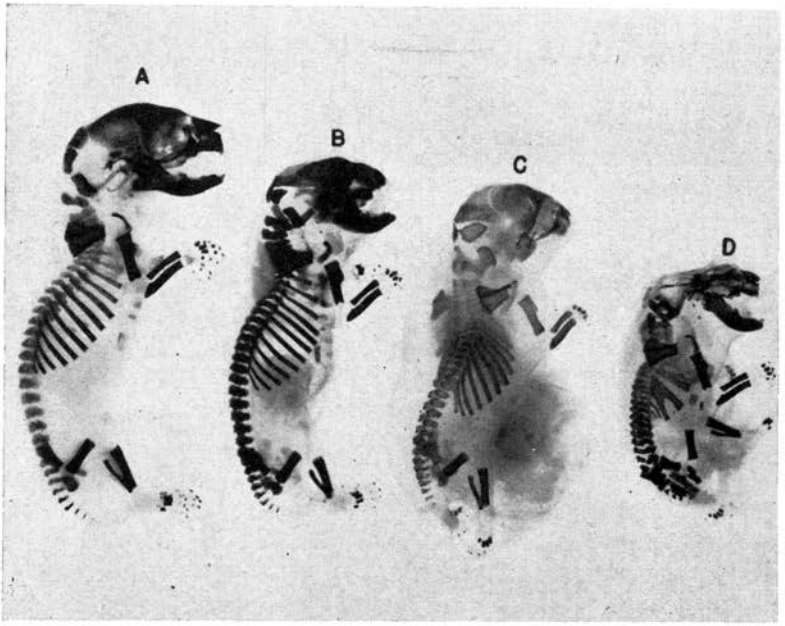

Fig. 2. Lateral skeletal views of representative "cleared" rat feti; illustrating
A, normal skeleton.
B, harelip and incomplete ossification of the parietal bones.
C, receding mandible.
D, absence of some of the cranial bones in exencephalus, and fusion of ribs.

exact appraisal may be possible. At present the data suggest that umbilical hernia, hydrocephalus, and exencephalus may occur more frequently on the 10, 9, and 11th days of supplementation, respectively.

In addition to the twelve types of abnormalities listed in Table III, there are those which involve various parts of the skeleton only. The data in Table IV summarize the incidence of gross skeletal abnormalities which appeared in 38 abnormal and 29 macroscopically normal feti from females comprising groups 2 through 10. Some of these abnormalities are illustrated in Fig. 2. The incidence of skeletal abnormalities which occurred in the obviously abnormal feti is much greater than in the case of the macroscopically normal appearing feti.

Gross skeletal abnormalities occurred in the frontal, parietal, supraoccipital, maxilla and mandible bones of the head; the ribs, sternebrae, and vertebral column of the trunk; and the digits of the anterior and posterior extremities.

Certain types of abnormalities occurred with surprisingly similar frequency in both the abnormal and macroscopically normal feti.

Table IV.

Frequency of gross skeletal abnormalities in cleared full-term rat feti from laparotomized avitaminosis E dams.

Part of body involved	Macroscopic classification			
	*Abnormal feti		*Normal feti	
	no.	%	no.	%
Head:				
Frontal bone	15	39		
Parietal bone	30	79	15	52
Supraoccipital bone	15	39		
Maxilla	5	13		
Mandible	1	3		
Trunk:				
Vertebral column	10	26		
Ribs	10	26		
Sternebrae	37	98	28	97
Upper extremities:				
Digits	5	13	7	24
Lower extremities:				
Digits	29	76	21	72
Total feti examined	38		29	

*Feti from dams given a single dose of 1.2 mg. of d,l-alpha-tocopherol acetate on the 9th, 10th, 11th, or 12th day of gestation.

They involved the sternebrae, 97 and 98 per cent; parietal bones, 52 and 79 per cent; and the digits of the lower extremities, 72 and 76 per cent (Table IV). Conspicuous among the abnormalities of the sternebrae was the variation in the number of ossification centers. These ranged from none to the normal number of six. Even when six sternebrae were present there were instances when the halves of each were separated, indicating retarded ossification. Also, when the sternebrae were fewer than six the halves frequently were separated terminating ultimately in their irregular fusion. The skeletal abnormality next most common occurred in the parietal bones. Parts of these were either absent or ossification was markedly subnormal. These conditions appeared more commonly in those feti exhibiting either hydrocephalus or exencephalus, although it was by no means limited to hydrocephalic or exencephalic feti. The third most frequently occurring abnormality appeared in the digits of the hind feet of both the abnormal and macroscopically normal feti. It was manifested principally by the absence of ossification in either the middle or both the middle and distal rows of digits.

Skeletal abnormalities appearing less frequently occurred in 1)

the frontal and supraoccipital bones where ossification was retarded or absent; 2) the spinal column where abnormal curvature to the right was more common than to the left; 3) the ribs where two or more on either the right or left side often were fused into a plexus-like mass; 4) the maxillae and mandibles which failed to attain their full length; and 5) others also listed in Table IV.

The foregoing presentation clearly demonstrates that erroneous timing of therapy in maternal avitaminosis E produces a variety of congenital abnormalities, many of which are similar or identical to those reported by other workers for other avitaminoses (Thomas and Cheng, 1952). Whether or not the results here reported are uncomplicated by allied nutritional shortcomings has not yet been demonstrated. The persistence of fairly large numbers of resorptions, and the failure of the feti to attain sizes at term comparable to those reached by the feti on the positive control ration in spite of vitamin E therapy suggest contributory complications. Data bearing on these points are being accumulated. Challenging are the questions of whether or not the severity of vitamin E depletion will 1) increase the incidence of abnormalities, and 2) shift the time during pregnancy when the administration of a single dose of vitamin will produce the greatest percentage of abnormal feti. It is our hope that the use later in this study of 1) a satisfactory physiological inhibitor of vitamin E, and 2) a basal vitamin E depleting ration composed entirely of synthetic ingredients, will aid in answering these questions.

Summary

The usual abnormality in reproduction ascribed to female rats in an advanced state of avitaminosis E is fetal resorption. Therapy per se will not always correct this disturbance, but sometimes will induce associated complications. Whereas vitamin E therapy will reduce the occurrence of resorptions it is unable, under certain circumstances, to prevent the occurrence simultaneously of congenital abnormalities. The timing of therapy greatly influences the incidence of morphological normality or abnormality of the products of conception. Adequate vitamin E therapy administered during the 1) early 2) middle or 3) late stages of gestation will produce 1) a very large percentage of normal feti, few resorptions and no congenital abnormalities; 2) many feti with gross external and skeletal abnormalities and many resorbed feti; and 3) essentially 100 per cent resorbed feti and none with congenital abnormalities; respectively. When therapy is administered on the 9, 10 or 11th day of gestation the uterine contents of a single conception frequent-

ly will include resorbed and dead feti, live feti having single or multiple morphological abnormalities, and macroscopically normal-appearing feti. The abnormalities resulting from avitaminosis E involve both the soft and bony tissues.

Most abnormalities observed to date fall into the following classifications: umbilical hernia, hydrocephalus, exencephalus, rigid ankle, receding mandible and maxilla, harelip, cleft palate, ectocardia, kinked tail, syndactylism, and gross changes involving numerous parts of the skeletal system. The skeletal abnormalities include disturbances such as complete or partial absence of certain bones, retarded ossification, curvature of the vertebral column and fusion of certain ribs. Umbilical hernia, hydrocephalus, exencephalus, and skeletal abnormalities involving the parietal bones, sternebrae, and digits of the posterior extremities occurred most frequently.

Detailed studies of some of the broader aspects of this research are underway.

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