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A study of the occurrence of teratogeny in vitamin E-deficient rats and associated abnormalities in blood and tissues

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A STUDY OF THE OCCURRENCE OF TERATOGENY IN VITAMIN E-DEFICIENT RATS AND ASSOCIATED ABNORMALITIES IN BLOOD AND TISSUES

by

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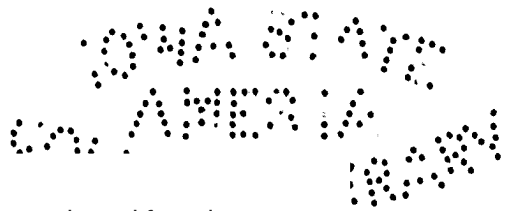

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I. INTRODUCTION

Vitamin E, one of the fat-soluble vitamins, has been shown to be essential to the well-being of several species of animals such as the mouse, rat, guinea pig, chick, lamb, goat, calf, rabbit, duckling and dog. It is widely distributed in nature and is comparatively stable. Even so, sporadic occurrences of disturbances due to vitamin E deficiency have been reported among certain farm animals. The predominant symptom of avitaminosis E in herbivorous animals is muscular dystrophy as exemplified by stiff-lamb disease. In poultry the predominant symptom is encephalomalacia; in the rat it is reproductive failure. The outward manifestations of vitamin E deficiency do not develop rapidly. Consequently, in many instances the effects on the organism are profound and for the most part irreparable.

Most of our knowledge about this vitamin and the effects of avitaminosis E have been obtained in the research laboratory. This has led to successful prophylaxis and treatment of both encephalomalacia in chicks and stiff-lamb disease in the field. More widely known, and mainly of academic interest, is the occurrence of fetal resorption in experimental rats exhaustively depleted of vitamin E. For instance, female rats thus depleted fail to produce young in spite of conception due usually to partial or total resorption of the

feti. An entirely new finding associated with this phenomenon is the production of live feti having few to numerous congenital abnormalities involving a variety of organs and tissues.

Teratogeny is defined as the production of congenital malformations or structural defects in the feti, and these usually are apparent for the first time at birth. The causes of such defects are either genetic or environmental. Some of the known congenital abnormalities due to inheritance are polydactylism, albinism, brachydactylism, cleft palate and hare lip. Among the environmental causes perhaps those associated with nutrition occur most frequently. Adequate nutrition of the embryo is an indispensable requirement for normal development. Inadequate consumption of one or more essential nutrients, namely, those which cannot be synthesized by the mother or the fetus, may produce pathological manifestations in the latter. However, not every type of maternal nutritional deficiency leads to congenital abnormalities. Tissue exhaustion of an essential element or general starvation of the mother immediately prior to conception often causes total sterility. Induced deficiencies attaining a maximum depletion during the various stages of gestation may result in fetal resorption, stillborn, or abortion of the feti. It appears that borderline deficiencies probably

are most favorable for the production of congenital abnormalities.

A borderline deficiency in rats can be produced in either of two ways. One is by administering during gestation a specific vitamin antagonist to rats receiving a ration adequate for normal nutrition. Another is by restricting female rats to a ration deficient in the vitamin for a sufficient length of time prior to mating and then fortifying the diet with the vitamin at a critical time during gestation. The first method has been employed by Nelson et al. (1952) while the second was used in this investigation.

Many congenital abnormalities have been produced in animals experimentally by omitting single nutritional elements from the diets of the mother. For instance, goiter can be produced experimentally in rat feti by restricting the mother to a diet deficient in iodine. Ewes fed ration deficient in copper often give birth to abnormal lambs exhibiting a condition known as "swayback". Among vitamin deficiencies, the congenital blindness in pigs due to vitamin A deficiency is an example. Experimental congenital abnormalities produced by a deficiency in one of the following vitamins; namely, A, D, thiamine, riboflavin, biotin, pyridoxine, pantothenic acid, niacin, folic acid and B_{12}, have been reported. However, until recently

(1952, 1953) no report has appeared with regard to the experimental production of congenital abnormalities due to maternal avitaminosis E.

Vitamin E, alpha-tocopherol, is a unique nutrient as far as rats are concerned because exhaustive maternal depletion always leads to reproductive failure even though coitus and implantation occur normally. Supplementing the deficient diet with adequate vitamin E even as late as during the early one fourth of the gestation period normal young are delivered. It is possible that when vitamin E supplementation is given during the organogenetic period of gestation, the deficiency state is only partially corrected and adversely affects the formation of the organs in the embryonic state. Whenever an attempt is made during this critical period to correct the effects of the deficiency, one or more of the various organs may resume growth. Consequently the mother may give birth to viable young with malformations. Whatever the theory may be, it is important to be able to define the conditions for the production of such congenital abnormalities due to vitamin E deficiency. Some of these were reported first by Thomas and Cheng (1952), and more recently by Cheng and Thomas (1953). A detailed study of the conditions giving rise to these abnormalities as well as the nature of the abnormalities induced formed the basis of this dissertation.

II. REVIEW OF LITERATURE

A. Vitamin E

Mattill and Conklin (1920) as early as 1920 reported that female rats fed on cow's milk could not bear or raise young even when the milk was supplemented with yeast and iron. Evans and Bishop (1922) demonstrated the existence of a vitamin whose deficiency caused this effect. At the time they called it "vitamin X", which was later changed to vitamin E (Sure, 1924). Volumes have been written on this vitamin since its first discovery. This review deals primarily with those aspects of the vitamin that have some bearing on the present investigation.

1. Nutritional and physiological importance

A fuller understanding of the nutritional and physiological importance of vitamin E has been a challenge of many scientists of different training and background, and it is still the goal of many investigators in the field. In the pages immediately following are presented the important results of published information that have a bearing on this phase of the review of literature.

a. Effect on growth. The effect of vitamin E on growth has been studied by a number of investigators. Some have found that vitamin E deficiency depressed growth while others have observed normal growth. Retardation of growth has been shown to be one of several characteristic manifestations of vitamin E deficiency in the rat. After two to four months on a vitamin E-deficient diet the growth of the rat reaches a plateau (Emerson and Evans, 1937; Olcott and Mattill, 1937; Nelson et al., 1940). The administration of vitamin E at any time after cessation of growth elicits a definite growth response.

When the guppy fish, Lebistes reticulatus, was reared on a vitamin E-deficient ration, growth was inhibited or stopped (Cumings, 1942). Results of the addition of vitamin E on growth of bacteria and frogs show that this vitamin enhances growth. When agar plates containing 3 mg. of vitamin E in a lecithin-oil-vitamin suspension were inoculated with Escherichia coli and incubated at 37°C for 24 hours, the resultant cultures had colonies which were larger and more vigorous than those grown on analogous E-free agar-lecithin-oil media. There were 1740 million bacteria per cc. for the vitamin medium as compared with 1280 for the non-vitaminized medium (Trasino and Traverso, 1947). Larvae of the frog, Bufo vulgaris, when treated daily with 1 mg. vitamin

E in olive oil solution, increased in growth leading to an earlier metamorphosis (Martella, 1949).

Dietary fat has a definite influence on the growth pattern. Large amounts of fat in E-deficient diets accelerate adolescent growth (Olcott and Mattill, 1937), and elimination of fat induces an earlier and more consistent growth plateau (Gottlieb et al., 1943).

Body growth in the mouse is not influenced by lack of vitamin E (Goettsch, 1942), which is in keeping with its greater resistance to deprivation of the vitamin.

b. Role of vitamin E in fetal nutrition. As early as 1922 Evans and Bishop showed that a lack of vitamin E in the diet of the rat resulted in failure of reproduction. Resorption of the feti occurred in the females, yet they appeared healthy; and maintained normal estrus rhythm, normal ability to conceive and normal implantation (Evans et al., 1927; Urner, 1931). Abnormality of the estrus cycle and failure of implantation followed only very prolonged deprivation when gross pathological changes were visible in the uterus (Wiesner and Bacharach, 1937; Martin and Moore, 1939; Kaunitz and Slanetz, 1947).

According to Evans (1932), vitamin E is not necessary for the early phases of reproduction. He showed that it was still possible to obtain normal termination of pregnancy by

the administration of vitamin E as late as the fifth day after insemination. Up to the tenth day of gestation the pregnant uterus appeared normal on gross examination, and serial sections of embryos also showed normal development (Urner, 1931). However, on the tenth day the pregnant uteri began to show changes due to vitamin E deficiency. They developed a softening of the implantation sites which showed a blue discoloration due to the blood in the amniotic cavity. Sections of the embryos revealed rarefaction of the mesenchyme and failure of the blood forming-tissues, the blood islands. There were changes in the ectoplacenta as evidenced by the more compact appearance of the fetal portion and failure of mesodermal elements. By the thirteenth day, necrosis began on the fetal surface. The decidua basalis retained its normal appearance until it degenerated on the fifteenth day. By the end of the 21st day only the implantation sites were retained indicating the preceding pregnancy (Urner, 1931).

Thus it has been found by study of the histopathology of "E-free-gestations" (Evans et al., 1927) that it is precisely those embryonic tissues important for fetal nutrition and metabolism which show abnormalities in their development. From the time of implantation on the 6th day of gestation until the appearance of the yolk sac on the 9th day all exchange of nutrients and waste products takes place through the embryotrophe filling the decidual cavity. From the time

of the appearance of the yolk sac substances going to and from the embryo must pass through this structure either by diffusion or special secretion. By this mechanism all exchanges are normally carried on until the 11th day when the penetration of the trophoblast by the umbilical vessels provide channels for close contact between the fetal and maternal blood. From this time on the function of nutrition and respiration begins to be performed more and more through the allantoic route even though the blood vessels on the yolk sac may be considered to perform similar functions.

The yolk sac in "E-free-gestations" shows a general flatness of structure and poor development of villi on its outer surface. It would seem reasonable to suppose that a larger part of the underdevelopment of "E-free-embryos" can be accounted for by starvation due to interference with paraplacental nutrition. Abnormalities in the yolk sac can also be held responsible for the early fetal deaths, namely, those which succumb before sufficient penetration of the trophoblast by the fetal capillaries could establish an efficient placental circulation.

Another abnormality which occurs in the yolk sac causes fetal death due to failure of the earliest hemopoietic organ; namely, the blood islands. For example, on the 12th day of gestation the blood islands in the yolk sac of the vitamin E-deficient embryo may be few or missing. Evidently the

splanchnic mesoderm of this organ becomes exhausted and is unable to produce new blood vessels. Meanwhile, the liver, now presenting a more or less solid mass of cells with hardly any sinusoids, can hardly fulfill its role as a hemopoietic organ. Also abnormal are the places of secondary multiplication of erythrocytes, the blood vessels of the embryo, and the allantois and the yolk sac all of which show a marked diminution in their content of blood cells; namely, 10 to 35 per cent of normal. Thus, the inhibition of fetal hemopoieses along with other changes in the mesenchyme is a particularly sensitive indicator of this special type of abnormality due to avitaminosis E.

c. Avitaminosis E and testicular degeneration. A survey of the literature leaves no doubt that nutritional adequacy of vitamin E is important for the normal function of the testicles. Mattill et al. (1924) were the first workers to investigate the pathological changes in the testes due to avitaminosis E. They found that there was a marked degeneration of the germinal epithelium and an increased proliferation of the interstitial tissue. In advanced stages of degeneration no spermatozoa were observed in the lumen of the tubules. When five to ten per cent of wheat germ or green lettuce leaves were added to the ration no degeneration of the testes occurred. Similar reports and additional abnormalities were reported subsequently by others. Evans (1925), and Evans

et al. (1927) reported sterility in male rats on vitamin E-free rations. They divided the development of male sterility into six stages according to the number, motility, and morphology of the spermatozoa in the vaginal plug after coitus, and the libidinousness of the animals. Mason (1925, 1926, 1933) published detailed description of the cytological changes taking place in the degeneration of the seminiferous tubules. He divided testicular degeneration into five stages entirely on the basis of morphological criteria. He also pointed out that although testicular degenerations due to avitaminoses A and E might be alike morphologically, the degeneration caused by avitaminosis A could be repaired ad integrum while that caused by avitaminosis E seldom, if ever, could be repaired. The testicular atrophy and progressive degenerative changes in the seminiferous tubules due to vitamin E deficiency have also been observed in male birds (Adamstone and Card, 1934; Adamstone, 1942), and in male rabbits (Chevrel and Cormier, 1948).

d. Relation to muscular dystrophy. Goettsch and Pappenheimer (1931) first described a muscular dystrophy of nutritional origin in guinea pigs and rabbits and did not attribute the disease to a lack of any of the vitamins known at the time. Morgulis and Spencer (1936a, 1936b) and Morgulis et al. (1938) suggested that at least two factors might be involved, one water-soluble and the other probably

identical with vitamin E. Later it was demonstrated that vitamin E was the sole limiting factor in this dietary disease (Mackenzie and McCollum, 1940). In the rat, Evans and Burr (1928) showed that vitamin E deficiency in the mothers was the cause of paralysis of the suckling young. This paralysis was shown to be associated with a degeneration of the muscle cells (Olcott, 1938) which could be prevented by the administration of alpha-tocopherol (Goettsch and Pappenheimer, 1941).

The histopathological changes in muscular dystrophy are essentially those known as focal, hyaline, and waxy or Zenker's degeneration. They are characterized by loss of striations, multiplication and irregular distribution of sarcolemma nuclei, and swelling of the sarcoplasm which in turn becomes vacuolated and structureless. Edema and inflammatory reactions in the interstitial connective tissues together with calcification of necrotic muscle fibers may occur.

The muscle lesions in adult rats are developed very gradually. They cannot be repaired but can be arrested at any stage by vitamin E therapy. Furthermore, they are never the primary cause of death of the animal, and usually show relatively little histological evidence of repair in association with degenerative changes.

In addition to the species already mentioned; namely, guinea pigs, rabbits and rats, muscular dystrophy has been produced in cattle and calves (Hjarre and Lilleengen, 1936; Blaxter et al., 1951, 1952) goats and sheep (Madsen et al., 1935; Davis and Maynard, 1938; Willman et al., 1946; Marsh, 1946; Whiting et al., 1949; Culik et al., 1951), hamsters (Houchin, 1942), ducks, goslings (Pappenheimer and Goettsch, 1934; Pappenheimer et al., 1939), tree kangaroos (Goss, 1940), dogs (Brinkhous and Warner, 1940), monkeys (Mason and Telford, 1947) and the cold-blooded vertebrate, the guppy fish (Cumings, 1942). Necrosis of striated musculature constitutes one of the most common manifestations of vitamin E deficiency in vertebrate species.

e. The metabolic changes in avitaminosis E. When rabbits are fed a vitamin E-free diet there is an absolute as well as relative loss of creatine in skeletal muscle, however, no demonstrable loss of it in either the heart or brain (Goettsch and Brown, 1932). The creatine and glycocyamine metabolism in vitamin E deficient rabbits has been studied by Melville and Hummel (1951). Creatinuria and loss of creatine from the muscle tissue usually preceded the external signs of paralysis and histological alteration. Simultaneously the amounts of creatine in the liver, kidney and blood increased four to five times that present in control animals. On the other hand, glycocyamine undergoes

very little alteration. Skeletal muscle strips from rabbits on a vitamin E-deficient diet respired at an abnormally rapid rate even before symptoms of muscle dystrophy were noted (Roderuck et al., 1949; Hummel and Melville, 1951). The rate of oxygen uptake increased as the dystrophy progressed. The glutamine level and transaminase activity of muscle from vitamin E-deficient guinea pigs and rabbits were lower than that of muscles from normal controls (Roderuck et al., 1949).

Feuer and Frigyes (1951) showed that the myosin and adenosine triphosphate content of dystrophic muscle of E avitaminotic rabbits was found to be reduced to 25 per cent of normal, while the adenosine triphosphatase increased. There was no qualitative difference found between the actin, myosin and myokinase isolated from normal and dystrophic muscle. Breakdown of adenosine triphosphate was increased in the homogenates of dystrophic muscle. According to these authors, vitamin E facilitates the formation of myosin and inhibits the accumulation of magnesium-activated adenosine triphosphatase.

The relationship of vitamin E to nucleic acid metabolism has been studied by Young and Dinning (1951). They found that vitamin E-deficient rabbits excreted much greater quantities of allantoin than comparable control animals. This elevated allantoin excretion was reduced to normal by the

oral administration of alpha-tocopherol acetate. It was also shown that skeletal muscle from vitamin E-deficient rabbits contained more ribonucleic acid and desoxyribonucleic acid than did the tissues from control animals. The concentration of desoxyribonucleic acid in the liver was increased but that of ribonucleic acid was not significantly altered by the deficiency. It would appear that the turnover rate of nucleic acids is accelerated in vitamin E deficiency, especially in skeletal muscle.

In vitamin E-deficient rats there was a diminished urinary creatinine excretion (Hove and Hardin, 1952). This reduction in creatinine excretion correlated significantly with the increased creatine excretion of the deficient animals. Also, the cholesterol content of the muscle was significantly increased, but the increase in total lipid was less marked (Heinrich and Mattill, 1943). In brain tissue the cholesterol was increased considerably, especially that of free cholesterol.

f. Changes in the circulatory system. In vitamin E deficiency changes in the circulatory system have been observed. These changes will be discussed according to their effect on the heart, the blood vessels and the blood itself.

(1) Heart. Myocardial damage due to vitamin E deficiency has been described in a number of animals including cats, dogs (Agduhr, 1927), rabbits (Houchin and Smith, 1944;

Bragdon and Levine, 1949; Gatz and Houchin, 1946, 1951), rats (Freire, 1941; Mason and Emmel, 1945; Ruppel, 1949; Lecoq and Isidor, 1949), cattle(Gullickson and Calverley, 1946) and guinea pigs (Freire and Magalhaes, 1943). In studying the effect of cod liver oil poisoning, Agduhr (1926) noted that the outer wall of the right ventricle of cattle was changed extensively into connective tissue in most cases, and these changes began sub-endocardially. Houchin and Smith (1944) observed signs of severe myocardial damage in vitamin E-deficient rabbits. These signs were: (1) a greatly increased sensitivity to posterior pituitary extracts; (2) a high resistance to the toxic effects of cardiac glycosides; and (3) probable cardiac dilatation as revealed by thoracic X-ray films. In experiments with posterior pituitary extracts, the vitamin E-deficient rabbits succumbed to doses much smaller than those which were well tolerated by the normal control animals. In experiments with lethal cardiac stimulants, such as digitoxin and ouabain, the vitamin E-deficient animals lived several days longer than the normal controls. These authors concluded that sudden death of vitamin E-deficient rabbits in an advanced stage of muscular dystrophy is due directly to myocardial failure.

Histological studies of the heart of vitamin E-deficient rabbits revealed a degeneration of the cardiac muscle (Gatz

and Houchin, 1946). The J and Q bands were absent in the major portion of the heart tissues. In scattered areas of the experimental tissue the striated appearance was maintained in some fibers though the Q band appeared narrower than in the control tissue. Intercalated discs remained apparent in some of the fibers which failed to exhibit other striations. The branched anastomosing structure of the cardiac muscle was maintained. All these changes concurred with the metabolic signs mentioned above.

Further detailed histological, physiological and biochemical studies with rabbits have shown some very interesting results (Gatz and Houchin, 1951). There was an increase in the muscle metabolism as evidenced by an increased oxygen consumption of the cardiac muscle strips and by the lengthened Q T interval in cardiac muscle electrocardiogram of vitamin E-deficient rabbits. Histologically, the affected cardiac muscle fibers underwent marked contraction as determined by the appearance of constriction bands. They became hyaline in appearance and exhibited basophilia. Fluid droplets appeared in the sarcoplasm between the myofibrils. As the droplets coalesced, the Q bands were separated and presented a granular appearance. Eventually the affected fibers consisted of the sarcolemma which surrounded the coalesced droplets and the muscle nuclei. Then an interstitial edema caused the separation of the cardiac fibers, and the

polymorphonuclear cells and histocytes made their appearance and invaded the necrotic area. Those portions of the heart showing necrosis may be listed as follows: papillary muscle, ventricular walls and septum, and the atrial walls. The Purkinje fibers and the neurons and the nerve fibers of the cardiac plexus appeared unaffected.

Studies made of the hearts of vitamin E-deficient cats, dogs, rats and guinea pigs are much less extensive than those for rabbits, but they all confirm, in one way or another, the findings obtained with rabbits.

(2) The blood vessels. Another effect of vitamin E is to prevent exudation (Tusini and Montorsi, 1950) and capillary fragility (Ames et al., 1951) in the circulatory system. The usual symptoms of vitamin E deficiency in chicks are encephalomalacia and exudative diathesis. Both of these abnormalities are caused by changes in capillary permeability and fragility. According to Adamstone (1947) the gross lesions found in nutritional encephalomalacia consist of a general disintegration of the cerebellum during which time it takes on a greenish-yellow discoloration, hemorrhage and edema. Rarely do lesions occur in the cerebrum. Microscopically, degeneration of the neurons was characterized by pyknosis and shrinkage. The whole lesion was considered to be typical of ischaemic necrosis. In studying the histopathology of nutritional encephalomalacia

in chicks, Wolf and Pappenheimer (1931) observed proliferation of the capillary endothelium, and hemorrhage and thrombosis of many capillaries. These findings leave little doubt that circulatory disturbances, such as edema with separation and disruption of cellular and fibrillar elements of the cerebellum, together with degeneration and necrosis of Purkinje cells and cells of the granular layer, are among the primary causes of the abnormalities observed in this syndrome.

Bird and Culton (1940) found that generalized edema is the chief clinical symptom in exudative diathesis. Intraperitoneal accumulation of fluid was rare, but sometimes accumulated fluid caused considerable distension of the pericardium. The edema was associated with hemorrhage in the fatty layers beneath the skin (Dam and Glavind, 1938, 1939). The accumulation of fluid resulted from "sterile inflammation" and hyperemia of the capillaries.

Mason (1943) examined many viable vitamin E-deficient feti during the 16th day of gestation. Many were normal, but others were pale with scattered areas in which the blood vessels were distended with stagnant blood. The superficial vessels were first affected in the following regions: cranium, external ear, shoulder and dorsolateral portions of the trunk. Capillary petechiae were found in these areas and plexiform dilatations of the venous channels. The large

venous trunk in the neck was frequently distended. Sites of large extravasations of blood in the walls and floor of the cerebral vesicles were observed. It is interesting to note that Mason found these lesions in feti in which normal mitoses were still present in other organs. Thus Mason has emphasized the importance of the vascular lesions in causing fetal death and resorption through asphyxia.

(3) Blood. The effects of various factors such as riboflavin, nicotinic acid, pyridoxine, pantothenic acid, iron, copper, cobalt, tryptophan, lysine, phenylalanine and isoleucine in animal diets on erythropoiesis have been ably and extensively reviewed by Cartwright (1947), who cites 665 references. However, in his review no mention was made of the effect of vitamin E deficiency on the blood picture or on blood chemistry. The literature on this is rather scarce. In the early studies on cod-liver oil toxicity, in which white mice, rats, rabbits, calves, pigs, cats and dogs were used, Agduhr (1927) observed some changes in the blood picture. When large doses of cod-liver oil, more than 1 ml. per kilogram body weight, were added to the food of the animals, there was a decrease in the number of erythrocytes and a concurrent increase in hemoglobin. Leukocytosis developed in all the animals studies. This condition was due to an increase of polymorphonuclear cells and occurred in

all animals except the calves where instead there was increase of lymphocytes. As the cod-liver oil dosing continued the leukocytosis disappeared and was followed by leukopenia, in which the cells were reduced to one half or one third of the normal level. As has been mentioned earlier cod-liver oil may act as an antivitamin E substance. Changes in the blood picture resulting from the administration of cod-liver oil might well be the same as that obtained from vitamin E deficiency.

In their studies of vitamin E deficiency in eight monkeys, Filer et al. (1949) routinely examined their blood for hemoglobin, red cells, white cells and hematocrit. Except for a possible leukopenia, the data failed to show any differences between the control and deficient groups. These same workers also made analyses for blood glucose and non-protein nitrogen, plasma proteins, and tocopherol. The data failed to show differences that would enable one to distinguish the deficient animals from the controls, except by the level of plasma tocopherol which was about 0.58 mg. per 100 ml. in the control compared to 0.18 mg. per 100 ml. in the deficient animals.

Recently Dinning et al. (1951) reported leukocytosis which was due primarily to the elevation in the number of granulocytes in acute vitamin E deficiency in the monkey.

This same phenomenon also was observed in vitamin E-deficient rabbits (Dinning, 1952). The total number of white blood cells increased from the normal level of about 10,000 to over 20,000 per cu. mm. and the granulocytes increased from 3,500 to about 18,000 per cu. mm. There was no difference in the red cell count, hemoglobin or hematocrit.

Changes in the chemistry of blood due to vitamin E administration have been reported by Prosperi and Ragazzini (1950). They studied changes in concentration of some constituents in the blood of fifteen children ranging in age from 6 to 12 years who had received single doses of 50 or 200 mg. alpha-tocopheryl acetate. They found that the levels of inorganic phosphorus and creatine had decreased, whereas that of glucose remained essentially unchanged. These observations suggested to the authors that tocopherol functions by inhibiting the breakdown of phosphocreatine thereby slowing the process of phosphorylation necessary for the utilization of glycogen.

g. Anatomical changes in avitaminosis E. The nutritional avitaminosis E results in a number of morphological changes other than those connected with the reproductive system. The muscular and nervous systems together with liver, kidney, tooth and adipose tissue show different lesions and changes as a result of vitamin E deficiency. The following is a resume of the pertinent literature to date.

(1) Smooth muscle. As far as smooth muscles are concerned, only changes in the uterus, gut or gizzard have been reported. The effect of vitamin E on the uterus of ovariectomized rabbits has been studied by Tusini and Vandelli (1951). These authors found that high doses of vitamin E stimulated proliferation of the vascular system in the uterus of ovariectomized does. The uteri from untreated castrates were pale and atrophic, but those from castrates that had been given vitamin E were turgid and hyperemic.

In guppy fish raised on a vitamin E-free diet large masses of extraneous reticular tissue developed in the gut, sometimes blocking the lumen of the tube (Cumings, 1942). In turkeys restricted to a vitamin E-deficient diet nutritional myopathy of the gizzard developed (Jungherr and Pappenheimer, 1938). Lesions appeared in the gizzard muscle whereas the mucosa lining remained intact. In "crazy chicks" disease (Dunlop, 1932) sarcoma of the reticulum cells developed in the vitamin E-deficient chicks following ulceration of the intestine (Adamstone, 1941c).

(2) Nervous system. Mention has been made of the changes in the cerebellum in connection with encephalomalacia. In addition to edema and small hemorrhages, there is degeneration, necrosis, pyknosis and shrinkage of the Purkinje cells and those of the granular layer of the cerebellum (Wolf and

Pappenheimer, 1931). Also, degeneration of the nerve fibers, accompanied by formation of blister-like vesicles on the fibers occurs (Adamstone, 1947). Only the central nervous system is affected, and since the peripheral nervous system is not affected true paralysis does not occur (Adamstone, 1941a).

To evaluate conflicting reports in the literature Luttrell and Mason (1949) made a detailed histological study of the spinal cord of vitamin E-deficient rats which showed paresis at 9 to 12 months of age. They found that the posterior columns (fasciculi cuneatus and gracilis) and proximal parts of the posterior roots of the cervical, thoracic, lumbar segments of the spinal cord consistently showed demyelinization, gliosis and distortion of the axon pattern. There were no significant alterations in the pyramidal tract, the cerebellum or the cerebrum. Gliosis of the spinal cord also has been observed in vitamin E-deficient calves (Blaxter et al., 1951).

(3) Liver. Adamstone (1941b) noted that the liver of vitamin E-deficient chicks at autopsy frequently was found to be swollen, friable, and marked with dark-brown spots. In the vitamin E-deficient guppy, Cumings (1942) observed clumping of the chromatin of the nuclei or formation of signet ring nuclei in the liver, pancreas, and spleen.

Hove *et al.* (1949) reported the production of a fatal disease in rats characterized by centrilobular or massive liver necrosis and lung hemorrhage whenever they were restricted to a diet containing 10 per cent casein. Protection against this disease was given by either alpha-tocopherol or by increasing the level of casein to 16 per cent. This finding was confirmed by Abell *et al.* (1950) who produced hepatic necrosis in rats restricted to an avitaminotic E diet containing only seven per cent protein. Also, it was observed that loss of cytoplasm and basophilic granules occurred before necrosis. In the areas around the central veins stainable lipid increased while the content of glycogen decreased. Biochemical changes in the liver during necrosis were studied by Abell and Beveridge (1950). They found that necrotic livers increased in dry and wet weight, water and free cholesterol, and decreased in phospholipid and glycogen.

In the last stages of vitamin E deficiency in the rabbit steatosis of the liver developed (Chevrel *et al.*, 1951). Total glycogen decreased as also did the ribonucleotides in the regions of fatty infiltration. Histochemical staining showed evidence of increased alkaline phosphatase activity in the endothelium of the centrolobular veins and adjacent sinusoids. These histochemical data indicate the importance of E avitaminosis as a factor in functional disturbances of the liver.

(4) Kidney. In studying "crazy chick" disease Dunlop (1932) also observed nephritis with its characteristic albuminous degeneration of the kidneys, and interstitial hemorrhages. Many rats maintained on a vitamin E-free ration develop hyaline degeneration of the convoluted tubules of the kidneys, unaccompanied by any inflammatory reaction of interstitial tissue or of the glomeruli (Martin and Moore, 1938, 1939).

(5) Tooth. According to Irving (1942) vitamin A deficiency results in depigmentation of the teeth of rats. On the contrary, in vitamin E deficiency there is no whitening of the incisor teeth. Normally the enamel organ regresses as the rat grows older. In young rats the ameloblasts usually are columnar and the epithelial papillae are quite large and pronounced. In vitamin E-deficient rats a premature and abnormal degeneration of the enamel organ occurs in the middle third of the incisor teeth. There is either a disappearance of epithelial papillae and shrinkage of ameloblasts to an ill-arranged cubical layer, or a sudden disappearance of all layers of the enamel organ only to be replaced by fibrous tissues. Other investigators also have reported that there was depigmentation of the teeth during vitamin E deficiency in the rat (Granados et al., 1949, 1950; Dam et al., 1950;

Glavind et al., 1950; Granados and Dam, 1950; Moore, 1950; Pindborg, 1950).

(6) Adipose tissue. Brown pigment occurs ultimately in the adipose tissue of chicks fed rations deficient in vitamin E and containing unsaturated fat (Dam, 1944). The same changes occur in the adipose tissues of the rat (Mason et al., 1946; Granados et al., 1949). Brownish discoloration of the fatty tissue of pigs which were reared on rations containing fat from fish or other marine products has been known for a long time. This disturbance was experimentally produced in pigs by feeding a ration containing much fish scrap and fish oil (Gorham et al., 1951) or feeding a ration deficient in vitamin E and containing 5 per cent of cod-liver oil (Robinson and Coeg, 1951). A similar abnormality was reported to occur sporadically in mink kits (Hartsough and Gorham, 1949), and was prevented by feeding fresh horse meat (Mason and Hartsough, 1951), or alpha-tocopherol (Gorham et al., 1951). The same disturbance was produced in mink by feeding rations containing raw linseed oil (Lalor et al., 1951).

2. Occurrences in natural foods and feedstuffs

Occurrence of the E vitamins is widespread in nature. They are found most abundantly in the germ of vegetative seeds and in the green leafy parts of plants. Employing a bioassay procedure involving the rat, Palmer et al. (1940) classified feedingstuffs as being rich, poor or almost lacking in vitamin E. The importance of grass as a source of vitamin E for farm animals was demonstrated by Cabell and Ellis (1942). Not all roughages contain vitamin E. Recently Van der Kaay et al. (1949) have shown that wheat straw and rye straw are devoid of vitamin E. Of all the natural products, perhaps wheat germ oil contains the most total tocopherols (Quaife, 1948; Brown, 1952b). Next in the order of richness in total tocopherols are corn oil (Quaife, 1948), rice bran oil (Fisher, 1945), cottonseed oil and soybean oil (Brown, 1952b). Among the oils that have been studied olive oil (Quaife and Harris, 1946) and grapeseed oil (Herraiz and Herrero, 1949) seem to contain the least vitamin E. The distribution of the different tocopherols in these oils is quite characteristic for each oil. Thus, all the tocopherol present in sunflower seed oil is in the form of alpha-tocopherol (Brown, 1952b) while only about 70 per cent of the tocopherols in wheat germ oil, cottonseed oil

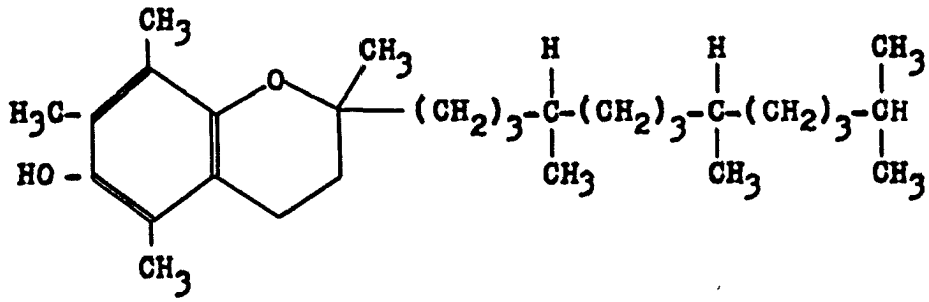
and rice bran oil is present as alpha-tocopherol. Beta-tocopherol occurs only in wheat germ oil (Emerson, 1938; Moss and Drummond, 1938; Todd et al., 1937; Quaife, 1948; Brown, 1952b). Gamma-tocopherol occurs predominantly in corn oil and soybean oil (Emerson et al., 1939; Brown, 1952b) while delta-tocopherol is found only in soybean oil (Quaife, 1948; Brown, 1952b).

Up to now our knowledge on the distribution of the individual tocopherols is rather incomplete, but with the application of paper chromatography (Emmerie, 1949; Brown and Blaxter, 1951; Brown, 1952a, 1952b) and micro-methods (Quaife et al., 1949), information of this kind is likely to increase rapidly in the future.

3. Chemistry and synthetic products

The chemistry of vitamin E has been reviewed by Underbjerg (1939) and Gustafson (1941). In the monograph of the monumental contributions on vitamin E Evans et al. (1927) describe some of the chemical and physiological properties of the vitamin as well as the nature of the reproductive failure due to its absence in the diet. Subsequently, Evans et al. (1936) isolated it from the non-saponifiable fraction

of the wheat germ oil and named it tocopherol, implying an alcohol concerned with the bearing of the young. Its chemical structure was definitely formulated by Fernholz (1937) as follows:



Alpha-tocopherol: $C_{29}H_{50}O_2$: Mol. wt. 430.69

It consists of a methylated chromane ring containing the phytyl group as a substituent on carbon 2 and a hydroxyl group on carbon 6 para to the chromane oxygen.

Four tocopherols have been isolated and synthesized. These are alpha-, beta-, gamma-, and delta-tocopherol. Karrer *et al.* (1938), Smith *et al.* (1938) carried out the synthesis of alpha-tocopherol through condensation of trimethyl hydroquinone and phytyl bromide with or without zinc chloride. Naturally occurring alpha-tocopherol and its synthetic equivalent have the same absorption spectrum and reducing properties. The allophanates, 2,4-dinitrobenzoates, and nitrophenylurethanes of the synthetic substance when

mixed with the same derivates of naturally occurring alpha-tocopherol show no depression of melting points thus indicating that they were identical chemically. The optically inactive synthetic compound can be resolved into 2 optically active forms by means of bromocamphorsulfonic acid. A single dose at 3 mg. level was shown to have definite biological activity (Evans et al., 1938).

The constitution of natural beta-tocopherol was demonstrated by synthesis and by comparison of derivatives with those of the natural compound to be 5,8-dimethyl tocol (Karrer and Fritzsche, 1939). Gamma-tocopherol is identical except that the methyl group in the 5-position is not present (Jacob et al., 1939). Synthetic d,l-gamma-tocopherol has 30 per cent of the antidystrophic potency of natural d,gamma-tocopherol. Delta-tocopherol is 8-methyltocol and was isolated recently from soybean oil by Stern et al. (1947). In attempting to synthesize the tocopherols a large number of compounds were prepared which have biological properties similar to that of vitamins E (Werder and Moll, 1938; John and Günther, 1938; Evans et al., 1938). Some of these are much simpler in chemical constitution than the naturally occurring tocopherols. However, none of these synthetic compounds has greater biological activity than either the synthetic or the naturally occurring tocopherols.

In addition to the tocols different salts have been derived from them such as fat-soluble d,l-alpha-tocopherol acetate and alpha-tocopherol palmitate and water-soluble d,alpha-tocopheryl disodium phosphate, alpha-tocopherol calcium succinate and alpha-tocopherylamine hydrochloride (Hickman, 1943). One milligram of the acetate salt has been designated as one international unit (I. U.). The practical advantage of the water-soluble salts is that they can be used safely for intermuscular or intravenous injections. Intermuscular injection of d,alpha-tocopheryl phosphate effected a slower but more prolonged cure of muscular dystrophy than the oral administration of d,l-alpha-tocopherol acetate. Harris et al. (1947) reported that ulcer-like lesions which develop in the stomachs of rats recovering from deficiencies of vitamin A, vitamin B_6, or essential fatty acids could be cured by oral administration of alpha- or gamma-tocopherol, but not by injection of alpha-tocopheryl phosphate.

Alpha-tocopherol is sensitive to light (Karrer and Keller, 1939) and is oxidized by air at room temperature. This is not true of the acetate of d,l-alpha-tocopherol (Isler, 1938). The principal tocopherol oxidation products are tocopheryl quinone (chromane ring opened) with an intermediate free radical and an epoxide and a chromane-5,6-quinone, sometimes known as the red quinone, in which the

vulnerable methyl group on carbon 5 has been replaced by oxygen. Since the quinone formed is not reconverted into vitamin E on reduction, Michaelis and Wollman (1949) have sought some other oxidation product capable of functioning in atypical oxidation-reduction process. By dissolving tocopherol in a mixture of alcohol, ether and pentane, freezing it to a vitreous mass with liquid air and irradiating it with ultra-violet rays, they obtained an orange-red colored semiquinone radical of tocopherol by removal of one electron only. This process does not open the side ring and implies reversible oxidation-reduction. This significant finding places tocopherol in the class of reversible oxidation-reduction systems and reveals a system analogous to other vitamins such as riboflavin, nicotinic acid and thiamine which act as coenzymes, or prosthetic group of enzymes concerned with specific dehydrogenation. Another reversible oxidation product of alpha-tocopherol, namely, alpha-tocopheroxide, has been prepared by oxidizing alpha-tocopherol with ferric chloride in the presence of 2,2'-bipyridine (Boyer, 1951). This reversibly oxidized product was isolated by use of chromatographic methods and was found to differ from alpha-tocopherol in containing one more oxygen atom.

According to Boyer (1951), almost all compounds showing vitamin E activity in the rat sterility test are substituted chromane derivatives. The requisite substituents of the chroman ring are: (a) one or more alkyl groups on the carbocyclic ring, (b) a hydroxyl group free or esterified in position six, and (c) a short or long side chain in position two. Based upon the structural requirements for vitamin E activity and from the fact that alpha-tocopheroxide has biological activity, however, only one thirtieth that of alpha-tocopherol, Boyer surmised that the biological function of alpha-tocopherol involves reversible oxidation and reduction. Both semi-quinone and alpha-tocopheroxide are intermediate compounds in the reaction involving the conversion of tocopherol and tocopheryl quinone. Probably the production of the semi-quinone of Michaelis and Wollman is the first step in the formation of alpha-tocopheroxide.

4. Therapeutic value in avitaminosis E

Muscular dystrophy has been produced in calves by Blaxter et al. (1951, 1952) when fed rations consisting of dried skimmed milk, lard, minerals, and arachis oil to which vitamins A and D had been added. This condition in calves was prevented by subsequently administering alpha-tocopherol

acetate. Wheat bran and unextracted wheat germ meal were effective in reducing the incidence of a naturally occurring disease in sheep, characterized by stiffness in the lambs (Willman et al., 1940). Later, Willman et al. (1945, 1946) showed that "stiff-lamb" disease could be both prevented and cured by giving alpha-tocopherol to the lambs only, or to both the lactating ewes and lambs. Whiting et al. (1949) confirmed these findings. In addition they demonstrated that the disease could not be prevented by supplementing the avitaminotic diet of pregnant ewes with tocopherols. Furthermore, differences in the total tocopherol content of the ewes' rations did not appear to account for differences in incidence of the disease. Using a diet similar to that employed by Blaxter et al. (1951), Culik et al. (1951) experimentally produced the disease in lambs and effectively alleviated all clinical signs in three to five days by administering alpha-tocopherol. However, degeneration of the muscles was evident when the lambs were killed twelve to twenty days later, indicating that muscle repair proceeds slowly or possibly not at all.

Nutritional encephalomalacia in chicks was first produced by Pappenheimer and Goettsch (1931), and is often referred to as "crazy chick disease". Also, it has occurred in farm flocks of ducklings and turkeys (Pappenheimer et al.,

1939). A study of the occurrence of encephalomalacia in percentile relation to all diagnostic poultry lots received during the years 1936 to 1951 in Connecticut reveals that the yearly incidence in chicks ranges from 1 to 6 per cent (Jungherr et al., 1952). The greatest incidence during any one year is usually during the warm season. The disease can be prevented by the administration of vitamin E (Dam et al., 1938; Pappenheimer et al., 1939). Exudative diathesis (Dam and Glavind, 1938), another vitamin E deficiency disease in chicks, also can be cured by administration of alpha-tocopherol. Encephalomalacia and exudative diathesis may occur separately or simultaneously depending on the composition of the diet.

Besides calves and lambs, nutritional muscular dystrophy also occurs in guinea pigs, rabbits (Goettsch and Pappenheimer, 1931), and rats (Evans and Burr, 1928). It can be cured in these animals by the administration of alpha-tocopherol alone (Mackenzie and McCollum, 1940; Goettsch and Pappenheimer, 1941).

B. Congenital Abnormalities Attributed to Nutritional Deficiencies

Congenital malformations are structural defects present at birth. They may be gross or microscopic, on the surface of the body or within it, familial or sporadic, hereditary or nonhereditary, single or multiple. There are many factors concerned in the production of such abnormalities, such as genetic factors (Gruenberg, 1943), nutritional factors, chemical factors (Needham, 1942), endocrine factors (Greene, 1942), actinic factors (Job, 1935), infectious factors (Albauch, 1945), and mechanical factors (Grosser, 1938). For the present investigation only the nutritional factors involved in the production of congenital abnormalities will be reviewed.

1. Those related to the skeletal system

The fetal skeletal system often shows many congenital abnormalities due to nutritional deficiencies. In fact, the different gross malformations are most noticeable. The nutrients that affect the normal development of the bones are vitamins A, D, riboflavin, biotin, pantothenic acid, folic acid as well as the minerals calcium, manganese,

copper, and iodine. Later on, it will be shown from results of this investigation that vitamin E is also involved in the skeletal development. The detailed findings of other investigators will be reviewed below.

a. Gross malformations. Zilva et al. (1921) in studying the anomalies resulting from maternal vitamin A deficiency in pigs observed varying degrees of abnormality in the hind limbs. Malformation was particularly pronounced in two cases in which the hind limbs were thin and tail-like.

When the maternal diet is deficient in vitamin D, infantile ricket can occur at the beginning of the neo-natal period according to the report of Wallis (1938), and Swanson and Iob (1938). Gross congenital malformations were induced in approximately one third of the offspring of rats of the Sprague-Dawley and Baltimore strains reared and maintained on a vitamin D deficient diet (Warkany and Nelson, 1940, 1941, 1942a, 1942b; Warkany, 1943). The malformations in the young at birth consisted of abnormally short mandibles, deformed extremities, syndactylism, short tail, reduction in size of hind legs, absence of tibiae, shortened fibulae, fusion of ribs to form a plexus-like mass, and the absence of some centers of ossification of the sternum (Warkany et al., 1943). The addition of vitamin D to the maternal diet prevented these malformations. By alternately breeding the

same female on vitamin D-deficient and vitamin D supplemented diets abnormal and normal litters could be produced alternately (Warkany and Nelson, 1942a).

Riboflavin deficiency in pregnant rats also causes congenital skeletal malformations comparable to those produced in maternal vitamin D deficiency (Warkany and Schraffenberger, 1943, 1944a; Warkany, 1944; Schroeder, 1950; Gilman et al., 1952). Chondrification begins in the rat on the 14th and 15th day of fetal life. Malformations of this type do not originate before the 13th day nor after the 15th day of gestation (Warkany, 1944). Therefore, the 13- or 14-day represents a critical period in the development of the rat embryo in which the presence or absence of sufficient riboflavin has a decisive influence on the development of the embryo. In the chick micromelia and prognathism were observed in the embryos as a result of riboflavin deficiency in the diet of the hen (Romanoff and Bauerfeind, 1942).

The effect of biotin deficiency on embryonic development of the chick was investigated by Cravens et al. (1944) and Couch et al. (1948). Among the abnormal manifestations were skeletal abnormalities of the beak, scapula and hind limbs. These deformities were prevented by adding biotin to the diet or by injecting biotin into eggs from hens fed the low biotin diet (Couch et al., 1948).

Besides the vitamins mentioned above, pantothenic acid and folic acid also are concerned in skeletal development. In maternal pantothenic acid deficiency, defective limbs were sometimes observed in the young rat (Boisselot, 1948; Lefebvres-Boisselot, 1951). Flattened head, missing or under-developed lower beaks and toes in the chick embryos from eggs of folic acid deficient hens were reported by Karnofsky et al. (1949) and Sunde et al. (1950). Syndactylism occurred in young rats born to folic acid deficient dams (Nelson et al., 1952).

The minerals that are concerned with normal development of the bones are iodine, manganese, calcium and copper. According to Smith (1917), pigs from dams fed an iodine deficient diet had hoofs that were under-developed and brittle. Congenital chondrodystrophy in chick embryos was produced by a manganese deficiency in the diet of the hen (Lyons and Insko, 1937; Caskey and Norris, 1940). The importance of manganese in the development of bone in the young of albino female rats was studied by Barnes et al. (1941). The abnormalities were mainly of three kinds: (1) abnormally short tibiae; (2) excessively thin epiphysis at the proximal end of the tibiae and (3) disproportionality between width and length of the tibiae at the proximal end. Stuart (1945) and Burke and Stuart (1948) observed a retardation of ossification

in the newborn infant when the diet of the mother was poor in calcium. Cunningham (1946) noted abnormal fragility in the bones of lambs born to ewes receiving ration deficient in copper.

Some gross malformations due to deficiency of some unidentified nutritional factor or factors were also reported in the literature. Micromelic chick embryos having relatively short bones in the antero-posterior axis of the skull and in the tarso-metatarsi frequently occurred in eggs laid by hens while receiving diets lacking in some unidentified nutritional factor or factors (Byerly et al., 1935; Landauer, 1936). Syndactylism, and talipes of an unidentified nutritional origin were reported in swine (Ross et al., 1944; Cunha et al., 1944). Good quality alfalfa meal was shown to carry a factor or factors that would correct the deficiency of the basal corn-soybean ration.

b. Perosis. Congenital perosis in the chick developed when hens were fed a low biotin diet (Couch et al., 1948), or when hens were fed a vitamin B_{12} deficient ration (Olcese et al., 1950).

c. Teeth. The structure of the teeth of young albino rats (Mellanby, 1939, 1941) and guinea pigs (Wolbach and Howe, 1933) became defective when the maternal diet was deficient in vitamin A. The initial effect of vitamin A

deficiency is upon the enamel organ. The ameloblasts respond earliest by atrophy. Then the remainder of the organ atrophies. Finally metaplasia and calcification and in the guinea pigs ossification occur. Atrophy and depolarization of odontoblasts follow enamel organ changes. The odontoblasts survive longest on the labial side where in long continued experiments gross deformities in the incisors of rats resulted from absent or defective dentine formation. These findings were later confirmed by Godlewski (1948). Burke and Stuart (1948) observed retardation of calcification of the teeth of the newborn when the diet of the pregnant woman was poor in calcium.

2. Those related to the muscular and nervous systems

Some kinds of muscular and nervous disorders of the young occur as a result of nutritional deficiencies prevalent during pregnancy. Some involve either the muscular or the nervous system while others may involve both of these systems. A review of the more pertinent literature follows.

a. Muscular incoordination and paralysis. Muscular incoordination and paralysis are often observed in the offspring of mothers fed deficient diets. Goettsch and Pappenheimer (1941) and Pappenheimer (1948) reported muscular

dystrophy in the newborn rat from vitamin E-deficient mothers. This was confirmed by Callison and Orent-Keiles (1951) who noted that the paralyses were either the flaccid or the spastic type and affected the fore or hind limbs, but rarely did both types appear in the same animal. Richardson and Hogan (1946) also observed muscular incoordination in the hydrocephalous rats produced from mothers fed a diet deficient in folic acid.

Congenital paralysis agitans of an unidentified nutritional origin in swine was reported by Ross et al. (1944). Leg weakness in pigs due to maternal thiamine, riboflavin, and choline deficiency was noted by Ensminger et al. (1947). Histological examinations revealed varying degrees of necrosis of the muscle fibers of the pig due to vitamin E deficiency (Adamstone et al., 1949).

Congenital myoatrophy in the legs of chicks due to maternal vitamin B_{12} deficiency was reported by Olcese et al. (1950).

b. Epileptiform convulsions. Recently Nelson and Evans (1951), in their study of the effect of pyridoxine on reproduction in the rat, observed epileptiform convulsions in some of the young born by dams restricted to a pyridoxine deficient ration. Whether these convulsions were due to muscular or nervous disorders was not investigated.

c. Ataxia. A congenital chronic ataxia in chicks caused by a manganese deficiency in the maternal diet was studied by Caskey and Norris (1940) and Caskey et al. (1944). It was found to be usually a tetanic spasm of the opisthotonic type as had been observed earlier by Byerly et al. (1935). Histologically the brain of the ataxic chick did not show any evidence of injury, but the lipid, total phosphorus and phosphatase content were less than in the brain of the normal chick. Later Couch et al. (1948) also observed congenital ataxia in chicks whenever the maternal diet was deficient in biotin.

Cunningham (1946) reported that ewes which had been grazing on copper-deficient peat pasture for two or three years gave birth to lambs that showed ataxia. This symptom is analogous to the enzootic ataxia of lambs reported in Australia (Bennetts and Chapman, 1937) or the "swayback" of England.

d. Beri-beri. Lockhart et al. (1943) found that the requirement for thiamine in women was trebled during pregnancy. Inadequate ingestion of thiamine caused polyneuritis of gestation in the mother (Stähler, 1937). Congenital beri-beri was reported by Van Gelder and Darby (1944). The infant with this condition was cyanotic and aphonic with extreme tachycardia and a greatly enlarged heart. The

frequency of beri-beri in infants in the Orient, especially in Japan, appears to be due to a maternal deficiency. Goldstein (1948) observed a case of severe congenital beri-beri which responded very favorably to the administration of the vitamin.

e. Hydrocephalus. This malformation is characterized by an enlarged and dome-shaped head. The relationship between maternal nutrition and the occurrence of hydrocephalus in the newborn has been established by several investigators. It was first observed in pigs from vitamin A-deficient sows (Zilva et al., 1921). Hyde (1940) noted the occurrence of hydrocephalus in a group of experimental rabbits whose diet lacked hay and kale. Richardson and Hogan (1946), and Richardson and DeMottier (1947) feeding experimental diets deficient in folic acid to pregnant rats found that the incidence of this congenital anomaly was 1.5 to 1.7 per cent. O'Dell et al. (1948) observed that about 2 per cent of the young rats from dams fed a folic acid-deficient diet developed this brain condition which could be prevented by addition of folic acid to the maternal diet. Hogan et al. (1950) showed that when a folic acid inhibitor, crude methylfolic acid, was added to the diet, approximately 20 per cent of all the young born were hydrocephalic. Recently O'Dell et al. (1951) reported that after female rats became depleted

of vitamin B_{12} the incidence of hydrocephalus among their offspring was 28 per cent. The addition of a vitamin B_{12} concentrate to the diet or the injection of crystalline vitamin B_{12} in the dams during the early stages of gestation prevented the occurrence of this abnormality in the young.

f. Congenital exencephaly and pseudencephaly. Maternal pantothenic acid deficiency in the rat gave rise to exencephaly and pseudencephaly (Boisselot, 1948; Lefebvres-Boisselot, 1951). It is interesting to note that maternal hypervitaminosis A induced as high an incidence of congenital exencephaly as 54 per cent in newborn rats (Cohlan, 1953). This congenital cranial defect appeared regularly in maternal hypervitaminosis A.

g. Degeneration of the nervous system. Engel et al. (1940) reported that one of the first changes which appeared in the chick embryo, due to riboflavin deficiency in the hen's ration, was a degeneration of the myelin sheath in sciatic nerve. This occurred in about 60 per cent of the surviving embryos. This degeneration of the sciatic nerve could be prevented by injecting 50 µg. of riboflavin into the egg on the first day of incubation.

Lesions involving the central nervous system occur in lambs having "swayback" induced by a dietary copper deficiency (Bennetts and Chapman, 1937; Dunlop and Wells, 1938).

Widespread symmetrical degeneration occurred in the white matter manifested by cavities filled either with a transparent gelatinous substance or fluid (Innes, 1935). When the ewes were given access to salt licks containing 1 per cent copper in the form of copper sulfate during pregnancy the lambs were usually free of the disease.

h. Congenital deafness or deaf-mutism. Mellanby (1938) observed a case of deafness due to maternal vitamin A deficiency in dogs. The auditory as well as trigeminal and facial nerves were squeezed by bone hypertrophy at the various foramina, thus causing deafness. Also, a deficiency of iodine in the diet of the pregnant woman induced deaf-mutism in the young (Murray and Wilson, 1945).

i. Congenital abnormalities of the eye. It has been repeatedly shown that congenital blindness in animals may be caused by a maternal nutritional deficiency. Hart and Guilbert (1933) reported eye lesions in calves from vitamin A-deficient cows. Blindness in cattle associated with a constriction of the optic nerve was reported by Moore et al. (1935) in their extensive review of the literature concerning this subject. Constriction of the optic nerve was associated with stenosis of the optic canal, papillary edema and probably increased intracranial pressure (Moore, 1939). Wolbach and Bessey (1941) showed that these nervous lesions were

caused by an imbalance between the growth of the optic nerve and that of the surrounding bony structures.

Hale (1933, 1935, 1937), Cunha et al. (1944) and Marq and Hennaux (1948) reported the occurrence of anophthalmos and microphthalmos in pigs from sows that had been fed a ration deficient in vitamin A. In Mason's (1935) study of reproduction in vitamin A-deficient rats, he observed fetal death, prolonged gestation and difficult parturition, but no congenital malformation of the offspring. Cannon (1940) also reported failure to produce congenital anomalies in young rats whose mothers were depleted of vitamin A. Warkany and Schraffenberger (1944b) and Jackson and Kinsey (1946), however, reported congenital blindness and malformations of the eyes induced in rats by maternal vitamin A deficiency. Histological examinations of such blind and deformed eyes revealed fusion of eyelids and cornea, absence of anterior chamber, iris and ciliary body, disorganization or folding and eversion of the retina, persistence of the choroidal fissure, and the presence of the retrolenticular membrane. These findings were later confirmed (Warkany and Roth, 1948; Wilson and Barch, 1949; Schroeder, 1950). Recently congenital abnormalities of the eyes of young rats littered by vitamin E-deficient mothers were observed by Callison and Orent-Keiles (1951). They reported such conditions as eyes

smaller than normal, eyes which fail to open, blood clots in the eyes, and white membranes behind the pupil. It is suggested that vitamin E may play an important role in the prophylaxis of retrolental fibroplasia by virtue of its anti-oxidant action.

Congenital cataract due to maternal niacin deficiency was observed by Pike (1951). When the diet was supplemented with 10 mg. per cent niacin the eyes of the young were normal. Absence or underdevelopment of the eye was observed in rats from pantothenic acid deficient mothers (Lefebvres-Boisselot, 1951). Coloboma was reported in the young littered by rats fed a folic acid deficient diet (Giroud and Lefebvres-Boisselot, 1951). Morgagnian-type cataracts appeared in the eyes of some of the young as a result of maternal folic acid deficiency (Nelson et al., 1952). In chick embryos from hens fed folic acid deficient rations (Karnofsky et al., 1949) small eyes with enlarged anterior chamber were observed. Ingestion of excesses of certain vitamins also will produce congenital abnormalities of the eye. Gross defects in eye development were observed in young rats born to females that received an excessive intake (35,000 I. U. daily from second, third, or fourth to the sixteenth day post coitus) of vitamin A during gestation (Cohlan, 1953). The work of Bannon et al.

(1945) showed that cataracts appeared in the newborn rat when the mother received a diet consisting mainly of galactose.

j. Diaphragmatic hernia. Andersen (1941) first noted the high incidence of diaphragmatic hernia in the young of rats bred while receiving a diet deficient in vitamin A. This was later confirmed by Warkany and Roth (1948) who reported that in a number of animals the right dorso-lateral portion of the diaphragm had not developed and also that a lobe of the liver occasionally protruded into the pleural cavity.

There is a genetic basis for some diaphragmatic hernias. The incidence of this anomaly differed with different strains of rats fed the same vitamin A-deficient diet. The incidence was 0.9 and 18.9 per cent in the Long-Evans and stock strains of rats (Andersen, 1949). The results of this investigator suggest that the expression of a genetic trait might be enhanced or suppressed by means of the diet during pregnancy.

k. Umbilical hernia. In maternal riboflavin deficiency in the sow umbilical hernia occurs in the pig (Ensminger et al., 1947). Exteriorization of the viscera was observed in chick embryos as a consequence of folic acid deficiency in the maternal diet (Karnofsky et al., 1949). Both umbilical hernia and ectocardia were reported in the young from rats

fed a diet deficient in the same vitamin (Giroud and Lefebvres-Boisselot, 1951).

3. Those related to the circulatory and uro-genital systems

The circulatory and urogenital systems of the fetus are sensitive to the maternal deficiency of the different nutrients also. They respond by becoming abnormal either physiologically or morphologically. The detailed conditions are reported below.

a. Anemia. Lepkovsky et al. (1938) reported that a deficiency of riboflavin in the diet of the hen induced anemia in the chick embryo. Also, anemia was observed in young rats born to dams in a state of folic acid deficiency (Giroud and Lefebvres-Boisselot, 1951; Nelson et al., 1952).

b. Hemorrhage and edema of the embryos. The occurrence of hemorrhage in vitamin E-deficient chick embryos (Adamstone, 1931) and rat feti (Mason, 1943) was mentioned earlier in this review. However, Adamstone et al. (1949) could not find any evidence of spontaneous hemorrhage in the embryos of pregnant gilts restricted to a vitamin E-deficient ration.

Brown et al. (1947) showed that in the absence of lard and vitamin K in the diet of female rats during gestation fatal cerebral hemorrhage occurred in 35 to 74 per cent of

their young. However, the coagulation time of the blood of the mother and the young was normal. One could assume then that a substance was necessary for maintaining capillary resistance. This substance was apparently lacking whenever the diet did not contain lard or vitamin K.

Dietary folic acid deficiency in pregnant rats produced hemorrhage in the feti (Giroud and Lefebvres-Boisselot, 1951) as well as edema (Nelson et al., 1952). Also, edema was observed in the chick embryos of hens fed a ration deficient in folic acid (Karnofsky et al., 1949). Edema in the chick embryo was observed as a result of riboflavin deficiency in the hen's diet (Lepkovsky et al., 1938; Romanoff and Bauernfeind, 1942). Also, in the young from riboflavin deficient rats edema was sometimes observed (Ensminger et al., 1947). In maternal pantothenic acid deficiency edema and hemorrhage were observed in young rats (Lefebvres-Boisselot, 1951). In maternal vitamin B_{12} deficiency the legs of the chick embryos become hemorrhagic and atrophic (Olcese et al., 1950).

c. Cardiovascular malformations and ectocardia. Cardiovascular malformations in the offspring of vitamin A-deficient rats were first observed by Warkany and Roth (1948) who showed that the heart muscle retained the spongy structure of the fetal condition. Wilson and Warkany (1949) and Wilson and Baroh (1949) made a detailed study of these conditions.

In addition to the highly trabeculated spongy appearance, several other abnormalities were observed, namely, failure of interventricular septum to close, right subclavian artery arising distally, reversed asymmetry of the dorsal aorta, double arch of the dorsal aorta, absence of the fourth arch, absence or bilateral persistence of the ductus arteriosus, and absence of the pulmonary artery. The incidence of these anomalies in the abnormal embryos studied was about 60 per cent. All of these defects were more common in younger feti, indicating that they were associated with, but not necessarily responsible for early fetal death. It was concluded that all of these malformations were the result of interference with normal developmental processes occurring on or subsequent to the 12th day of gestation.

In addition to the abnormalities mentioned above, others are reported in the literature. Smith (1917) showed that the heart of pigs with prenatal iodine deficiency had a persistent foramen ovale. This defect could be prevented by giving potassium iodide to the pregnant sow. Hydropericardium was observed by Zilva et al. (1921) in pigs from vitamin A-deficient hogs, and by Karnofsky et al. (1949) in chick embryos as a result of folic acid deficiency in the hen's diet. Ectocardia was observed in the feti of rats fed

a folic acid deficient diet (Giroud and Lefebvres-Boisselot, 1951).

d. Acute uremia. This phenomenon somewhat resembles the "inborn errors of metabolism" except that it is curable. When pregnant rats were maintained on a vitamin B_{12}-deficient ration, 50 per cent of the young developed acute fatal uremia within 36 to 48 hours following birth. The concentration of blood urea had risen to values ranging from 150 to 300 mg. per 100 ml. (Schultze, 1949). This syndrome was prevented by a single subcutaneous injection of 0.05 µg. of vitamin B_{12} into the young at birth.

e. Malformations of the uro-genital tract. Hale (1933, 1935) noted incomplete migration of the kidney in the young of vitamin A-deficient hogs. Lepkovsky et al. (1938) noted that a deficiency in riboflavin in the diet of the hen induced a degeneration of mesonephros in the chick embryo. Evidence of vitamin A deficiency in rat feti was first presented by Wilson and Warkany (1947). They observed epithelial keratinization in the lower genito-urinary tract of feti older than 18 days gestational age. This keratinization was preceded by partial hyalinization of the cytoplasm and the appearance of keratohyalin granules in the surface cells.

Congenital malformations in the uro-genital tract of the rat induced by maternal vitamin A-deficiency were studied

by Warkany and Roth (1948), Wilson and Warkany (1948) and Wilson and Barch (1949). The incidence of these anomalies in the 42 fetal and newborn embryos studied was 7.5 per cent. These consisted of hypoplasia of renal parenchyma, renal ectopia, ectopic ureteric openings, incomplete development of the Müllerian ducts, abnormal retention of heterologous genital ducts in both sexes, aplasia of vagina and male accessory glands, faulty differentiation of the urogenital sinus, horse-shoe kidneys, cryptorchidism, hypospadias, and atresia of genital ducts and ureters.

The beginnings of testis degeneration were observed in boar pigs littered by gilts that had been restricted to a vitamin E-deficient ration (Adamstone et al., 1949). Also, genital abnormalities were observed in the young from rats fed a pantothenic acid deficient diet (Lefebvres-Boisselot, 1951).

Retarded development of the kidneys, with cystic dilatation of the collecting tubules, was observed in the young rat due to maternal folic acid deficiency (Nelson et al., 1952).

4. Miscellaneous

Many congenital abnormalities reported in the literature as due to maternal nutritional deficiencies are difficult to classify. Consequently they are grouped under this category. A brief review of these diverse abnormalities is given below.

a. Goiter. Smith (1917), Hart and Steenbock (1918) and Murray and Wilson (1945) noted that an iodine deficiency during the gestation period of the sow caused functional retardation and hyperplasia of the fetal thyroid, resulting in arrested development of the feti. According to these authors, tremendous activity of the fetal thyroid during the later stages of the intra-uterine life is essential for the normal development of the fetus.

Congenital goiter has been reported in chicks (Gassner and Wilgus, 1940). These authors noted that the average size of the thyroid glands of baby chicks hatched from eggs laid by hens receiving a goitrogenic ration were two to three times that of glands from chicks hatched from the eggs of hens receiving iodine supplementation.

b. Congenital scurvy. The majority of the animal species synthesize ascorbic acid. The question of the requirement of the embryo for this vitamin involves only the guinea pig, monkey and man. Scorbutic lesions have been

observed in the guinea pig fetus as a result of maternal vitamin C deficiency (Hess, 1917; Ingier, 1915; Reyher et al., 1928). Jackson and Park (1935) presented the first absolutely authentic report of congenital scurvy in man. They observed the scorbutic lattice in the bone near cartilage.

c. Liver changes. Liver changes were observed in chick embryos as a result of riboflavin deficiency of hens (Romanoff and Bauernfeind, 1942). Some damage was found in the liver of young pigs littered by gilts fed a vitamin E-deficient ration by Adamstone et al. (1949).

d. Malformation of the lung. In a study of cross sections of the trunk region of young rats from vitamin A-deficient mothers, Warkany and Roth (1948) noted that the lungs were under-developed. They failed to grow forward thereby retaining their fetal position behind the heart. The pleural chambers were seen to border the pericardial cavity posteriorly and laterally, but did not surround it. This same condition was observed in the young rats from folic acid deficient dams (Nelson et al., 1952). The lungs were small and compact instead of being spongy and expanded.

e. Hairlessness and retardation in hair or feather growth. Nordfeldt (1945), as quoted by Boisselot (1949) noted that the growth of hair was retarded in some of the

pigs littered by avitaminotic A sows. The young of riboflavin deficient female rats sometimes were hairless (Ensminger et al., 1947). Lepkovsky et al. (1938) noted the appearance of abnormal down in the chick embryo as a result of riboflavin deficiency in the diet of the hen. Absence of feathers was observed in chick embryos due to folic acid deficiency in the hen's diet (Karnofsky et al., 1949). Murray and Wilson (1945) reported that maternal iodine deficiency induced hairlessness in the young. This confirmed the previous findings of Smith (1917) and Hart and Steenbock (1918) that the hairless pig malady was occasioned by a subnormal assimilation of iodine by the sow.

f. Congenital cleft palate and hare lip. Cleft palate is due to a lack of fusion of the palatine processes of the maxillae and the horizontal processes of the palatine bones, whereas hare lip is caused by an absence of union of the medial nasal process and maxillary process. Hale (1933, 1935) observed cleft palate and hare lip in the pigs from vitamin A-deficient sows. On the other hand, excessive vitamin A intake during gestation also sometimes induced hare lip and cleft palate in the young pigs (Cohlan, 1953). Similar defects were observed in young rats littered by females receiving diets deficient in vitamin D (Warkany et al., 1943), riboflavin (Gilman et al., 1952; Warkany and

Schraffenberger, 1943), and folic acid deficiency (Giroud and Lefebvres-Boisselot, 1951; Nelson et al., 1952).

It is apparent from the numerous instances cited in the foregoing that the composition of the maternal diet previous to and during gestation is one of the important causes of congenital abnormalities. In general, the congenital malformations result from arrested or abnormal development of the embryonic "anlagen" as indicated by incompletely developed organs, by the abnormal position of organs, or by fusion of the anlagen. Experimental studies have revealed that anomalies of nutritional origin depend upon the occurrence of one or more maternal nutritional deficiency during the critical period when the development and differentiation of the embryonic anlagen are taking place. When prolonged excessively maternal deficiencies give rise to sterility due to resorption, abortion, or both. On the other hand, when dietary deficiencies occur after the anlagen already have become differentiated, they are without effect in producing congenital anomalies. Finally, if nutritional deficiencies occur in sufficient intensity during the critical period, they will retard or induce local modifications of development.

III. EXPERIMENTAL

The first objective in pursuing this study was to demonstrate the production of avitaminosis E congenital abnormalities. This having been done, steps were undertaken to define more precisely the conditions for their production. Next, attempts were made to learn ways of increasing their incidence. Finally, the abnormal effects produced were investigated from the view points of hematopoiesis, histology and anatomy. The detailed procedures will be described forthwith.

A. Composition of the Rations, and Management of the Rats

1. Composition of the rations

The only animals used in this study were albino rats and they were of the Sprague-Dawley strain. The investigation was confined exclusively to females. These were weaned from their dams and allocated to experimental rations when they attained a weight of approximately 40 grams each. Prior to weaning they and their dams received ad lib. a stock ration having the composition given in Table 1.

Table 1

Composition of Ration Used to Produce Weanling Rats

Ingredients	Gm.
Yellow corn-ground	58.50
Wheat midlings	10.00
Butter milk-dried	13.25
Linseed oil meal	10.00
Tankage-60% digestion	8.25
Alfalfa meal-dehydrated	5.00
Sodium chloride	0.50
Bonemeal-steamed	0.50
Vegetable oil-Wesson*	2.00

*Includes 200,000 units vitamin A concentrate per pound.

The experimental rations were compounded periodically in batches of a size which would be consumed in approximately, but never less than, two weeks. All batches were aged for two weeks in a granular state. Granulation was accomplished by forcing each batch through a sieve made of one eighth inch hardware cloth. The pasty nature of the rations, due to the large amount of lard present, made granulation easy. Aging at room temperature for two weeks in a granular state enhanced the inactivation of any residual vitamin E present.

The composition of each experimental ration used is shown in Table 2.

Table 2

Composition of Rations Used to Produce Avitaminosis E in Female Rats

Ingredients	Ration I %	Ration II %	Ration III %
Dextrin	49	49	49
Lard	22	22	22
Casein	18	18	18
Dried brewer's yeast	5	5	5
Salt mixture*	4	4	4
Cod liver oil	2	2	2
Tri-o-cresyl phosphate	0	0.025**	0.100**

*U. S. P. #XIV.

**Mg./gram of ration.

2. Management of the rats

The production of the weanling females and their use in experimentation was done in rooms maintained at approximately 76°F and 50 per cent relative humidity by means of air conditioning equipment. Since the first series of experiments lasted about nine months and the second nearly a year, this constant environment was very helpful in minimizing contagion, and irregularities in reproduction due to seasonal changes which might otherwise have occurred.

When the nursing young females reached approximately 40 grams each, they were weaned, marked for identification, and allotted to standard metal animal cages designed to minimize coprophagy. When the rats were small six were placed in each cage. As they grew larger fewer were housed in a cage to avoid crowding. Fresh ration and water were given daily. Occupied cages, droppings pans, and feeding cups and drinking fountains were replaced by clean equipment frequently in keeping with good sanitation and experimental procedure.

All females were weighed weekly until they weighed 200 grams each, at which time they were mated and weighed weekly during gestation. All females were mated to "proven" males in the following manner. Females of breeding age were placed

with adult males late each afternoon in the mating cages where they remained until morning. Then all males were removed, and the females were vaginal smeared for signs of positive mating. The presence of either the vaginal plug or sperm in the vaginal smear was positive evidence of coitus. The day on which the sperm was found was considered tentatively as the zero day of gestation. This procedure was followed routinely every day as long as there were females to be mated. When positive coitus had been established the particular female was weighed and allotted randomly to receive one of the several treatments under way at the time.

Detection of implantation was determined by noting the presence of red blood cells in vaginal smears made during the 11th to 15th day after breeding. This is a positive sign of conception. Whereas live weights taken periodically during gestation give a general idea of the nature of the pregnancy, much more specific information is obtained by examining the uterus and its contents following laparotomy. Laparotomies were performed routinely on the 21st day of each gestation. The procedure followed and the information collected are given below. During the 21st day of gestation each female was heavily desensitized by etherization during which time a median ventral longitudinal incision was made on the abdomen to expose fully the viscera. The uterine horns were excised

quickly, the extraneous adipose and connective tissues removed, and the uterus and its contents weighed together. Then an incision was made along the anti-mesometrial pole of the uterus and its contents examined for number of implantation sites, degree and number of resorptions, if any, and number of live normal and abnormal feti. Weights were taken of the uterus and its contents, the empty uterus, live normal and abnormal feti singly and collectively. The normal and abnormal feti were either used for hematological studies, preserved in formalin, or processed for skeletal and histologic studies.

3. Allocation of the rats

Allocation of all females to the different ration treatments was at random following proof of coitus. In Series I there were 11 groups of rats and each received a different dietary treatment as shown in Table 3.

Series II consisted of 25 groups of pregnant rats. The females were randomized according to the plan presented in Table 4.

Table 3

Series I. Number and Size of Groups and Days during Gestation Vitamin E Was Given Orally

Group no.	Rats in group no.	Time of gestation tocopherol given* day
1	21	-
2	25	4
3	23	5
4	22	6
5	19	7
6	21	8
7	32	9
8	27	10
9	28	11
10	20	12
11	35	--

*Single dose of 1.2 mg. d,l-alpha-tocopherol acetate given orally on day indicated. All groups given ration I ad lib. excepting group 11 which received stock ration ad lib. only.

Table 4

Series II. Number and Size of Groups on Each of the Four Rations and Level of Vitamin E Given Orally during Certain Days of Gestation

Group no.	Rats in group no.	Ration no.	Supplementation d,l-alpha-tocopherol acetate mg.	Time of gestation tocopherol given day
21	24	I	-	-
22	20	I	1	8
23	20	I	2	8
24	20	I	4	8
25	20	I	1	10
26	20	I	2	10
27	20	I	4	10
28	20	I	1	12
29	19	I	2	12
30	20	I	4	12
31*	18	I	2	1,2,3,4,5
32	19	I	2	1,2,3,4,5
41	20	II	-	--
42	20	II	1	10
43	20	II	2	10
44	19	II	4	10
45*	17	II	2	1,2,3,4,5
46	17	II	2	1,2,3,4,5
51	20	III	-	--
52	20	III	1	10
53	20	III	2	10
54	20	III	4	10
55*	14	III	2	1,2,3,4,5
56	20	III	2	1,2,3,4,5
33	23	Stock ration		

*2 mg. of d,l-alpha-tocopherol acetate given orally each week from weaning to positive mating.

B. Macroscopic and Histologic Procedures

1. Alizarin method

The method is used primarily to aid in visualizing the degree of calcification in the bones. The following procedure was used.

a. Each live fetus was immersed in 95 per cent alcohol for 96 hrs.

b. Then place in acetone for 96 hrs. to remove fat.

c. Next place in 95 per cent alcohol again for 48 hrs. to remove acetone.

d. Bleach in 95 per cent alcohol containing H_2O_2 until desirable. The H_2O_2 solution was made up by mixing 1 part of 3 per cent H_2O_2 with 9 parts of 95 per cent alcohol.

e. Place in 1 per cent aqueous KOH solution for 72 hrs., or till bones become clearly visible.

f. Transfer directly to a dilute solution of alizarin in KOH, which was made up by mixing 1 part of alizarin with 10,000 parts of 1 per cent aqueous KOH, for 24 hrs.

g. Complete clearing process in Mall's solution, which consists of 79 parts of distilled water, 20 parts of glycerine and 1 part of KOH.

h. Dehydrate in 40, 60, 80, 95, and 100 per cent glycerine.

i. Store in white glycerine with a few crystals of thymol as preservative.

2. Histologic method

For histologic studies the fixatives used were Bouin's solution and Heidenhain's Susa solution. After fixation and proper washing the tissues were stored in 70 per cent alcohol. For embedding the following procedure was followed:

a. 70 per cent alcohol.

b. 80 per cent alcohol 2 changes, 24 hrs. each.

c. 95 per cent alcohol 2 changes, 12 hrs. each.

d. 100 per cent alcohol 2 changes, 2 hrs. each.

e. 100 per cent alcohol plus xylene, 30 min.

f. Xylene, 30 min. to 1½ hr., noting time to clear specimen.

g. Xylene plus soft paraffin, 30 min.

h. Soft paraffin, 2 changes, 30 min. each to 3 hrs. depending on size of tissue.

i. Hard paraffin, 2 changes, 30 min. each.

j. Imbed in pure hard paraffin in paper boxes.

Since the feti were comparatively large the regular long method was adopted instead of the shorter time-saving one. After cooling the paraffin blocks were trimmed and mounted on the microtome stage. Sections 7.5 to 10 microns in thickness were cut and mounted on slides by using albumin fixative and water. After thorough drying the slides were stained.

For staining the routine hematoxylin and eosin method was used. The procedure used follows:

a. Deparaffinize slides in xylene, 10 min.

b. Pass to 100 per cent alcohol, 1 min.

c. 0.5 per cent colloidin in a 1:1 mixture of 100 per cent alcohol and ether, 1 min.

d. 67 per cent alcohol, to set the colloidin, 2 min.

e. 50 per cent alcohol, 1 min.

f. Distilled water, 1 min.

g. Hematoxylin, 2 to 5 min.

h. Distilled water, 1 min. to differentiate the staining.

i. Wash in tap water till sections were blue.

j. 0.5 per cent aqueous eosin, 1 min.

k. Distilled water, 1 sec.

l. 95 per cent alcohol, 1 min.

m. 100 per cent alcohol, 1 min.

n. 100 per cent alcohol plus ether, to remove the colloidin coating.

o. Xylene, to clear the sections, 1 min.

p. Mount in Canada balsam.

C. Hematological Procedures

Many of the live abnormal feti were lighter colored than the normal feti and were suspected of being anemic. Therefore, a comparison was made between the blood of normal full term feti from dams fed a normal ration or a vitamin E-deficient ration with weekly oral supplementation of the vitamin, and abnormal full term feti from avitaminotic E dams. Blood samples for these comparisons were obtained from single feti at term by cutting the umbilical cord and collecting the blood on a watch glass. Red cell, white cell, and differential white counts and hemoglobin values were determined.

1. Enumeration of the red blood corpuscles

The principle of this method is based on the accurate dilution of a measured quantity of blood with a fluid which is isotonic with the blood and which will prevent its coagulation. A dilution of 1 to 200 usually is necessary because

the corpuscles in normal blood are so concentrated they scarcely can be distinguished in a counting chamber.

In making the erythrocyte count, blood was drawn by suction into a Thoma red pipet to the 0.5 mark. The blood adhering to the outside of the pipet was next wiped off and the diluent, Hayem's solution, was drawn in until it filled the bulb and reached the mark 101. After the desired quantity of solution had been drawn into the pipet, it was held horizontally and shaken for about one-half minute in order to secure thorough mixing. The cover-glass was next placed on the Levy counting chamber with improved Neubauer double ruling. Several large drops of fluid were expelled from the pipet and discarded in order to remove the fluid in the capillary portion of the pipet which had not come in contact with the blood. A small quantity of diluted blood was then placed between the cover-glass and the ruled platform of the counting chamber.

After a few minutes had been allowed for the blood to settle, the slide was examined for even distribution of the cells in the chamber. If the distribution was satisfactory, the cells were counted using the high-power objective of the microscope. The number of cells in the 4 corner groups of 16 squares and in one central group in the central square millimeter of the slide was recorded. Since the dilution

was 1 to 200, the total number of cells found in five groups of 16 squares was multiplied by 10,000 in order to give the number of cells per cu. mm. of blood for the following reasons: The smallest squares on the slide have an area of 0.0025 sq. mm. and are 0.1 mm. deep, being thus 0.00025 cu. mm. in volume. Since 80 such squares are counted, a volume of 0.00025 x 80 or 0.02 cu. mm. has been covered. In order to give the value per cu. mm., the number of cells counted must be multiplied by 1/0.02 or 50. However, since the dilution was 1 to 200, the multiplication factor is 50 x 200 or 10,000. The average of two counts from two separate counting chambers was taken as one reading.

2. Enumeration of white blood corpuscles

Enumeration of the white blood corpuscles was carried out according to the same principles used in counting the erythrocytes except for appropriate modifications. The blood was diluted less since the cells were not as numerous. A special pipet permitting dilutions of 1 to 20 was used. The diluent was a 0.5 per cent solution of acetic acid which was colored with 0.1 per cent methylene blue enabling the leukocytes to be seen more readily. The cells were counted in five one millimeter squares of the counting chamber.

Distribution of the cells in the counting chamber was considered satisfactory when the differences between the cell counts in the 5 squares were not more than 8, provided the total count was near that for normal blood. The number of cells found in 5 squares was multiplied by 40 to find the total number of leukocytes per cu. mm. (x 20 for the dilution, x $\frac{1}{1 \times 1 \times 0.1 \times 5}$ for the volume counted). The final count was obtained by averaging two counts made in two separate chambers.

3. Differential count of the leukocytes

The procedure used in making differential counts of the leukocytes was as follows. Samples of blood were smeared on slides by means of a rubber ruler. After drying in the air the slides were covered with twenty drops of Wright's stain for one minute. Then twenty drops of distilled water were added to the contents of the slide. The staining required four minutes to complete. Excess stain was then "floated" off in a horizontal position by washing repeatedly with distilled water. Next, they were washed in tap water for 30 seconds until the color became pinkish. Then they were air dried and examined in immersion oil. Not fewer than 200 cells were counted in each examination. The values for lymphocytes,

monocytes, neutrophiles, eosinophiles and basophiles were arrived at by averaging duplicate counts made from duplicate slides.

4. Hemoglobinometry

Hemoglobin was determined by the oxyhemoglobin method since it was thought to be the best method for determining hemoglobin (Wintrobe, 1951). The oxyhemoglobin formed with ammonia is very stable. The hemoglobin reading may be read with equal accuracy any time during a period of twelve hours after the determination (Bell et al., 1945). Furthermore, the optical density of the solution is of the same order as that of other hemoglobin derivatives, and the presence of as much as even 20 per cent of abnormal pigments in the blood introduces no significant error. The dilute ammonium hydroxide was made up by adding 4 ml. of concentrated ammonium hydroxide to 996 ml. of distilled water. Ten ml. of this solution was placed in a test tube followed by the addition of 20 cu. mm. (0.02 ml.) of blood. Colorimetric readings were made with a Klett-Summerson photoelectric colorimeter equipped with a 540 mμ filter. Hemoglobin concentration was read directly from a graph which related optical density to grams of hemoglobin per 100 ml. blood.

The amount of blood usually obtainable from the umbilical cord of each abnormal fetus was smaller than the 20 cu. mm., the amount required by standard procedures. Consequently, white pipets were calibrated gravimetrically (Wintrobe, 1951) by weighing the water drawn up to the 0.1 mark of the pipet and comparing its weight to that of water in the 20 cu. mm. pipet. From the average of duplicate determinations the volume was calculated to be 2.14 cu. mm. in one white pipet, and 2.15 cu. mm. in another. Thus, in the determination of hemoglobin in the abnormal feti 2.14 or 2.15 cu. mm. of blood was used instead of the 20 cu. mm. as specified by standard procedures. Since the amount of blood was reduced, 5 ml. of the dilute ammonium hydroxide instead of 10 ml. was used to obtain satisfactory readings with the colorimeter. For both of these modifications a corresponding conversion factor was used in the final calculations. Whenever possible duplicate samples were taken. Data obtained from the standard and modified procedures agreed very closely. For example, when determining the hemoglobin in the same sample of blood, the standard procedure which requires 20 cu. mm. of blood gave a reading of 81.1 while the modified procedure calling for only 2.15 cu. mm. of blood gave a reading of 81.9. The difference between the values obtained by these two procedures

was only 0.9 per cent, which is within the range of experimental error.

The colorimeter was calibrated using the pyridine-hemochromogen method of Rimington (1942). One hundred milligrams of recrystallized dried hemin was transferred to a 250 ml. volumetric flask and made to volume with N/10 sodium hydroxide. One ml., 2 ml., 3 ml., and 5 ml. of the hemin solution were transferred to 100 ml. flasks and diluted to volume with N/10 sodium hydroxide. Then 10 ml. of each of these dilutions were transferred in duplicate to matched test tubes. Next, 2 ml. pyridine (C.P.) plus a small amount of sodium hydrosulfite ($Na_2S_2O_4$, C.P.) were added to each tube. These four pairs of tubes contained 0.04, 0.08, 0.12 and 0.20 mg. hemin respectively. The optical density of these solutions was related to quantity of hemin by making colorimetric readings for each solution using a 540 mμ filter. These values were plotted on the logarithmic scale of semi-log paper whereas milligrams of hemin would be indicated on the ordinary scale.

The standard curve for oxyhemoglobin for normal whole rat blood was determined. A given amount of this blood was variously diluted with ammonium hydroxide as follows:

a. 0.2 ml. blood, diluted to 50 ml.

b. 0.2 ml. blood, diluted to 100 ml.

c. 0.2 ml. blood, diluted to 250 ml.

d. 0.2 ml. blood, diluted to 500 ml.

The blood was thoroughly washed into the flasks. These solutions of oxyhemoglobin were then read against a water blank at 540 mμ.

The pyridine hemochromogen was prepared in triplicate by treating 10 ml. of a 1:500 dilution of normal blood in N/10 NaOH with 2 ml. pyridine and a pinch of sodium hydrosulfite. The optical density of the solution was then measured in the colorimeter at 540 mμ. The value of this solution in terms of milligrams of hemin (X) is determined by consulting the standard hemin curve.

The molecular weight of hemin is 651.4. On the basis of an iron content of 0.339 per cent, hemoglobin has a minimal molecular weight of 65,888. Then since there are 4 molecules of hemin in hemoglobin, the factor for converting hemin to hemoglobin is $\frac{65,888}{651.4 \times 4} = 25.2$. Consequently the quantity of hemoglobin in the original sample of blood (Y), expressed in grams per 100 ml., equals: $\frac{X \times 500 \times 10 \times 25.2}{1000}$. Calculated on the basis of this obtained value for solution (b) solutions (a), (c) and (d) should correspond, respectively to 2Y, $\frac{Y}{2.5}$, and $\frac{Y}{5}$ grams of hemoglobin.

To relate the optical density of the oxyhemoglobin solutions to grams of hemoglobin per 100 ml., the colorimeter

readings are plotted on the logarithmic scale of semi-log paper and the grams of hemoglobin are indicated on the ordinary scale. The straight line represents the scale of values for hemoglobin, expressed as grams per 100 ml., corresponding to the optical densities of solutions of oxyhemoglobin prepared by diluting 20 cu. mm. of blood to 10 ml. with dilute ammonium hydroxide.

IV. RESULTS AND DISCUSSION

A. Verification of Relationship of Teratogeny to Avitaminosis E in the Rat

The most obvious abnormality in reproduction associated with female rats in an advanced state of avitaminosis E is fetal resorption. This disturbance always has been easily overcome by vitamin E therapy during gestation. However, it has now been demonstrated that improper timing of therapy during gestation leads to other complications. The most striking of these is the production of congenital abnormalities. Several kinds of these were produced originally in this laboratory about ten years ago quite by accident in connection with another phase of research involving vitamin E. Time and facilities then did not permit any study of the circumstances giving rise to these abnormalities. Consequently, the first task in this present study was to verify these earlier observations. Once their reproducibility had been established they then could be investigated in detail.

Three groups of female rats which had been restricted to vitamin E depleting ration I (Table 2) were mated to proven males. During the 8th, 9th, or 10th day of each gestation the females were given 1.2 mg. of d,l-alpha-tocopherol

acetate via stomach tube. All females were laparotomized on the 21st day of gestation, and the contents of the uteri examined. The findings are reported in Table 5.

Table 5

Confirmation of Avitaminosis E Teratogeny in Rats

Group*	Time of therapy, day**	Pregnancies, no.	Females having abnormal feti at term, no.
1	8	21	0
2	9	32	9
3	10	27	7
Total	--	80	16

*Females in all groups received a basal vitamin E depletion ration ad lib. from weaning time.

**Day of gestation 1.2 mg. d,l-alpha-tocopherol acetate was given orally.

Note that abnormal feti occurred at term in a goodly percentage of those females which had been given tocopherol on the ninth and tenth days of gestation, and that none appeared when similar treatment was given on the eighth day.

These are not chance observations since they are based on a fairly sizable number of conceptions. Teratogeny due to avitaminosis E is a reality. The writer next proceeded to study the cause and effects of this nutritional disturbance in more detail. These may be classified generally into factors affecting the incidence of avitaminosis E teratogeny, characterization of the fetal abnormalities, and hematological studies of the blood of affected feti.

B. Factors Affecting the Incidence of Teratogeny in Rats

It was pointed out in the review of literature that there appears to be a short interval during the stage of embryonic growth when certain environmental disturbances can alter or retard development of the embryos and result in live deformed feti. Presumably conditions may prevail in certain avitaminoses which lead to this type of abnormality. This may be the basic cause of teratogeny in avitaminosis E. Several factors would appear to exert a definite rôle in determining the extent and severity of the occurrence of these abnormalities. Data dealing with the more obvious of these follow. They relate to such factors as: 1) timing of therapy, 2) different levels of therapy, and 3) extent of vitamin E depletion as influenced by time, or the use of an

antagonist to vitamin E, or the effect of both operating simultaneously.

1. Relationship of timing of therapy

Several groups of mated avitaminosis E females were given 1.2 mg. d,l-alpha-tocopherol acetate by stomach tube in one dose on different days of the gestation period. Whether or not normal or abnormal feti were present at term depended upon which days supplementation was administered. The data obtained are summarized in Table 6. Note that abnormal feti occurred in females which had been given tocopherol on the 9, 10, 11 or 12th day of gestation, and that only normal feti occurred when identical therapy was administered on the 4, 5, 6, 7 or 8th day of gestation. Obviously, 1.2 mg. of d,l-alpha-tocopherol is adequate to prevent the occurrence of malformed feti if given prior to the 9th day of gestation and that identical amounts of the vitamin given immediately following the 11th day of gestation resulted in a marked decrease in congenitally abnormal feti. Instead, the feti died in utero and had undergone various degrees of advanced resorption by term. Apparently, there is a critical short interval of time in the middle of each gestation during which a therapeutic dose of vitamin E, that would be adequate if given

Table 6

Relationship of Incidence of Teratogeny to Timing of d,l-alpha-Tocopherol Acetate Therapy during Gestation

Group[a] no. and ration fed	Day of gestation 1.2 mg. tocopherol fed	Conceptions	Uteri containing at term[b]	
			Abnormal live feti	Normal live feti
	day	no.	no.	no.
1 I	Neg.	21	0[c]	0[c]
2 I	4	21	0	21
3 I	5	23	0	23
4 I	6	22	0	22
5 I	7	19	0	19
6 I	8	21	0	21
7 I	9	32	9	23
8 I	10	27	7	20
9 I	11	28	7	21
10 I	12	20	2	18
11 S	Pos.	35	0	35

[a]I denotes depleting ration I (Table 2) and S, stock colony ration (Table 1).

[b]Normality or abnormality based on macroscopic morphological appearance.

[c]Feti and placentae partially or completely resorbed.

earlier in the gestation, is unable to prevent the occurrence of congenital abnormalities. The reason for this may be found when one recalls the development of the vitamin E-deficient rat embryo on the 10th day post coitus (Urner, 1931). Up to and including the 9th day of gestation, no abnormality in development was observed. However, on the 10th day, the vitamin E-deficient rat embryo revealed beginning rarefaction of the mesenchymal tissues. The heart contained a few scattered fetal blood cells. The yolk sac showed only a few small blood islands. On the whole, the development was retarded when compared with the control embryos. It is conceivable that supplementation with an adequate amount of vitamin E at this time could prevent the inception of degeneration in some embryos and allow them to develop normally to term, and simultaneously in cases of borderline deficiency, congenital malformations would result due to interruption of local differentiation and development. Whether the disturbance is due primarily to improper timing or can be prevented by increasing the therapeutic dose was next determined.

2. Effect of therapeutic level of vitamin E

Thus far the data, Tables 5 and 6, show that avitaminosis E teratogeny occurs largely when therapy is administered

during the 9, 10, or 11th day of gestation. A study of whether this period of greatest incidence could be shifted or narrowed materially by varied therapy was next undertaken. Levels of d,l-alpha-tocopherol acetate ranging from 1 to 4 mg. per rat was fed by stomach tube during the 8, 10 or 12th day of gestation to female rats which had been restricted to ration I since they were weaned. The results are summarized in Table 7, and presented graphically in Figure 1. Note that comparatively few of the females which received therapy on the 8th or 12th day produced abnormal feti. In marked contrast, a much larger number of females that had been given similar therapy on the 10th day of gestation gave birth to abnormal feti. These data essentially confirm those presented in Tables 5 and 6.

Apparently, the peak of incidence is not influenced significantly by levels of 1, 2, or 4 mg. of d,l-alpha-tocopherol acetate, whether given on the 8, 10, or 12th day of gestation. When 1 mg. of d,l-alpha-tocopherol is given early in gestation to avitaminosis E females, it barely protects against complete resorption of all feti by term. On the other hand, 2 and 4 mg. levels afford ample protection under similar experimental conditions. Even though an increase to 4 mg. was 400 per cent above the approximate minimal protective dose against fetal resorption, therapeutic

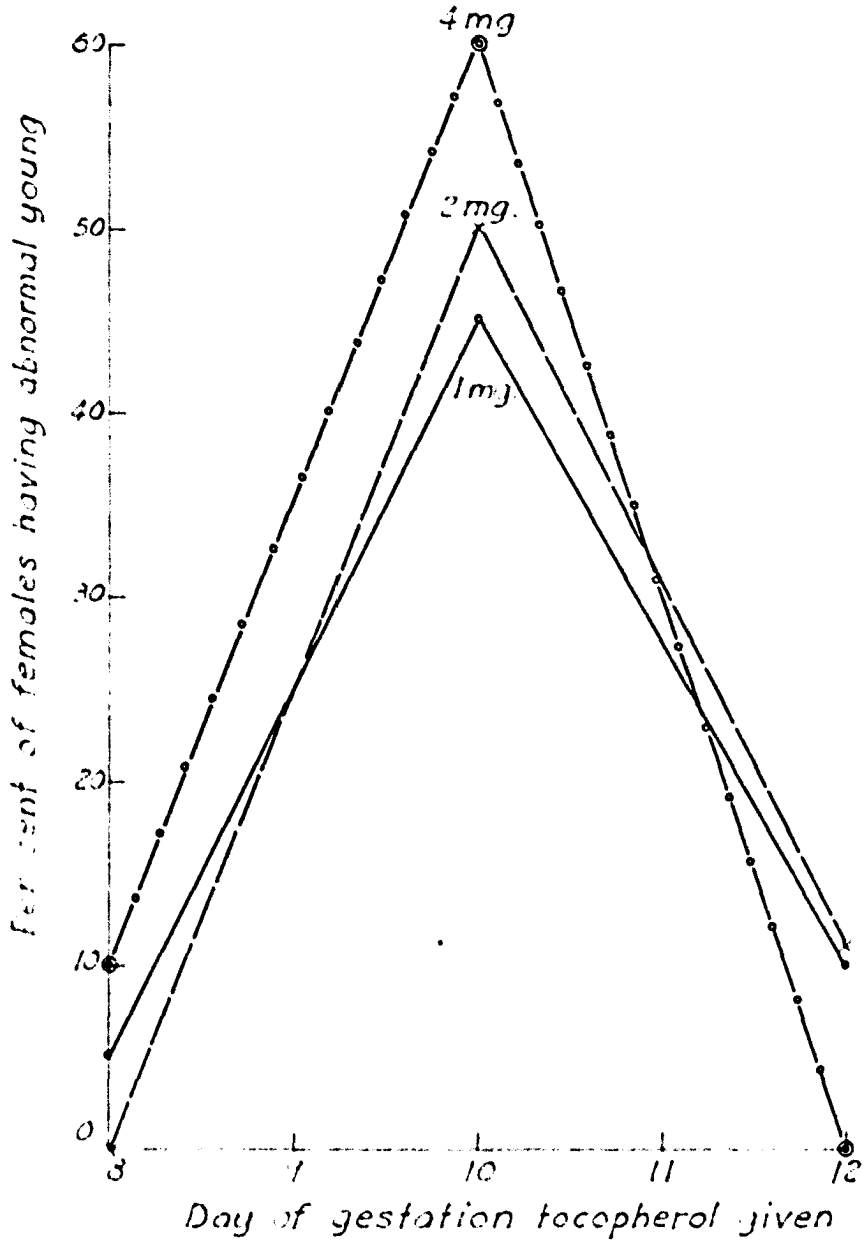


Figure 1

Relationship of Time and Level of d,l-alpha-Tocopherol Acetate Therapy during Gestation to Incidence of Congenital Abnormalities in Rats Fed Ration I

Table 7

Effect of Therapeutic Level of d,l-alpha-Tocopherol Acetate Fed during Gestation on the Incidence of Teratogeny

Group[a] no. and ration fed		Day of gestation tocopherol fed / day	Level of tocopherol fed on day(s) indicated / mg.	Abnormal preg. / Normal preg. / no./no.	Abnormal feti / Normal feti / no./no.	Abnormal pregnancies / %	Abnormal feti / %
21	I	Neg.	0	0/24	0/0	0	0
22	I	8	1	1/19	1/56	5	2
23	I	8	2	0/20	0/71	0	0
24	I	8	4	1/19	1/99	5	1
25	I	10	1	9/11	20/17	45	54
26	I	10	2	10/10	30/18	50	62
27	I	10	4	12/8	27/42	60	39
28	I	12	1	2/18	2/1	10	67
29	I	12	2	2/17	5/4	11	56
30	I	12	4	0/20	0/21	0	0
31[b]	I	1,2,3,4,5	2	0/18	0/155	0	0
32	I	1,2,3,4,5	2	0/19	0/148	0	0
33	S	Pos.	0	0/23	0/228	0	0

[a]I denotes depleting ration I (Table 2) and S, stock colony ration (Table 1).

[b]Each female received weekly by stomach tube 2 mg. d,l-alpha-tocopherol from weaning to mating.

levels of 1, 2, and 4 mg. produced comparable teratogenic results when administered on the 8, 10, or 12th day of gestation. Evidently avitaminosis E teratogeny cannot be circumvented or its severity appreciably reduced by increasing the therapeutic level of tocopherol when administered during the critical period of embryonic development.

3. Relationship of extent of depletion to incidence of teratogeny

In studies involving the depletion of animals of a specific vitamin, increase in the severity of avitaminosis proceeds roughly proportional to time, other conditions being equal. This principle operates to a certain point as ultimately the law of diminishing returns has to be reckoned with. Early in this study, as has been true with many others, attempts were undertaken to determine the principal factors involved in the consistent production of a high percentage of congenital abnormalities. Up to this point, time of gestational therapy is a very important factor, yet the percentage incidence is variable and not large in many instances. On the other hand, other things being equal, differences in tocopherol therapy ranging from 1 to 4 do not affect incidence significantly. It seemed that

conditions contributing to rapid or severe depletion of vitamin E might enhance the incidence of abnormality. Two alternatives were possible; namely, prolonged restriction of the rats to depleting rations, or restriction to a depleting ration to which had been added an antagonist to vitamin E, such as tri-o-cresyl phosphate. The latter alternative seemed preferable and its practical acceptability was investigated. Before presenting these data, those having a bearing on the first alternative will be discussed.

a. Effect of length of time females are limited to the depletion rations. In the course of this study many hundreds of conceptions were produced under a variety of controlled conditions. Although no statistical analysis was undertaken to correlate incidence of teratogeny with extent of depletion, the voluminous data gathered have been organized in the hope of shedding light on this phase and are presented in Table 8. Probably more critical comparisons can be made amongst those groups which received therapy on the 10th day of gestation (Figure 2). In group 8 of Table 8 abnormal feti occurred in gestations ranging from period II through V with the greatest number occurring in period III. In group 7 all abnormalities occurred in period V. In groups 25, 26, and 27 the largest number of abnormal gestations occurred in periods II and III, I and II, and II respectively.

Table 8

Relationship of Incidence of Teratogeny to Length of Time from Wea
Females Were Restricted to Avitaminosis E Depletion Ration

Group	Therapy[a]	Ration[b] no.	Total no. of conceptions	Total days by periods prior to conception that restricted to depleting rations							
				I	II	III	IV	V	VI	VII	
				49-58	59-68	69-78	79-88	89-98	99-108	109-118	11
				Total number of conceptions per period and the which abnormal live feti were present at							
				No tri-o-cresyl phosphate							
1	0	I	21	3	6	3	2	1	3	2	
2	4/1.2	I	25	2	4	1	3	0	8	1	
3	5/1.2	I	23	3	4	6	2	0	6	1	
4	6/1.2	I	22	1	2	4	3	2	7	2	
5	7/1.2	I	19	1	3	5	1	3	3	2	
6	8/1.2	I	21	0	2	3	6	0	7	2	
7	9/1.2	I	32 (10)	0	4	3	4	17 (10)	2	1	
8	10/1.2	I	27 (8)	2	2 (2)	15 (4)	6 (1)	1 (1)	1	0	
9	11/1.2	I	28 (7)	12 (7)	4	5	4	0	1	2	
10	12/1.2	I	20 (2)	10 (2)	1	2	2	1	3	0	
21	0	I	24	9	9	3	1	2	0	0	
22	8/1	I	20 (1)	6 (1)	7	5	0	2	0	0	
23	8/2	I	20	7	5	7	1	0	0	0	
24	8/4	I	20 (1)	4	5	7 (1)	4	0	0	0	
25	10/1	I	20 (9)	3 (1)	8 (3)	6 (3)	1	2 (2)	0	0	
26	10/2	I	20 (10)	6 (5)	7 (4)	4 (1)	2	0	1	0	
27	10/4	I	20 (12)	2 (2)	13 (7)	2 (2)	3 (1)	0	0	0	
28	12/1	I	20 (2)	5	10	2 (1)	1	1 (1)	1	0	
29	12/2	I	19 (2)	4 (1)	2	11 (1)	0	2	0	0	
30	12/4	I	20	3	10	5	2	0	0	0	
31[d]	2x5	I	18	1	8	7	1	0	0	0	
32	2x5	I	19	6	4	6	3	0	0	0	

[a]Numerator denotes day(s) in gestation therapy given; denominator, mg tocopherol given at that time.

[b]Described in Table 2.

[c]Numbers not in parentheses denote total conceptions, those in paren numbers in which one or more live malformed feti occurred.

[d]Each female received weekly by stomach tube 2 mg. d,l-alpha-tocophe from weaning to mating.

Table 8

hip of Incidence of Teratogeny to Length of Time from Weaning That emales Were Restricted to Avitaminosis E Depletion Rations

Total no. of conceptions	Total days by periods prior to conception that females were restricted to depleting rations								
	I	II	III	IV	V	VI	VII	VIII	IX
	49-58	59-68	69-78	79-88	89-98	99-108	109-118	119-128	129-138
	Total number of conceptions per period and the number in which abnormal live feti were present at term[6]								
	No tri-o-cresyl phosphate								
21	3	6	3	2	1	3	2	1	0
25	2	4	1	3	0	8	1	4	2
23	3	4	6	2	0	6	1	1	0
22	1	2	4	3	2	7	2	1	0
19	1	3	5	1	3	3	2	1	0
21	0	2	3	6	0	7	2	1	0
32 (10)	0	4	3	4	17 (10)	2	1	0	1
27 (8)	2	2 (2)	15 (4)	6 (1)	1 (1)	1	0	0	0
28 (7)	12 (7)	4	5	4	0	1	2	0	0
20 (2)	10 (2)	1	2	2	1	3	0	1	0
24	9	9	3	1	2	0	0	0	0
20 (1)	6 (1)	7	5	0	2	0	0	0	0
20	7	5	7	1	0	0	0	0	0
20 (1)	4	5	7 (1)	4	0	0	0	0	0
20 (9)	3 (1)	8 (3)	6 (3)	1	2 (2)	0	0	0	0
20 (10)	6 (5)	7 (4)	4 (1)	2	0	1	0	0	0
20 (12)	2 (2)	13 (7)	2 (2)	3 (1)	0	0	0	0	0
20 (2)	5	10	2 (1)	1	1 (1)	1	0	0	0
19 (2)	4 (1)	2	11 (1)	0	2	0	0	0	0
20	3	10	5	2	0	0	0	0	0
18	1	8	7	1	0	0	0	1	0
19	6	4	6	3	0	0	0	0	0

lenotes day(s) in gestation therapy given; denominator, mg. dl-alpha- n at that time.

in Table 2.

t in parentheses denote total conceptions, those in parenthsses the h one or more live malformed feti occurred.

s received weekly by stomach tube 2 mg. d,l-alpha-tocopherol acetate mating.

Table 8 (Continued)

Relationship of Incidence of Teratogeny to Length of Time from Weanir
Females Were Restricted to Avitaminosis E Depletion Rations

Group	Therapy[a]	Ration[b] no.	Total no. of conceptions	Total days by periods prior to conception that fer restricted to depleting rations							
				I	II	III	IV	V	VI	VII	VII
				49-58	59-68	69-78	79-88	89-98	99-108	109-118	119-1
				Total number of conceptions per period and the nu which abnormal live feti were present at t							
				Tri-o-cresyl phosphate (25 µg./gm. of ration)							
41	0	II	20	6	5	6	3	0	0	0	0
42	10/1	II	20 (9)	5(4)	5(1)	5(2)	2(1)	2	1(1)	0	0
43	10/2	II	20 (15)	4(3)	7(7)	5(2)	2(1)	1(1)	1(1)	0	0
44	10/4	II	19 (11)	1(1)	9(6)	4(1)	4(2)	1(1)	0	0	0
45[d]	2x5	II	17	1	3	2	4	5	1	0	0
46	2x5	II	17	3	7	4	2	1	0	0	0
				Tri-o-cresyl phosphate (100 µg./gm. of ration)							
51	0	III	20	2	11	4	3	0	0	0	0
52	10/1	III	20 (9)	2	6(4)	9(4)	1	0	0	0	2
53	10/2	III	20 (12)	1(1)	9(7)	8(4)	2	0	0	0	0
54	10/4	III	20 (8)	3(2)	8(3)	6(2)	1	0	2(1)	0	0
55[d]	2x5	III	14	3	3	3	3	2	0	0	0
56	2x5	III	20	8	5	6	0	1	0	0	0

[a]Numerator denotes day(s) in gestation therapy given; denominator, mg. alpha-tocopherol given at that time.

[b]Described in Table 2.

[c]Numbers not in parentheses denote total conceptions, those in parenth numbers in which one or more live malformed feti occurred.

[d]Each female received weekly by stomach tube 2 mg. d,l-alpha-tocopher from weaning to mating.

Table 8 (Continued)

ıip of Incidence of Teratogeny to Length of Time from Weaning That ımales Were Restricted to Avitaminosis E Depletion Rations

Total no. of conceptions	Total days by periods prior to conception that females were restricted to depleting rations								
	I	II	III	IV	V	VI	VII	VIII	IX
	49-58	59-68	69-78	79-88	89-98	99-108	109-118	119-128	129-138
	Total number of conceptions per period and the number in which abnormal live feti were present at term[c]								
	Tri-o-cresyl phosphate (25 µg./gm. of ration)								
20	6	5	6	3	0	0	0	0	0
20 (9)	5(4)	5(1)	5(2)	2(1)	2	1(1)	0	0	0
20 (15)	4(3)	7(7)	5(2)	2(1)	1(1)	1(1)	0	0	0
19 (11)	1(1)	9(6)	4(1)	4(2)	1(1)	0	0	0	0
17	1	3	2	4	5	1	0	0	1
17	3	7	4	2	1	0	0	0	0
	Tri-o-cresyl phosphate (100 µg./gm. of ration)								
20	2	11	4	3	0	0	0	0	0
20 (9)	2	6(4)	9(4)	1	0	0	0	2(1)	0
20 (12)	1(1)	9(7)	8(4)	2	0	0	0	0	0
20 (8)	3(2)	8(3)	6(2)	1	0	2(1)	0	0	0
14	3	3	3	3	2	0	0	0	0
20	8	5	6	0	1	0	0	0	0

denotes day(s) in gestation therapy given; denominator, mg. d,l-
. given at that time.

in Table 2.

ɔt in parentheses denote total conceptions, those in parentheses the
n one or more live malformed feti occurred.

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mating.

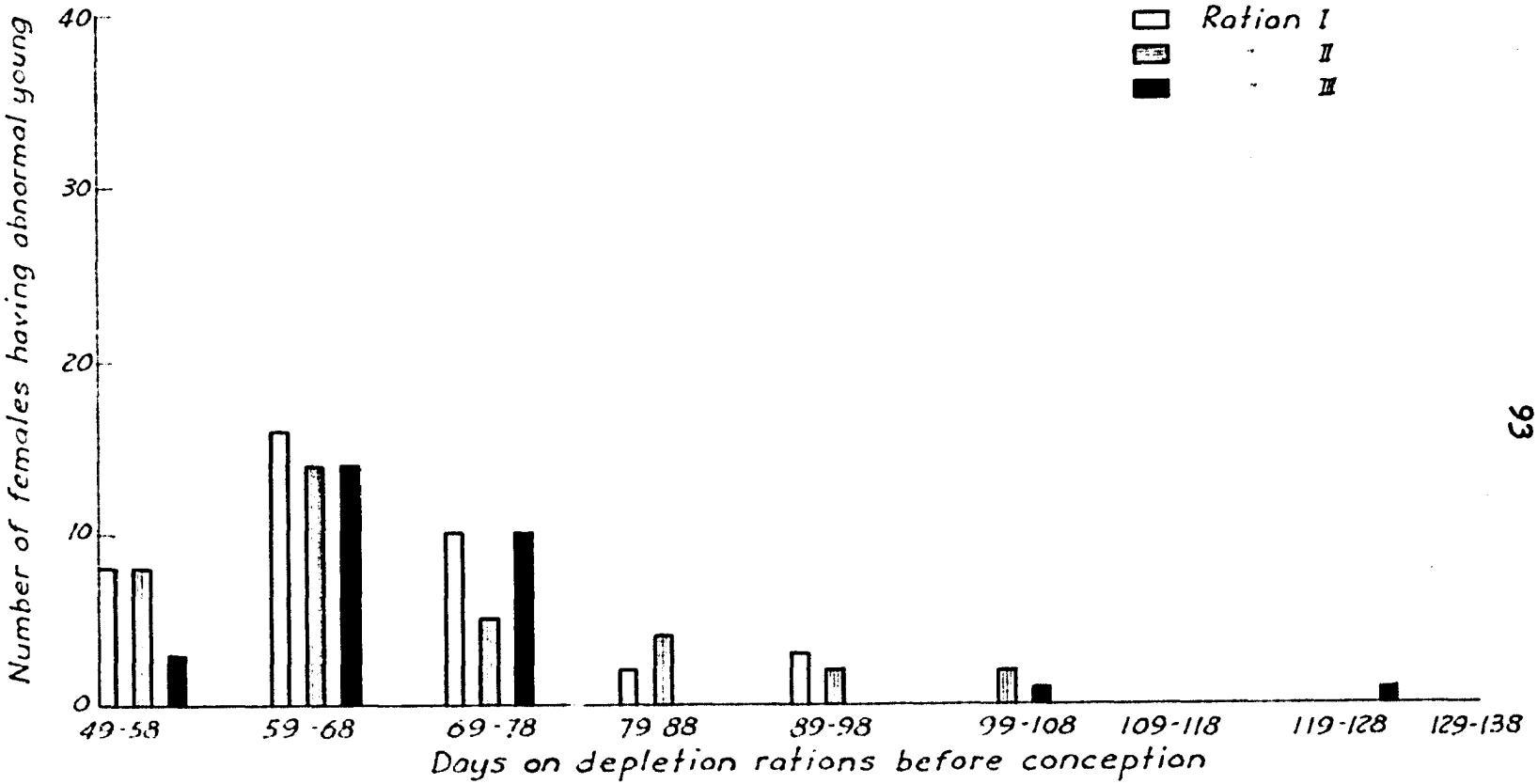


Figure 2

Relationship of Length of Time Restricted to Vitamin E-Deficient Rations to Incidence of Teratogeny in Female Rats Receiving Doses of Alpha-Tocopherol on the 10th Day of Gestation

In groups 42, 43 and 44 of Table 8 the largest number of abnormal pregnancies appeared in periods I, I and II, and II respectively. In groups 52, 53, and 54 abnormalities predominated in periods II and III, II and III, I, II, and III respectively. On their face value these data indicate that periods I, II and III are about equally effective in producing congenital abnormalities. From Table 8 it is difficult to know how much significance to attach to the data in periods V through IX. Excepting group 7, relatively few conceptions occurred after period V. However, the percentage of abnormal pregnancies averaged higher than those for periods I through IV. Admittedly the data represent a multiplicity of treatments, which tend to confound the picture. Yet no single treatment or combination of treatments can be singled out as pointing definitely to a positive correlation between prolongation of depletion and incidence of teratogeny.

b. Effect of the vitamin E antagonist, tri-o-cresyl phosphate (TCP). Other investigators have reported that tri-o-cresyl phosphate (TCP) behaves as a biological antagonist to vitamin E (Bloch and Hottinger, 1943; Meunier et al., 1947; Draper et al., 1952; Hove, 1953). In their studies growth was used as the criterion of effectiveness. Conversely, it is conceivable that vitamin E can nullify the antagonistic effects of TCP when the levels of intake of both are optimal

for such a demonstration. If this principle applies in reproduction as in growth, then the proper combination of tocopherol and TCP could be determined which might aid greatly in increasing the incidence of teratogeny in avitaminosis E gestational therapy. The data in Table 9 and Figure 3 have been assembled with a view to shedding some light on this point.

These data will be interpreted from several angles; namely, the effect of increasing the therapeutic dose of tocopherol in the presence or absence of TCP on the total number of live and abnormal live feti at term, and the effect of prolonged feeding of TCP on 1) the number and percentage of normal and abnormal feti, and 2) the number and percentage of normal and teratogenic pregnancies. Using these criteria, let us examine the data. Had the same levels of tocopherol been administered to the females not later than the 5th day of gestation the total number of live feti would have increased, within limits, with each increased dosage of tocopherol. Even though therapy was delayed to the tenth day of gestation in the case of all three rations and presumably complicated with teratogeny, there was an increase in total number of live feti with each increase in dosage of tocopherol. Also, the same effect occurred between dosage and number of live normal feti. These observations indicate

Table 9

Effect of Prolonged Feeding of Tri-o-cresyl Phosphate Prior to Matir on the Incidence of Teratogeny among Rats Fed Different Levels of d,l-alpha-Tocopherol Acetate on the 10th Day of Gestation

Ration[a] no.	Level of tocopherol given mg.	Group no.	Abnormal preg. / normal preg. no./no.	Abnormal live feti / normal live feti no./no.	Total no. of feti no.	Pregnancies Abnormal %	Pregnancies Normal %	Live Abnorma %
I	1	25	9/11	20/17	37	45	55	54
	2	26	10/10	30/18	48	50	50	62
	4	27	12/8	27/42	69	60	40	39
Total			31/29	77/77	154			
II	1	42	9/11	13/10	23	45	55	57
	2	43	15/5	38/19	57	75	25	67
	4	44	11/8	23/53	76	58	42	30
Total			35/24	74/82	156			
III	1	52	9/11	21/9	30	45	55	70
	2	53	12/8	21/25	46	60	40	46
	4	54	8/12	20/29	49	40	60	41
Total			29/31	62/63	125			

[a]Rations described in Table 2. I, II and III denote none, 25 µg./gm. 100 µg. of tri-o-cresyl phosphate per gram of ration respectively.

Table 9

of Prolonged Feeding of Tri-o-cresyl Phosphate Prior to Mating
he Incidence of Teratogeny among Rats Fed Different Levels of
l,l-alpha-Tocopherol Acetate on the 10th Day of Gestation

l of herol en	Group no.	Abnormal preg. / normal preg. no./no.	Abnormal live feti / normal live feti no./no.	Total no. of feti no.	Pregnancies Abnormal %	Pregnancies Normal %	Live feti Abnormal %	Live feti Normal %
	25	9/11	20/17	37	45	55	54	46
	26	10/10	30/18	48	50	50	62	38
	27	12/8	27/42	69	60	40	39	61
		31/29	77/77	154				
	42	9/11	13/10	23	45	55	57	43
	43	15/5	38/19	57	75	25	67	33
	44	11/8	23/53	76	58	42	30	70
		35/24	74/82	156				
	52	9/11	21/9	30	45	55	70	30
	53	12/8	21/25	46	60	40	46	54
	54	8/12	20/29	49	40	60	41	59
		29/31	62/63	125				

described in Table 2. I, II and III denote none, 25 µg./gm., and
i-o-cresyl phosphate per gram of ration respectively.

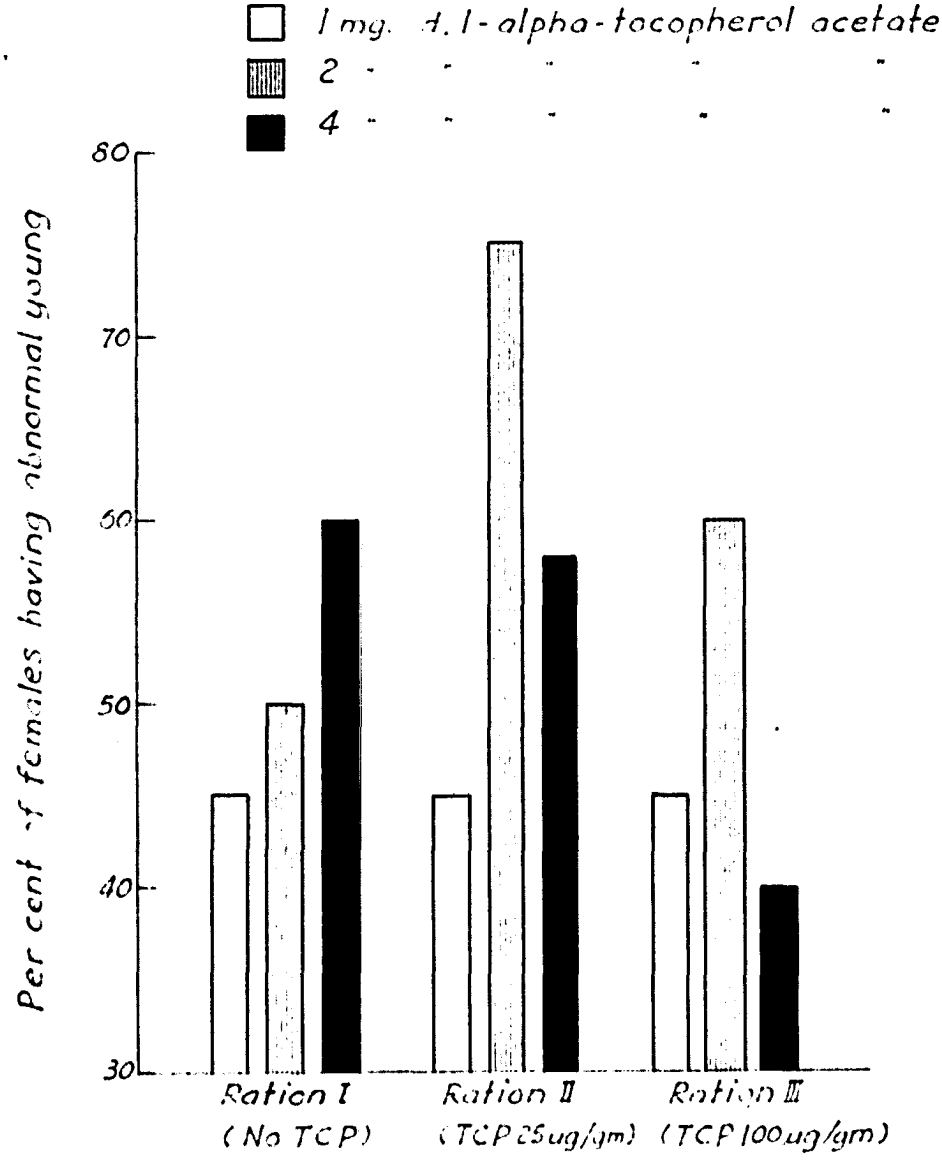


Figure 3

Effect of Tri-o-cresyl Phosphate (TCP) Feeding and Different Levels of d,l-alpha-Tocopherol Acetate Supplementation during the 10th Day of Gestation on the Incidence of Teratogeny in Female Rats

that the range of levels of tocopherol were rather well chosen for this study.

Next, let us review the data as to whether they support or refute the hypothesis that TCP might increase the incidence of teratogeny. On the basis of numbers of normal and abnormal pregnancies there is no difference in the results from the three rations at the 1 mg. level of tocopherol. At the 2 mg. level there were more abnormal and fewer normal pregnancies with the rations containing TCP (Figure 3), whereas at the 4 mg. level of tocopherol the trend is to fewer abnormal and more normal pregnancies with increase in TCP (Table 9). Judged on the basis of total pregnancies for each ration, disregarding level of tocopherol, there is no evidence of an increase in abnormal and simultaneous decrease in normal pregnancies due to inclusion of TCP in the basal depleting ration I. The data on percentages of pregnancies obviously agree with the trends just noted.

Similar comparisons based on numbers of live feti at term also can be made. When the three rations are compared at the 1 mg. level of tocopherol, the numbers of abnormal feti do not indicate a trend. However, on the basis of percentages of abnormal live feti there is a trend upward; namely, 54, 57, and 70, suggesting that TCP was effective in increasing the incidence of teratogeny. If instead, a

comparison is made similarly but on the basis of normal feti, there is a trend downward for both number and percentage of feti with increased ingestion of TCP; namely, 17, 10 and 9, and 46, 43 and 30 respectively. Identical comparisons for abnormal feti made at the 2 mg. level of tocopherol indicate a trend downward both numerically and percentagewise; viz., 30, 38 and 21, and 62, 67 and 46 respectively. When the data for the 2 mg. level of tocopherol are similarly compared for normal feti, the trend in number and percentage of feti is upward essentially; namely, 18, 19 and 25, and 38, 33 and 54 respectively. At the 4 mg. level the trend is downward for number of both normal and abnormal feti; namely, 42, 53 and 29, and 27, 23 and 20 respectively. Percentagewise, there does not seem to be any trend at the 4 mg. level with increase in TCP. When the three rations are compared on the basis of total number of feti, the trend is downward for both number of normal and abnormal feti; namely, 77, 74 and 62; 77, 82 and 63 respectively.

For TCP to be judged effective in increasing avitaminosis E teratogeny both the number and the percentages of abnormal feti and pregnancies should increase with increased intake of TCP at the two lower and possibly all three levels of tocopherol. The results at the 1 and 2 mg. levels of tocopherol probably should be more indicative because the tocopherol

feedings are nearer the marginal level necessary to prevent fetal resorptions.

The data in Table 9 do not lend themselves to easy interpretation largely because of the variability inherent in them. Criteria have been used to evaluate the data on the basis of each level of tocopherol, and noting whether they indicate trends favorable, unfavorable or indecisive as regards the effectiveness of TCP. At the 1 mg. level there are no unfavorable trends. However, at the same time the amount of favorable and indecisive data tend to off-set each other. At the 2 mg. level the numbers of instances indicating favorable and unfavorable trends are equal. At the 4 mg. level there is only one instance indicating a favorable trend, two indecisive, and five unfavorable. In view of the variability and the failure of the data in Table 9 to point consistently to any trend, it is doubtful that TCP is effective in increasing the incidence of teratogeny in avitaminosis E.

The following discussion cites certain bases for arriving at this tentative deduction. The highest therapeutic dose of tocopherol may have been so large as to mask any anti-vitamin E effect of TCP. Possibly the feeding levels of TCP were too small to exert pronounced effects. However, caution had to be exercised in this regard as there was

danger from feeding too much of it. Also, to be reckoned with is the fact that at the 1 mg. level of tocopherol the data may have been confounded to some extent by the tendency for fetal resorptions to occur. Furthermore, it is not unreasonable to speculate that all females on the TCP-free ration were extensively depleted by the time of mating and consequently the effects of all three rations on teratogeny were equalized. Probably the factor contributing most to the indecisiveness of the results in Table 9 is the timing of gestational therapy. The 10th day of pregnancy lies very close to the peak of teratogenic incidence in the critical period of the gestation of rats, and consequently the incidence of teratogeny may not be significantly altered regardless of the level of tocopherol therapy when administered at this time.

4. Effect of different dietary treatments on the growth of female rats from weaning to sexual maturity

a. Growth from weaning through the eighth week on the rations. The nourishment of the fetus usually is most directly affected by the quality and quantity of the diet of the mother during gestation. Also, it is important to pay careful attention to the pregestational nourishment of the female

in nutritional studies of pregnancy. To determine the extent to which certain experimental conditions followed in this study might have adversely affected each initial pregnancy, a record was kept of the growth of each female from weaning to mating, and of the changes in the dam's live weight during each pregnancy, and the weight of her uterus and its contents. These data are summarized in Tables 10 and 11.

Table 10 summarizes the pregestational growth response of comparable groups of females on the stock colony ration and the basal depleting rations with and without tocopherol supplementation. Obviously the females which received the depleting rations did not attain the size that those fed the stock ration did. It should not be inferred from this that the depleting rations were necessarily nutritionally deficient in other factors than vitamin E. Note in Table 10 that the stock females gained much more weight in comparison with the other groups during the first two or three weeks after weaning and that beginning with the fourth week all the weekly live weight gains were quite similar.

This was to be expected because at weaning time the stock colony positive control group was continued on the same ration, while all other weanling females were transferred to the relatively unpalatable, high fat depletion rations.

Table 10

Growth of Young Female Rats during the Eight Weeks Following Weaning When Fed Ad Lib. Vitamin E Depleting Rations Unsupplemented or Supplemented with d,l-alpha-Tocopherol Acetate

Duration of feeding period week	Ration numbers and treatments, and average weekly weights of groups of females[a]						
	I (21)[d]	I/t[b] (21)	II (21)	II/t (21)	III (21)	III/t (21)	S[c] (24)
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0	38	37	38	38	38	39	38
1	55	56	57	56	55	55	55
2	68	69	71	68	72	63	82
3	88	82	88	82	89	82	114
4	103	93	107	91	106	95	133
5	120	108	123	104	121	110	149
6	137	123	140	117	134	124	162
7	150	137	156	132	149	136	174
8	159	146	165	142	159	148	183
8	163[e]	---	159[e]	---	162[e]	---	---

[a]I, II and III denote depleting rations (Table 2) containing none, 25 µg. and 100 µg. tri-o-cresyl phosphate per gram of ration respectively.

[b]Denotes weekly supplementation by stomach tube with 2 mg. of tocopherol per rat.

[c]S denotes stock colony ration.

[d]Figures in parenthesis denote number of rats.

[e]Figures are averages of 230, 111, and 110 rats that received rations I, II and III respectively.

Table 11

Effect of Avitaminosis E Regimes and d,l-alpha-Tocopherol Acetate Therapy in Female Rats Prior to and during Pregnancy on Maternal, Uterine, and Fetal Increases in Weight

Group no.	Conceptions no.	Day of gestation supplement fed day/mg.	Av. wt. of females at Weaning gm.	Av. wt. of females at Coitus gm.	Av. wt. increase during gestation: Female gm.	Female less uterus and its contents at term gm.	Uterus and its contents gm.	Live feti at term: Normal gm.	Live feti at term: Abnormal gm.
Females fed depleting ration I (no tri-o-cresyl phosphate)									
1	21	---	47	198	26	25.1	0.9	---	--
2	25	4/1.2[a]	47	193	49	35.5	13.5	4.4/27[b]	--
3	23	5/1.2	49	200	46	33.8	12.2	3.9/37	--
4	22	6/1.2	46	193	48	32.9	15.1	4.6/38	--
5	19	7/1.2	47	194	51	33.8	17.2	4.1/43	--
6	21	8/1.2	44	194	52	29.9	22.1	4.7/59	--
7	32	9/1.2	47	197	44	30.2	13.8	4.2/45	3.9/15[b]
8	27	10/1.2	48	196	41	34.5	6.5	3.5/2	2.8/23
9	28	11/1.2	47	193	51	42.4	8.6	3.6/14	3.2/19
10	20	12/1.2	43	189	44	41.5	2.5	3.1/1	3.3/2
11[c]	35	---	47	213	105	35.7	69.3	5.2/347	--

[a]Numerator denotes day, denominator mg. of d,l-alpha-tocopherol acetate fed.

[b]Denominator denotes total number of feti, numerator their average weight.

[c]Females fed stock ration (see Table 1).

Table 11 (Continued)

Effect of Avitaminosis E Regimes and d,l-alpha-Tocopherol Acetate Therapy in Female Rats Prior to and during Pregnancy on Maternal, Uterine, and Fetal Increases in Weight

Group no.	Conceptions no.	Day of gestation supplement fed day/mg.	Av. wt. of females: Weaning gm.	Av. wt. of females: Coitus gm.	Av. wt. increase during gestation: Female gm.	Female less uterus and its contents at term gm.	Uterus and its contents gm.	Live feti at term: Normal gm.	Live feti at term: Abnormal gm.
Females fed depleting ration I (no tri-o-cresyl phosphate)									
21	24	---	37	166	28	26.8	1.2	---	---
22	20	8/1[a]	38	178	56	35.6	20.4	4.2/56[b]	4.3/1[b]
23	20	8/2	39	176	57	29.9	27.1	4.6/71	---
24	20	8/4	39	177	60	28.6	31.4	4.6/99	3.2/1
25	20	10/1	38	178	46	33.2	12.8	4.2/17	2.6/20
26	20	10/2	38	179	47	31.3	15.7	3.9/18	3.1/30
27	20	10/4	39	186	60	37.0	23.0	4.3/42	3.1/27
28	20	12/1	39	181	40	36.8	3.2	3.3/1	2.3/2
29	19	12/2	38	179	36	31.3	4.7	3.8/4	2.2/5
30	20	12/4	38	180	45	36.4	8.6	4.2/21	---
31[c]	18	1,2,3,4,5/2	37	176	56	1.5	54.5	4.5/155	---
32	19	1,2,3,4,5/2	38	179	74	23.0	51.0	4.9/148	---
33[d]	23	---	38	184	112	44.7	67.3	5.1/228	---

[a]Numerator denotes day, denominator mg. of d,l-alpha-tocopherol acetate fed.

[b]Denominator denotes total number of feti, numerator their average weight.

[c]Each female also received weekly by stomach tube 2 mg. d,l-alpha-tocopherol acetate from weaning to mating.

[d]Females fed stock ration (see Table 1).

Table 11 (Continued)

Effect of Avitaminosis E Regimes and d,l-alpha-Tocopherol Acetate Ther
Female Rats Prior to and during Pregnancy on Maternal, Uterine,
and Fetal Increases in Weight

Group no.	Conceptions no.	Day of gestation supplement fed day/mg.	Av. wt. of females: Weaning	Av. wt. of females: Coitus	Av. wt. increase during gestatio: Female gm.	Female less uterus and its contents at term gm.	Uterus and its contents gm.	Live fet: Normal gm.
Females fed depleting ration II (tri-o-cresyl phosphate, 25 µg./gm								
41	20	---	38	174	32	30.2	1.8	---
42	20	10/1[a]	39	175	49	39.6	9.4	4.1/1(
43	20	10/2	39	182	55	37.9	17.1	4.0/1
44	19	10/4	39	178	60	35.1	24.9	4.4/5
45[c]	17	1,2,3,4,5/2	38	187	51	0.9	50.1	4.5/1
46	17	1,2,3,4,5/2	38	176	82	29.6	52.4	4.9/1
33	23	---	38	184	112	44.7	67.3	5.1/2
Females fed depleting ration III (tri-o-cresyl phosphate, 100 µg./gm								
51	20	---	38	182	34	32.4	1.6	---
52	20	10/1	39	177	45	33.3	11.7	3.9/9
53	20	10/2	37	179	60	42.4	17.6	4.1/2
54	20	10/4	39	182	52	34.4	17.6	4.5/2
55[c]	14	1,2,3,4,5/2	39	174	53	7.5	45.5	4.1/1
56	20	1,2,3,4,5/2	39	181	72	19.9	52.1	4.8/1
33	23	---	38	184	112	44.7	67.3	5.1/

[a]Numerator denotes day, denominator mg. of d,l-alpha-tocopherol a
given.

[b]Denominator denotes total number of feti, numerator their averag

[c]Each female also received weekly by stomach tube 2 mg. d,l-alpha-
acetate from weaning to mating.

Table 11 (Continued)

Vitaminosis E Regimes and d,l-alpha-Tocopherol Acetate Therapy in le Rats Prior to and during Pregnancy on Maternal, Uterine, and Fetal Increases in Weight

Day of gestation supplement fed day/mg.	Av. wt. of females		Av. wt. increase during gestation				
	Weaning	Coitus	Female gm.	Female less uterus and its contents at term gm.	Uterus and its contents gm.	Live feti at term Normal gm.	Abnormal gm.
fed depleting ration II (tri-o-cresyl phosphate, 25 µg./gm of ration)							
---	38	174	32	30.2	1.8	---	---
10/1[a]	39	175	49	39.6	9.4	4.1/10[b]	2.8/13[b]
10/2	39	182	55	37.9	17.1	4.0/19	2.9/38
10/4	39	178	60	35.1	24.9	4.4/53	2.6/23
1,2,3,4,5/2	38	187	51	0.9	50.1	4.5/139	---
1,2,3,4,5/2	38	176	82	29.6	52.4	4.9/136	---
---	38	184	112	44.7	67.3	5.1/228	---
ed depleting ration III (tri-o-cresyl phosphate, 100 µg./gm. of ration)							
---	38	182	34	32.4	1.6	---	---
10/1	39	177	45	33.3	11.7	3.9/9	3.4/21
10/2	37	179	60	42.4	17.6	4.1/25	2.9/21
10/4	39	182	52	34.4	17.6	4.5/29	3.0/20
1,2,3,4,5/2	39	174	53	7.5	45.5	4.1/113	---
1,2,3,4,5/2	39	181	72	19.9	52.1	4.8/155	---
---	38	184	112	44.7	67.3	5.1/228	---

tor denotes day, denominator mg. of d,l-alpha-tocopherol acetate

nator denotes total number of feti, numerator their average weight.

emale also received weekly by stomach tube 2 mg. d,l-alpha-tocopherol weaning to mating.

Consequently, these weanling females adjusted less rapidly immediately following weaning than did the positive control group on stock ration. Therefore, it is incorrect to assume from the data in Table 10 that the depleting rations were nutritionally inferior, except for vitamin E, to the stock colony ration. Blumberg (1935), Kaunitz and Johnson (1946), and Martin (1937) reported vitamin E-deficient ration depressed growth in early adolescence. However, Emerson and Evans (1937), and Olcott and Mattill (1937) observed no retarding effect with similar rations.

The data in Table 10 clearly demonstrate that the two levels of tri-o-cresyl phosphate used in this investigation did not adversely affect growth. On the other hand, the data in the same table reveal slight differences in growth between the tocopherol supplemented and unsupplemented groups. The difference consistently favored the non-tocopherol groups. Whether or not these differences are significant would be interesting to know. What is most important in this study is the fact that they are not large. Blumberg (1935) reported that the growth of male rats restricted to a vitamin E-depleting ration was retarded slightly when fortified gavagely once a week with wheat germ oil equivalent to 2 mg. of tocopherol. Even though the sex and the supplement used by Blumberg were different, it is

interesting that the retardation of growth he obtained was slight, as in this study.

b. Increases of maternal, uterine and fetal weight during pregnancy. The data in Table 11 have been assembled to indicate the extent, if any, to which the various vitamin E-depleting and subsequent supplementing regimes affected the weight of the dams and their uterine contents during gestation. It should be pointed out that the data in groups 1 to 11 and 21 to 56 of Table 11 were collected simultaneously. In all these tables the comparable weights at weaning time and coitus were quite uniformly alike. In each series of groups the average gain in live weight during gestation made by the negative control group was the smallest, while that gained by the positive control group was the largest. The performance of these groups is in full agreement with numerous similar comparisons published elsewhere.

It is apparent from Table 11 that time of gestational therapy with single doses of 1, 1.2, 2 or 4 mg. of tocopherol does not markedly affect the average increase in weight of the females per se (excluding weight of uteri and their contents). On the other hand, the average weight of the uteri and their contents within comparable groups differed considerably, being consistently 1) smallest

for the negative control groups, 2) greatest for the positive control groups, and 3) intermediate for the groups that were given gavagely single doses of tocopherol.

Still referring to these tables, the average weight of the live feti at term differed markedly depending on 1) size of the dose of tocopherol, and 2) simultaneous occurrence of normal feti in the uterus. The feti almost invariably were largest when the uterus contained only normal feti. In most instances, except when the number of total feti was too small to permit fair comparisons, the feti increased in size with increased dosage of tocopherol, at least within the limits of 1 to 4 mg. of tocopherol. Almost without exception the smallest feti produced in this study were those exhibiting congenital abnormalities.

The data in Table 11 do not demonstrate any adverse effect on maternal change in weight per se during gestation or in size of feti at term due to the inclusion of TCP at levels of 25 or 100 ug. per gram of basal depleting ration.

c. Effect of prolonged pregestational administration of tocopherol on body weight change of the dams and their feti during pregnancy. It has been pointed out that continuous feeding of tocopherol from weaning to coitus slightly retards growth. Presumably this effect would continue through

pregnancy and even could adversely affect the growth of the feti. Table 11 of the preceding section presents data bearing on these points. All females in certain groups; namely, 31, 45 and 55, received 2 mg. of tocopherol weekly during growth and daily during the first five days of gestation. The females in certain other groups; namely, 32, 46 and 56, received identical treatment except that no tocopherol was administered during growth. In every instance, whether or not the rations contained TCP, those females which received tocopherol during growth gained considerably less body weight per se than those which received tocopherol only during gestation. Actually, they did very little more than maintain their weights. Also, pregestational feeding of tocopherol, as carried out in these experiments, seemed to slightly retard growth of the feti; viz. 4.5, 4.5, and 4.1 compared to 4.9, 4.9 and 4.8 grams respectively. Admittedly these differences are small, but they are considered significant in view of the large numbers of feti involved in each group, and the fact that their nutrition was exclusively of maternal origin.

At this point it is well to consider the effect of these same dietary treatments on the number of implantation sites. The groups which received gavagely alpha-tocopherol during growth showed a slightly larger number of sites compared to

those groups which received supplementation only during gestation although the difference is not significant. The average number of implantation sites per female in group 45 was 8.8 while that for those in group 46 was 8.4. This trend was repeated among the females in the groups on ration III, for here again the average number of implantation sites was 8.7 for group 55 as compared to 8.5 for group 56. While the number of implantation sites in all these positive control groups fell within the normal range of 9 to 11, it was smaller than the 10.7 observed in the stock ration control (group 33). Kaunitz and Slanetz (1947) observed that alpha-tocopherol had an effect on implantation. Failure of implantation was observed after the eighth month in females receiving a vitamin E low purified diet without tocopherol supplement. With continuous tocopherol administration, the implantation rate was normal even after being continued on the ration for a year.

In view of the foregoing, it seems likely that although the prolonged pregestational administration of alpha-tocopherol seemed to have a slight beneficial effect over administration only during the first five days of gestation with regard to the number of implantation sites, it slightly depressed the body weight increase of the mother and that of the feti during pregnancy.

C. Detection of Abnormalities during Development of the Fetus

Since the initial stages of congenital abnormalities occur prior to the termination of each gestation, preliminary examinations were made with the view to learning how early in fetal development these abnormalities could be observed. Several avitaminosis E females that had been gavaged on the 10th day of gestation were laparotomized as early as the 15th and 16th day of gestation. All live feti were removed and observed and then fixed in Bouin fixative and preserved in 70 per cent alcohol for further study. The results are summarized in Table 12. Judged on the basis of crown-rump length and body weight, the abnormal feti of the 16th day of gestation were the equal of the normal feti of the 15th day. In other words, they were retarded in development by approximately one day. In every instance the abnormal feti were definitely smaller in physical dimensions and in weight than the normal feti of corresponding stage.

One 15-day fetus with induced teratogeny showed exencephalus, oblique facial cleft due to the failure of union between a maxillary process and the nasal processes, and non-fusion of the maxillary and mandibular processes. Another abnormal fetus of the same stage of development

Table 12

Early Detection and Extent of Anatomical Abnormalities in Feti Prior to Term Following Vitamin E Therapy during Gestation

Indices of normality or abnormality	Dietary treatment and day of gestation feti examined			
	15th day		16th day	
	Normal[a]	E-deficient[b]	Normal[a]	E-deficient[b]
Av. live wt. (gm.)	0.29	0.13[c]	0.44	0.30[c]
Crown-rump length (mm.)	13.4	10.9	15.3	13.3
Anatomical abnormalities				
Skeletal tissues	none	multiple	none	multiple
Soft tissues	none	multiple	none	multiple

[a]Dams received only stock ration (Table 1) from weaning until time of these examinations.

[b]Dams received depletion ration III (Table 2) from weaning time and 2 mg. d,l-alpha-tocopherol acetate during the 10th day of gestation.

[c]Average for four feti, all other figures are the averages of five feti.

showed curvature of the spinal column and umbilical hernia. The 16-day abnormal feti showed exencephalus and umbilical hernia.

Whereas these data do not indicate exactly how early during gestation these congenital abnormalities occur, there is no reason to question their appearance as early as the 15th day of gestation. Future embryological, chemical and histochemical investigations may reveal how much earlier during gestation these malformations occur, and also might shed light on the exact mechanisms whereby a borderline vitamin E deficiency during gestation exerts its teratogenic effects.

D. Hematological Study of the Blood of Feti

In many instances, the nutritional status of an animal is reflected by the cellular and chemical composition of its blood. For example, ingestion of nutritionally inadequate amounts of cobalt results in anemia. On the other hand, ingestion of small amounts of cobalt in excess of that normally required for good nutrition produces polycythemia, while the ingestion of much larger amounts depressed the formation of red blood cells (Cartwright, 1947).

Dinning (1951, 1952) observed leukocytosis in vitamin E-deficient monkeys and rabbits and attributed it to an elevation of the number of granulocytes (neutrophiles, eosinophiles and basophiles). Impairment of the development of the erythropoietic system in the feti from vitamin E-deficient female rats starts about the 10th day of gestation, and soon thereafter causes fetal death and its subsequent resorption if therapy is not properly timed (Urner, 1931). In order to shed light on the effect of a combination of maternal avitaminosis E and delayed gestational therapy on fetal erythropoiesis, a study of the blood picture of many feti variously affected was undertaken. It included the determination of the hemoglobin concentration of the blood, the erythrocyte count, the total white blood cell count and the differential counts of the lymphocytes, monocytes, eosinophiles, basophiles and neutrophiles.

The results of these hematological studies are summarized in Tables 13, 14, 15, 16, 17, and graphically illustrated in Figures 4 and 5. The data for many group comparisons were analyzed statistically (Snedecor, 1946) and the results are presented in Table 18. In order to avoid confusion in the presentation and discussion of these data, reference to the feti will be by means of their maternal group number. Thus, group 33 refers to the feti from dams which were fed stock

Table 13

The Hemoglobin Content and Cytological Composition of the Blood of Full Term Live Normal Feti from Dams Reared and Maintained on Stock Colony Ration

Fetus no.	Hemoglobin gm. %	Cytological composition						
		RBC count /c. mm.	WBC count /c. mm.	Lymphocytes %	Neutrophiles %	Eosinophiles %	Basophiles %	Monocytes %
646-1[a]	17.7	1,925,000	6,280	94.0	3.0	0.5	2.0	0.5
646-2	17.6	2,445,000	10,020	77.5	18.5	3.0	1.0	0
646-3	16.2	2,700,000	6,480	86.0	8.0	4.5	0.5	1.0
646-4	18.5	2,085,000	8,520	85.5	6.5	3.5	4.0	0.5
646-5	18.1	2,680,000	6,260	81.5	14.5	1.0	3.0	0
656-1	20.0	2,295,000	5,540	77.5	17.5	2.0	2.5	0.5
656-2	21.5	2,495,000	4,340	88.5	7.0	3.5	1.0	0
656-3	18.1	2,000,000	4,880	78.0	12.5	5.0	4.5	0
656-4	18.3	2,460,000	6,980	81.5	12.0	4.0	2.5	0
656-5	17.7	2,820,000	3,820	84.5	6.5	6.0	2.5	0.5
656-6	17.4	2,555,000	4,720	88.5	6.0	1.5	4.0	0
667-1	16.7	2,850,000	4,620	83.5	11.5	3.0	1.5	0.5
Av.	18.2± 1.41[b]	2,443,000± 310,000	6,038± 1,820	83.9± 5.04	10.3± 4.88	3.1± 1.65	2.4± 1.3	0.3± 0.25

[a]Number identifies fetus; -1, and its dam; 646. Group no. 33.

[b]Standard deviation.

Table 14

The Hemoglobin Content and Cytological Composition of the Blood of Live Full Term Normal Appearing Feti from Dams Reared and Maintained on a Vitamin E-Depleting Ration Supplemented with d,l-alpha-Tocopherol Acetate

Fetus no.	Hemoglobin gm. %	RBC count /c. mm.	WBC count /c. mm.	Cytological composition: Lymphocytes %	Neutrophiles %	Eosinophiles %	Basophiles %	Monocytes %
From dams that received 2 mg. tocopherol daily during first 5 days of gestation								
690-1[a]	17.5	2,395,000	11,900	82.0	11.5	4.0	0	2.5
690-2	17.5	2,230,000	9,920	91.5	7.0	0	0	1.5
690-3	17.7	1,701,000	8,480	89.0	8.5	1.0	0	1.5
690-4	16.6	2,165,000	7,000	81.5	13.0	3.5	0.5	1.5
Av.	17.3± 0.49[b]	2,123,000± 297,000	9,325± 2,090	86.0± 5.0	10.0± 2.6	2.1± 1.9	0.12	1.8± 0.50
Same as above and in addition 2 mg. weekly prior to mating								
511-1[c]	17.6	2,245,000	8,960	62.5	34.0	2.0	0	1.5
511-2	16.4	2,570,000	10,390	88.0	15.5	1.0	0.5	1.0
645-1	16.9	2,605,000	9,340	58.5	26.5	2.5	1.0	11.5
645-2	15.6	2,760,000	11,000	89.0	9.0	1.5	0	0.5
591-1	16.8	2,130,000	10,840	90.0	8.5	0	0	1.5
591-2	17.5	2,575,000	15,940	96.0	3.5	0	0	0.5
591-3	16.4	2,125,000	16,640	87.5	6.0	3.0	1.0	2.5
591-4	16.7	1,790,000	15,940	87.0	7.5	3.0	1.0	1.5
Av.	16.7± .64	2,350,000± 328,000	12,380± 3,220	82.3± 13.8	13.8± 10.9	1.6± 1.1	0.44± 1.22	2.6± 3.7

[a]Number identifies fetus; -1, and its dam; 690. Group 32.

[b]Standard deviation.

[c]Number identifies fetus; -1, and its dam; 511. Group 31.

Table 15

The Hemoglobin Content and Cytological Composition of the Blood of Live Full Term Normal Appearing Feti from E-Deficient Dams Which Received Gestational Therapy That Resulted in Both Normal and Abnormal Feti in the Same Uterus

Fetus no.	Hemoglobin gm. %	Cytological composition						
		RBC count /c. mm.	WBC count /c. mm.	Lymphocytes %	Neutrophiles %	Eosinophiles %	Basophiles %	Monocytes %
651-1[a]	12.9	1,920,000	11,490	95.5	3.5	0.5	0	0.5
651-2	15.0	2,495,000	18,500	91.5	6.0	1.0	0.5	1.0
651-3	16.1	1,785,000	17,600	94.0	2.5	0.5	0.5	0.5
651-4	14.6	2,395,000	7,420	82.5	13.0	1.0	0	3.5
665-1	17.6	2,355,000	7,560	93.5	4.0	0.5	0.5	1.5
665-2	17.8	2,725,000	10,530	91.0	7.0	0	1.0	1.0
665-3	16.4	2,325,000	5,020	88.0	6.0	0	0.5	5.5
613-1	12.2	1,690,000	14,200	92.0	5.5	0.5	0	2.0
607-1	16.4	3,040,000	15,080	91.0	4.5	0.5	1.0	3.0
607-2	15.2	2,365,000	6,780	75.5	18.0	0.5	0.5	5.5
705-1	17.3	2,255,000	8,020	88.0	11.0	0	0	1.0
Av.	15.6± 1.83[b]	2,305,000± 380,000	11,100± 4,630	89.5± 5.81	7.4± 4.73	0.45± 0.25	0.41± 0.272	2.3± 1.86

[a]Number identifies fetus; -1, and its dam; 651. Groups 26, 43 and 53.

[b]Standard deviation.

Table 16

The Hemoglobin Content and Cytological Composition of the Blood of Live Full Term Abnormal Feti from E-Deficient Dams Which Received Gestational Therapy That Resulted in Both Normal and Abnormal Feti in the Same Uterus

Fetus no.	Hemoglobin gm. %	Cytological composition						
		RBC count /c. mm.	WBC count /c. mm.	Lymphocytes %	Neutrophiles %	Eosinophiles %	Basophiles %	Monocytes %
631-1[a]	13.2	925,000	11,900	53.0	33.0	0.5	1.0	12.5
643-1	6.4	450,000	13,800	92.5	4.0	0	0.5	3.0
643-2	8.4	2,250,000	7,620	83.0	8.5	0	0.5	8.0
643-3	7.9	1,535,000	8,500	89.5	7.0	0.5	0	3.0
601-1	10.0	1,250,000	5,000	94.0	3.5	0.5	1.0	1.0
651-1	12.8	1,865,000	5,500	87.5	8.0	0.5	1.0	3.0
665-1	14.3	1,000,000	6,280	94.0	4.0	0.5	0.5	1.0
666-1	0.2	1,220,000	13,600	75.0	15.0	0.5	1.0	8.5
613-1	10.6	1,140,000	13,380	88.5	8.0	0.5	2.5	5.0
613-2	11.8	1,155,000	7,900	94.5	4.0	0	0	1.5
697-1	13.3	765,000	10,760	91.5	5.5	0	1.5	1.5
697-2	1.9	761,000	6,760	91.0	5.5	0	1.0	2.5
607-1	17.4	2,180,000	16,820	96.0	2.0	1.0	0	1.0
Av.	11.1± 5.34[b]	1,268,900± 551,000	9,832± 3,780	86.9± 11.6	8.3± 8.12	0.34± 0.23	0.80± 0.57	3.96± 3.58

[a]Number identifies fetus; -1, and its dam; 631. Groups 26, 43 and 53.

[b]Standard deviation.

Table 17

Summary of the Average Hemoglobin and Cytological Values Presented in Tables 13, 14, 15 and 16

Description of feti examined	Identification[a] of ration regime of dams / Group	No. of feti	Hemoglobin gm. %	Cytological composition: RBC count /c. mm.	WBC count /c. mm.	Lymphocytes %	Neutrophiles %	Eosinophiles %	Basophiles %	Monocytes %
Normal	33	12	18.2± 1.41	2,443,000± 310,000	6,038± 1,820	83.9± 5.04	10.3± 4.88	3.1± 1.65	2.4± 1.3	0.3± 0.25
Normal	32	4	17.3± 0.49	2,123,000± 297,000	9,325± 2,090	86.0± 5.0	10.0± 2.6	2.1± 1.9	0.12	1.8± 0.5
	31	8	16.7± 0.64	2,350,000± 328,000	12,380± 3,220	82.3± 13.8	13.8± 10.9	1.6± 1.1	0.44± 1.22	2.6± 3.7
Normal	(26-43-53-N)	11	15.6± 1.83	2,305,000± 380,000	11,100± 4,630	89.5± 5.81	7.4± 4.73	0.5± 0.25	0.41± 0.27	2.3± 1.86
Abnormal	(26-43-53-Abn)	13	11.1± 5.34	1,269,000± 551,000	9,832± 3,780	86.9± 11.6	8.3± 8.12	0.3± 0.23	0.8± 0.57	3.96± 3.58

[a]See Tables 2 and 4 for details.

Table 18

Student's "t" Test of the Differences between the Average Hemoglobi
Cytological Values for the Groups of Feti Summarized in Table 1

Identification between	Value of "t" and level of significance for differences average hemoglobin and cytological values						
	Hemoglobin gm. %	RBC count /c. mm.	WBC count /c. mm.	Lymphocytes %	Monocytes %	Eosinophiles %	Ba phi
33 & 32	t=1.66 P>.10	t=.575 P>.50	t=30.2 P<.01[a]	t=.721 P<.50	t=7.22 P<.01[a]	t=1.01 P<.40	t=3 P<.
33 & 31	t=2.79 P<.02[c]	t=.643 P>.50	t=5.65 P<.01[a]	t=.037 P>.50	t=2.19 P<.05[b]	t=2.20 P<.05[b]	t=4 P<.
33 & (26-43-53-N)	t=3.84 P<.01[a]	t=0.09 P>.50	t=3.52 P<.01[a]	t=2.48 P<.05[b]	t=3.70 P<.01[a]	t=5.26 P<.01[a]	t=5 P<.
33 & (26-43-53-Abn)	t=4.48 P<.01[a]	t=6.48 P<.01[a]	t=3.16 P<.01[a]	t=0.825 P>.40	t=3.54 P<.01[a]	t=5.99 P<.01[a]	t=4 P<.
32 & 31	t=1.63 P>.10	t=1.16 P>.20	t=1.70 P>.10	t=.511 P>.50	t=.424 P>.50	t=.556 P>.50	t=1 P>.
32 & (26-43-53-N)	t=1.79 P<.10	t=.830 P>.40	t=.748 P>.40	t=1.06 P>.30	t=.518 P>.50	t=2.80 P<.02[c]	t=1 P>.
32 & (26-43-53-Abn)	t=2.40 P<.05[b]	t=2.93 P<.01[a]	t=.255 P>.50	t=.148 P>.50	t=1.17 P>.20	t=3.47 P<.01[a]	t=3 P<.
31 & (26-43-53-N)	t=1.61 P>.10	t=.262 P>.50	t=.670 P>.50	t=1.57 P>.10	t=.243 P>.50	t=2.87 P<.02[c]	t= P>.
31 & (26-43-53-Abn)	t=3.04 P<.01[a]	t=4.94 P<.01[a]	t=1.57 P>.10	t=.810 P>.40	t=.824 P>.40	t=3.65 P<.01[a]	t=3 P>.
(26-43-53-N) & (26-43-53-Abn)	t=2.66 P<.02[c]	t=5.18 P<.01[a]	t=0.743 P>.40	t=0.674 P>.50	t=1.39 P>.10	t=1.13 P>.20	t= P<

[a]Significant at 1% level.

[b]Significant at 5% level.

[c]Significant at 2% level.

Table 18

s "t" Test of the Differences between the Average Hemoglobin, and ological Values for the Groups of Feti Summarized in Table 17

ion	Value of "t" and level of significance for differences between average hemoglobin and cytological values							
	Hemo-globin gm. %	RBC count /c. mm.	WBC count /c. mm.	Lympho-cytes %	Mono-cytes %	Eosino-philes %	Baso-philes %	Neutro-philes %
	t=1.66 P>.10	t=.575 P>.50	t=30.2 P<.01[a]	t=.721 P<.50	t=7.22 P<.01[a]	t=1.01 P<.40	t=3.46 P<.01[a]	t=.115 P>.50
	t=2.79 P<.02[c]	t=.643 P>.50	t=5.65 P<.01[a]	t=.037 P>.50	t=2.19 P<.05[b]	t=2.20 P<.05[b]	t=4.14 P<.01[a]	t=.984 P>.30
3-	t=3.84 P<.01[a]	t=0.09 P>.50	t=3.52 P<.01[a]	t=2.48 P<.05[b]	t=3.70 P<.01[a]	t=5.26 P<.01[a]	t=5.0 P<.01[a]	t=0.145 P>.50
3-	t=4.48 P<.01[a]	t=6.48 P<.01[a]	t=3.16 P<.01[a]	t=0.825 P>.40	t=3.54 P<.01[a]	t=5.99 P<.01[a]	t=4.06 P<.01[a]	t=0.74 P>.40
	t=1.63 P>.10	t=1.16 P>.20	t=1.70 P>.10	t=.511 P>.50	t=.424 P>.50	t=.556 P>.50	t=1.13 P>.50	t=.671 P>.50
3-	t=1.79 P<.10	t=.830 P>.40	t=.748 P>.40	t=1.06 P>.30	t=.518 P>.50	t=2.80 P<.02[c]	t=1.41 P>.10	t=1.02 P>.30
3-	t=2.40 P<.05[b]	t=2.93 P<.01[a]	t=.255 P>.50	t=.148 P>.50	t=1.17 P>.20	t=3.47 P<.01[a]	t=1.80 P<.10	t=.404 P>.50
3-	t=1.61 P>.10	t=.262 P>.50	t=.670 P>.50	t=1.57 P>.10	t=.243 P>.50	t=2.87 P<.02[c]	t=.15 P>.50	t=1.75 P<.10
3-	t=3.04 P<.01[a]	t=4.94 P<.01[a]	t=1.57 P>.10	t=.810 P>.40	t=.824 P>.40	t=3.65 P<.01[a]	t=1.21 P>.20	t=1.31 P>.20
N) & Abn)	t=2.66 P<.02[c]	t=5.18 P<.01[a]	t=0.743 P>.40	t=0.674 P>.50	t=1.39 P>.10	t=1.13 P>.20	t=2.09 P<.05[b]	t=0.324 P>.50

ificant at 1% level.

ificant at 5% level.

ificant at 2% level.

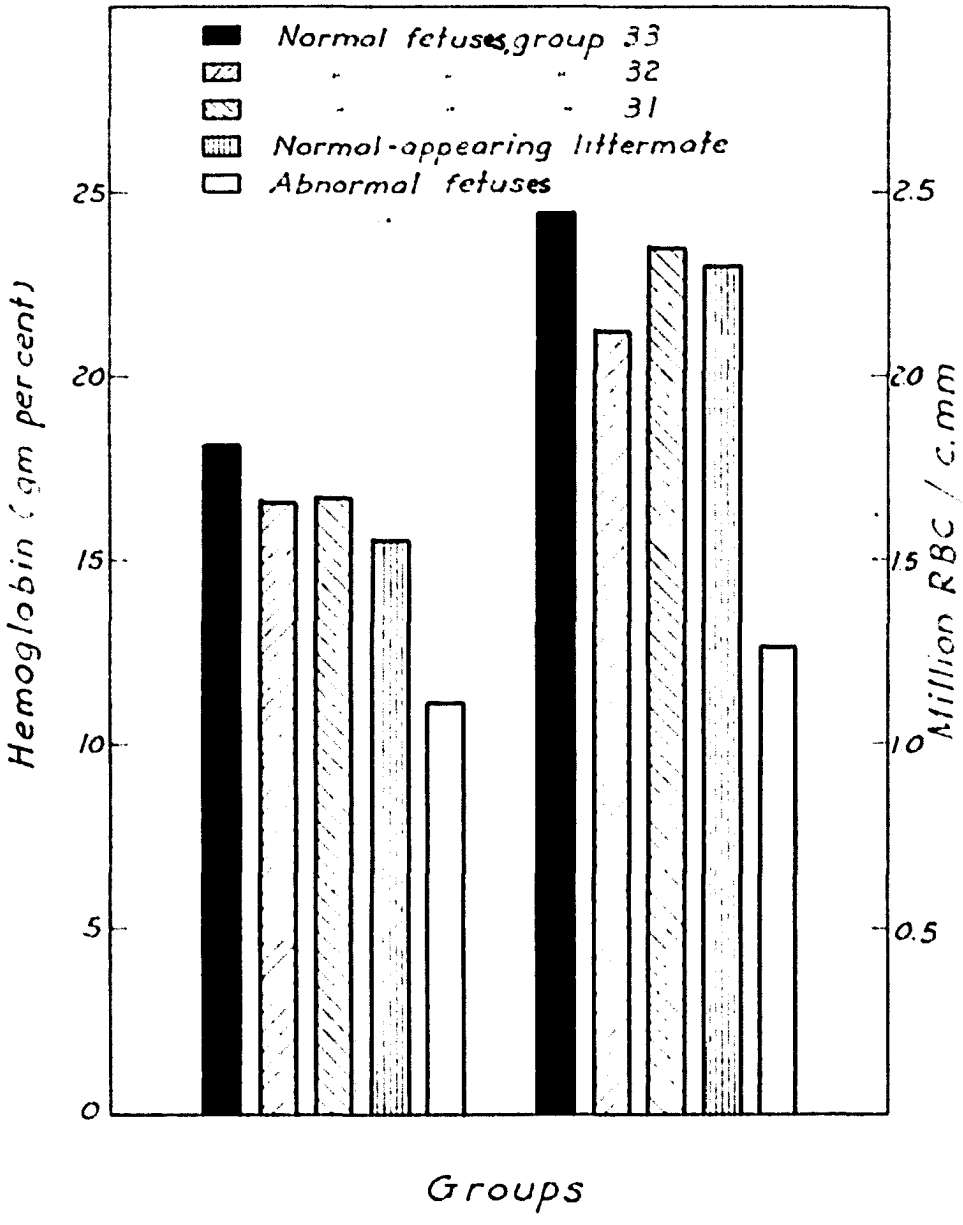


Figure 4

The Red Blood Cell Picture of the Rat Feti from Normal and Vitamin E Deficient Dams That Had Received Gestational Therapy

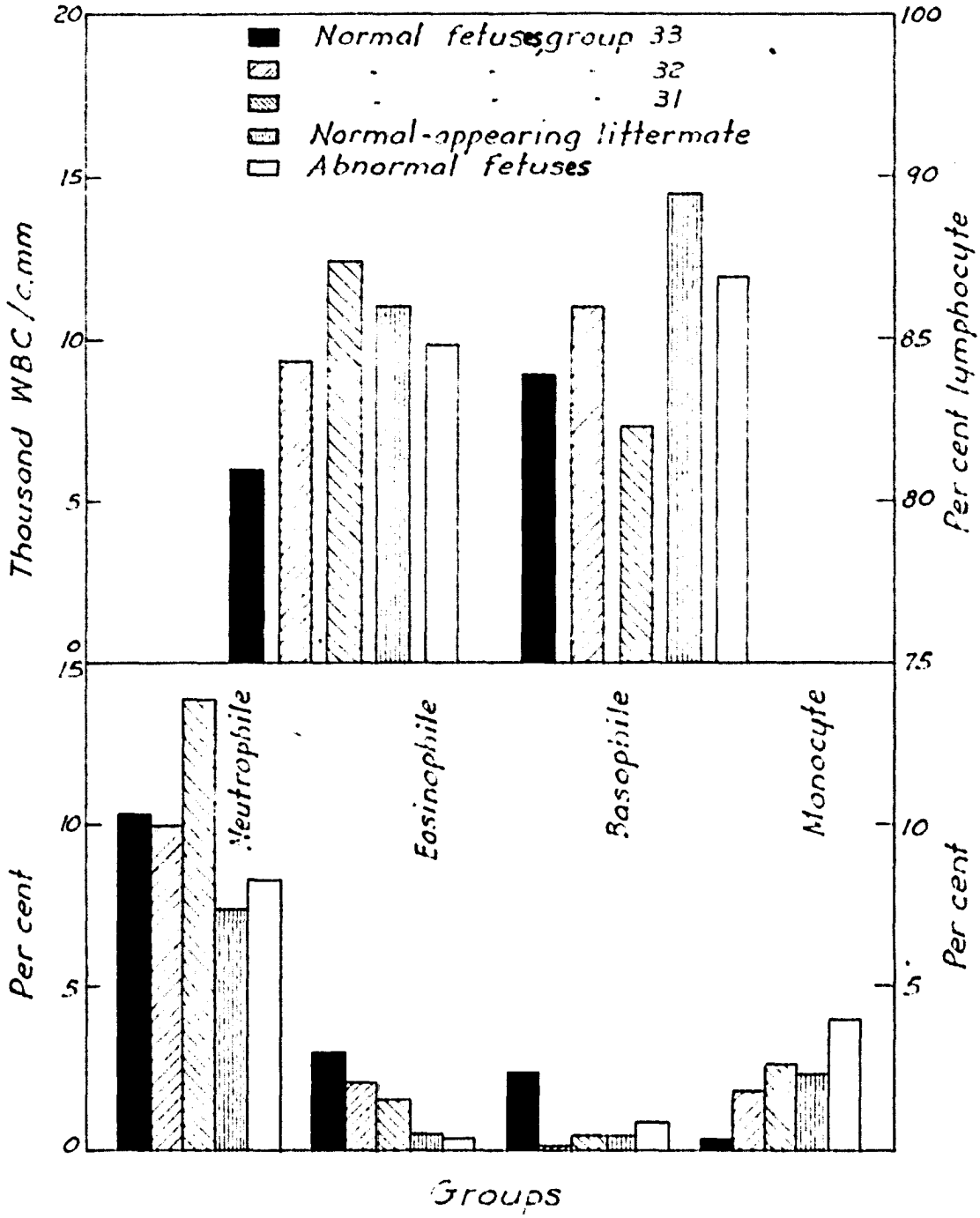


Figure 5

The White Blood Cell Picture of the Rat Feti from Normal and Vitamin E Deficient Dams That Had Received Gestational Therapy

colony ration during the entire course of the experiment. Group 31 designates the feti from therapeutically treated positive control dams; namely those which were fed vitamin E-depleting ration I from weaning to the termination of their initial gestations, and received simultaneously 2 mg. d,l-alpha-tocopherol acetate weekly during growth and daily during the first five days of gestation. Group 32 designates the feti from dams treated similarly to those in group 31, except no tocopherol was given during growth. Group (26-43-53-N) designates the normal-appearing feti from dams in groups 26, 43 and 53 (Table 4) fed vitamin E-deficient rations I, II and III respectively, and given one feeding of 2 mg. tocopherol during the 10th day of gestation. Group (26-43-53-Abn) designates the abnormal feti from the same groups.

1. Hemoglobin values

It is apparent from Tables 13, 16, 17 and 18 and Figure 4 that the hemoglobin (11.1 gm. per 100 ml.) of the abnormal feti was significantly lower than that (18.2 gm. per 100 ml.) of the normal feti from the stock ration control group. The variation was very great, ranging from 0.2 to 17.4 gm. per 100 ml., and seemed to be inversely proportional to the severity of abnormality. Also, the hemoglobin of the

abnormal feti was lower than that of the normal-appearing feti from either the therapeutic positive control groups (31 and 32), or the deficient groups (26, 43 and 53). The differences between the average hemoglobin values of group 33 and (26-43-53-Abn), and 31 and (26-43-53-Abn) were significant at 1 per cent level. The difference between group 32 and (26-43-53-Abn) was significant at 5 per cent level, while that between groups (26-43-53-N) and (26-43-53-Abn) was significant at 2 per cent level only (Table 18). The difference between the hemoglobin values of groups 33 and 31 was significant at 2 per cent level, whereas that between groups 33 and (26-43-53-N) was significant at 1 per cent level. The differences in average hemoglobin between groups 33 and 32, 32 and 31, 32 and (26-43-53-N), and 31 and (26-43-53-N) were not significant statistically (Table 18).

Thus, the analyses of these data show that the average hemoglobin of the abnormal feti was markedly lower than that of the normal feti from the stock ration controls (group 33), the therapeutic positive controls (groups 32, 31), and even the normal-appearing littermates (Table 17, Figure 4). When compared to the hemoglobin of the feti from stock ration rats, the values from the therapeutic positive controls (groups 32, 31) were numerically lower. However, only the value for group 31 was significantly lower than that for

group 33 (Table 18). Also, it is of interest to note that the normal-appearing feti (26-43-53-N) had significantly lower average hemoglobin than group 33.

The normal value of hemoglobin for blood from the peripheral vessels of rats is 12.2 grams per cent according to Donaldson (1924). In maternal folic acid deficiency in rats, 10 to 13 gm. per cent hemoglobin was reported as normal (Nelson et al., 1952). In the present experiments the grams per cent hemoglobin of feti from dams fed stock colony ration (group 33) was 18.2, while that of feti from groups 32 and 31 was 17.3 and 16.7 respectively (Table 17). The hemoglobin of abnormal feti in maternal folic acid deficiency was 1 to 3 gm. per 100 ml. (Nelson et al., 1952), whereas in this investigation that of abnormal feti from vitamin E-deficient dams was 11.1 gm. per 100 ml. (Table 17). This enormous difference may be explained by the fact that folic acid is concerned with erythropoiesis, and its lack during fetal growth affects the red blood cell picture very drastically.

2. Red blood corpuscles

The erythrocyte counts were lowest in the abnormal feti, the average being about 1.27 million per cu. mm. (Table 16). The differences between the average RBC count of this group

of abnormal feti and those of group 33 (2.44 million per cu. mm.), group 32 (2.12 million per cu. mm.), group 31 (2.35 million per cu. mm.), and group (26-43-53-N) (2.3 million per cu. mm.) all were significant at 1 per cent level (Table 18), again reflecting the very anemic condition of these abnormal feti. The differences in RBC counts between the other groups were not significant statistically.

The normal RBC count for day-old rats and feti at term has been reported to be 2 to 3 million per cu. mm. (Donaldson, 1924; Farris and Griffith, 1949; Nelson et al., 1952). The RBC count for the normal feti corresponded to these; namely, that in group 33 was 2.44, which is in good agreement with the published data.

3. White blood corpuscles

As for the leukocyte counts, the blood of the feti from dams fed stock colony ration (group 33) showed the lowest number of cells, averaging about 6000 per cu. mm. (Tables 13, 17; Figure 5). This figure which should be considered normal for feti from our colony of rats is higher than the 3200 given as normal for the new-born rats reported by Farris and Griffith (1949). The dietary treatments of the other four groups raised the number of leukocytes, indicative of

leukocytosis. The differences between the average total WBC count of group 33 and those of groups 32, 31, (26-43-53-N), and (26-43-53-Abn) were significant at 1 per cent level (Table 18). The differences between the other groups were not significant. The leukocytosis in these abnormal feti essentially confirms Dinning's finding of leukocytosis in vitamin E-deficient monkeys and rabbits.

a. Lymphocytes. The highest percentage of lymphocytes (89.5 per cent) was encountered in the blood of group (26-43-53-N)(Tables 15, 17). The percentage of lymphocytes in the blood of the abnormal feti was higher than that in the blood of groups 33, 32, and 31. However, since the variation in the percentage of lymphocytes of the blood of the abnormal feti was large (Table 16), and the group differences were relatively small, the differences mentioned above were not significant. The difference between the percentages of lymphocytes in the blood of group 33 and (26-43-53-N) was significant at 5 per cent level, while those between all other groups were not significant (Table 18). In short, lymphocytosis actually occurred only in the feti of group (26-43-53-N)(Table 18). Lymphocytosis usually accompanies rickets and malnutrition in infants and children (Wintrobe, 1951). It is highly improbable that the conditions

favorable to lymphocytosis in rickets also exist in maternal vitamin E malnutrition.

b. Monocytes. The percentage of monocytes in the blood of the feti of group 33, like the total white cell count, was significantly lower than those of all other groups (Table 17, Figure 5). The difference between groups 33 and 31 was significant at 5 per cent level, while those between groups 33 and 32, 33 and (26-43-53-N), 33 and (26-43-53-Abn) were significant at 1 per cent level. However, the differences between the other groups were not significant statistically (Table 18). The reason for this monocytosis is not readily explainable. The fact that monocytes can be derived from lymphocytes (Wintrobe, 1951; Cowdry, 1946) might offer a plausible explanation. Since there was general lymphocytosis, it is conceivable that some of the lymphocytes were changed into monocytes, resulting in an increase of the latter.

c. Eosinophiles. Eosinopenia would appear to be a condition of the blood associated with avitaminosis E. The eosinophiles in the blood of the feti of all groups were fewer than those in group 33, in which the percentage of eosinophiles was 3.1 (Table 17, Figure 5). The difference between the percentages of eosinophiles of groups 33 and 31 was significant at 5 per cent level, while those between group 33 and (26-43-53-N), 33 and (26-43-53-Abn), 32 and

(26-43-53-Abn), and 31 and (26-43-53-Abn) were significant at 1 per cent level (Table 18).

Since eosinophiles are normally more abundant in tissue fluid than in blood and are found within the epithelial lining of the bowel, the respiratory tract and the skin, an important role in detoxification has been attributed to them (Wintrobe, 1951). Eosinophiles are also reported to decrease after the injection of adrenocorticotrophic hormone (ACTH) in humans with unimpaired adrenal function (Hill *et al.*, 1948). During stress there is usually a pronounced eosinopenia (Selye, 1949) together with an increase in the total white cell count (leukocytosis). It might be that the vitamin E deficiency acted as a stress agent in the feti, thus causing eosinopenia observed in this investigation.

d. Basophiles. Compared to the blood of the feti from group 33, there were fewer basophiles in all the groups of feti from avitaminosis E dams (Table 17, Figure 5). The differences in the percentages of basophiles between group 33 and the other four groups were significant at 1 per cent level, while that between the abnormal and normal littermates was significant at 5 per cent level (Table 18).

From the fact that basophiles as well as the mast cells of the connective tissues appear in greater numbers only during the healing phase of inflammation or in chronic

inflammation, and from the observation that their metachromatic granules contain heparin, it is thought that they function in inflammation by delivering anti-coagulants to facilitate absorption or to prevent clotting of blood and lymph in the obstructed tissue (Ehrich, 1950). The significance of the decrease of basophiles found here, if any, cannot be ascertained right now.

e. Neutrophiles. There were no significant differences between any of the groups of feti in regard to the percentage of neutrophiles in each (Tables 17, 18). However, when viewed in terms of absolute number of neutrophiles, neutrophilia may have occurred in group 31.

The foregoing survey of the blood picture of feti from avitaminosis E dams leaves little doubt that their blood was significantly altered in composition. This is notably the case as regards hemoglobin content and cytological composition. Undoubtedly significant difference also could be revealed in chemical composition.

To recapitulate briefly, the blood of the abnormal feti was significantly lower in hemoglobin and RBC count. In other words, these feti were definitely anemic. Also, leukocytosis occurred judged by their significantly higher total WBC count, and monocytosis too, compared to feti from dams fed the stock colony ration. The abnormal feti had a

significant eosinopenia when compared with those from the three positive control groups. Also, they had a significantly lower percentage of basophiles compared to feti from the stock ration control, but strangely enough and difficult to reconcile, this value was significantly higher than that of their normal-appearing littermates. There was no significant change in the percentage of lymphocytes or neutrophiles. Therefore, the leukocytosis may have been due principally to monocytosis, and slight lymphocytosis. The leukocytosis, eosinopenia and slight lymphocytosis fit into the blood picture of the alarm reaction of Selye (1949). Consequently, the data suggest that the stress pattern induced by maternal vitamin E deficiency and subsequent delayed gestational therapy may have been primarily responsible for the changes noted here in the blood picture of the abnormal feti.

The blood picture of the normal-appearing feti parallels that of their littermate abnormal feti with one significant exception; namely, the occurrence of lymphocytosis in the former. The rest of the picture, such as significantly lower hemoglobin, leukocytosis, monocytosis, eosinopenia, and basopenia, was the same as that of the abnormal feti. Compared to the therapeutic positive controls, the normal-appearing

feti showed only significant eosinopenia (Table 18). The RBC count of the latter was not significantly different from that of the feti from the stock ration control group. Therefore, even though these feti appeared normal macroscopically, they showed deviations from the normal in their blood picture.

When the therapeutic positive control group (32) is compared with the stock ration control group (33), three significant differences in their blood picture stand out. Group 32 developed leukocytosis, monocytosis, and basepenia whereas the feti in group 33 did not (Table 18). There were five significant differences between groups 33 and 31. Group 31 developed anemia, leukocytosis, monocytosis, eosinopenia and basopenia compared to group 33 (Table 18). However, there were no significant differences in the blood picture between the therapeutic positive control groups 31 and 32 judged by any of the eight criteria used (Table 18). Thus, these data seem to indicate that the conditions in the therapeutic positive control groups that ensured the production of normal appearing young to the exclusion of abnormal littermates were not conducive to the maintenance of a normal blood picture.

E. Nature of the Anatomical Abnormalities at Term

This study of avitaminosis E teratogeny might have been undertaken exclusively from the nutritional point of view. This did not seem to be advisable. Since this investigation was the first of its kind to be undertaken, it was deemed feasible to study simultaneously the nutritional factors involved in its production and the nature of the fetal anatomical abnormalities. In regard to the latter, an effort was made to determine 1) the nature of the macroscopic differences by employing gross observations and physical measurements, and 2) certain microscopic differences by means of the histological sections. The results are reported in the following pages.

1. Macroscopic

Macroscopic abnormalities can be studied without the help of any lens as they are usually observed by the naked eyes. They are the most obvious and striking abnormalities that can be observed by any layman. Physical measurements can give first approximations as to the degree and severity of the abnormalities. Measurements and observations of gross

abnormalities are important, but only complementary to and therefore cannot take the place of histological examination.

a. External appearance.

(1) Incidence of the gross abnormalities. The principal types of gross fetal abnormalities which were observed are listed in Tables 19, 20 and 21, and are illustrated in Figures 6 and 7. They were umbilical hernia, rigid ankle, exencephalus, hydrocephalus, hare lip, curved spine, receding maxillae, edema, kinked tail, ectocardia, syndactylism, cleft palate, taillessness, receding mandible, protruding mandible, and "elephant trunk" like nose. These abnormalities can be broadly classified as those involving the head, the trunk region, and both the head and trunk region. In those cases involving slight abnormalities of the head, the brain protruded through the top of the cranium and was covered only by a thin membrane. At other places in the head, small protrusions occurred which appeared transparent under the skin giving every indication of being hydrocephalic (Figure 6, A). In the more severe cases of cranial abnormality, part of the roof of the brain was exposed and the head gave the appearance of having receded (Figure 6, D). In these instances the brain appeared to have developed normally whereas the growth of the bony cranium had become retarded, thereby forcing the brain to the outside through the cranial sutures.

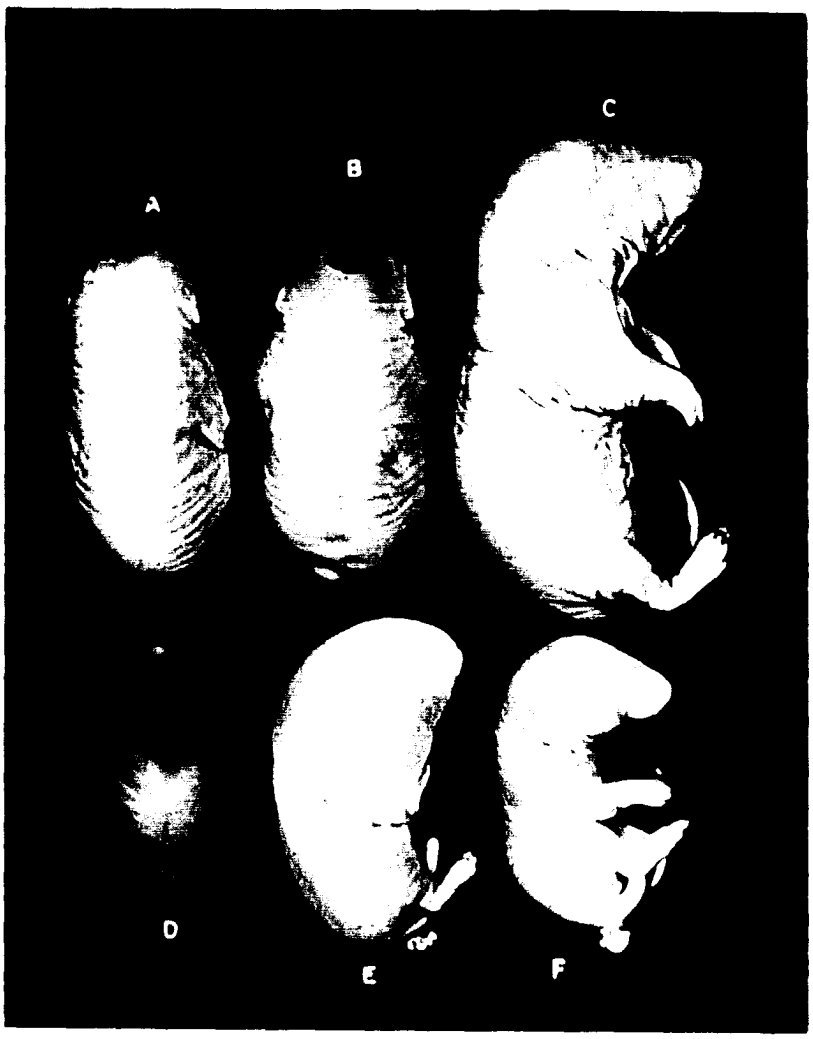


Figure 6

Dorsal and Lateral Views Illustrating Certain Gross Congenital Abnormalities in Full-Term Live Feti Removed after Laparotomy from Avitaminosis E Rats That Had Been Given 1.2 mg. d,l-alpha-Tocopherol Acetate Therapy on the 9, 10, 11 or 12th Day of Gestation. A, Hydrocephalus; B, Mild Exencephalus; C, Normal Control; D, Exencephalus; E, Edema; F, Agnathus

Figure 7

Representative Specimens of 21-Day Old Abnormal Rat Feti from Vitamin E-Deficient Dams Fed 1.2 mg. d,l-alpha-Tocopherol Acetate on the 9, 10, 11 or 12th Day of Gestation. A, Kinked Tail; B, Unilateral Hare Lip; C, Ectocardia; D, E, F, Syndactylism; A, C, D, F, Exencephalus; A through F, Umbilical Hernia

Regarding the formation of the face, the fusion of the mandibles was complete in every instance except in the case of agnathus (Figure 6, F) where the mandibles were missing. The abnormalities that did occur were in the maxillae and premaxillae. In some instances the fusion of the premaxillae was complete, but apparently they were under-developed causing the tongue to appear to protrude from the mouth (Figure 7, D, F). In other instances unilateral (Figure 7, B) or bilateral hare lip or complete absence of union of the medial nasal process and maxillary process was observed. The eyes were present in those cases which had milder head abnormalities, but were indistinguishable or absent in the more severe cases.

A common manifestation of avitaminosis E teratogeny is umbilical hernia. In less severe cases only loops of the intestine protruded from the vicinity of the umbilical cord (Figure 7, B). In severe cases, however, the intestine, lobes of the liver, spleen, kidneys and even heart (Figure 7, C), or even a piece of the lung might protrude from the abdominal wall in the region of the umbilical opening (Figure 7, A, C, D, E, F). This type of umbilical hernia might have been due to a continuation of the fetal condition, or to the possibility that the development of the abdominal cavity and the posterior

part of the body had not kept pace with that of the viscera, thus forcing them to remain outside the abdominal cavity.

In the most severe conditions of multiple abnormalities, combinations of head and umbilical deformities (Figure 7, A, C, D, F) were observed together with such other abnormalities as syndactylism (Figure 7, D, E, F) curved spine (Figure 7, A, C, E, F), rigid ankle (Figure 7, D) or taillessness.

Data relating the incidence of several types of external abnormalities to time of tocopherol therapy during gestation are presented in Table 19. There were no abnormal feti in the litters of dams that received 1.2 mg. d,l-alpha-tocopherol acetate on the 4, 5, 6, 7 or 8th day of gestation. Also, none appeared in the positive or negative control litters. Abnormal feti were found only when the dams received the vitamin supplementation on the 9, 10, 11 or 12th day of gestation. 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 10 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 respectively. The data suggest that umbilical hernia, hydrocephalus, and exencephalus may occur more frequently on the 10, 9, and 11th day of gestation, respectively. The fact that

Table 19

Relationship of Time of d,l-alpha-Tocopherol Acetate Gestational Ther to the Incidence and Distribution of Different Types of Congenital Abnormalities in Maternal Avitaminosis E

Type of abnormality	Number of abnormalities by each day of gestational therapy v d,l-alpha-tocopherol acetate per rat									
	Neg.	4[a]	5	6	7	8	9	10	11	12
	0/21[b]	0/25	0/23	0/22	0/19	0/21	15/32	23/27	19/28	2/20
Umbilical hernia	0	0	0	0	0	0	1	17	9	1
Hydrocephalus	0	0	0	0	0	0	11	2	4	1
Exencephalus	0	0	0	0	0	0	2	5	10	1
Rigid ankle	0	0	0	0	0	0	0	6	4	0
Receding maxilla	0	0	0	0	0	0	0	4	5	0
Hare lip	0	0	0	0	0	0	1	4	1	0
Edema	0	0	0	0	0	0	0	2	3	0
Cleft palate	0	0	0	0	0	0	0	3	0	0
Ectocardia	0	0	0	0	0	0	0	0	3	0
Kinked tail	0	0	0	0	0	0	0	0	3	0
Syndactylism	0	0	0	0	0	0	0	3	0	0
Receding mandible	0	0	0	0	0	0	0	1	1	0
Total	0	0	0	0	0	0	15	47	43	3

[a]Day during gestation females in each group received the tocopherol by stomach tube.

[b]Numerator denotes total number of live abnormal feti at term, denc number of pregnancies in each group

Table 19

nship of Time of d,l-alpha-Tocopherol Acetate Gestational Therapy
he Incidence and Distribution of Different Types of Congenital Abnormalities in Maternal Avitaminosis E

Number of abnormalities by each day of gestational therapy with 1.2 mg. d,l-alpha-tocopherol acetate per rat											
Neg. 0/21[b]	4[a] 0/25	5 0/23	6 0/22	7 0/19	8 0/21	9 15/32	10 23/27	11 19/28	12 2/20	Pos. 0/35	Total abnormal 59/273
0	0	0	0	0	0	1	17	9	1	0	28
0	0	0	0	0	0	11	2	4	1	0	18
0	0	0	0	0	0	2	5	10	1	0	18
0	0	0	0	0	0	0	6	4	0	0	10
0	0	0	0	0	0	0	4	5	0	0	9
0	0	0	0	0	0	1	4	1	0	0	6
0	0	0	0	0	0	0	2	3	0	0	5
0	0	0	0	0	0	0	3	0	0	0	3
0	0	0	0	0	0	0	0	3	0	0	3
0	0	0	0	0	0	0	0	3	0	0	3
0	0	0	0	0	0	0	3	0	0	0	3
0	0	0	0	0	0	0	1	1	0	0	2
0	0	0	0	0	0	15	47	43	3	0	108

ring gestation females in each group received the tocopherol acetate ube.

tor denotes total number of live abnormal feti at term, denominator, egnancies in each group

hydrocephalus occurred more frequently in the feti when the vitamin E supplementation was given on the 9th day of gestation confirms in a way the previous findings of Job et al. (1935). These investigators found that a hydrocephalic condition could be produced in young rats when exposed to 36 to 90 r of X-rays on the 9th day of gestation. These findings support the "critical moment" of development hypothesis in which it is claimed there is a limited and relational time in development when a given anlage may be influenced more than others (Stockard, 1921). The fact that not all individuals of one litter were affected in the same manner indicates that there were slightly different stages of development at the time of the treatment as evidenced by Huber's work (1915).

Table 20 summarizes the incidence of various gross fetal malformations produced when dams limited to depleting ration I (Table 2) received different levels of tocopherol therapy on the 8, 10 or 12th day of gestation. In the 86 abnormal feti produced there were 230 gross anomalies observed. A large majority of them, namely, 89 per cent, appeared in feti from dams that had received therapy on the 10th day of gestation. Approximately 10 per cent appeared in feti from dams given their supplement on the 12th day, and less than 1 per cent appeared in dams that received supplementation on the 8th day.

Table 20

Relationship of Level of d,l-alpha-Tocopherol in Gestational Therapy to Incidence and Distribution of Congenital Abnormalities in Maternal Avitaminosis E

Type of abnormality	Number of abnormalities by each day of gestational therapy with levels of d,l-alpha-tocopherol acetate											
	Neg.	8th day			10th day			12th day			1,2,3,4,5	
	0	1[a]	2	4	1	2	4	1	2	4	5x2[b]	5x2[c]
	0/24[e]	1/20	0/20	1/20	20/20	30/20	27/20	2/20	5/19	0/20	0/18	0/19
Umbilical hernia	0	1	0	1	20	27	23	2	3	0	0	0
Rigid ankle	0	0	0	0	10	14	13	1	1	0	0	0
Exencephalus	0	0	0	0	5	4	8	2	4	0	0	0
Curved spine	0	0	0	0	9	8	6	0	0	0	0	0
Hydrocephalus	0	0	0	0	4	3	10	0	0	0	0	0
Receding maxillae	0	0	0	0	4	4	4	1	2	0	0	0
Hare lip	0	0	0	0	3	5	3	0	0	0	0	0
Edema	0	0	0	0	1	4	1	1	2	0	0	0
Syndactylism	0	0	0	0	1	3	1	1	1	0	0	0
Ectocardia	0	0	0	0	0	1	0	1	1	0	0	0
Kinked tail	0	0	0	0	0	2	1	0	0	0	0	0
Cleft palate	0	0	0	0	1	0	0	0	0	0	0	0
Receding mandible	0	0	0	0	0	1	0	0	0	0	0	0
Taillessness	0	0	0	0	0	0	0	0	1	0	0	0

[a]Amount in mg. of the tocopherol acetate given each female by stomach tube day or days noted.

[b]Two mg. of the tocopherol acetate given orally weekly to each female prior to positive mating. Also, each female was given 2 mg. of the tocopherol daily the first five days of pregnancy.

[c]Same as for b except the tocopherol was given only during pregnancy.

[d]Positive control. Females fed stock ration ad lib. at all times.

[e]Numerator denotes total number of live abnormal feti at term, denominator of pregnancies in each group.

Table 20

.p of Level of d,l-alpha-Tocopherol in Gestational Therapy to the ıce and Distribution of Congenital Abnormalities in Maternal Avitaminosis E

ır of abnormalities by each day of gestational therapy with different levels of d,l-alpha-tocopherol acetate

8th day			10th day			12th day			1,2,3,4,5th			Total abnormal
1[a]	2	4	1	2	4	1	2	4	5x2[b]	5x2[c]	Pos.[d]	
1/20	0/20	1/20	20/20	30/20	27/20	2/20	5/19	0/20	0/18	0/19	0/23	86/263
1	0	1	20	27	23	2	3	0	0	0	0	77
0	0	0	10	14	13	1	1	0	0	0	0	39
0	0	0	5	4	8	2	4	0	0	0	0	23
0	0	0	9	8	6	0	0	0	0	0	0	23
0	0	0	4	3	10	0	0	0	0	0	0	17
0	0	0	4	4	4	1	2	0	0	0	0	15
0	0	0	3	5	3	0	0	0	0	0	0	11
0	0	0	1	4	1	1	2	0	0	0	0	9
0	0	0	1	3	1	1	1	0	0	0	0	7
0	0	0	0	1	0	1	1	0	0	0	0	3
0	0	0	0	2	1	0	0	0	0	0	0	3
0	0	0	1	0	0	0	0	0	0	0	0	1
0	0	0	0	1	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	1	0	0	0	0	1

mg. of the tocopherol acetate given each female by stomach tube on the d.

the tocopherol acetate given orally weekly to each female from weaning ng. Also, each female was given 2 mg. of the tocopherol daily during ays of pregnancy.

r b except the tocopherol was given only during pregnancy.

ontrol. Females fed stock ration ad lib. at all times.

denotes total number of live abnormal feti at term, denominator, number n each group.

The gross abnormalities tabulated in Table 20 were similar to those reported in Table 19, except that taillessness appeared once in a fetus whose dam had received 2 mg. of supplement on the 12th day of gestation. Note that the data in Table 20 confirm the previous observation based on the data reported in Table 19, namely, that when supplementation was administered on the 10th day, the most common malformation was umbilical hernia (Tables 19 and 20).

The data in Table 21 summarize the effect of prolonged feeding of different levels of tri-o-cresyl phosphate (TCP) on the incidence of gross abnormalities in feti from dams that received tocopherol therapy on the 10th day of gestation. With respect to the incidence of congenital abnormalities in the different groups on ration II with 25 µg. TCP per gram of ration, it is seen that there was a total of 47 different gross anomalies in the group that received 1 mg., 125 in the group that received 2 mg., and 94 in the group that received 4 mg. of tocopherol supplementation respectively.

Reported in Table 21 are two gross malformations not mentioned in previous tables; namely, protruding mandibles and "elephant trunk" nose. These all appeared in feti from dams that had received 4 mg. of tocopherol supplementation on the 10th day of gestation. Also, it should be noted in Tables 20 and 21 that all the feti were partially or

Table 21

Effect of Including the Vitamin E Antagonist Tri-o-cresyl Phosphate in Depleting Ration for Female Rats from Weaning through Pregnancy, and c pherol Acetate Gestational Therapy on the Incidence and Distributic Different Types of Congenital Abnormalities

Type of abnormality	Number of abnormalities by different treatments													
	25 µg. tri-o-cresyl phosphate/gm. of ration II								100 µg. tri-o-cresyl phos of ration II					
	Mg. of d,l-alpha-tocopherol acetate given on 10th day of													
	Neg.	1[a]	2	4	5x2[b]	5x2[c]	Pos.[d]	Total	Neg.	1[a]	2	4	5x2[b]	5x2[c]
	0[e]/20	15/20	39/20	24/19	0/17	0/17	0/23	78/136	0/20	22/20	27/20	20/20	0/14	0/20
Umbilical hernia	0	15	39	24	0	0	0	78	0	22	27	20	0	0
Rigid ankle	0	7	28	17	0	0	0	52	0	10	11	11	0	0
Exencephalus	0	8	10	13	0	0	0	31	0	8	13	14	0	0
Hydrocephalus	0	1	14	6	0	0	0	21	0	6	3	5	0	0
Hare lip	0	6	7	7	0	0	0	20	0	7	1	1	0	0
Curved spine	0	1	12	4	0	0	0	17	0	0	3	4	0	0
Receding maxillae	0	2	3	5	0	0	0	10	0	2	2	6	0	0
Edema	0	1	4	5	0	0	0	10	0	2	1	4	0	0
Kinked tail	0	3	3	3	0	0	0	9	0	1	1	1	0	0
Ectocardia	0	1	2	3	0	0	0	6	0	0	3	2	0	0
Syndactylism	0	1	3	2	0	0	0	6	0	0	0	0	0	0
Taillessness	0	0	0	2	0	0	0	2	0	0	0	1	0	0
Cleft palate	0	1	0	0	0	0	0	1	0	1	0	0	0	0
Receding mandible	0	0	0	1	0	0	0	1	0	0	1	1	0	0
Protruding mandible	0	0	0	1	0	0	0	1	0	0	0	0	0	0
"Elephant trunk" nose	0	0	0	1	0	0	0	1	0	0	0	0	0	0

[a]Amount in mg. of the tocopherol acetate given each female by ston the day or days noted.

[b]Two mg. of the tocopherol acetate given orally weekly to each fen weaning to positive mating. Also, each female was given 2 mg. of the t daily during the first five days of pregnancy.

[c]Same as for b except the tocopherol was given only during pregna

[d]Positive control. Females fed stock ration ad lib. at all times.

[e]Numerator denotes total number of live abnormal feti at term, de number of pregnancies in each group.

Table 21

Including the Vitamin E Antagonist Tri-o-cresyl Phosphate in an E-Ration for Female Rats from Weaning through Pregnancy, and of Toco-Acetate Gestational Therapy on the Incidence and Distribution of Different Types of Congenital Abnormalities

	Number of abnormalities by different treatments															
	25 µg. tri-o-cresyl phosphate/gm. of ration II								100 µg. tri-o-cresyl phosphate/gm. of ration III							
	Mg. of d,l-alpha-tocopherol acetate given on 10th day of gestation															
	Neg.	1[a]	2	4	5x2[b]	5x2[c]	Pos.[d]	Total	Neg.	1[a]	2	4	5x2[b]	5x2[c]	Pos.[d]	Total
	0[e]/20	15/20	39/20	24/19	0/17	0/17	0/23	78/136	0/20	22/20	27/20	20/20	0/14	0/20	0/23	69/137
	0	15	39	24	0	0	0	78	0	22	27	20	0	0	0	69
	0	7	28	17	0	0	0	52	0	10	11	11	0	0	0	32
	0	8	10	13	0	0	0	31	0	8	13	14	0	0	0	35
	0	1	14	6	0	0	0	21	0	6	3	5	0	0	0	14
	0	6	7	7	0	0	0	20	0	7	1	1	0	0	0	9
	0	1	12	4	0	0	0	17	0	0	3	4	0	0	0	7
	0	2	3	5	0	0	0	10	0	2	2	6	0	0	0	10
	0	1	4	5	0	0	0	10	0	2	1	4	0	0	0	7
	0	3	3	3	0	0	0	9	0	1	1	1	0	0	0	3
	0	1	2	3	0	0	0	6	0	0	3	2	0	0	0	5
	0	1	3	2	0	0	0	6	0	0	0	0	0	0	0	0
	0	0	0	2	0	0	0	2	0	0	0	1	0	0	0	1
	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	1
	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	2
	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0

t in mg. of the tocopherol acetate given each female by stomach tube on days noted.

g. of the tocopherol acetate given orally weekly to each female from positive mating. Also, each female was given 2 mg. of the tocopherol g the first five days of pregnancy.

as for b except the tocopherol was given only during pregnancy.

ive control. Females fed stock ration ad lib. at all times.

ator denotes total number of live abnormal feti at term, denominator, regnancies in each group.

completely resorbed in the negative control groups, and there was a complete absence of abnormal young in the positive control groups.

The incidence and variety of gross abnormalities in the different groups which were fed ration III containing the higher level of TCP (Table 21) were about the same as those observed in the experiments reported in Tables 19 and 20. The dams that received 1 mg. of tocopherol supplementation during gestation produced feti having 59 gross abnormalities compared to 47 in the corresponding group on ration II. However, fewer abnormalities occurred in the groups which received 2 or 4 mg. of tocopherol supplementation compared to similar groups that were fed ration II. This might be interpreted as to indicate that the level of 100 µg. TCP per gm. ration was too high. If this were true, it might have exhausted the depleting ration of its residual vitamin E content to such an extent that supplementation with 2 or 4 mg. of tocopherol during gestation was insufficient to counteract the effect of TCP.

(2) Condition of the feti at term. In studies of reproduction involving vitamin E, consideration should be given to the extent of fetal resorptions and the distribution of normal and abnormal feti. Data relating to these points are presented in detail in Tables 22, 23, 24 and 25.

Table 22

Condition of the Feti at Term in Avitaminosis E Rats Fed Ration I Supplemented with 1.2 mg. d,l-alpha-Tocopherol Acetate on Different Days during Gestation

Group no.	Day of gestation and no. of females administered tocopherol: day	no.	Incidence of various conditions of feti: Dead: Resorbed: no.	%	Dead: Not resorbed: no.	%	Live: Normal[a]: no.	%	Live: Abnormal[a]: no.	%	Average live Normal[a] gm.
1	Neg.[b]	21	208	100	0	0	0	0	0	0	---
2	4	25	196	85	8	3.3	27	11.7	0	0	4.4
3	5	23	208	83.5	4	1.6	37	14.9	0	0	3.9
4	6	22	208	82	8	3.0	38	15.0	0	0	4.6
5	7	19	147	75.8	4	2.0	43	22.2	0	0	4.1
6	8	21	150	69.8	6	2.8	59	27.4	0	0	4.7
7	9	32	244	78.7	6	1.9	45	14.5	15	4.9(25)[c]	4.2
8	10	27	231	88.9	4	1.5	2	0.7	23	8.9(23)	3.5
9	11	28	232	86.6	3	1.1	14	5.2	19	7.1(58)	3.6
10	12	20	211	98.6	0	0	1	0.5	2	0.9(67)	3.1
11	Pos.[b]	35	24	6.4	0	0	347	93.6	0	0	5.2

[a]Normality or abnormality based on macroscopic morphological a

[b]Negative control (ration I, Table 2) and positive control (st ration, Table 1).

[c]Figures in parentheses are percentages of abnormal feti on th live feti only.

Table 22

on of the Feti at Term in Avitaminosis E Rats Fed Ration I and Supplemented with 1.2 mg. d,l-alpha-Tocopherol Acetate on Different Days during Gestation

Day of station	No. of males administered copherol	Incidence of various conditions of feti								Average wt. of live feti	
		Dead				Live				Normal[a]	Abnormal[a]
		Resorbed		Not resorbed		Normal[a]		Abnormal[a]			
ay	no.	no.	%	no.	%	no.	%	no.	%	gm.	gm.
g.[b]	21	208	100	0	0	0	0	0	0	---	---
4	25	196	85	8	3.3	27	11.7	0	0	4.4	---
5	23	208	83.5	4	1.6	37	14.9	0	0	3.9	---
6	22	208	82	8	3.0	38	15.0	0	0	4.6	---
7	19	147	75.8	4	2.0	43	22.2	0	0	4.1	---
8	21	150	69.8	6	2.8	59	27.4	0	0	4.7	---
9	32	244	78.7	6	1.9	45	14.5	15	4.9(25)[c]	4.2	3.9
0	27	231	88.9	4	1.5	2	0.7	23	8.9(23)	3.5	2.8
1	28	232	86.6	3	1.1	14	5.2	19	7.1(58)	3.6	3.2
2	20	211	98.6	0	0	1	0.5	2	0.9(67)	3.1	3.3
s.[b]	35	24	6.4	0	0	347	93.6	0	0	5.2	---

rmality or abnormality based on macroscopic morphological appearance.

ative control (ration I, Table 2) and positive control (stock colony able 1).

ures in parentheses are percentages of abnormal feti on the basis of only.

Table 23

Condition of the Feti at Term in Avitaminosis E Rats Fed Ration I Supplemented with Various Levels of d,l-alpha-Tocopherol Acetat on Different Days during Gestation

Group no.	Day of gestation and no. of females administered tocopherol: day	no.	Level of tocoph. mg.	Incidence of various conditions of feti: Dead: Resorbed: no.	%	Dead: Not resorbed: no.	%	Live: Normal[a]: no.	%	Live: Abnormal[a]: no.	%	Av: No.
21	Neg.[b]	24	0	254	100	0	0	0	0	0	0	
22	8	20	1	149	71.9	2	0.8	56	26.9	1	0.4(2)[f]	
23	8	20	2	121	60.5	8	4.0	71	35.5	0	0	
24	8	20	4	121	54.8	0	0	99	44.8	1	0.4(1)	
25	10	20	1	168	81.2	2	0.9	17	8.2	20	9.7(54)	
26	10	20	2	149	74.2	4	1.9	18	9.0	30	14.9(62)	
27	10	20	4	156	69.0	1	0.5	42	18.6	27	11.9(39)	
28	12	20	1	209	98.6	0	0	1	0.4	2	1.0(67)	
29	12	19	2	185	94.9	1	0.5	4	2.1	5	2.5(56)	
30	12	20	4	167	88.9	0	0	21	11.1	0	0	
31	Pos.[c]	18	2x5	4	2.5	0	0	155	97.5	0	0	
32	Pos.[d]	19	2x5	16	9.7	0	0	148	90.3	0	0	
33	Pos.[e]	23	0	18	7.3	0	0	228	92.7	0	0	

[a]Normality or abnormality based on macroscopic morphological ap

[b]Negative control (Ration I, Table 2).

[c]Positive control. Each female received weekly by stomach tube alpha-tocopherol acetate from weaning to mating and during first fiv gestation.

[d]Positive control. Each female received 2 mg. d,l-alpha-tocoph during first five days of gestation.

[e]Positive control; stock colony ration, Table 1.

[f]Figures in parentheses are percentages of abnormal feti on the live feti only.

Table 23

n of the Feti at Term in Avitaminosis E Rats Fed Ration I and mented with Various Levels of d,l-alpha-Tocopherol Acetate on Different Days during Gestation

of ion . of s ad- ered erol no.	Level of tocoph. mg.	Incidence of various conditions of feti								Average wt. of live feti	
		Dead				Live					
		Resorbed		Not resorbed		Normal[a]		Abnormal[a]		Normal[a]	Abnormal[a]
		no.	%	no.	%	no.	%	no.	%	gm.	gm.
24	0	254	100	0	0	0	0	0	0	---	---
20	1	149	71.9	2	0.8	56	26.9	1	0.4(2)[f]	4.2	4.3
20	2	121	60.5	8	4.0	71	35.5	0	0	4.6	---
20	4	121	54.8	0	0	99	44.8	1	0.4(1)	4.6	3.2
20	1	168	81.2	2	0.9	17	8.2	20	9.7(54)	4.2	2.6
20	2	149	74.2	4	1.9	18	9.0	30	14.9(62)	3.9	3.1
20	4	156	69.0	1	0.5	42	18.6	27	11.9(39)	4.3	3.1
20	1	209	98.6	0	0	1	0.4	2	1.0(67)	3.3	2.3
19	2	185	94.9	1	0.5	4	2.1	5	2.5(56)	3.8	2.2
20	4	167	88.9	0	0	21	11.1	0	0	4.2	---
18	2x5	4	2.5	0	0	155	97.5	0	0	4.5	---
19	2x5	16	9.7	0	0	148	90.3	0	0	4.9	---
23	0	18	7.3	0	0	228	92.7	0	0	5.1	---

lity or abnormality based on macroscopic morphological appearance.

ive control (Ration I, Table 2).

ive control. Each female received weekly by stomach tube 2 mg. d,l- herol acetate from weaning to mating and during first five days of

ive control. Each female received 2 mg. d,l-alpha-tocopherol acetate t five days of gestation.

ive control; stock colony ration, Table 1.

es in parentheses are percentages of abnormal feti on the basis of nly.

Table 24

Condition of the Feti at Term in Rats Restricted from Weaning throu Pregnancy to a Vitamin E-Depleting Ration Containing a Low Level Tri-o-cresyl Phosphate and Supplemented with Various Levels of d, alpha-Tocopherol Acetate on the Tenth Day of Gestation

Group no.	Day of gestation and no. of females administered tocopherol: day	no.	Level of tocoph. mg.	Incidence of various conditions of feti: Dead: Resorbed no.	%	Dead: Not resorbed no.	%	Live: Normal[a] no.	%	Live: Abnormal[a] no.	%	Ave Nor g
41	Neg.[b]	20	0	197	100	0	0	0	0	0	0	-
42	10	20	1	151	84.8	4	2.3	10	5.6	13	7.3(57)[f]	1
43	10	20	2	127	66.5	7	3.6	19	10.0	38	19.9(67)	1
44	10	19	4	84	51.9	2	1.2	53	32.7	23	14.2(30)	1
45	Pos.[c]	17	2x5	10	6.7	1	0.6	139	92.7	0	0	1
46	Pos.[d]	17	2x5	6	4.2	0	0	136	95.8	0	0	1
33	Pos.[e]	23	0	18	7.3	0	0	228	92.7	0	0	

[a]Normality or abnormality based on macroscopic morphological app

[b]Negative control (Ration II, Table 2).

[c]Positive control. Each female received weekly by stomach tube alpha-tocopherol acetate from weaning to mating and during first five gestation.

[d]Positive control. Each female received 2 mg. d,l-alpha-tocophe during first five days of gestation.

[e]Positive control; stock colony ration, Table 1.

[f]Figures in parentheses are percentages of abnormal feti on the live feti only.

Table 24

n of the Feti at Term in Rats Restricted from Weaning through cy to a Vitamin E-Depleting Ration Containing a Low Level of resyl Phosphate and Supplemented with Various Levels of d,l-lpha-Tocopherol Acetate on the Tenth Day of Gestation

of ion . of s ad- ered erol no.	Level of tocoph. mg.	Incidence of various conditions of feti								Average wt. of live feti	
		Dead				Live					
		Resorbed		Not resorbed		Normal[a]		Abnormal[a]		Normal[a]	Abnor-mal[a]
		no.	%	no.	%	no.	%	no.	%	gm.	gm.
20	0	197	100	0	0	0	0	0	0	---	---
20	1	151	84.8	4	2.3	10	5.6	13	7.3(57)[f]	4.1	2.8
20	2	127	66.5	7	3.6	19	10.0	38	19.9(67)	4.0	2.9
19	4	84	51.9	2	1.2	53	32.7	23	14.2(30)	4.4	2.6
17	2x5	10	6.7	1	0.6	139	92.7	0	0	4.5	---
17	2x5	6	4.2	0	0	136	95.8	0	0	4.9	---
23	0	18	7.3	0	0	228	92.7	0	0	5.1	---

lity or abnormality based on macroscopic morphological appearance.

lve control (Ration II, Table 2).

lve control. Each female received weekly by stomach tube 2 mg. d,l-herol acetate from weaning to mating and during first five days of

lve control. Each female received 2 mg. d,l-alpha-tocopherol acetate t five days of gestation.

ive control; stock colony ration, Table 1.

es in parentheses are percentages of abnormal feti on the basis of nly.

Table 25

Condition of the Feti at Term in Rats Restricted from Weaning thr
Pregnancy to a Vitamin E-Depleting Ration Containing a High Lev
of Tri-o-cresyl Phosphate and Supplemented with Various Levels
of d,l-alpha-Tocopherol Acetate on the Tenth Day of Gestation

Group no.	Day of gestation and no. of females administered tocopherol: day	no.	Level of tocoph. mg.	Incidence of various conditions of feti: Dead: Resorbed no.	%	Not resorbed no.	%	Live: Normal[a] no.	%	Abnormal[a] no.	%	Av: No.
51	Neg.[b]	20	0	217	100	0	0	0	0	0	0	
52	10	20	1	157	83.5	1	0.5	9	4.8	21	11.2(70[f])	
53	10	20	2	133	71.1	8	4.3	25	13.4	21	11.2(46)	
54	10	20	4	147	73.9	3	1.5	29	14.6	20	10.0(41)	
55	Pos.[c]	14	2x5	9	7.3	0	0	113	92.7	0	0	
56	Pos.[d]	20	2x5	15	8.8	0	0	155	91.2	0	0	
33	Pos.[e]	23	0	18	7.3	0	0	228	92.7	0	0	

[a]Normality or abnormality based on macroscopic morphological app

[b]Negative control (Ration III, Table 2).

[c]Positive control. Each female received weekly by stomach tube alpha-tocopherol acetate from weaning to mating and during first five gestation.

[d]Positive control. Each female received 2 mg. d,l-alpha-tocopha during first five days of gestation.

[e]Positive control; stock colony ration, Table 1.

[f]Figures in parentheses are percentages of abnormal feti on the live feti only.

Table 25

on of the Feti at Term in Rats Restricted from Weaning through
ancy to a Vitamin E-Depleting Ration Containing a High Level
ri-o-cresyl Phosphate and Supplemented with Various Levels
l,l-alpha-Tocopherol Acetate on the Tenth Day of Gestation

of tion	Level of tocoph.	Incidence of various conditions of feti								Average wt. of live feti	
		Dead				Live					
		Resorbed		Not resorbed		Normal[a]		Abnormal[a]		Normal[a]	Abnormal[a]
o. of s ad-tered herol no.	mg.	no.	%	no.	%	no.	%	no.	%	gm.	gm.
20	0	217	100	0	0	0	0	0	0	---	---
20	1	157	83.5	1	0.5	9	4.8	21	11.2(70)[f]	3.9	3.4
20	2	133	71.1	8	4.3	25	13.4	21	11.2(46)	4.1	2.9
20	4	147	73.9	3	1.5	29	14.6	20	10.0(41)	4.5	3.0
14	2x5	9	7.3	0	0	113	92.7	0	0	4.1	---
20	2x5	15	8.8	0	0	155	91.2	0	0	4.8	---
23	0	18	7.3	0	0	228	92.7	0	0	5.1	---

lity or abnormality based on macroscopic morphological appearance.

ive control (Ration III, Table 2).

ive control. Each female received weekly by stomach tube 2 mg. d,l-herol acetate from weaning to mating and during first five days of

ive control. Each female received 2 mg. d,l-alpha-tocopherol acetate t five days of gestation.

ive control; stock colony ration, Table 1.

es in parentheses are percentages of abnormal feti on the basis of nly.

Those in Table 22 summarize the outcome of fertilized embryos implanted in avitaminosis E females given 1.2 mg. tocopherol one day during gestation beginning with the fourth and ending with the twelfth day. When therapy was given on the 4, 5, 6, 7 or 8th day of gestation, the number of feti which were dead and morphologically normal at term was small. At the same time a much larger number were live and normal, and a very much larger number were completely or partially resorbed. The percentage of resorptions ranged from 100 in the negative control to about 70 in the group receiving tocopherol therapy on the 8th day of gestation. In none of these groups (1 through 6) of females were congenital abnormalities observed. On the other hand, when identical supplementation was administered on the 9, 10, 11 or 12th day of gestation, 15, 23, 19 and 2 feti respectively were abnormal showing single or multiple abnormalities. Simultaneously, the number of normal and dead feti decreased while that of resorptions was surprisingly large. Interesting is the observation that even in positive control females which were fed stock colony ration resorptions occurred in about six per cent of the implantations. The average weight of the feti (5.2 gm.) in the positive control group raised on stock colony ration was larger than that for live full-term

morphologically normal or abnormal feti from females which had received the basal ration I and supplementation.

Similarly Table 23 summarizes the outcome of pregnancies in avitaminosis E females that were given different levels of tocopherol therapy on the 8, 10 or 12th day of gestation. Therapy at the 1 mg. level given the dams on the 8th day of gestation produced less than 1 per cent of dead and normal feti at term, about 27 per cent live and normal, less than 1 per cent live and abnormal, and about 72 per cent resorbed. Increasing the therapeutic level of tocopherol to 2 mg. increased the percentage of dead and normal feti to 4, and that of live and normal to about 36. Simultaneously, the percentage of resorption was decreased from 72 to 61. Finally, increasing the tocopherol therapy to 4 mg. increased the normal and live feti to 45 per cent and reduced the percentage of resorption to about 55. The percentage of abnormal feti was insignificant.

When the same levels of tocopherol were administered on the 10th day of gestation, similar results were obtained except for the following notable and important differences; namely, a large decrease in the number of live normal feti and a very large increase in the number of abnormal live feti. When the same three levels of tocopherol were given on the 12th day of pregnancy, the number of live normal feti

was still further reduced, that of live abnormal feti reduced only a few per cent, and the number of resorptions increased. Attention is called to additional trends and relationships in the data of Table 23. On each of the three days the number of resorptions decreased, whereas the number of live normal feti increased with each increase in the therapeutic dose of tocopherol. Also, the data show that the later the day during gestation on which identical therapy was given, the higher the percentage of resorption and the lower the percentage of normal live young. The maximum number of resorptions occurred in the negative control and the minimum in the positive control groups. Conversely, the maximum number of live normal feti occurred in the positive control and the least in the negative control groups. No abnormal live feti occurred in either the negative or positive control groups. In comparable groups of uteri, the live normal feti were most always larger than littermate abnormal feti.

How the distribution of resorbed, live normal and abnormal feti was affected by the inclusion of TCP in the depleting ration I is presented in Tables 24 and 25. Table 24 summarizes the data obtained by prolonged feeding of the lower level of tri-o-cresyl phosphate. It is sufficient to note that the data for the 10th day of therapy are strikingly

similar in magnitude and direction of trends to those reported in Table 23. Apparently the inclusion of 25 µg. TCP per gm. ration has not altered the picture significantly.

Table 25 summarizes the data obtained by the prolonged feeding of 100 µg. TCP per gm. ration. The results differ in some details from those presented in the two previous tables. When 1 mg. of tocopherol was given on the 10th day, the percentage of resorption was about 84. The percentages of dead and live normal feti were 0.5 and about 5, respectively. The percentage of abnormal live feti was about 11 in terms of all the implantation sites, and 70 in terms of only the total live feti. When 2 mg. of tocopherol was fed, the resorption was reduced to 71 per cent. The percentages of both dead and live normal feti were increased to about 4 and 13, respectively. The percentage of abnormal live feti remained the same in terms of total implantation sites, but was reduced to 46 in terms of total number of live feti. When 4 mg. of tocopherol was given, the resorption remained essentially the same, namely 74 per cent. The percentage of dead normal young was reduced to about 2, while that for normal live young was increased slightly to about 15. The percentage of live abnormal young was slightly reduced to 10 in terms of total implantation sites, and 41 in terms of total live feti. The percentages of resorption for the

positive controls were 7.3 in group 55 and 8.8 in group 56, as compared to 6.7 and 4.2 in the corresponding groups on ration II.

Comparing the results of Tables 23, 24 and 25, it can be stated that at 1 mg. level of tocopherol supplementation on the 10th day of gestation, the feeding of tri-o-cresyl phosphate seemed to have raised the actual percentage of resorption from about 81 on ration I to 85 on ration II and 84 on ration III. The percentage of live normal feti was reduced from 8 on ration I to about 6 on ration II and 5 on ration III. The percentages of abnormal live young in terms of total implantation sites were 10, 7 and 11 for rats on rations I, II and III, respectively. When 2 mg. tocopherol therapy was given, the percentages of resorption for rats on rations I, II and III were 74, 67 and 71, respectively. The percentage of dead normal young was increased from 2 on ration I to 4 on rations II and III. The percentages of the normal live young were 9, 10 and 13, while those for abnormal live young were 15, 20 and 11 for rats on rations I, II and III, respectively. When 4 mg. vitamin E therapy was given, the percentages of resorption were 69, 52 and 74 for rats on rations I, II and III, respectively. The percentages of dead normal young were again increased by tri-o-cresyl phosphate feeding as evidenced by the increase from 0.5 per cent

for rats on ration I to 1 per cent for those on ration II and 1.5 per cent for those on ration III. This seems to be a consistent finding. The percentages for the normal live young were 19, 33 and 15 and those for the abnormal live young were 12, 14 and 10 for rats on rations I, II and III, respectively.

b. Crown-rump lengths. Another index of fetal development is what embryologists call "crown-rump" length. This is the measured distance between the poll and the base of the tail. In this study crown-rump lengths were determined on sizable samples of feti representative of various classifications. The data obtained are presented in Table 26. The abnormal feti varied greatly in their development. Those with only head abnormalities had about the same crown-rump length, 36 mm., as their normal-appearing littermates (37.5 mm.). Those with umbilical hernia were more retarded in their development along the antero-posterior axis, their average length being only 30.1 mm. Those with both head and abdominal malformations were most retarded in their development, since their average crown-rump length was 26.2 mm., only 69 per cent of that of their normal littermates, and 63 per cent of that of the normal feti of stock ration control. Also, note that even the normal-appearing littermates of abnormal feti were below normal in their development

Table 26

Comparison of Crown-Rump Lengths between Normal and Various Abnormal Feti Removed after Laparotomy at Term from Initial Gestation Females Reared on Normal or Vitamin E-Depleting Rations Supplemented during Gestation with d,l-alpha-Tocopherol Acetate

Description of feti and maternal dietary treatment	Feti examined	Average crown-rump length	Degree of normality
	no.	mm.	%
Normal (stock ration)	25	41.4	
Abnormal (depletion, then gestational therapy)			
a, Appearance normal	32	37.5	91
b, Head abnormal	20	36.0	83
c, Abdominal abnormalities	25	30.1	72
d, Head and abdominal abnormalities	25	26.2	63

judged by their average crown-rump length, which was about 91 per cent of that of the normal feti from stock ration control.

c. Skeletal malformations observed in cleared specimens. Attention already has been called to certain skeletal abnormalities associated with avitaminosis E teratogeny. Only

those readily visible from the exterior of such feti have been mentioned. Others less obvious were present but could not be readily detected until after the soft tissues had been rendered essentially transparent and the skeleton simultaneously stained. This was accomplished by the so-called "alizarin method" described in the section on procedures. Treatment in this manner brings the skeleton into clear relief, thus making it possible to detect abnormalities of the internal structures. Specimens treated in this manner are termed "cleared".

Twenty five abnormal and 19 macroscopically normal feti were cleared. It should be emphasized here that these normal feti appeared normal externally and were littermates to feti with obvious gross abnormalities. All were from avitaminosis E females that had been given 1.2 mg. d,l-alpha-tocopherol acetate on 9, 10 or 11th day of gestation. Typical examples of several skeletal abnormalities observed are illustrated in Figures 8, 9 and 10. Data summarizing the types of skeletal abnormalities which occurred in the 44 cleared feti are presented in Table 27.

Obviously the skeleton is affected adversely in many parts, the incidence being greater in the manifestly abnormal feti than in the macroscopically normal-appearing feti. Gross skeletal abnormalities and lack of ossification

Table 27

Incidence of Gross Skeletal Abnormalities in Full Term Live Feti Rem after Laparotomy from Avitaminosis E Rats Given d,l-alpha-Tocophe Acetate Therapy on the 9, 10 or 11th Day of Gestation

Portion of body involved	Frequency of abnormalities by day of therapy[a]												
	9th day				10th day				11th day				Tota
	Abnormal[b]		Normal		Abnormal[b]		Normal		Abnormal[b]		Normal		Abnor
	10[c]		8		10		2		5		9		2
	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.
Head bones:													
Frontal					5	50			4	80	1	11	9
Parietal	9	90	1	13	9	90	1	50	4	80	4	44	22
Supra-occopital					3	30			4	80			7
Maxilla					3	30							3
Mandible					1	10							1
Trunk bones:													
Vertebrae					7	70			2	40			9
Ribs					8	80			2	40			10
Sternebrae	4	40	1	13	10	100	1	50	4	80	7	77	18
Fore legs:													
Phalanges	1	10			1	10			1	20	2	22	3
Hind legs:													
Phalanges	9	90	2	25	8	80	2	100	4	80	6	66	21
Total	23	85	4	15	55	93	4	7	25	56	20	44	113

[a]Each pregnant female was given 1.2 mg. d,l-alpha-tocopherol ace day of gestation indicated.

[b]Abnormal denotes feti exhibiting one or more gross skeletal mal

[c]Number of feti examined.

Table 27

of Gross Skeletal Abnormalities in Full Term Live Feti Removed Laparotomy from Avitaminosis E Rats Given d,l-alpha-Tocopherol Acetate Therapy on the 9, 10 or 11th Day of Gestation

Frequency of abnormalities by day of therapy[a]															
9th day				10th day				11th day				Total (9,10,11)			
Abnormal[b]		Normal		Abnormal[b]		Normal		Abnormal[b]		Normal		Abnormal[b]		Normal	
10[c]		8		10		2		5		9		25		19	
no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%	no.	%
				5	50			4	80	1	11	9	36	1	5
9	90	1	13	9	90	1	50	4	80	4	44	22	88	6	32
				3	30			4	80			7	28		
				3	30							3	12		
				1	10							1	4		
				7	70			2	40			9	36		
				8	80			2	40			10	40		
4	40	1	13	10	100	1	50	4	80	7	77	18	72	9	47
1	10			1	10			1	20	2	22	3	12	2	11
9	90	2	25	8	80	2	100	4	80	6	66	21	84	10	53
23	85	4	15	55	93	4	7	25	56	20	44	113	80	28	20

pregnant female was given 1.2 mg. d,l-alpha-tocopherol acetate on the ation indicated.

mal denotes feti exhibiting one or more gross skeletal malformations.

r of feti examined.

Figure 8

Dorsal View of the Skeletons of Full Term Rat Feti Removed by Laparotomy from Dams Maintained under Normal or Vitamin E-Deficient Dietary Regimes

A, Normal--note general symmetry and density of the bones.

B, Abnormal--note specifically, incomplete ossification of parietal bones, curvature of the spinal column to the left, fusion of ribs on the left side, and smaller size.

C, Abnormal--note specifically, absence of roof of the cranium, curvature of spinal column to the right, fusion of ribs on the right side and smaller size.

Figure 9

Lateral View of the Skeletons of Full Term Rat Feti Removed by Laparotomy from Dams Maintained under Normal or Vitamin E-Deficient Dietary Regimes

A, Normal.

B, Abnormal--note specifically, hare lip and retarded ossification of parietal bone.

C, Abnormal--note specifically the absence of the mandible.

D, Abnormal--note specifically, absence of roof of the cranium and the fusion of ribs.

Figure 10

An Illustration of the Effect of Avitaminosis E on the Number and Orientation of the Sternebrae, as Seen from a Ventral View of the Skeletons of Full Term Rat Feti Removed by Laparotomy from Their Dams

A, Normal--usual number and orientation of sternebrae.

B, Abnormal--five sternebrae.

C, Abnormal--four pairs of separated sternebrae.

D, Abnormal--separated and irregular sternebrae.

F, Abnormal--two pairs of separated sternebrae.

occurred in the frontal, parietal, occipital, maxilla and mandible bones of the head; the ribs, sternebrae, and vertebral column of the trunk; and the phalanges of the anterior and posterior extremities. Abnormalities of the parietal bones occurred most frequently. Parts of these were either absent or ossification was markedly retarded. Abnormalities of the parietal bones appeared in 90 per cent of the hydrocephalic feti from dams that received tocopherol supplementation on the 9th day, and in 90 and 80 per cent of the exencephalic feti from dams which had received tocopherol supplementation on the 10th or 11th day of gestation, respectively. The incidence of abnormalities in the parietal bones of the normal-appearing feti from dams that received vitamin E therapy on the 9, 10 or 11th day of gestation were 13, 50 and 44 per cent, respectively. The cumulative incidence in the parietals of the abnormal feti was 88 per cent and that for the normal-appearing feti was 32 per cent.

Abnormalities in the phalanges of the hind feet occurred next most frequently. Absence of ossification in either the middle or both the middle and the distal rows of digits was the principal manifestation. Generally, ossification of the digits of these 21-day old feti was retarded, and, in effect, corresponded more nearly to that of a 19-day old normal control fetus. The incidence of this retarded

ossification of the phalanges was 90, 80 and 80 per cent in the abnormal feti from dams that received tocopherol therapy during the 9, 10 or 11th day of gestation, respectively. The incidence in the normal feti of the corresponding groups was 25, 100 and 66 per cent respectively. The overall incidence for the three groups of abnormal feti was 84 per cent, while that for the three groups of normal feti was 53 per cent.

The third most frequently occurring abnormality affecting the skeleton involved the sternebrae. The incidence of abnormality was 100, 80 and 40 per cent in the abnormal feti from dams that received the tocopherol supplementation on the 10, 11 or 9th day of gestation, respectively. The incidence in the normal-appearing feti of the corresponding groups was 50, 77 and 13 per cent, respectively. When the three groups are considered together, the overall incidence for the abnormal feti was 72 per cent, as compared to 47 per cent in the normal-appearing littermates.

In the rat fetus the normal number of sternebrae is six. Conspicuous among the abnormalities of the sternebrae was the variation in the number of ossification centers (Figure 10). These ranged from none to the normal number of six. In some instances, even when six sternebrae were present, the halves of each would be separated, indicating retarded ossification. Also, when the sternebrae were fewer

than six, the halves frequently were separated terminating ultimately in their irregular fusion (Figure 10, D). For the most part, the development of the sternebrae corresponded to that of a 19-day old normal control fetus. This observation is based on comparisons with stock colony rat feti ranging in gestational age from 15 to 21 days which had been similarly cleared and stained with alizarin red.

Skeletal abnormalities which occurred less frequently appeared in the following descending order of overall frequency in, 1) the ribs, where two or more on either the right or left side often were fused into a plexus-like mass (Figure 8, C; Figure 9, D); 2) the vertebral column, where abnormal curvature to the right was more common than to the left (Figure 8, B, C); 3) the frontal and occipital bones, where ossification was retarded or absent (Figure 9, D); 4) the maxillae (Figure 9, B) and mandibles (Figure 9, C), which showed non-fusion or failed to attain their full length; and 5) the phalanges of the front feet, which showed fusion or retarded ossification (Table 27).

d. Weights of organs. Differences in the weights of various organs, when determined at the end of gestation, can be used to give some conception of the comparative development of the feti. Typical data indicating the effect of tocopherol gestational therapy on the developmental retardation

of various organs are presented in Table 28. Note that at autopsy most organs of the feti which had head or abdominal abnormalities were lighter in weight than those of the normal stock colony control feti. The organs most affected were: heart, 40 per cent of normal weight; lungs (46 per cent); and the eyeballs, (53 per cent). The other organs; namely, liver, thyroid, stomach, brain, thymus, adrenals, intestines and kidneys were 53, 58, 60, 64, 65, 78, 81 and 98 per cent of normal, respectively. The spleen and testes were slightly larger than normal. By comparison, it is interesting to note that the kidneys of avitaminosis E feti were not as drastically affected as those in feti from dams subjected to folic acid deficiency (Nelson et al., 1952). In a small number of avitaminosis E feti, abnormalities of the kidneys were observed during the dissection. Out of 10 feti dissected, three showed malposition or malformation of the kidney. In one fetus the left kidney was embedded under the peritoneum. In another, there was complete fusion of the kidneys resulting in an irregular horse-shoe shaped organ. In a third, both kidneys appeared reddish in color and the right one was cystic.

Also, the adrenal glands showed irregularities. Sometimes one or both were missing. At other times they appeared to be similar to a red ball filled with capillaries. In

Table 28

Effect of Gestational Tocopherol Therapy in Avitaminosis E on the Weights of Various Organs Dissected from Teratogenic Feti

Organs	Normal feti[a]		Abnormal feti		Degree of normality
	no.	Av. wt. mg.	no.	Av. wt. mg.	%
Body weight		5200		2800	54
Testes	10[b]	4.3	10[b]	5.0	116
Spleen	10	3.7	10	4.0	108
Kidney	10[b]	23.8	12[b]	23.3	98
Intestines	10	136.2	10	110.7	81
Adrenals	10[b]	1.8	11[b]	1.4	78
Thymus	10	8.8	10	5.7	65
Brain	10	221.7	10	140.7	64
Stomach	10	81.0	10	48.7	60
Thyroid	10	34.2	10	19.7	58
Liver	10	344	10	182.7	53
Eyeballs	10[b]	25.7	10[b]	12.7	50
Lungs	10	160.7	10	74.3	46
Heart	10	61.0	10	24.4	40

[a]Stock colony ration control feti.

[b]In terms of pairs.

only four out of ten feti were the adrenals normal morphologically.

2. Histologic

Up to this point it has been demonstrated conclusively that ample tocopherol therapy administered during a certain critical interval in the gestation of avitaminosis E rats produced some live feti having gross congenital abnormalities. Equally conclusive is the evidence that the gross abnormalities range in severity from barely perceptible to extensive, and they may involve simultaneously one or more of the soft tissues and portions of the skeleton. These gross abnormalities are outward manifestations of abnormal circumstances, involving the cellular structures of the various tissues concerned, and the body fluids which nourish them and regulate their behavior. Therefore, this study was expanded to include a histological examination of various tissues and organs per se, and in relation to each other, hopeful that it would indicate avenues of approach which would be fruitful in understanding more completely the irregularities basically responsible for avitaminosis E teratogeny.

Different feti and portions of others were sectioned serially at 5 to 10 microns lengthwise and crosswise.

Subsequently, every tenth section was mounted on a slide and processed for examination and study. In the case of certain organs; namely, eyes, kidney or heart, the procedure used in selecting sections differed slightly in that only the best contiguous sections were mounted.

a. Longitudinal sections. Compared to the normal controls (Plate I, Figure 11), the length of the abnormal feti ranged from about one-half (Plate II, Figure 14) to slightly less than normal (Plate I, Figure 12). When umbilical hernia was the main malformation, the development of the posterior part of the body was stunted more drastically than the anterior part (Plate I, Figure 12). In this particular instance only the urogenital organs remained in the abdominal cavity, as the remainder of the viscera had been extruded through the umbilical opening. Also, the depth of the body was reduced to about half of normal judged by the widths of the diaphragms (Plate I, Figures 11 and 12). When the abnormality was confined to the head as shown in Plate II, Figure 13, the cranial as well as the facial development was retarded. When head and the abdominal malformations were present in the same fetus (Plate II, Figure 14), both the anterior and posterior parts of the body were retarded in development, especially the posterior part.

Also, differences in the brains and their lateral ventricles can be seen from these longitudinal sections. These will be described later in more detail. Normally, the cerebrum is thick and the lateral ventricle comparatively small (Plate I, Figure 11). However, the opposite prevails in the abnormal fetus; namely, the cerebrum, whenever present, was relatively thin and the lateral ventricle was large (Plates I and II, Figures 12 and 13), indicating a hydrocephalic condition (Whitley, 1952). There are instances where the major portion of the brain was missing (Plate II, Figure 14), viz.; only the floor and part of the diencephalon adjacent to the hypophysis were present. It is not improbable that avitaminosis E interferes with the progress of certain embryonic organizers. According to Weiss (1939), the development of the brain depends upon the amount of the chordamesoderm, or the head-organizer present (Needham, 1942). Likewise, the development of the posterior part of the body depends upon the tail-organizer. Thus, it seems that in some feti there might not have been enough of the head and/or tail organizer material in the embryo to induce normal development of the brain and the face, or the posterior part of the body.

b. Cross sections. Comparable cross sections of the head, thoracic and abdominal regions of the body were made

to compare the various histologic differences between the normal and abnormal feti. Comparisons of the cross sections of the head will be reported later under the section on brain. Only observations on the thoracic and abdominal regions will be presented now.

(1) Thoracic region. Cross sections of the thoracic region of normal and abnormal feti are shown in Plates III and IV, Figures 15, 16 and 17. Numerous abnormalities can be seen in Figures 16 and 17. Edema is very apparent in Plate III, Figure 16. The development of the musculature is much reduced in this fetus. Its back is concave due to a deficiency in back muscles and lipoidal tissue, while that of the normal control is convex in contour. Contraction of the ribs (Plate III, Figure 16) resulted in reduced depth and width of the thoracic region of the abnormal fetus compared to the control (Plate III, Figure 15). This same factor produced a compactness or non-expansion of the lung (Plate III, Figure 16), and resulted in the extrusion of the heart and one lobe of the lung to the exterior of the body due to diaphragmatic hernia. In addition, the development of the skin and the hair follicles was retarded. Both the epidermis and dermis of the skin were thinner and less differentiated than those of the control. The hair follicles were very sparse in the skin of the abnormal fetus. The

space between the skin and the ribs was occupied mostly by connective tissue.

In Plate IV, Figure 17, the spinal column is shown to be twisted to the right. The shape of both the spinal cord and the vertebrum is quite abnormal. Also, the heart occupies an abnormal position just underneath the skin instead of being confined to the bony thoracic basket as in Plate III, Figure 15. This might be an intermediate location between the normal position of the heart and ectocardia (Plate III, Figure 16). Similar malformations have occurred in other avitaminoses. Fetal abnormalities of the heart and lungs, and diaphragmatic hernia have been reported in maternal avitaminosis A (Andersen, 1941; Warkany and Roth, 1948) and in folic acid deficiency (Nelson et al., 1952; Giroud and Lefebvres-Boisselot, 1951).

(2) Abdominal region. A typical cross section of the abdominal region of a normal rat fetus is shown in Plate V, Figure 19. The normal contour of the body at this region is round. The vertebral column, kidneys, stomach, intestine, spleen and lobes of the liver as well as the umbilical cord are shown in their proper relation to each other. In contrast, the corresponding section of the abnormal fetus (Plate V, Figure 18) shows the contour to be slightly concave, only the vertebral column and diminutive kidneys to be in the

abdominal cavity, and the rest of the viscera lying outside the body. An exceedingly unusual situation can be observed in Plate VI, Figure 20. In this abdominal section the lung can be seen as well as the urinary bladder, gonad, rectum and part of the intestine. A piece of the liver lies outside the body. One plausible explanation is that diaphragmatic hernia occurred causing the lung to protrude posteriorly into the abdominal cavity. Figure 21 of Plate VII shows a mild case of umbilical hernia involving only the intestines. This section is much smaller than that of the normal (Plate V, Figure 19) and shows the lobes of the liver to be occupying a comparatively predominant place.

Figure 23 of Plate VIII shows a normal section through the scrotal region. The normal contour of the body in this region is essentially round also. The corresponding region in an abnormal fetus is shown in Figure 22, of the same plate. The striking difference between these figures is in the size of the spinal cords. That in the abnormal fetus is much larger than normal. This might be due to the fact that the abnormal fetus was shorter than normal due to under-development of the abdominal region, and consequently the spinal cord was not stretched and thinned out enough. Or it simply might be another manifestation of the deleterious effect of maternal avitaminosis E on the nervous system of

the fetus. Less prominently observed in this cross section is the lateral compression of the entire abdominal region. Also, there is some indication of hypospadias in this abnormal fetus. More work would be necessary to establish this point definitely.

Thus, it has been shown by means of the cross sections of the trunk region of normal and abnormal rat feti that there are numerous malformations in the latter, the most striking of which is the dislocation of the visceral organs due to umbilical and diaphragmatic hernia. The umbilical hernia noted in avitaminosis E is due to the incomplete closure of the body wall along its mid-ventral line. Diaphragmatic hernia could have been caused by imperfect development of the pleuro-peritoneal membrane resulting in the persistence of an opening usually on the left side. Both umbilical and diaphragmatic hernia in the full-term feti can be said to be due to either a continuation of the fetal abnormality or a retarded and imperfect development of the membranes and muscles derived from the mesoderm. The latter may be more plausible in view of Urner's (1931) observation that the effect of maternal vitamin E deficiency in the rat embryo is a reduction of the mesodermal tissue.

c. Tissue sections.

(1) Brain. Both the nervous system and the sensory epithelia are derived from portions of the primitive integument of the embryo. The basis of the nervous system is the thickened strip of ectoderm called the neural plate along the mid-dorsal line of the embryo. This tissue is determined neurally by induction from the chorda-mesoderm (Weiss, 1939). In amphibians this induction occurs at the gastrula stage. Generally speaking, the wall of the neural tube is composed of three layers: 1) an innermost ependymal (or germinal) layer, the cells of which retain the power of cell division; 2) a middle nucleated mantle layer, derived by proliferation and migration of the innermost cells; and 3) an outer non-cellular marginal layer into which the nerve fibers grow. In the wall of the pallium of the cerebral hemisphere are differentiated the ependymal, mantle and marginal layers typical of the neural tube in general. However, during embryonic development neuroblasts migrate from the ependymal and mantle layers into the superficial marginal layer giving rise to layers of pyramidal and other cells typical of the cerebral cortex. Therefore, the marginal layer became the cerebral cortex of the brain.

According to Bonin (1950) and Sugita (1917) the cellular layers in the wall of the pallium of the new-born rat fetus

are: the molecular or outermost layer, the pyramidal layer, the ganglionic layer, the fusiform layer, the transitional layer, and the germinal or innermost layer (Plate XI, Figure 26 and Plate XII, Figure 27). The molecular layer contains only the horizontal cells of Cajal which send their dendrites and axon horizontally along the surface of the cerebral cortex (Bonin, 1950). In the pyramidal layer the perikaryon of the neurons is triangular, with the long axis perpendicular to the surface of the cortex. The ganglionic layer is composed of cells which are round and somewhat larger than the pyramidal cells. Their apical processes are all directed ectad. In this layer also a relatively large number of small round cells, very probably can be seen. The fusiform layer is composed of spindle-shaped cells, together with many short pyramidal and granular cells. It can be subdivided into two sublayers by a band which is poor in cells. The number of cells in the ental sublayer at the time of birth is very much greater, and they are larger and stain better than the cells in the ectal sublayer which has already in it polymorphous cells destined to become ganglion cells (Sugita, 1917). The transitional layer consists of cells with small nuclei and relatively rich protoplasm. Their arrangement suggests every stage of rotation. This layer disappears when the rat is five days old. The germinal layer is

equivalent to the aforementioned ependymal layer having cells possessing the power of mitosis. The pallial wall of the full-term rat presents a unique appearance by having the transitional layer. The cells which are becoming ganglionic cells have originated from the germinal cells lying in the ventricular wall and have migrated from there to their final position in the cortex. In the rat brain, cell migration still is in progress at the time of birth, giving rise to the existence of the transitional layer.

In the brain of the therapeutic normal-appearing fetus (Plate XII, Figure 27) all these cellular layers appear to be about two thirds as wide as those in the normal control (Plate XI, Figure 26). However, it appears that the cells are packed more closely together per unit area in the normal-appearing cerebral cortex than in that of the normal fetus. Therefore, it might be inferred that even though differentiation may have been retarded, cell proliferation appeared to be normal.

A cross section of the cerebral cortex of a normal rat fetus is shown in Plate IX, Figure 24. Here the pallial wall is much thicker than that of the normal-appearing fetus (Plate X, Figure 25). A cross section of the brain of a normal rat in the region of the pineal body is oval in shape, and has greater width than depth (Plate IX, Figure

24) while the brain of a normal-appearing rat fetus at this level is compressed laterally (Plate X, Figure 25). Also, differences in the contour of the lateral ventricles between normal and normal-appearing feti are evident. In the normal brain it is more curved laterally due to the development of the corpus striatum while in that of the normal-appearing fetus it shows only a faint indication of curvature on the inside due to the absence of corpus striatum at this level.

In exencephalus the brain seemed disorganized and displaced, and sometimes even hemorrhagic in places (Plate XIII, Figure 29). In this particular section the exposed cerebral cortex was twisted to the left. Both the cerebellum and the medulla oblongata were asymmetrical in appearance; as also were the fourth ventricle and lateral foramina. Part of the right cochlea can be seen, while the left one appears to be missing. In the corresponding section of a normal rat fetus both cochleae are in evidence (Plate XIII, Figure 28). Also, in the normal section some evidence of the degree of ossification of the supra-occipital bone is indicated. In the section illustrating exencephalus (Plate XIII, Figure 29) the epidermis and dermis of the skin with the hair follicles failed to extend into the region where the brain was exposed. The whole phenomenon did not appear to be a resorption, but rather an eruption of the brain to the exterior of the

cranium. The development of the cranial bones at this place must have been lacking or arrested for this type of exencephalus to occur. A type of pseudencephaly due to the segregation of an induced translocation in mice was traced back to the open neural plate stages (Needham, 1942). In this latter instance, it appears that the notochord and mesoderm in the head region remain adhered to the unduly abundant neural tissue much longer than in normal embryos. As a result, there is a failure in the closure of the neural folds so that the whole of the fore-brain, mid-brain and hind-brain remains open. Whether the brain abnormalities encountered in avitaminosis E teratogeny were due to the same cause has not been ascertained. However, it probably would not seem too far-fetched to assume that some such abnormal development might have occurred in the case of exencephalus.

The longitudinal section of the cerebellum of the normal-appearing rat fetus (Plate XIV, Figure 30) shows that the cerebellar lobes are fewer and smaller than those in the normal control (Plate XIV, Figure 31). Also, the shape is slightly different. However, the widths of the two cerebellar cortices are essentially the same. This retardation in the development of the cerebellum may have some similarity to chick encephalomalacia caused also by vitamin E deficiency. In chick encephalomalacia the histological changes in the

cerebellum embrace edema, degeneration and necrosis of the Purkinje cells and the small cells of the granular layer, petechial hemorrhage, hyaline degeneration, and capillary thrombosis.

(2) Eye. The materials for the development of the eye come from three sources. The optic nerve and retina are derivatives of the fore-brain. The lens arises from the ectoderm of the head; whereas the accessory tunics such as sclera, and the mechanism of accommodation such as the ciliary muscles, differentiate from the adjacent mesenchyme. In many abnormal feti studied the eyes were absent. In those having eyes, some were only slightly while others were very sub-normal in size (Plate XV, Figure 32B). The vitreous body seemed to be reduced in amount and the ciliary and pupillary muscles appeared missing even in those eyes that appeared nearly normal in size. In extremely abnormal cases, the eyes were rudimentary and fetal in condition (Figure 32 A) compared to the normal (Figure 32 C). In these cases the lens was tiny and the retina was not formed. There was no differentiation of the cornea, anterior chamber, iris, and ciliary body as was observed in maternal vitamin A deficiency by Warkany and Roth (1948). Neither was there any evidence of retrolenticular membrane or coloboma in any of the abnormal eyes examined. The abnormal eye conditions observed

here add emphasis to the findings of Callison and Orent-Keiles (1951). They observed abnormalities in the eyes of weanling rats born to vitamin E-deficient females and restricted post-partum to similar deficient rations.

(3) Muscles. There are three kinds of muscles comprising the muscular system; namely 1) skeletal, chiefly attached to the skeleton; 2) cardiac, localized in the myocardium of the heart; and 3) smooth, found principally in the walls of the viscera, glandular ducts and blood vessels. All three differentiate from formative myoblasts, originating in the middle germ layer, namely, the mesoderm. Myoblasts in becoming skeletal muscles are differentiated out of myotomes. Those forming cardiac muscles are differentiated out of splanchnic mesoderm of the myocardium, and those becoming smooth muscles are derived from unspecialized mesenchyme. The myoblasts in their early state seem to interconnect in a syncytial manner. Fibrillae, coursing lengthwise in the elongating myoblasts soon make their appearance. They are commonly described as differentiating through the linear arrangement and union of cytoplasmic granules. The primitive fibrils multiply by splitting and thus tend to group in bundles. The fibrillae of cardiac and skeletal muscles acquire alternate dark and light bands, the former being thickened regions. The living myofibrils are easily

demonstrated by the use of polarized light. It has been claimed that the myofibrils are composed almost entirely of myosin and that the cross striations are due to different arrangements of the molecules of this protein in the succeeding segments. The dark bands are birefringent zones and they alternate with clear isotropic zones. In these cells only a small part of the cytoplasm; namely, the sarcoplasm, retains its embryonic, undifferentiated, homogeneous state and lies between the myofibrillae and particularly around the nucleus. In smooth muscles these myofibrillae are homogeneous and birefringent. In the musculature of the vitamin E-deficient animals the skeletal and cardiac muscles are mostly affected. Therefore, the manner in which they may have been affected in this study was given some attention.

(a) Skeletal muscles. A type of muscular dystrophy characterized by hyaline degeneration, necrosis and fibrosis is associated with vitamin E deficiency (Blaxter et al., 1951; 1952). This investigation provided an opportunity to learn to what extent changes in the skeletal muscles similar to those reported by Blaxter et al. occurred in the abnormal feti produced in this study. In general, the muscles were under-developed in the abnormal feti compared to those of the normal. Specifically, several layers of intercostal muscles covered the pleural cavity of the

normal feti. However, in the abnormal feti abnormal amounts of connective tissue interspersed with the few muscle fibers encased the pleural cavity. Also, the abnormal feti had comparatively few back muscles, and these had striations which could hardly be observed (Plate XVI, Figure 34). In contrast, the striations of the muscle fibers of the normal feti showed very distinctly (Plate XVI, Figure 33). On the other hand, neither necrosis nor fibrosis occurred in the muscle fibers of the abnormal feti. Thus, maternal vitamin E deficiency affects skeletal muscles of the feti by retarding their development and differentiation rather than affecting them pathologically.

(b) Cardiac muscles. Instances have been reported wherein vitamin E-deficient dairy cattle have died suddenly presumably from heart failure (Gullickson and Calverley, 1946). Upon autopsy the hearts were found to be defective. Consequently, it was deemed advisable to learn whether maternal vitamin E deficiency in the rat had any adverse effect on the fetal heart. In general, it was found that the size of the heart of an abnormal fetus was much smaller than that of the control rat (Plate XVII, Figures 35 and 36). More specifically, the muscles of some of the abnormal hearts appeared spongy and trabeculated even right underneath the epicardium (Plate XVII, Figure 36).

Furthermore, the septum between the ventricles was observed to be incomplete. This might be an advanced stage of the transient fetal condition in which the interventricular foramen remains open. These defects are similar to those observed in maternal vitamin A deficiency (Wilson and Warkany, 1949).

The cells of the myocardium of the ventricular wall of the abnormal fetus were observed to be irregular in their arrangement (Plate XVIII, Figure 38) compared to those of the heart of a normal rat (Plate XVIII, Figure 37). The striations were apparent in some muscle fibers and indistinct in others. Even though the striations were indistinct in some fibers, the intercalated discs were quite evident. A similar observation was made by Gatz and Houchin (1946) in the heart of vitamin E-deficient rabbits. Also, the branched anastomosing appearance of the cardiac muscle was maintained. On the other hand, large sinuses containing red blood corpuscles were more numerous in the abnormal heart (Figure 38) than in the control (Figure 37). Thus, it seems that the abnormal appearance and behavior of the cardiac and skeletal muscles of congenitally abnormal rat feti may be attributed to their under-development and lack of differentiation.

(4) Lung. The respiratory system has its origin in the endoderm of the floor of the gut just caudal to the

pharyngeal pouches. The original single lung bud soon begins to bifurcate giving rise eventually to the three right and two left lobes. The fetal lung is lined completely with endodermal cuboidal epithelium. Also, there is a network of capillaries around the alveoli underneath the epithelium resting on reticular fibers. Scattered among these are some fibroblasts or macrophages. According to one theory, as development proceeds the adjoining capillaries push through the epithelium so that the lining soon becomes discontinuous and the epithelium largely disappears.

Careful study of sections of the lungs of both normal (Plate XIX, Figure 39) and abnormal (Plate XIX, Figure 40) feti obtained in this study indicate that the lung of the abnormal fetus was under-developed and non-expanded. The lumen of the alveolus of the abnormal lung tissue was lined with several layers of cells, compared to two or three layers in the case of the normal lung tissue. Furthermore, the cells comprising the epithelium of the limiting membrane of the normal alveolus were much more flattened, or less cuboidal than those of the abnormal, thus indicating that the development of the lung of the abnormal feti was very much retarded.

(5) Liver. The liver is a ventral outgrowth from the gut endoderm in the region of the future duodenum. The

early epithelial liver cords become the definitive trabeculae around which the endothelium of the broad sinusoids becomes closely applied. During fetal development the blood cells are actively differentiating between the hepatic cells and the covering endothelium. In its early growth upward around the gut, the wings of the liver come to enclose and interrupt the nearby vitelline veins. After this occurs, only sinusoids interconnect the supplying portal vein and the draining hepatic vein. At first relatively apart, these two venous trees grow steadily as the liver expands and thus progressively approach each other in a dovetailing manner. The regularity of this system of branching is responsible for creating the characteristic hepatic lobules from the parenchyma and sinusoids. Each lobule is surrounded by several terminal branches of the portal vein and is drained by a single central hepatic vein.

In two thirds of the abnormal feti examined the livers showed some degree of abnormality. Most prevalent was the hemorrhagic or necrotic condition illustrated in Plate XX, Figure 42. Thus, the nuclei of the normal liver cells stained bluish with hematoxylin (Plate XX, Figure 41) while those of the abnormal cells in the affected region failed to become stained. These latter cells were uniformly yellowish in appearance, being eosinophilic. This condition could be

called central hemorrhage or necrosis, in which the cells about the central veins of the lobules were affected. These were the cells least favored with arterial blood, and most subjected to passive congestion due to failure of the blood to leave the liver freely via the hepatic vein (Cowdry, 1946). Massive liver necrosis often appears in mature rats in a state of vitamin E deficiency. Consequently, it is not surprising to find in gestational vitamin E malnutrition that the fetal liver also shows signs of central hemorrhage or necrosis.

(6) Kidneys. The embryos of mammals develop first a functionless pronephros reminiscent of those functional in such adult forms as Amphioxus and certain lampreys, and then a mesonephros resembling those functional in adult fishes and amphibians, and lastly the permanent kidney, the metanephros. The essential parts of the permanent kidney are the renal corpuscles (glomeruli and Bowman's capsules), secretory tubules and collecting tubules. The ureter, renal pelvis, calyces and collecting tubules are all derived from a bud growing off the mesonephric duct. On the other hand, the secretory tubules and Bowman's capsules differentiate from the caudal end of the nephrogenic cord which arises from the mesoderm originally. Secretory and collecting portions

then unite secondarily to complete the continuous uriniferous tubules.

The kidneys of the abnormal rat feti studied were abnormal in a number of ways. They were smaller (Plate XXI, Figure 44) than those of the control (Plate XXI, Figure 43). In the feti with umbilical hernia the kidneys occupied a ventro-central position in the abdominal cavity, and presented a more vacuolated appearance (Plate XXI, Figure 44). Actually this appearance was due to enlarged sinusoids (Plate XXII, Figure 46). In the abnormal kidney there were fewer glomeruli, convoluted tubules and collecting tubules (Plate XXI, Figure 44; Plate XXII, Figure 46) than in the normal (Plate XXI, Figure 43; Plate XXII, Figure 45). Also, it appears that the secondary union of the collecting tubules with the convoluted tubules might be lacking (Figure 46). Although the external diameter of the existing collecting tubules of the abnormal kidney was about the same as that of the normal, the internal lumen of the former was slit-like and very much reduced. This was probably due to retarded growth, or to the taller cuboidal cells lining the lumen (Plate XXII, Figure 46). The abnormalities observed in this study might be due to arrested instead of aberrant development.

Although "horse-shoe" kidney was noticed in ten per cent of the macroscopic dissections, it was not observed

in tissue sections. Abnormalities of fetal kidneys were reported for maternal vitamin A deficiency (Wilson and Warkany, 1948) and folic acid deficiency (Nelson et al., 1952).

Thus, it is seen that the fetal abnormalities produced by maternal avitaminosis E gestational therapy affected both the skeletal and soft tissues in a multiplicity of ways. These abnormalities resemble many of those cited in the previous section on review of literature due to other vitamin deficiencies. One plausible explanation may be that they are all produced by the same block in the biochemical mechanism (Hogan, 1953). One might suppose that under conditions of borderline deficiency, an embryo would begin to develop abnormally at a certain critical stage if some specific enzyme were present in too minute a quantity; for example, X + tocopherol + phosphate → enzyme. Many vitamins, such as riboflavin, thiamine, nicotinic acid, have been proven to be coenzymes or prosthetic groups of enzymes. Although vitamin E has not been associated with any enzyme so far, scattered reports of the effect of vitamin E upon enzyme systems have appeared from time to time (Eddy, 1949). From the fact that vitamin E can be reversibly oxidized and reduced (Michaelis and Wollman, 1949), it may be surmised that the day may come when tocopherol might be definitely

proven to be part of a reversible oxidation reduction enzyme system, and that its effect upon teratogeny might be due to a block in the biochemical mechanism during the critical period (9 to 12th day in rat) of embryonic development.

PLATES

In the interest of minimizing redundancy in the wording of the titles of the plates mention is made here of the fact that all abnormalities pictured are of live 21-day old rat feti or parts thereof produced by administering to vitamin E-deficient females d,l-alpha-tocopherol acetate therapy during the interval in each gestation which has been demonstrated in the foregoing to be critical in the production of congenital abnormalities.

ABBREVIATIONS

a, anus
al, alveolus
an, anastomosis of fibers
au, auricle
as, alveolar sac
b, back muscle
bb, supra-occipital bone
br, bronchus
bv, bicuspid valve
bw, body wall
c, cerebrum
ca, cochlea
cc, cerebral cortex
ck, cortex of kidney
cm, cardiac muscle
co, colon
cq, connective tissue
cs, cardiac sinus
ct, collecting tubule
cv, centrum of vertebrum
ch, chorioid plexus
d, diaphragm
da, dorsal aorta
db, dark band
dg, dorsal ganglion
e, eye
ea, epithelial cell
el, eye lid
en, endothelial cell of capillary
ep, epicardium
er, erythrocyte
es, esophagus
f, fibroblast
fa, fibrilla
fl, fusiform layer
g, gonad
gl, ganglionic layer
gs, glomerulus
gt, genital tubercle
h, hypophysis
he, hemorrhage
ht, heart
hl, hind limb
i, intestine
is, interventricular septum
jl, fore limb
k, kidney
ks, corpus striatum
l, lung
lb, light band
lc, liver cell
li, liver
lf, lateral foramen
lv, left ventricle
m, muscle
md, Mullerian duct
mf, muscle fiber
mk, medulla of kidney
ml, molecular layer
mo, medulla oblongata
my, myocardium
n, nucleus
o, ovary
p, pia mater
pa, portal canal
pc, pericardium
pk, pelvis of kidney
pl, pyramidal layer
py, pyramid of kidney
q, 4th ventricle
ql, germinal lay
r, rib
re, rectum
rf, fusion of ri
rl, retinal laye
rv, right ventri
s, sternebra
sa, stomach
sc, spinal cord
sd, sinusoid
se, squamous epi cell
sg, salivary gla
sh, sinusal horn
sk, skin
t, tongue
tc, thoracic cav
te, testis
th, thymus
tl, transitional
ts, tela subcuta
tv, tricuspid va
u, urethra
ub, urinary blad
um, umbilical co
ur, ureter
v, sclera
vc, vertebral co
vt, ventricle
w, cornea
x, cerebellum
xc, cerebellar c
xm, cerebellar m
y, lobe of cereb
z, lens
zm, ciliary musc

ABBREVIATIONS

is of fibers
sac
s
ipital bone
valve
cortex
kidney
uscle
e tissue
inus
g tubule
f vertebrum
plexus
rta
nglion
l cell
al cell of
y
m
te

fl, fusiform layer
g, gonad
gl, ganglionic layer
gs, glomerulus
gt, genital tubercle
h, hypophysis
he, hemorrhage
ht, heart
hl, hind limb
i, intestine
is, interventricular septum
jl, fore limb
k, kidney
ks, corpus striatum
l, lung
lb, light band
lc, liver cell
li, liver
lf, lateral foramen
lv, left ventricle
m, muscle
md, Mullerian duct
mf, muscle fiber
mk, medulla of kidney
ml, molecular layer
mo, medulla oblongata
my, myocardium
n, nucleus
o, ovary
p, pia mater
pa, portal canal
pc, pericardium
pk, pelvis of kidney
pl, pyramidal layer
py, pyramid of kidney
q, 4th ventricle

ql, germinal layer
r, rib
re, rectum
rf, fusion of rib
rl, retinal layer
rv, right ventricle
s, sternebra
sa, stomach
sc, spinal cord
sd, sinusoid
se, squamous epithelial cell
sg, salivary gland
sh, sinusal horn
sk, skin
t, tongue
tc, thoracic cavity
te, testis
th, thymus
tl, transitional layer
ts, tela subcutanea
tv, tricuspid valve
u, urethra
ub, urinary bladder
um, umbilical cord
ur, ureter
v, sclera
vc, vertebral column
vt, ventricle
w, cornea
x, cerebellum
xc, cerebellar cortex
xm, cerebellar medulla
y, lobe of cerebellum
z, lens
zm, ciliary muscle

Plate I

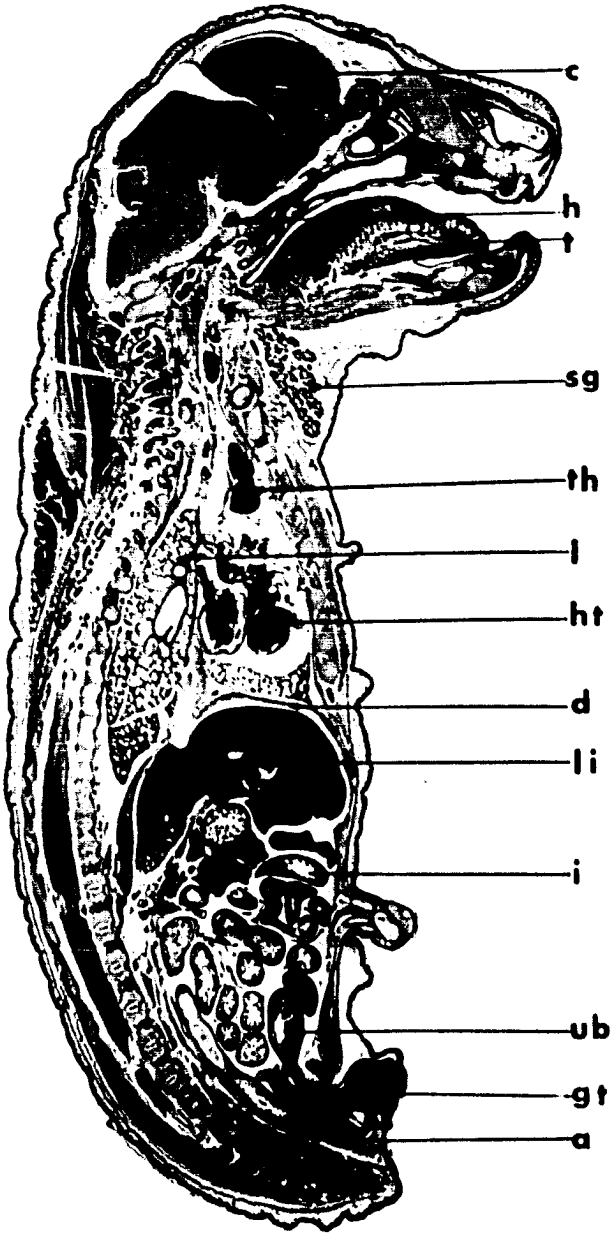
Figure 11

Longitudinal Section of a Normal Rat Fetus
Bouin, H & E, x5

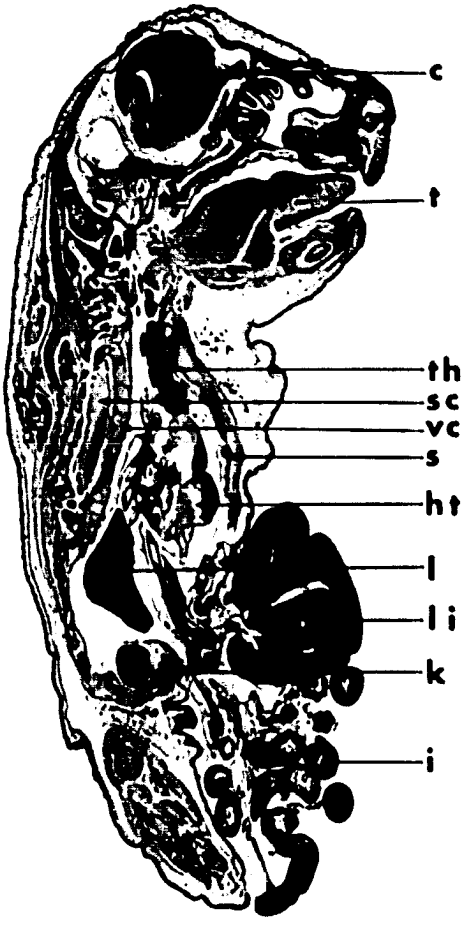
Figure 12

Longitudinal Section of an Abnormal Rat Fetus
Bouin, H & E, x5

Note particularly umbilical hernia, and under-development of the posterior part of the body.



11



12

Plate II

Figure 13

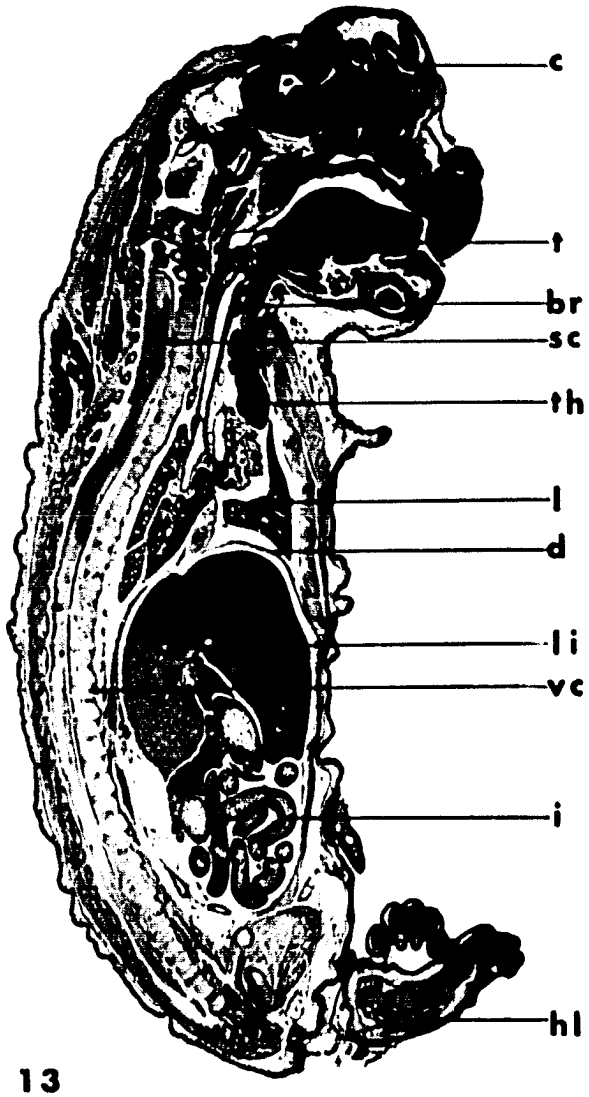
Longitudinal Section of an Abnormal Rat Fetus
Bouin, H & E, x5

Note particularly exencephalus and under-development of the cranium and face.

Figure 14

Longitudinal Section of an Abnormal Rat Fetus
Susa, H & E, x5

Note particularly diminutive size, edema, exencephalus, absence of face, and retarded growth of the posterior part of the body.



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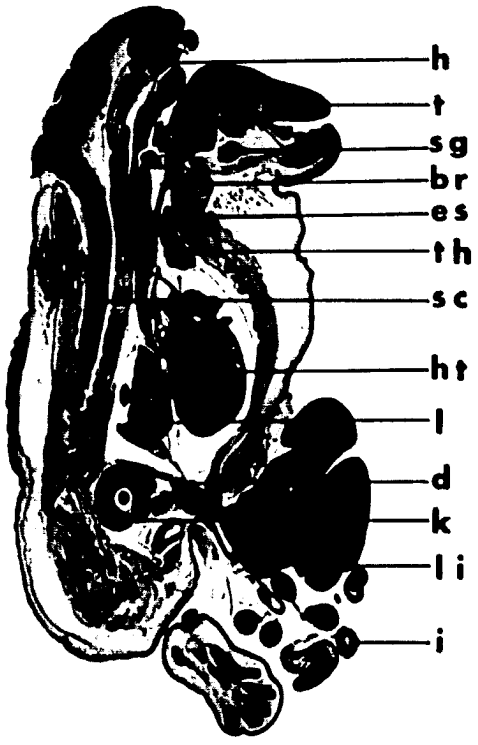

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Plate III

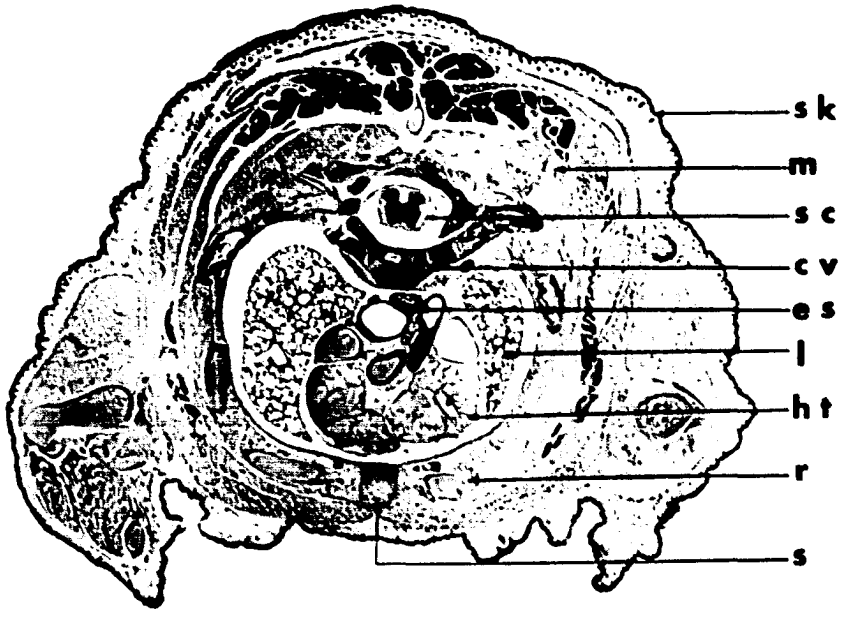
Figure 15

Cross-Section of the Thorax of a Normal Rat Fetus
Susa, H & E, x8

Figure 16

Cross-Section of the Thorax of an Abnormal Rat Fetus
Bouin, H & E, x8

Note particularly extrusion of the heart and right lobes of the lung, incomplete separation of the ventricles, edema, retarded development of the skin and musculature, and crowding of the ribs.



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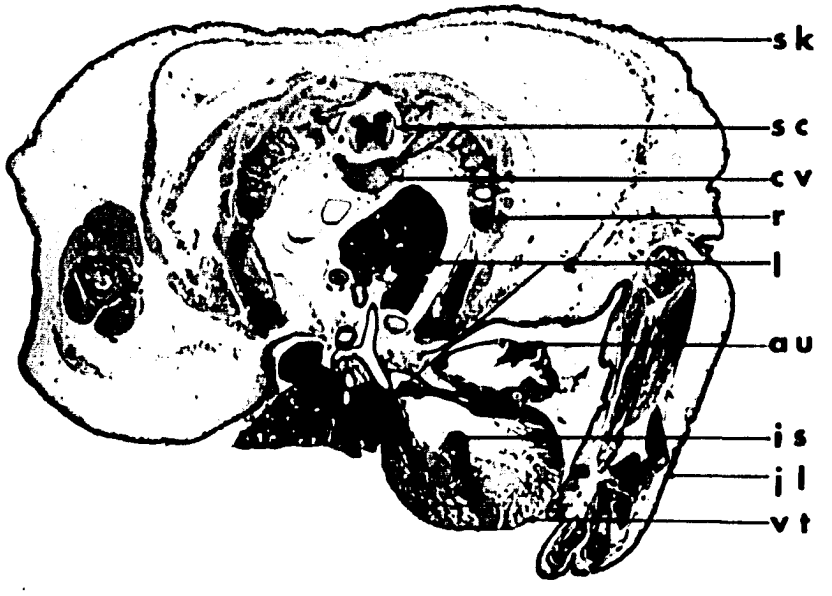

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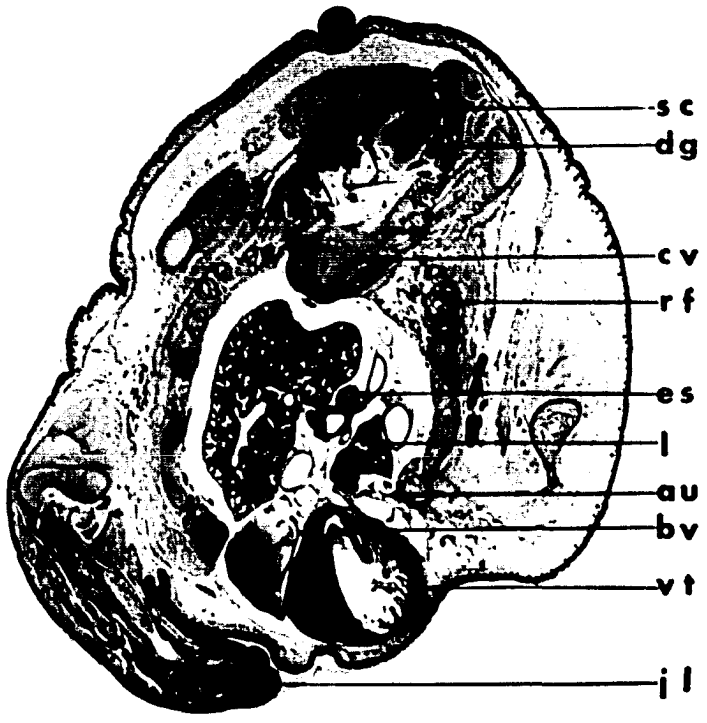
Plate IV

Figure 17

Cross-Section of the Thorax of an Abnormal Rat Fetus
Susa, H & E, x8

Note particularly twisted spinal cord, fusion of ribs, and unusual location of the heart in being directly underneath skin of the ventral body wall.



17

Plate V

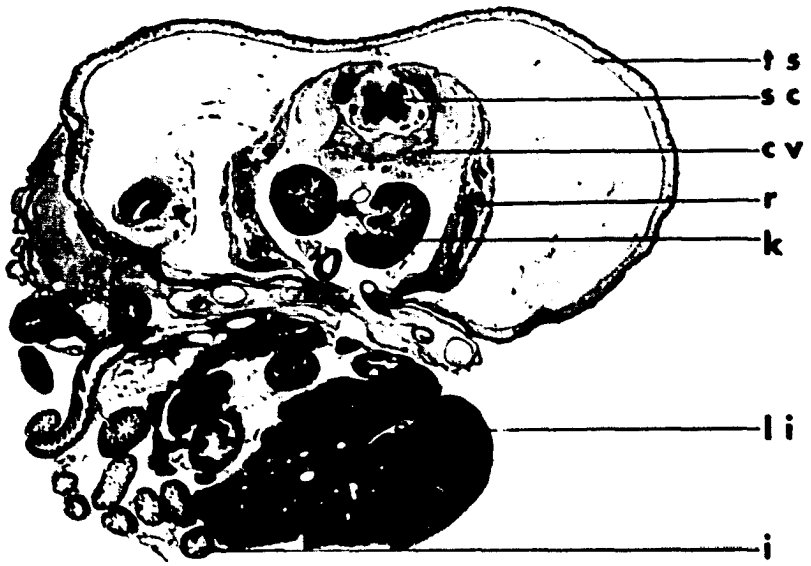
Figure 18

Cross-Section of the Abdomen of an Abnormal Rat Fetus
Bouin, H & E, x8

Note particularly the smallness of the abdominal cavity, unusual presence of ribs, the abnormal ventro-central position of the kidneys, edema, poor development of skin and musculature, and the extrusion of the viscera.

Figure 19

Cross-Section of the Abdomen of a Normal Rat Fetus
Susa, H & E, x8



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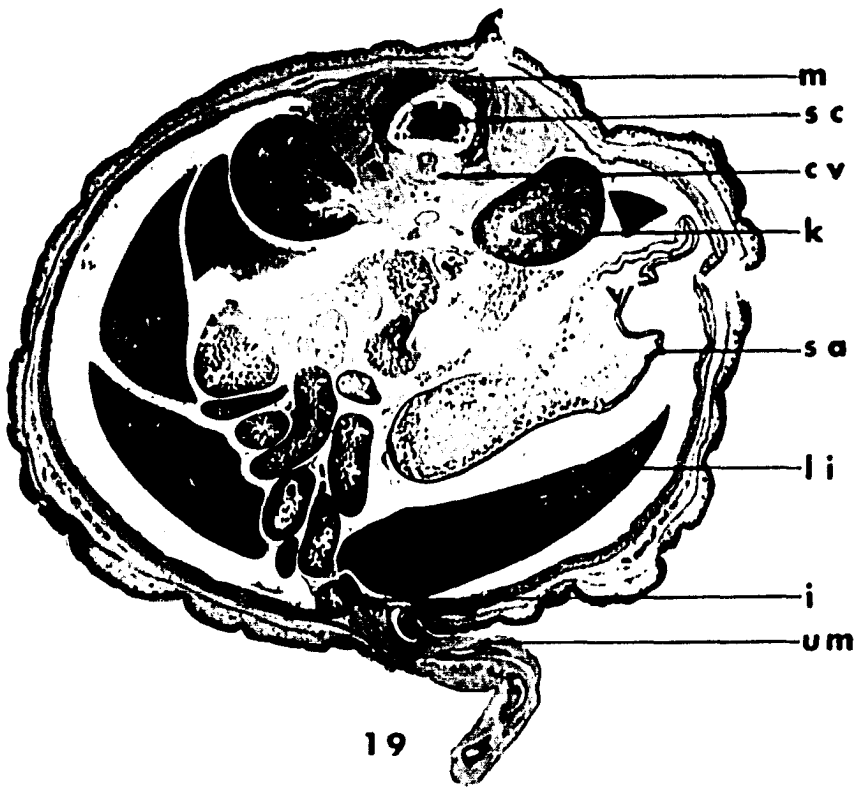


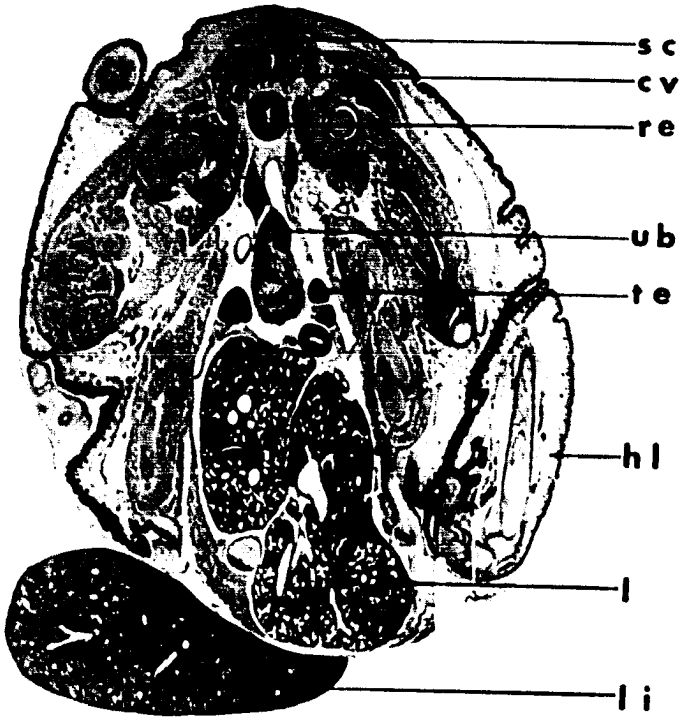
19

Plate VI

Figure 20

Cross-Section of the Posterior Part of the Abdomen of an Abnormal Rat Fetus
Susa, H & E, x8

Note particularly the dislocation of the lobes of the lung by being in the abdominal instead of the thoracic cavity.



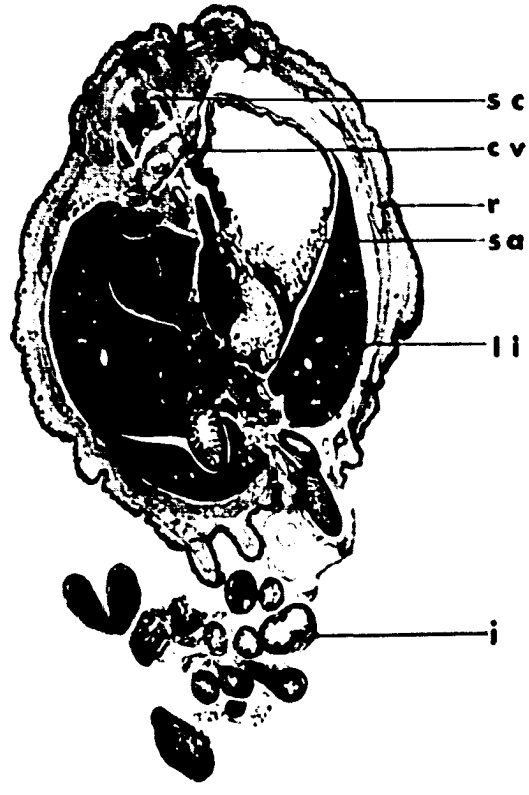
20

Plate VII

Figure 21

Cross-Section of the Abdomen of an Abnormal Rat Fetus
Susa, H & E, x8

Note particularly that the umbilical hernia involves only the intestines.



21

Plate VIII

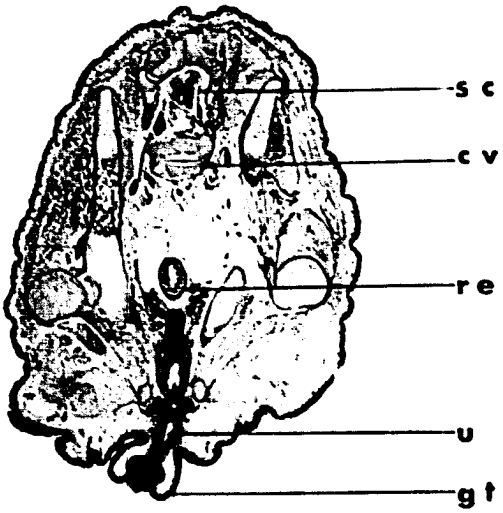
Figure 22

Cross-Section of the Pelvic Region of an
Abnormal Male Rat Fetus
Susa, H & E, x8

Note particularly the indications of hypospadias, and the comparatively large spinal cord.

Figure 23

Cross-Section of the Pelvic Region of a
Normal Male Rat Fetus
Susa, H & E, x8



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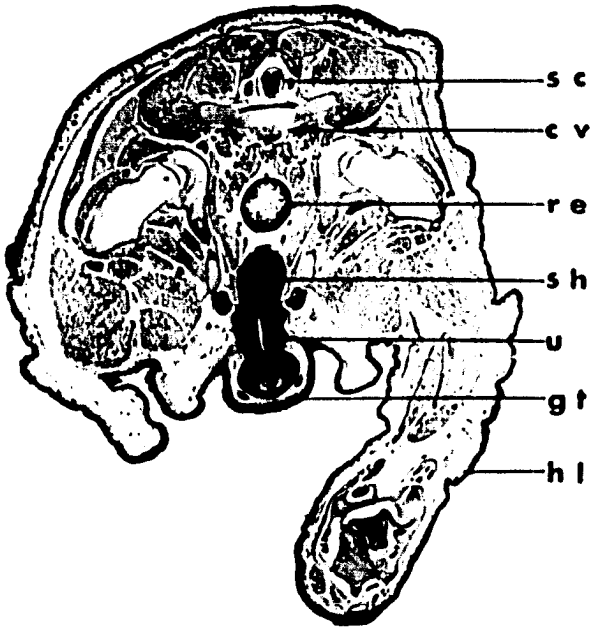


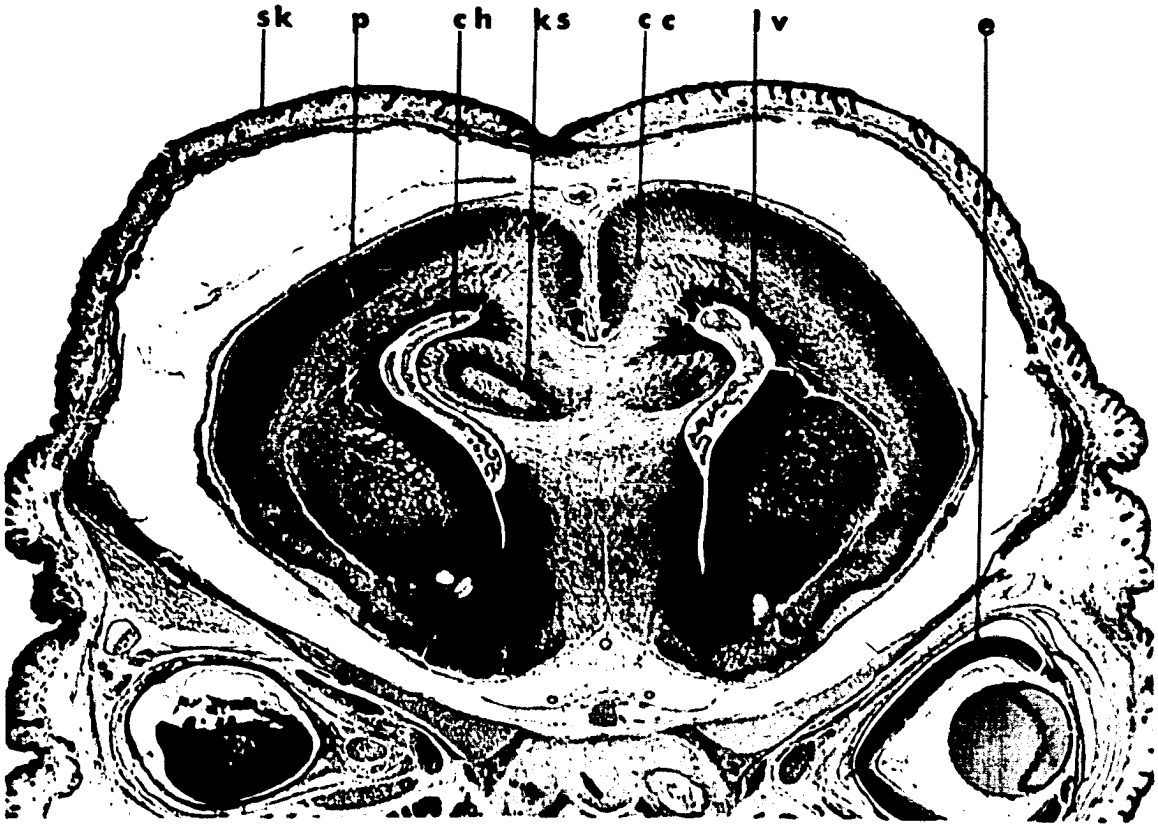
23

Plate IX

Figure 24

Cross-Section of the Anterior Part of the Cerebrum
of a Normal Rat Fetus
Bouin, H & E, x19

Note particularly the thick cerebral cortex and comparatively small lateral ventricles.



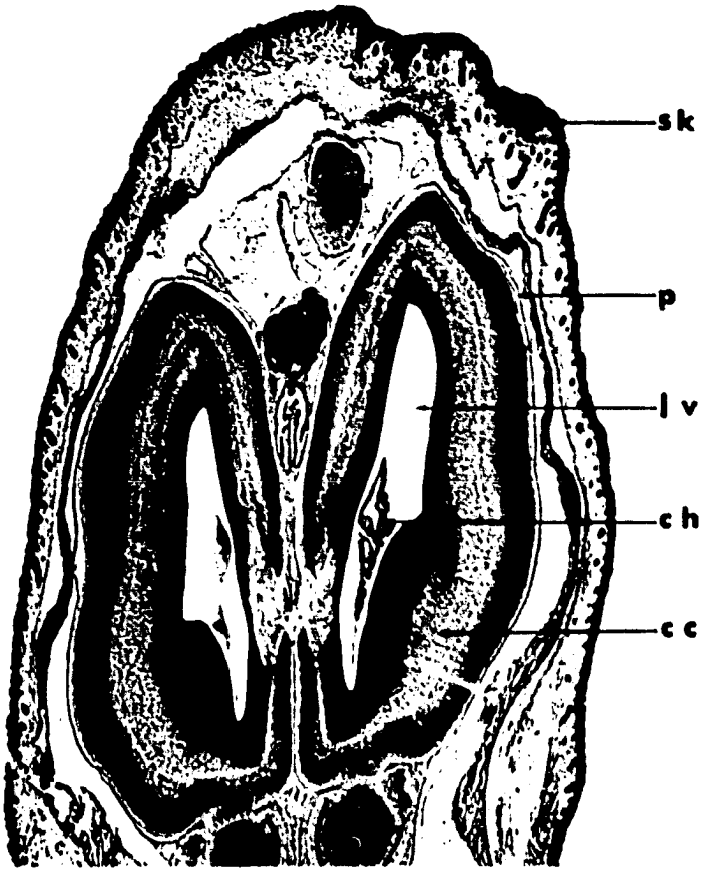
24

Plate X

Figure 25

Cross-Section of the Anterior Part of the Cerebrum
of a Macroscopically Normal Rat Fetus
Bouin, H & E, x19

Note particularly the thin cerebral cortex and comparatively large lateral ventricles.

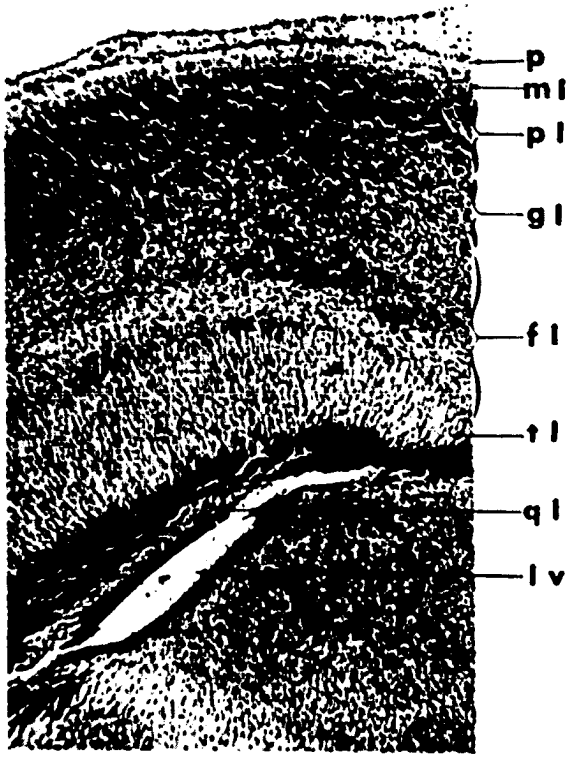


25

Plate XI

Figure 26

Longitudinal Section of the Cerebral Cortex
of a Normal Rat Fetus
Susa, H & E, x82



26

Plate XII

Figure 27

Longitudinal Section of Cerebral Cortex of a Macroscopically Normal-Appearing Rat Fetus
Susa, H & E, x82

Note particularly the thinness of the different cellular layers and the enlarged lateral ventricle illustrative of chorioid plexus.

27

Plate XIII

Figure 28

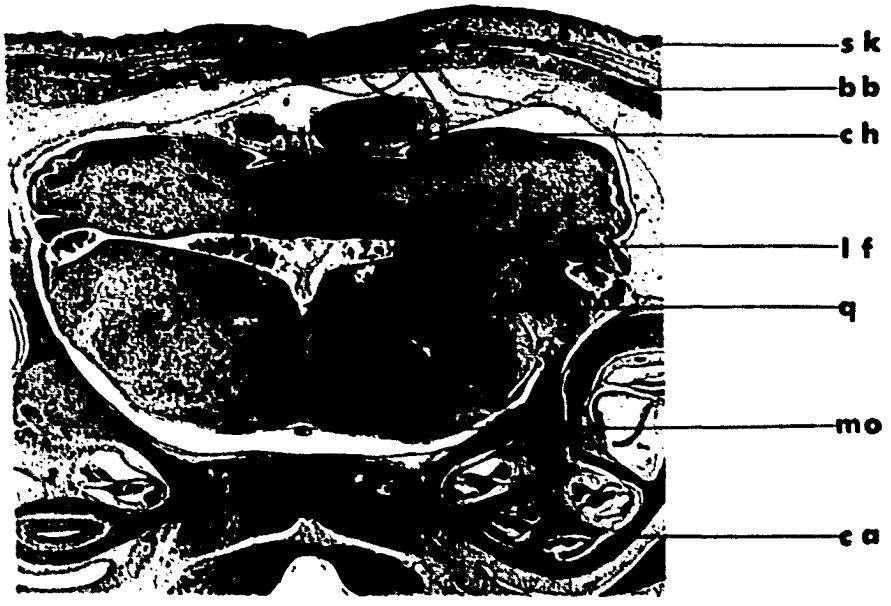
Cross-Section of the Medulla Oblongata of a Normal Rat Fetus
Susa, H & E, x 19

Note particularly the 4th ventricle, lateral foramina, and choriod plexus.

Figure 29

Cross-Section of the Medulla Oblongata of an
Abnormal Rat Fetus
Susa, H & E, x19

Note particularly the irregular appearance of this region as a whole, the asymmetry of the 4th ventricle and the lateral foramina, and the exencephalic condition showing a part of the cerebral cortex twisted to the left.



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29

Plate XIV

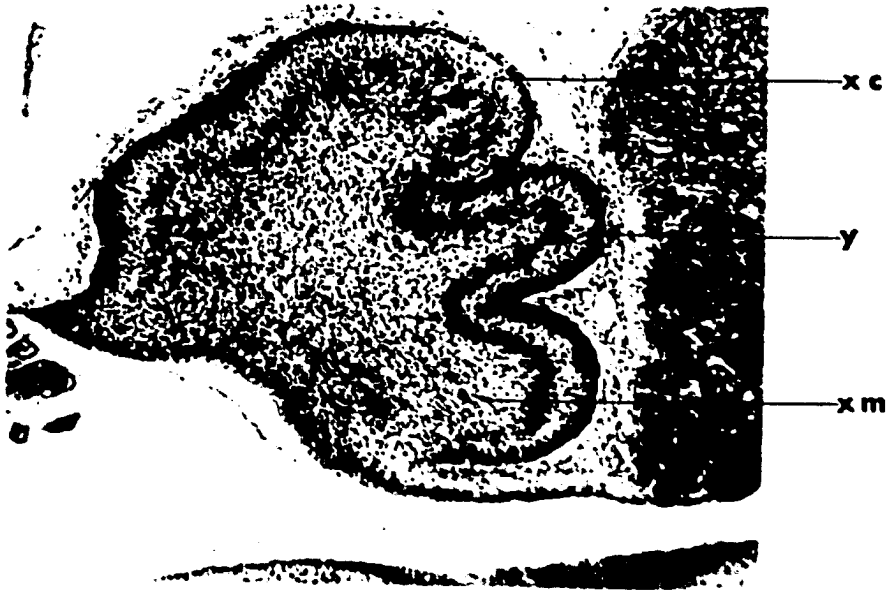
Figure 30

Longitudinal Section of the Cerebellum of a
Macroscopically Normal-Appearing Rat Fetus
Bouin, H & E, x82

Note particularly the subnormal size and lesser number of cerebellar lobes.

Figure 31

Longitudinal Section of the Cerebellum of a
Normal Rat Fetus
Bouin, H & E, x82



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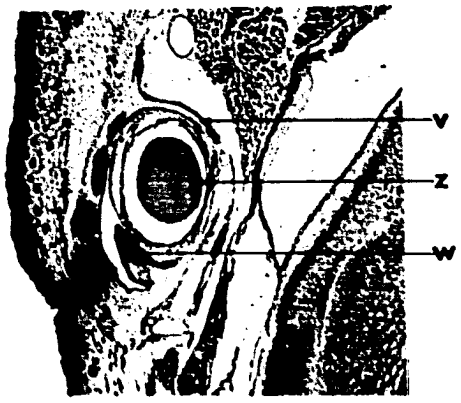
31

Plate XV

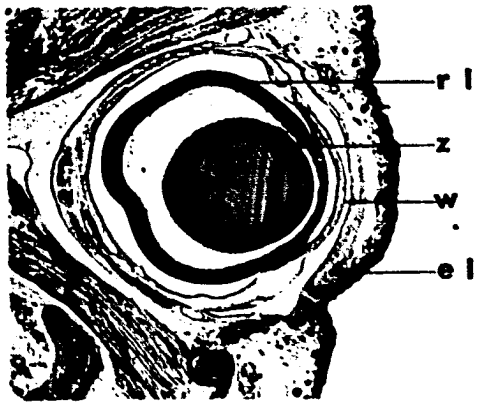
Figure 32

Longitudinal Sections of the Eyes of Abnormal (A & B) and Normal (C) Rat Feti
Susa, H & E, x 19

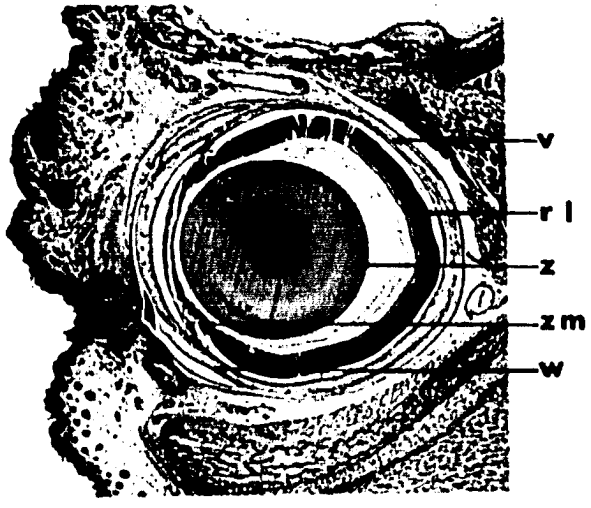
Note particularly the incomplete development of the cornea, the lack of differentiation of the lens; the absence of retina, anterior chamber, iris and ciliary body; and the subnormal sizes in A and B.



A



B



C

Plate XVI

Figure 33

Longitudinal Section of the Skeletal Muscle from the Back of a Normal Rat Fetus
Susa, H & E, x870

Note particularly the clarity of the striations here compared to Figure 34.

Figure 34

Longitudinal Section of the Skeletal Muscle from the Back of an Abnormal Rat Fetus
Susa, H & E, x870

Note particularly indistinctness and/or absence of striations.

33

34

Plate XVII

Figure 35

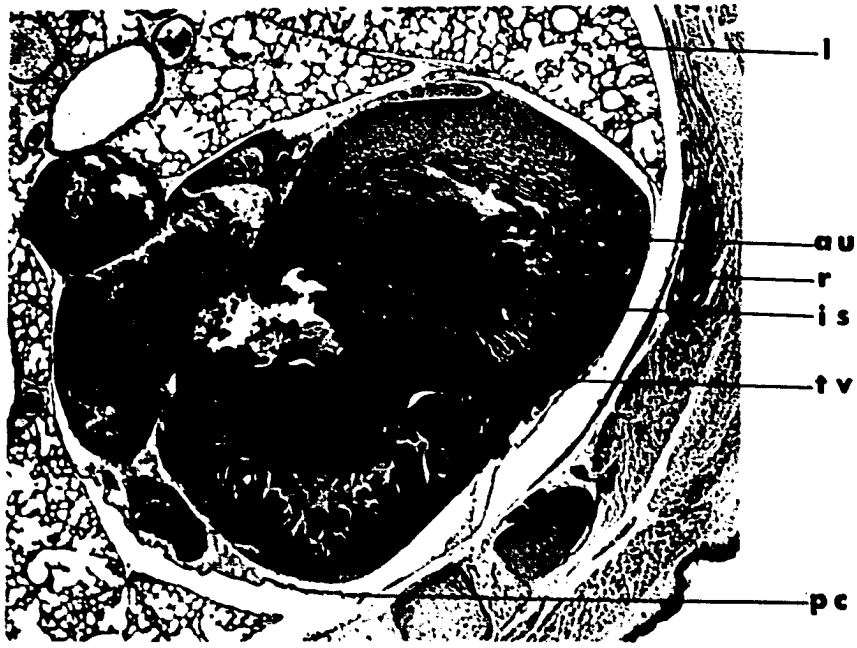
Cross-Section of the Heart of a Normal Rat Fetus
Susa, H & E, x19

Note particularly the compact myocardium, the complete septum between the ventricles, and the large size.

Figure 36

Cross-Section of the Heart of an Abnormal Rat Fetus
Bouin, H & E, x19

Note particularly the spongy appearance of the myocardium, the incomplete septum between the ventricles and the small size.



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36

Plate XVIII

Figure 37

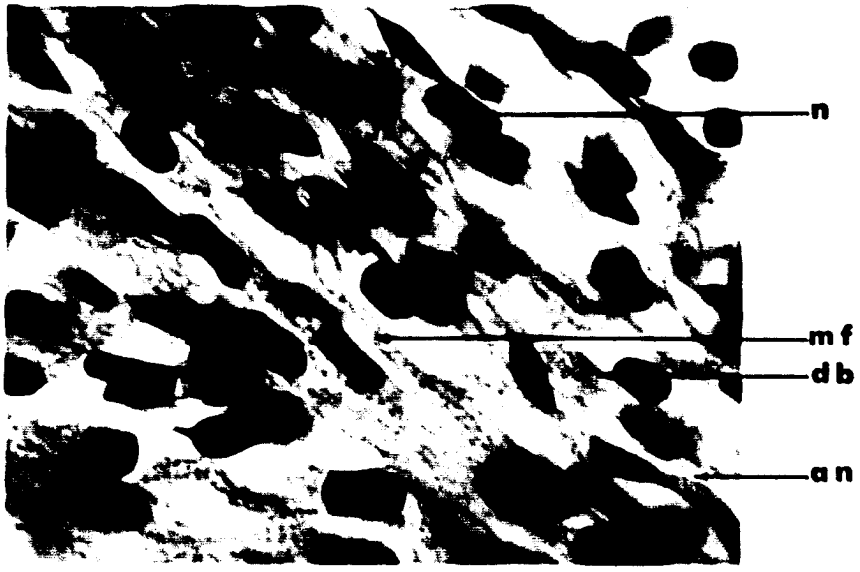
Longitudinal Section of the Myocardium of the Ventricular Wall of a Normal Rat Fetus
Susa, H & E, x870

Note particularly the regular arrangement of the cells, and striations.

Figure 38

Longitudinal Section of the Myocardium of the Ventricular Wall of an Abnormal Rat Fetus
Bouin, H & E, x870

Note particularly the irregular arrangement of the cells and striations, and large sinuses containing red blood corpuscles.



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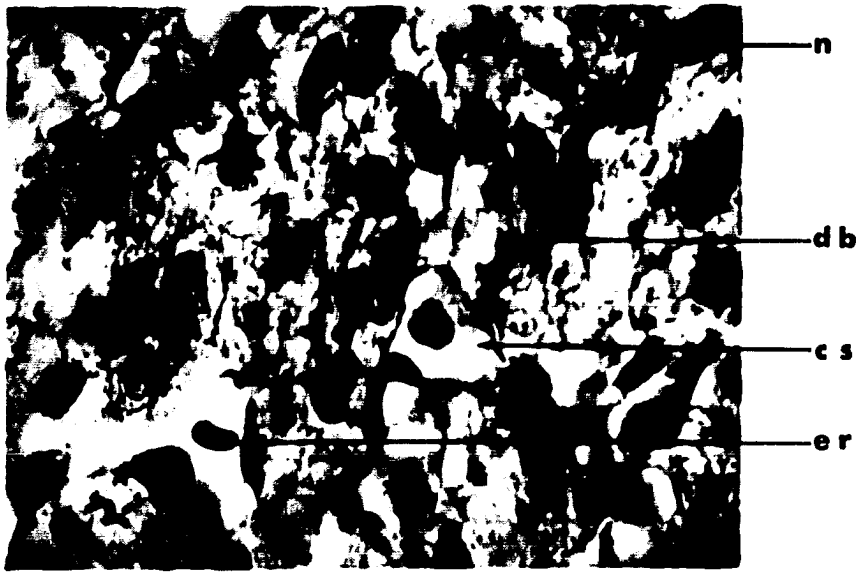


38

Plate XIX

Figure 39

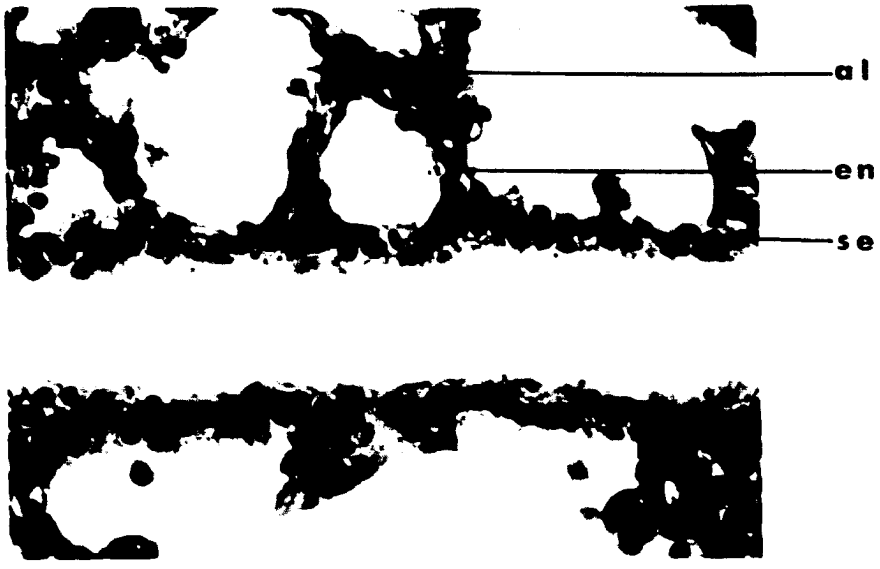
Cross-Section of a Part of the Lung of a Normal Rat Fetus
Susa, H & E, x415

Note particularly the alveoli surrounded by 2 or 3 layers of cells.

Figure 40

Cross-Section of a Part of the Lung of an Abnormal Rat Fetus
Bouin, H & E, x415

Note particularly the contracted condition of the alveoli with an abnormally large number of layers of cells surrounding them.



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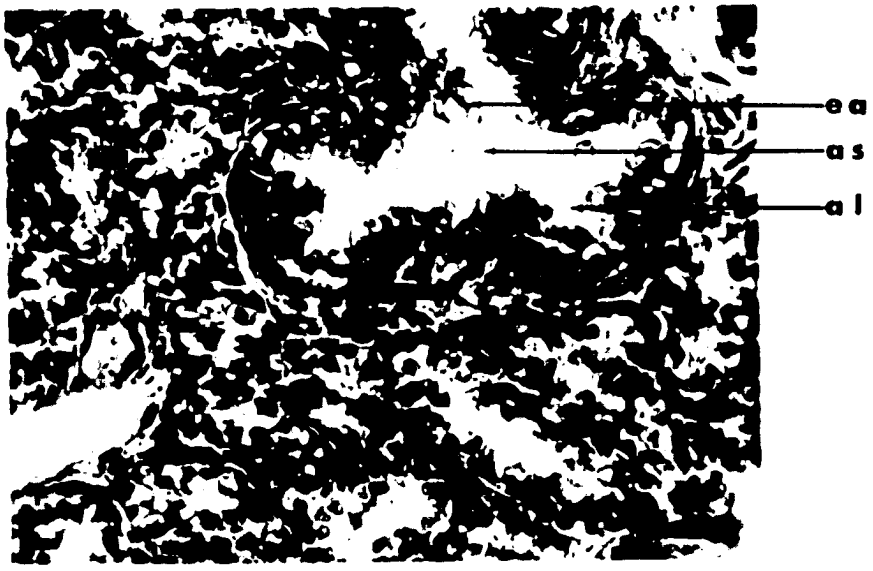


40

Plate XX

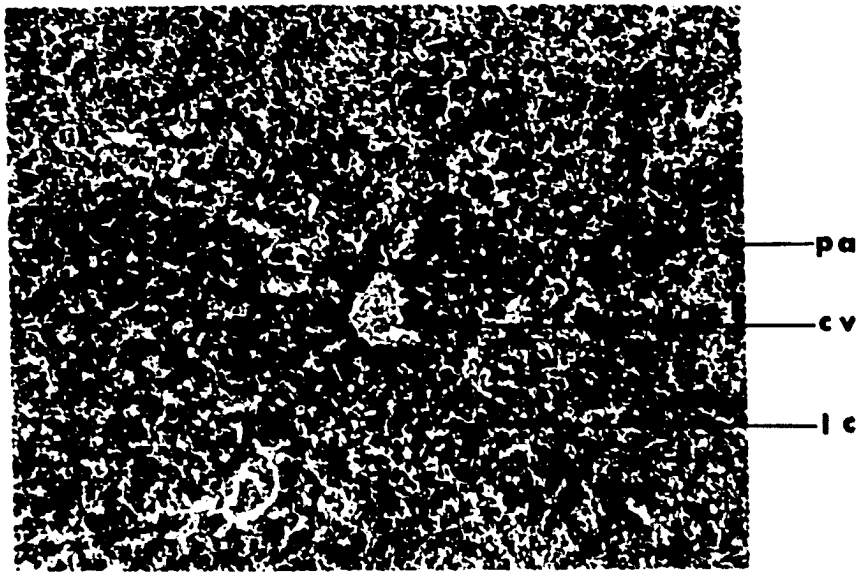
Figure 41

Cross-Section of a Part of the Liver of a Normal Rat Fetus
Susa, H & E, x82

Figure 42

Cross-Section of a Part of the Liver of an
Abnormal Rat Fetus
Bouin, H & E, x82

Note particularly the hemorrhagic and necrotic condition in the central area of each liver lobule surrounding the central vein.



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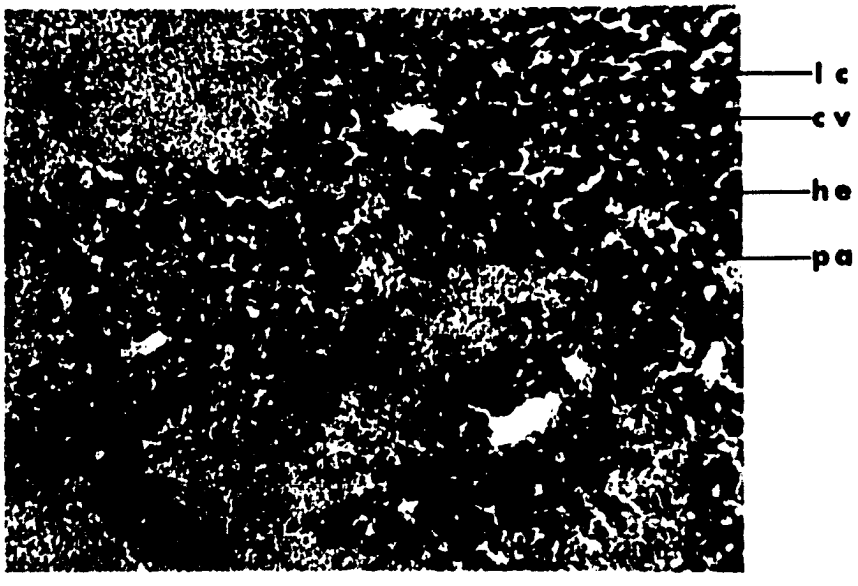


42

Plate XXI

Figure 43

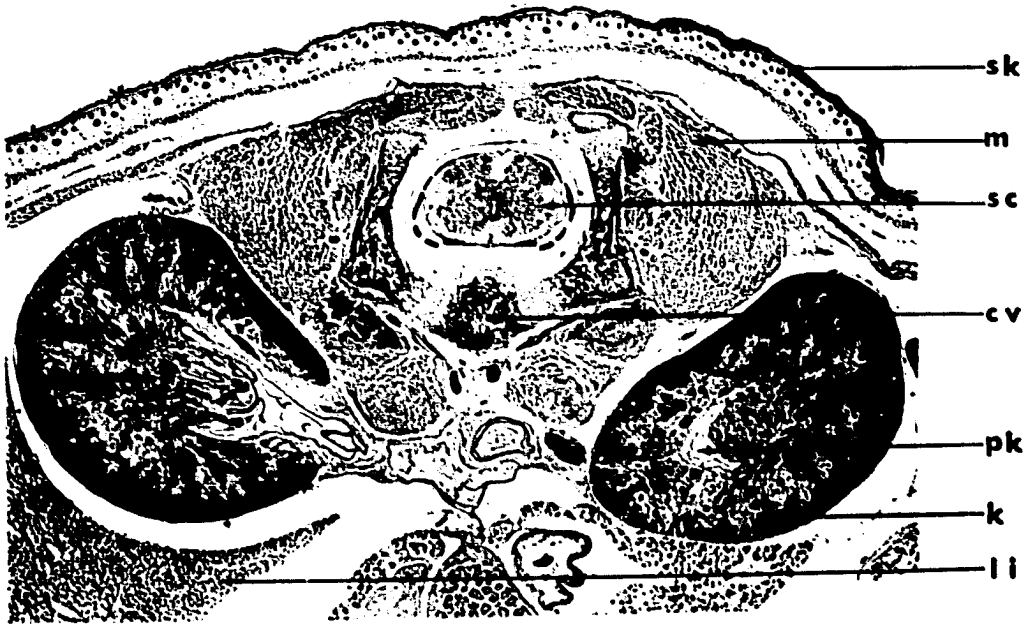
Cross-Section of the Kidneys of a Normal Rat Fetus
Susa, H & E, x19

Note particularly their size, shape, relative location, and development.

Figure 44

Cross-Section of the Kidneys of an Abnormal Rat Fetus
Bouin, H & E, x19

Note particularly their small size, ventro-central position, under-development of the collecting tubules, and enlarged sinusoids.



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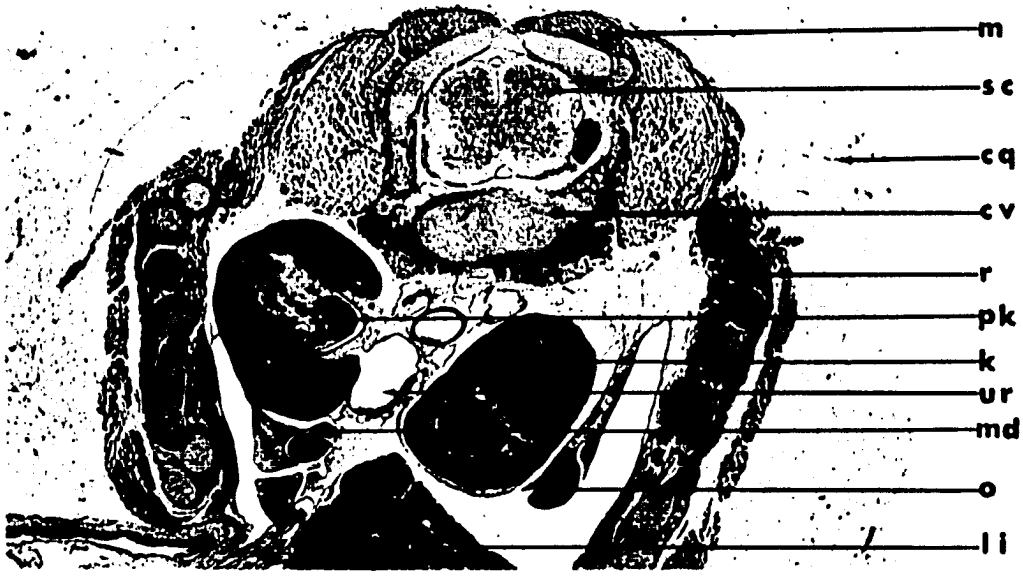

44

Plate XXII

Figure 45

Cross-Section of the Pelvis of the Kidney of a
Normal Rat Fetus
Susa, H & E, x82

Note particularly the development of the collecting tubules.

Figure 46

Cross-Section of the Pelvis of the Kidney of an
Abnormal Rat Fetus
Bouin, H & E, x82

Note particularly the relatively fewer collecting tubules, their under-development and the slit-like appearance of their lumens; and the much enlarged sinusoids.

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V. SUMMARY

This dissertation deals with the production of congenital abnormalities in vitamin E-deficient female rats that had received tocopherol supplementation during certain period of gestation. Experiments were performed using 36 groups of Sprague-Dawley strain rats of about 20 each to determine the conditions under which congenital abnormalities can be produced, and the factors affecting the incidence of teratogeny. For this purpose four kinds of rations were used; namely, stock colony ration, basal vitamin E-depleting ration (ration I), basal ration plus 25 ug. tri-o-cresyl phosphate (TCP) per gram of ration (ration II), and basal ration plus 100 ug. TCP per gram of ration (ration III). Also, the results of some macroscopic, histologic and hematological studies of the normal and abnormal 21-day old gestational age rat feti are described.

The usual abnormality in reproduction ascribed to female rats on vitamin E-deficient ration in an advanced state of avitaminosis E is fetal resorption. Therapy per se does not always correct this disturbance, but sometimes induces associated complications. Though vitamin E therapy reduces the occurrence of resorptions, it is unable, under certain circumstances, to prevent the simultaneous occurrence of congenital abnormalities. The timing of therapy greatly

influences the incidence of morphological normality or abnormality of the products of conception. Thus, only when therapy was given on 9, 10, 11 or 12th day of gestation could abnormal feti be produced. When identical therapy was given on the 4 through 8th day, only live normal young were obtained. The peak of teratogenic incidence seems to be near the 10th day of gestation. This peak was not shifted by increasing the therapeutic level of tocopherol from 1 to 4 mg.

The relationship of extent of depletion to incidence of teratogeny was next studied from two viewpoints, namely, effect of the length of time female rats were restricted to the de letion rations, and effect of the vitamin E antagonist, tri-o-cresyl phosphate (TCP). Due to the small number of animals that had been on the rations longer than 79 days, analyses of the data regarding the effect of length of time female rats were restricted to the depletion rations on the incidence of teratogeny were inconclusive. Most female rats gave rise to abnormal young when they had been on the rations for 8 to 11 weeks, but the percentage of females having abnormal young was not as high as that of the few females that had been on the rations for longer periods of time. Data from experiments with the vitamin E antagonist, TCP, are variable and do not show any consistent trend in increasing the incidence of abnormality. However, under the experimental conditions employed, the

administration of 2 mg. tocopherol on the 10th day of gestation to rats on vitamin E-depleting ration containing 25 µg. TCP per gram of ration produced more abnormal pregnancies with a larger number of abnormal young than any other combination tried.

The effect of different dietary treatments on the growth of female rats from weaning through gestation was noted. The growth of female rats on vitamin E-deficient rations was slightly depressed compared to stock ration control due to the less rapid adjustment of the rats to the comparatively unpalatable, high fat rations. Tri-o-cresyl phosphate at the low levels used in this investigation did not affect growth appreciably. However, the rats with weekly supplementation of tocopherol from weaning to mating showed consistently slightly subnormal growth. This pregestational feeding of tocopherol depressed the body weight gain of the dams and slightly retarded the growth of the feti during gestation.

Data on increases in maternal, uterine and fetal weight show that gestational therapy of 1, 1.2, 2 or 4 mg. of tocopherol does not markedly affect the maternal weight per se, but it does affect the average weight of the uteri and their contents, which was smallest for the negative control groups, greatest for the positive control groups and intermediate for the groups given single doses of tocopherol. In general,

the average weight of uterine contents increased with increasing dosage of tocopherol. No adverse effect on maternal body weight increase per se or size of feti was observed due to the TCP ingestion.

Conditions of the feti regarding the number and percentage of resorptions, dead feti, live normal feti and live abnormal feti, and the average weight of live normal and abnormal feti from dams on stock colony ration, and vitamin E-depleting rations supplemented with or without TCP are reported. The number of resorptions decreased with each increase in therapeutic dosage of tocopherol, with the maximum number of resorptions (100 per cent) occurring in the negative control and the minimum in the positive control groups. The number of live normal feti increased with each increase in tocopherol supplementation, with the maximum number occurring in the positive control groups. Live abnormal feti occurred largely only when vitamin E therapy was administered during the 9, 10 or 11th day of gestation. The average weight of the stock colony feti was heavier than that for either full-term macroscopically normal or abnormal feti from dams on the E-depleting rations which had received gestational therapy.

The abnormal feti were detected as early as the 15th day of gestation. At this early date their development was

delayed by approximately one day. Most gross abnormalities observed in full-term abnormal feti fall into the following macroscopic classifications: umbilical hernia, hydrocephalus, exencephalus, rigid ankle, receding mandible and maxillae, hare lip, cleft palate, ectocardia, kinked tail, syndactylism, taillessness, edema, protruding mandible, and "elephant trunk" nose. The data show that hydrocephalus, umbilical hernia, and exencephalus occurred most frequently when tocopherol supplementation was given on the 9, 10 or 11th day of gestation, respectively. Measurements of the crown-rump lengths of 127 feti ranging from normal to severely abnormal showed that in general, the latter was shorter in length.

The skeletal abnormalities observed in 44 cleared specimens include disturbances such as complete or partial absence of certain cranial bones in exencephalus and hydrocephalus, retarded ossification of many bones including the sternebrae and phalanges, curvature of the vertebral column and fusion of certain ribs. It is interesting to note that of all the abnormalities observed in cleared feti from dams that received tocopherol supplementation on the 9, 10 or 11th day of gestation, one fifth occurred in macroscopically normal-appearing feti.

Comparisons of the average dissected organ weights from ten normal and ten abnormal feti showed, in the order of

descending severity, that the following organs were affected: heart, lungs, eyeballs, liver, thyroid, stomach, brain, thymus, adrenals, intestines and kidneys. The spleen and testes were about normal in weight.

Preliminary histologic studies reveal marked retardation in development of heart, lungs and kidneys and a less marked arrest in development of practically all other organs and tissues of the abnormal feti. The thickness of the cerebral cortex of the normal-appearing fetus was reduced, while the lateral ventricles were larger than normal. In exencephalus the brain was disorganized and displaced. The eyes of the abnormal feti were either missing, small and lacking in differentiation, or subnormal in size. The striations of the skeletal muscles were indistinct or faint. The cardiac muscles were irregularly arranged with striations apparent in some and indistinct in others. The lungs were non-expanded with many layers of cells surrounding the alveoli. The liver of some abnormal feti showed central hemorrhage and necrosis while the kidneys showed enlarged sinusoid with retarded development of the glomeruli, convoluted and collecting tubules.

Hematological studies of the hemoglobin content, red blood cell count, total white blood cell count and differential count on 48 feti from abnormal, normal-appearing, and

positive control groups reveal that the abnormal feti were definitely anemic. They had a significantly lower hemoglobin value and red blood cell count than the other four groups. Also, they showed significant leukocytosis and monocytosis together with basopenia. They had a significant eosinopenia compared to the three positive control groups. Compared to the normal-appearing littermates they had a significantly higher percentage of basophiles in addition to a significantly lower hemoglobin and red blood cell count.

The normal-appearing feti had significant lymphocytosis, leukocytosis, monocytosis, eosinopenia and basopenia and significantly lower hemoglobin value compared to stock colony ration feti. However, the only difference in the blood picture between the normal-appearing feti and the therapeutic positive control groups was that the percentage of eosinophiles of the former was significantly lower than that of the latter.

VI. CONCLUSIONS

The foregoing experiments warrant the following conclusions:

1. Teratogeny due to maternal avitaminosis E gestational therapy is an established fact.

2. An important factor affecting the incidence of teratogeny is the timing of the therapy during gestation.

3. The greatest incidence of teratogeny occurs when therapy is delayed to the tenth day of gestation.

4. Reasonably prolonged depletion with or without moderate amounts of tri-o-cresyl phosphate does not affect the incidence of teratogeny.

5. Differences in the therapeutic amount of d,l-alpha-tocopherol acetate ranging from 1 to 4 mg. per rat do not significantly affect the incidence of teratogeny when administered on the 8, 10 or 12th day of gestation.

6. The deformed feti show a multiplicity of abnormalities macroscopically, microscopically and hematologically.

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