CXCIII. THE EFFECT OF VITAMIN E DEFICIENCY ON THE RAT

I. DURATION OF GESTATION

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It has been known for some time [Kirkham, 1916; Brambell, 1937] that if small rodents or shrews are mated during the *post partum* oestrus so that lactation and gestation are simultaneous, then provided that the number of young suckled exceeds a minimum, which is characteristic for each species and is 5 for the rat, gestation is prolonged. There is no direct correlation between the duration of gestation and the number of young suckled, but a rat suckling 9 young may have a gestation period lasting 10 days beyond the normal period.

It has been noticed, in the course of a number of vitamin E assays, that among the proved sterile animals which had been given a small dose of vitamin E, gestation was often prolonged beyond the normal 22 days characteristic of our stock. This prolongation was so consistent among the rats suffering from severe vitamin E deficiency that a further investigation of the problem was undertaken, which has already been the subject of a preliminary communication [Barrie, 1937, 3].

Two diets were used throughout the experiment:

Diet E. 5.	Fat-free casein	2000 g.
	Rice starch	6000 "
	Lard	800 "
	Cod liver oil	200 "
	Salt mixture	500 "
	Yeast extract	180 I.U. vitamin B ₁ per 100 g. diet

This diet contains no detectable amount of vitamin E and 635 animals fed on it from weaning all resorbed in their first gestation.

The second diet, E. 4, differed from E. 5 in that light white casein was used instead of fat-free casein. However, animals fed on it were usually capable of producing two litters before the resorption typical of vitamin E deficiency occurred. That the E. 4 diet contained inadequate vitamin E, even for the first of these litters, was shown by the fact that the young produced became abnormal and usually died within 23 days after birth [Barrie, 1937, 1, 2].

In the experiments which are reported here, all the test animals were mated with males which had been fed on a diet rich in vitamin E and been proved fertile. Successful implantation of the ova was verified by the finding of the placental sign (detection of blood in the vagina) between the 9th and 13th days after mating. A daily record of the weight and condition of the animals was kept from the occurrence of the placental sign and was continued either until the birth of the young, or until resorption was so advanced that the weight had fallen considerably. In those cases in which young were born, a daily record was kept of the weight and condition of the litter.

The test animals were divided into 5 groups:

1. *Negative controls.* These were not specially selected and were the sterile and untreated representatives of the colony of vitamin E-free rats. These rats were fed on the vitamin E-free diet E. 5 from weaning, and in spite of positive mating followed by successful implantation, the foetuses were resorbed *in utero* in the first gestation.

2. *Animals severely deficient in vitamin E.* This was a group of 22 rats fed on the E. 4 diet until approximately 1 month before mating when they were transferred to diet E. 5. These animals usually produced a living litter but were never able to rear it.

3. *Animals slightly deficient in vitamin E.* This was a group of 40 fed on the diet containing a small but still inadequate amount of vitamin E (diet E. 4). Although able to produce living litters, they were seldom able to rear them.

4. *Positive controls.* This was a group of 27 fed on the vitamin E-free diet (E. 5), but on mating they were given a large dose of a vitamin E concentrate. The adequacy of the dose was shown by the ability of the doe to rear her litter.

5. *Stock rats.* This was a group of 50 rats selected at random from our normal breeding stock. They were fed on a diet containing an adequate amount of vitamin E. These animals all reared their litters and the few deaths which occurred among the young were usually due to the mother becoming fierce and killing them.

Table I

Group	No. rats in group	Length of gestation (days)			No. young born	No. born dead	No. young reared
		Average	Longest	Shortest			
2	22	25.3 (corrected 24.8)	32	23	71	43	1
3	40	23.0 (corrected 22.5)	25	19	242	24	62
4	27	23.04 (corrected 22.54)	25	22	175	17	119

Fig. 1. Plotted to show the relative distribution of the gestation period. The largest group was arbitrarily selected as 100 in each case. The hatched columns represent gestations which were shorter than normal, the hollow columns those of normal duration, and the black columns those in which the duration was beyond the normal range.

Table I gives the length of gestation and the live and dead litter rates in animals of groups 2, 3 and 4. Since the animals were weighed and examined only once daily, at approximately 11 a.m. on each day, the litters born were anything up to 24 hr. old when first observed. In order to correct for this, one half day has been deducted from the average gestation period for each group. It will be seen that the positive controls had a gestation period varying between 22 and 25 days with an average of 23 days, or 22.5 after making the correction. This

period is the same as that of our normal breeding stock (see also Hain [1934]). The normal range must, however, be taken to lie between 21 and 23 days, as a variation of one day could not be considered abnormal.

The animals which were suffering from a slight deficiency of the vitamin (group 3) had a gestation period of 19–25 days, 67.5 % of them falling within the normal range of 22–23 days; those of group 2, which were suffering from a much more severe deficiency of vitamin E, had a gestation period which lasted as long as 32 days in one case, and the duration was normal in only 31.82 % of the animals. Fig. 1 shows these results scaled down so that the relative spreads in each group can be compared. The weighted mean of groups 3 and 4 is 23 days, while that of group 2 is 25.3 days (both uncorrected).

These results give a definite indication that the length of gestation bears an inverse relation to the amount of vitamin E in the animal's diet.

Although the majority of the animals of group 3 which were suffering from a slight deficiency of vitamin E had a gestation period of normal duration, their deficiency of vitamin E was shown by the low survival rates of the litters.

Table II

Mother	No. of litters averaged	Av. no. born per litter			Av. no. reared
		Total	Born alive	Born dead	
Group 5	50	7.84	7.84	0.00	7.24
	First litter in each case				
Group 4	27	6.48	5.87	0.63	4.40
Group 3	40	6.05	5.99	0.06	1.55
Group 2	22	3.23	1.28	1.95	0.04
Group 1	635	0.00	0.00	0.00	0.00
	pregnancies				

Table II gives the average number of young born to animals in each of these groups, and again a difference is seen: the animals receiving adequate vitamin E (group 4) had litters averaging 6.5 young with an average of 0.6 still-births per litter. Those receiving less vitamin E (group 3) had litters of approximately the same number, but the still-birth rate was slightly higher and the litters were not reared, while the animals of the second group which had not received any vitamin E for a month before mating had very small litters averaging 2.14 live- and 1.27 still-births per litter. In spite of the smaller number born, the mothers were unable to rear them. Among our normal breeding stock, which is fed on a diet rich in vitamin E, we find that the average number of young born is 7.84 and in the 50 selected at random there were no still-births. Among these normal breeding stock rats there are a few which never give birth to more than 5, even in the first gestation, while others have as many as 14. Old breeding rats frequently have small litters, but if the number born is fewer than 5, we consider the animal unsuitable for breeding. Hence it may be concluded that if a vitamin E-deficient rat is dosed with vitamin E and has a litter of 5, the dose given is not necessarily smaller than that which produced 7 in another rat, but if the number in the litter is less than 5 and the animal is young and in good condition, there is an indication that the dose given was inadequate; this supposition is borne out by the fact that such small litters are seldom reared by vitamin E-deficient rats.

Fig. 2 shows typical weight curves (*A*) of a resorbing rat, (*B*) of a rat given a large dose of vitamin E and (*C*) of a rat suffering from severe vitamin E deficiency. These three rats were chosen because they were all at approximately the same weight on the 13th day of pregnancy. The three curves are very similar

until the 16th day, from which date the dosed rat (136) gained weight until full term. This weight curve is typical of those given by the 27 positive controls. The resorbing rat and the rat partially deficient in vitamin E ran together until the 17th day; the latter rat then started to gain weight more rapidly until the birth of the litter on the 32nd day of pregnancy.

Fig. 2. Rat 136 dosed with vitamin E; rat 77 given inadequate vitamin E; rat 1052 given no vitamin E.

A pregnant rat deficient in vitamin E, which is also receiving a diet containing no vitamin E, gains weight slowly from the time of implantation until about the 15th day; after this stage the weight gradually decreases, and there may be a haemorrhage, possibly comparable with abortion, at any time from the 16th day onwards (Fig. 2, rat 1052). Only one rat has been taken as an example, as the rats become pregnant at different weights and it would not be desirable to make a composite curve. The curve for this single rat agrees with that of Evans [1927] and is typical of over 800 obtained during this and other experiments. Placentation is often early in these rats and if it is detected on the 9th day of gestation and is followed by continuous bleeding it is fairly certain that resorption will follow; in fact, the time and type of placental sign found give a fairly accurate forecast of the course of pregnancy. Tables III, IV and V illustrate this point.

Rat 697 is representative of group 4, rat 658 had very little reserve of vitamin E and is therefore comparable with rats of group 2, while rat 115 had been fed on the vitamin E-free diet from weaning and belonged to the negative control group 1. Comparison of these three records shows that when pregnancy takes a normal course there is very little bleeding.

To the rat whose gestation period lasted 32 days, only 4 young were born and these were well above the average birth weight, weighing 8 g. each instead of the normal 4.5 g. In a few cases, animals have died or have been killed at some stage during the pregnancy and the *post mortem* findings have given the explanation of this prolonged gestation. One of these animals was 19 days

Table III. *Rat 697 fed on vitamin E-free diet, but given a dose of 2.5 ml. vitamin E concentrate on mating. This concentrate was known to be active in a dose of 1.5 ml.*

Stage of pregnancy days	Wt. g.	Vaginal smear
13	184	Faint trace of blood
14	184	Mucus and blood
15	186	Mucus and blood
16	189	No blood
17	191	No blood
18	196	No blood
19	202	No blood
20	211	Trace of blood. Foetuses could be felt
21	220	More blood than preceding day
22	222	Unchanged
23	229	Occult blood, no fresh blood
24	Litter of 6	—

Table IV. *Rat 658 fed on vitamin E-rich diet for 4 months then on vitamin E-free diet for 3 months before mating. The animal had practically run out of vitamin E*

Stage of pregnancy days	Wt. g.	Vaginal smear
10	188	Trace of blood
11	189	Trace of blood
12	Not examined	—
13	187	Trace of blood
14	195	Mucus and blood
15	196	Very faint trace of blood
16	200	Fresh blood
17	202	Very faint trace of blood
18	206	Quite a lot of blood
19	Not examined	—
20	203	Mucus
21	212	Mucus
22	216	Trace of blood. Foetuses felt
23	221	Faint trace of blood
24	224	Faint trace of blood
25	Litter of 3 (2 dead)	—

Table V. *Rat 115 fed on vitamin E-free diet from weaning. First pregnancy*

Stage of pregnancy days	Wt. g.	Vaginal smear
10	180	Trace of blood
11	180	Trace of blood
12	Not examined	—
13	183	Trace of blood
14	180	Large amount of blood
15	186	Large amount of blood
16	181	Fresh blood
17	178	Bleeding profusely
18	176	Blood and mucus
19	Not examined	—
20	175	Bleeding profusely
21	175	No blood
22	176	Fresh blood
	Litter resorbed	

pregnant when it died and the remains of 7 resorbing fetuses could be seen together with two apparently normal but very undersized ones which would probably have been born at some time after 21 days' gestation. Similar conditions have been seen in animals which have reached the 24th day of gestation. In a preparation made from a 23 days' pregnant vitamin E-deficient rat which had been given a small dose of vitamin E, resorption of three fetuses was well advanced in the right horn of the uterus, while in the left horn two others were developing, and one was probably being resorbed. This evidence, together with that of the weight curves (Fig. 2), shows that resorption of the litter is not an "all or none" process and that there are varying degrees of vitamin E deficiency. Partially deficient animals resorb some of their fetuses and develop the others so that the number carried to term is smaller than would normally be the case.

It seems probable that owing to the process of resorption, the development of the remaining fetuses is retarded and in consequence pregnancy is prolonged. This is shown very clearly in Fig. 2 where the weight curve of the rat which had a 32 days' gestation first follows the line of the resorbing rat and then leaves it to make normal daily increases in weight until the birth of the litter.

Similar prolongations of gestation have been observed before in the rat, the mouse and the shrew, but under very different circumstances. Kirkham [1916; 1918] found that this prolonged gestation in the mouse was associated with delayed implantation and that the retardation occurred in the blastula stage of the embryo. There was no evidence of resorption of any of the fetuses. The phenomenon is therefore hardly comparable with that observed in the vitamin E-deficient rat, in which implantation is often earlier than normal and is followed by resorption of some of the fetuses. The prolonged gestation in both these and the lactating animals is very probably due to some impairment of the normal function of the corpus luteum. It is here that a connexion between the two sets of results seems most probable, if the function of the anterior pituitary gland is controlled by vitamin E. This theory is strongly supported by the work of Rowlands & Singer [1936], who showed that the luteinizing activity of the pituitary of vitamin E-deficient rats is about half that of the normal rat. Verzář & Kokas [1931] showed that the coat of the vitamin E-deficient male rat changed to the type characteristic of hypophysectomized animals and later Verzář [1937] summarized the published work on vitamin E which gives support to this theory. Nelson [1933] found castration changes in the anterior pituitary of vitamin E-deficient male rats and Barrie [1937, 2] described degeneration of the acidophils and basophils of the anterior pituitary of the vitamin E-deficient female rat and ascribed the abnormal condition of the young born to partially vitamin E-deficient rats to anterior pituitary involvement. It can therefore be taken that the vitamin E-deficient animals used in these experiments, in which gestation was prolonged, were suffering from anterior pituitary deficiency. It seems probable that this deficiency was sufficiently severe to impair the function of the corpus luteum, although so far no morphological evidence of such impairment in vitamin E deficiency has been found.

SUMMARY

In the rat partially deficient in vitamin E the length of gestation bears an inverse relation to the amount of vitamin E in the diet. Such partially deficient animals are unable to develop all the ova implanted, with the result that some of the fetuses are resorbed *in utero*.

It is suggested that the prolongation of gestation caused by vitamin E deficiency is due to the resorption of some of the foetuses delaying the development of the others and that this condition is an indirect effect, through the ovary, of anterior pituitary deficiency.

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