CXCIV. THE EFFECT OF VITAMIN E DEFICIENCY ON THE RAT

II. LACTATION

By MARY MARGARET OGILVY BARRIE

*From the Physiological Laboratories of The British Drug Houses
Limited, London, N. 1*

(Received 17 June 1938)

THE accepted characteristic of vitamin E deficiency is resorption of the foetuses *in utero* before the completion of gestation. In ordinary circumstances, absolute deficiency of a vitamin must be a very rare occurrence and for this reason the characteristics of partial deficiency are probably more readily observed.

Vitamin E is in some way connected with the normal function of the anterior pituitary gland [Verzár, 1937; Barrie 1937, 1] and when one considers the diversity of function of this gland it is not surprising that the effects of even a partial deficiency of the vitamin are widespread.

If a female rat is fed on a diet which contains inadequate vitamin E, she may produce a living litter after mating with a normal male but is unable to rear it because the young become abnormal and die within 21 days of birth. This abnormality was described in an earlier paper [Barrie, 1937, 1] and an apparently similar condition was observed by Evans [1928] and found by him to be preventable if the young were dosed with wheat germ oil within 15 days of birth. In a preliminary communication [Barrie, 1937, 2] the result of an experiment was given in which it was found that this abnormality of the young, the onset of which occurred about 18 days after birth, was due to some deficiency in the mother's milk. The experiment has been repeated and confirmed.

Rats which had been proved to be sterile were mated and given a small dose of a vitamin E concentrate on the day of mating. The activity of the concentrate used had been previously determined and the dose given was known to be adequate for the production of a litter, but inadequate for the rearing of it. In one experiment the litter was exchanged for a normal stock litter born on the same day, in another experiment the exchange was made at 3 days, and in further experiments at 5 and 6 days after birth. In every case it was found that the animals fed by the vitamin E-deficient mother became abnormal, while those fed by the stock mother grew normally.

Full details of the first of these interchange experiments are given in Table I.

On the 16th day the young fed by the vitamin E-deficient rat were all definitely affected, although her own males were weaker than the females she was fostering. At this stage of the experiment the stock mother's own males were killed as she was a very small rat and a litter of 10 seemed too much for her; she was therefore left with only the fostered females from the vitamin E-deficient rat. At 17 days the young fed by the vitamin E-deficient rat were definitely abnormal; they were dragging their hind legs and their forepaws were contracted and on the following day the condition of the males was so bad that they were killed. The fostered females reached the same condition on the 21st day but the females born to the vitamin E-deficient mother and fed by the stock mother grew normally and were weaned when 21 days old.

Table I. *Rat 214, partially deficient in vitamin E; litter of 8, 3 ♂ 5 ♀; total wt. 43 g. 6 days after birth total wt. 77 g., all apparently normal. The 5 ♀ exchanged for 4 stock ♀ born on the same day; the vitamin E-deficient mother then had 4 stock ♀ and her own 3 ♂, the stock mother had 5 vitamin E-deficient ♀ and her own 5 ♂*

Days after birth	Fed by vitamin E-deficient mother				Fed by stock mother			
	Wt. ♂ g.			(Fostered) Wt. ♀ g.	♂	(Fostered)	Wt. ♀ g.	
6	10	10	10	11 11 11 11	Not weighed	10	9	9 10 9
9	14	13	14	12 12 12 13		12	12	12 12 12
14	19	19	19	18 18 18 18		17	18	18 17 18
16	20 20 20 One has weak hind legs Another has front paws contracted	20 19 20 20 Two have weak hind legs			♂'s killed because the litter was too large	17 18 19	16 18	Normal
17	22 22 20 Two very abnormal*	22 22 22 22 No change						Normal
18	19 22 18 Condition much worse, killed	23 23 23 23 All have weak hind legs				18 20 20	18 18 20	All active and normal
21		32 27 25 1 dead others very weak and dragging hind legs				25 26 27	24 27	
22		All dying, killed				28 28 31	26 29	Normal, weaned

* Fur soft and cottony, hind legs dragging, fore feet contracted; animals very weak.

A very similar result was obtained when the exchange was made at 5 days, but in this case the males and not the females were exchanged. The result was further confirmed by giving to another vitamin E-deficient mother 4 stock young in addition to her own 2. The young were 3 days old when given to the vitamin E-deficient mother and they and her own litter became abnormal and died when between 20 and 22 days old.

In a further case a vitamin E-deficient rat had a litter of 6 which died when 12 days old and the mother was given 2 stock rats, aged 8 days, to foster. Both of these were reared.

In a somewhat similar experiment a sterile vitamin E-free rat was dosed with vitamin E concentrate though it was still fed on the vitamin E-free diet. This rat had a litter of 8, 5 of which were born dead, and one of which died later, leaving 2 which grew very slowly and died when 16 days old. The mother was given 4 stock young aged 3 days which she fed for 8 days, then 2 of them were returned to their own stock mother and 3 other stock young, aged 4 days, were given to the vitamin E-deficient mother, who was thus fostering 2 stock rats aged 11 days and 3 aged 4 days. The older two developed normally, as did the two which had been returned to their stock mother after being fostered by the vitamin E-deficient mother for 8 days. The 3 younger animals fostered by the vitamin E-deficient rat developed the characteristic leg weakness when 18 days old.

The exchange experiments show that the milk of the vitamin E-deficient rat is lacking in some substance essential for the normal growth and development of the suckling young, but that if the young are fed on normal rat milk until they

are 8 days old they obtain sufficient of this substance to last them through the rest of their suckling period. In view of the experiments of Evans [1928], in which he found that the onset of muscular weakness in the young was prevented if they were dosed with a source of vitamin E before they were 15 days old, it can safely be presumed that this missing factor in the milk is the vitamin, and the foregoing experiments suggest that the young of these deficient rats are normal at birth since they can be reared by a normal mother receiving vitamin E in her diet. The subsequent history of these and other fostered rats is being followed and will be the subject of a future publication.

In all the experiments the mother was fed on a vitamin E-free diet throughout but was dosed with just sufficient vitamin E on mating to enable her to produce a living litter. During the gestation and lactation periods the animal received no further dose of the vitamin. In one experiment the animal had been given a larger dose of vitamin E on mating, yet was unable to rear her own litter, 5 of which were born dead, one of which died soon after and the remaining 3 of which were weaklings and died when 16 days old. At this stage the mother evidently had sufficient vitamin E in her milk to fulfil the requirements of normal healthy sucklings for she was able to rear two stock young which she fostered, but this evidently exhausted her store of vitamin E as she failed to rear a further 3 given 8 days later.

From this it may be inferred that vitamin E is not stored for any length of time. In this case the dose given was large but the animal had exhausted her supply by the time she was feeding the third litter. If, however, the dose given is large enough, the animal stores sufficient vitamin E to enable her to complete her pregnancy and to feed and rear her litter. This was shown by an experiment in which two normal stock rats, which had been fed from weaning on an adequate diet containing plenty of vitamin E, were mated and from the day of mating fed on the vitamin E-free diet; both rats had normal gestations, one had a litter of 10 and the other 11. Both fed and reared their litters so that they must have stored sufficient vitamin to carry them through this period. The experiments previously described showed that at birth a rat, whether born of a normal or of a vitamin E-deficient mother, has no store of vitamin E, or so little that it is insufficient to carry it through the suckling period. If this is the case then the abnormality which becomes obvious at about 18 days after birth must be due to vitamin E deficiency and should, if taken in the early stages, be curable by this vitamin. To test this point a sterile rat was mated and given a dose of vitamin E concentrate and had a litter of 6, one of which died. Four of the remaining 5 were given small doses of an active oil from the 6th day onwards. On the 16th day the negative control was weak and the hind feet were contracted while the dosed animals were normal. The negative control became progressively worse; its front feet were affected on the 17th day and on the 21st day it was in a very weak condition, was unable to move and had lost weight. At this stage it was given 0.06 ml. of an oil active in the adult rat in a dose of 0.8 ml.; the following day its condition was worse. The dose was repeated on the 23rd day and on the 24th day there was a marked improvement. On the 25th day the animal was moving normally. No further dose was given and the rat was put on the vitamin E-free diet and died 4 days later. The animals which were dosed from the 6th day onwards remained normal throughout. The experiment was repeated with another litter, but in this case the negative control was dosed 2 days after the onset of the leg weakness and it made a complete recovery.

In another experiment a litter of 9 was born to a vitamin E-deficient mother and was left untreated until 3 days after the onset of the symptoms. At this stage

6 of the litter were alive and all were badly affected, showing both fore and hind leg weakness. All 6 were given several doses of 0.06 ml. of a vitamin E concentrate of which the adult rat dose was 0.31 ml. The details of the experiment are given in Table II.

Table II. *Progress of abnormal young rats dosed with vitamin E concentrate*

Days after birth	No. of animals	Wt. (g.)	Dose (ml.)	Condition
21	6	28-32	0.04	All badly affected, legs weak, feet contracted, hind legs dragging
22	6	—	0.04	Unchanged
23	6	—	0.04	"
24	6	—	0.06	5 showed improvement, 1 recovered
26	6	26-36	0.06	1 still very bad, 2 practically recovered, 1 improved but feet still contracted, 2 normal
27	6	26-43	0.06	2 unchanged, 1 still weak in left hind foot, 3 normal
28	4	28-45	—	Normal, put on vitamin E-free diet
	2	—	0.06	Still slightly weak
29	2	31 and 50	0.06	Unchanged
30	2	33 and 52	0.06	Slight improvement
31	2	—	0.06	"
33	2	46 and 60	—	Normal, though left hind foot of one still contracted
34	2	—	—	Both put on vitamin E-free diet, the affected animal gradually regained use of left hind foot

This result was repeated and confirmed with a litter of 6 and again with the 5 survivors of a litter of 7 of which 4 recovered within 10 days and the 5th died on the day following the first dose.

DISCUSSION

Both Evans [1928] and Morelle [1931] described a paralysis in the suckling young of rats deprived of vitamin E. They found that the paralysis affected the hind but never the fore limbs. The mortality was approximately 30%. Evans found that the condition was preventable if the young were treated with a vitamin E concentrate before they were 15 days old. Morelle found that it was prevented by the addition of vitamin E to the mother's diet up to 10 days after mating. Evans made no histological examination, but Morelle examined the nervous system and could find no degeneration. The animals described by Barrie [1937, 1] were obviously comparable, although the condition was much more severe and affected both fore and hind legs and caused carpopedal spasm and convulsions in the final stages. The mortality among these animals was 100%. As in the animals described by Morelle, no nervous degeneration was found, but there was thyroid hypoplasia and involvement of the anterior pituitary.

The present series of experiments, together with the work of Evans and Morelle, shows quite clearly that this paralysis is due to deficiency of vitamin E. The young are apparently normal at birth and under normal conditions would obtain the amount of vitamin E they require from the mother's milk, but when the mother is deprived of the vitamin she does not secrete it in the milk, and unless the young are dosed with vitamin E they suffer from its deficiency and paralysis ensues. This paralysis cannot be caused by any severe lesion as it is fairly readily cured by administration of a large dose of the vitamin. It is

reasonable to suppose that the mode of action of vitamin E is the same in the young as in the adult rat, in which case this paralysis is very probably connected in some way with failure of the function of the anterior pituitary.

It has been known for some time [Evans, 1924; Sure, 1926; Barrie, 1937, 2] that deprivation of vitamin E may cause total or partial failure of lactation, but this failure cannot be due to total lack of milk since the young in the present experiments have all been fat and those that have died have been examined and their stomachs have been found to be full of milk. It may therefore be concluded that although vitamin E in the mother's diet may influence the amount of milk secreted, even if the total amount of milk is adequate still more of the vitamin must be added to the diet before the amount secreted in the milk will be adequate for the requirements of the young.

A very interesting experiment in this connexion was performed by Müller [1936], who found that if sterile vitamin E-deficient rats were fed on human milk the sterility could be cured if the mothers from whom the milk was obtained had been fed on a diet containing vitamin E. This shows that the secretion of vitamin E in human milk is governed by the same factors as in the rat, and although a large volume of clinical evidence for the efficacy of vitamin E in the treatment of habitual abortion is now accumulating, it may well be that this vitamin has a far greater range of clinical application.

I wish to acknowledge the help of my assistant Miss I. Harley, throughout the experiment. My thanks are also due to Dr F. H. Carr and Dr S. W. F. Underhill for their encouragement, and to the directors of the British Drug Houses Ltd., for permission to publish this work.

REFERENCES

- Barrie (1937, 1). *Lancet*, ii, 251.
— (1937, 2). *Nature, Lond.*, **140**, 426.
Evans (1924). *Science*, **60**, 20.
— (1928). *J. biol. Chem.* **76**, 273.
Morelle (1931). *C.R. Soc. Biol., Paris*, **108**, 804.
Müller (1936). *Schweitz. Med. Wschr.* **66**, 1164.
Sure (1926). *J. biol. Chem. Proc.* **67**, xlv.
Verzár (1937). *Handb. biol. ArbMeth.* **5**, 1269.