CXXII. THE EFFECT OF VITAMIN A DEFICIENCY ON THE DEVELOPMENT OF THE RETINA AND ON THE FIRST APPEARANCE OF VISUAL PURPLE.

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ONE of the earliest effects of vitamin A deficiency is a failure in the regeneration of visual purple in the retina [Fridericia and Holm, 1925; Tansley, 1931; Yoshiere, 1925]. The deficiency may also cause damage to the outer limbs of the rods which contain the visual purple [Tansley, 1934]. It was thought, therefore, that by depriving very young animals of vitamin A during the period when the retina, and particularly the outer limbs of the rods, are still developing, it might be possible, not only to inhibit the production of visual purple, but also to produce retinæ with abnormal, or perhaps no, outer limbs to their rods.

Rats were chosen for this study partly because much is known about their vitamin requirements and partly because their eyes only become mature at a relatively late date after birth. The retina is not fully developed until the rat is 15 days old and visual purple does not appear until at least 12 days after birth and often later [Tansley, 1934]. Dann [1932] found that the young rats of mothers deprived of vitamin A possessed no reserves of the vitamin in the liver at birth. It was, therefore, thought that it might be possible to obtain animals which were deficient in vitamin A during the crucial first 2 weeks of life when the outer parts of the retina are developing.

Methods.

The mother rats were taken from the Lister Institute breeding stock. This stock has an exceptionally good diet and therefore the mothers probably had adequate vitamin A reserves at the beginning of the experiments. The vitamin A-deficient diet used subsequently was that usually employed at the Lister Institute. It was made up as follows:

Caseinogen (Prideaux's)	...	...	...	100 g.
Wheat starch	...	...	...	250 „
Hardened cottonseed oil (irradiated)	...			75 „
Salt mixture	...	...	...	25 „
Dried yeast	...	...	...	92 „
Lemon juice	...	...	...	25 ml.
Potassium iodide (6.4 g. per litre)	...	...		0.33 „
Copper sulphate (15.25 g. per litre)	...	...		1.0 „
Water	...	...	...	350 „

Three series of experiments were done. In the first the mothers on the ordinary stock diet were mated at about 200 g. weight and received the deficient diet in the 2nd week of pregnancy. They were then about 4 months old. No

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vitamin A was given during the latter half of the pregnancy period or during lactation. The rats were allowed to suckle the whole litter. In the second series the mothers were given the deficient diet when very much younger, as soon as they had reached about 60 g. weight. This would be at about 5-6 weeks old. After 4 weeks, or sooner if they began to decline in weight, 1 γ β -carotene in coconut oil was given daily; this dose is just sufficient to maintain non-pregnant rats on the deficient diet described above. These animals were allowed to mate as soon as they would after going on the deficient diet. This occurred 8-34 days later but most often after 15-20 days. In the third series the procedure was the same as in the second, except that 2 γ β -carotene daily were allowed. In both series II and III the carotene was replaced by 2 g. daily of wheat germ as soon as the placental sign appeared (usually about the 10th-15th day of pregnancy). This material contains only small amounts of vitamin A but has been found to assist lactation and in series III it was continued until the young were weaned. In this series only two young rats from each litter were left with the mother.

In series II and III vaginal smears were taken daily from the mother rats both before mating and during pregnancy.

The young were killed at varying ages during lactation and their eyes were sectioned and stained in order to detect the characteristic changes found to occur in the retina in vitamin A deficiency. These changes are shown in a shortening and ragged appearance of the outer limbs of the retinal rods together with a marked thinning at the junction of the outer and inner limbs. There is also a failure to produce visual purple which may be detected by fixing the eyes in platinum chloride. This fixative colours the visual purple in the rods yellow and no yellow reaction is given by the retinae of vitamin A-deficient animals [Tansley, 1934]. In the case of animals killed at 15 days of age or older, one eye was treated with platinum chloride as a test for the presence of visual purple.

Those young rats which were reared to the stage of weaning then received the vitamin A-deficient diet until they showed a serious decline in weight. Some were then killed and their eyes examined, while litter-mates were given cod-liver oil until the weight began to recover, when these were also killed and the eyes investigated.

The eyes of control young rats from the Lister stock were also examined at 12 and 15 days of age in order to confirm the normal course of development of visual purple and of the rods in the retina.

The fixatives used were Zenker, Bouin and Flemming without the acetic acid (F. W. A.), and the stains were Mallory's triple connective tissue stain and Mallory's phosphotungstic acid haematoxylin. Stern's [1905] method was used as the test for visual purple.

Results.

Series I. In three cases the experiment failed. In one case the mating did not result in pregnancy and in two cases the mother rats would not eat enough of the deficient diet. One of the latter cast a litter of 10 of which 8 were dead, and failed to suckle the remaining 2. The other was transferred to the stock diet 5 days before parturition; 6 of the litter of 11 were born dead, but the other 5 were satisfactorily reared and all their retinae developed normally.

Four rats produced and suckled litters of 9-13 young. Although of normal weight at birth, these young were all under weight at weaning, the average weight at 21 days being only 27 g. as compared with about 40 g. for the normal young of this stock. The results of the histological examinations of the retinae are given below with those of series III.

Series II. Fifteen rats became pregnant but none was successful in casting and rearing her litter. All these rats had an abnormally long gestation period and none took less than 6 hours over the actual labour. The normal gestation time in the rat is 21–22 days and labour should take about 2 hours. Of the above 15 rats, 1 cast a litter on the 22nd day of pregnancy, 2 on the 23rd day, 5 on the 24th day, 3 on the 25th day, 3 on the 26th day and 1 on the 28th day. One rat was 6 hours in labour, 8 took 12–24 hours, 3 over 24 hours and 1 took 2 days. Two rats died after 2 days in labour, having produced dead litters of 2 and 3 respectively and having several foetuses still in the uterus. The 15 rats produced at least 99 young, of which 85 were born dead and 14 alive. The figure for the dead young is probably too low since nearly all the bodies were partially eaten when found and it is likely that some were entirely destroyed. The average size of litter was, however, much below normal. Two of the mother rats died 3 and 4 days after the uterus was emptied. None of the young which were born alive survived more than 2 days.

This condition seems to be due, at least in part, to uterine inertia. In several cases the uterine muscle was found *post mortem* to be quite flaccid. The symptoms are probably due to the vitamin A deficiency, since a daily dose of 2γ β -carotene, as given to the mothers in series III, was found to prevent their appearance. Similar results were noticed by Mason [1935] in rats deprived of vitamin A. In some of his rats the deficiency was severe enough to prevent the continuation of pregnancy beyond the 2nd week. Although early death and resorption of all the foetuses was not found in the experiments described above, this probably took place to some extent since at least 9 of the 15 litters were abnormally small.

Series III. Nine rats became pregnant in this series. The resulting litters, which contained an average of 10 young rats, were all cut down to 2. Although, judging by the condition of the retinae, these young were, on balance, more deficient than those of series I, the gain in weight during suckling was better, the weight at weaning being 30–35 g. This indicates that the very poor growth shown by the young of series I was chiefly due to lack of food and not entirely to any transference of vitamin A deficiency from the mother to the young.

In series II and III, where vaginal smears were taken daily until littering, it was found that in no case was the deficiency severe enough to cause a constant appearance of cornified cells. Of the rats in series II, 4 had normal cycles before mating and a constant anoestrous smear after mating. Of the remaining 11 rats, 4 had frequent cornified cells in the vagina both before and after mating though not sufficient to obscure the oestrous cycle. The other 7 rats tended to show a prolonged oestrous smear before mating but were normal afterwards.

In series III all but 2 rats produced normal smears throughout the experiment. Of these 2, one had occasional cornified cells in the smears taken during pregnancy while the other showed a cornified smear lasting 2–3 days instead of the normal 1 day before mating.

A general review of the three series of experiments described above (see Table I) leads one to the following conclusions.

(1) A complete absence of vitamin A from the food of a pregnant rat, which had had a good diet for the first 4 months of life, did not affect its ability to complete a pregnancy successfully. If the deficiency was continued during lactation, however, insufficient milk was secreted for the satisfactory rearing of a large litter. A normal rat of this stock is quite able to rear a litter of 10 to a weight of approximately 40 g. per individual young rat at 21 days. In the cases here studied the milk seemed to be deficient in quality as well as quantity since all the young rats of the first series showed signs of vitamin A deficiency.

Table I. *Summary of history of rats before and after pregnancy.*

Series	No. of mothers	Wt. and age at beginning of exp. g.	Wt. at beginning of pregnancy g.	Diet	No. which reared young	No. of young reared	Av. wt. of young at weaning g.
I	7	ca. 200 (ca. 4 months)	ca. 200	Vitamin A-deficient during second half of pregnancy and lactation	4	41*	27
II	15	ca. 60 (ca. 5-6 weeks)	89-144	Vitamin A-deficient, supplemented after ca. 4 weeks with 1 γ β -carotene daily. Carotene replaced by 2 g. wheat germ after 15th day of pregnancy	0†	0	—
III	9	ca. 60 (ca. 5-6 weeks)	90-143	As in series II but supplement of 2 γ β -carotene instead of 1 γ	9	18‡	30-35

* Litters not reduced.

† All mothers showed prolonged gestation and difficult labour.

‡ Litters reduced to 2.

(2) A small daily ratio of 1 γ of β -carotene, which is sufficient to maintain a non-pregnant rat on a vitamin A-deficient diet was not enough to enable a pregnant animal to cast its litter.

(3) A very small increase in the extra carotene provided, to 2 γ daily, not only allowed normal reproduction to take place but also made it possible for the mother to rear a small litter. The milk, however, did not contain sufficient vitamin A to protect the young from the retinal abnormalities which follow a deficiency in this vitamin.

General histological examination of the young rats. All the young from series I and III, as well as those which were not partially eaten from series II, were well developed and of normal weight (4-5 g.) at birth. The lungs, kidneys and eyes of many of the young from series II and III were examined histologically at birth for signs of keratinisation or abnormal development, and with the exception of one litter from series II, all were found to be normal. The only abnormal litter was one of 11 cast by a rat from series II at intervals in a labour lasting over 6 hours. Of these 7 were born alive and 5 dead, but none survived till the next day. Of the living young 3 were very blue and made violent gasping efforts about once a minute. On examination the lungs were found to be very full of blood and almost solid with a few large cavities. The general appearance suggested that something had prevented the proper expansion of the greater part of the lung with the movements of the chest wall but that the pull had been enough to produce abnormally large cavities where the tissue could expand. The circulation through the lung had obviously been established.

Examination of the retinae of the young rats from series I and III. The eyes of 59 young rats were examined at ages from 3 to 61 days. No changes from the normal development of the retina were detected during the first 12 days of life, that is until the first appearance of the outer limbs of the rods. After this period the development of the outer limbs tended to be retarded but was never prevented altogether. Of 23 rats killed between 12 and 21 days, 12 showed almost normal morphological development of the retina although only 4 had perfectly healthy outer limbs. No visual purple was produced before 21 days of age and even then the amount was far below normal. In normal young rats visual purple can usually be detected between the 12th and 15th days. All the deficient

retinae except those from 2 rats showed the characteristic lack of differential staining of the outer limbs.

Of the 59 young rats suckled by deprived mothers 12 were weaned on to the vitamin A-deficient diet. Of these one developed a small corneal ulcer 36 days after weaning and another severe xerophthalmia of both eyes 35 days after weaning. In all the others the eyes remained healthy externally. Of these 12 rats 5 received cod-liver oil, 2 for 3 days, 1 for 5 days and 2 (of which the young rat with xerophthalmia was one) for 6 days before being killed.

The 7 rats which did not receive cod-liver oil were killed between 44 and 58 days of age. All the retinae showed typical symptoms of vitamin A deficiency with poorly stained, unhealthy outer limbs to the rods and thinning at the junction of outer and inner limbs. Dosing with cod-liver oil for only 3 days was sufficient to restore the staining reactions but even 6 days' treatment was not enough to repair the damage to the structure of the outer limbs. In the rat with xerophthalmia which was killed after receiving cod-liver oil for 6 days the retina had completely disappeared in one eye and was seriously affected in the other. However, in the better eye the rods stained normally where they were still present, indicating that even when the structure of the retina is severely damaged, administration of vitamin A is able to restore the normal staining reactions.

DISCUSSION.

The results of these experiments are rather disappointing since it seems doubtful whether the young rats examined were suffering from a severe deficiency during the actual period of retinal development. The effect of vitamin A deficiency in upsetting the mechanism of labour makes it difficult to obtain young rats seriously deficient at birth while the effect on lactation adds a further complication.

It does appear, however, that partial deprivation of vitamin A will postpone the first appearance of visual purple and, in some cases, prevent its formation in young suckling rats. These experiments also indicate that the first effect of vitamin A deficiency on the developing retina is shown on visual purple formation and that the damage to the rod structure is a later effect. Thus, although visual purple is not found in unhealthy rods [Tansley, 1934], it would seem probable that vitamin A affects the production of visual purple directly rather than by interference with the normal health and structure of the rods. This idea is in harmony with Wald's conception of the visual purple system. He believes that visual purple is formed by a direct combination of vitamin A with protein [1935], and the results described above may certainly be explained on this theory.

SUMMARY.

1. An attempt was made to obtain young rats which were deficient in vitamin A during the first 3 weeks of life.

2. Pregnant rats subjected to too severe vitamin A deficiency were found to show a prolonged gestation period and an abnormal and difficult labour. They also failed to suckle their litters.

3. It was therefore found possible to induce only a moderate, and not a severe, deficiency in young rats during the suckling period.

4. In such young rats the development of visual purple was retarded and in some cases prevented, while the effect on the structure and development of the rods was not so marked.

5. The part played by vitamin A in the visual purple system is discussed.

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