

THE EFFECT OF THE DURATION OF VITAMIN-A DEFICIENCY IN FEMALE RABBITS UPON THE INCIDENCE OF HYDROCEPHALUS IN THEIR YOUNG

BY

J. W. MILLEN and D. H. M. WOOLLAM

From the Department of Anatomy, University of Cambridge

When female rabbits are subjected to an experimental vitamin-A deficiency before mating, their offspring show signs of a nervous disorder from three to six weeks after birth (Millen, Woollam, and Lamming, 1953; Lamming, Woollam, and Millen, 1954). This disorder was attributed to hydrocephalus which was present in the young animals. In a subsequent paper (Millen and others, 1954) it was reported that a congenital hydrocephalus was constantly found in young stillborn rabbits when their dam had been maintained on a vitamin-A deficient diet for periods of from 14 to 38 weeks before mating.

The initial observations on the production of hydrocephalus by a maternal vitamin-A deficiency were made on a small number of litters from an inbred strain of rabbits. Attention was drawn to the need for the substantiation of these findings (Annotation, *Brit. med. J.*, 1954). In order, therefore, to study more closely with larger numbers this relationship between maternal vitamin-A deficiency and the incidence of hydrocephalus in the young a colony of rabbits was established in 1953. In this paper the effect of maternal hypovitaminosis A upon the incidence of hydrocephalus in the young in a large number of litters is reported.

Materials and Methods

Rabbits aged 4 or 5 months were purchased from accredited dealers at monthly intervals. These does were of mixed breeds, including Dutch and Flemish rabbits and crosses. In addition some albino rabbits from the general laboratory stock were included in the experiment. It may be noted here that although litters with hydrocephalus were obtained from all the breeds used, the majority of the litters came from the smaller rabbits of the Dutch type. Five males of different varieties were employed for breeding purposes.

The rabbits were housed in standard metal cages and transferred to wooden breeding hutches one week before the expected date of parturition. Sawdust was used for both cages and hutches, and wood wool was supplied as nesting material. The animals were fed on

a vitamin-A deficient pellet diet made according to the formula given by Lamming and others (1954). Water was supplied *ad libitum*. All adult animals received a weekly supplement by mouth of 50 mg. α -tocopheryl acetate and 1,500 i.u. vitamin D in 2 ml. arachis oil. The control animals received in addition 7,500 μ g. vitamin A weekly in arachis oil. The males received the normal laboratory diet.

No attempt was made to breed from does on the deficient diet (deficient rabbits) until they were at least 9 months old and had been on the diet for 12 weeks or longer. Surviving rabbits from litters born to deficient dams were separated from their mothers after four weeks and fed on the same diet. Some of these young animals were subsequently killed for examination but others are still surviving in the colony. Up to the present 59 rabbits have been used in the experiment.

The presence or absence of hydrocephalus in the young rabbits was determined either by sectioning the head after freezing or by dissection after fixation. Histological examination of the material is being carried out with the object of determining the mechanism producing the hydrocephalus and it is intended to publish a report on this in a subsequent communication.

Cerebrospinal fluid pressure measurements were made on a small number of the young born to deficient dams. The pressures were measured by cisternal puncture and recorded by means of a bubble manometer (Griffith and Farris, 1942).

An outbreak of snuffles due to *Pasteurella leipsepticans* occurred in the colony in December, 1954. This disease has been responsible for the deaths of a number of the experimental animals. Apart from this outbreak the deficient animals generally remained in good condition, although xerophthalmia has appeared in some does and others have shown a tendency to develop conjunctivitis.

Results

The Incidence of Hydrocephalus in the Colony.—

Thirty-two deficient animals were successfully mated. These produced, in first pregnancies, 32 litters of which one was aborted before term. In the 31 litters carried to term 142 young were born and, of these, 82 showed varying degrees of hydro-

Duration of deficiency in weeks before mating	Number of Young	CONDITION OF YOUNG			
		% Hydrocephalic	% Normal	% Still Alive	% Still Alive
12-15	16	12.5	12.5	12.5	12.5
16-19	15	13.3	13.3	13.3	13.3
20-23	72	18.0	18.0	18.0	18.0
24-28	39	22.5	22.5	22.5	22.5

FIG. 1.—Diagram illustrating the incidence of hydrocephalus in first litters.

cephalus at post-mortem examination. In 42 young the ventricles of the brain appeared normal on section, and a further 18 are still alive. The length of time on the deficient diet before mating varied from 12 to 28 weeks. No hydrocephalus was found in 23 control young from six litters.

The results are shown in Fig. 1. The most important points which emerge are the following.

1. Dams which had been on the deficient diet for 24 to 28 weeks before mating produced many more hydrocephalic young than those which had been on the deficient diet for shorter times.

2. Of the young recorded as hydrocephalic two, born to a 24 weeks deficient doe, appeared normal at birth and were killed when 41 days old. At necropsy hydrocephalus was found in both these

animals. Three litter mates of these animals are still alive.

3. Twenty-four "normal" young (including seven with increased cerebrospinal fluid pressures) were born to five does which in subsequent litters gave birth to hydrocephalic young.

4. In 27 litters the young in any one litter were either all normal or all hydrocephalic.

Measurement of the Cerebrospinal Fluid Pressure.—Cerebrospinal fluid pressure measurements made on seven young gave readings which ranged from 120 mm. to 265 mm. of water. (The average cerebrospinal fluid pressure in the rabbit is less than 100 mm. of water.) These animals were not hydrocephalic when examined later at necropsy.

Post-mortem Findings.—There was no difficulty in distinguishing the small, slit-like ventricles of the normal young (Fig. 2) from the grossly dilated ventricles seen in the hydrocephalic young (Fig. 3). In none of the hydrocephalic young examined after death was any congenital abnormality discovered other than the presence of hydrocephalus. There was no evidence of bony overgrowth in the skull. On the contrary the calvaria was very thin in the young which were stillborn or survived only a few weeks.

Discussion

The effects of a vitamin-A deficiency in the diet upon the nervous system have been frequently recorded. They include muscular incoordination and weakness, paresis, fits, and sometimes death. The mechanism by which the lesions of the nervous system are produced, however, has been a subject of controversy. Theories have ranged from a

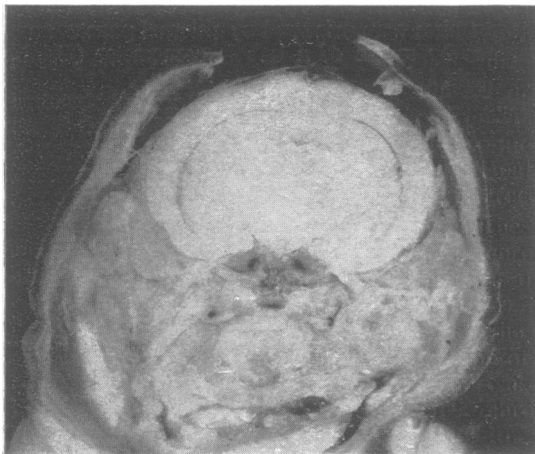


FIG. 2.—Coronal section through head of control rabbit aged 1 day. $\times 2.5$.

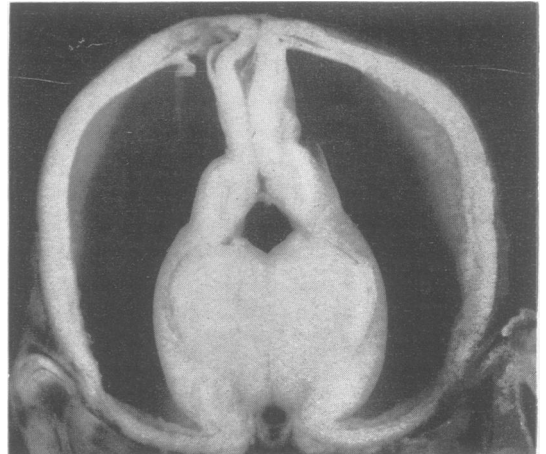


FIG. 3.—Coronal section through head of hydrocephalic rabbit aged 20 days. $\times 2.5$.

primary degeneration of the nervous system (Rigdon, 1952) to the implication of a toxic factor allied to ergot (Mellanby, 1926). Current opinion has tended to accept the view that the lesions of the nervous system are due to a disturbance of normal bone growth, itself an immediate consequence of vitamin-A deficiency. There have been two conflicting views on the nature of the disorganization. Mellanby (1938) considered that there was an overgrowth of apparently normally formed bone in certain places, whilst Wolbach and Bessey (1941) held that the fault lay in the cessation of endochondral bone formation. The present experiments would appear to show that there is a direct relationship, in rabbits, between the duration of the maternal hypovitaminosis A and the production of hydrocephalic young. There is no evidence in this study that the production of the hydrocephalus is in any way dependent upon a disturbance of bone growth. These observations make it necessary to reconsider the causes of the nervous disorders found in association with a vitamin-A deficiency in other animals.

The experiments reported in this paper suggest that the majority of the young will appear normal at birth when the degree of maternal vitamin-A deficiency is slight. It is probable, however, that many of these young have increased cerebrospinal fluid pressures. This view is supported not only by the limited number of pressure measurements made during this study but also by observations on chicks (Woollam and Millen, 1955). Some of the apparently normal young may have a minor degree of hydrocephalus at birth which produces no clinical signs and remains undiscovered until the animal either dies or is killed for examination. After more prolonged maternal deficiencies more live young can be diagnosed as hydrocephalic at birth, and, as might be expected, there are more stillborn young with hydrocephalus. Indeed, the longer the mother has been deprived of vitamin A the greater the proportion of hydrocephalic to normal live young and the greater the number of hydrocephalic stillborn.

These studies support the view that an increased cerebrospinal fluid pressure is one of the earliest signs of hypovitaminosis A (Woollam and Millen, 1955). In the absence of any evidence of pressure on the central nervous system due to a disorganization of bone growth, it must be concluded that the increase in the intraventricular pressure of the cerebrospinal fluid is responsible for the production of the hydrocephalus. Whether hydrocephalus appears or not depends largely, according to this interpretation, upon the period of growth during

which the rise in pressure occurs. If the pressure rises early in gestation hydrocephalus will be present at birth. This results in stillbirth or in the production of young with enlarged and deformed heads produced by a hydrocephalus which progresses rapidly and leads to the early death of the animal. If the severity of the hypovitaminosis is less and the rise in pressure begins later in development or, perhaps, occurs more slowly, ossification of the calvaria proceeds more or less normally. Once this ossification is relatively complete it hinders or prevents further enlargement of the head. Under these circumstances an increased cerebrospinal fluid pressure is present at birth and moderate degrees of hydrocephalus may be discovered at necropsy several months later. The essential factor which determines the appearance of a gross hydrocephalus seems to be the state of ossification of the skull at the time when the rise in cerebrospinal fluid pressure is initiated.

The hypothesis that the primary factor in the pathogenesis of the condition is an over-production of cerebrospinal fluid (Millen and others, 1954) receives additional corroboration from the results of the present experiments. Moreover, only by a hypothesis such as this can the results of experiments be explained in which increased cerebrospinal fluid pressures were recorded in vitamin-A deficient animals and rapid falls in pressure occurred after the restoration of the vitamin (Helmboldt, Jungherr, Eaton, and Moore, 1953; Sorensen, Kowalczyk, and Hentges, 1954). Indeed so sensitive to vitamin-A deficiency is the cerebrospinal fluid pressure that Sorensen and others (1954) suggest it may be used as a guide to the onset of deficiency. Such a sensitivity would be extremely difficult to explain on the basis of any hypothesis which involves gross bony changes or the presence of an aqueductal stenosis as the cause of the increase in the cerebrospinal fluid pressure.

Summary

The relationship between the duration of maternal vitamin-A deficiency in rabbits and the incidence of hydrocephalus in their young has been studied.

Thirty-one litters were born at term to deficient does in first pregnancies. The length of time on the vitamin-A deficient diet for these does before mating varied from 12 to 28 weeks.

One hundred and forty-two young were born of which 82 were hydrocephalic. Eighteen are still alive and 42 showed no hydrocephalus.

The proportion of hydrocephalic young was much greater in litters from animals which had been

fed on the diet for 24 to 28 weeks before mating than from those so fed for 12 to 15 weeks.

Increased cerebrospinal fluid pressures have been recorded in seven young which were not hydrocephalic.

These results are discussed in relation to previous accounts of the effects of hypovitaminosis A in animals.

We wish to thank Professor J. D. Boyd for his advice and encouragement in the preparation of this paper; Miss I. Dean and Miss A. Smith for technical assistance; and Mr. J. F. Crane for the photographs.

This work has been supported by a grant from the Nuffield Foundation, which is gratefully acknowledged.

REFERENCES

- Annotation (1954). *Brit. med. J.*, 1, 809.
 Griffith, J. Q., and Farris, E. J. (1942). *The Rat in Laboratory Investigation*. Lippincott, Philadelphia.
 Helmboldt, C. F., Jungherr, E. L., Eaton, H. D., and Moore, L. A. (1953). *Amer. J. vet. Res.*, 14, 343.
 Lamming, G. E., Woollam, D. H. M., and Millen, J. W. (1954). *Brit. J. Nutr.*, 8, 363.
 Mellanby, E. (1926). *J. Physiol. (Lond.)*, 61, xxiv.
 ——— (1938). *Ibid.*, 94, 380.
 Millen, J. W., Woollam, D. H. M., and Lamming, G. E. (1953). *Lancet*, 2, 1234.
 ———, ———, ——— (1954). *Ibid.*, 2, 679.
 Rigdon, R. H. (1952). *Arch. Path. (Chicago)*, 53, 239.
 Sorensen, D. K., Kowalczyk, T., and Hentges, J. F. (1954). *Amer. J. vet. Res.*, 15, 258.
 Wolbach, S. B., and Bessey, O. A. (1941). *Arch. Path. (Chicago)*, 32, 689.
 Woollam, D. H. M., and Millen, J. W. (1955). *Nature (Lond.)*, 175, 41.