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Hydrocephalus in Young Rabbits Associated with Maternal Vitamin A Deficiency

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After earlier studies on the effect of incipient vitamin A deficiency on reproduction in the female rabbit (Lamming, Salisbury, Hays & Kendall, 1954*a*, *b*) the present studies were undertaken to determine the effect of incipient vitamin A deficiency in the dam on development and growth of the young.

Many published accounts have appeared of the effect of vitamin A deficiency on farm and laboratory animals during the period of growth (in pigs, Hart, Miller & McCollum, 1916; Hughes, Lienhardt & Aubel, 1929–30; Dunlop, 1934; Hostetler, Foster & Halverson, 1935; Kellermann, Schulz & Thomas, 1943; in cattle, Hart, Steenbock, Humphrey & Hulce, 1924–5; Moore, Huffman & Duncan, 1935; Moore & Sykes, 1941; in sheep, Eveleth, Bolin & Goldsby, 1949; in rabbits, Phillips & Bohstedt, 1938).

In most accounts convulsion, paralysis and incoordination have been noted as signs. Already 30 years ago Hart *et al.* (1924–5) reported that calves from cows with vitamin A deficiency were often born dead or died soon after birth. Later Moore *et al.* (1935) described blindness, xerophthalmia and partial paralysis in calves born from vitamin A-deficient cows, as well as in calves receiving poor-quality roughage from the age of 3 months.

The cause of these signs has been much debated. After examination of vitamin A-deficient calves Moore *et al.* (1935) concluded that the blindness was due to injury of the optic nerve at the optic foramina, presumably owing to pressure by the surrounding bone, although they were reluctant to accept the view that vitamin A deficiency as

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such could be responsible for the bony abnormalities. However, they did suggest that intracranial pressure might be raised during vitamin A deficiency, and later Moore & Sykes (1941) by taking cerebrospinal-fluid pressure measurements confirmed that the cerebrospinal-fluid pressure was raised. Mellanby (1938–9) working with vitamin A-deficient puppies reported that abnormal bone growth was responsible for nerve lesions causing night-blindness, papilloedema and optic atrophy, and Moore (1939), though accepting this explanation, considered that the bony changes might be secondary to some other undetermined factor and stressed the importance of the increased intracranial pressures he had observed. In a further series of papers Mellanby described disorganization of osteoblast and osteoclast activity after vitamin A deficiency in support of his earlier views that abnormalities of bone growth were responsible for the lesions of the nervous system (cf. Mellanby, 1944).

MATERIALS AND METHODS

All except one of the rabbits used in this experiment were from an inbred strain kept as a closed colony since 1924 and separated for colour in 1944. They were housed in wooden cages with sawdust and received a pellet ration containing no detectable carotene (less than 1 μg carotene/g diet) for about 14 weeks before mating. This ration was modified from that used by Lamming *et al.* (1954*a*) because of the unavailability of white maize in England and consisted of groundnut meal 17·5, beet pulp 10, oatmeal 27, wheat feed 13, molasses 3, linseed meal 23, salt 0·5, iodized minerals 2·0 and dried yeast 4 parts by weight.

Weekly food intakes were recorded for each parent animal until the young began feeding and individual recordings became impossible. All adult animals received weekly by mouth 1 ml. arachis oil containing 50 mg α-tocopheryl acetate and 1500 i.u. vitamin D as Radiostol (British Drug Houses Ltd.). The adult control animals received in addition 7500 μg vitamin A acetate in the arachis oil. From 2 weeks of age the young rabbits from both deficient and control groups received 0·5 ml. arachis oil containing 25 mg α-tocopheryl acetate and 750 i.u. vitamin D orally. The adults were weighed at weekly intervals and most of the young were weighed at approximately 2-day intervals.

The results reported here concern investigations into neurological abnormalities in the young of nine litters from eight dams with incipient vitamin A deficiency (hereafter called the deficient dams), compared with the young of nine litters from eight dams receiving the same ration and adequate vitamin A (hereafter called the control dams). It should be noted that young from the control dams received no vitamin A besides that obtained from the dam's milk. Details of the reproductive performance of dams, neonatal mortality and growth rate in the young of these and additional animals are being prepared for publication later.

After death the skulls of the young rabbits were sectioned in the coronal plane with the brain *in situ*. The optic nerves and surrounding tissues were always examined and the relationship of the brain to its meninges and bony coverings was carefully investigated. In a few animals 'Dag' colloidal carbon was injected into the lateral ventricles during life and the hind brain and spinal cord were examined for colloidal carbon at

autopsy to decide whether or not the hydrocephalus was of the non-communicating type and, if so, at what point the blockage had occurred. Transverse sections of the mid-brain were made to determine the dimensions of the cerebral aqueduct. At each stage of the investigation control rabbits of similar age were examined for comparison.

RESULTS

It was noted that young kindled and reared by does with incipient vitamin A deficiency developed a nervous disorder at different periods (3–6 weeks) after birth. The first sign of the nervous disorder was the appearance in one animal of paralysis of the hind-quarters and head retraction. The remaining animals in the same litter and the animals of a second litter developed head retraction (Pl. 1), but in a third litter the animals became emaciated without head retraction occurring. Radiological examination suggested that the underlying cause might be hydrocephalus and additional litters were used to study the condition further. A preliminary report on the first sixteen young examined has already been published (Millen, Woollam & Lamming, 1953) together with evidence supporting the conclusion that the condition was not due to a genetic factor. In the remaining six litters some animals died without signs and others were killed as soon as signs appeared.

Table 1. *Incidence of hydrocephalus and other signs in young vitamin A-deficient rabbits with parallel observations on control animals*

	Young from vitamin A-deficient dams					Young from control dams
	Hydrocephalus present	Mild hydrocephalus present	Hydrocephalus not noted	No record on brain	Total	Total
No. of animals	24	2	9	14*†	49	45
Blood-plasma vitamin A (μg/100 ml.)	—	—	—	—	—	12·80 ± 11·59 (23)
Liver vitamin A (μg/g)	0·80 ± 0·61 (11)	—	0·62 ± 0·46 (8)	1·48 ± 1·70 (3)	—	2·75 ± 2·38 (28)
No. died before 10 days old	—	—	—	9	9	4
No. died	10	1	8	3	22	4‡
No. killed	14	1	1§	2*	18	37
No. with head retraction	12	1	—	3*†	16	0
No. with convulsions	3	—	—	—	3	2‖
No. emaciated	10	1	—	2	13	0

Figures in parentheses indicate the number of analyses contributing to the means and their standard errors.

* Includes two animals in which a sagittal section of the skull was made.

† Includes one animal in which the entire skull was cleared free of tissue.

‡ See text, p. 365.

§ Animal killed for observation when litter sisters showed head retraction and hydrocephalus.

‖ See text, p. 366.

The results are presented in Table 1. Whereas all young from vitamin A-deficient dams had shown typical signs, or were dead, 74 days after birth, no similar signs were observed in young from control dams. Of forty-five young from control dams, four animals were allowed to develop terminal deficiency and they died at 98, 110, 123 and 130 days respectively. Of forty-nine young from deficient dams, nine died before the

young emerged from the nest and were not available for study. Of the remaining forty animals two died before the deficiency was diagnosed, and three were examined in such a way that no information is available to indicate the presence or absence of hydrocephalus, although all three showed head retraction before slaughter (see Table 1). Twenty-four animals had severe hydrocephalus, two had mild hydrocephalus and nine showed no signs of the condition at death. Thus hydrocephalus was recognized in twenty-six of thirty-five young examined. There was a high incidence of head

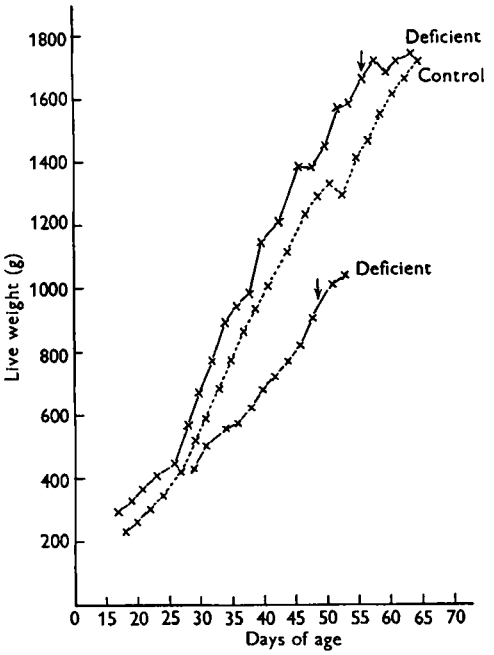


Fig. 1

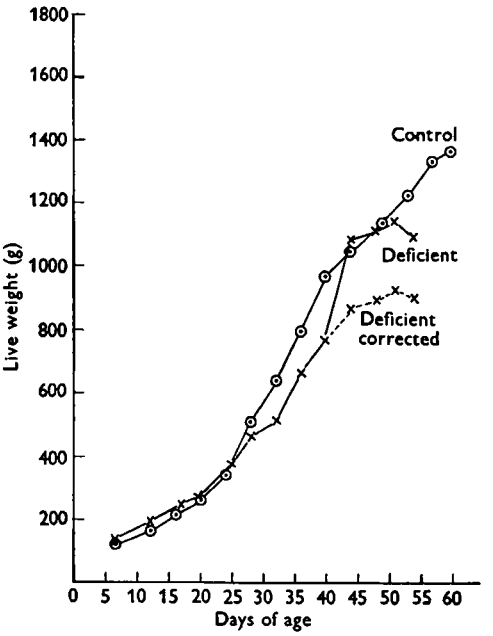


Fig. 2

Fig. 1. Growth rate of two vitamin A-deficient rabbits with hydrocephalus showing head retraction before maximum live weight was achieved and of a control rabbit for comparison. Arrow indicates head retraction.

Fig. 2. Average growth rate of young from vitamin A-deficient and control rabbits. At approximately 40 days of age, ten young from deficient dams died or were killed. The apparent increase in growth rate at this age is an artifact and the dotted line shows the corrected average weight of the deficient group.

retraction and emaciation in the hydrocephalic group (Table 1). No control animals had hydrocephalus and none showed any evidence of head retraction, emaciation or paralysis, although two animals that died, of the four allowed to develop to terminal vitamin A deficiency, showed convulsions before death.

The onset of signs in young from deficient dams was often rapid and it often occurred before maximum live weight was reached (Fig. 1). Average growth rates for young of deficient dams compared with young of control dams (Fig. 2) show that there was little difference between the two groups before the 4th week of age.

An account of the typical signs noted during the early studies has already been given (Millen *et al.* 1953). In animals with severe hydrocephalus the coronal sections of the

cerebral hemispheres showed gross dilatation of the lateral and third ventricles (Millen *et al.* 1953). Frequently the cerebullum had herniated through the foramen magnum, although there appeared to be no deformity of the foramen magnum or of the base of the skull. Transverse sections of the cerebral aqueduct showed considerable stenosis of the aqueduct (Millen *et al.* 1953). In those animals in which colloidal carbon was injected into the lateral ventricles, it was absent from the fourth ventricle and spinal canal. This finding indicates that stenosis of the cerebral aqueduct was preventing the normal flow of cerebrospinal fluid from the third ventricle to the fourth ventricle and subarachnoid spaces. In those animals in which hydrocephalus was well marked, the optic nerves showed distinct constrictions in the region of the optic foramina (Millen *et al.* 1953). At no stage of the investigations was any abnormality observed in the young from control dams examined for comparison.

The vitamin A levels of animals from deficient and control groups are given in Table 1. When deficient animals were killed it was often not possible to collect blood for analysis. In the deficient animals that showed no evidence of hydrocephalus there were slightly lower average liver vitamin A levels, although individual animals in this group had higher liver vitamin A levels than the average for the hydrocephalic group. The considerable variation in the levels of blood-plasma and liver vitamin A in the control group is due to the fact that those killed later in life had lower levels than those used for comparison during the early stages. Further investigations will be necessary to show what levels are required to prevent the occurrence of these signs.

DISCUSSION

The high incidence of hydrocephalus in young from vitamin A-deficient dams and the absence of hydrocephalus in young from control dams indicate the importance of adequate vitamin A for normal development of the brain and its associated and related structures. The clinical accounts of the effects of vitamin A deficiency in young farm animals are remarkably similar to the description given here and suggest that the underlying pathological condition may well be similar.

Moore *et al.* (1935) reported that vitamin A-deficient calves held their heads in a peculiar position and Moore & Sykes (1941) later confirmed that the cerebrospinal-fluid pressure was raised in growing and adult cattle suffering from vitamin A deficiency. By measuring this pressure in a number of calves Moore, Sykes, Jacobson & Wiseman (1948) were able to distinguish between daily carotene intakes of about 28 i.u. and of about 32 i.u./lb. body-weight. Hart *et al.* (1916) noted that vitamin A-deficient pigs developed paralysis, that the vertebral canal contained much fluid and that the spinal cord appeared to be under pressure. The account of Milne (1942), who described a congenital deformity in pigs born to two sows receiving no green food, is remarkably similar to the present account. These sows produced normal piglets when green food was added to their diet. Mellanby (1938–9) noted the occasional occurrence of hydrocephalus in vitamin A-deficient puppies, but did not seem to attach much significance to it, and Moore & Sykes (1941) also reported dilatation of the ventricles in a vitamin A-defioient calf. de Schweinitz & De Long (1934) described a condition of blindness

and papilloedema in Guernsey calves, accompanied by oedema of the cerebral hemispheres and dilatation of the perivascular spaces. Previous studies have been confined to detailed examination of the peripheral nerves, with little attention to the gross relationships of the brain and spinal cord during vitamin A deficiency, and the condition of aqueductal stenosis in their material may well have been overlooked by previous observers.

Mellanby (1944) has maintained that the nervous signs typical of vitamin A deficiency in the puppy are a direct result of disorganization of bone formation, leading to bone overgrowth and compression of the central nervous system. However, Wolbach & Hegsted (1952*a*, *b*), working with vitamin A-deficient chicks and ducklings, concluded that the nervous signs they observed were due to retardation and suppression of endochondral bone growth, which resulted in compaction of the developing brain and spinal cord. They rejected a suggestion by Rigdon (1952) that the signs were not related to abnormalities of bone growth, but rather to a primary degeneration of the brain and cord.

Moore & Sykes (1941) and Moore *et al.* (1948) have reported increase in the cerebrospinal-fluid pressure in the bovine due to vitamin A deficiency, without reaching any conclusions as to how it occurs. The present investigations suggest that the increase in cerebrospinal-fluid pressure may well be the primary cause of the nervous signs in the vitamin A-deficient rabbit: they are almost invariably accompanied by aqueductal stenosis and consequent hydrocephalus. The extent of the hydrocephalus alone is sufficient to explain the severe signs, although the possibility of additional nerve degeneration has not been entirely ruled out. Whether or not the increased cerebrospinal-fluid pressure produces the aqueductal stenosis or is itself a consequence of the stenosis has yet to be determined. In any event the occurrence of aqueductal stenosis in animals suffering from vitamin A deficiency may well be the limiting factor that, once established, prevents vitamin A therapy from producing complete recovery from the nervous signs.

The results of this investigation also suggest that the papilloedema and compression of the optic nerves observed by Mellanby (1938–9) in puppies and Moore (1939) in calves may well be a direct result of an increased pressure in the subarachnoid spaces in the region of the optic foramina.

SUMMARY

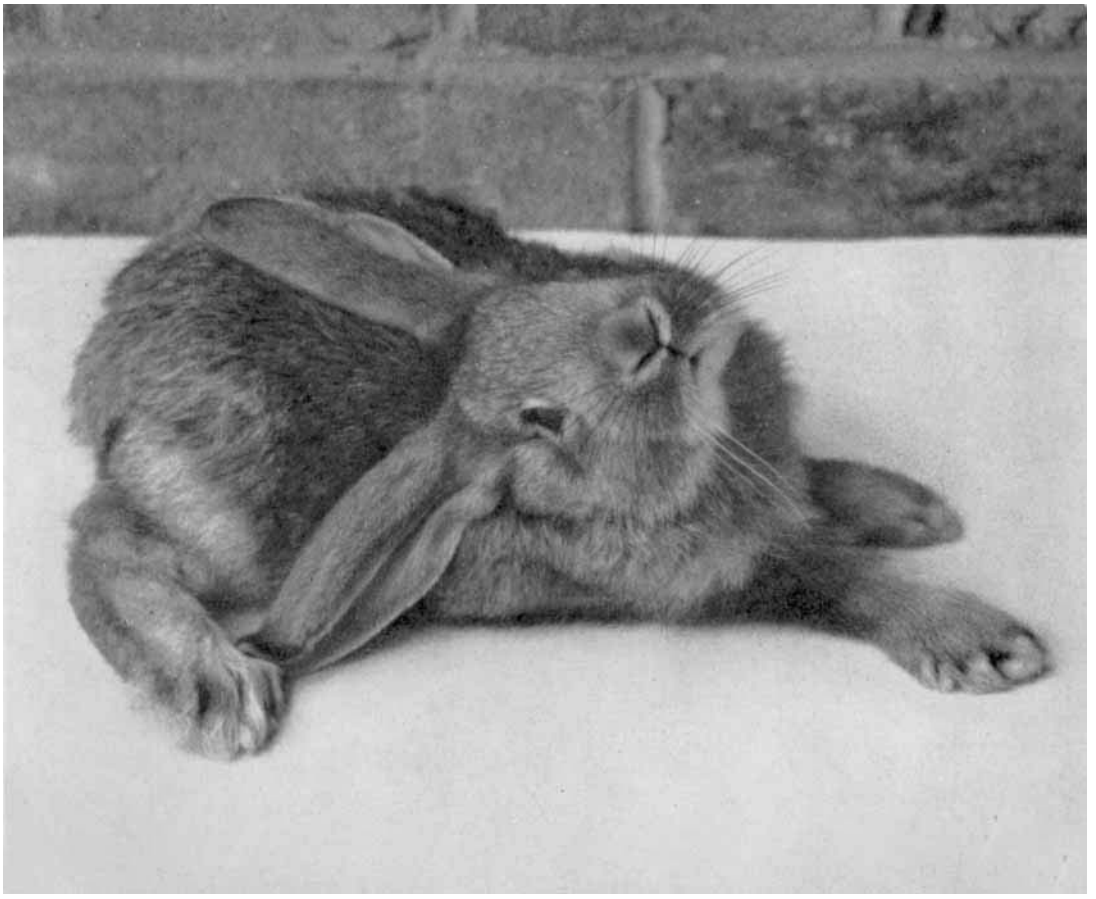
1. Young rabbits produced by dams with incipient vitamin A deficiency developed convulsions, paralysis and head retraction 21–74 days after birth.

2. A high proportion (twenty-six out of thirty-five) of the vitamin A-deficient young had hydrocephalus with stenosis of the cerebral aqueduct.

3. The possibility is discussed that the increased cerebrospinal-fluid pressure produced the constriction of the optic nerves observed in these animals.

4. No abnormalities were seen in young from dams receiving the same diet with adequate vitamin A.

5. The relationship of these findings to reports of vitamin A deficiency in growing farm animals is discussed.



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EXPLANATION OF PLATE

Head retraction as shown by many young rabbits from vitamin A-deficient dams.