The relationship between hypovitaminosis A and the cerebro-spinal-fluid pressure in the chick: an experimental study

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There is general agreement that, although severe cases of vitamin A deficiency are encountered only on rare occasions amongst the human populations of western countries, minor degrees of hypovitaminosis A may be widespread. When a detailed survey was made of the effects of hypovitaminosis A in man (Hume & Krebs, 1949), the results seemed to indicate that minor degrees of vitamin A deficiency were of little clinical importance apart from their relationship to night-blindness. Since then, however, it has been repeatedly shown that the induction of a maternal hypovitaminosis A in laboratory animals is a most effective method of producing congenital malformations in the young (see review by Warkany, 1954). A most significant finding is that, whereas gross degrees of maternal vitamin deficiency result in abortion or in the resorption of the litter early in pregnancy, the birth of deformed young occurs only when the mother is subjected to a minor degree of hypovitaminosis (Warkany, 1947). In animal experimentation it has been found that the critical period at which a maternal vitamin deficiency is effective in producing the birth of malformed young is that which corresponds to the 2nd and 3rd months of pregnancy in man. It would seem that a supply of vitamins sufficient for the normal development of the foetus can only be assured if the mother has adequate stores of the vitamin at the time of conception. If a hypovitaminosis is not removed until the pregnancy is clearly evident, the probability is that it will then be too late to correct its deleterious effects upon the development of the foetus. It might be argued that normal human foetal development is not necessarily dependent upon nutritional considerations important to the normal development of other mammals. This argument apart, there are good reasons for assuming that the presence of minor degrees of hypovitaminosis A in women may be of considerable significance. In this connexion it is important to note that the experiments mainly responsible for the impression that vitamin A deficiency was not a serious problem in this country (Hume & Krebs, 1949) were performed on male conscientious objectors. The question whether vitamin A deficiency was of importance to human reproduction was not under consideration, and there has been no large-scale nutritional survey of the effects of hypovitaminosis A in the female. It is surprising therefore that, in the many accounts that have appeared of the effects of vitamin A deficiency in farm and laboratory animals, emphasis has mainly been laid on the end-results of the process, and, although there is now a voluminous literature on the effects of extreme degrees of deficiency, few observations have been made on the early stages of experimental hypovitaminosis A.

Of all the manifestations of vitamin A deficiency, the effects on the nervous system

have proved the most difficult to explain and have aroused the most controversy. In animals the result of a gross deficiency is to produce a syndrome whose principal features may be summarized as follows: weakness and muscular incoordination appear, to be followed soon by paralysis and convulsions and finally by death. Various views have been expressed as to the relationship between vitamin A deficiency and this syndrome. It has been postulated that hypovitaminosis A has a primary effect on the nervous system (Rigdon, 1952). Indeed at one time Mellanby regarded the condition as akin to subacute combined degeneration of the cord in man, but later, modifying his views, held abnormal bone growth to be responsible for the condition. He considered that the nervous system was damaged by the direct pressure of abnormal bony excrescences (Mellanby, 1938–9, 1944). Neither of Mellanby's concepts satisfied Wolbach & Hegsted (1952), who believed that the nervous signs of the deficiency were due to the compression of a normally developing central nervous system by a skeleton that was the subject of a retarded and suppressed endochondral bone growth.

The constant occurrence of hydrocephalus in young rabbits born to does subjected to experimental hypovitaminosis A (Millen, Woollam & Lamming, 1953, 1954; Lamming, Woollam & Millen, 1954) suggested that there might be a relationship between disturbance in the circulation of the cerebrospinal fluid and the effects of vitamin A deficiency on the nervous system. Suggestions of such a relationship had appeared sporadically in the earlier literature. Mellanby (1938–9) had noted the occasional occurrence of hydrocephalus in vitamin A-deficient puppies, Moore & Sykes (1941) had reported dilatation of the ventricles in a vitamin A-deficient calf and de Schweinitz & Long (1934) had described the occurrence of blindness and papilloedema in Guernsey calves in which oedema of the cerebral hemispheres and dilatation of the perivascular spaces had been observed *post mortem*. For these reasons it appeared that an experimental study of the cerebrospinal-fluid pressure at the early stages of vitamin A deficiency in the experimental animal might throw light on features of the nervous syndrome of hypovitaminosis A.

EXPERIMENTAL

In order to obtain information on the relationship between hypovitaminosis A and the cerebrospinal-fluid pressure, measurements of the pressure were made on chicks of varying ages subjected to different nutritional conditions. Because no adequate information existed as to the normal cerebrospinal-fluid pressure of birds, it was found necessary to determine whether there was any variation with age in the cerebrospinal-fluid pressure of the chick on a normal diet. The other measurements were made on chicks born either to vitamin A-deficient hens (group 3) or to hens on a normal diet (group 2).

The régime and nutrition of the experimental chicks

Group 1. These chicks were obtained from a commercial hatchery where they had been reared on a standard chick diet. Fifty chicks in all were used, there being ten birds of each of the following ages: 50, 75, 100, 125, 150 days. The pressures were measured immediately on arrival from the hatchery.

Group 2. Batches of day-old chicks on arrival from a commercial hatchery were placed in a partitioned brooder; one side of the brooder was labelled 'experimental' (group 2*a*) and the other 'control' (group 2*b*). The total number of chicks in group 2*a* was thirty-five and in group 2*b* thirty. All the chicks received the following diet: white maize 56, wheat middlings 25, casein 12, dried-yeast powder 4, calcium carbonate 1, bone flour 1, salt 1, parts.

To each lb. of the mash 180 i.u. of vitamin D_3 were added and the diet was also supplemented with vitamin K and riboflavin. This diet contained 1·6 p.p.m. carotene. The experimental chicks (group 2*a*) and the control chicks (group 2*b*) received identical treatment, with one exception: the control chicks received 1500 μg vitamin A acetate twice weekly by mouth.

Group 3. The experiments in this group differed from those in group 2 inasmuch as the chicks were obtained from hens that themselves had been subjected to the effects of vitamin A deficiency. Laying hens were placed on a diet containing no detectable amount of provitamin A, and the eggs were collected daily and date-marked. To obtain fertile eggs, a cock was introduced into each individual hen's pen for a few hours every other day; this cock received a normal diet. When a sufficient number of eggs had been collected, the labelled eggs were placed in divided trays in an incubator and allowed to hatch. Although nearly all the eggs developed to maturity, there were considerable losses through failure of the mature chicks to hatch. After hatching, the chicks were placed in a brooder and received the same diet as the chicks in the experiments with group 2*a*. The deficient hens eventually went off lay, but the period of some months during which they did lay was long enough to provide a series of chicks with graded degrees of vitamin A deficiency at hatching. These chicks could be compared with the chicks of group 2*a*, which had been obtained from a large commercial hatchery and could be regarded as of equal vitamin A status at hatching.

All the chicks of groups 2 and 3 were wing-tabbed to facilitate identification and were weighed twice weekly.

Measurements of the cerebrospinal-fluid pressure

After the chicks in groups 2 and 3 had been in the brooder for various periods of time, the cerebrospinal-fluid pressures were measured by cisternal puncture, using the apparatus described by Jeffers & Griffith (1942). The same technique was used for measuring the pressures of the chicks of group 1 immediately on arrival from the hatchery.

Other investigations on the chicks

On three occasions, after the cerebrospinal pressure had been measured, colloidal carbon was introduced into the lateral ventricles of chicks of group 2*a* to outline the cerebrospinal-fluid pathways. All the chicks were killed a few hours after the cerebrospinal-fluid pressure was measured, and the cadavers were preserved in formalin. A general post-mortem examination was conducted on each specimen. Detailed dissection of the brain and spinal cord under the Greenough microscope was made in a number of specimens, and special attention was paid to the relations of the bony coverings to the brain and spinal cord.

RESULTS

Health and post-mortem findings in the chicks

All the chicks of groups 2 and 3 showed normal gain in weight, and there was no difference in the weight gain between those on the vitamin A-deficient diet alone (groups 2*a* and 3) and those on the same diet receiving a supplement of vitamin A (group 2*b*).

None of the chicks showed any sign of neurological disease, and at autopsy no abnormality of the bones of the skull or vertebral column was detected.

On the three occasions when it had been injected into the lateral ventricle of a chick from group 2*a*, colloidal carbon was found to have passed freely through the ventricular system into the subarachnoid space and to have filled up the meningeal cuffs around the emerging spinal nerves.

The cerebrospinal-fluid pressure in the three groups of chicks

Group 1. The mean cerebrospinal-fluid pressure for each age-group of ten chicks is given below:

Age of chicks (days)	Cerebrospinal-fluid pressure (mm water)
50	105
75	95
100	110
125	102
150	100
	Mean 102·4

These findings indicate that the normal cerebrospinal-fluid pressure in the chick does not increase with age.

Group 2*a*. The figures for the pressures in this group have been statistically analysed with the result shown below:

Age of chicks (days)	No. examined	Mean cerebrospinal-fluid pressure* (mm water)
30	4	108 ± 16·8
62	21	157 ± 7·3
88	7	180 ± 12·6
125	3	212 ± 19·3

* Value with its standard error. (Standard errors are based on a pooled estimate of variance within groups.)

There was a constant increase of pressure with age, of estimated amount 1·03 mm/day with standard error 0·243 (31 degrees of freedom, $P < 0{\cdot}001$).

Group 2*b*. The cerebrospinal-fluid pressures of this group of control chicks ranged from 90 to 105 mm of water, with a mean of 98.

Group 3. The findings in this group have been subjected to statistical analysis with the following results.

Analysis of the figures showed that there was a regression, $\beta = 0{\cdot}987 \pm 0{\cdot}346$ mm water/day, of p, the cerebrospinal-fluid pressure, on the total number of days for which the deficient diet had been fed to hens and chicks. This regression is the same as that calculated on the basis of the experiments with group 2*a* ($\beta = 1{\cdot}031 \pm 0{\cdot}243$ mm water/day, regression of p on number of days).

It followed therefore that the same rate of increase in the cerebrospinal-fluid pressure is produced by depriving the hen of vitamin A before the egg is laid as by depriving the chick after hatching (Fig. 1).

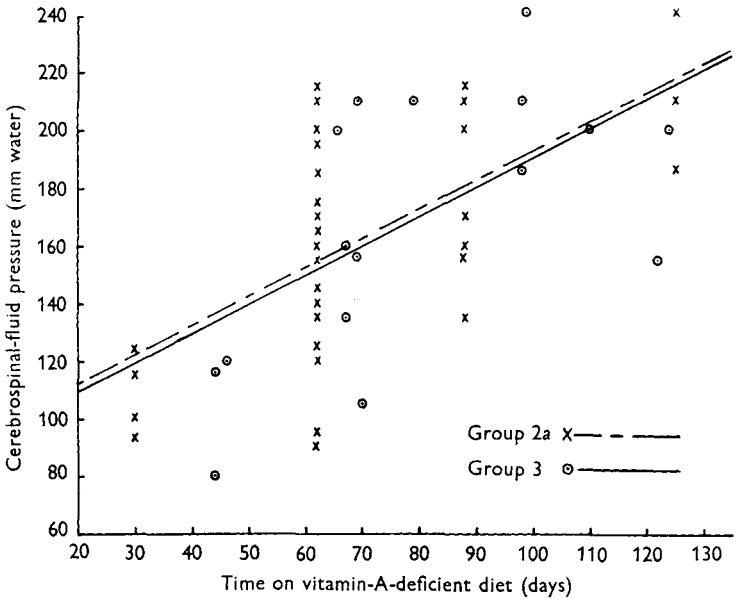

Fig. 1. Relationship between cerebrospinal-fluid pressure and duration of vitamin A deficiency. Group 2*a*: the cerebrospinal-fluid pressure of chicks hatched from eggs laid by hens on a normal diet is plotted against the time the chicks were maintained on a diet deficient in vitamin A. Group 3: the cerebrospinal-fluid pressure of chicks hatched from eggs laid by hens on a vitamin A-deficient diet is plotted against the time the chicks were maintained on a diet deficient in vitamin A added to the time the mother was on the deficient diet before the egg was laid.

The pooled estimate of the regression as $\beta = 1$ was taken. In order to discover whether the cerebrospinal-fluid pressures of chicks born to vitamin A-deficient mothers and themselves reared on a deficient diet were greater than those of chicks fed on a deficient diet but born to mothers fed on a normal diet, the mean cerebrospinal-fluid pressure of each group was calculated at a standard age of 50 days:

Group 2*a*, mean = 141·31 mm water; group 3, mean = 174·28 mm water.

The difference is significant at the 1% level.

Thus the chicks hatched from eggs laid by hens that had been fed on a deficient diet and therefore already deficient in vitamin A at birth showed higher cerebrospinal-fluid pressure than those also fed on a deficient diet from birth but hatched from eggs laid by hens on a normal diet (Fig. 2).

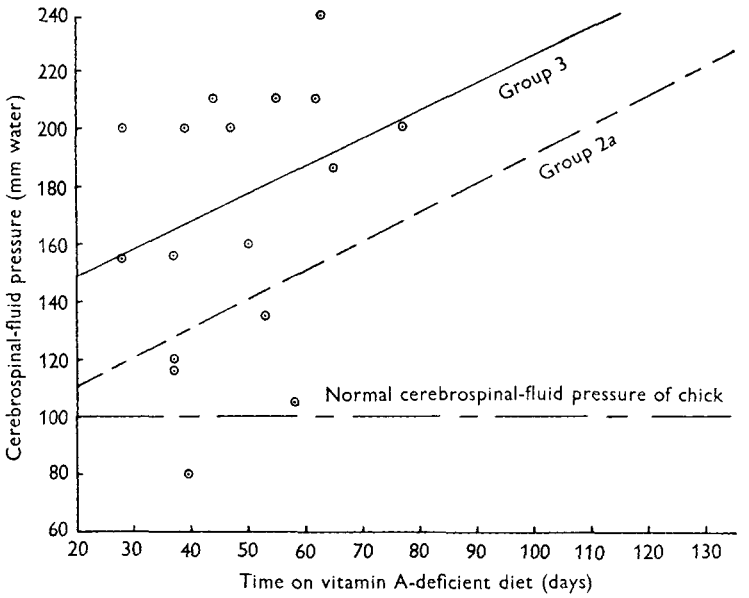

Fig. 2. The cerebrospinal-fluid pressure of the two groups of chicks is contrasted; both were reared on diets deficient in vitamin A. Group 2*a* were hatched from eggs laid by hens on a normal diet, group 3 from eggs laid by hens on a vitamin A-deficient diet. The regression line for group 2*a* is taken from Fig. 1 and the individual points for this group are not shown.

DISCUSSION

The findings of the present investigation suggest that the effect of a minor degree of hypovitaminosis A, insufficient to produce signs of neurological disturbance, is to cause a rise in the pressure of the cerebrospinal fluid. In the production of this rise it appears that a maternal deficiency of sufficient severity is as effective as a deficiency in the diet of the chick after hatching.

Raised cerebrospinal-fluid pressures in vitamin A deficiency had previously been reported in the calf (Moore & Sykes, 1940), the sheep (Eveleth, Bolin & Goldsby, 1949) and the pig (Sorensen, Kowalczyk & Hentges, 1954). Our findings in chicks differed from those previously reported in mammals in that the pressure was found to be raised in an otherwise perfectly healthy animal with no signs of neurological or other disturbance. It must be emphasized that the chicks on this diet were indistinguishable from the control animals both during life and at post-mortem. In this respect they differed markedly from the birds, described by Wolbach & Hegsted (1952), that on a more deficient diet proceeded to show the classic neurological signs of hypovitaminosis A within a short time.

It might appear difficult to reconcile the present findings with those of other workers on the effects of hypovitaminosis A. Before attempting to do so, it is important to note certain points that lie in the background of all nutritional research and have a peculiar and potent significance in experiments on vitamin A deficiency.

First, it may be suggested that the effects of a severe vitamin A deficiency are widespread, acting on all the tissues of the body. Through this action, hypovitaminosis A

may be responsible for creating what is effectively a mixed deficiency by interfering with the absorption and metabolism of other vitamins and essential amino-acids, so that what has been accepted as the syndrome of vitamin A deficiency may in reality be a composite picture of the effects of a mixed dietary deficiency on the central nervous system. It is reasonable to assume that the effect is more likely to occur when severe degrees of deficiency are induced. Further, the role and activity of a vitamin in the body is related both qualitatively and quantitatively to the presence of other constituents of the diet. Not only may the absence of the vitamin be expected to affect the metabolism of these other constituents of the diet, but any modification of their quantitative relationships may adversely affect the metabolism of the vitamin itself.

For these reasons it is most significant that there has been no general agreement as to a standard formula for the diet to be used in experiments on vitamin A deficiency, the diets tending to differ in the constituents of low vitamin A content used. Frequently experiments have not been controlled by the use of animals on the same diet with supplementation with pure vitamin A, but have made use of so-called 'normal' diets of a totally different constitution as controls. This is particularly significant inasmuch as the experiments have entailed the death of the animal after a period of somewhat prolonged ill-health, for it is precisely under such conditions that one might expect the general nutrition of the animal to be of the utmost importance.

Another point to be raised in relation to the study of the nervous signs of hypovitaminosis A is that it is not justifiable to compare observations made on different animal species without taking into account the differing anatomical relationships between the central nervous system, the cerebrospinal fluid and the skeletal structures in individual species. For example, the lateral ventricles, although forming the greater part of the ventricular cavities in man, form a minor part only in the rat (Woollam, 1952). It would seem also that the choroid plexuses of the lateral ventricles, which are generally regarded as being mainly responsible for the production of the cerebrospinal fluid in man, probably play a secondary role to the plexuses of the fourth ventricle in lower animals, such as the chick. When the fact that the choroid plexuses protrude through the lateral foramina of Luschka into the general subarachnoid space is taken into account, it can be seen that, whereas a general increase in the activity of the choroid plexuses in the production of the cerebrospinal fluid would lead in man and the higher animals mainly to an increase in the intraventricular fluid, in the lower animals such as the chick it would lead almost exclusively to an increase in the extraventricular fluid. In the light of these anatomical facts it is interesting to note that none of the chicks hatched from eggs of vitamin A-deficient hens developed hydrocephalus as did rabbits born to vitamin A-deficient dams (Millen *et al.* 1953, 1954), and it is significant in this matter to note that it does not appear that hydrocephalus from any cause has ever been reported in birds.

It is theoretically possible for a raised cerebrospinal-fluid pressure to be produced in three ways: by overproduction or underabsorption of the fluid or by some obstruction being offered to its flow. The absence of any bony abnormality or any sign of displacement of the normal relationships between the central nervous system and its

bony coverings in the experimental chicks suggests that no obstruction was present. When colloidal carbon was introduced into the lateral ventricle of the experimental chick it travelled freely through the ventricular system to escape into the subarachnoid space and to fill up the meningeal cuffs around the emerging spinal nerves, in a fashion parallel to that described as occurring in the rabbit by Field & Brierley (1948) and in the rat by Woollam & Millen (1953). This finding indicates that no obstruction was offered to the passage of the cerebrospinal fluid, and also that the area of the meningeal cuffs was at least freely available for absorptive purposes.

For these reasons it is suggested that the cause of the increased cerebrospinal-fluid pressure in the chicks on a diet with a low content of vitamin A was overproduction of the cerebrospinal fluid. This finding agrees with the mechanism suggested to explain the occurrence of hydrocephalus in the young of rabbits whose dams had been subjected to experimental hypovitaminosis A (Millen *et al.* 1954; Millen & Woollam, 1956). Though it is now clear that a raised cerebrospinal-fluid pressure is the first sign of vitamin A deficiency in the chick, it does not necessarily follow that overproduction of cerebrospinal fluid is responsible for all the neurological aspects of the syndrome of vitamin A deficiency, although the possibility that it is obviously merits further investigation.

SUMMARY

1. The cerebrospinal-fluid pressure was measured in fifty chicks hatched from eggs laid by hens on a normal diet and themselves reared on a normal diet. There was no significant variation in the cerebrospinal-fluid pressure over the range of from 50 to 150 days of age. The average value for the cerebrospinal-fluid pressure was 102 mm of water.

2. When chicks hatched from eggs laid by mothers on a normal diet were reared on a diet containing only 1·6 p.p.m. carotene, a rise of cerebrospinal-fluid pressure occurred, estimated as 1·03 mm/day.

3. Chicks hatched from eggs laid by hens maintained on a vitamin A-deficient diet and themselves reared on a deficient diet showed higher cerebrospinal-fluid pressures than those reared on the same diet but hatched from eggs laid by hens on a normal diet.

4. The level of the cerebrospinal-fluid pressure was found to be related to the total number of days for which hen and chick were receiving the deficient diet. The same rate of increase of cerebrospinal-fluid pressure was achieved by depriving the hen of vitamin A before the egg was laid as by depriving the chick for an equivalent period after hatching.

5. The rise of cerebrospinal-fluid pressure was not associated with the presence of any sign of neurological disturbance, nor was any bony abnormality found at post-mortem examination.

6. It is considered that this finding can best be explained as a direct result of the effect of vitamin A deficiency on the production of the cerebrospinal fluid.

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The effects of chronic undernutrition and of total starvation on growing and adult rats

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People interested in human nutrition have always held that there was some inherent difference between the metabolism in, and the reactions of the body to, chronic undernutrition and complete deprivation of food. Their view is mainly based on the fact that oedema is a serious presenting problem in undernutrition, whereas it is said never to appear in people who are eating no food at all. There have been suggestions also from animal work that the effects on the weight and composition of the organs are different. Jackson (1915*a*, *b*), for example, found that the liver of the adult rat lost less weight during chronic undernutrition than during acute starvation. A comprehensive review of the older work was made by Jackson (1925), and Keys and his collaborators (Keys, Brožek, Henschel, Mickelsen & Taylor, 1950) have brought the subject up to date.