STUDIES IN VITAMIN A AVITAMINOSIS IN THE CHICK*

BY C. A. ELVEHJEM AND V. F. NEU

(From the Department of Agricultural Chemistry, University of Wisconsin, Madison)

(Received for publication, April 13, 1932)

The rat has been used almost exclusively as the laboratory animal in vitamin A studies. The results obtained with rats have been so consistent that little consideration has been given to the use of other animals. In fact the characteristic development of ophthalmia in the rat has led to considerable neglect in the study of other physiological changes occurring during vitamin A avitaminosis. Since chickens do not develop ophthalmia uniformly when deprived of vitamin A, investigations concerning the incidence of other symptoms during this dietary deficiency in the chick become necessary.

The early work on the fat-soluble vitamin requirements of chicks was reviewed by Emmett and Peacock (1) in 1923. From their own work they concluded that a very large percentage of the chicks fed a vitamin A- (vitamins A and D were not differentiated at that time) deficient diet developed symptoms of ophthalmia. Upon autopsy of their animals they found the presence of urates in the tubules, kidneys, and at times on the surface of the heart, liver, and spleen. Beach (2) had previously reported similar symptoms in a disease which he classed as poultry nutritional roup. Emmett and Peacock concluded that both conditions are the same and that poultry nutritional roup is due to the absence of the fat-soluble vitamins in the ration.

The following year Hart, Steenbock, Lepkovsky, and Halpin (3) reported their observations on the vitamin A requirement of chicks when the antirachitic factor was supplied in the form of ultra-violet light. They concluded as did Emmett and Peacock*

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.
that growing chickens require vitamin A for normal development, but they failed to observe ophthalmia in the chicks suffering from vitamin A deficiency.

In 1927 Cruickshank, Hart, and Halpin (4) in their studies on the vitamin A and vitamin D content of cod liver meals described a typical vitamin A deficiency in chicks reared on a diet of 92 parts of white corn, 5 parts of yeast, 2 parts of CaCO₃, 1 part of NaCl, and skim milk ad libitum. The symptoms exhibited were: a staggering gait, a loss of control accompanied by emaciation and weakness, and a general ruffled condition of the feathers. They stated that the incidence of ophthalmia was very rare. Other workers (5, 6) have reported similar difficulties in producing ophthalmia in the chick. It is evident, therefore, that ophthalmia cannot be used as a criterion for the diagnosis of vitamin A deficiency in this species.

Cruickshank, Hart, and Halpin found kidney lesions in practically all of the chickens that died during the experiment. The kidneys were pale in color due to the accumulation of large amounts of urates in these organs. The ureters were distended and filled with urates. Some of the birds killed at the termination of the experiment, although in a very weak condition, displayed only slight kidney lesions. Cruickshank, Hart, and Halpin made no attempt to follow the uric acid content of the blood. The addition of 2 per cent cod liver oil to the basal ration prevented this condition and allowed the development of normal chicks.

Hughes, Lienhardt, and Aubel (7) reported that pigs, chickens, and cows, kept on vitamin A-deficient diets, developed, without exception, a marked nervous disorder characterized by blindness, incoordination, and spasms. Histological examinations of nerve tissue from the pig showed a degeneration of the nerve bundles of the spinal cord, optic, sciatic, and femoral nerves. Although they reported no histological examinations of the chicken, they suggested that the nerve degeneration in this animal is similar to that found in the pig.

Seifried (8) made extensive histological studies on chicks suffering from vitamin A avitaminosis but he did not study the nerve changes. He states, however, that there is a general keratinization of all parts of the respiratory tract, the olfactory region, and the
upper alimentary tract, especially the mucous glands and their ducts.

Capper, McKibbin, and Prentice (9) used the chicken to demonstrate that the fowl as well as the rat can convert carotene into vitamin A. They used chickens which were intended for another experiment and the development of vitamin A deficiency was unexpected since the basal ration contained some yellow corn. The chickens were approximately 16 weeks old before a deficiency developed. These workers described the symptoms in chickens as follows: "Their sense of balance appeared to be disturbed and they walked with difficulty and with a staggering gait." Deposits of urates were found around the heart, liver, and other organs, and the disease was diagnosed as visceral gout. The livers were found to contain no vitamin A. Cod liver oil concentrates prevented or cured this condition.

A survey of these early papers on the relation of vitamin A to the nutrition of the chick has brought to mind a number of questions, which we have attempted to answer by the work reported in this paper. Are the symptoms described by Cruickshank, Hart, and Halpin, and Capper, McKibbin, and Prentice, specific for vitamin A deficiency, and can conditions be standardized sufficiently to use the chick for vitamin A studies? Does the uric acid content of the blood increase during vitamin A deficiency, and are the symptoms reported dependent upon these blood changes? Is the uric acid content of the blood dependent upon kidney degeneration?

**EXPERIMENTAL**

Day old white Leghorn chicks were used for all the experimental work reported in this paper. However, we have used chickens taken from the general poultry flock when 6 weeks old with equal success. These older birds developed typical symptoms of vitamin A deficiency in a period of 3 weeks when placed on a vitamin A-low ration. In our earlier work we used a ration consisting of 97 parts of white corn, 2 parts of CaCO₃, 1 part of NaCl, and skim milk ad libitum, together with irradiation, for the production of vitamin A deficiency. Chicks placed on this diet when 1 day of age failed to grow very well and died before typical symptoms developed and before they were old enough to supply
sufficient blood for analysis. Hart, Steenbock, Lepkovsky, and Halpin (3) obtained considerable growth on this ration in the presence of sunlight, but their chicks were kept on shavings rather than on screens. This ration also requires the use of liquid milk, and is undoubtedly somewhat deficient in the B vitamins.

The ration used by Hart, Kline, and Keenan (10) for the production of rickets in chicks under standardized conditions has been found so successful that we attempted to modify this ration sufficiently to make a standard diet for the production of vitamin A deficiency. The yellow corn was replaced by white corn, the yeast was increased to 2 per cent, and the chicks were irradiated to supply vitamin D. The basal ration was compounded in the following proportions.

58 parts ground white corn
25 " wheat middlings (standard)
12 " crude casein
1 part common salt
1 " precipitated calcium carbonate
1 " " phosphate
2 parts dried yeast (Northwestern)

The corn, middlings, and casein were mixed in relatively large quantities, but the salts and yeast were added weekly to smaller portions. The chicks were given this ration, together with water ad libitum, and were irradiated 20 minutes three times weekly. They were kept in small pens in our animal room where there is no direct sunlight. The pens were equipped with wire screen floors and small electrically heated brooders.

When day old chicks were placed on this ration they grew at a fairly normal rate for a period of 3 weeks. Between the 3rd and 4th weeks growth ceased and often there was a decided decline in weight during the 4th week. The affected birds exhibited a staggering gait and a general incoordination of their movements. As the condition progressed they appeared very drowsy and moved only when disturbed. There was considerable tendency for them to crouch on their haunches in order to maintain their equilibrium. In advanced stages the animal lay on one side, and the head fell forward. The feathers were very ruffled and there was some soreness around the eyes, but there was no typical ophthalmia. The beak and shanks were extremely colorless. There was no decided
decrease in appetite and often the animals made considerable effort to reach the feed even when in a very weakened condition. Chicks in the same group became affected with extreme uniformity. These conditions lasted for only a few days, when death ensued. Most of the birds were dead before the end of the 5th week, while many of them succumbed before the end of the 4th week. Every chick which we have placed on this diet has developed, without exception, these specific symptoms.

The growth curves for three chicks fed the basal ration are plotted in Chart I. The growth curves and the symptoms exhibited by these birds are typical of all chicks fed this ration. The records of two chicks reared on the same ration plus 2 per cent cod liver oil are included for comparison. The excellent growth and normal development of these chicks demonstrates that the basal ration furnishes other factors in sufficient quantity.

Autopsies were performed on all the animals that died or were killed for samples of blood. Especial attention was given to the accumulation of urates in the kidneys and renal tubules. In many cases the tubules were greatly distended due to the accumulation of urates. The kidneys were also filled with urates, in some cases to such an extent that these organs appeared almost white. However, in a number of cases the chicks would exhibit incoordination when there was no evident accumulation of urates in the urinary tract.

The uric acid content of the blood of a large number of vitamin A-deficient and normal chicks was determined, but the results from only one series of experiments will be given in detail. 80 chicks were divided into eight groups of ten chicks each and fed as follows: Group 1, basal ration; Group 2, basal ration plus 2 per cent cod liver oil; Group 3, basal ration prepared with corn, middlings, and casein which had been heated at 95° for 72 hours; Group 4, same as Group 3 plus 2 per cent cod liver oil; Group 5, basal ration in which the corn was decreased to 52 parts and the casein increased to 18 parts; Group 6, same as Group 5 plus 2 per cent cod liver oil; Group 7, basal ration in which the corn was decreased to 46 parts and the casein increased to 24 parts; Group 8, same as Group 7 plus 2 per cent cod liver oil. This arrangement not only allowed us to study the uric acid content of the blood of vitamin A-deficient and normal chicks, but gave us an oppor-
tunity to determine the influence of the protein intake on the distribution of uric acid in the blood. Groups 3 and 4 were included to determine whether heating would decrease any of the remaining vitamin A in the ration sufficiently to alter the usual results.

Chart I. Growth records of three chicks fed the basal ration alone and two chicks fed the basal ration plus 2 per cent cod liver oil.

All the chicks placed on the basal or the modified basal ration developed the typical symptoms of vitamin A deficiency in the usual length of time. The chicks fed the heated ration did not develop the symptoms at a faster rate, which demonstrates that the basal ration must be very low in vitamin A. The chicks on
Weight gm.

CHART II. Average growth records of the chickens in Groups 2, 4, 6, and 8. All groups received 2 per cent cod liver oil. Group 2 received the basal ration which contained 12 parts of casein; Group 4, the same ration heated at 95° for 72 hours; Group 6, the basal ration which contained 18 parts of casein; and Group 8, the basal ration which contained 24 parts of casein.
Vitamin A Avitaminosis in Chicks

the high protein diet did not differ in their reactions from those on the unmodified basal ration.

The average growth curves for the chicks in the groups receiving cod liver oil are plotted in Chart II. It is readily seen that the changes in the basal ration had little effect on the growth of the chicks in the presence of sufficient vitamin A. The growth of

<table>
<thead>
<tr>
<th>Age</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-6</td>
<td>4.6</td>
<td>5.9</td>
<td>5.2</td>
</tr>
<tr>
<td>6-9</td>
<td>3.8</td>
<td>6.6</td>
<td>5.1</td>
</tr>
<tr>
<td>9-12</td>
<td>4.4</td>
<td>5.4</td>
<td>4.9</td>
</tr>
<tr>
<td>12-15</td>
<td>3.7</td>
<td>6.8</td>
<td>5.9</td>
</tr>
</tbody>
</table>

TABLE II
Effect of Protein Intake on Uric Acid Content of Blood of Normal Chicks

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Ration</th>
<th>Uric acid per 100 cc. whole blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>12 parts casein</td>
<td>4.8</td>
</tr>
<tr>
<td>4</td>
<td>12 &quot; (heated)</td>
<td>4.9</td>
</tr>
<tr>
<td>6</td>
<td>18 &quot;</td>
<td>4.8</td>
</tr>
<tr>
<td>8</td>
<td>24 &quot;</td>
<td>5.4</td>
</tr>
</tbody>
</table>

the animals on the heated ration was slightly less than the other groups and the early increment in the weights of the birds receiving 18 per cent casein was the greatest, although the increase for the chicks on the lowest level of casein was equally as good after the 3rd week.

The blood for analysis was obtained from the external jugular, and the uric acid content was determined by Benedict's method (11). In Table I are shown the average figures for normal chicks, produced on the basal ration plus 2 per cent cod liver oil. The results indicate that the average figure for chicks up to 12 weeks
of age is 5.0 mg. per 100 cc. of blood. There is a tendency for the uric acid to increase slightly with age.

The effect of the protein intake on the uric acid content of blood in the presence of sufficient vitamin A is given in Table II. Again the average figures check very well with the normal of 5 mg. per 100 cc. The figure for the chicks on the highest casein level is slightly higher but probably not enough to be significant. In the case of the vitamin A-deficient chicks, the uric acid content of the blood was found to be more closely related to factors other than the protein intake.

In Table III we have given the uric acid values for a number of

<table>
<thead>
<tr>
<th>Age of chick</th>
<th>Uric acid per 100 cc. whole blood</th>
<th>Eye</th>
<th>Locomotion</th>
<th>Kidney and ureters</th>
<th>Age of chick</th>
<th>Uric acid per 100 cc. whole blood</th>
<th>Eye</th>
<th>Locomotion</th>
<th>Kidney and ureters</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
<td>mg.</td>
<td></td>
<td></td>
<td></td>
<td>days</td>
<td>mg.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>5.8</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>34</td>
<td>22.5</td>
<td>+</td>
<td>+</td>
<td>+++++</td>
</tr>
<tr>
<td>25</td>
<td>8.7</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>36</td>
<td>6.7</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>11.8</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>36</td>
<td>6.9</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>4.8</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>36</td>
<td>6.9</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>9.8</td>
<td>-</td>
<td>+</td>
<td>++++</td>
<td>36</td>
<td>7.1</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>26</td>
<td>30.2</td>
<td>+</td>
<td>+++</td>
<td>+++++</td>
<td>36</td>
<td>8.6</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>4.4</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>36</td>
<td>9.7</td>
<td>-</td>
<td>+</td>
<td>+++++</td>
</tr>
<tr>
<td>27</td>
<td>4.8</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>41</td>
<td>44.4</td>
<td>+</td>
<td>++</td>
<td>+++++</td>
</tr>
<tr>
<td>27</td>
<td>5.1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>43</td>
<td>8.1</td>
<td>-</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>27</td>
<td>7.1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>43</td>
<td>9.3</td>
<td>-</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>24.6</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>47</td>
<td>7.1</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>28</td>
<td>7.7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>47</td>
<td>11.3</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>29</td>
<td>11.5</td>
<td>-</td>
<td>+</td>
<td>+++</td>
<td>48</td>
<td>25.3</td>
<td>+</td>
<td>++</td>
<td>+++++</td>
</tr>
<tr>
<td>32</td>
<td>5.4</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>48</td>
<td>29.6</td>
<td>+</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>32</td>
<td>6.7</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>59</td>
<td>7.8</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>32</td>
<td>7.2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>60</td>
<td>37.0</td>
<td>+</td>
<td>+</td>
<td>++++</td>
</tr>
</tbody>
</table>

Eye. - indicates normal; +, some soreness.
Locomotion. - indicates normal; +, difficulty in walking; ++, unable to stand.
Kidney and Ureters. - indicates no abnormal amount of urates; +, small amount; ++, large amount; ++++, very large amount; ++++, kidneys impaired and ureters distended with urates.

In Table III we have given the uric acid values for a number of
individual vitamin A-deficient chicks. The gross symptoms and autopsy findings for each animal are also included. It is evident that a number of interesting conclusions can be drawn from these results. The uric acid figures vary from the normal (5 mg. per 100 cc. of blood) to values as high as 44 mg. per 100 cc. of blood. The uric acid values cannot be correlated with the age of the chick. There is a tendency for the uric acid values to become greater as the degree of incoordination progresses. However, a number of the chickens exhibited great difficulty in locomotion before the uric acid value had increased much above normal, which indicates that the incoordination noted is not due to an increased uric acid content of the blood.

It is more likely that the incoordination observed is due to nerve degeneration. Hughes et al. (7) have shown that this is the case in the pig, and Mellanby (12) has recently reported that young puppies kept on vitamin A-deficient diets exhibit degenerative changes in the spinal cord in the form of demyelination of the nerve fibers. Studies on the cord changes in the chick are now in progress.

The high uric acid content of the blood of a number of the chickens indicated that the normal elimination of uric acid is disturbed during vitamin A deficiency. A general correlation was noted between the uric acid content of the blood and the degree of kidney hypertrophy. This led us to make a histological comparison of the kidneys of normal and vitamin A-deficient chicks. Whole kidneys were removed carefully from the surrounding tissue in each chick, after autopsy, and fixed immediately in Bouin's fluid. After fixation the tissue was hardened in graded alcohol, cleared, and embedded in paraffin. Cross-sections from each lobe of individual kidneys were mounted and stained with iron-hematoxylin-eosin.

Sections from the kidneys of chicks exhibiting extreme vitamin A deficiency showed in every case a slight nephrosis and an occasional parenchymatous degeneration. In most cases the degeneration was in the proximal convoluted tubules, while the distal portion of the collecting tubules and ducts of Bellini had undergone considerable dilatation. The cytoplasm of the cells in the proximal part of the tubule was clear in the basal portion while the granular material was massed in the periphery or distal portion
of the cell. The basal portion often contained fat globules. Some of the tubules contained leucocytes, giant cells, and cellular débris; others, considerable colloidal material. Practically all the sections showed hyaline deposits in both arteries and glomeruli. Kidneys from chicks with large amounts of uric acid in the blood showed the most marked degeneration.

These results demonstrate that definite pathological changes occur during vitamin A deficiency, which suggests that the uric acid metabolism is not disturbed, but that the structure of the kidney is damaged sufficiently to prevent the normal elimination of uric acid. In this respect it is interesting to refer to the work of McCarrison (13) on the relation of vitamin A deficiency to urinary calculi. He reports the incidence of renal calculi in 22 per cent of the rats raised on diets compounded from food in common use by the people of India. This condition was prevented by the addition of vitamin A. He states that no tissue suffers more severely from want of vitamin A than that of the urinary tract and suggests that the keratinized epithelium from this tract may form the nidus around which calculus deposition occurs.

Since birds make no attempt to destroy uric acid and also convert the greater part of their urea to uric acid for elimination, the uric acid content of the blood of this species is more readily affected by degenerative changes in the kidney than the blood of other animals. In the rat the amount of uric acid excreted is very small. This fact undoubtedly explains the results reported by Osborne and Mendel (14) and van Leersum (15); namely, that the urinary calculi observed in rats on a vitamin A-deficient diet consist chiefly of calcium salts.

Man occupies an intermediate position between birds and reptiles and mammals other than man in regard to the destruction of uric acid. The changes observed in the uric acid elimination in the chick during vitamin A deficiency, therefore, are probably more applicable to man than those observed in other animals.

**SUMMARY**

1. A standard ration for the production of vitamin A avitaminosis in the chick is described.

2. Growth curves for chicks reared on this ration alone and on the basal ration plus 2 per cent cod liver oil are given.
3. The typical symptoms of vitamin A deficiency in the chick are described.

4. The uric acid content of the blood of normal chicks is approximately 5.0 mg. per 100 cc. of whole blood. The amount of uric acid in the blood is independent of the protein intake.

5. The uric acid content of the blood of vitamin A-deficient chicks varies from normal values to amounts as high as 44 mg. per 100 cc. of blood.

6. The kidney undergoes definite pathological changes during severe vitamin A avitaminosis. The amount of uric acid in the blood is dependent upon the degree of kidney damage. The degree of incoordination is independent of the uric acid content of the blood.

7. Vitamin A deficiency does not disturb uric acid metabolism, but does injure the structure of the kidney sufficiently to prevent normal elimination of uric acid.

We are indebted to Mr. Paul H. Phillips for assistance in the examination of the histological sections.

BIBLIOGRAPHY
STUDIES IN VITAMIN A
AVITAMINOSIS IN THE CHICK
C. A. Elvehjem and V. F. Neu

J. Biol. Chem. 1932, 97:71-82.

Access the most updated version of this article at http://www.jbc.org/content/97/1/71.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/97/1/71.citation.full.html
#ref-list-1