THE NUTRITIONAL REQUIREMENT OF THE CHICKEN.

VI. DOES THE CHICKEN REQUIRE VITAMIN C?*

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In 1922 (1) we stated that the water-soluble and antiscorbutic vitamin requirement of chicks can be met by the amounts of these substances contained in a cereal grain and skimmed milk. Further experiments in chick feeding have fully confirmed this statement. On rations made from the grains supplemented with raw liquid skimmed milk or with milk powder no evidence of scurvy in the chicken develops. However, from such experiments it is always possible to conclude that sufficient antiscorbutic vitamin was contained in the ration to protect the chick, although such a ration would not protect the guinea pig from scurvy, unless the milk consumption was liberal.

In order to study the problem more fully, it was decided to rear chicks on rations either freed from the antiscorbutic vitamin by heat or on rations made of purified food materials and presumably free from vitamin C. Should there be an accumulation of the antiscorbutic vitamin in the liver of chicks so reared, then such evidence would strongly support the idea that the chick does not require vitamin C preformed in the diet in the same sense as does the guinea pig, but in all probability can build it from certain dietary complexes.

The method used in testing the accumulation of vitamin C in

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chicken livers was to feed these livers at different levels of intake to scurvy guinea pigs. This method had already been used by Parsons (2), Parsons and Hutton (3), and Leptovsky and Nelson (4) in their study of the accumulation of vitamin C in rat livers. Recently Carrick and Hauge (5) have used this method in studies of the accumulation of vitamin C in the livers of chicks fed scurvy diets and reached conclusions similar to those announced by us earlier (1) and also in agreement with the facts presented in this paper.

EXPERIMENTAL.

The accumulation of vitamin C in the livers of animals on scurvy rations may be explained in several ways; it may represent a power on the part of the animal to concentrate vitamin C in its liver when present in minute amounts in the ration; it may represent a power on the part of the animal to activate some inactive precursor; or it may represent a power on the part of the animal to synthesize this vitamin. The experimental animals whose livers are to be tested should be on the scurvy ration the shortest time possible so as to provide the least opportunity for concentration in their livers of any of this vitamin that may be contained in the ration. Carrick and Hauge (5) are the only workers who have published data on the potency of chicken livers in respect to vitamin C when fed on a scurvy diet. They used a group of cockerels of the American variety which had been fed on a ration consisting of degerminated yellow corn, tankage, yeast, and a salt mixture. The birds fed on this ration were used in other experiments on polyneuritis, but were completely cured before being used for the scurvy test. The length of time the chickens were on the experimental ration is not stated, but this is a very important factor in such studies. We cannot by any means say that yellow corn, tankage, and yeast are absolutely free from vitamin C. That guinea pigs succumb to scurvy on such a ration simply shows that there is insufficient vitamin C present in the ration to protect guinea pigs from scurvy; it does not disprove the presence in the ration of minute amounts of vitamin C which might very well be concentrated in chicken livers over a long period of feeding.

On November 29 a group of Plymouth Rocks was started on the "Wisconsin Chick Ration" (6) consisting of 80 parts of ground
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yellow corn, 20 parts of standard wheat middlings, 5 parts of raw bone, 5 parts of pearl grit (crushed marble), 1 part of common salt, and skimmed milk _ad libitum_. The skimmed milk was autoclaved for 1 hour at 15 pounds pressure in order to destroy vitamin C. In addition the birds were irradiated 10 minutes daily with ultra-violet light to prevent leg weakness or rickets.

On this ration, which can be considered a fairly good scorbutic ration, the chickens grew very well and on February 11, the feeding of livers from these chickens began. The chickens were on the Wisconsin Chick Ration for 73 days when the liver feeding began; they were killed daily; their livers were removed and passed through a wire screen to remove the connective tissue and vascular materials and the remaining liver pulp fed to scorbutic guinea pigs from a syringe at levels of 1 to 3 gm. daily. The guinea pigs were brought down with scurvy on our scurvy-producing ration consisting of 69 parts of rolled oats (coarsely ground), 25 parts of alfalfa meal heated for 30 minutes at 15 pounds steam pressure, 5 parts of crude casein, and 1 part of common salt.

The guinea pigs receiving 1 gm. of liver daily showed some improvement but were not completely cured nor did they gain weight (see Chart I); after 17 days of liver feeding they still showed signs of scurvy, indicating that 1 gm. of this chicken liver fed daily was just on the scorbutic border-line and somewhat insufficient for a complete cure. The guinea pigs receiving 3 gm. of liver were cured at once, as can be seen in Chart I.

The results secured with the above ration of natural materials, particularly the grain portion, is open to the same criticism which we made of the results obtained by Carrick and Hauge (5); namely, that the ration may have contained traces of vitamin C which could be concentrated in the liver of the chickens. It has been pointed out by Eddy, Kohman, and Carlsson (7) and by Cavanough, Dutcher, and Hall (8) that natural food materials may be subjected under certain conditions to high temperatures for considerable lengths of time without destroying vitamin C. Consequently, we resorted to the use of a ration composed of purified food materials and which would be as free from vitamin C as can be prepared in any considerable quantity. Further, it is entirely possible to grow chickens on purified diets in which the presence of vitamin C must be greatly reduced, if present at all.
On March 10 a group of Rhode Island Reds was started on the following synthetic ration:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrin</td>
<td></td>
<td>65</td>
</tr>
<tr>
<td>Casein</td>
<td></td>
<td>18</td>
</tr>
</tbody>
</table>

CHART 1

Recovery of Guinea Pigs from Scurvy When Fed Chicken Livers

Dextrin ........................................... 65
Casein ......................................... 18
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Yeast ........................................................ 10
Salt Mixture 32 .............................................. 5
Agar ............................................................ 2
Saponified cod liver oil (equivalent to 5 per cent of raw oil).

The dextrin was prepared by moistening corn-starch with water and heating it for 1 hour at 15 pounds pressure. It was then dried at 60–70°C. for at least 2 weeks and ground. The agar was dried at 60–70°C. for 2 months and ground. The casein was extracted with 95 per cent alcohol for 1 week by letting it soak in the alcohol in large percolators. The alcohol was drawn from the casein every day and a fresh supply immediately added. This was continued for 1 week. The casein was then dried at 60–70°C. for 24 hours. The yeast was dried brewers' yeast. The temperature to which it had been exposed was unknown to us. The cod liver oil was saponified by boiling 275 cc. of the oil in 500 cc. of 20 per cent KOH, cooling, and diluting with 2000 to 3000 cc. of water. The mass was then extracted with ether, two extractions of about 500 cc. each being made. Portions of the ether extract were evaporated on the ration in order to make the amount introduced equivalent to approximately 5 per cent as raw oil. Salt Mixture 32 (9) was used in the make-up of the ration.

It was felt that this ration was as free from vitamin C as can at present be conveniently prepared in any quantity and yet obtain fairly good growth of chicks. Every constituent of the ration with the exception of the salts had been subjected to considerable heat in the presence of oxygen, which is an effective combination for the destruction or inactivation of vitamin C. The chickens grew rather slowly on this ration and were somewhat irregular in size and weight. On June 2 (84 days after the chicks were started) they were killed daily as needed and their livers fed to scorbutic guinea pigs. The chickens weighed about 300 to 600 gm. with an average of about 450 gm. when killed. As can be seen from Chart I, 3 gm. of the liver fed daily cured scorbutic guinea pigs at once and 1 gm. of the liver fed daily improved scorbutic guinea pigs sufficiently so that they even gained slightly in weight; but when discontinued they still showed unmistakable signs of scurvy. It is fairly certain then that livers from chickens fed on a purified diet as free from vitamin C as it was possible to prepare readily in considerable amounts and suitable for the
growth of chickens contained as much vitamin C as livers from chickens fed on a diet of natural foodstuffs. Apparently the chicken, like the rat, does not need vitamin C preformed in the diet in the same sense as does a guinea pig; but its presence in large amounts in the livers of chickens even on synthetic diets indicates that it plays some rôle in its metabolism. Whether the chicken synthesizes this vitamin from an inactive precursor or from some other organic complex cannot at present be stated.

SUMMARY.

1. Chicks fed a ration of grains and heated skimmed milk, presumably free or at least very low in its content of vitamin C, did not suffer from scurvy. Further, their livers were an abundant source of vitamin C which, when fed at a level of 3 gm. daily, cured guinea pigs suffering from scurvy.

2. Chicks fed a ration of purified food materials—dextrin, casein, salts, etc.—did not develop scurvy. The livers from these chickens were also potent sources of vitamin C.

BIBLIOGRAPHY.

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