PHOSPHORUS PARTITION IN THE BLOOD SERUM OF LAYING HENS*

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Much work has been done during recent years in investigating the calcium partition in the blood of laying hens. From this work an attempt has been made to determine the nature of the non-diffusible calcium. Although Laskowski (1) and Benjamin and Hess (2) investigated the partition of inorganic phosphorus in connection with the study of calcium in the serum of the laying hen, little has been done concerning the partition of total phosphorus.

Since it has been shown by Hughes (3) that the total phosphorus as well as the calcium increases during the laying season, it was deemed advisable to investigate more thoroughly the partition of phosphorus in the serum of the laying hen.

EXPERIMENTAL

The blood serum of laying hens, non-laying hens, and mature males was analyzed for total, lipoid, total acid-soluble, inorganic, and protein phosphorus, and for calcium. The ultrafiltrate of the serum was analyzed for inorganic phosphorus.

The samples were obtained by heart puncture. The ultrafiltrates of the serum were obtained by the method described by Nicholas (4). Calcium was determined by the method of Roe and Kahn (5) and inorganic phosphorus by Youngburg and Youngburg's method (6).

The organic fractions of phosphorus were determined colorimetrically by a revised method (to be published) in which the samples were dry ashed with magnesium nitrate. By protein

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Phosphorus is meant the fraction which remains in the trichloroacetic acid precipitate after repeated extractions with a 10 per cent trichloroacetic acid solution and a boiling alcohol-ether mixture. Iron determinations were made according to the method described by Elvehjem (7).

**DISCUSSION**

Data are presented on three groups of chickens: laying hens, non-laying hens, and males. The separation of the hens into laying and non-laying groups is based chiefly on the concentration of calcium in the serum rather than their egg production record. No doubt there are several birds included in the group of laying hens whose egg production is quite low.

**TABLE I**

*Average of Results of Analyses of Chicken Sera*

The results are expressed as mg. per 100 cc. of serum.

<table>
<thead>
<tr>
<th></th>
<th>No. of birds</th>
<th>Phosphorus</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total - Lipid Acid-</td>
<td>Total - Inorganic</td>
<td>Ultrafilterable</td>
<td>Ultrafilterable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lipoid soluble</td>
<td>Lipid soluble</td>
<td>Inorganic</td>
<td>Inorganic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>7</td>
<td>13.2</td>
<td>7.7</td>
<td>5.7</td>
<td>-0.16</td>
<td>5.4</td>
<td>3.6</td>
<td>11.2</td>
</tr>
<tr>
<td>Non-laying hens</td>
<td>4</td>
<td>11.4</td>
<td>6.7</td>
<td>4.1</td>
<td>0.60</td>
<td>3.8</td>
<td>2.5</td>
<td>11.9</td>
</tr>
<tr>
<td>Laying hens</td>
<td>12</td>
<td>35.2</td>
<td>20.1</td>
<td>5.4</td>
<td>9.7</td>
<td>5.0</td>
<td>1.8</td>
<td>20.0</td>
</tr>
</tbody>
</table>

From Table I it can be seen that in the serum of males and non-laying hens, the total phosphorus is practically equal to the sum of the lipid and acid-soluble fractions. This conforms with the results given for the sera of mammals.

The analyses of sera of laying hens show a marked difference in that the total phosphorus is much greater than the sum of the lipid and acid-soluble fractions. This indicates the presence of an additional fraction, most likely a phosphoprotein. Protein phosphorus as determined on the serum compared closely with the value calculated by subtracting the sum of the lipid and acid-soluble phosphorus from the total phosphorus. In five samples of sera of laying hens the protein phosphorus averaged 10.52 mg. per 100 cc., while the calculated value was 10.33 mg.
Another difference noted is the large increase in lipoid phosphorus in the serum of laying hens over that of males or non-laying hens. These differences are not surprising since the yolk of the egg contains a large amount of lecithin and phosphoprotein (vitellin).

In a paper appearing shortly after the completion of this work, Heller, Paul, and Thompson (8) gave results which showed that the total and lipoid phosphorus of the blood plasma of the hen increase during the laying season. However, they apparently overlooked the possibility of an additional phosphorus fraction, since they attempted to account for the increase in total phosphorus only by the increase in lipoid phosphorus.

While attempting to determine the nature of the phosphoprotein of the serum of the laying hen, we noticed that its properties resembled those given by Jukes and Kay (9) for vitellin. The protein phosphorus of the serum is quite resistant to acid hydrolysis, being only slowly hydrolyzed by concentrated hydrochloric acid at room temperature. It is also resistant to pepsin hydrolysis. It was found that, although the bulk of precipitated protein was much less in the pepsin-hydrolyzed serum than in the untreated serum, the protein-phosphorus fraction remained practically unchanged.

According to Hugounerq and Morel (10), the pepsin-resistant fraction of vitellin contains 1 part of iron to 19 of phosphorus. Analyses of the pepsin-resistant protein fractions of six samples of serum of the laying hen gave an average of 1 part of iron to 13.8 of phosphorus. However, the pepsin-resistant fraction of rooster serum also contained a small amount of iron, even though the phosphorus was negligible. Serological tests gave some indication that the vitellin of the egg yolk is similar to the phosphoprotein of hen serum. These tests are being continued to determine more definitely the relation between the two proteins.

If the phosphoprotein of hen serum is vitellin, it would be of interest to know of the manner in which it is transferred to the yolk; i.e., whether it is transferred directly or undergoes hydrolysis and resynthesis. Jukes and Kay (11) also have shown a similarity between the levitin of the yolk and fowl serum and Fraser, Jukes, Branion, and Halpern (12) have traced diphtheria antitoxin from the serum of a duck, through the egg yolk, to the serum of the hatched duckling.
The results given in Table I also show that the percentage of ultrafiltrable inorganic phosphorus of laying hens is about one-half that of males and non-laying hens, even though the total inorganic phosphorus may be equal. This is in agreement with the results of Laskowski (1), although it is difficult to understand how the small increase in non-filtrable inorganic phosphorus could account for the large increase of non-filtrable calcium in the serum of the laying hen, as he has suggested.

Although in general the protein and lipoid fractions of phosphorus increase along with the total calcium, no definite relation could be found between the non-filtrable calcium and the two phosphorus fractions. The work is being continued in an attempt to determine whether the phosphoprotein or phospholipids may not account for a part of the non-filtrable calcium of the serum of the laying hen.

SUMMARY

The total phosphorus of the serum of the laying hen is about three times that of males and non-laying hens.

The lipoid phosphorus of laying hens is also about three times that of males and non-laying hens.

The inorganic and acid-soluble fractions differ only slightly in the three groups of chickens.

The percentage of ultrafiltrable inorganic phosphorus in the serum of laying hens is about one-half that for roosters and non-laying hens.

In the serum of males and non-laying hens the total phosphorus is equal to the sum of lipoid and acid-soluble fractions. In the serum of the laying hen the total phosphorus is greater than the sum of the lipoid and acid-soluble fractions, indicating the presence of a phosphoprotein.

The phosphoprotein in the serum of the laying hen was found to have properties similar to those of vitellin. Serological tests give some indication that the vitellin of the egg yolk is similar to a protein in the serum of laying hens.

BIBLIOGRAPHY

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