The inorganic calcium and phosphorus content of the blood serum has been of interest in mineral metabolism studies for many years. With an increased knowledge of the importance of these ions in certain deficiency diseases, numerous investigations have been made of the calcium-phosphorus ratios existing in these conditions. In nutritional disturbances in chickens an even greater problem is presented due to the occurrence of various bone maladies not recognized in mammals and for which no satisfactory explanation has been offered by the calcium-phosphorus balances. It, therefore, becomes necessary to seek further explanation of these conditions. In a previous article from this laboratory (1), it was reported that the phosphorus content of the blood of the fowl is peculiar in that it is much greater than that of mammals and the inorganic phosphorus content of the serum, as usually determined, composes only a small part of the whole. The theory was offered that other fractions possibly contribute to bone formation as well as the inorganic phosphorus. In the case of the calcium content an equally unique condition exists. In mammals this ion changes very little throughout the life cycle but in fowls the calcium increases over 100 per cent at the time of egg production. The fact that the total calcium content consists of several fractions has been reported by Benjamin and Hess (2), by Correll and Hughes (3), and by this department (4). The possibility that
Blood Ca and P in Chicken

changes may occur in some of the fractions which would not be indicated by the usual determinations has suggested a partition study throughout the complete life cycle of the hen.

EXPERIMENTAL

300 white Leghorn chickens of the same strain and age were secured from the college Poultry Department. These were placed in brooders, and from this group two lots of 50 healthy female chickens were selected, placed in pens, and fed an all mash ration composed of cereal grains supplemented with animal and vegetable proteins, vitamins, and adequate amounts of calcium and phosphorus in the postulated correct proportions. Analyses of the rations showed the protein, calcium, and phosphorus percentages to be 17, 1.21, and 0.776, respectively. Records of food consumption, growth curves, and frequent examinations were made so that none but healthy, active birds were kept in the lots.

The blood was drawn in all cases by heart puncture. For phosphorus determinations it was delivered into chilled oxalate-coated flasks and separated into plasma and cells by immediate centrifuging according to methods described in a preceding article (5). This procedure was followed because it has been demonstrated that there are changes in the phosphorus fractions if the serum is secured by slow coagulation. The method of analysis was similar to that outlined in a previous publication (1). The blood for calcium studies was similarly drawn but permitted to coagulate. The serum so obtained was analyzed by the methods outlined by Benjamin and Hess (6).

These analyses were repeated once a month or oftener beginning at the time the chickens were 1 month of age and continuing through the periods of growth, egg production, and subsequent molting. The results, which are graphically illustrated, present interesting and surprising data which demonstrate that the usual determination of only the inorganic calcium and inorganic phosphorus contents of the serum fails to present a true picture of the changes taking place in the blood of the fowl.

In Chart I are curves showing the fractions of phosphorus determined both in the cells and plasma throughout the experimental period. These percentages have been calculated in terms of parts of the total blood content and are represented as ordinates...
in the figure. The abscissa divisions show ages in terms of months. It will be noted that there is a gradual increase in the total phosphorus for the first 5 months and then a rapid rise at the time of production, this high level being held during the entire production period with some fluctuations and dropping quickly as production ceases and molting season approaches. This condition is true for
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total phosphorus of both cells and plasma. An examination of the other fractions reveals that this increase is due to the lipid fraction of both cells and plasma, while the inorganic and acid-soluble fractions remain rather fixed at all ages. We have previously called attention to the fact that this inorganic fraction, which is usually used in blood studies, does not give the true picture of phosphorus conditions. An examination of the above data reveals that the fraction which is responsible for changes must be the more complex lipid fraction. Had the inorganic fraction only been determined, as is the usual custom in making such studies, little noticeable change would have been observed throughout the entire life cycle, yet certain profound changes must have been taking place to cause the fluctuations as indicated by the lipoid phosphorus curves. These findings are somewhat contradictory to the theory often expressed that the inorganic fraction is responsible for calcification. Possibly the lipid could be considered as a storage form, but the quick changes occur so nearly at the same time as egg production that this theory is difficult to explain.

The blood for calcium studies was also obtained by heart puncture but was delivered into chilled flasks where it was permitted to coagulate normally. After 2 hours the coagulum was broken up and placed in tubes and the serum separated by centrifuging. The method of making calcium distribution determinations as outlined by Benjamin and Hess (6) was followed in detail.

The samples for analysis were obtained every 2 weeks from the time the chickens were 4 weeks old until they had passed through production and into the molting stage. It is apparent that the total calcium varies from 12 or less mg. per 100 ml. of serum for non-laying chickens to 24 mg. for laying hens. 2 weeks after production had started the total calcium was still rising slowly, as is illustrated in Curve 1 of Chart II. Curves 2 and 3 for protein-bound and total adsorbable calcium show the same general trend. However, when the total adsorbable calcium is divided into its components, the non-filtrable adsorbable calcium (Curve 5) and the filtrable adsorbable calcium (Curve 4), it is found that marked changes are taking place. The non-filtrable adsorbable complex is not present in significant quantities in the young hen, but makes its appearance about 8 weeks before egg production begins, at which time about 2 mg. per 100 ml. are present. At the time of
production this value ascends rapidly to over 8 mg. The converse is true of the filtrable adsorbable calcium, the degree of variation being much less, which drops from 6 to less than 4 mg. per 100 ml.

(Curve 4). Curve 6 shows only a slightly upward trend for the ionic fraction with values varying from 2 to 4 mg. From these results it appears that the non-filtrable adsorbable complex form of cal-
Blood Ca and P in Chicken

cium is the most closely associated with the process of egg-shell formation and it must be the fraction which is physiologically active and therefore responsible directly or indirectly for the larger portion of calcium used in egg production. If this fraction is the one that contributes to shell production there is evidence that it may also contribute to various forms of ossification. Again, as in the case of the determination of total inorganic phosphorus of the blood serum, the inorganic calcium as commonly determined does not fully picture all the changes taking place in the animal so that the determination of various fractions would better picture conditions accompanying malformation of bone.

The error is not as noticeable as with the phosphorus, however, as the inorganic calcium of serum measures practically all the calcium of the blood, since the cells are almost devoid of calcium and practically all forms are transformed into and accounted for by the usual method of determining inorganic calcium. This is not the case with phosphorus, as more phosphorus is present in cells than in serum and any hemolysis taking place causes the serum to be contaminated by the phosphorus of the cells. Furthermore, the common method of determining inorganic phosphorus accounts for only a small part of that present in plasma, as was more fully explained in previous articles.

Studies of Osteoporotic Chicks

An abnormal condition, often encountered in chicks kept in brooders, referred to by poultrymen as hock disease, porosis, or slipped tendons, and thought to be due to a deficient or unbalanced diet, has been extensively studied in this country. The symptoms in certain respects resemble those of rickets; consequently, many attempts have been made to diagnose this condition by blood and bone analyses as has been done in the study of rickets.

Many calcium and phosphorus ratios and distributions have been made in this laboratory during the past 3 years. It was found necessary to make the determinations reported in the first section of this paper due to unexplained changes in the blood analyses of the normal group. It having been established that there are normal changes taking place, caused by age, sex, and other factors, calcium and phosphorus distribution studies were
made of groups of normal and osteoporotic chickens of definite sex and age, using methods previously mentioned. The averages of five or more sets of analyses are presented in Tables I and II. Table I lists the calcium distribution of the blood of normal and osteoporotic chickens of the same age and origin. A comparison of the two sets of analyses is of interest because the total calcium contents of the serum of the two are apparently similar, although changes are noted in certain fractions. These changes are sig-

**Table I**

*Calcium Distribution of Blood of Normal and Osteoporotic Chickens*

<table>
<thead>
<tr>
<th>Group of chickens</th>
<th>Total Ca per 100 ml. serum</th>
<th>Protein-bound mg.</th>
<th>Adsorbable mg.</th>
<th>Adsorbable, filtrable mg.</th>
<th>Adsorbable, non-filtrable mg.</th>
<th>Ionized remainder mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal.............</td>
<td>15.1</td>
<td>2.9</td>
<td>8.5</td>
<td>2.0</td>
<td>6.5</td>
<td>3.7</td>
</tr>
<tr>
<td>Osteoporotic.......</td>
<td>14.0</td>
<td>2.9</td>
<td>7.6</td>
<td>4.4</td>
<td>3.2</td>
<td>3.5</td>
</tr>
</tbody>
</table>

**Table II**

*Phosphorus Distribution of Blood of Normal and Osteoporotic Chickens*

Values are given in mg. of P per 100 ml. of blood.

<table>
<thead>
<tr>
<th>Group of chickens</th>
<th>Total P</th>
<th>Cell P</th>
<th>Plasma P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Lipid</td>
<td>Inorganic</td>
</tr>
<tr>
<td>Normal.............</td>
<td>115.3</td>
<td>100.0</td>
<td>6.4</td>
</tr>
<tr>
<td>Osteoporotic.......</td>
<td>119.6</td>
<td>106.8</td>
<td>6.3</td>
</tr>
</tbody>
</table>

significant because the same trends are displayed by individual distributions within each group.

While the methods have not had sufficient application to make positive statements as to their value in predicting the occurrence of this malady, it is believed that information obtained by such analyses may be used in making changes in the rations in order to eradicate such conditions.

Table II presents the average phosphorus distributions of the cells and plasma of normal and abnormal 8 week-old chickens.
In general, statements made in regard to the calcium distributions hold for the phosphorus data, except that in the case of the phosphorus analyses the changes are observed in the totals and there are small changes in the inorganic fraction of the plasma, the only determination ordinarily made in most laboratories. It is believed that with the accumulation of such data, there will be found a close correlation between phosphorus and calcium partitions and abnormal bone developments.

**SUMMARY**

1. Analyses of complete calcium and phosphorus distribution in chicken blood have been made at regular intervals throughout the life cycle of the chicken.

2. It has been observed that there are definite changes not only in the totals but in certain partitions at various periods.

3. The variations in different fractions are not proportional, the adsorbable filtrable calcium decreasing at the time of egg production, while the other fractions greatly increase.

4. The totals for both calcium and phosphorus show pronounced increases at the time of egg production and return to former levels at molting time.

5. Comparison of the distributions of the blood of normal chickens and of those afflicted with osteoporosis reveals a significant tendency toward regular changes in certain fractions of both calcium and phosphorus, indicating the possibility of correlating such analyses and ossification.

**BIBLIOGRAPHY**


CHANGES IN THE BLOOD CALCIUM AND PHOSPHORUS PARTITION DURING THE LIFE CYCLE OF THE CHICKEN

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