Visceral lymphomatosis is both an infectious and a neoplastic disease. A method for the identification of the infectious stage has not been developed, but the study of plasma phosphatase activity suggests that the activity of plasma from chickens in which neoplastic lymphoid tumors are present may be significantly different from that of nonlymphomatous birds. We are concerned in this study with three separate and distinct phosphatases; acid phosphatase, alkaline phosphatase, and adenosine triphosphatase.

It is well known (8) that the presence of a tumorous condition sometimes produces considerable changes in the enzyme pattern or in the concentration of certain components of the blood. This change in enzyme pattern has served as an excellent diagnostic tool in detecting the presence of certain types of neoplastic growths. A striking increase in acid phosphatase (acidPH) levels occurs in the plasma of patients with cancer of the prostate (9), and it has been shown that increased alkaline phosphatase (alkPH) activity frequently accompanies osteolytic and lymphoid tumors (5, 11, 16). Mommaerts et al. (13, 14), studying the plasma adenosine triphosphatase (ATPase) activity of chickens with erythromyeloblastic leukemia, found that a close relationship exists between enzymatic activity and infectivity of the plasmas. They found no activity in the plasma from normal birds.

**MATERIALS AND METHODS**

Two inbred lines (6 and 15), of Single-Comb White Leghorn chickens, developed at this Laboratory (19), served as a source of plasma from normal, disease-free birds. The data were analyzed as a single unit, since a comparison of lines showed no significant differences.

Line 15 chickens which had been inoculated at 1 day of age with strain RFL 12 lymphomatous liver filtrate (1) and which had developed lymphomatosis were the sources of plasmas from tumor-bearing chickens.

Blood samples were obtained by pricking the cubital vein and allowing the blood to be drawn into Pyrex capillaries 1.5—2.0 mm. × 100 mm. Twenty-four hours before taking blood, the capillaries were rinsed with phenol-free heparin. The residual heparin coating the lumen of the capillaries was sufficient to prevent clotting. This procedure minimizes the possibility of heat damage to the blood cells and the subsequent release of intercellular phosphatase. The cells were sedimented in a refrigerated centrifuge (5 minutes at 5,000 × g). Frequent blood samples can be taken from the same series of birds by this method without producing excessive trauma.

In determining alkaline phosphatase activity, 1—5 μl of cell-free plasma was pipetted into 15-ml Pyrex conical centrifuge tubes containing the phosphate substrate. Dilution of the plasma was avoided by varying the amount of plasma used. The volume used in obtaining the final reading varied inversely with the phosphatase activity; i.e., when the activity was found to be high (5—25 days post-hatching), only 1 μl of plasma was used; and, conversely, as the activity decreased, the amount of plasma was increased accordingly. This permitted the percentage of transmission readings to fall within the 85—70 per cent range. If differences between duplicate readings exceeded 5 per cent, the procedure was repeated.

A modification (15) of the King-Armstrong phenyl phosphate method was used in determining the acidPH and alkPH activity. All analyses were made immediately after taking the blood sample. The enzyme activator, magnesium chloride, was added to the phenyl phosphate substrate in sufficient quantity to give a final concentration...
of 0.01 M. A Coleman spectrophotometer (Model 6A) was used to determine the percentage of transmission at 660 m\(\lambda\). ATPase activity was measured by the micro-method of Green et al. (7), who demonstrated a close linear relationship between the reaction time of this micro-method and the amount of phosphorus liberated from adenosine triphosphate, as determined electrochemically.

RESULTS

**Acid phosphatase.**—Plasma acidPH determinations were made on 100 normal and 42 tumor-bearing chickens. The acidPH activity varied in the normal, noninfected chickens from a low of 0.12 to a high of 0.4 acidPH unit, expressed as \(\mu g\) of phenol/\(\lambda\) plasma with an average of 0.33 acid PH unit. Determinations on the tumor-bearing chickens gave a range of 0.2 to 0.42 acidPH unit, with an average of 0.33 unit. Since there was no significant difference in the ranges or means, it would appear that plasma acidPH activity was not influenced by the presence of lymphoid tumors.

**Alkaline phosphatase.**—The alkaline phosphatase activity of the plasma from 660 normal chickens, ranging from 1 to 500 days in age, is presented graphically in Chart 1. The activity is expressed as \(\mu g\) of phenol\(^1\)/\(\lambda\) plasma in 1 hour/\(\lambda\) plasma. The points on the curve are averages, and the ranges are indicated by the vertical bars. The number of samples involved is indicated by the figures at each plotted point.

The activity was low at hatching—4 \(\mu g\) phenol/\(\lambda\) plasma—but began to rise on the 2nd day. It reached a peak of 81 \(\mu g\) between the 9th and 16th day, then began to fall rapidly again, so that it reached a low level of 2.5 \(\mu g\) phenol/\(\lambda\) plasma by 100 days of age. From 100 days to 500 days the alkaline phosphatase remained constant. At these low levels there was little variation among individual birds, but during the period of rapid growth after hatching, the phosphatase values varied over a wide range.

The alkPH activity in the plasma of the 42 tumor-bearing chickens is presented in the graph of Chart 2 along with the mean alkPH activity of normal chickens at 100, 125, and 250 days of age. The data show that the enzyme activity in 37 of the 42 birds was much lower than that of normal birds. The remaining five gave activities the same as or much higher than those of the normal chickens.

**Adenosine triphosphatase.**—Plasma ATPase activities in terms of minutes required to shift the pH of the substrate mixture from 8.2 to 6.6 by 3\(\lambda\) of plasma are summarized in Table 1. The data show that in the plasma of all except two of the 42 tumor-bearing birds the pH was reduced in less than 20 minutes, whereas in none of the 297 normal controls did this occur in the same period. When the incubation period was extended to 30 minutes, a positive test for ATPase activity was obtained in two controls. When the

\(^1\) Conversion factors: 1 \(\mu g\) phenol is equal to 1 alkaline phosphatase unit and equal to 0.33 acid phosphatase unit.
time was further extended to 2 hours, it was found that the plasma of all chickens tested, normal or tumor-bearing, had a positive reaction for ATPase activity.

To check on the possible effect of atmospheric CO₂ and the possibility that the plasma had a naturally low pH, the following controls were added to each ATPase determination: (a) salt solution, brom thymol blue, ATP, but no plasma—a reduction in pH, upon incubation, would indicate absorption of CO₂ from the air; (b) salt solution, brom thymol blue, 3 λ of plasma, but no ATP—in this case any lowering of pH would be due to the plasma, since there are no acidic ions present in the incubation medium. It was found that no change in pH occurred unless the substrate (ATP) and the plasma were present in the incubating media. Hence, all changes in pH were due entirely to the release of —PO₄ ions from the ATP, and there is strong evidence that this release was mediated by a dephosphorylating enzyme (ATPase).

**DISCUSSION**

The alkaline phosphatase activity of most of the tumor-bearing chickens was significantly below the average and fiducial limits of that for the normal birds of a comparable age, although a few exceptions to this were obtained. A previous histochemical study of normal and lymphomatous livers (Lesher and Lucas, unpublished data) showed that alkaline phosphatase accumulates in the tumor areas and is reduced in the adjacent liver cells. This low plasma alkPH activity of tumor-bearing chickens may be due to the high demand of the rapidly proliferating tumor cells for alkPH, with the result that very little is allowed to escape and circulate in the plasma. A second possibility is that the neoplasm interferes with the normal metabolism of the liver sufficiently to result in a reduction in the transfer of alkPH to the plasma.

The striking changes in plasma phosphatase activity during the early development period following hatching merit special attention. Alkaline phosphatase is believed to have a close relationship to the calcification of bone (2, 5, 16, 18). Kay (11) found that the first histological signs of ossification and the first chemically detectable appearance of phosphatase activity were almost simultaneous. Serum or plasma phosphatase activity closely parallels changes in bone phosphatase; i.e., changes brought about by normal embryonic and post-embryonic bone formation and pathological conditions affecting bone.

If plasma phosphatase is closely correlated with bone phosphatase (6, 10) and if the latter is involved in calcification, then bone formation should parallel the pattern of plasma phosphatase activity. Very little information on bone formation in the chicken is available beyond that published by Latimer (12), based on skeletal weights at widely separated ages following hatching. An analysis of Latimer's curve for bone weight, which may be considered to be a delayed measure of osteogenic activity, revealed that the ratio of bone weight to body weight increases for the period shortly after hatching up to 25 days of age and then declines to 80 days, after which period a constant ratio is maintained. Latimer's data lack information on the period immediately following hatching, which is a critical one for the plasma phosphatase activity; but beyond this point there is a fair measure of agreement between the two curves, and from this comparison it might be inferred that phosphatase activity roughly parallels osteogenic activity. It is also possible that the rapidly growing post-embryonic tissues may also contribute to the high activity immediately following hatching.

Adenosine triphosphatase presented an entirely different picture in that it was greatly increased in chickens having visceral lymphomatosis. This finding is similar to that of Mommaerts et al. (13, 14) for the plasma of chickens having another type of leukemia;—namely, erythromyeloblastosis. The shift in pH of the substrate mixture containing plasma of normal birds after a 2-hour incubation period may indicate the presence of small amounts of this enzyme in plasmas of normal birds, although Eckert et al. (3) concluded that it was absent in such plasmas. Furthermore, there may be some question as to the specificity of the micro test after a 2-hour incubation period.

It has been conclusively demonstrated (3, 7, 14, 17) that the adenosine triphosphatase activity in plasmas of birds having erythromyeloblastosis is an intrinsic part of the virus particle causing this disease. Whether or not this activity is also a

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**TABLE 1**

| TIME REQUIRED FOR INDIVIDUAL PLASMA FROM NORMAL AND LYMPHOMATOUS CHICKENS TO LOWER THE PH TO 6.6 |
|---|---|---|
| **NO. OF PLASMA SAMPLES** | **TIME (MINUTES)** | **1 HOUR OF INCUBATION** |
| Tumor-bearing chickens | | |
| 42 | 4 | 12 | 10 | 14 | 0 | 2 |
| Normal chickens | 297 | 0 | 0 | 0 | 0 | 2 | 295 |

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**Lesher and Burmester—Plasma Phosphatase Activities in Chickens**
characteristic of the virus causing visceral lymphomatosis remains to be determined. It would appear significant that, on the basis of the use of 3\textsuperscript{a} of plasma and a 20-minute incubation period, 95 per cent of the lymphomatous plasmas gave a positive reaction, while all 297 plasmas from normal birds gave a negative reaction.

SUMMARY

A study was made of the activity of acid phosphatase, alkaline phosphatase, and adenosine triphosphatase in the plasma of normal and lymphomatous chickens.

The acid phosphatase activity of plasma of all normal and lymphomatous chickens tested was very low, with no significant differences between the two types.

A study was also made of the alkaline phosphatase of normal chicken plasma for the period from hatching to 500 days of age. It was found that alkaline phosphatase activity increased rapidly in the chick plasma immediately following hatching, reaching a peak between the 5th and 16th day, then gradually decreasing by 100 days to a low level which was maintained to 500 days of age. Alkaline phosphatase activity of plasma from lymphomatous chickens was found to be considerably lower than that of the nonlymphomatous chickens.

High levels of adenosine triphosphatase activity were found in plasma of 40 of the 492 lymphomatous birds tested, while none of 297 normal birds had a reaction in 20 minutes; however, after an incubation period of 2 hours, all plasma gave a positive reaction.

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Plasma Phosphatase Activities of Normal and Lymphomatous Chickens

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