Plasma Changes in Fowls with a Transmissible Multiple Lymphoma

Laura E. Krejci, Morris H. Ross, and Lucile Sweeny

(From the Biochemical Research Foundation, Newark, Delaware)

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Few studies have been made of the blood proteins of tumor-bearing animals. Sanders, Huddleson and Schaible (8) studied the pooled serum of chickens infected with various types of fowl leukosis, and Sharp, Taylor, Beard and Beard (9) the serum of papillomatous rabbits. Changes in the serum proteins were found by both groups of investigators.

In the present investigation the Tiselius method (11) was utilized to follow the effect of a rapidly growing and metastasizing transmissible multiple fowl lymphoma on the plasma proteins of individual chickens. A sharp decrease in albumin was found to accompany growth of the lymphoma. Although liver damage resulting from metastasis of the lymphoma seemed a plausible basis for this decrease, liver damage resulting from exposure to carbon tetrachloride vapors failed to produce a comparable change.

When spontaneous regression of the lymphoma occurred, there was an abrupt increase in gamma globulin. The injection of inactive lymphoma tissue, which produced no tumor growth, gave rise to plasma changes similar to those observed during regression; these changes were not produced by injection of normal liver tissue. Two chickens which proved immune to repeated implantation of active lymphoma tissue showed no significant change in plasma composition.

MATERIALS AND METHODS

Chickens.—University of Delaware line-bred White Rock chickens of the fourth generation were used for all experimental work. Families had been selected on the basis of high production factors and high viability throughout 14 weeks of age, and bred for use as broilers and efficient egg production. No cases of spontaneous lymphomatosis were observed during the entire experimental period in the fowls obtained from this source. The chickens were maintained on a diet of Purina mash for young chicks, scratch feed and pellets for older chickens, and water and grits ad libitum.

Tumor and tumor characteristics.—Fowl lymphoma tissue of strain RPL 16 was obtained from the U. S. Regional Poultry Research Laboratory, East Lansing, Michigan. The strain was carried through 26 transfers with no loss of virulence.

The characteristics of this transplantable strain of lymphoid tumor have been described in detail by Burmester and Prickett (2). The strain was derived from a case of spontaneously occurring lymphomatosis at the U. S. Regional Poultry Research Laboratory. It is characterized by multiple lymphoid tumors, uniformity of reaction, and high virulence. Burmester and Prickett (2) reported that all of the birds inoculated with the strain developed tumors, only about 6 per cent of which regressed, and the remainder of which proved rapidly fatal.

Transfer procedure.—A portion of the actively growing breast tumor and a portion of lymphomatous liver were removed aseptically and minced with fine scissors. The resultant tissue mash was diluted with twice its volume of sterile physiological saline and filtered through a double layer of sterile gauze. Inoculations were made into the right pectoral muscle with 0.5 ml. of the resultant cell suspension. Tumors and livers from a number of donors were macerated together whenever a large number of chickens were to be inoculated. Transfers were made every 7 days.

Electrophoretic analyses.—Electrophoretic analyses were made on plasma samples from individual chickens. Food was withheld for 24 hours before bleeding to eliminate suspended fats in the plasma except when the weakened condition of the animals made it inadvisable. Ordinarily 5 or 6 ml. of blood were drawn by cardiac puncture and centrifuged in tubes containing 0.8 mgm. of dry lithium oxalate as anticoagulant; when successive blood samples were drawn at short intervals, these quantities were halved. The plasma was diluted immediately with 3 (occasionally 2, 4, or 5) volumes of diethyl barbiturate buffer solution of pH 8.6, ionic strength 0.1, and dialyzed against two changes of the same buffer solution. Electrophoretic measurements were made either in the single section Tiselius cell or in the lower half of the double cell, at a potential gradient of about 12.0 V/cm. Photographs were taken by both the Svensson (10) and Longsworth (5) methods for 4 different periods of migration. Protein concentrations were computed from area measurements made on enlarged tracings of the four Svensson diagrams, corrected for the delta and epsilon effects, and expressed in refractive increments.
One hundred and fifty-five electrophoretic analyses were made on the plasmas of 108 chickens: 60 on the plasmas of 43 tumor-inoculated chickens, 49 on plasmas of 36 normal chickens, and the remainder on plasmas of chickens subjected to various types of treatment. In the graphical presentation of the data (Figs. 1 to 6) the concentrations of alpha and beta globulin and of fibrinogen and gamma globulin have been plotted together as the sums, both to simplify the graphs and to eliminate the effect of compensating errors resulting from poor resolution.

**PLASMA PROTEIN CONCENTRATIONS OF NORMAL CHICKENS: INFLUENCE OF AGE AND SEX**

The plasma protein concentrations of healthy, uninoculated chickens at ages of 28, 61-2, 75, and 91 days are given by the solid black symbols in Fig. 1. It is evident that the composition of chicken plasma may vary between fairly wide limits at each of the age levels investigated. While the concentration range for the fraction alpha plus beta globulin was about the same at all 4 age levels, the mean for the fraction gamma globulin plus fibrinogen was somewhat lower at 28 days than later. The mean albumin concentration dropped appreciably between 28 and 61 days, but appeared to rise slightly between 61 and 91 days.

The sex differences found in mature chickens (6) were not apparent at 91 days (Fig. 1). The concentration ranges were wider for females, however, than for males (Fig. 1).

**PLASMA CHANGES PRODUCED BY INOCULATION WITH ACTIVE TUMOR TISSUE**

The effect of tumor growth on the protein composition of the plasma is shown best by the data on successive blood samples from individual chickens. Normal chickens inoculated with tumor cell suspension, 5 at the age of 61 days and 4 at the age of 75 days, were bled just before inoculation and twice weekly thereafter until death or sacrifice; 5 chickens 61 days old served as controls and were bled at the same times. In Figs. 2, 3, and 4 the plasma protein concentrations are plotted against the time in days following inoculation; points referring to the same chicken are connected by straight lines. The isolated points in the diagrams refer to single determinations on additional tumor-bearing chickens.

Tumor growth.—No significant change or trend in the protein composition of the plasma of normal chickens was disclosed as the result of successive bleedings (Fig. 2). On the other hand, the six chickens that developed tumors proving fatal within a week to 10 days of inoculation (Fig. 3) showed without exception an abrupt decrease in albumin concentration. Of the 3 inoculated chickens sur-

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**Fig. 1.—Plasma protein concentrations: ▲ ● ■, normal chickens; ○ △, tumor-resistant chickens; × □, chickens injected at the age of 52 days with minced normal liver tissue.**

**Fig. 2.—Effect of successive bleedings on the plasma protein concentrations of normal chickens.**

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viving the 16-day period of observation (Fig. 4), two (A and B), which developed large tumors, also showed this decrease; the third (C), which developed only a small tumor, did not. The albumin concentration, therefore, was consistently reduced during rapid tumor growth. A second group of fowls that were inoculated at the somewhat younger age of 47 days, but for which the plasma composition prior to inoculation was not determined, also appeared to exhibit reduced concentrations of albumin 8 days after inoculation (Fig. 3, A and B, isolated points).

In still younger chickens inoculated at ages of 28, 33, or 34 days, and in 2 inoculated at 45 and 82 days respectively, rapid tumor growth was accompanied by low concentrations not only of albumin, but of the globulins as well (Fig. 3C, isolated points). While the albumin concentrations appeared to become progressively lower with time following inoculation, the concentrations of the globulins, particularly the alpha globulin, showed a tendency to rise, with the result that the alpha globulin fraction ultimately dominated the plasma pattern.

Tumor regression.—Of the 3 inoculated chickens that survived the 16 day period of observation (Fig. 4), two (C, which developed a small tumor, and B, which developed a large tumor) began to exhibit tumor regression about ten days after inoculation. Regression was in both instances accompanied by an abrupt increase in the concentration of gamma globulin. This sharp increase was not shown by the third chicken (Fig. 4A), the tumor of which remained large to the end.

A similar contrast in plasma composition resulting from tumor regression, as distinct from further
tumor development, was shown by 2 pairs of chickens, 1 inoculated at 47 days, the other at 75 days. Fourteen days after inoculation 1 member of each pair gave evidence of tumor growth, the other of tumor regression. The latter pair (Fig. 4B, 14 days) showed an elevated content of gamma globulin; the former pair (Fig. 4A, 14 days) did not.

The chickens represented by the isolated points in Fig. 4, C all exhibited early, rapid, and complete regression. The trend toward higher albumin and lower globulin concentrations in these chickens reflects the tendency for the plasma pattern to return to normal when regression is complete.

The plasma composition of the chickens in which delayed tumor regression occurred at 16 and 21 days is plotted in Fig. 4, B. While the changes accompanying this condition were similar in direction to those accompanying earlier regression, they were in general more profound. Elevation of the globulin levels was not restricted to the gamma globulin, but embraced the alpha plus beta globulin fraction as well.

**Tumor-resistant chickens.**—Two chickens proved resistant to repeated inoculations with active tumor tissue. Plasma obtained on the 67th day, following inoculations on the 42nd and 55th days, showed no departure from the normal (Fig. 1, O △, 67 days). A third inoculation on the 75th day, a fourth inoculation on the 98th day 4 days after x-irradiation with 300 r, and a fifth inoculation on the 116th day immediately after x-irradiation with 600 r, caused in one of the chickens no change of plasma composition with respect to the earlier bleeding, but in the other a reduction in albumin (Fig. 1, O △, 133 days).

**INOCULATION WITH INACTIVE TUMOR TISSUE**

A specimen of tumor tissue RPL 17 from the U. S. Regional Poultry Research Laboratory proved inactive. This strain was carried through three passages without demonstrating tumors macroscopically at necropsy 10 to 12 weeks after inoculation. Plasma obtained from the animals of the first passage showed abnormalities in protein composition similar to those found during tumor regression: reduced concentrations of albumin and increased concentrations of gamma globulin (Fig. 5). These deviations from the normal were less pronounced in the chickens of the second transfer, and still less in those of the third. The average albumin:globulin ratio rose from 0.378 for the first transfer to 0.479 for the second and 0.549 for the third.

**INJECTION OF NORMAL LIVER TISSUE**

To test whether liver tissue contaminating the inactive tumor tissue used as inoculum might have contributed to the plasma changes, several chickens were injected with minced normal liver tissue. None of these chickens gave evidence for a drop in albumin concentration, and only one showed a significant increase of gamma globulin (Fig. 1, X □, 91 days).

**LIVER DAMAGE**

To determine whether the plasma changes accompanying tumor growth were the result of liver damage resulting from metastatic infiltration, 14 chickens were exposed to carbon tetrachloride vapors thrice daily for 12 days during a 2 week period. Two ounces of carbon tetrachloride were used for each animal, and the vapors were administered in a wooden box, about 4,000 cu. in. in capacity, equipped with a small motor-driven fan and a window for observation. Immediately at the onset of anaesthesia (usually after about ten minutes) the animal was removed. At necropsy the livers were enlarged and very pale, in color a light brown, and the kidneys appeared to be somewhat reduced in size.

In spite of the liver damage, none of the five
chickens surviving the treatment showed a decrease in albumin concentration (Fig. 6). The reduced albumin concentrations which accompanied tumor development, therefore, appear not to have been directly attributable to concomitant liver damage.

- **Fig. 6.**—Effect of exposure to carbon tetrachloride vapors on the plasma protein concentrations. Points referring to the same chicken are connected by lines. Solid black symbols: untreated chickens. Line symbols: chickens exposed to carbon tetrachloride vapors at the times indicated by arrows.

**X-IRRADIATION OF TUMOR-BEARING CHICKENS**

X-irradiation of tumor-bearing chickens decreased the rate of growth of the tumor. The doses used were apparently of no therapeutic value, however, for the irradiated animals died earlier than the non-irradiated controls. Although too few electrophoretic experiments were carried out to warrant definite conclusions, the gamma globulin concentrations appeared to be increased, as in normal rats (13).

**DISCUSSION**

All but two of the 328 chickens inoculated with the RPL 16 strain of lymphoma developed tumors. Unless spontaneous regression occurred, the tumors proved fatal within 2 or 3 weeks.

The changes in plasma composition which accompanied the development and the regression of the lymphomas were consistent and reproducible. Rapid growth of the tumors was accompanied by a reduction in albumin concentration; in very young chickens, or in chickens which for some other reason were apparently more susceptible, there was a reduction of all the globulins as well. When tumor regression occurred, there was an abrupt rise in the concentration of gamma globulin, which appeared to be greater the longer regression was delayed, and which occasionally included the alpha and beta globulins as well. Complete regression appeared to be followed by a return to the normal. The plasma changes following inoculation of inactive tumor tissue were similar to those found during tumor regression.

The factors directly responsible for the albumin depletion accompanying rapid tumor growth are difficult to assess. While the reduction in albumin concentration might reasonably be attributed to the failure or impairment of albumin synthesis as a result of liver damage caused by massive tumor infiltration, it was not duplicated in chickens subjected to liver damage by exposure to carbon tetrachloride vapors. The severe depletion of albumin during tumor growth may be due to increased utilization of the albumin, or to competition for the materials from which it is synthesized, by the rapidly dividing tumor cells for their growth, and by the lymphocytes evoked by the host in response to the disease.

During regression the lymphoidal-like elements that compose the tumor undergo destruction. The lymphocytes and lymphocytic tissue elaborated by the host in response to the disease may also be destroyed. Recent evidence indicates that lymphocytes and lymphocytic tissue are a source of gamma globulin (3), which can be liberated into the plasma through adrenal cortical stimulation or by direct destruction of the tissue (13). Penetrating radiations are known to cause lymphocyte destruction (12, 7), and increased concentrations of gamma globulin have been observed in rats following large doses of x-rays (13) and in chickens following irradiation with neutrons (4). The large increase in gamma globulin which was found to accompany tumor regression may thus have been a direct result of the spontaneous destruction of the lymphoidal-like cells and lymphocytes produced during tumor growth.

The few chickens which exhibit regression and thus survive inoculation with the lymphoma appear to be immune to further implantation of the same strain (1). The extent to which immune bodies compose the gamma globulin produced during regression has not yet been determined. Sanders, Huddleson, and Schaible (8) attributed the elevated concentrations of gamma globulin in their leucosis-affected chickens to the formation of an antibody which they called the L component. Since in the
chicken, however, gamma globulin is the plasma constituent most susceptible to change, even in pathological conditions which do not elicit antibody formation (4), the increase during regression may consist to a large extent of non-specific protein.

SUMMARY

Electrophoretic analyses were made of the blood plasma of chickens inoculated with the RPL 16 strain of lymphoma. Rapid tumor growth was consistently accompanied by a reduction in albumin concentration, occasionally by a reduction in the concentrations of the globulins as well. The concentration of gamma globulin rose abruptly when tumor regression occurred; complete regression appeared to be followed by a return to the normal. The inoculation of inactive tumor tissue caused plasma changes similar to those found during tumor regression.

REFERENCES

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