AN ELECTROPHORETIC STUDY OF SERUM AND PLASMA FROM NORMAL AND LEUCOSIS-AFFECTED CHICKENS

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AN ELECTROPHORETIC STUDY OF SERUM AND PLASMA FROM NORMAL AND LEUCOSIS-AFFECTED CHICKENS

by

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An Electrophoretic Study of Serum and Plasma from Normal and Leucosis-Affected Chickens

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AN ELECTROPHORETIC STUDY OF SERUM AND PLASMA FROM NORMAL AND LEUCOSIS-AFFECTED CHICKENS.*

It has been observed by poultry pathologists that when chickens are affected with leucosis they may show one or more pathological manifestations. It is a debated question whether the etiological agent is the same in all the known manifestations of the disease. In erythroblastic leucosis, according to Johnson (16), all stages of red cells appear in the peripheral blood, in the capillaries of the visceral organs, and in the sinusoids of the marrow. In granuloblastic leucosis all stages of immature granulocytes appear in the peripheral blood as well as extravascular proliferation in many of the visceral organs. In the other types of the disease there may be an increase of lymphocytes and hemocytoblasts in the peripheral blood. When these types of cells proliferate or infiltrate in the nerves or central nervous system, the iris, or the visceral organs, the disease is termed neuralymphomatosis, ocular lymphomatosis, and visceral lymphomatosis, respectively.

*This study represents a cooperative project of the Chemical Section and the Bacteriological Section of the Michigan State Agricultural Experiment Station and the Poultry Research Laboratory of the United States Department of Agriculture. The material used in the study was furnished by the Poultry Research Laboratory, and the work was carried out in the Brucellosis Laboratory of the Department of Bacteriology.

In neural lymphomatosis paralysis of the legs, wings, or neck may be observed. The involved nerve is yellowish-gray or reddish-gray in color and enlarged, due to the presence of many mononuclear cells. Ocular lymphomatosis is characterized by a gray iris; small, irregular pupil; asymmetrical pupillary vacillations; and the carriage of the head. The iris and ciliary body are thickened by the presence of many mononculear cells. Subcutaneous, muscular, or visceral tumors are present in visceral lymphomatosis. Malignant mononuclear cells are aggregated in foci or are diffused throughout the organs or tissues involved. In osteopetrotic lymphomatosis the bones are enlarged, dense, and hard with a rough, irregular, porous surface. The enlargement of the bones results from the formation of new abnormal bone. A chicken affected with erythrogranuloblastosis is usually dull, weak, and anemic; it usually has an enlarged cherry-red liver, spleen, and kidney and many immature cells of the erythrocytic and granulocytic series in the blood.

The terms used in describing the types of the disease and the pathological changes by which they are characterized were adopted by a conference of investigators held at the U. S. Regional Poultry Research Laboratory (3) (7).

Investigators differ as to whether each of the various types of leucosis is caused by the same or a different agent. It was thought, therefore, that a study of the blood serum and plasma proteins of affected chickens by the moving boundary method in the Tiselius electrophoresis apparatus might furnish revealing information and aid in clarifying the present state of knowledge pertaining to the different types of the disease and also show whether there are changes that characterize affected chickens from normal ones.

Electrophoretic analysis by the moving boundary method finds its widest application in the study of changes that occur in the blood proteins accompanying diseases. Characteristic changes in the relative composition of protein components of serum have been noted in many diseases and pathological disturbances in man and animals by this method (4) (18) (29) (30).

PROCEDURE

Chickens of the single combed White Leghorn breed from 15 to 18 weeks of age were used. All the chickens were killed by electrocution and exsanquination and then examined for evidence of infection. Blood was collected from two to nine chickens and pooled. They were usually starved 24 hours previous to bleeding. For plasma samples the blood was collected in 1/100 volume of a 25-per cent solution of sodium citrate. The protein of the serum or plasma sample was determined by the refractive differential method of Siebenmann (26), and the sample adjusted to a protein concentration of 2 per cent with buffer solution. The diluted sample was placed in cellophane tubing and dialyzed against three changes of buffer at 3°C. The final dialysate was saved and used in filling the electrode vessels and cell.

The buffer solution used on all samples consisted of diethyl barbituric acid and sodium hydroxide adjusted to a pH 8.6 and ionic strength of 0.1. Longsworth (14), in a study of 12 buffers, found none superior to this one for the electrophoretic study of human serum and plasma. In this study phosphate buffers of pH 7.7 and ionic strength of 0.1 or 0.2 were found inferior to the barbiturate buffer.

Conductance measurements were made at 0°C. on the buffer and protein after final dialysis. The Shedlovsky type conductivity cell was used (18) (25) in a Dewar vessel containing crushed ice and water.

Electrophoresis was carried out in the modified Tiselius apparatus described by Longsworth and MacInnes (15). The "schlieren scanning method" of Longsworth (13) (18) was used in photographing the moving boundaries. Electrophoresis was carried out at 0°C. and at a potential gradient of 6 to 7 volts per centimeter. The time varied from 10,000 to 12,000 seconds.

The first objective was to obtain the electrophoretic patterns for normal chicken serum and plasma and to establish the mobilities and ratio of normal protein components. After determination of the normals, a comparison was made of serum from chickens showing different pathological manifestations of the leucosis complex. Later, serum samples were obtained from chickens at intervals following injection with a lymphoid tumor and also from chickens injected with this same tumor inactivated so that it consistently failed to produce any manifestations of the disease.

EXPERIMENTAL AND RESULTS

A. Electrophoretic patterns of normal chicken serum and plasma.

Serum and plasma samples were obtained from apparently normal chickens which showed no symptoms or lesions of any disease. Electrophoretic diagrams of these samples are shown in Figs. 1, 2, 3, 4, and 17. Mobilities and composition calculated from the patterns of the descending boundaries are sent forth in Table 1.

San Clemente (22), in his report on patterns, mobilities, and composition of normal, or more correctly, negative pullorum-agglutinating chicken serum, used a phosphate buffer of pH 7.78 and 0.2 ionic strength. The protein components showed lower mobilities and less separation than in barbital buffer. Although good separation of α_1 and α_2 was not attained in all samples in barbital buffer, pH 8.6 and 0.1 ionic strength, no evidence of separation of the two was found in the buffer of lower pH.

The very pronounced γ-globulin disturbance is present in the patterns of all chicken serum and plasma. It appears as a tall spike on all descending patterns and as a small peak on ascending patterns. According to Longsworth, et al. (17) (18) a similar disturbance in the β-globulin is due to convection, resulting from reaction in the neighborhood of the boundary following electrophoretic separation of the constituents. He has shown that at least one of the β-globulins of normal serum has an affinity for lipid material which migrates with the β-globulin. Abramson (1) has discussed the physics of the β-anomaly and other possible explanations.

The position of the disturbance with respect to the mobilities of the β-and γ-globulin is not always the same on the ascending and descending patterns (Figs. 6, 11, and 14).

It may be noted that the total protein in the normal serum samples varied from 2.19 to 3.74 grams per 100 ml. The fibrinogen concentration,

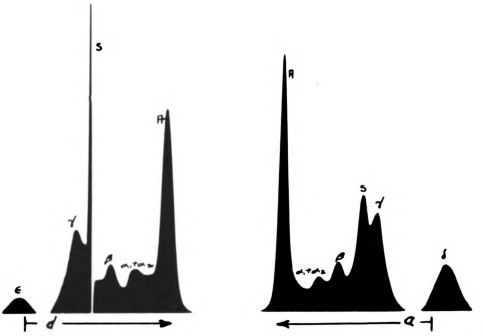

Figure 1. Electrophoretic patterns of normal chicken serum. The patterns were obtained after electrophoresis for 10,000 seconds at 6.03 volts per cm.

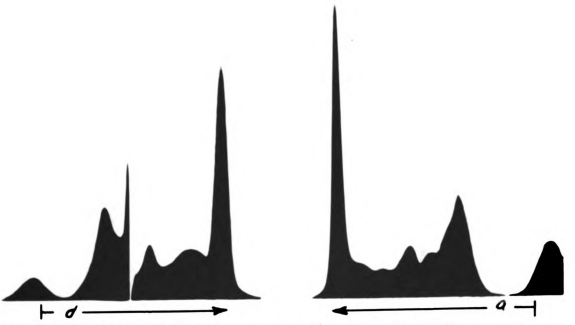

Figure 2. Electrophoretic patterns of normal chicken serum. The patterns were obtained after electrophoresis for 12,000 seconds at 6.09 volts per cm.

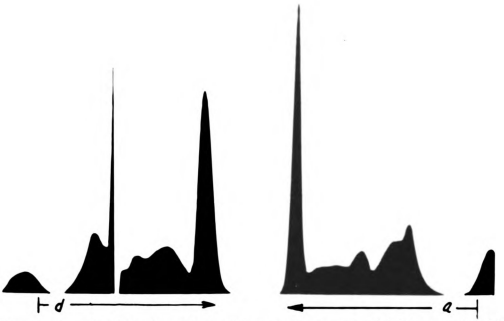

Figure 3. Electrophoretic patterns of normal chicken serum. The patterns were obtained after electrophoresis for 11,200 seconds at 6.75 volts per cm.

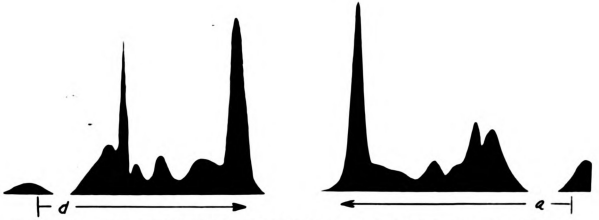

Figure 4. Electrophoretic patterns of normal chicken plasma. The patterns were obtained after electrophoresis for 12,150 seconds at 7.16 volts per cm.

Table 1

Mobility and relative concentration[1] of protein components in normal chicken serum and plasma

Fig. No.	Sample	Mobilities ($\mu^- \times 10^5$)						Gm/100 ml.		Concentrations per cent total area						Composition					
		A	α_1	α_2	β	ϕ	γ	T.P.[2]	Alb.	A	α_1	α_2	β	ϕ	γ	A/G	α_1/A	α_2/A	β/A	ϕ/A	γ/A
1	Serum	6.0	4.5		3.5		2.0	3.74	1.61	43.1	15.6		16.0		25.3	0.76	0.36		0.37		0.59
2	Serum	5.9	4.9		3.5		2.0	2.19	0.92	41.9	20.0		11.7		26.5	0.72	0.48		0.28		0.63
3	Serum	5.8	4.4		3.3		1.9	3.13	1.46	46.5	22.3		11.2		20.0	0.87	0.48		0.24		0.43
17	Plasma	6.0	5.0	4.4	3.6	2.5	2.0	2.48	1.23	49.7	13.4	5.5	9.6	10.6	11.3	1.25	0.27	0.11	0.19	0.21	0.23
4	Plasma	6.1	5.0		3.7	2.6	1.9	2.55	1.08	42.2	15.3		9.9	16.4	16.2	1.02	0.36		0.24	0.39	0.39

1 Calculations from descending patterns
2 T. P. = Total Protein

calculated from percentage of total area in the two normal plasma samples, was 10.6 and 16.4 per cent.

Fig. 2 shows the patterns of serum from apparently normal chickens. However, since the γ-globulin peak shows a slight bulge in the region where a new component was found in serum from leucosis-affected chickens, there is a possibility that the sample was from affected chickens, or at least a portion of the composite sample was from an affected chicken.

B. Electrophoretic patterns of serum from leucosis-affected chickens.

Studies of the various manifestations of the avian-leucosis complex have resulted in differences of opinion as to whether all are manifestations of the same fundamental disease and due to a common causative agent.

Olsen (19) found that none of three experimental strains produced only one type of leucosis but that "the transmissible agent of fowl leukosis is not responsible for conditions known as lymphocytoma or neurolymphomatosis gallinarum." In experiments by Stubbs (27) no tumors were produced, and no paralysis occurred in chickens injected with either blood or tissues from birds showing erythroleucosis and myeloid leucosis. Brandly and associates (2) observed that in six serial passages with "strain 3", lymphomatosis-osteopetrosis, 40.8 per cent of the chickens injected developed various manifestations of lymphomatosis and of osteopetrosis. Of the affected chickens, 21.9 per cent showed evidence of osteopetrosis. In the contact and non-contact controls 21.2 per cent showed lesions of lymphomatosis, but only one control chicken showed osteopetrosis.

The results of Lee, Wilcke, et al. (11) (12) indicate a unity of etiology of erythroleucosis, myeloid leucosis, lymphocytoma, irites, and neurolymphomatosis gallinarum. They reported that the disease in all its

manifestations may be transmitted to healthy chicks by injection of tissue suspensions of affected organs, by injection of cell-free filtrates, by direct pen contact, or by rearing chicks on contaminated litter. In another experiment 80 per cent of the chicks hatched from eggs coming from matings of males and females affected with the iritis form of leucosis succumbed to some form of leucosis complex, that is, iritis, lymphocytoma, myeloid leucosis, or osteopetrosis. In similar experiments (6) (21) others have reached the same conclusions.

In working with a naturally occurring strain of neural lymphomatosis with leucemic tendencies Hall, et al. (5) reported that after 17 serial passages through young chicks the strain became an apparently pure leucosis type. There was a marked reduction in the incubation period and an increase in the percentage of takes. Thus, the agent itself may undergo changes with regard to its pathological manifestation and its qualities.

Serum samples for electrophoretic study were obtained from typical cases of neurolymphomatosis, visceral lymphomatosis, osteopetrotic lymphomatosis, and erythrogranuloblastosis. Electrophoretic patterns of these samples (Figs. 5, 6, 7, and 9) show, in addition to the five normal proteins components, a peak which is designated as the "L" component. The new component is closely associated with γ-globulin and moved with that component in electrophoresis of the neurolymphomatosis serum. Since the "L" component was apparently masked on the descending patterns by the boundary disturbances, calculations of mobility and relative composition were made from the ascending pattern. According to Longsworth (16), close agreement of mobility on ascending and descending patterns may be obtained by using an equation which includes the ratio of area of the ascending peak to the area of the descending peak and correction for the ϵ-boundary. This equation could be applied only to those

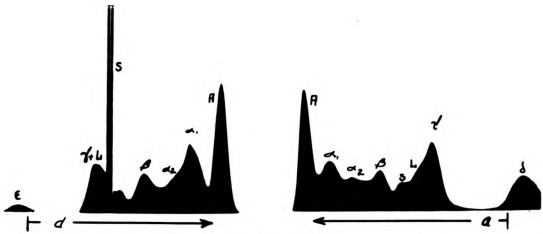

Figure 5. Electrophoretic patterns of composite serum from chickens showing visceral lymphomatosis. The patterns were obtained after electrophoresis for 12,000 seconds at 6.95 volts per cm.

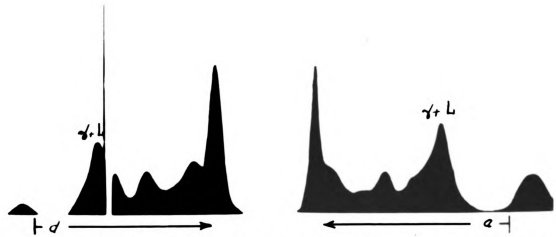

Figure 6. Electrophoretic patterns of composite serum from chickens showing neurolymphomatosis. The patterns were obtained after electrophoresis for 12,000 seconds at 6.84 volts per cm.

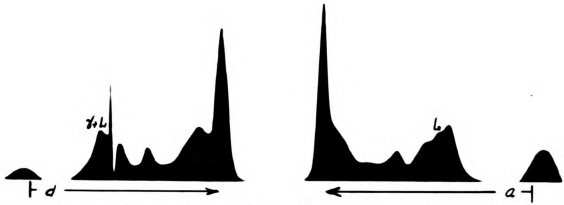

Figure 7. Electrophoretic patterns of composite serum from chickens showing osteopetrotic lymphomatosis. The patterns were obtained after electrophoresis for 12,000 seconds at 6.69 volts per cm.

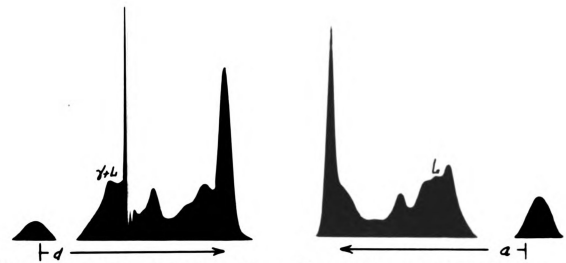

Figure 8. Electrophoretic patterns of composite serum from chickens showing erythrogranuloblastosis. The patterns were obtained after electrophoresis for 12,100 seconds at 6.53 volts per cm.

Table 2

Mobility of the "L" component in chicken serum

Fig. No.	Sample origin	No. of days after injection	Ascending ($u^- \times 10^5$)	Ascending (corrected) ($u^- \times 10^5$)	Descending ($u^- \times 10^5$)
5	Visceral lymphomatosis		2.7		
7	Osteopetrotic lymphomatosis		3.0		
8	Erythrogranulo-blastosis		3.0		
9	Transmissible tumor	3	3.2		
11	Transmissible tumor	7	3.1	2.8	2.7
13	Transmissible tumor	13	3.0		
14	Transmissible tumor	17	2.8	2.5	2.3
15	Transmissible tumor	19			2.6
20	Inactivated transmissible tumor	15	2.9	3.0	2.6 Average = 2.55

Table 3

Relative composition[1] of protein components in serum

and plasma from normal and leucosis-affected chickens

Fig. No.	Sample Origin	Gm/100 ml.		Concentrations per cent total area							Composition						
		T.P.[2]	Alb.	A	α_1	α_2	β	ϕ	L	γ	A/G	α_1/A	α_2/A	β/A	ϕ/A	L/A	γ/A
1	Normal	3.74	1.85	49.4	10.9		13.2			26.6	0.98	0.22		0.27			0.54
2	Normal	2.19	0.91	41.7	8.3	3.8	12.8			32.5	0.72	0.20	0.11	0.31			0.78
3	Normal	3.13	1.45	47.8	8.5	7.8	14.8			21.1	0.92	0.18	0.16	0.31			0.44
17	Normal (3)	2.48	1.17	47.2	15.0		10.9	14.1		13.7	1.22	0.32		0.22	0.30		0.28
16	Transmissible tumor (3)	3.25	0.91	28.0	9.0	6.2	8.6	27.4		20.9	0.39	0.31	0.22	0.31	0.98		0.75
5	Visceral lymphomatosis	3.15	0.90	28.5	17.4	11.3	14.3		11.8	16.7	0.40	0.62	0.40	0.50		0.42	0.59
6	Neurolymphomatosis	3.28	0.99	30.1	14.6	4.8	13.2		37.2		0.43	0.48	0.16	0.44		1.23	
7	Osteopetrotic lymphomatosis	3.04	1.05	34.7	20.4	6.9	11.7		9.2	17.1	0.53	0.59	0.22	0.34		0.27	0.49
8	Erythrogranulo-blastosis	3.18	1.06	33.2	15.2	3.6	14.1		10.5	23.4	0.50	0.46	0.10	0.43		0.32	0.76
9	3 days after injection with tumor	2.91	1.25	43.1	16.5		10.3		13.1	17.1	0.76	0.39		0.24		0.30	0.40
10	5 days " "	3.58	1.38	38.5	14.0		15.7		31.8		0.63	0.36		0.41		0.83	
11	7 days " "	3.17	1.04	32.9	10.1	9.9	15.9		10.7	20.5	0.49	0.31	0.30	0.48		0.33	0.62
12	9 days " "	2.93	1.02	35.0	12.4		14.9		26.7		0.54	0.43		0.51		0.92	
13	13 days " "	2.30	0.84	36.3	12.6	10.0	12.1		10.7	18.4	0.57	0.35	0.27	0.33		0.29	0.51
14	17 days " "	3.18	1.26	39.7	12.1		14.6		10.5	23.2	0.66	0.31		0.37		0.26	0.58
15	19 days " "	3.12	1.19	38.3	10.3	6.7	11.5		33.2		0.62	0.27	0.18	0.30		0.87	
19	7 days after injection with inactivated tumor	3.13	1.31	41.9	18.2		14.2			25.8	0.72	0.43		0.34			0.62
20	15 days " "	3.44	1.41	41.0	7.3	5.4	15.8		11.9	18.6	0.70	0.18	0.16	0.36		0.29	0.45
21	19 days " "	2.86	1.25	43.6	6.5	6.7	13.0		30.3		0.77	0.15	0.15	0.30		0.69	

1 Calculations from ascending patterns-uncorrected
2 T.P. = Total Protein
3 Plasma

patterns in which the area of the "L" component could be measured on both ascending and descending patterns.

In order to make comparisons of mobility of the "L" component calculations were made on all ascending patterns and are included in Table 2. The average value is 2.9×10^{-5}. In a later experiment the "L" component was visible on the descending patterns, and more accurate mobility data were obtained from these.

Included in Table 3 are relative compositions of components of all normal and pathological serum studied. A comparison of normal serum with visceral, neuro-, and osteopetrotic lymphomatosis, and erythrogranuloblastosis shows significant changes in composition. There was an average decrease in albumin content from a normal 46.3 to 32.2 per cent; the α-globulins increased from 13.1 to 20.0 per cent, and γ-globulin decreased from 26.7 to 19.1 per cent. In one sample, neurolymphomatosis, the "L" component moved with the γ-globulin which accounted for 37.2 per cent of the total protein. The "L" component represented an average of 10.5 per cent of the total protein.

Changes in concentrations of the components resulted in the alteration of the albumin/globulin ratio from 0.87 to 0.46.

C. Electrophoretic patterns of serum obtained at intervals after injection with active transmissible tumor.

Following the observation of the presence of the "L" component in serum from chickens showing all the different manifestations of the avian leucosis complex, the next logical step was to correlate the appearance and development of symptoms of leucosis with the appearance of the "L" component in the blood. This study was performed on the blood of chickens

affected with a transmissible lymphoid tumor. The strain used was the one recovered by Olsen (20) and termed "R.P.L. 12" by the Regional Poultry Research Laboratory. Tumors are produced in the muscle tissues of chickens within five days after injection and in the visceral organs after a longer period of time.

Pectoral tumors obtained from three chickens were minced and an equal volume of 0.85-per cent NaCl added. One ml. of this suspension was injected intramuscularly in susceptible chickens. Composite serum samples for electrophoresis were obtained from three chickens at one day intervals after injection. The last was collected on the nineteenth day.

The chickens killed on the third day after injection showed no symptoms or internal lesions. However, the electrophoretic patterns of the serum (Fig. 9) show the "L" component present. The composition data shown in Table 3 indicate a change in the relative compositions of protein components. The albumin content decreased from a normal 46.3 to 43.1 per cent, and γ-globulin from a normal 26.5 to 17.1 per cent.

Small tumors were found present in the pectoral muscle in two of the three chickens examined on the fifth day. An increase in γ-globulin to 31.8 per cent of the total protein indicates that the "L" component moved as a fraction of γ-globulin (Fig. 10). A further decrease in albumin to 38.5 per cent occurred.

From the seventh to the nineteenth day tumors in the pectoral muscles increased in size in all chickens, and intradermal tumors also were present. The "L" component is present in all ascending patterns, as a separate fraction in the seventh, thirteenth, and seventeenth day samples (Figs. 11, 13, and 14), and as a component in γ-globulin in the nineteenth day sample (Fig. 15). The albumin content dropped to a minumum of 32.9 per

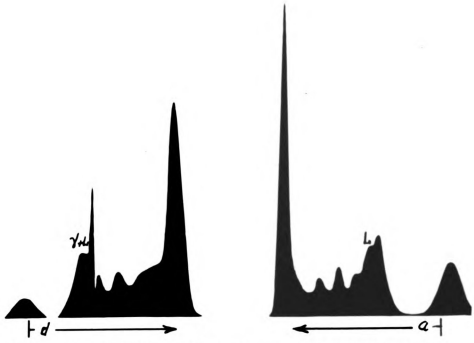

Figure 9. Electrophoretic patterns of composite serum collected from chickens three days after injection with transmissible tumor. The patterns were obtained after electrophoresis for 10,220 seconds at 6.19 volts per cm.

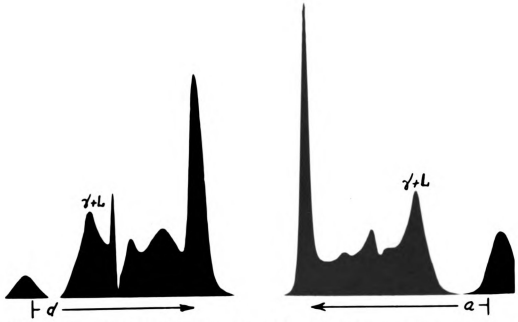

Figure 10. Electrophoretic patterns of composite serum collected from chickens five days after injection with transmissible tumor. The patterns were obtained after electrophoresis for 12,000 seconds at 5.99 volts per cm.

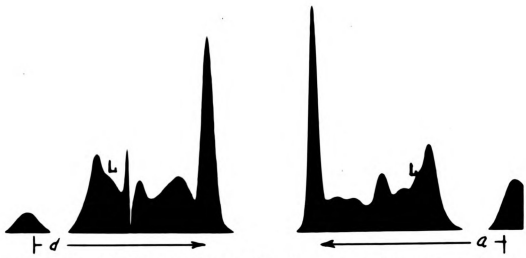

Figure 11. Electrophoretic patterns of composite serum collected from chickens seven days after injection with transmissible tumor. The patterns were obtained after electrophoresis for 11,500 seconds at 6.42 volts per cm.

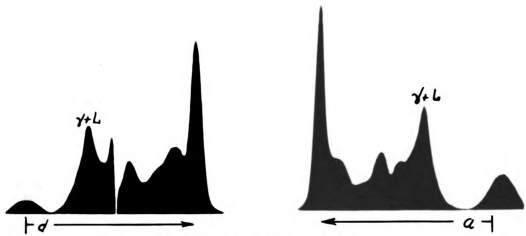

Figure 12. Electrophoretic patterns of composite serum collected from chickens nine days after injection with transmissible tumor. The patterns were obtained after electrophoresis for 12,000 seconds at 5.78 volts per cm.

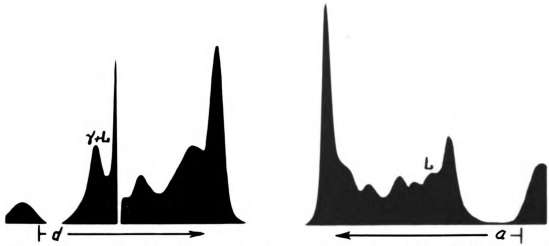

Figure 13. Electrophoretic patterns of composite serum collected from chickens thirteen days after injection with transmissible tumor. The patterns were obtained after electrophoresis for 12,260 seconds at 6.33 volts per cm.

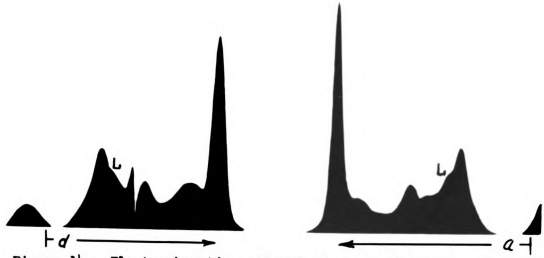

Figure 14. Electrophoretic patterns of composite serum collected from chickens seventeen days after injection with transmissible tumor. The patterns were obtained after electrophoresis for 12,000 seconds at 6.57 volts per cm.

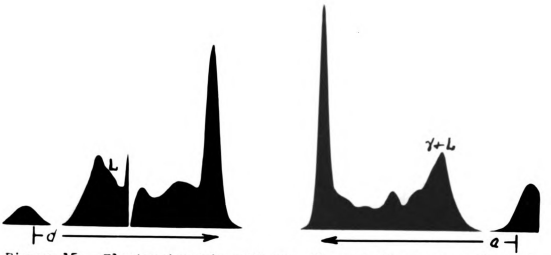

Figure 15. Electrophoretic patterns of composite serum collected from chickens nineteen days after injection with transmissible tumor. The patterns were obtained after electrophoresis for 12,000 seconds at 6.39 volts per cm.

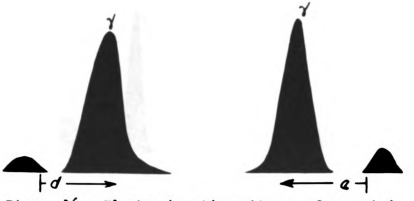

Figure 16. Electrophoretic patterns of a protein fraction removed from composite serum by precipitation with 15 per cent Na_2SO_4. Samples were collected from chickens nineteen days after injection with transmissible tumor. The patterns were obtained after electrophoresis for 12,000 seconds at 6.38 volts per cm.

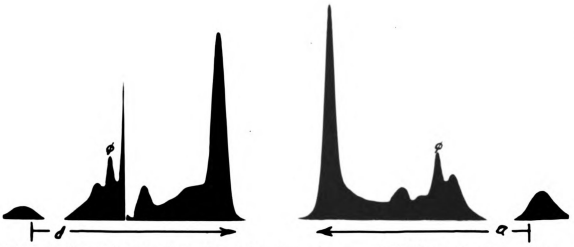

Figure 17. Electrophoretic patterns of normal chicken plasma. The patterns were obtained after electrophoresis for 12,000 seconds at 6.3 volts per cm.

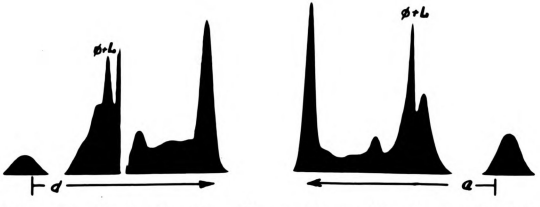

Figure 18. Electrophoretic patterns of composite plasma collected from chickens injected with transmissible tumor. The patterns were obtained after electrophoresis for 12,000 seconds at 6.61 volts per cm.

cent on the seventh day. The albumin/globulin ratio showed a gradual decrease from 0.76 on the third day to a minumum 0.49 on the seventh.

Calculations on the ascending patterns (Table 2) show the average mobility of the "L" component to be -3.0×10^{-5} as compared with the average -2.9×10^{-5} in the earlier studies of serum from chickens showing different types of the avian leucosis complex. The "L" component occurs as a separate peak in both ascending and descending patterns shown in Figs. 11 and 15; a more accurate mobility value for the "L" component was calculated from these patterns, giving an average of -2.5×10^{-5} cm^2 $sec.^{-1}$ $volt^{-1}$.

The early appearance of the "L" component in the serum and the early shift in composition indicate that there is a rapid change in the blood serum proteins of chickens affected with the transmissible tumor agent..

Two electrophoretic patterns of plasma samples from apparently normal chickens (Figs. 4, and 17) were compared with the patterns of a plasma sample from chickens 19 days after injection with a tumor producing agent (Fig. 18). The comparison revealed the same decrease in the albumin/globulin ratio that was observed in serum (Table 3). The normal plasma contained 14.1 per cent fibrinogen; the increase to 27.4 per cent of this component in the leucosis plasma indicates that the "L" component moved at approximately the same rate as fibrinogen.

D. Electrophoretic patterns of serum obtained at intervals after injection with inactivated transmissible tumor.

The inactivated material was prepared from the tumor-producing agent described in Section C, by rapidly freezing to -76°C and thawing twice.

As in the previous experiment, a composite serum sample for electrophoretic study was obtained from three chickens at one day intervals after injection. Tissues taken at necropsy showed no evidence of tumor formation in any of the chickens injected with the inactivated material.

The electrophoretic pattern of the serum sample collected on the seventh day after injection (Fig. 19) is apparently normal. Calculations on the ascending pattern (Table 3) show a decrease in the albumin below that observed in the normal. The fifteenth day sample (Fig. 21) shows the presence of the "L" component, accounting for 11.9 per cent of the total protein in the sample. There is close agreement between the mobility value -2.6×10^{-5} of the "L" component in this serum with that found in serum samples from affected chickens which was -2.5×10^{-5}. The relative compositions of the serum with respect to albumin remained virtually the same as that found on the seventh day, but the γ-globulin decreased to 18.6 per cent.

The electrophoretic patterns of the sample collected on the nineteenth day after injection (Fig. 22) show the "L" component present and moving with the γ-globulin as evidenced by an increase of that component to 30.3 per cent of total protein.

In Table 4 are sent forth the comparative mobility, composition, and ratio values of the proteins in a composite serum sample from normal chickens, one from chickens injected with active tumor agent, and one from chickens injected with inactivated transmissible tumor agent. These values, as calculated from the descending patterns, are more accurate than those calculated for the ascending patterns and shown in Table 3.

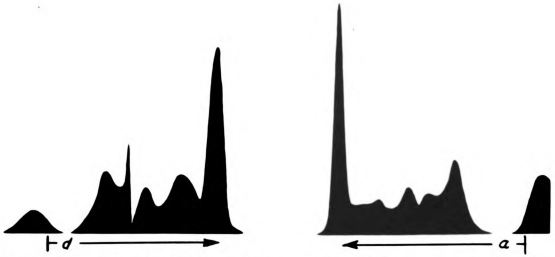

Figure 19. Electrophoretic patterns of composite serum collected from chickens seven days after injection with inactivated transmissible tumor. The patterns were obtained after electrophoresis for 12,620 seconds at 5.88 volts per cm.

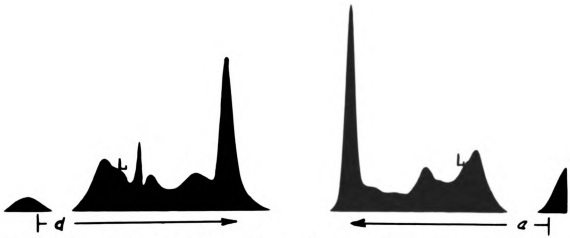

Figure 20. Electrophoretic patterns of composite serum collected from chickens fifteen days after injection with inactivated transmissible tumor. The patterns were obtained after electrophoresis for 12,330 seconds at 6.47 volts per cm.

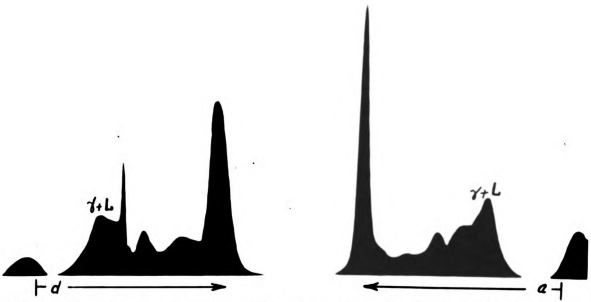

Figure 21. Electrophoretic patterns of composite serum collected from chickens nineteen days after injection with inactivated transmissible tumor. The patterns were obtained after electrophoresis for 12,000 seconds at 6.64 volts per cm.

Table 4

Mobility and relative composition[1] of protein

in three types of chicken serum

Fig. No.	Type of Serum	Mobilities ($u^{-} \times 10^5$)					Gm/100ml.		Concentrations per cent total area					Composition				
		A	$\alpha_1+\alpha_2$	β	L	γ	T.P.[2]	Alb.	A	$\alpha_1+\alpha_2$	β	L	γ	A/G	$\alpha_1+\alpha_2$/A	β/A	L/A	γ/A
2	Normal	5.9	4.9	3.5		2.0	2.19	0.92	41.9	20.0	11.7		26.5	0.72	0.48	0.28		0.63
15	Transmissible tumor	6.0	4.8	3.6	2.6	2.1	3.12	1.24	39.9	21.9	10.1	9.2	18.9	0.66	0.55	0.25	0.23	0.47
20	Inactivated transmissible tumor	6.0	5.0	3.5	2.6	2.0	3.44	1.46	42.3	17.8	11.3	9.5	18.9	0.73	0.42	0.27	0.23	0.45

1 Calculations from descending patterns
2 T.P. = Total Protein

DISCUSSION

The electrophoretic analysis of the proteins in serum from chickens affected with leucosis revealed the presence of a protein component which is not found in normal chicken serum. The new component is closely associated with γ-globulin and has been designated by the letter "L".

The "L" component in blood serum samples taken from chickens that showed gross pathological conditions of leucosis represented approximately 10 per cent of the total serum proteins. It does not seem likely that the protein of the leucosis agent would be present in blood serum in such a high concentration. By crude electrophoretic methods, Lee and Wilcke (10) studied the filtrate of a tumor-producing material and reported that the causative agent of fowl leucosis migrated to the cathode at pH 4.01 to 6.01 and to the anode at pH 7.01 to 9.01. They found that the causative agent was inactivated at a pH of 4 and 9.

Previous electrophoretic studies of blood serum have shown that the antibody proteins are closely associated with γ-globulin and migrate as a component of that globulin or as a separate fraction of a mobility slightly greater than γ-globulin (22) (23) (28) (31). Tiselius (29) found that the pneumococcus carbohydrate-precipitating serum-antibody produced in the horse migrated as a new component, and a similar antibody produced in the rabbit appeared as an addition to the normal γ-globulin. The new component was no longer present in horse serum after the antibody had been removed by homologous type specific polysaccharide or by a heavy suspension of specific pneumococci. In the rabbit antiserum the amount of γ-globulin increased to 56 per cent of the total protein as compared with 17 per cent in normal serum. Pneumococcus antibody was shown by Van der Scheer and associates (30) to migrate with the γ-globulin component of

serum from 14 out of 15 hyperimmunized horses. In the one exception, shortly after hyperimmunization, the antibody protein migrated as a separate component between β-and γ-globulins. The mobility was -2.1×10^{-5} cm^2 sec.$^{-1}$ volt^{-1} in a .02M phosphate buffer solution of pH 7.6. This mobility is very close to that calculated for the "L" component in serum from leucosis affected chickens. The mobility of the "T" component demonstrated in diphtheria and in tetanus antitoxin from the horse was between that of β-and γ-globulins (32).

Shapiro and associates (24) separated a viscous protein from the blood plasma of a patient with multiple myeloma; the pattern of the extra protein appeared between β-and γ-globulin and moved with fibrinogen.

It is postulated that the new "L" component described herein is of the nature of an antibody protein and occurs in all types of the avian leucosis complex. Also, it may be produced by the injection of an inactivated transmissible tumor.

Due to the position of the γ-globulin disturbance noted in all chicken serum, it is not improbable that one would find evidence of the "L" component in serum from normal chickens or from chickens that have been exposed to infective material.

The occurrence of the "L" component in serum after the injection of inactivated tumor material suggests further investigations into its immunological and diagnostic possibilities. The presence of the new component suggests that it might be possible to develop an active immunity against the disease or that the antibody-like substance may be used in the development of a diagnostic test for the detection of the disease.

Production of neutralizing antibodies in serum against leucosis agents has been demonstrated by the protective value of such a serum against the disease. According to Kabat and Furth (8), the injection of materials from fowl tumors into rabbits will produce virus-neutralizing antibodies in rabbits. Lee (9) produced protective antiserum by the immunization of ducks and turkeys with repeated injections of leucosis material. The antiserum obtained protected susceptible chickens against disease-producing doses of leucosis agent. The results of his experiments lend support to the hypothesis that the "L" component found in the serum of chickens affected with different forms of leucosis is antibody protein. Lee states, "Neutralization of the agent of one type or form of the disease by duck or turkey serum produced by a series of injections of infectious material from lesions of a different form or type of the complex more closely indicates the presence of a single etiology."

Since the electrophoretic patterns of chicken serum show that the "L" component is closely associated with γ-globulin, it was thought possible that the salting out of the γ-globulin might also remove the antibody protein. This was attempted by adding 30-per cent Na_2SO_4 to an equal volume of serum from chickens showing leucosis tumors. The precipitated globulin was centrifuged, washed, dissolved, and dialyzed against 0.85-per cent sodium chloride solution. The protein solution was diluted to a protein concentration of 2 per cent. The electrophoretic pattern of this fraction as shown in Fig. 16 indicates apparently pure γ-globulin. There was no separation of the "L" component.

SUMMARY

A comparative electrophoretic analysis by the moving boundary method has been made of the proteins in serum and plasma from normal chickens and from chickens affected with avian leucosis. The average mobilities of the constituents in normal serum and plasma, as found under the conditions employed in this study, are as follows: albumin, -5.9×10^{-5}; α_1,-globulin, 5.0×10^{-5}; α_2,-4.4×10^{-5}; β,-3.5×10^{-5}; γ,-2.0×10^{-5}; fibrinogen, -2.5×10^{-5} cm^2 $sec.^{-1}$ $volt^{-1}$. The average relative concentrations of the components in normal serum with respect to total protein are: albumin, 43.8 per cent; $\alpha_1 + \alpha_2$-globulin, 19.3 per cent; β, 13.0 per cent; γ, 23.9 per cent.

A new component, designated the "L" component, was found in serum from chickens affected with the several forms of leucosis. The new protein component has a mobility -2.55×10^{-5} cm^2 $sec.^{-1}$ $volt^{-1}$ and represents approximately 10 per cent of the total protein in the serum.

The relative composition of the components in leucosis serum (Table 3) as compared with the normal showed a decrease in albumin and γ-globulin and an increase in α-globulins. The electrophoretic patterns in which the new component failed to migrate as a separate peak show a very high concentration of γ-globulin. This fact would indicate a close relationship between the "L" component and γ-globulin.

A series of blood samples drawn from chickens at intervals after injection with an active transmissible tumor agent shows the presence of the "L" component as early as the third day. Chickens injected with the inactivated tumor agent also show the presence of the "L" component but not until the fifteenth day after injection.

Attempts to separate the new protein component from γ-globulin by salting out methods were unsuccessful.

The presence of the "L" component in the serum from leucosis-affected chickens has immunological and diagnostic significance.

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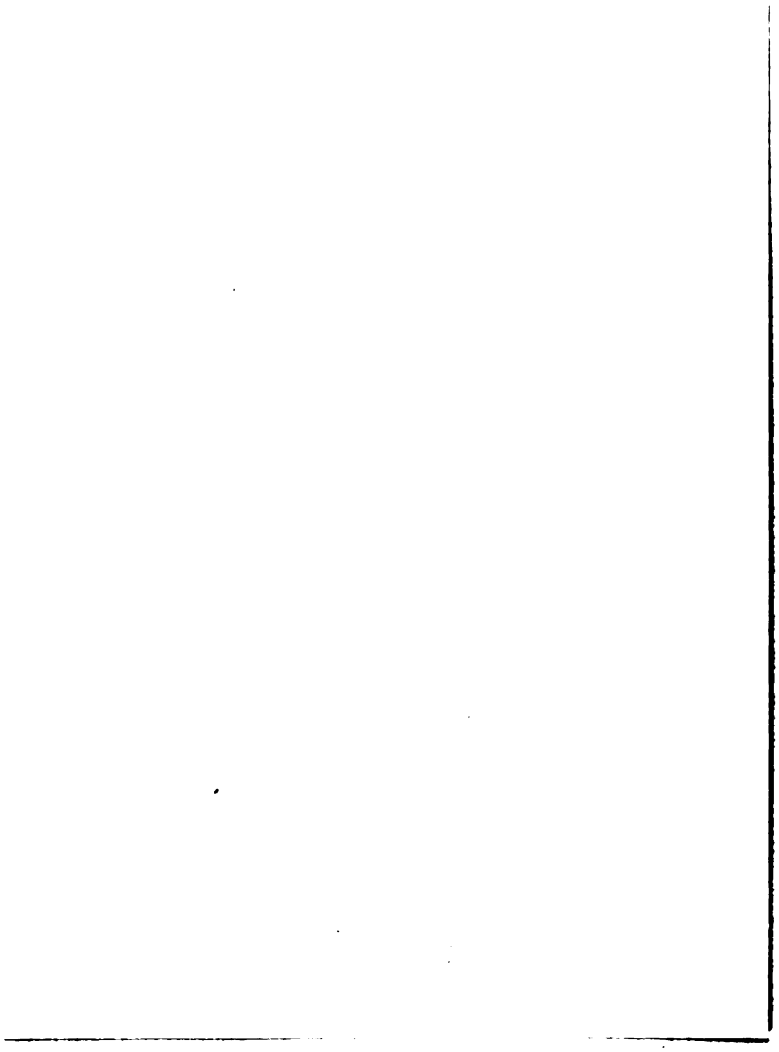

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An electrophoretic study of serum and plasma from normal and leucosis-affected chickens.