calandra (47), *Motacilla a. alba* (34), *Lanius minor* (96), *Phylloscopus* sp. (63), *Acrocephalus* sp. (39), *Hirundo r. rustica* (26), *Upupa e. epops* (39), *Alcedo a. atthis* (11), *Streptopelia t. turtur* (501), *Cursorius c. cursor* (16), and 16 other species (totaling 33 specimens), represented by one to five specimens each.

The human diseases with which *Hyalomma m. marginatum* and *Haemaphysalis punctata* have been epidemiologically or experimentally associated in the areas from which the ticks in question originate are Crimean hemorrhagic fever, Q fever, tularemia, tick typhus, and brucellosis. These species are also vectors of several pathogens causing diseases in domestic animals.

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23 September 1960

Electrophoretic Analysis of Young Alligator Serum

Abstract. Serum from 30 young alligators was examined by free boundary, starch block, and cellulose acetate strip electrophoresis. The patterns obtained showed that the alligator serum proteins differ significantly from those of other chordates in that an α-globulin is the major component. The average ratio of α-globulin to albumin in the serum is 3.4. In this respect it resembles serum of human beings with renal disease in which the α-globulin ratio to albumin ratio reaches 3.0.

Upon making an electrophoretic analysis of alligator serum for another study, we noted that the pattern (Fig. 1) was significantly different from other chordate patterns (*1*) in that the major component is not albumin but an α-globulin. Since then, we have examined the sera from over 30 individual animals, and in all cases, we have obtained patterns similar to the one shown.

Table 1. Mobility and percentage composition of alligator serum proteins as measured from ascending patterns of free-boundary electrophoresis. Mobilities are average of nine measurements showing mean deviation. Percentage composition of six individual alligators, measured by a planimeter, is given.

Component	Mobility (10^{-5} cm^2/v sec)	Relative composition (%)
Albumin	7.69 ± 0.34	13.88 ± 2.74
α_1-Globulin	5.54 ± 0.28	32.59 ± 3.19
α_2-Globulin	4.41 ± 0.24	15.27 ± 3.60
β_1-Globulin	3.58 ± 0.28	21.88 ± 3.71
β_2-Globulin	2.10 ± 0.14	11.40 ± 3.55
γ-Globulin	0.51 ± 0.11	4.93 ± 1.55

The average ratio of α-globulin to albumin in alligator serum is 3.4. In this respect it resembles serum of human beings with renal disease in which the ratio of α-globulin to albumin reaches 3.0 (*2*).

The presence of such large globulin-to-albumin ratios can be misleading in electrophoretic analysis, since it is possible to conclude, on the basis of mobilities, that a component is present in the serum which migrates ahead of the albumin, such as is observed in synovial fluid (*3*). Therefore, the following experimental results are presented in support of our conclusion that the major (33 percent) component of alligator serum behaves as an α-globulin.

The serum was obtained from young alligators (*Alligator mississipiensis*), approximately 60 cm long, by cardiac puncture. Both fresh and stored frozen sera were used in this study, and no significant differences in electrophoretic behavior were observed between them. Serum was examined by moving-boundary electrophoresis in the Perkin-Elmer model 38 apparatus, by starch block electrophoresis, and by the cellulose acetate strip method of Kohn (*4*). Prior to analysis, serum was dialyzed against several changes of sodium diethylbarbiturate buffer, *p*H 8.6, with constant stirring for 48 hours at 4°C. The ionic strength of the buffer for the moving boundary and starch electrophoresis was 0.1; that for the acetate strip method, 0.05.

The ascending patterns from the moving-boundary analysis were used for all mobility and percentage composition measurements because the ascending pattern gave better resolution between the components. A typical pattern is shown in Fig. 1; Table 1 gives the mobilities and the percentage composition of the various components. For purposes of identification the peaks were arbitrarily labeled in order of decreasing mobility as albumin, α_1, α_2, β_1, β_2, and γ-globulin. In order to study the sedimentation behavior of the leading components, fractionation was carried out by zone electrophoresis with potato starch, washed three times in distilled water and once in buffer, as the medium. One-centimeter sections were extracted with 6 ml of 1 percent sodium chloride and examined in the Spinco model E analytical ultracentrifuge at 56,100 rev/min at 25°C. The albumin fraction had one component with an $S_{20,w}$ value of 4.1 *S*. The α-globulin fraction contained a major component (90 percent) with an $S_{20,w}$ value of 16.6 *S* and a minor component, $S_{20,w}$ of 3.4 *S*. These sedimentation coefficients are similar to those reported for other serum albumins and α-globulins (*5*). The 16.6 *S* component of the α-globulin fraction could account for most of a major sedimentation component of alligator serum (13.9 *S* in serum).

The alligator albumin and α-globulin fractions also were added to rabbit serum and examined by the cellulose acetate method of Kohn (*4*), by which a relatively large separation between the albumin and globulins is obtained. It was found that alligator albumin migrated with the rabbit albumin, and the alligator α-globulin with the rabbit globulin. Further experiments were carried out with the moving-boundary apparatus in which it was found that bovine serum albumin (Armours No. 2266), when added to alligator serum, moved with the alligator albumin. Hyaluronic acid (Nutritional Biochemicals) also was added to alligator serum, because the mucin clot obtained under certain conditions indicated its possible presence (*6*). However, the added hyaluronic acid migrated ahead of the alligator albumin (mobility of 8.2×10^{-5} at *p*H 8.6).

Finally, it was found that the alligator globulin precipitates out in 30–40 percent saturated ammonium sulfate solutions while the albumin is soluble at 50 percent saturation.

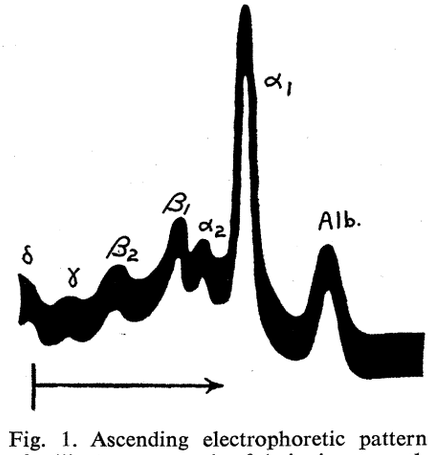

Fig. 1. Ascending electrophoretic pattern of alligator serum in 0.1 ionic strength diethylbarbiturate buffer of *p*H 8.6 after 120 minutes at 6.0 volt/cm.

The relative solubilities in ammonium sulfate, the respective sedimentation coefficients, and the relative mobilities with respect to rabbit serum, bovine serum albumin, and hyaluronic acid of the two major and leading electrophoretic components of alligator serum has led to identification of the leading component with the serum albumin and the second and largest components with serum α-globulin (*7*).

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Alteration of Plasma Proteins at Metamorphosis in the Lamprey (Petromyzon marinus dosatus)

Abstract. Coincident with metamorphosis in the lamprey (*Petromyzon marinus dosatus*), a new, more rapidly moving plasma protein component appears. In the mature lamprey this new component is of major quantitative importance. With paper electrophoretic mobility as the sole criterion, the new component is considered to be an alpha globulin rather than albumin.

The striking anatomical alterations which occur during metamorphosis in vertebrates may be accompanied by equally striking, if less overt, biochemical changes. A number of such biochemical changes have been reported recently. These include the retinal pigments in various vertebrates, including the sea lamprey, and the serum proteins and nitrogen metabolism in the tadpole and the frog (*1*). In this communication we report another example of a biochemical change—an alteration in the serum proteins which accompanies metamorphosis in the sea lamprey, *Petromyzon marinus dosatus.*

Specimens of Petromyzon (*2*) were shipped by air to Washington, D.C. in insulated jugs containing ice water. All the animals were healthy and vigorous upon arrival. The larvae were 3 to 5 in. long, while the newly transformed adults were 5 to 6 in. long. These specimens had been obtained during the winter months. The mature adults (obtained in May) were captured as they were going upstream, and these animals were approximately 15 in. long.

The blood from the larvae and the newly transformed adults was obtained after parental administration of 50 μg of heparin to each animal. Twenty minutes after heparin injection the tail was amputated and the blood was aspirated from the caudal artery into a polyethylene tube with an inside diameter of 0.86 mm; 30 to 100 μl of blood was obtained from each animal. After the blood had been drawn into the polyethylene tube, the tube was folded in the shape of a U, inserted in a small-bore centrifuge tube, and centrifuged for 2 minutes. The plasma was separated by cutting the plastic tube just above the junction of cells and plasma. Blood was obtained from the mature adults by puncture of a caudal vessel either with a heparinized syringe or after administration of 1.0 mg of heparin to the animal. In all, samples were taken from at least ten larvae and newly transformed adults and three mature adults.

Paper electrophoresis was performed on 18-μl samples by using a Durrum-type cell and Veronal buffer (*p*H 8.6) with constant current of 2.5 ma for 18 hours. The resulting voltage was about 80. The strips were stained with bromphenol blue, and the density was analyzed with a Spinco Analytrol.

Paper-electrophoretic strips of plasma from a larva, a newly transformed adult lamprey, a mature adult lamprey, and a normal human being are shown in Fig. 1. In the newly transformed adult there appeared a small amount of protein with a higher electrophoretic mobility than any component seen in the larva. In the mature adult this new component constituted an important percentage of the plasma proteins. It did not, however, have the electrophoretic mobility of human albumin. In certain mature adult specimens there was a marked increase, seemingly related to hemolysis, in the amount of a more slowly moving component.

Human serum albumin has been used as a reference for labeling the electrophoretic components (Table 1), although it is clear that electrophoretic mobility alone is inadequate for categorizing such components. The major component in larval lamprey plasma is considered to be an alpha globulin. In adult lamprey plasma, the two strongest bands are considered to be an alpha-1 globulin and an alpha-2 globulin. In the mature lamprey the alpha-1 and alpha-2 globulins are approximately equal in concentration. It is clear that these are two separate components rather than a single large component. This scheme indicates the apparent lack in lamprey plasma of a component with the same electrophoretic mobility as mammalian albumin.

Table 1. Plasma protein components of the lamprey.

Specimen	Protein (%)				
	Albumin	Globulin			
		α_1	α_2	β	γ
		Human being			
	59	4	10	11	17
		Lamprey			
Larva	0	70		8	22
New adult	0	6	71	10	13
Mature adult	0	37	45	10	8

Wald demonstrated the changes in the retinal pigments which accompany metamorphosis in the lamprey (*1*). The development of the thyroid gland from an exocrine to an endocrine gland is another example of metamorphosis in the lamprey (*3*). To this can now be added the development of a new protein component in the plasma of the metamorphosing lamprey. This component has a greater electrophoretic mobility than any component in the larval animal. It is important to note, however, that even the adult lamprey lacks plasma albumin. This finding is consistent with the observation that certain elasmobranchs also lack a plasma component comparable to human

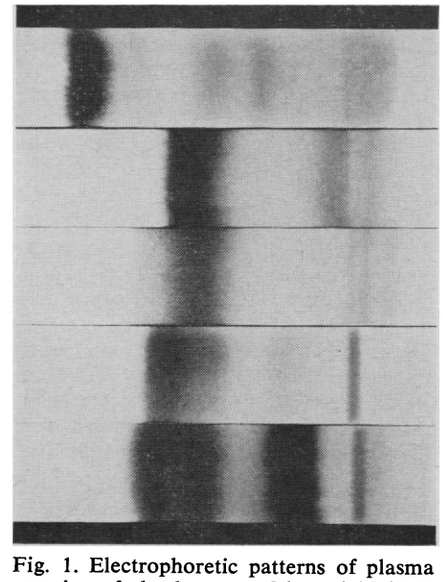

Fig. 1. Electrophoretic patterns of plasma proteins of the lamprey. The origin is to the right. The top strip represents, for comparison, 6 μl of human serum; the second strip, 18 μl of larval lamprey plasma; the third or middle strip, 18 μl of plasma from a newly transformed adult; the fourth strip, 18 μl of plasma from a mature adult lamprey; and the bottom strip, 24 μl of hemolyzed plasma from a mature adult lamprey.