Paper Electrophoretic Patterns of Human Serum Proteins Compared with Those of Lower Forms.* (26343)

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Although the patterns of sera from mammals have a general resemblance in number of components and their electrophoretic properties(1,2), this apparently does not apply or include(3,4,5,6,7) those from lower vertebrates (Elasmobranchi, Teleosts, Amphibia, Reptilia, Aves). Proteins with electrophoretic properties similar to human serum albumen were not observed in the shark(3,4,8) and in more than one genus and family of turtles(4,5,7). In this paper comparative observations were extended and include patterns from widely divergent forms. With each run of a given species, samples of human serum were included which were always taken from the same individual. Volumes of sera, time, ionic strength, voltage, amperage, paper strips, buffers, fixing and staining of proteins, and densitometer adjustments for recording were kept as uniform as possible. Each serum profile (Fig. 7) was prepared from average values taken from 3 or more individuals of a given species. From this study as summarized (Fig. 7) one can obtain qualitative information on relative concentration of proteins in 10 lambda samples from each of the various species. Number of components, their percentages, and migration can be compared with those of human serum.

Method and materials. Mobility studies with reference to pH levels were done on serum components of the shark (*S. acanthias*), goosefish (*L. piscatorius*) and human (Fig. 1). One tenth molar monobasic (KH_2PO_4) and dibasic (Na_2HPO_4) phosphates were combined in proportions to provide buffers at pH 5.4, 6.0, 7.0 and 7.8. Ten lambda samples from each of the 3 species were run simultaneously in a Durrum electrophoretic cell at the given pH levels. After 8 hours at 12 milliamperes and 110 volts, paper strips were removed; dried at 125°C for 30 minutes; stained with bromphenol blue for 6 hours; rinsed with 5% glacial acetic; dried at 125°C for 15 minutes and briefly exposed to ammonia vapor. The strips were then read with a self-recording densitometer. Distances in millimeters of the various components from point of application were recorded relative to pH (Fig. 1).

To provide serum patterns in which the electrically separable components were shown in higher contrast and in which relative proportions of each were readily recorded with the densitometer, 10 lambda serum samples were run with barbiturate buffer (pH 8.6, ionic strength 0.075) for 16 hours at 6 milliamperes and 110 volts. Paper electrophoretic strips were processed as mentioned above. A strip of human serum was included with each

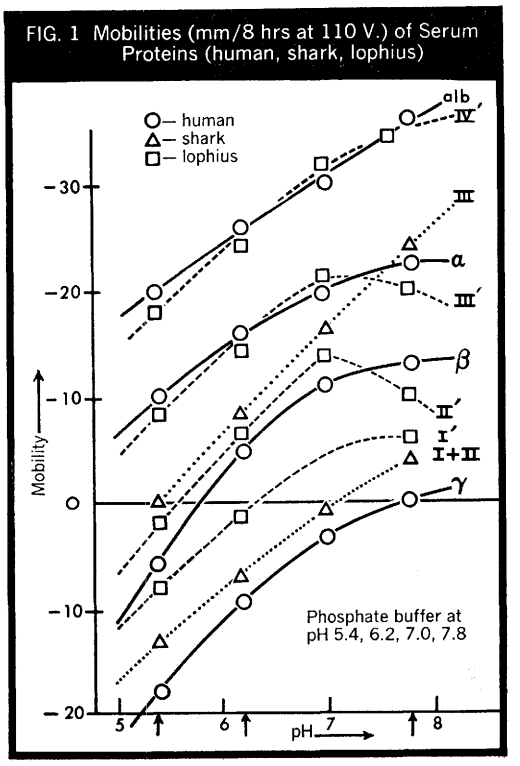

FIG. 1 Mobilities (mm/8 hrs at 110 V.) of Serum Proteins (human, shark, lophius)

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serum of a given species to be studied. Percentages and mobilities of components in an unknown serum were then compared with the gamma, beta, alpha and albumen components of the human serum as a frame (or grid) for points of reference (Figs. 2, 5, 7).

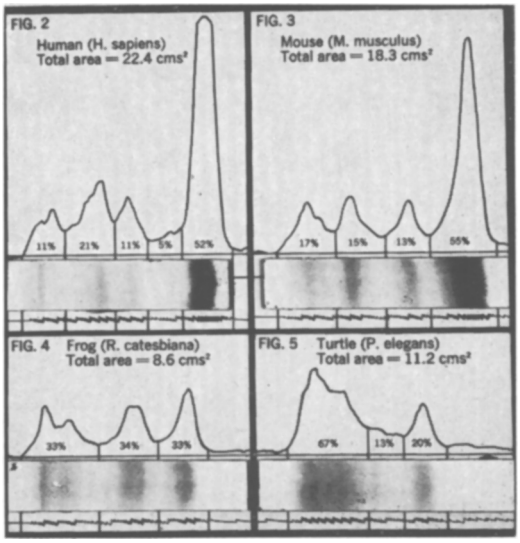


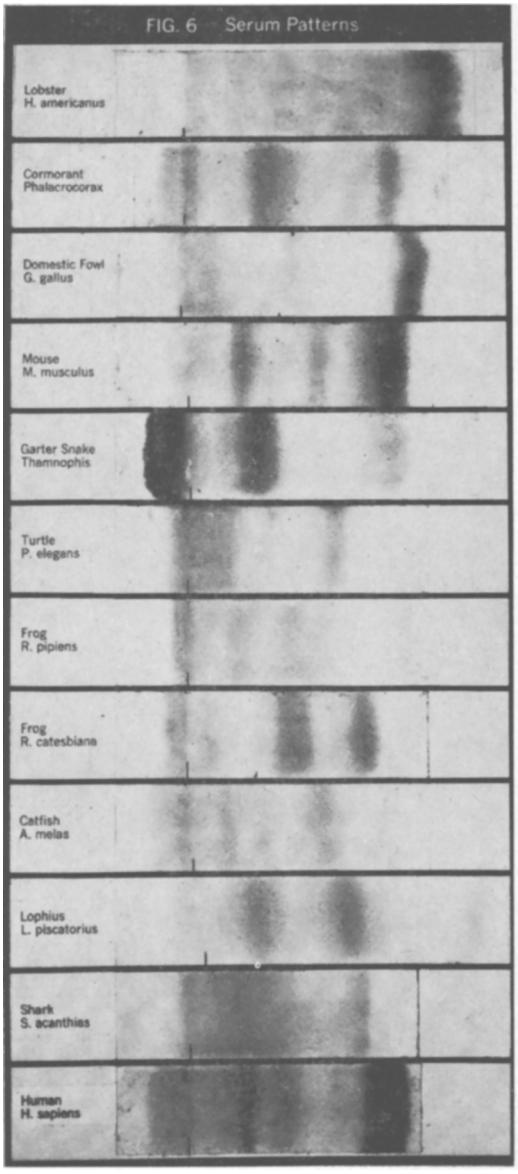
FIG. 2-5.

The components of each serum studied were numbered in sequence from the point of application toward anode as I to the final number of III to V.

Results. Mobilities of serum components of shark, goosefish and human were plotted in millimeters relative to pH 5.4, 6.2, 7.0 and 7.8 (Fig. 1). Four serum components of the human maintain their identities. Components I and II of shark serum as shown (Fig. 7), move at these pH levels as a single unit, and were found in proximity to human gamma. Shark (III) was found in relative positions to human alpha and beta. Four components of goosefish (Lophius) are found at these pH levels. Retention of electrophoretic properties of the components of the 3 sera at various levels of pH would indicate that each exists as a natural entity.

To prepare serum patterns in which the relative proportions of each component stand out in good contrast, 10 lambda samples of each serum were applied to paper strips and run in barbiturate buffer (pH 8.6) for 16 hours at 6 milliamperes and 110 volts. With each run of a given species, 2 or more strips of human serum were included. A comparison of the serum patterns follow.

Shark (Squalus acanthias). Blood samples were taken from tail veins of 18 sharks (6 ♂, 12 ♀), which had been taken from Frenchmen's Bay, Me., and supplied through the courtesy of Dr. Roy Forster. Three components were observed in most of the serum samples (Fig. 6, 7). Component II, 70% to 90% (Avg 82%) was located as compared with human serum in the position



of gamma, beta and alpha globulins (Fig. 7). Component III, 4% to 22% (Avg 16%) in a position relative to human albumen and alpha^2. In all patterns a small component 1, 2%-4% (Av. 3%) was found within 15 millimeters from point of application toward the cathode (Fig. 2). Sera from 8 animals with recently ingested food (stomach contents) were turbid. Component III was as high as 10%-22% in these sera, and as low as 4%-8% in the others. Patterns from male and female were similar.

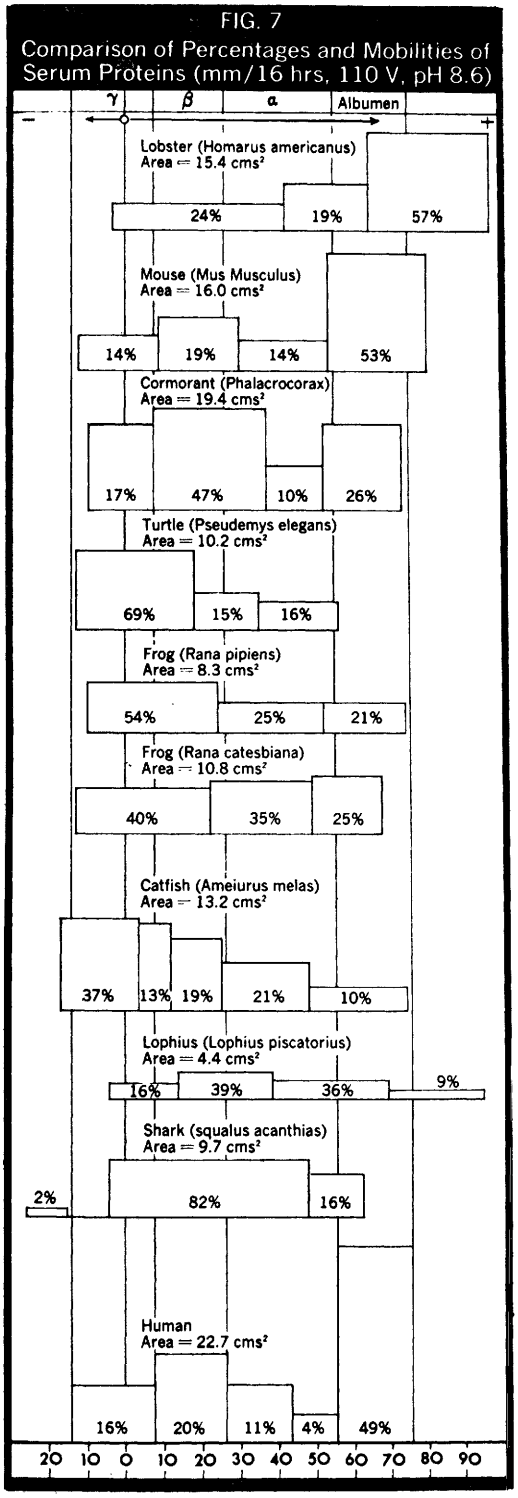


FIG. 7
Comparison of Percentages and Mobilities of Serum Proteins (mm/16 hrs, 110 V, pH 8.6)

The total stainable protein from 10 lambda samples of 18 animals as measured with the analytrol gave an average value of 9.7 cm^2 (Fig. 7). This value was approximately 43% of the total area of 22.7 cm^2 obtained from an equivalent volume of human serum. These values show the low level of protein in shark serum, and indicated that most of the proteins (78%-96%) had mobilities within the range of human globulins.

Goosefish (*Lophius piscatorius*). Blood samples were taken from renal veins of 8 animals. Four components were observed in the serum samples (Fig. 6, 7). The largest component (II) 39% to 44% (Av. 39%) had a mobility comparable to the range of beta and alpha^2 (Fig. 7). Component III, 28% to 41% (Avg. 36%) was found at levels of alpha^1 and albumen; I, 6% to 16% (Avg 13%) related to the range of gamma and beta; IV, 5% to 10% (Avg 9%) within and beyond the range of migration for human serum albumen. Total stainable protein of 10 lambda samples from 8 animals averaged 4.4 cm^2. This value was approximately 20% of the total area of 22.7 cm^2 obtained from an equivalent volume of human serum.

Catfish (*Ameiurus melas*). Eight catfish (3 ♀, 5 ♂) were used for obtaining serum from blood taken from tail veins. Five serum components were observed (Fig. 6, 7). The largest component (I, 33% to 40%) (Avg 37%), was within a range of mobility comparable to human gamma globulin; component II, 8%-14% (Avg 13%) within the range of gamma and beta; component III, 16%-22% (Avg 19%) was relative in migration to beta globulin; IV, 16% to 24% (Avg 21%) to the alpha complex; and V, 4%-12% (Avg 6%) to alpha^2 and albumen. Total

stainable protein of 10 lambda samples averaged 13.2 cm^2; 13.2/22.7 or 58% of amount obtained with the same volume of human serum.

Frog (*Rana catesbiana*). Blood samples were taken from the atria of 4 adult males. Three serum components were observed (Fig. 4, 6, 7). Component I was the largest; 32% to 47% (Avg 40%). Mobility was within the general level of human gamma and beta globulins. Component II, 32% to 40% (Avg 35%) was comparable in mobility to the alpha complex; III, 19% to 35% (Avg 25%) with a mobility within the levels of alpha^1 and extended into the range for human serum albumen. Total stainable protein of 10 lambda samples gave an average area of 10.8 cm^2; 10.8 22.7 or 48% of the amount obtained with an equal volume of human serum.

Frog (*Rana pipiens*). Blood was taken from the atria of 5 frogs and pooled. This procedure was done for each lot of 5 frogs in September, December and January. Total areas of stainable protein of 10 lambda samples in the same sequence were 17.7 cm^2, 11.3 cm^2 and 8.3 cm^2 respectively. This indicates that total serum protein decreases during the winter months. However, a comparison of the percentages of the 3 protein components in each of the 3 lots of pooled sera indicated that the relative proportions of the 3 components to each other remained approximately the same. Pooled sera of September frogs were used for comparative study (Fig. 6, 7). Component I was the largest, 46% to 56% (Avg 54%); with a mobility within the range of gamma and beta globulins. Component II, 20% to 30% (Avg 25%) within the mobility of the alpha complex; III, 15% to 25% (Avg 21%) was within the range for alpha^1 and albumen. Total stainable protein in 10 lambda samples of September frogs was 8.3 cm^2, or 8.3/22.7; 36% of an equivalent amount of human serum.

Turtle (*Pseudemys elegans*). Blood was taken from the atria of 3 males. Animals had been in laboratory tanks for 6 weeks without feeding. Three serum components were observed (Fig. 5, 6, 7). Component I was the largest; 59% to 76% (Avg 69%). The mobility of this component was at the level of human gamma and beta globulins. Component II, 10% to 26% (Avg 15%) was found within the levels of beta and alpha^2; III, 14% to 18% (Avg 16%) a mobility within the range of the alpha complex. Total stainable protein of 10 lambda samples gave an average area of 10.2 cm^2; 10.2/22.7 or 45% of the amount obtained with human serum.

Cormorant (*Phalacrocorax*). Blood was taken from the wing veins of 3 animals and combined in one volume. Four serum components were observed. Component II was the largest (Fig. 6, 7), 47%, with a mobility within the range of human gamma and alpha^2 globulins. Component I, 17% was comparable to human gamma globulin in mobility; III, 10%, within the level of the alpha complex; IV, 26%, within the levels of human albumen. Total stainable protein in a pattern of 10 lambda of serum gave an average area of 19.4 cm^2; 19.4/22.7 or 81% of the amount obtained from an equivalent volume of human serum.

Mouse (*Mus musculus*). Blood samples from the atria of 3 Swiss mice were combined into one volume. Total of 6 volumes from 18 mice were used as sources of 6 serum samples. Four serum components were observed with percentages and mobilities comparable to human serum, except for the alpha which did not separate as 2 components (Fig. 3, 6, 7). Component IV was the largest, 49% to 56% (Avg 53%). Mobility of this component was similar to human albumen. Component I, 10% to 16% (Avg 14%) was similar in mobility to human gamma globulin; II, 16% to 24% (Avg 19%) similar to gamma globulin; III, 12% to 20% (Avg 14%) within the range for the human alpha complex. Total stainable protein in a 10 lambda sample gave an average area of 16.0 cm^2; 16.0/22.7 or 70% of the amount obtained with an equivalent amount of human serum.

Lobster (*Homarus americanus*). Lobster haemolymph was collected from the pericardal sinus of 8 specimens. Each was filtered and then applied as 10 lambda samples to electrophoretic paper strips, and processed

by the same methods as sera from vertebrates. Three components were observed which in their mobilities were not comparable to those of vertebrate sera (Fig. 6, 7). Component III, 55% to 60% (Avg 57%) was the largest component, with a mobility within and beyond the limits human albumen. Component I, 16%-30% (Avg 24%) was within levels of gamma, beta and alpha globulins. II, 17% to 25% (Avg 19%) within levels for alpha and albumen. Total stainable protein of 10 lambda samples averaged 15.4 cm^2; 15.4/22.7 or 68% of the equivalent value for human serum.

Discussion. Total stainable protein of 10 lambda serum samples from various species, as measured with a densitometer and recorded in cm^2, was highest for the human. Lower values were found for sera of lophius, shark, frog (*R. pipiens*) and turtle which were 20, 43, 36 and 45% respectively of human serum (Fig. 7). The frog (*R. catesbiana*), catfish (*A. melas*), lobster, mouse and cormorant were 48, 58, 68, 70 and 81% respectively.

Approximately half of the stainable protein of mouse or human serum was albumen (Fig. 2, 4, 6, 7). A similar albumen-like component was not found in the turtle (*P. elegans*). The serum component most proximal to the anode observed in patterns of shark (*S. acanthias*), lophius and frogs (*R. pipiens, R. catesbiana*) extended only over a portion of the range of mobility of human albumen (Fig. 7).

The globulin components of mouse serum patterns present a picture of general similarity in the relative proportions of each and in their mobilities to those of the human (Fig. 2, 4, 7). Serum pattern of mammalian species as reported in the literature have a remarkable resemblance(1,2). Although the serum patterns of cormorant and domestic fowl differ in relative proportions of globulins from those of the human (Fig. 6, 7), mobilities of gamma globulin and albumen approximated a similar range.

A component which moves some distance from the point of application toward the cathode was observed in serum patterns of turtle, frogs, catfish, lophius and shark (Fig. 7) and in this respect resembles human gamma globulin. However, the range of mobility and proportionate amounts present, differ so that they cannot be said to be comparable. A striking difference in the pattern of the garter snake is shown in Fig. 6. In this species the largest component is a fraction which has a mobility comparable to human gamma globulin. This observation was in agreement with that of Dessauer(9).

The serum pattern of lobster is in no way comparable to those of the vertebrates. It has a resemblance to the pattern of *Carcinus maenas* which was described as 3 components (10). The gamma globulin fraction as observed in mammals was not found in lobster serum.

Conclusions. The paper electrophoretic pattern of human serum has a general resemblance to other mammalian species in number of components and their electrophoretic properties. Globulin and albumin fractions of avian species (domestic fowl, cormorant) resemble in mobility those of the human. Serum components of lower vertebrates differed in their electrophoretic properties from those of the human and thus are not comparable. The serum pattern from an anthropod, *H. americanus,* was completely different from the patterns obtained from vertebrates.

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