SOME EVOLUTIONARY PATTERNS IN FISHES' BLOOD [1]

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Electrophoretic analyses of serum protein fractions of some fishes and certain other animals have been presented by Deutsch and Goodloe (1945), Deutsch and McShan (1949), Engle *et al.* (1958), Irisawa and Irisawa (1954), and Moore (1945). In general it was shown that there are characteristic, reproducible, species differences in the plasma proteins and differences between the lower and higher animals. Deutsch and McShan give data on the blood plasmas of 16 fresh-water fishes and Engle *et al.* gave data on 20 species of marine fishes. Both groups of authors reported that gamma globulin fractions in the plasma of teleost fishes were absent or diminished, but Engle *et al.* found them high in the elasmobranchs.

Sulya *et al.* (1961) presented plasma protein data derived by paper electrophoresis from 183 specimens of 26 species of fishes from the Gulf of Mexico, and this is the most extensive series of data on fishes presented so far. In that report the techniques are described, with a discussion of certain physiological and clinical implications.[2] Gamma globulin was found in all teleosts and in some it was quite high. Data were given by species and there was some discussion of differences between the various fishes.

We propose here to call attention to certain facts of evolutionary significance, derived from Sulya *et al.* (1960 and 1961), and to present part of the unpublished data.

The Data

The electrophoretic patterns were illustrated by Sulya *et al.* (1961), but the tables, which list the plasma protein components of each species, were too long for publication. Therefore, those data have been deposited as Document 6454 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. They may be purchased as photoprints or on 35-mm. film by advance payment of $1.25 by check or money order made out to Chief, Photoduplication Service, Library of Congress.

The data presented in Table I are average values for the various Orders of fishes, arranged in a series from the generalized to the more specialized, according to the classification of Berg (1947). In general this arrangement conforms to the sequence from lower to higher fishes, as presently understood. The original data were the mean values for all specimens of the given species. The species were then

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[2] Sulya *et al.* (1961) have been criticized for not offering full information regarding quantification, especially staining of the strips. For that reason we wish to state here, somewhat out of context, that the procedure used was Spinco "Procedure A" in which the dye is bromphenol blue.

TABLE I

Plasma protein distribution in fishes arranged in the order of increasing evolutionary development

Order	Number of species	Total protein gms. %	Albumin gms. %	Globulins gms. %				Other plasma globulins
				α_1	α_2	β_1	γ_1	
Lamniformes (sharks)	3 (4)*	2.18			.695	.805	.539	.143γ_2
Lepidosteiformes (gars)	3 (5)*	2.87		.125	.344	1.386	1.011	
Clupeiformes (herring-like fishes)	4 (63)*	3.59	.082	.988	1.144	.810	.421	.122γ_2
Siluroidea (catfishes)	2 (10)*	2.94	.688	.226	.429	.546	1.159	
Mugiliformes (mullets)	2 (23)*	3.78	.550	.893	1.127	.378	.811	
Perciformes (perch-like fishes)	12 (78)*	3.70	.291	.661	.889	.723	.658	.048α_1(2) .108α_2(2) .041β_2 .230γ_2

* The number in parenthesis represents the number of animals.

all given equal rank, regardless of the numbers of specimens, and the means for the species values are presented as the values for the Orders in the table. Thus 12 values were averaged for the Perciformes and only two for the Mugiliformes.

The writers had hoped to utilize the data on fish plasma previously published, along with our own, so as to have a more extensive series of fishes in the tables. However, previous studies were carried out by the moving boundary electrophoresis while our own were made by paper electrophoretic methods, and there is a basic difference between the patterns obtained by the two methods. In the moving boundary procedure, the pattern is obtained from the refractive index gradient relative to cell level and the pattern represents, therefore, all refracting components irrespective of their chemical composition. On the other hand, the paper electrophoresis method measures only those components which combine with the staining dye. The data obtained by the two methods may be similar, in general, but not necessarily so. In some instances we observed fish plasma cholesterol values that were very high relative to higher vertebrates. In these cases the lipids may be expected to contribute to refraction, thus giving the associated fractions higher values in the moving boundary procedure.

DISCUSSION

Of the four major groups of living fishes (Elasmobranchii, Holocephali, Dipnoi and Teleostomi) we have representatives only of the first and the last.

However, these two Classes are widespread and numerous (which is the reason we have specimens), and the other two are somewhat side-issues to the main stem of evolution in fishes. It should also be recalled that the Teleostomi are divided into two subclasses, the Crossopterygii and the Actinopterygii, the first being represented among living fishes only by *Latimeria,* of South Africa and Madagascar, and we have no specimens. The main line of evolution of the higher vertebrates probably passed through the Crossopterygii, but this group is an evolutionary side issue in fishes and in fact it is virtually extinct. Therefore, the data presented cover representatives along the main stem of evolution in fishes, as represented by living species, somewhat better than might appear at first glance. One large gap concerns the most primitive of the Actinopterygii, the sturgeons and paddlefishes.

The table shows that there is gradual increase in the percentage of total plasma protein from lower to higher fishes. Only the Clupeiformes are out of sequence. In high protein content of the plasma the Clupeiformes are similar to the advanced rather than to the lower fishes. This Order corresponds largely to the Isospondylii of Jordan (1923) and previous workers. The Perciformes (Acanthopterygii, of Jordan and others), the most specialized fishes, have about a 70% higher content of plasma proteins than the elasmobranchs. There is considerable variation between the species and among the Perciformes the range was 1.68 to 6.19 grams of plasma proteins per 100 ml. of plasma. The plasma protein content of fishes is low as compared to mammals.

Irisawa and Irisawa (1954) first showed that albumin was not present in the sera of two elasmobranchs, a skate and a shark. Drilhon and Fine (1959) made similar observations on two species of sharks, both by electrophoretic and reagent methods. Sulya *et al.* (1961) found the same condition in three other species of sharks. Additionally, Table I shows that there was no albumin in the plasma of three species of gars. These are fresh-water fishes which sometimes enter brackish waters, where all of our specimens were caught. Furthermore, there was no albumin in the plasma of two (*Dorosoma cepedianum* and *D. petenense*) of the four clupeid fishes examined. Two of the twelve species of perciform fishes examined (*Cynoscion arenarius* and *Pogonias cromis*) also lacked albumin in the blood plasma. Analbuminemia seems to be the common condition among the lower fishes; it gradually becomes less prevalent as the fishes become more specialized but does not entirely disappear, and a small per cent of the higher fishes lack albumin in the blood plasma. Thus analbuminemia among fishes seems to be a primitive characteristic. However, Deutsch and McShan (1949) reported albumin in the plasma of sturgeons, the most primitive of living Teleostomi, and that group merits further attention.

The table shows that the sharks, gars, catfishes and mullet had four globulin components in the plasma, and the clupeids have five. It is clear that the perciform fishes, with eight types of globulin, have generally more complex plasma proteins than the lower groups.

Species data show that ten of the perciform fishes had four or five plasma globulin fractions. The croaker, *Micropogon undulatus,* had only two fractions, but the speckled squeteague, *Cynoscion nebulosus,* had eight. These two fishes are considered by most authorities to be in different sub-families of the family

Sciaenidae. One of the gars and one of the sharks were found to have only two globulin fractions; the other two species in each group contained three and four fractions.

In terms of average numbers of globulin fractions per species for each Order, there is an increasing complexity up the evolutionary scale, but again the Clupeiformes are out of sequence. The mean numbers of globulin fractions per species are: sharks 3, gars 3, clupeids 4.5, catfishes 3.5, mullets 4, percoids 4.5.

Species differences are clearly reflected in the plasma protein complexes. For instance, *Dorosoma petenense* possesses gamma-2 globulin, but *D. cepedianum* does not, and the latter has over 11 times as much alpha-1 globulin as the former; one of the catfishes has alpha-1 globulin, but the other does not; the striped mullet has ten times as much albumin as the silver mullet and only one-fourth as much gamma-1 globulin. On the other hand, the white squeteague, *Cynoscion arenarius,* and the black drum, *Pogonias cromis,* have remarkably similar plasma proteins.

Berg (1947) separated the Mugiliformes from the Perciformes because they have abdominal pelvic fins, which is a primitive characteristic. In general the blood plasma proteins of the mullet are less complex than in the Perciformes and similar to certain species of the catfishes and clupeids. This gives some corroborative evidence to the correctness of Berg's separation of the Mugiliformes from the Percomorphi. On the other hand, the plasma proteins of the mullets are also similar to certain perciform species of the families Carangidae, Sciaenidae and Scombridae. Thus, the difference between the plasma proteins of lower and higher fishes is a general character of the groups which does not always hold true when certain species are compared.

The Clupeiformes studied here have a higher plasma protein content and greater protein complexity than would be expected from their position on the evolutionary scale. On the other hand, they show primitive affinities in that half of the four species examined possess no albumin, and the other two only have small amounts.

Sulya *et al.* (1960) confirmed previous reports on the high electrolyte content in the blood of marine elasmobranchs. They found that the three major electrolytes, Na, K and Cl, were about 66% higher in the plasma of sharks than in the teleosts. They also found that the total cholesterol, including cholesterol esters, in plasma of sharks and gars is comparable to the levels in higher vertebrates, 173–151 mg.%, respectively, but in all the remaining teleosts it was considerably higher (Clupeiformes 663, Siluroidea 367, Mugiliformes 560 and Perciformes 606). These are mean figures for the Orders and species variations are averaged out. It was pointed out that there seems to be an association of plasma cholesterol content and concentrations of certain plasma proteins. Resemblances of the normal blood in fishes to the blood of the nephrotic rat and of man with biliary cirrhosis were noted. It appears that the normal condition in fishes' blood is comparable in some ways to certain pathological conditions of mammals, including man. This point is significant, not only from the evolutionary standpoint, but as indicating a new line of approach to the understanding of certain types of pathology of the kidney and liver of mammals. In this regard, it should be noted that Baril *et al.* (1961) called attention to resemblances of the serum of young alligators to that of humans with renal disease.

Summary

1. A study was made, by electrophoretic techniques, of the plasma proteins of 26 species of elasmobranch and teleostome fishes from the northern Gulf of Mexico.

2. A general increase was found in the amount of plasma proteins from lower to more specialized fishes. Elasmobranchs lacked albumin in the plasma. This condition of analbuminemia also held true for all the gars and half of the Clupeidae studied; it generally disappeared in the more specialized fishes, but not entirely, and was found in two species of the Perciformes. There was a gradual increase in the number of globulin fractions of the plasma, from lower to higher fishes, among the Orders of fishes, which did not always hold true at species levels.

3. Closely related species could be distinguished by the presence or absence and varying amounts of plasma proteins, but there were exceptions and certain species in the same family could only be differentiated doubtfully on that basis.

4. The plasma proteins of the fishes examined showed a trend of increasing complexity from the generalized to the more specialized fishes.

5. The content of the chief electrolytes, Na, K and Cl, in the plasma of marine sharks was found to be much higher than in teleosts, in confirmation of earlier workers.

6. Total cholesterol in the plasma of sharks and gars was found in the same range as in mammals, but in the remaining teleost fishes it was a great deal higher.

7. Relations between cholesterol and plasma proteins in the blood of fishes resemble pathological conditions in mammals, including man.

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