Plasminoptysis and Gelation of Erythrocytes in Coagulation of Blood of Freshwater Bony Fishes

By Ken Wolf

Studies on Blood of freshwater bony fishes indicated that the coagulation reaction was similar to that of mammalian blood. A notable difference was reported for the rapidity with which fish blood coagulated. The process was reported to be swift; clotting times ranged from 10 seconds to about 1 minute, with the mean being about 30 seconds. Convincing explanations for the rapid clotting were lacking. For example, the inorganic composition of trout blood and human blood were found to be very similar. With one exception, fibrinogen levels for these fishes were comparable to or less than that of man. Such fibrinogen levels were inconsistent with the rapid clotting. Two of these reports also noted a deficient prothrombin activity.

An unusual, rapid reaction of fish erythrocytes with water has been observed. It is probable that the reaction accounts for the speed of fish blood coagulation, and that it is highly significant in hemostasis in these fishes in their normal environment. The purpose, therefore, of this investigation was: (1) to elucidate the unusual response of fish blood cells to water, (2) to ascertain the validity of the speed of clotting of fish blood cells in water, and (3) to look for similar reactions in the blood of other aquatic vertebrates.

Methods

Briefly, the methods involved obtaining blood of freshwater bony fishes, amphibians and reptiles and exposing thoroughly washed blood cells to water and to certain aqueous solutions. Clotting time for fish blood was determined by the usual clinical methods and by a modification intended to simulate the aqueous environment.

Blood was obtained from hatchery-raised rainbow trout (Salmo gairdneri), brown trout (Salmo trutta), eastern brook trout (Salvelinus fontinalis), wild bullfrogs (Rana catesbeiana) and wild painted turtles (Chrysemys picta) by cardiac puncture. The largest fish were occasionally anesthetized by immersion in or spraying the gills with a solution of tricaine methanesulfonate. A 2 per cent solution of sodium citrate was usually used (1:5) as an anticoagulant. On some occasions 100 mg. per cent heparin was used (1:10). Anticoagulants were dissolved in aqueous 1 per cent NaCl. Sodium chloride alone, however, at 1, 3 or 5 per cent would not prevent clotting of fish blood. Blood was separated by centrifugation. Leukocytes and thrombocytes were largely removed with the plasma, and as determined by thin films stained with Wright's stain, the final cell pack was composed almost exclusively of erythrocytes. Cells were from The Eastern Fish Disease Laboratory, U.S. Department of the Interior, Bureau of Sport Fisheries and Wildlife, Kearneysville, W. Va.

Mr. C. E. Dunbar shared in the observations which led to this study; his technical assistance is gratefully acknowledged. Drs. R. E. Lennon, A. M. Phillips Jr., G. L. Hoffman, A. Shelokov, G. Brecher, L. B. Jaques, N. R. Shulman, and L. M. Tocantins read and commented on the manuscript. Dr. Tocantins kindly viewed the reaction of fish erythrocytes in water and identified the reaction as being similar to that of platelet agglutination.

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washed free of plasma* in several changes of cold (8 C.) 2 per cent sodium citrate in
in water with 1 per cent sodium chloride. Washed erythrocytes were left in just enough
washing solution to make a thick but still fluid suspension. Such suspensions were added
to distilled or deionized water to form the clot-like material described below.

Qualitative chemical analyses of the washed clot-like material were made according
to methods given by Hawk et al. Coagulation time was determined on blood of fingerling
rainbow trout which had an average total length of 105 mm. and an average weight of 11
Gm. The blood was obtained by severing the caudal peduncle, the method most practical
and most frequently used with fish of this size. When the capillary tube method was used,
the ends of the tubes were touched to the blood which welled from the caudal artery.
When the slide method was used, one drop of blood was placed on a dry part of the slide and
a second drop was permitted to fall into a 4 drop pool of water on the opposite end of the
slide.

RESULTS

Plasma-free erythrocytes of trout reacted almost instantly when dropped
into water, and an entire mass of erythrocyte suspension metamorphosed
completely within a minute or less. Microscopic examination showed that the
erthrocytes swelled and burst on contact with water. Nuclear membranes
usually burst before cell membranes. The cell contents lost their refractile
appearance and became homogeneous. When the mass was stirred gently
most of the hemoglobin was leached, but there remained a large volume
(two- to threefold volume increase) of coherent, pink, transparent, gel-like
material (fig. 1). The rapid reaction occurred in 100 mg. per cent aqueous
heparin but not in 100 mg. per cent heparin prepared in 1 per cent sodium
chloride. Similarly, the reaction did not occur in 2 per cent aqueous sodium
citrate. Cells which were frozen and held at −20 C. would, upon thawing,
react the same as unfrozen material. When frozen cells were thawed, de-
hydrated and ground, the resulting powder did not react with water and swell.

Whole blood of trout also formed a clot-like mass rapidly when it was added
to distilled or deionized water. The reaction could be demonstrated in spring
water which had a calcium content generally in excess of 350 parts per million,
but at times the reaction failed to occur in tap water from a well source.

Washed blood cells of the bullfrog were more resistant to plasmoptysis.
Only after a reaction time of a minute or two could some coagulum be
gathered from frog cells in water. This coagulum was indistinguishable in
appearance from that which resulted from fish cells. It formed more slowly,
however, and tended to dissolve with ease.

Of the vertebrate classes represented in this work, reptile erythrocytes
were most resistant to plasmoptysis. When washed turtle cells were added to
water, they quickly agglutinated, but 15 to 20 minutes were required for
the transformation to the gelatinous state.

Blood cells of human and of sheep origin were subjected to the same wash-

*In order to eliminate the possibility of “adsorbed plasma,” washed erythrocytes were
held in 1 per cent trypsin in a phosphate-buffered saline (pH 7.6) for 5 hours at 19 C.
Control trypsin solutions digested fowl plasma clots within one hour. With regard to their
subsequent reactions with water, there was no observable difference between washed
tROUT erythrocytes and washed trypsinized trout erythrocytes.
STUDIES ON ERYTHROCYTES OF FRESHWATER FISH

Fig. 1.—A (Left), Plasma-free cells of trout blood, when mixed with water, formed a gelatinous material which adhered to the wooden applicator. (Right) Sheep cells subjected to the same treatment hemolyzed but produced no gel.

B (Left), Adhesive and cohesive nature of the gel formed from trout cells in water was demonstrated by harvesting the mass on an applicator. (Right) No such material could be harvested from hemolyzed sheep erythrocytes.

When given to blood cells from trout. The mammalian blood cells hemolyzed in water but did not produce a coagulum (fig. 1).

Coagulation time for blood of fingerling rainbow trout as determined by the capillary tube and slide methods was very short. When the capillary tubes were broken at 15 second intervals, coagulation time for 10 fingerling rainbow trout ranged from 30 to 60 seconds, with the mean being 42 seconds. Coagulation time of 10 additional fish with the slide method was considerably shorter; it ranged from 15 to 30 seconds, with the mean being 16.5 seconds. With use of the standard criterion for coagulation time, that of an initial fibrin shred, preliminary trials to find if differences existed between clotting time of fish blood in air and in water were made. It was difficult to decide when the first shred of material occurred in water. Usually, it was picked up later than from blood of the same fish which was on the dry end of the slide. Complete coagulation was identified readily, and was judged to have
TABLE 1.—Completed Coagulation Time for Rainbow Trout Blood Tested Simultaneously by the Slide Method in Air and in Water Held on a Slide

<table>
<thead>
<tr>
<th>Fish No.</th>
<th>Air*</th>
<th>Time in seconds</th>
<th>Water†</th>
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<tr>
<td>1</td>
<td>60</td>
<td>60</td>
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<tr>
<td>2</td>
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<tr>
<td>Mean</td>
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<td>67</td>
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*The order in which blood was placed on the slide was changed for each fish.
†One drop of blood was added to a 4 drop pool of spring water.

occurred when the entire drop of blood cohered as a mass that could be freely moved about with the point of a needle. As herein defined, complete coagulation occurred far more rapidly in water than in air (table 1).

The coagulum which resulted from the action of water upon trout erythrocytes was incompletely digested by pepsin but completely digested in trypsin. It was identified as being conjugated protein. It had a mucin-like quality, but the presence of phosphorus and purine base radicals indicated nucleoprotein.

**DISCUSSION**

Clotting time of fish blood has been reported to be much less than that of mammals. The present study confirmed this and also found that with the usual clinical methods, the clotting time of fish blood, like human blood, was shorter when determined by the slide method than when determined with the capillary tube. In the presence of water, coagulation was completed in about two-thirds the time required for it to occur in air (table 1). The rapidity of the reaction in water could not be found in nor attributed to the plasma; it was most like the rapidity of the reaction of erythrocytes with
It is considered likely, therefore, that the reaction of erythrocytes with water is in fact the first and possibly the most effective factor in blood coagulation among freshwater bony fishes in their normal milieu.

Fish have only one-fifth to one-fourth the relative blood volume of mammals, and rapid coagulation and hemostasis probably have survival value. Similarly, predator attraction is perhaps minimized by the rapidity of the processes. The quick clotting and participation of erythrocytes may well represent the primitive condition among vertebrates.

The speed of the clotting reaction was less pronounced in amphibian erythrocytes and almost lacking in erythrocytes from a reptile. The decrease in response from fish to reptile might reflect the altered need for hemostasis as the aquatic environment was forsaken for the terrestrial.

No reports of gelation of erythrocytes or of erythrocyte karyoplasm have been found for vertebrates. The reaction of fish erythrocytes bore a similarity to the arthropod hemolymph “cell coagulation” of Howell, Loeb and Yeager et al., which Gregoire recently reviewed. The cells of the fish were very fragile. They gave up contents which rapidly became clot-like, and which certainly contributed to coagulation. Gregoire himself found no “cell coagulation.” The reaction of fish erythrocytes with water was also similar to the described agglutination and “viscous transformation” of human blood platelets. Interestingly enough, platelet agglutination was inhibited by citrate, just as citrate inhibited or prevented fish erythrocyte gelation. Similarly for both, heparin itself had little or no effect on their reaction. Ferguson found that hypertonic solutions were better platelet preservers than hypotonic solutions.

SUMMARY

1. A clot-like material which results from very rapid plasmoptysis and hydrophyllic swelling of karyoplasm of erythrocytes from freshwater fish was reported. This attribute was found to a lesser degree in erythrocytes from an amphibian, but it was almost lacking in those of an aquatic reptile.

2. Rapid clotting of fish blood with the usual clinical methods was confirmed in this work. Fish blood clotted more quickly with the slide test than with the capillary tube test. In the presence of water, complete coagulation of fish blood occurred in only 65 per cent of the time required for clotting to be completed in air. This speed is attributed to the water-erythrocyte reaction, and not to a quality of the plasma.

3. An attempt was made to correlate the findings with the need for hemostasis and blood coagulation that is peculiar to aquatic vertebrates.

SUMMARIO IN INTERLINGUA

1. Es reportate le formation de un substantia coaguloide, resultante ab un rapidissime plasmoptysis e le tumescentia hydrophile del caryoplasma de erythrocytos ab pisces de aqua dulce. Iste characteristica esseva trovate a grados minus marcate in le erythrocytos de un amphibio; illo esseva quasi absente in le erythrocytos de un reptile aquatic.

2. Le hic reportate studios confirmava le reportos de altere autores de un
rapide coagulation de sanguine de pisce sub le conditiones del usual methodos clinic. Le sanguine de pisce se coagulava plus rapidemente in tests a lamina que in tests a tubo capillar. In le presentia de aqua, un coagulation complete de sanguine de pisce occurreva in 65 pro cento del tempore requirite pro le completion del coagulation in aere. Iste rapiditate es attribuite a un reaction inter le aqua e le erythrocytos e non a un qualitate special del plasma.

3. Es facite le tentativa de correlationar le constatationes del presente reporto con le requirimentos de hemostase e de coagulation sanguinee sub le conditiones de vita normal de vertebratos aquatic.

REFERENCES

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