Coagulation of Catfish Blood.* (29869)

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(Introduced by R. H. Wagner)
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The coagulation of mammalian blood results from interactions of numerous factors (1). During the past several years much has been learned about the coagulation process, but with each new advance the process is found to be more complex. It would be advantageous to have a biologic system in which coagulation of blood would occur with a minimum of factors. On the assumption that the complex coagulation system developed during phylogenesis, we have turned our attention to primitive genera. In the present study, various parameters of coagulation were investigated in the blood of a fresh water catfish (*Ameiurus nebulosus*).

Materials and methods. Fish used in this study were caught by means of a trotline. The fish weighed 0.5 to 1.5 kg and were bled immediately or kept alive in a wire cage submerged in a fresh water lake until blood was to be obtained. Blood was collected from the cardinal venous system or by direct cardiac puncture, and was either used immediately or was added to one-ninth volume 0.1 M sodium oxalate.

Whole blood clotting time was determined at 28°C by a modified Lee-White procedure (2), and blood was subsequently observed for evidence of clot retraction and clot lysis. Packed cell volume(3) and plasma fibrinogen (4) were determined. Prothrombin was determined by the 2-stage method using dog fibrinogen(5). One-stage clotting tests were performed at 28°C. Reagents were placed in a glass tube (10 × 75 mm) in the following order: 0.1 ml plasma, 0.1 ml thromboplastin solution, and 0.1 ml 0.02 M calcium chloride. The time elapsing from addition of calcium until a visible fibrin clot appears was recorded. The thromboplastins used were acetone dehydrated brain extract(6) from human, canine, rabbit or catfish; beef lung extract(5); or crude cephalin(7). The clotting time of recalcified plasma was obtained by substituting 0.154 M NaCl for thromboplastin.

To determine the effect of catfish plasma on human plasmas known to be deficient in certain plasma procoagulants, 0.05 ml human plasma was added to 0.05 ml catfish plasma and to this mixture was added 0.1 ml crude cephalin and 0.1 ml 0.02 M $CaCl_2$. In control studies, 0.05 ml normal human plasma or saline was used in place of the catfish plasma.

Results. The whole blood clotting time of blood from 8 catfish averaged 4.6 minutes (range 3 to 6 min). A solid clot always formed, clot retraction was complete by one hour, and no visible lysis occurred in 24 hours.

Calcium chloride solutions of varying concentration from 1.0 M to 0.007 M were used to determine the optimal calcium concentration for one-stage clotting studies of oxalated fish plasma. When the packed cell volume of whole blood was in the usual range (24-30%), addition of 0.02 M $CaCl_2$ resulted in the shortest clotting times.

One-stage clotting tests done on fish plasma were dependent upon the type of thromboplastin used. In Table I, the results of studies of catfish plasma are compared with human plasma as well as normal and hemophilic canine plasma. All procedures were done at 28°C since fish plasma was found to deteriorate rapidly at higher temperatures. Note that tissue thromboplastin prepared from catfish brain acted as a partial thromboplastin with canine plasma. In other studies, $BaSO_4$-treated normal dog plasma added to hemophilic dog plasma shortened the clotting time to the normal range. Barium sulfate-treated catfish plasma had no accelerator effect on either normal or hemophilic dog plasma. To obtain clotting times greater

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TABLE I. One-Stage Clotting Times of Plasmas of Catfish, Human, Normal Dog, and Hemophilic Dog with Various Thromboplastins at 28°C.

Thromboplastin	Clotting time (sec‡)			
	Catfish*	Normal dog†	Hemophilic dog†	Human†
None (recalcified clotting time)	80	250	400	300
Rabbit brain	60	9	9	15
Human "	51	9	9	15
Crude cephalin	55	47	88	85
Catfish brain	11	45	95	91

* Mean of 10. † Mean of 5. ‡ Nearest whole number.

than 180 seconds, 200 mg $BaSO_4$/ml of catfish plasma were necessary.

The prothrombin activity determined by the 2-stage method was dependent on the type of thromboplastin used. Only a trace of prothrombin activity could be detected in fish plasma when tissue thromboplastin of human brain, rabbit brain or beef lung was used, whereas with fish brain thromboplastin, a mean prothrombin value of 60 units per ml was found. When dog plasma was tested in the 2-stage system using catfish brain thromboplastin, prothrombin values were 90-100% of those obtained using more conventional thromboplastins.

Fibrinogen levels of catfish plasma ranged from 124 to 394 mg%, with a mean value for 5 samples of 260 mg%. Catfish plasma clotted rapidly with addition of bovine, human or canine thrombin. Thrombin evolving from clotting catfish plasma caused rapid clotting of canine fibrinogen.

The effect of catfish plasma on the partial thromboplastin time of several human plasmas each known to be deficient in one of the plasma procoagulants was determined. Using crude cephalin prepared from human brain, catfish plasma had no acceleratory effect on the long partial thromboplastin time of human plasmas deficient in factor V, factor VIII, factor IX, factor X, or factor XII.

Discussion. The present studies extend previous observations indicating a species specificity of prothrombin and thromboplastin(8,9). These studies suggest that prothrombin may be inert in heterologous systems. An alternate possibility is suggested by the work of Seegers, who has presented evidence that the end product of bovine prothrombin depends upon the manner of activation and that some of the intermediates of prothrombin activation do not yield thrombin with thromboplastin and calcium(10). With heterologous thromboplastins, prothrombin activation could stop with one of the inactive intermediates.

Complete or tissue thromboplastins cause rapid clotting; they become partial thromboplastins as they become less concentrated and the one-stage clotting time lengthens(7). Considerable emphasis has been placed on apparent functional differences between tissue thromboplastin (extrinsic prothrombin activator) and partial thromboplastin (intrinsic prothrombin activator)(11). Presumably during the clotting process "intrinsic prothrombin activator" interacts with certain procoagulants to generate potent clot accelerating activity(11). These procoagulants appear to be absent in catfish plasma, yet rapid clotting of catfish plasma occurs with catfish brain thromboplastin, a partial thromboplastin when tested with dog plasma. It is of interest that catfish brain thromboplastin of the same concentration acts as a complete thromboplastin or partial thromboplastin depending on the type of plasma used.

Failure of catfish plasma to correct deficiencies in human plasma could indicate that these procoagulants are not present and have no function in the plasma of the catfish. It appears to be equally possible that these factors are present, but there is little or no cross-reactivity in heterologous clotting systems. It was shown several years ago that it is difficult to demonstrate factor VIII activity in human plasma with the partial thromboplastin time assay using canine hemophilic plasma as a substrate(7). Cox, Lanchantin and Ware have described differences in accelerator globulin of human and bovine origin(12), Doolittle has described species

differences of fibrinogen(13), and Lewis has noted quantitative differences of various coagulation factors(14). Although we can show no procoagulant activity of catfish plasma when tested in deficient human or canine systems, these studies cannot be interpreted unequivocally to indicate the absence of similar but species-specific procoagulant factors. It does not seem possible to arrive at a definitive conclusion by adding plasma from one species to a clotting system which is capable of detecting "accelerators" of another species.

Summary. The blood of the fresh water catfish (*Ameiurus nebulosus*) clots rapidly. There appears to be species specificity of the prothrombin-thromboplastin reaction with both the one- and two-stage methods. Catfish brain thromboplastin causes rapid clotting of catfish plasma, but acts as a partial thromboplastin with canine plasma. Catfish plasma does not correct the long partial thromboplastin time of human plasmas deficient in factor V, factor VIII, factor IX, factor X, or factor XII.

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Characterization of Chick Embryo Interferon Induced by a DNA Virus. (29870)

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Interferon(1) is elaborated by animal cells in response to stimulation by viral and perhaps other substances. First definitive purification and biochemical characterization of an interferon was accomplished in this laboratory(2,3), employing interferon elaborated by chick embryo infected with an RNA virus, influenza A (RNA-interferon). The present report extends these biochemical studies to include the characterization of interferon elaborated from chick embryo cells by a DNA virus, herpes simplex. Additionally, development of technology for more precise estimate of isoelectric points for interferon is presented. The essential qualitative identity of the interferons induced by these two widely different viruses in cells of the same host species supports the present concept that interferon is a host-specific substance synthesized under the host genetic code rather than under the viral code.

Materials and methods. In vitro *assay for interferon activity.* The method(2) used previously was modified slightly. By the present procedure, serial 2-fold dilutions of the interferon sample were diluted in medium 199 containing 1% calf serum and antibiotics and 1 ml amounts were added in quadruplicate to monolayer primary cell cultures of whole chick embryo. After overnight incubation at