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The Prothrombin Conversion Rate in Various Species.*

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The rate at which thrombin forms in freshly drawn blood is known to depend upon the amounts of prothrombin and thromboplastin available. It is the purpose of the present article to show that in addition to these factors there is an element of species specificity. Even with comparable levels of prothrombin and thromboplastin, the conversion of prothrombin into thrombin is much more rapid in the plasmas of dog and rabbit than in those of man and guinea pig. This implies differences in the convertibility of prothrombin in the blood of these species. This sluggish conversion of prothrombin in man is a contributory factor in making man susceptible to a large number of hemorrhagic diseases.

The blood of dog or rabbit, when placed at once in 14 mm test tubes, clots in about 6 minutes, while the blood of man or of guinea pig, under like conditions, clots in about 11 minutes (Column 2, Table I). Data given in this same table show that this striking difference between the 2 groups is due to difference in the rate at which prothrombin is converted into thrombin.

It is easily shown that the above difference is not due to relative scarcity of thromboplastin in the blood of man and guinea pig. Thus, to each type of plasma we have added constant amounts of a very powerful preparation of thromboplastin. This addition is far in excess of the amounts normally present in clotting blood, and by this addition we have largely eliminated any relative inequality in the amount of thromboplastin. The plasma thus enriched with thromboplastin clotted quite rapidly when recalcified (Column 3), but the specific difference in speed of conversion still persisted. The plasma of dog and rabbit clotted in 9-10 seconds; those of man and guinea pig in 32-33.

In these plasmas, rich in thromboplastin, one can stop the conversion of prothrombin at varying intervals by the addition of oxalate to remove the calcium. By this technic one can study the entire curve of prothrombin conversion, and obtain further proof of the slow conversion in plasmas of man and guinea pig. Thus, in

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TABLE I.
Prothrombin conversion under various conditions.*

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
		Undiluted plasma plus concentrated thromboplastin (lung extract)			Dilute plasma (5 prothrombin units per cc) plus dilute thromboplastin (lung extract)	
Species	Clotting time of whole blood min	Total clotting time sec	"Latent period" of thrombin formation sec	Prothrombin conversion time (85-95% complete) sec	"Latent period" of thrombin formation sec	Prothrombin conversion time (100% complete) sec
Dog	6	9	4	23	10	90
Rabbit	6	10	4	30	21	90
Man	11	32	16	60	45	225
Guinea pig	11	33	18	75	40	240

**Reagents for Table I:* Oxalated plasma, as described previously.[3] Lung extract made by extracting ground beef lung 48 hours at 5°. Extract then cleared in centrifuge and neutralized (pH 7.3). Fibrinogen prepared as previously described,[3] but all steps carried out at 5°, using 1/5 sat. ammonium sulphate.

Column 3: Mix 5 vol. oxal. plas. + 5 vol. lung extr. + 1 vol. $CaCl_2$ (1.2%) + 4 vol. saline (0.9% NaCl).

Column 4: Same mixture as in column 3. After varying periods of incubation (0-20 sec.) add 3 vol. potassium oxalate (1.85%), and determine minimal incubation which will produce enough thrombin to form a clot.

Column 5: Repeat experiments of column 4, but incubate 15-90 seconds, then determine in each case the residual prothrombin.[2,3] Determine the incubation time required to convert 85-95% of the original prothrombin into thrombin.

Column 6: Mix 3 vol. diluted plasma (5 prothrombin units per cc) + 3 vol. lung extr. (dil. 1-16 with saline) + 2 vol. acacia (15% in saline) + 2 vol. $CaCl_2$ (1.0%) + 3 vol. saline. Incubate varying periods (0-45 sec.), add 2 vol. oxalate (1.85%) + 3 vol. fibrinogen. Determine minimal incubation which will produce enough thrombin to form a clot.

Column 7: Same incubation mixture as in column 6. Incubate varying periods (60-300 sec.). Add 3 vol. fibrinogen, but no oxalate. Determine shortest incubation period which will give a maximal yield of thrombin.

Column 4, it is seen that in these species thrombin did not appear in the mixture in measurable amounts until 16-18 seconds had elapsed after the addition of calcium to initiate the clotting process. In the plasmas of dog and rabbit, this "latent period" was only 4 seconds, however. The species difference persists throughout the entire period of prothrombin conversion. In Column 5 are data which show that in plasmas of man and guinea pig 60-75 seconds must elapse before prothrombin conversion is 85-95% complete; in plasmas of dog and rabbit, on the other hand, only 23-30 seconds were required to bring about this degree of conversion.

Several years ago Quick also made the observation that, on adding thromboplastin, rabbit plasma clots rapidly, while human plasma

clots slowly. He ignored the possibility that there might be a difference in the ease with which prothrombin is converted into thrombin in the 2 species, and he assumed, instead, that there is a 5-fold difference in amount of prothrombin to account for the difference in clotting time.[1] He proposed, in fact, to use these accelerated clotting times as a method for determining the relative amounts of prothrombin in various plasmas. We have developed, instead, a 2-stage titration procedure for prothrombin determinations,[2, 3] and with it we have eliminated conversion speed as a variable. With this method we have shown that the prothrombin level is almost identical in the 2 species.[4] Quick's clotting time test is of value to the clinician as a presumptive test of prothrombin activity, but for purposes of research a 2-stage method must remain the standard for the quantitative determination of prothrombin.

The slow conversion of prothrombin of man and guinea pig can also be demonstrated under a different set of circumstances. If the plasma be diluted as a preliminary step, one might expect to eliminate the effect of inhibitory substances which could affect the conversion rate. Antithrombin, for example, loses most of its activity even with moderate dilution. In experiments cited in Columns 6-7, Table I, we have diluted the plasma of all 4 species 35-70 fold prior to adding calcium and thromboplastin. The plasma of guinea pig normally contains about 185 units of prothrombin per cubic centimeter; that of man 295 units; that of rabbit 310, and that of dog 350 units.[4] In making the 4 dilutions, saline was added until each mixture contained 5 prothrombin units per cubic centimeter. On adding calcium and thromboplastin each received further dilution and the final level was 1 unit per cubic centimeter. Thus, in addition to diluting inhibitors, we have also completely eliminated variations in the amount of prothrombin. Under these conditions the prothrombin conversion was still found to be more rapid in the plasmas of dog and rabbit than in those of man and guinea pig. The "latent period" of thrombin formation was 10-21 seconds in the plasmas of dog and rabbit; 40-45 in those of man and guinea pig. Pro-

[1] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501; Quick, A. J., *Am. J. Physiol.*, 1936, **114**, 282; Quick, A. J., *J. A. M. A.*, 1938, **110**, 1658.

[2] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667.

[3] Smith, H. P., Warner, E. D., and Brinkhous, K. M., *J. Exp. Med.*, 1937, **66**, 801.

[4] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1939, **125**, 296.

thrombin conversion was complete in 90 seconds in the first 2 species; in 225-240 seconds in the last 2.

One is perhaps not justified in concluding that the prothrombin of one species is chemically different from that of another. One might assume, instead, that other substances could vary in such a way as to favor conversion in one species or impede conversion in another. It is significant, however, that partial purification of the prothrombin by ammonium sulphate fractionation does not eliminate the difference. The globulin precipitate, rich in prothrombin, is largely free of the known inhibitors of clotting.[2, 5] In experiments with dialyzed solutions of the globulin fraction we have found that the prothrombin conversion rate is still far more rapid in the canine than in the human material.

The difference in conversion rate between the species studied is not referable to any difference in the type of thromboplastin used, for in all cases the thromboplastin was obtained by saline extraction of ground beef lung. We have experiments to show that essentially identical results are obtained on using thromboplastin derived from dog lung, or by the use of homologous thromboplastins in each case. On using cephalin as a thromboplastin we have obtained slower conversion in every case, but here, too, the conversion was almost twice as rapid in plasmas of dog and rabbit as in those of man and guinea pig.

We have performed similar experiments to those of Table I on a wide variety of other vertebrates, including cats, rats, fowls, turtles and fish. When the plasmas were diluted to identical prothrombin concentrations, the conversion rate of the prothrombin almost always approximates that of dog prothrombin. The sluggish conversion in the plasma of man and guinea pig must be considered to be a handicap in the control of hemorrhage, for hemostasis depends upon covering the denuded surface promptly with a film of fibrin and platelets. If a clot is slow in forming, the blood is washed away before an effective clot can form. Many other factors influence the tendency to bleed, but the unfavorable factors, when multiple, are additive.

Summary. The rate at which prothrombin is converted into thrombin is much slower in the plasmas of man and guinea pig than in those of other vertebrates studied. The sluggish conversion of prothrombin in man is no doubt a factor which aggravates hemorrhagic diseases.

[5] Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.