Comparative Thromboplastin Activity of Acetone-Dehydrated Rabbit and Human Brain.* (22210)

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The clotting factor present in various tissue extracts is commonly designated as thromboplastin and its potency is usually measured by the one-stage prothrombin time. With the development of prothrombin consumption time(1), an alternative method for estimating thromboplastic activity became available. Plasma from a severe hemophiliac which is almost completely devoid of thromboplastinogen and, therefore, of thromboplastic activity can be employed as assay medium. Prothrombin consumption in this plasma resulting from addition of various tissue extracts can be regarded as a measure of their thromboplastic activity. To make the procedure independent of hemophilic plasma, which is not always obtainable, a substitute was sought. Serum from normal platelet-poor plasma was found fairly satisfactory. It appears to contain all essential clotting factors except fibrinogen and thromboplastinogen when prepared under carefully standardized conditions. The two tissues studied were acetone-dehydrated rabbit and human brain because they are used most widely as reagents for the one-stage prothrombin time.

Methods. Prothrombin time. The method as previously described was employed and thromboplastin was prepared by means of dehydration with acetone(2). *Prothrombin consumption time.* One ml of blood was transferred to test tube which was placed in water bath at 37°C. Fifteen minutes after formation of a solid clot, the tube was centrifuged 1 minute at 2000 rpm and replaced in water bath for 45 minutes. Prothrombin remaining in serum was determined by the one-stage test previously described(3). When testing activity of thromboplastin preparations, 0.05 ml of the extract was mixed with 1 ml of hemophilic plasma (or serum obtained from normal platelet-poor plasma) and prothrombin consumption determined as before. *Serum for assaying thromboplastic activity.* Human blood collected with silicone-coated syringes and needles was transferred to centrifuge tubes also treated with silicone. The tubes were placed in ice bath for 30 minutes and then centrifuged at 3°C in an angle centrifuge for 40 minutes at 10000 rpm. Plasma which was nearly platelet-free was carefully removed and transferred to glass container placed in a water bath at 37°C. As the fibrin formed, it was promptly removed by wrapping it about a glass stirring rod. After all fibrinogen was removed, the serum was placed again in water bath at 37°C for 10 minutes and then for 30 minutes at 3°C. During this period residual thrombin becomes neutralized and thromboplastinogen disappears almost completely. No detectable diminution of prothrombin, labile and stable factors and PTC (plasma thromboplastin component) was observed. It was stored at –20°C.

Results. Thromboplastic activity measured by prothrombin time of human and rabbit brain dehydrated with acetone. It was shown previously that physiological saline extracts of acetone-dehydrated rabbit brain varying in concentration from 1½ to 5% gave a prothrombin time of 12 seconds(4).

TABLE I. Effect of Varying Concentrations of Aqueous Extract of Acetone-Dehydrated Rabbit and Human Brain on the Prothrombin Time.

	Prothrombin time, sec.			
	Rabbit brain		Human brain	
Conc. of extract, %	Normal plasma	Dicumarol plasma	Normal plasma	Dicumarol plasma
.5	14½		14	
1	14	26	13½	32
2	12	26	12	30
4	12	28	12	30
6	12	28	13	34
8	13	36	14½	44

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TABLE II. Variation of Thromboplastic Activity in Various Types of Thromboplastin Preparations When Tested on Normal and Stable Factor Deficient Plasmas.

Thromboplastin preparation	Prothrombin time, sec. Normal plasma	Plasma deficient in stable factor
Rabbit brain	12	45
Human "	12	50
Dog "	21	67
Pig "	23	68
Russell viper venom	14	13½
Commercial preparation I	11½	25
Idem II	19	27
" III	14	32
" IV*	12	50

* Made from rabbit brain.

The results recorded in Table I show that the same prothrombin time is obtained with 2 to 4% extracts of acetone-dehydrated human brain. At higher concentrations, human brain extract has a depressing effect which is somewhat greater than that of rabbit brain. This has also been observed by Stein and Abrahams(5). Both human and rabbit brain extracts give very nearly the same prothrombin times on plasma from patients receiving Dicumarol.

To compare thromboplastic activity of acetone-dehydrated rabbit and human brain with other preparations of thromboplastin, the prothrombin time was done with various types of thromboplastin using normal plasma and plasma from patient with stable factor deficiency. The results are recorded in Table II. It is to be noted that only acetone-dehydrated human and rabbit brain extracts gave comparable results on normal and stable factor deficient plasmas. Of various commercial preparations of thromboplastin, only one, which is made from acetone-dehydrated rabbit brain, gave results which were similar to those obtained on human and rabbit brain processed according to the technic of Quick.

Thromboplastic activity of acetone-dehydrated rabbit and human brain as tested by prothrombin consumption time. When increasing amounts of extracts of rabbit brain are added to plasma from a severe hemophiliac, a proportionate increase in serum prothrombin time occurs as shown in Table III. When serum, which is obtained from normal platelet-poor plasma, is substituted for hemophilic plasma, the results are very similar showing that the latter can be used successfully in place of hemophilic plasma.

On comparing the thromboplastic activity of rabbit and human brain by adding the same quantity of extract of each to the assay serum and determining resulting consumption of prothrombin, a similar but slightly greater potency was observed as shown in Table III. Brain extracts of other species have less thromboplastic activity than human and rabbit brain when measured either by prothrombin time or prothrombin consumption time and it can be seen from the results in Table IV that as prothrombin time increases, prothrombin consumption time decreases. Russell viper venom is the exception in that it produces a fairly short prothrombin time but does not cause high consumption of prothrombin. The poor thromboplastic activity of chicken brain can be attributed to species

TABLE III. Comparison of Hemophilic Plasma and Serum from Platelet-Poor Plasma as Assay Media for Thromboplastic Activity of Rabbit Brain Extract.

Conc. of rabbit brain, %	Prothrombin consumption time, sec. Hemophilic plasma	Serum from platelet-poor plasma	
.0	8	8	(8)*
.2	9	9	(10)
.5	9	12	(18)
1	16	15½	(27)
2	25	20	(42)
4	34	31	(50)
6	38	35	(63)

* Figures in parentheses are the prothrombin consumption times when human brain extract was employed.

TABLE IV. Thromboplastic Activity of Various Tissue Extracts as Measured by Prothrombin Time and Prothrombin Consumption Time.

Source	Prothrombin time, sec.	Prothrombin consumption time, sec.
Rabbit brain*	12	36
Human "	12	39
Dog "	20	24
Pig "	23	19
Chicken "	57	9
Russell viper venom	14	17½

* A 2% aqueous extract was employed.

TABLE V. Effect of Heating on Thromboplastic Activity of Rabbit and Human Brain.

	Prothrombin time, sec.	Prothrombin consumption time, sec.		
		Normal platelet-poor plasma	Hemophilic plasma	Serum from platelet-poor plasma
Rabbit brain:				
Heated to 50°C	12	74	34	39
" " 60°C for 20 min.	27	28	8	15
" " " " 60 "	32	12	8	8
Human brain:				
Heated to 50°C	12	94	49	50
" " 60°C for 20 min.	16	62	18	19
" " " " 60 "	24	13	8	8

specificity since it is highly potent when added to avian blood(6).

Action of heat on thromboplastic activity of rabbit and human brain extract. No activity is lost when either human or rabbit brain is heated at 50°C but at 60°C a marked loss of activity occurs especially in rabbit brain extract(7). Human brain is more resistant to heat, and even after 20 minutes at 60°C, it still produces prothrombin consumption when added to hemophilic blood. (Table V).

Discussion. Acetone-dehydrated brain extract is admittedly a crude thromboplastin but when properly prepared either from rabbit or human brain, it has remarkably constant activity as measured by the one-stage prothrombin time. In the present study it has been shown that rabbit and human brain preparations also have almost the same activity when determined by their effect on prothrombin consumption test. Since no method is known for preparing a purified thromboplastin which has an activity equal or superior to rabbit brain, it seems desirable to accept the latter as a temporary standard of reference. Contamination with blood can be reduced to a minimum when rabbit brain is carefully cleared of visible blood vessels. The product is, therefore, devoid of labile and stable factors which shorten prothrombin time of plasmas in which these factors are deficient. The reagent is, therefore, particularly valuable when prothrombin time is employed for estimating the over-all prothrombin activity of plasma. This is especially important in controlling Dicumarol therapy since this drug diminishes both prothrombin and stable factor. High concentrations of either rabbit or human brain exert an inhibitory effect on prothrombin time but for the concentration recommended (200 mg in 5 cc saline solution), the error is negligible.

Rabbit brain extract heated to 60°C for 20 minutes looses its holothromboplastic properties but becomes a suitable reagent for differentiating hemophilia and hemophilia-like diseases from thrombocytopenia and thrombasthenia since its addition does not correct the hemophilic group but does correct the latter conditions. Human brain cannot be substituted since it is too heat-resistant.

Summary. Rabbit and human brains, when dehydrated with acetone, yield a product which has a high, constant and practically equal thromboplastic activity as measured by the one-stage prothrombin time and by prothrombin consumption time using hemophilic plasma as the assay medium. Serum prepared from normal platelet-poor plasma can be substituted for hemophilic plasma. Human brain extract differs from rabbit brain in that it has a greater inhibitory effect at high concentration and is more resistant to heat.

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