VARIATION IN EXTERNAL MECHANISM OF PROTHROMBINASE FORMATION IN DIFFERENT SPECIES OF VERTEBRATES

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The recalcification time and prothrombin time were studied in vertebrates of different species by the use of homologous and heterologous brain thromboplastins. Indices of the recalcification time in cold-blooded vertebrates were much higher than in warm-blooded animals. Rabbit and human thromboplastins of cold-blooded animals had little or no activity in heterologous plasms and they significantly shortened the recalcification time only in homologous plasma.

The rate of prothrombinase formation under the influence of tissue thromboplastins shows considerable variation in vertebrates of different species. This suggests that the external (priming) mechanism of blood clotting differs in its significance in the formation of hemostasis in these animals. According to some reports homologous brain thromboplastins are more active, other conditions being equal, than heterologous [3-5, 7-9]. Meanwhile tissue thromboplastins of some animals have low activity when tested on homologous or heterologous plasmas.

More fundamental qualitative differences have also been found between the action of different brain thromboplastins, especially under pathological conditions. In one form of hemophilia (Bm hemophilia) the prothrombin time is lengthened if bovine thromboplastin is used in the reaction whereas the use of human, rabbit, and other animal thromboplastins gives normal results of this test. The reaction with bovine thromboplastin is used for the differential diagnosis between B and Bm hemophilias. However, the mechanisms of the different actions of the tissue thromboplastins have not yet been explained.

In the investigation described below a comparative study was made of the external mechanism of prothrombinase formation when homologous and heterologous brain thromboplastins in fish (Serranus scriba), frogs (Rana ridibunda), turtles (Testudo horsfieldi), chickens and hens (Gallus gallus), rabbits (Oryctolagus cuniculus), bulls (Bos taurus), pigs (Sus domesticus), and cats (Felis domestica) are used.

EXPERIMENTAL METHOD

Dry brain thromboplastin was prepared from the gray matter of the bovine, equine, and porcine brains and from the whole brain of fishes, frogs, turtles, chickens, hens, cats, and rabbits by the method described by Baluda et al. [1]. Before the experiments the samples of brain thromboplastin were diluted in 0.85% sodium chloride solution (mammals and birds) or in 0.65% salt solution in the case of cold-blooded animals (100 mg thromboplastin to 5 ml physiological saline). The activity of each sample was tested on homologous and heterologous plasmas of all the animals listed above. Experiments were carried out on the oxalated plasma of fishes (30), frogs (48), turtles (13), chickens (51), hens (25), rabbits (31), bulls (50), pigs (56), and cats (33). Blood was taken from the heart of all the animals and from the heart and marginal vein of the ear of the rabbits. The blood was stabilized with 0.1 N sodium oxalate solution in the ratio of 9:1. The blood was then centrifuged for 15 min at 2,500 rpm and the plasma separated. The recalcification time of the resulting

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TABLE 1. Mean Recalcification and Prothrombin Times in Different Animals With the Use of Homologous and Heterologous Brain Thromboplastins (M± m)

Species of animals in whose plasma tests were carried out	Recalcification time (in sec)	Prothrombin time (in sec) with different specimens of thromboplastin									
		fishes	frogs	turtles	chickens	rabbits	pigs	bulls	horses	cats	human
Fishes	180±2,0	96±0,7	179±1,9	—	272±3,0	226±2,2	210±2,0	210±2,0	229±1,8	218±1,6	—
Frogs	164±1,2	—	52±0,4	—	—	101±0,7	122±0,7	80±0,7	175±2,6	80±1,5	—
Turtles	180±2,5	42±1,3	65±1,2	28±0,7	89±1,7	67±1,6	90±1,2	72±1,0	87±2,0	78±2,0	—
Chickens	115±1,0	—	97±0,3	—	28±0,2	68±0,5	86±0,4	88±0,5	100±0,6	81±0,4	—
Rabbits	27±0,3	—	34±0,2	—	—	11±0,1	24±0,2	23±0,5	29±0,2	23±0,2	14±0,1
Pigs	55±0,5	—	—	—	—	25±0,1	30±0,1	25±0,1	30±0,1	27±0,1	25±0,2
Bulls	95±0,8	—	55±0,5	—	—	22±0,1	31±0,1	44±0,3	60±0,4	37±0,1	37±0,7
Cats	58±0,6	—	30±0,3	—	—	20±0,3	25±0,3	30±0,3	29±0,3	25±0,2	18±0,1

specimens of plasma was determined by the method of Bergerhof and Roca and the prothrombin time by Tugolukov's method using all the prepared thromboplastins. The index of acceleration of coagulation (IAC) under the influence of thromboplastin was calculated by the following equation:

$$IAC = \frac{\text{Recalcification time}}{\text{Prothrombin time (for each thromboplastin)}}$$

EXPERIMENTAL RESULTS

The results given in Table 1 show that the recalcification times in cold-blooded vertebrates and chickens were much greater than in warm-blooded animals. These results suggest differences in the kinetics of blood coagulation in the vertebrates. Of all the tissues thromboplastins studied, rabbit thromboplastin was most active, for when tested on both homologous and heterologous plasma it gave the shortest prothrombin time. The thromboplastins of all the other warm-blooded animals possessed approximately equal activity when tested on different plasmas. The only exceptions were bovine and chicken thromboplastins. When all thromboplastins were used the prothrombin time with samples of plasma from many species of animals was very long and differed only slightly from the recalcification time. Thromboplastins obtained from the brain of cold-blooded animals had little or no activity. The prothrombin time when they were used was frequently longer even than the recalcification time determined in the plasma of the same animals. These thromboplastins significantly shortened the recalcification time only in homologous plasma.

Analysis of results in Table 1 shows that the prothrombin time in homologous plasma was by no means always shorter than in heterologous plasma. For example, bovine thromboplastin, when tested on the plasma of rabbits, pigs, and cats, caused more rapid coagulation than when tested on bovine plasma. The plasmas of rabbits and cats were more sensitve to human thromboplastin. The same results were observed when frog thromboplastin was used.

These results indicate that the functioning of the external mechanism of prothrombinase formation depends not so much on whether the thromboplastin and substrate plasma are from the same species as on the species-specific variations in the kinetics of the enzymes system of blood coagulation.

This state of affairs is clearly illustrated by comparison of the indices of acceleration of blood coagulation by different thromboplastins (Table 2). Brain thromboplastins of rabbits, pigs, bulls, and cats caused the greatest degree of acceleration of coagula-

TABLE 2. Index of Acceleration of Blood Clotting for Different Animals with the Use of Homologous and Heterologous Brain Thromboplastins

Species of animals in whose plasma tests were carried out	Indices when different brain thromboplastins were used									
	fishes	frogs	turtles	chickens	rabbits	pigs	bulls	cats	horses	human
Fishes	1,8	1,0	—	0,66	0,8	0,86	0,86	0,83	0,82	—
Frogs	—	1,0	—	—	1,6	1,3	2,0	2,0	0,9	—
Turtles	4,2	2,7	6,4	2,0	2,7	2,0	2,5	2,3	2,0	—
Chickens	—	1,2	—	4,1	1,7	1,3	1,3	1,4	1,2	—
Rabbits	—	0,8	—	—	2,5	1,1	1,1	1,1	0,9	—
Pigs	—	—	—	—	4,3	3,1	2,2	2,0	1,8	2,2
Bulls	—	1,8	—	—	4,3	3,1	2,5	2,7	1,6	2,7
Cats	—	1,9	—	—	2,9	2,1	1,9	2,1	2,0	3,2

tion of bovine plasma while human thromboplastins occupied the same position as regards cat plasma. Only certain thromboplastins showed maximal activity when tested on homologous plasma (chicken and bovine thromboplastin).

These results show that the kinetics of the external mechanism of prothrombinase formation depends not only on whether the tested samples of thromboplastin and substrate plasma are of the same species, but also on differences in the coagulating activity of thromboplastin from different animals and also, possibly, on the sensitivity of the clotting system of the blood of animals of the same species to them.

The fact will be noted that animals with the longest recalcification time most frequently showed a longer prothrombin time with individual thromboplastins, including during testing of homologous tissue thromboplastins. For example, bovine plasma showed low sensitivity to thromboplastin from cats, bulls, horses, man, and frogs. This suggests that the cause of the unequal activity of the various tissue thromboplastins must be sought primarily in typological species-specific differences in the blood clotting systems.

LITERATURE CITED

1. V. P. Baluda, V. N. Malyarovskii, and I. A. Oivin, Laboratory Methods of Investigation of the Blood Clotting System [in Russian], Moscow (1962).
2. Ya. I. Vygovskaya, Abstracts of Proceedings of the 40th Plenum of the Scientific Council of the Central Institute of Hematology and Blood Transfusion [in Russian], Moscow (1961), p. 69.
3. B. A. Kudryashov, L. I. Murav'eva, and L. D. Ulitina, Dokl. Akad. Nauk SSSR, 77, 673 (1951).
4. B. A. Kudryashov, L. I. Murav'eva, and L. D. Ulitina, Dokl. Akad. Nauk SSSR, 84, 563 (1952).
5. B. I. Kuznik and A. D. Naumov, in: Proceedings of the 10th Congress of the I. P. Pavlov All-Union Physiological Society [in Russian], Moscow-Leningrad (1964), p. 438.
6. G. Sh. Labakhua, M. V. Tordiya, and E. Sh. Kirguradze, Abstracts of Proceedings of a Scientific Session of the Institute of Experimental and Clinical Surgery and Hematology, Academy of Sciences of the Georgian SSR [in Russian], Tbilisi (1961), p. 69.
7. C. H. Bigland and C. C. Triantaphyllopoulos, Am. J. Physiol., 200, 1031 (1961).
8. E. Hecht, Biochem. Z., 326, 325 (1955).
9. G. I. Svet-Moldawsky, Nature, 197, 4864 (1963).