コイのプロトロンビン時間

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Short Paper

Prothrombin Time of Common Carp Blood

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We reported in a previous paper[1] that it was necessary to eliminate the effects of contact factors in the measurement of clotting times, particularly for the determination of prothrombin time (PT). When eliminating the effects of contact factors, the PT of common carp blood was found to be 232±101 s using a commercially available PT reagent prepared from the rabbit brain, "Activated thromboplastin" (International Reagent Corp., Tokyo). This value was very high compared with those described in other reports.[2,3] As it has been reported that the interaction of the tissue factor and clotting time of plasma is species-specific,[3,4] the above high PT value seemed to result from a heterologous tissue factor. In the present study we used homologous brain extract as a tissue factor for the determination of PT.

The brain extract was prepared according to the method of Ahmad *et al.*[5] Brains were removed and washed with 0.85% saline three times. Pooled brains were homogenized with equal volumes of saline. The material was centrifuged twice at 2,700×g for 20 min at 4°C, and the supernatant was saved. The total protein of this extract was determined to be 26–29 mg/m*l*, by biuret analysis using a bovine albumin (Sigma) as a standard.

Procedures for the taking of blood and for preparing the citrated plasma were described in a previous paper.[1] Equal volumes of brain extract solution and M/40 calcium chloride solution were mixed and kept in a water bath at 37°C. One-tenth m*l* of plasma was pipetted into a polystyrene tube (10×72 mm) and placed in a 37°C water bath for 3 min. Then 0.2 m*l* of brain extract-calcium chloride mixture was added and a stopwatch was started. The clotting time was taken as the time when a fibrin clot was formed.

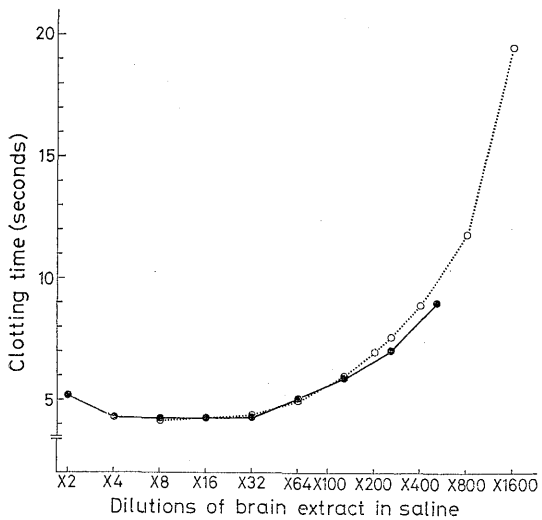

Fig. 1. Relationship between concentration of brain extract and PT. The solid and dotted curves were obtained from different lots of plasma samples and different lots of brain extracts.

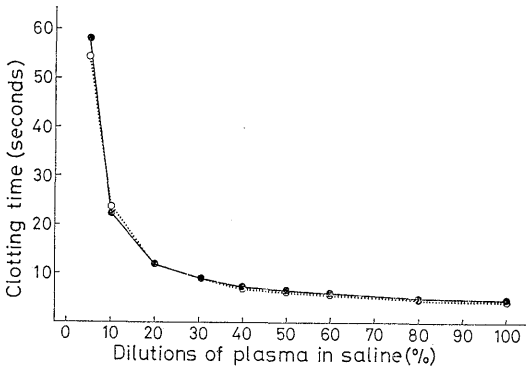

Fig. 2. The PT of graded dilutions of plasma in saline. The solid and dotted curves were obtained from different lots of plasma samples.

Two-fold dilutions of the brain extract in 0.85% saline were prepared and PT was determined using each dilution. The clotting times were plotted against the dilution on single logarithmic graph paper (Fig. 1). The two curves shown in Fig. 1 were obtained from different plasma samples and different lots of brain extract, and they revealed similar trends. No significant difference was observed in the clotting times in dilutions from 1:4 to 1:32, and prolongations were observed in dilutions of more than 1:64. The original brain extract (1:2 dilution) revealed a rather slight prolongation of clotting time. Therefore, the 1:20 dilution seemed optimal as a tissue thromboplastin for the purposes of evaluating prothrombin activities and saving the amount of brain extract.

The PT was determined for 10 plasma samples using the 1:20 dilution of the brain extract. Plasma samples were taken from fish weighing 415–562 g that were kept in glass tanks for a few weeks and fed a commercial pellet food. The clotting times ranged from 4.3 to 5.2 s, and the mean±SD was determined to be 4.8±0.3 s. This indicates that it is essential to use a homologous tissue factor for the determination of PT in fish blood.

Graded dilutions of pooled citrated plasma in saline were prepared, and PT was determined for each dilution using the same lot of brain extract (1:20 dilution). The clotting time was taken as the time when a fibrin clot formed, but in high dilutions of plasma (1:10 and 1:20 dilution) the first appearance of a fibrin web was taken as the clotting time. The two curves shown in Fig. 2 were obtained from different lots of pooled citrated plasma, and they revealed almost the same trend.

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