does not seem possible to explain the protective effect in shocked dog by any action in the cardiovascular system in normal animals.

The experiments do not exclude the possibility of a pharmacodynamic antagonism for one of the hypothetical toxic substances responsible for shock.

Received May 9, 1950. P.S.E.B.M., 1950, v74.

Prothrombin Utilization during Clotting: Comparison of Results with the Two-Stage and One-Stage Methods.* (17927)

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It has been recognized for decades that prothrombin disappears from blood during clotting, but that traces of this clotting factor may persist in serum for days(1). With the development several years ago of the 2-stage method for determining prothrombin in fibrinogen-free systems(2), further studies of prothrombin and the dynamics of clotting became possible. One of us, using this method, determined the rate of prothrombin consumption during clotting of normal, hemophilic and platelet-poor bloods(3,4). Originally, the one-stage test could be used only for the study of plasma, since no separate source of fibrinogen was provided. Recently this procedure has been modified so that it can be applied to serum(5). With the modified one-stage test, serums from normal, hemophilic and platelet-poor bloods have been investigated by several workers. While the results seemed to confirm in a general way the findings with the 2-stage test, certain discrepancies were apparent.

The present study was undertaken to compare prothrombin utilization during clotting, as indicated by the 2 methods, and to determine, if possible, the basis for the discrepancies observed. Simultaneous one-stage and 2-stage determinations of prothrombin activity were performed on a series of bloods during and after clotting. Normal bloods, both human and dog, and a group of slowly clotting specimens—canine hemophilic blood, human platelet-poor plasma and normal blood clotting in silicone-treated containers—were studied.

Materials and methods. Three normal human subjects, 6 normal kennel dogs and 3 hemophilic dogs(6) were used in these experiments. Blood was collected by venipuncture in 2 dry syringes. Four ml of blood from the first syringe were mixed with 0.5 ml 3.2% sodium citrate solution and the control plasma obtained by centrifugation. Blood from the second syringe was distributed immediately into a series of 10x75 mm dry glass tubes calibrated to 2 ml (28°C). Periodically, 0.25 ml of 3.2% sodium citrate was added to each tube and the citrated serum (or plasma) obtained by centrifugation (about 3000 g for 3 minutes). For the silicone experiments, silicone-treated needles, syringes and calibrated tubes were used(7). Platelet-poor human plasma was obtained as described previously(7). One-stage and 2-stage pro-

* This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institutes of Health, U. S. Public Health Service.

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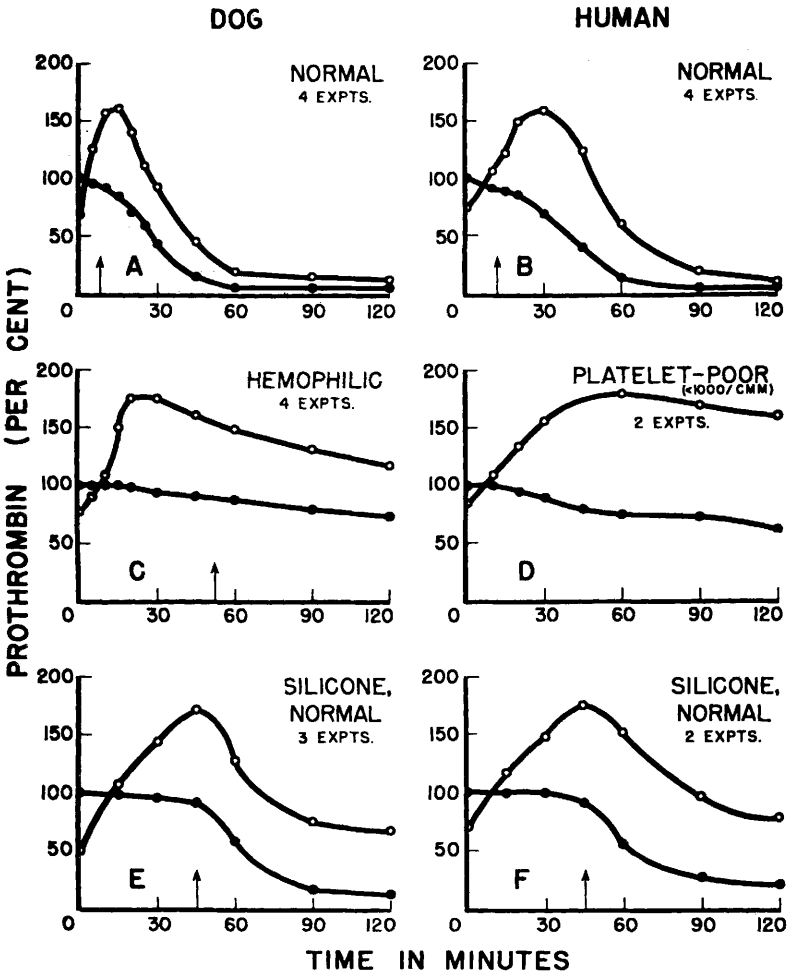

FIG. 1.

Comparison of one-stage and 2-stage prothrombin values in clotting blood. Mean one-stage determinations indicated by ○ (upper curve), mean 2-stage determinations by ● (lower curve). Arrows indicate mean clotting time (modified Lee-White method).

thrombin determinations and a test for thrombic activity were performed on all samples. Modified one-stage prothrombin times(8) were determined by mixing 0.1 ml fresh thromboplastin solution (Difco), 0.1 ml fresh normal homologous plasma treated with $BaSO_4$(9), 0.2 ml 0.02M $CaCl_2$ solution and 0.1 ml citrated test sample (37°C) in the order named. The experimental samples were tested 3½ to 5 minutes after citration, the control plasmas 40 minutes after venipuncture. Normal control dog plasma clotted between 8.2 and 8.6 seconds, human plasma between 14.6 and 15.2 seconds. Results of the one-stage test are expressed as % prothrombin activity, using plasma dilution curves(9) with the 100% values obtained from the control plasmas. For values higher than 100%, the sample was diluted to half-strength with $BaSO_4$-treated plasma before testing. Percentile yields were then doubled. Serum thrombic activity was determined

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simultaneously with the one-stage prothrombin tests by adding 0.1 ml citrated serum to 0.1 ml homologous $BaSO_4$-treated plasma and 0.3 ml 0.9% NaCl (37°C). Two-stage prothrombin determinations(2) were performed with added beef serum(10). Normal dog plasma contained 375 to 425 prothrombin units per ml, normal human plasma 275 to 325 units. Results are expressed as % of the appropriate control plasma.

Results. In Fig. 1 the results obtained during the first 2 hours of these experiments are summarized. As measured by the 2-stage method, the prothrombin content of the serum diminished progressively, but at varying rates in the different types of experiments. With the one-stage method, a period of hyperactivity in which the values exceeded 100% was noted soon after the blood was drawn. The serum prothrombin clotting times reached a minimum of about 6.0 seconds for the dog serum, and about 10.5 seconds for the human serum. The hyperactive phase was greatly protracted in the hemophilic and platelet-poor samples (Fig. 1, C and D). Data from 6 experiments on canine hemophilic blood, not included in the chart, showed that the period of hyperactivity lasted for 3 to 4 hours. In 2 experiments with platelet-poor human plasmas, this hyperactive period lasted 6 to 7 hours.

Little or no thrombin was found in the citrated serum samples. With the dog serum, no clots were observed in 4 hours. With the human serum, the thrombin clotting times varied greatly; a few samples clotted in 5 minutes, but most were not clotted in 4 hours.

With the original 2-stage prothrombin procedure(2), the incubation time required for maximum thrombin activity was regularly shorter for the serum samples than for the plasma samples. Optimal incubation times were determined carefully in several experiments, using Ac globulin-poor beef lung (Mg $(OH)_2$ treated) as thromboplastin. The optimal activation time for the human plasma was 5 minutes, compared to 3 minutes for serum of approximately the same prothrombin unitage. Although the rate of thrombin formation was more than 1½ times as fast in serum, prothrombin values were never higher than in the control plasma. Similar results were observed in canine experiments.

Discussion. During the early stages of these experiments, the one- and 2-stage procedures gave divergent results. The slower the clotting, the more prolonged was the period of divergence. After the peak of prothrombin activity observed by the one-stage method, the 2 curves tended to converge. However, the one-stage results remained consistently higher than the 2-stage values. The high serum activity observed by the one-stage test does not appear to be due to residual thrombin(11), but rather to the elaboration of an accelerator of prothrombin conversion during clotting(12,13,14). An attempt was made to obtain better agreement between the results with the 2 methods by recalculating the one-stage values on the descending portions of the curves. The highest one-stage result in % was made equivalent to the corresponding 2-stage value in %. The subsequent one-stage values then were adjusted proportionately. With this recalculation the one-stage values became almost identical with the 2-stage values. This suggests that if the one-stage procedure were modified further by addition of the serum accelerator factor, the discrepancies in the serum values obtained by the 2 methods might disappear. It will be noted that the peak of hyperactivity of serum in the hemophilic and platelet-poor samples (Fig. 1, C and D) is approximately the same as in the corresponding normal bloods. The hyperactivity of hemophilic serum was evident in the data presented previously by Quick(5). It was not recognized at the time however, and in retrospect, it is easy to see how this was interpreted as evidence for a "quantitative lack of conversion" of prothrombin dur-

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ing the clotting of hemophilic blood.

It will be noted in Fig. 1 that the initial one-stage prothrombin values were lower than those obtained with the control plasma. Thus, in the normal human experiments (Fig. 1 B), the prothrombin time of the control plasma was 14.8 seconds (100%), while in the experimental series the plasma prothrombin time was 16.2 seconds (74%). The chief difference between these 2 plasmas was the age at the time of testing. The one-stage plasma determination in the experimental series was made within 5 minutes after venipuncture, while in the control plasma the test was not made until 40 minutes after venipuncture. Other studies have shown that during the first 30 to 45 minutes after blood is collected, the prothrombin time of citrated plasma gradually shortens. These findings are in accord with those of Owren(12), and indicate the importance of the time elapsing between collection of blood and performance of the one-stage test. While prothrombin times longer than 11 or 12 seconds on normal human plasma by the usual method are generally attributed to a relatively inactive thromboplastin, they may actually be due to the prompt testing of freshly collected plasma. Further studies are needed to determine whether the changes in plasma prothrombin time immediately after venipuncture are due to the same factor(s) that causes hyperactivity of serum in the one-stage test.

Summary. 1. A comparison was made of the changes in prothrombin during clotting, as indicated by the one- and 2-stage methods.

2. By the 2-stage method, progressive disappearance of prothrombin from serum was observed. Prothrombin utilization was slower in human blood than in dog blood, and was delayed greatly in canine hemophilic blood, platelet-poor human plasma, and in blood clotting in silicone-treated glassware.

3. By the one-stage method, an initial period of hypoactivity in the plasma was followed by a hyperactive phase in the serum. At the peak of hyperactivity, "prothrombin" values were about 180% of the control plasma. In slowly clotting bloods, the hyperactive phase developed less rapidly and persisted for a longer period than in normal blood. The abnormally high serum prothrombin values obtained by the one-stage test appear to be due to the evolution and persistence of the recently recognized serum factor which accelerates thrombin formation. Apparently this factor does not influence the 2-stage serum prothrombin values.

Received April 18, 1950. P.S.E.B.M., 1950, v74.

A Diabetes Insipidus-like Condition Produced in Dogs by a Potassium Deficient Diet. (17928)

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It is well known that administration of desoxycorticosterone acetate, an indirect means of reducing the serum potassium level, results in the production of a syndrome in dogs of polydipsia and polyuria resembling diabetes insipidus(1-3). To our knowledge the effect of direct dietary withdrawal of potassium on the fluid exchange has not been reported.

Twelve dogs housed in metabolism cages were placed on basic diets* containing approximately 0.01% potassium. The details

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