Coagulation Defect in Horse Plasma.* (23099)

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Discrepancies in the explanation of the coagulation defect of horse plasma exist. Antihemophilic factor (AHF) is said to be present (1) or absent(2). We have recently reported an apparent lack of the Christmas factor (PTC)(3). Four out of 5 different horse plasma samples showed delayed thrombin formation, which could be restored to normal by addition of normal human serum. However, investigations with human hemophilic patients have revealed that other defects in the plasma thromboplastin system than a deficiency in PTC are restored by addition of normal serum(4). This observation prompted a re-investigation of the defect in horse plasma.

Materials and methods. The prothrombin time (Quick) was determined according to (5). The prothrombin-proconvertin complex was estimated by the Owrens method(6,7), using $BaSO_4$ absorbed plasma(8). The thrombin generation test was performed as described before(4). Barium sulfate absorbed serum, heated reabsorbed serum and platelet suspensions were prepared as described(9).

Results. The following results were obtained with blood from one horse. They are representative for the deficiency. Prothrombin time: 20 sec. (Normal human control: 19 sec.) Prothrombin-proconvertin (Owren): 90%. Platelet count: 136000 per mm^3 plasma. The thrombin generation was delayed and the thrombin concentration never reached high values (Fig. 1 A). Recalcification time (as estimated during thrombin generation test) was 3 min. 50 sec. Addition of 0.2 ml absorbed bovine plasma to 1 ml of horse plasma had only a slight effect on thrombin generation (Fig. 1B). Recalcification time was now 3 min. 30 sec. Addition of 0.2 ml platelet poor, frozen plasma from another deficient horse had no effect on thrombin formation (Fig. 1 C). After addition of 0.2 ml of its own frozen, platelet rich plasma, thrombin generation became almost normal (Fig. 1 D). Similar result was obtained also after addition of normal platelet containing human plasma (Fig. 1 E).

Fig. 2 shows thrombin generation after addition of 0.2 ml normal human serum (curve A), 0.2 ml twice absorbed normal human serum (curve B) and 0.2 ml twice absorbed human serum, previously heated for 30 min. at 56°C (curve C). In all these mixtures thrombin generation became normal. Recalcification time was 1 min. 45 sec., 2 min. and 2 min. 50 sec. respectively. Addition of 0.2 ml fresh plasma (curve D) or serum (curve E) from a patient with the Hageman trait had no effect on thrombin generation (Fig. 2).

Fig. 3 shows results obtained after storage of the horse plasma at –20°C for one night. Thrombin generation became completely normal after freezing the plasma with its normal content of platelets (curve A), whereas there was no improvement after freezing of the platelet poor plasma (15000 platelets per mm^3) (curve B). Curve C demonstrates thrombin generation in fresh horse plasma after addition of 0.2 ml of its own thrombin free serum. There was no improvement. Curves D and E show also thrombin generation in plasma from a patient deficient in PTC after addition of horse plasma (D) and horse serum (E) (0.2 ml). Thrombin generation in the PTC deficient plasma became completely normal. Thrombin generation in the Christmas plasma with no additives is demonstrated in Fig. 3 curve F.

Fig. 4 demonstrates effects of various platelet suspensions: Curve A was obtained after addition of 0.4 ml fresh human platelet

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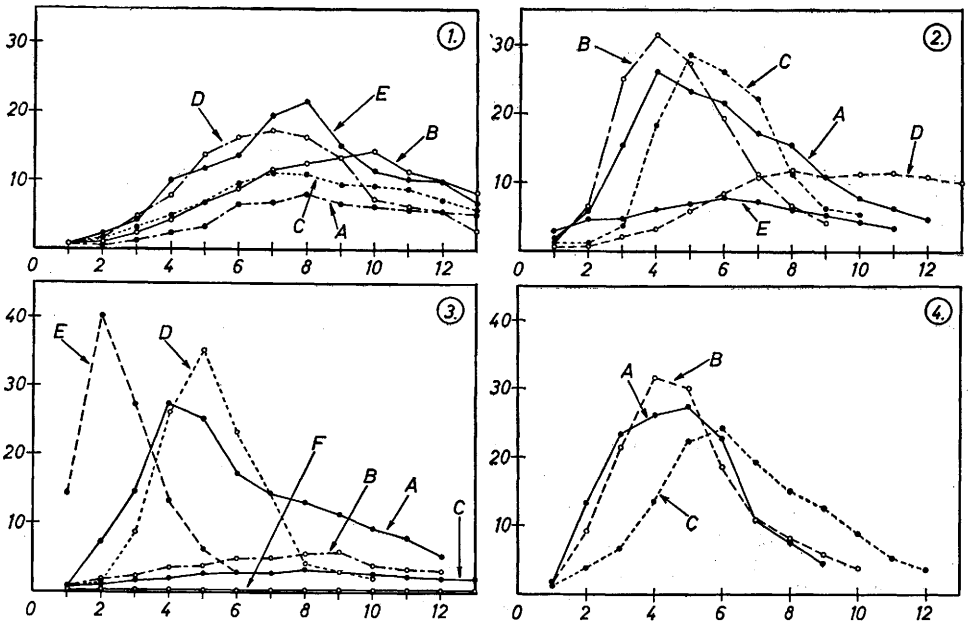

FIGS. 1-4. Thrombin generation in horse plasma. *Abscissa:* reaction time in min.; *Ordinate:* reciprocal clotting time of fibrinogen solutions expressed as 600/t (t in sec).

suspension (245000 platelets per mm^3). Curve B shows addition of 0.4 ml frozen human platelet suspension (272000 platelets per mm^3). Curve C shows addition of 0.4 ml of a fresh suspension of platelets from the horse plasma investigated here (156000 platelets per mm^3). Thrombin generation became normal in all these experiments.

Discussion. Samples of hemophilioid diseases, which yield normal thrombin generation after addition of normal serum, comprise more than one group of defects in the plasma thromboplastin system(4). One of these groups is deficient in PTC. The results described here show that the defect in horse plasma, though corrected by addition of serum, is different from PTC deficiency. Thrombin generation in horse plasma was improved after addition of normal human plasma. It was also improved by addition of a component in human serum, which is not adsorbed by $BaSO_4$ and resists heating at 56°C for 30 min. This factor was absent from the horse serum and from plasma or serum from a patient with the Hageman trait. These results indicate that the coagulation defect in this horse is caused by lack of a factor probably identical with the human Hageman factor. Thrombin generation in horse plasma is also improved by addition of a washed suspension of human platelets, fresh as well as frozen. It is even improved by addition of a fresh suspension of washed platelets from its own plasma. Similarly thrombin generation became normal after freezing of the platelet rich plasma with no further additives. Freezing of the platelet poor plasma had no significant effect on thrombin generation. Apparently absence of the Hageman factor can be compensated by the frozen or normal washed platelets. This phenomenon has recently been observed also in human plasma(10). The finding that the samples of horse plasma and horse serum here investigated were able to make normal thrombin generation in plasma from a supposed PTC deficient patient does not accord with our earlier observation(3). However, further investigations have shown this patient to be deficient in a factor which is present in reabsorbed normal human serum, and which therefore differs from PTC.

The sample of horse plasma previously used(3) did not yield a completely normal thrombin generation after freezing, as was found in the present sample. This observa-

tion and the finding mentioned before of a horse with completely normal thrombin generation, together with the reports of other authors indicate that horse, like man, presents various types of hemophilioid diseases. P. Barkhan (personal communication) has also observed different defects in the thromboplastin system of the various samples of horse plasma.

Summary. 1. The delayed thrombin generation in samples of horse plasma was made normal by 1) addition of normal human plasma, or 2) addition of reabsorbed heated human serum, or 3) addition of washed human or horse platelets, or 4) freezing of the horse plasma with its normal content of platelets. 2. These results indicate that the clotting defect of the horse plasma investigated is caused by lack of a factor similar to the Hageman factor. 3. Blood platelets, after washing or freezing, apparently substitute for the effect of the lacking plasma factor. 4. The coagulation defects in horse plasma apparently reflect various types of hemophilioid deficiencies.

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Comparison of Duration of Action of Progesterone and 17-α-Hydroxyprogesterone-17-n-Caproate. (23100)

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17-α-Hydroxyprogesterone(1), a progestationally ineffectual compound(2), has been esterified to yield 17-α-hydroxyprogesterone-17-n-caproate (HPC), a potent progestational substance(3). Junkmann(3) also demonstrated that when HPC was administered to rabbits in an ethyl lactate-castor oil vehicle, the progestational action was more prolonged than when progesterone was given in the same vehicle.

The present study was designed to verify Junkmann's findings and to determine the duration of action of HPC in benzyl benzoate-sesame oil, an oily vehicle more desirable for human therapy. Limited success has been reported previously by Plotz(4) in prolonging the activity of progesterone by vehicular alteration.

Materials and methods. Immature virgin female Flemish rabbits were primed with daily subcutaneous injections of 5 μg of estradiol in 0.5 cc of sesame oil for 6 days. Treatment was initiated on the morning of the seventh

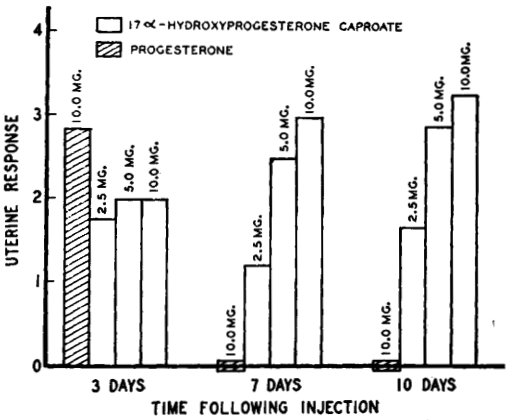

FIG. 1. Effect of progesterone and 17-α-hydroxyprogesterone caproate in ethyl lactate-castor oil on progestational changes in rabbit uterus (single subcutaneous inj).