EFFECT OF BLOOD PLATELETS ON PROTHROMBIN UTILIZATION OF DOG AND HUMAN PLASMAS

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It has been demonstrated, qualitatively, that the rate of blood coagulation is related to the presence of blood platelets. This relationship has been known to exist in bird blood since the investigations of Delezenne (1). In mammalian blood, however, conflicting data and opinions regarding the rôle of platelets in coagulation have been published during the past half-century. Only in the past decade has it been clearly demonstrated that platelets play an important rôle in the clotting process. In 1939 one of us (2) showed that prothrombin is slowly utilized in human blood if it is immediately centrifuged to reduce the number of platelets present during clotting. More recently, Jaques and co-workers (3) showed that platelet-poor plasmas have a prolonged clotting time. In their experiments, platelet alterations during the preparation of plasma were prevented by use of silicone-treated equipment. Later work demonstrated that plasma, carefully prepared with a silicone technique to minimize or prevent platelet rupture, clots very slowly or not at all when freed of platelets. Incoagulable platelet-free plasma has been obtained from both human and dog blood (4, 5).

The purpose of this investigation was to determine the platelet levels at which impairment of the clotting process appears, and to compare the platelet requirements of human and dog blood.

METHODS

The method for obtaining the blood and plasma was as follows: syringes, needles (16–18 gauge) and glassware were treated with a 10 per cent solution of a methychlorosilane (General Electric Dri-Film) in benzene. Blood was obtained from the external jugular vein in the dog and from the cephalic vein in the human. No samples were used in which difficulties were encountered in the venepuncture or in blood withdrawal, or in which there was evidence of lipemia. The blood was collected in a series of three syringes. In the first syringe, 4 to 6 ml. of blood were obtained for determination of the hematocrit. In the second syringe, 4 ml. of blood were obtained for platelet counts. And in the third, 45 to 50 ml. of blood were obtained without anticoagulant for the clotting studies. Blood from the last syringe was cooled rapidly in silicone-treated tubes in an ice bath. To reduce the number of platelets to the desired level, the blood was centrifuged for varying periods of time in angle centrifuges. For dog samples, a centrifugal force of about 1450 g for 2 to 15 minutes was used. For human samples the centrifugal force was about 1650 g for the same periods of time. To obtain platelet-poor plasmas containing less than 5,000 to 15,000 platelets per cu. mm., a centrifugal force of about 15,000 g was used. Collection and centrifugation of the blood were carried out in a constant temperature room (2°C).

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Two measures of the rate of clotting were used, the clotting time and the prothrombin utilization rate. In both tests, timing of the clotting process was started when the blood or plasma was placed in ordinary glass tubes. For the clotting time determinations, 1 ml. of whole blood or 0.5 ml. of native plasma was transferred with a silicone-treated needle and syringes to each of two dry 10 x 75 mm. glass tubes. For the determination of the rate of prothrombin utilization (2), blood or plasma was transferred in a similar manner to a series of tubes containing 0.15 ml. of imidazole buffer at pH 7.3 (6). At frequent intervals during the next 50 to 60 minutes, the contents of each of two tubes were mixed with 0.12 to 0.16 ml. of 3.2 per cent sodium citrate solution to stop the conversion of prothrombin to thrombin. Prothrombin determinations, using the two-stage method of Warner, Brinkhous and Smith (7, 8), were made promptly on the plasma or serum. The above procedures were carried out at 27° to 28° C.

Platelet counts on the whole blood were performed by a modification of Nygaard's method (9), using 4 parts of 3.2 per cent sodium citrate to one part of whole blood. After sedimentation of dog blood for 15 minutes, and of human blood for 30 minutes, supernatant plasma was transferred to a counting chamber and the platelets counted. Direct platelet counts were made on the native plasma specimens obtained by centrifugation. One of us (J. A. B.) performed all the counts, which were done in duplicate or triplicate.

RESULTS

Sixteen healthy adult dogs and 9 healthy adult human subjects, selected without regard to sex, were used. In all, 48 experiments were performed on dog blood and 32 experiments on human blood. The mean number of platelets for dogs was 383,000/cu. mm. of whole blood, or 658,000/cu. mm. of plasma, with a standard deviation of 82,000. The mean value for the human subjects was 285,000/cu. mm. of whole blood, or 549,000/cu. mm. of plasma, with a standard deviation of 59,000.

The results of one group of experiments with dog blood are given in figure 1 and table 1. This group exemplifies the experimental procedure followed throughout this work. The residual prothrombin content of the whole blood and plasma samples during the course of clotting is shown in the figure. It is seen that in the plasma with 58 per cent of the original number of platelets (curve II), the prothrombin disappeared...
at the same rate as in the whole blood (curve I). Plasmas containing 22 per cent of the original number of platelets or less (curves III and IV) showed a considerable retardation of the rate at which prothrombin disappeared. The plasma in which only a few platelets remained showed no loss of prothrombin during the experimental period (curve V).

To obtain a numerical expression of the extent of the retardation of clotting, a prothrombin utilization index was devised. This index represents the ratio of the amount of prothrombin utilized in the plasma to the amount utilized in the normal whole blood. The method of calculation of the index is shown in Table I. The interpolated points on the whole blood curve (fig. 1) at which 75, 50, and 25 per cent of the original prothrombin remained in the serum were selected. The times on the

| TABLE I. EFFECT OF PLATELET CONTENT OF PLASMA ON CLOTTING TIME AND PROTHROMBIN UTILIZATION INDEX |
|------------------------------------------------------|------------------|------------------|------------------|
| CURVE NO. (see fig. 1) | PLATELETS | CLOTTING TIME | PROTHROMBIN UTILIZED |
| SPECIMEN | Per cu. mm. of plasma | % No. relative to whole blood | | Minutes | |
| Whole blood | 650 | 100 | 9 | 25 | 50 | 75 | 150 or 1.0 |
| Plasma (3 min. centrifugation) | 380 | 58 | 10 | 25 | 50 | 75 | 150 |
| Plasma (4 min. centrifugation) | 140 | 22 | 14 | 8 | 15 | 31 | 54 or 0.36 |
| Plasma (150 min. centrifugation) | 10 | 12 | 12 | 0 | 0 | 0 | 0.05 |
| Plasma (200 min. centrifugation) | 0.3 | 0.3 | >35 | 0 | 0 | 0 | 0 |

| PROTHROMBIN UTILIZATION INDEX |
|------------------------------------------------------|------------------|
| 150 or 1.0 |
| 150 |
| 54 or 0.36 |
| 8 or 0.05 |
| 0 or 0.00 |

absissa were found to be 19, 28, and 35 minutes respectively. Then the corresponding prothrombin values on the plasma curves were obtained. The average ratio of the prothrombin utilized at the indicated times was then determined. Values of less than 1.0 indicate that clotting is impaired—the slower the clotting the lower the value of the prothrombin utilization index.

Figures 2 and 3 show the relationship between the platelet content of plasma and the rate of clotting. In the dog plasmas, it will be observed that an impairment of clotting occurred when the number of platelets was reduced below about 35 per cent of the number present in whole blood, or about 230,000 platelets per cu. mm. of plasma (fig. 2). In the human plasmas, on the other hand, no impairment of clotting was evident until the number of platelets was below about 25 per cent of the original value, or about 135,000 platelets per cu. mm. of plasma (fig. 3). When the platelets were reduced to a range of 5 to 15 per cent in dog plasma, frequently no prothrombin disappeared during the period of observation. In these cases, the prothrombin utilization index was 0. In human plasma, considerably greater reduction in the number of
platelets was required to prevent utilization of prothrombin in the experimental period.

The clotting time was a less sensitive index of changes in the course of clotting than was the prothrombin utilization rate. In human plasma, even with a reduction of platelet levels to 5 per cent of the original whole blood values, no prolongation of the clotting time was noted. Only when the platelets were at a level of about one per cent or lower, was a consistent delay in clotting time observed. In dog plasma, on the other hand, a prolongation of the clotting time was observed regularly when the platelet levels were reduced below about 15 per cent of the original values. With extremely low platelet counts in either type of plasmas, clotting generally did not occur during the 50 to 60 minute period of observation.

These results emphasize the need for platelets in clotting and indicate that normally platelets are present in numbers considerably in excess of minimal requirements for normal clotting. Roughly, there is a three-fold factor of safety in dog blood, and a four- or five-fold safety factor in human blood. The difference between the platelet needs of dog and human blood is more striking if considered in terms of platelet volume. Normally, according to Van Allen (10), the average volume percentage of platelets for dog blood is 1.04, for human blood 0.49. Thus, the minimal platelet volumes required for normal clotting would be about 0.36 ml/100 ml. of dog blood and about 0.12 ml/100 ml. of human blood. By volume, then, dog blood requires about three times as much platelet material as human blood, compared to about 1.7 times as many if considered in terms of platelet numbers.

The fundamental reason for the greater need for platelets in dog blood is not clear. Whether there are qualitative or quantitative differences in the platelet coagulant factors in the two species is not known. Earlier work has shown that the
plasma factor deficient in hemophilic blood is necessary for platelet utilization (4). It may be that the quantity of the anti-hemophilic principle in plasma determines the extent to which platelets are utilized in the clotting process. At any rate, our data indicate that normally the number of platelets is not the factor which determines the rate of coagulation, and only when their numbers are greatly reduced do they limit the speed of clotting.

A comparison of our findings on normal human plasma with results obtained on blood from patients with thrombocytopenia is of interest. In this disease, the clotting time is nearly always normal. Our data are in accord with this fact, since platelets rarely reach the low levels that would be required for a prolonged clotting time. Soulier (11) has used a modified two-stage method for the determination of residual prothrombin in the serum of a group of thrombocytopenic patients. Seventeen of his patients showed a retardation in the clotting process, as judged by high serum prothrombin levels. All of these patients had platelet counts below 81,000/cu. mm. This is in good agreement with our data, which indicate that the average critical level of platelets is equivalent to a value of about 71,000/cu. mm. of whole blood. On the other hand, there is less evident agreement between our data and those of Conley, Hartmann and Morse (12) and of Quick, Shanberge and Stefanini (13). These authors studied both blood from thrombocytopenia patients and platelet-poor normal plasmas. From their work, it would appear that only a moderate reduction in the number of platelets is sufficient to cause a delay in clotting. They used a one-stage technique for determination of serum prothrombin. This procedure results in erroneously high serum prothrombin values, due apparently to the fact that thrombin is formed more rapidly from prothrombin in serum than it is from prothrombin in plasma. De Vries, Alexander and Goldstein (14) have suggested that the difference in prothrombin convertibility in plasma and serum, as observed in the one-stage method, is due to the elaboration in serum of an accelerator of prothrom-
bin conversion. This factor, as well as other differences in technique, may account for these apparently divergent results.

**SUMMARY**

The platelet requirements for clotting in dog and human blood have been compared. The rate of prothrombin utilization was used to indicate the clotting capacity of plasmas containing varied numbers of platelets. The results indicate that in both plasmas platelets are present in great excess over minimal requirements, and that below critical platelet levels, clotting is impaired. Dog plasma requires more platelets for a normal clotting rate than does human plasma. In terms of platelet count, the requirements of dog plasma are about 1.7 times greater than they are in human plasma; in terms of platelet volume, the requirements are about 3 times greater.

**REFERENCES**

1. Delezenne, C. Arch. de Physiol. 29: 333, 1897.