

hand, only one Sunday intervened in the groups with vaginal opening at 29 and 30 days.

The necessary involvement of ovarian function to produce vaginal opening is indicated not only by the histological findings given above but by the fact that it was not achieved after 15 days of treatment 3 times daily in 6 immature castrates.

Since it was not possible to give adequate amounts of neostigmine to the hypophysectomized rats used in this study, the question of anterior pituitary involvement in this regard remains unsettled. Other workers(5), however, have been able to stimulate ovarian function in normal adults by direct application

of this drug to the hypophysis.

Summary. The daily subcutaneous administration of adequate doses of neostigmine to immature rats has resulted in a premature development of ovarian function with a precocious opening of the vagina.

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Received September 30, 1952. P.S.E.B.M., 1952, v81.

Plasma "Thrombocytopenic Factor" as Measured by Direct and Indirect Platelet Methods.* (19863)

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A prompt decrease in the blood platelets as measured by the indirect method occurred in 8 of 10 human subjects following the intravenous administration of normal human plasma. However, no decrease was observed when the platelets were evaluated by the direct citrate method.

Harrington *et al.*(1) demonstrated a "thrombocytopenic factor" in the blood and plasma of patients with idiopathic thrombocytopenic purpura (ITP) but observed no such effect of normal whole blood. Stefanini and Chatterjea(2) reported a temporary but significant decrease in platelets in 32 of 36 subjects when compatible blood plasma or serum from non-thrombocytopenic donors was transfused. These investigators measured the platelets by the indirect method of Dameshek(3). We previously confirmed the observations of Harrington *et al.* as to the thrombocytopenic effect of plasma from subjects with ITP as measured by the indirect method but observed no decrease in platelets as measured by the

direct citrate method(4). The effect of normal plasma was not studied.

Materials and methods. Fresh pooled normal plasma was obtained from the blood of normal donors. The blood was collected in acid citrate dextrose solution. Irradiated pooled human plasma was obtained commercially in the lyophilized state. Dextrose solution, 5% in H₂O, was used as a control to be certain that the platelet decrease was not due to a speed-shock reaction. See Table I for recipient data. No transfusion reactions occurred. All intravenous fluids were administered within 30 minutes. Platelets were counted at intervals (Fig. 1, 2) by the direct citrate method of Rees and Ecker(5) and the indirect method of Dameshek(3). The following platelet levels were considered normal: Rees and Ecker method, 225000 to 350000 per cu mm; Dameshek method, 500000 to 900000 per cu mm.

Results. a) *Effect of intravenous 5% dextrose in H₂O.* The two subjects were observed. There was no effect on the platelet levels as measured by either the direct or indirect methods.

* This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Services.

TABLE I. Data on 12 Subjects Receiving Transfusions of Pooled Plasma or Dextrose Solution.

Age	Sex	Diagnosis	Type of transfusion	cc administered
36	♀	Common duct obstruction	Fresh plasma	210
60	♂	Carcinoma of stomach		210
18	♀	Anxiety neurosis		350
39	♂	Metastatic carcinoma of liver		350
62	♂	Cirrhosis of liver		200
42	♀	" " "		300
57	♂	Duodenal ulcer (uncomplicated)		210
68	♂	Lobar pneumonia	Irradiated plasma	250
34	♂	Epilepsy		
64	♂	Emphysema		
22	♂	Epilepsy	5% dextrose in H ₂ O	
49	♂	Psychoneurosis		

b) *Effect of fresh pooled normal human plasma.* Seven recipients (subjects 1 to 7, Table I) were transfused with fresh normal human plasma. In 5 subjects there was a significant decrease in the circulating platelets as measured by the indirect method (Fig. 1). The thrombocytopenic effect was transient. No drop was noted in the platelets as measured by the direct method (Fig. 2).

c) *Effect of pooled irradiated lyophilized human plasma.* Three recipients were given pooled irradiated human plasma (subjects 8

to 10, Table I). Again the decrease in platelets as measured by the indirect method was noted (Fig. 1). No fall occurred in the platelet levels as measured by the direct method (Fig. 2).

Discussion. Apparently there is a "thrombocytopenic factor" in human plasma. It is present in the globulin fraction(1), stable at 56°C, not absorbed by either Seitz filters or ion-exchange resins, but is absorbed on $\text{Ca}_3(\text{PO}_4)_2$ gel(2). Blood or plasma from patients with ITP produces a prolonged de-

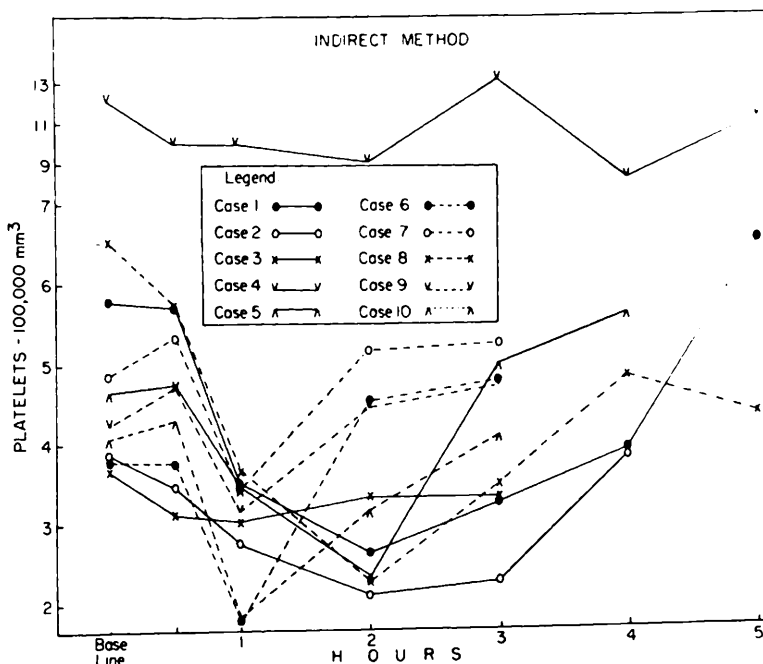


FIG. 1. Effect of pooled normal plasma transfusions on platelet counts by the indirect (Dameshek) method.

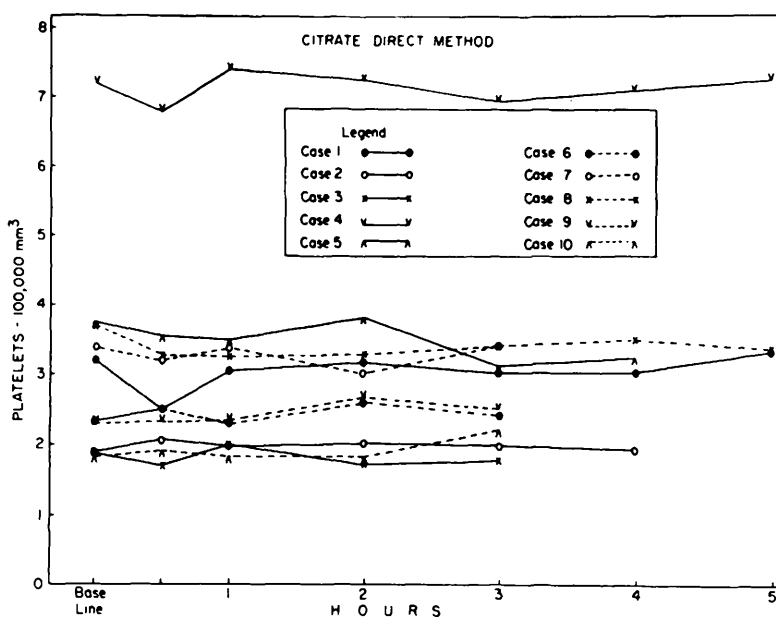


FIG. 2. Platelet values (direct method, Rees and Ecker) following transfusions with pooled normal plasma. Observations simultaneous with those in Fig. 1.

crease in platelets as measured by the indirect method(1,4). In our series(4) the most rapid return to normal occurred in 24 hours (1 case). In other subjects the decreased platelet level continued over a period of several days(1,4). The present study on the effect of normal plasma on platelets confirms the observations of Stefanini and Chatterjea(2) but is in disagreement with the observations of Harrington *et al.*(1). The decrease in platelets following the administration of normal plasma is rapid and transient, and not as prolonged as that following the transfusion of ITP plasma.

There is a tremendous variation in platelet values obtained by the direct and indirect technics. Wintrobe(6) states that incorrect high platelet levels can be caused by fragmentation without dissolution. Tocantins(7) reported that it would seem that figures above 500,000 per cu mm are seldom, if ever, obtained in cutaneous blood under normal conditions, and are perhaps the result of a defective technic. As we have previously stated (4) it is theoretically possible that the "thrombocytopenic factor" is a platelet-fragmentation inhibiting substance that can best be measured by correlating platelet values obtained by both direct and indirect methods. Our present studies show that it is present in both normal and ITP plasma. The basic

difference in the two technics is that in the direct method there is a greater dilution of the blood (1:200) and in the indirect method the dilution is less (1:5). Is the substance causing platelet-fragmentation diluted by the direct method to the extent that most of its mechanism of action is inhibited?

Conclusions. A temporary and significant decrease in platelets as measured by the indirect method occurred in 8 of 10 individuals following the transfusion of normal blood plasma. No decrease in platelets occurred as determined by the direct method of evaluating platelet levels. The possible cause for the differences in the results obtained by the direct and indirect methods is discussed.

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Received September 16, 1952. P.S.E.B.M., 1952, v81.