TABLE I.
Atherosclerotic Involvement of the Aorta in Rabbits Fed 0.5 g Cholesterol Daily for 184 Days.

Grade of involvement	0	1	2	3	4	Total affected
No. of rabbits	0	2	3	5	11	21
% of rabbits affected	0	9%	14%	24%	53%	100%

TABLE II.
Atherosclerotic Involvement of the Aorta in Rabbits Fed 0.5 g Cholesterol Daily for 184 Days, Then 1.0 g Choline Daily for 185 Days.

Grade of involvement	0	1	2	3	4	Total affected
No. of rabbits	17	4	2	0	0	23
% of rabbits affected	74%	17%	9%	0	0	26%

in 26% of animals. Seventy-four percent of animals were free of demonstrable aortic atherosclerosis.

Conclusion. Choline caused reabsorption of aortic atherosclerosis in the majority of rabbits whose lesions had been produced by cholesterol feeding.

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The Relationship of Heparin Activity to Platelet Concentration.

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(Introduced by G. S. Mirick.)
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An increased susceptibility of thrombocytopenic blood to heparin has recently been reported by Allen and his associates.[1] These authors found that following the addition of a standard concentration of heparin to blood, more protamine sulfate was necessary to restore the normal clotting time in thrombocytopenic blood than was required by normal controls. They interpreted this observation as suggesting that in thrombocytopenia an increased amount of a heparin-like substance may be present in the blood. Studies in our laboratory[2] on 12 patients with hemorrhagic diathesis associated with thrombocytopenia in no instance revealed evidence of a circulating anticoagulant. It therefore seemed quite plausible that the increased susceptibility of thrombocytopenic blood to heparin may be a direct result of the decreased number of platelets present.

Experiments. Using silicone-treated apparatus and high speed centrifugation (12,000 to 14,000 RPM, 17,500 to 22,000 g's) at low temperature (4°C), platelet-free plasma was obtained from normal individuals without the use of anticoagulants. This plasma could be kept fluid in iced silicone-treated tubes for at least several hours. Platelet-rich plasmas were obtained by a similar technic except that the blood was centrifuged only at 3000 RPM (about 1300 g) for 5 minutes. Platelet counts were performed on these plasmas. These two plasmas were mixed in different proportions and varying amounts of heparin* added. Clotting times of these plasmas in glass tubes were then determined using a 3 tube method at 37°C. Results are shown in the table.

These results clearly indicate that the

[1] Allen, J. G., Bogardus, G., Jacobson, L. O., and Spurr, C. L., *Ann. Int. Med.*, 1947, **27**, 382.

[2] Conley, C. L., Hartmann, R. C., and Morse, W. I., II, *Bull. Johns Hopkins Hosp.*, in press.

* Solution of heparin (sodium salt), Lederle Laboratories, Inc.

TABLE I.

Experiment 1.

Clotting Times of Plasma Samples Containing Varying Numbers of Platelets and Concentrations of Heparin as Indicated. The clotting times were determined simultaneously on samples derived from pooled plasma specimens. Clotting times of each of the 3 tubes are recorded.

	Clotting times in minutes of 1.0 ml portions in glass tubes at 37°C				
Heparin (mg per ml of plasma)	Whole blood (Platelets 190,000 per cmm)	"Platelet-rich" plasma (Platelets 420,000 per cmm)	"Platelet-free" plasma 90% "Platelet-rich" plasma 10% (Platelets 42,000 per cmm)	"Platelet-free" plasma 95% "Platelet-rich" plasma 5% (Platelets 21,000 per cmm)	"Platelet-free" plasma (Platelets 2 per cmm)
0	(1) 14 (2) 15 (3) 16	(1) 12 (2) 12 (3) 14	(1) 12 (2) 16 (3) 16	(1) 12 (2) 18 (3) 18	(1) 16 (2) 16 (3) 20
0.00025	(1) — (2) — (3) —	(1) — (2) — (3) —	(1) — (2) — (3) —	(1) 10 (2) 24 (3) 28	(1) 44 (2) 78 (3) Partial clot in 24 hrs.
0.0005	(1) — (2) — (3) —	(1) — (2) — (3) —	(1) 12 (2) 30 (3) 36	(1) 60 (2) 89 (3) 98	(1) No clots (2) in 24 hrs (3)
0.001	(1) 15 (2) 16 (3) 20	(1) 15 (2) 16 (3) 20	(1) 32 (2) 53 (3) 66	(1) 38 (2) Partial (3) clots in 24 hrs	(1) No clots (2) in 24 hrs (3)
0.01	(1) 7 hrs (2) No clots (3) in 24 hrs	(1) 51 (2) 113 (3) Partial clot in 24 hrs.	(1) No clots (2) in 24 hrs (3)	(1) No clots (2) in 24 hrs (3)	(1) No clots (2) in 24 hrs (3)

Experiment 2.

	Whole blood (Platelets 210,000 per cmm)	"Platelet-rich" plasma (Platelets 448,000 per cmm)	"Platelet-free" plasma 90% "Platelet-rich" plasma 10% (Platelets 44,800 per cmm)	"Platelet-free" plasma 95% "Platelet-rich" plasma 5% (Platelets 22,400 per cmm)	"Platelet-free" plasma (Platelets 4 per cmm)
0	(1) 11 (2) 15 (3) 16	(1) 4 (2) 8 (3) 10	(1) 10 (2) 14 (3) 17	(1) 10 (2) 14 (3) 16	(1) 10 (2) 16 (3) 17
0.00025	(1) — (2) — (3) —	(1) 10 (2) 14 (3) 24	(1) 10 (2) 14 (3) 21	(1) 19 (2) 37 (3) 60	(1) 23 (2) Partial clots (3) in 24 hrs
0.0005	(1) — (2) — (3) —	(1) 11 (2) 20 (3) 28	(1) 23 (2) 26 (3) 31	(1) 14 (2) 35 (3) 56	(1) Partial clots (2) in 24 hrs (3) No clot in 24 hrs
0.001	(1) 22 (2) 24 (3) 29	(1) 11 (2) 22 (3) 26	(1) 19 (2) 54 (3) 65	(1) 41 (2) 65 (3) 90	(1) Partial clot in 24 hrs (2) No clots in (3) 24 hrs
0.01	(1) 60 (2) No clots (3) in 24 hrs	(1) 41 (2) 93 (3) 138	(1) No clots (2) in (3) 24 hrs	(1) No clots (2) in (3) 24 hrs	(1) No clots (2) in (3) 24 hrs

TABLE I (Continued).
Experiment 3.

Heparin (mg per ml of plasma)	Clotting times in minutes of 1.0 ml portions in glass tubes at 37°C			
	"Platelet-rich" plasma (Platelets 208,000 per cmm)	"Platelet-free" plasma 25% "Platelet-rich" plasma 75% (Platelets 156,000 per cmm)	"Platelet-free" plasma 50% "Platelet-rich" plasma 50% (Platelets 104,000 per cmm)	"Platelet-free" plasma (Platelets 8 per cmm)
0	(1) 5 (2) 6 (3) 7	(1) 7 (2) 9 (3) 9	(1) 5 (2) 9 (3) 12	(1) 7 (2) 13 (3) 21
0.001	(1) 14 (2) 24 (3) 32	(1) 12 (2) 20 (3) 29	(1) 16 (2) 27 (3) 41	(1) 25 (2) 67 (3) 91
0.005	(1) 24 (2) 52 (3) 81	(1) 25 (2) 73 (3) 100	(1) 36 (2) 52 (3) 83	(1) No clots (2) in (3) 3 hrs
0.01	(1) 36 (2) 74 (3) 105	(1) 44 (2) 108 (3) No clot in 3 hrs	(1) 91 (2) No clots (3) in 3 hrs	(1) No clots (2) in (3) 3 hrs

delaying or inhibitory action of heparin on the clotting of normal plasma is inversely related to the concentration of platelets present.

Discussion. The interpretations of several clinical tests for detecting hypo- and hypercoagulability of blood have been based on rather uncertain theoretical grounds. One of these is the test devised by Allen and his associates[1] for determining the existence of an increased amount of a heparin-like substance in thrombocytopenic blood. Our observations show that the results of their tests can be explained by the variation in the platelet concentration alone.

The "heparin tolerance test"[3] has been proposed as a means of measuring the tendency to intravascular clotting. Although this test has been used in clinical studies, the variables which determine its results have been to a large extent unknown. Our experiments suggest that one factor which may influence the results of this test is the number of platelets present.

In tests in which there is a minimal agitation of normal platelet-free plasma, the addition *in vitro* of heparin to a final concentration of 0.0005 mg per ml completely inhibits coagulation in *glass* tubes at 37°C . Normal platelet-free plasma to which no anticoagulant has been added invariably clots when transferred to glass tubes at 37°C. It therefore seems reasonable that the amount of active heparin present in normal plasma is of the order of magnitude of 0.0005 mg per ml or less.

Conclusions. 1. The concentration of heparin required to inhibit or delay coagulation is directly related to the number of platelets present.

[3] de Takats, G., *Surg., Gyn., and Obs.*, 1943, **77**, 31.

2. The increased susceptibility of thrombocytopenic blood to heparin is a direct manifestation of the decreased number of platelets present and does not necessarily indicate the presence of a heparin-like substance.

3. One variable which may influence the results of the "heparin tolerance test" is the number of platelets present.

4. Our results suggest that the amount of active heparin present in normal plasma is very small and at least of the order of magnitude of 0.0005 mg per ml or less.

16694 P

Effects of Electro-Convulsive Shock on Pregnancy in the Rat.*

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In an investigation of the effects of electro-convulsive shock on the maternal behavior of the white rat, Rosvold[1] found that females did not bear litters if shocked within 12 to 15 hours after copulation and once daily for 14 days thereafter. The rats received 45 milliamperes for 0.2 second through alligator clips attached to the ears. This strength of current invariably induced a complete tonic-clonic convulsion.

Gross examination at autopsy suggested that the fetuses were being resorbed. No histological study of the uteri, ovaries, or pituitaries was made in the original experiments. Accordingly a series of experiments was repeated in order to permit microscopic study of these organs under various conditions with respect to the number of shocks and the time of their administration. This preliminary report is based on the study of 24 healthy nulliparous females, about 100 days of age in which mating was observed in the evening and vaginal plugs found on the following morning. The animals were divided into 3 groups and shocked according to the following schedules:

Group I. Five animals shocked once, 12 hours after mating. Three controls mated at the same time, but not shocked.

Group II. Five animals shocked once daily for 6 days beginning 12 hours after mating. Three controls mated at the same time, but not shocked.

Group III. Five animals shocked once daily for 11 days beginning 12 hours after mating. Three controls mated at the same time, but not shocked.

All animals were sacrificed with ether on the eleventh day after copulation and the uteri, ovaries, and pituitaries removed at once and fixed in Bouin's fluid. Table I indicates the results observed at autopsy. The uteri of all the controls contained normal embryos of the 11th day of development.

In the two uteri of Group II which showed resorption of the embryos, the remnants of the implantation sites were located on the antimesometrial side of the uterus. In all of the animals of Group III the implantation sites were located on the mesometrial side of the uterus and were reduced to from 1/2 to 1/5 the size of those in the uteri of control animals.

For this preliminary study a randomly selected implantation site from each animal was sectioned serially at 10 μ and stained with hematoxylin and eosin. Sections of ovaries were stained with hematoxylin and eosin, and pituitaries with Mallory-azan.

In the uteri of the two animals of Group II in which the conceptuses were undergoing resorption, the deciduae were apparently normal and contained many giant cells, but

* Supported by a grant from the Scottish Rite Fund, administered at Stanford University by Calvin P. Stone.

[1] Rosvold, H. E., Doctoral dissertation, Stanford University, 1948.