TABLE III. Attempts to Reverse the Implantation-Inhibiting Action of Estrone.*

Group No.	Compound	Dose (mg)	No. of rats	No. pregnant
I	Oil control	.1 ml	5	0
II	Progesterone	5 mg/day for 4 days from day 1	2	0
III	"	5 mg/day for 3 days from day 4†	6	0
IV	Synthetic progestin‡	1.0 mg on day 1	5	0
V	" "	1.0 mg/day for 3 days from day 1	5	0
VI	Synthetic protestin§	1.0 mg on day 1	5	0
VII	" "	1.0 mg/day for 3 days from day 1	4	0

* E_1 was administered (20 μg/rat) on day 1 of pregnancy.
† E_1 (20 μg/rat) given on day 4.
‡ 1, 2α-Methylene-6-chloro-6-dehydro-17α-hydroxyprogesterone-17-acetate.
§ 3β, 17α-Diacetoxy-6α-methylpregn-4-3en-20-one.

a single dose of 10 or 15 μg of estrone per rat administered on day 1 of pregnancy; a single dose of 20 μg per rat appears to cause complete inhibition. Doses of 20 μg per rat or higher cause a premature passage of tubal ova into the uterus with later expulsion from the uterus. This loss of ova and consequent lack of implantation cannot be counteracted by daily preimplantation doses of 5 mg progesterone, or 1 mg of the two synthetic potent progestins:

(1) 1,2α-methylene-6-chloro-6-dehydro-17α-hydroxyprogesterone-17-acetate;

(2) 3β-17α-diacetoxy-6α-methylpregn-4-en-20-one.

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Hypercoagulability and Thrombosis: Effect of Injected Thrombokinase and Adenosine Diphosphate on Established Microthrombi in Rabbits.* (29442)

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There has been an increasing tendency to implicate blood hypercoagulability in the pathogenesis of thrombotic disease. Although intravascular clots have been produced by a combination of increased blood coagulability and stasis, as in the studies of Wessler (1), there have been no reports claiming the production of structured thrombi similar to those found in human disease(2) resulting from alterations of the blood hemostatic components alone.

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In the present study, an attempt is made to evaluate further the effect of the hypercoagulable state. Since no suitable method has been devised to perform direct studies on thrombus formation in the presence of hypercoagulability alone, the technique adopted was a modification of the *in vivo* method described by Honour and Russell(3), in which established thrombi were studied for evidence of augmentation. Thrombi were produced by physical trauma to blood vessels, and hypercoagulability by intravenous infusions of thrombokinase. This coagulant (4) evidently corresponds to the activated form of factor X; it was previously designated "product I"(5). Additional experiments evaluated the effect of increased platelet agglutination induced by injections of adenosine diphosphate (ADP). The effects of these maneuvers on the preformed thrombi were observed by direct microscopy.

Methods and materials. Experimental animals were young male New Zealand rabbits weighing about 2 kg. Each of these animals was used for a single thrombus experiment; blood reagents were obtained from larger animals of the same strain. Thrombokinase was prepared as previously described(6) from a mixture containing a citrate eluate from barium sulfate used in the adsorption of serum, a solution of "euglobulin" obtained from aluminum hydroxide-adsorbed plasma, and calcium ion. The final product clotted recalcified and phosphatide-enriched rabbit substrate plasma in about 5-6 seconds(7), and contained traces of thrombin. It was stored at —20°, and was filtered through Whatman #1 filter paper prior to use. Adenosine diphosphate (ADP) was purchased as the sodium salt from Sigma Chemical Co., St. Louis, Mo.

Experimental thrombi were produced as follows: Animals were anesthetized with pentobarbital sodium. The abdomen was opened, and a suitable segment of small intestinal mesentery was draped on the end of an L-shaped lucite rod consisting of a bent cylinder ½″ in diameter. With a microscope light shining into the opposite end of the rod, this device served both as a support and a source of cold transillumination. Blood vessels chosen for study were about 0.5 mm in diameter. After careful removal of the covering fat and connective tissue, observations were made through a Bausch & Lomb dissecting microscope provided with a zoom lens. Thrombi were produced by fragments of dry ice with pointed ends applied to veins. The ice was held in contact with the vessel for about 30 seconds, then thawing was accomplished with a rinse of warm isotonic saline. This procedure was repeated at brief intervals on the same site until a thrombus was seen which had a thickness of about 1/5 the diameter of the vessel. About 5 freezings were usually required. In the first few minutes after completion of this process there was occasionally additional thrombus growth; therefore, the preparation was allowed to stabilize for 20 minutes under gauze sponges moistened with isotonic saline.

Thrombokinase was infused into the inferior vena cava for 100 minutes at a rate of 0.5 ml/minute, through a 15 gauge trochar held in place by silk ligatures. ADP in imidazole buffered saline at pH 7.3 was similarly infused at a concentration of 40 μg/ml. In addition, a similar concentration of ADP was administered into a tributary vein about 2 cm distal to the thrombus. This was accomplished through a 27 gauge needle positioned by a micromanipulator manufactured by the Line Tool Co., Allentown, Pa. This regional infusion was at the rate of 0.05 ml/minute for 3-4 hours.

Vessels were maintained under continuous microscopic observation during the period of infusion and for sufficient time thereafter to ascertain the subsequent sequence of events. Thrombus growth was graded by the following semiquantitative criteria:

0 —No change or decrease in size
± —Addition of tiny platelet clumps
1+—Slight growth
2+—Growth to diameter half that of vessel
3+—Almost complete occlusion of vessel
4+—Complete vessel occlusion at least temporarily

Plastic tube clotting times were performed on right auricular blood as previously described(8).

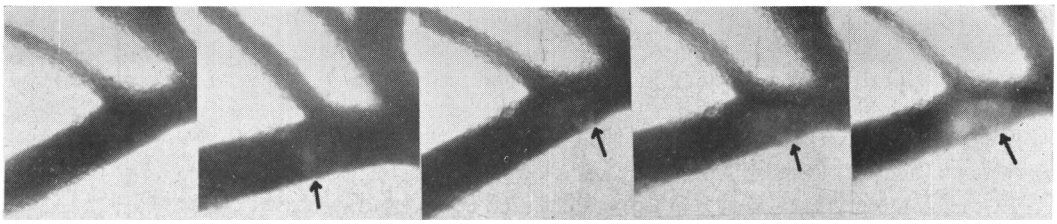

FIG. 1. Serial photomicrographs showing 3+ thrombus growth during regional ADP infusion. Photograph to left shows blood vessel before trauma; the second shows thrombus before ADP infusion; the rest illustrate thrombus growth under the influence of ADP. Magnification about 16.6 ×.

Results. In control animals given 10 ml of isotonic saline for 3-4 hours into a tributary vein, the following sequence of events was seen in 8 out of 10 experiments: Small fragments of the original thrombus were gradually swept off into the flowing stream of blood so that an hour after starting the infusion only a thin layer of platelets remained at the site of injury. No subsequent reaccumulation of platelets occurred. In 2 experiments there was minimal and reversible thrombus growth during the period of perfusion.

Infusion of thrombokinase into the inferior vena cava resulted in the production of a hypercoagulable state, as shown by marked shortening of the clotting times in plastic tubing. Several animals were tested during the infusion, and in each, clotting occurred within 10 minutes, compared to preinfusion values of about 90 minutes. As previously reported, defibrination was not produced(9), nor was there reduction in factors V, VIII, or fibrinogen. The results obtained with thrombokinase infusions were identical to those with isotonic saline; no augmentation of thrombus growth was demonstrated. Similarly, administration of ADP into the inferior vena cava was without effect on the local thrombi, although occasional clumps of platelets could be seen passing rapidly through the vessel.

Local irrigation of the thrombi with ADP via the regional tributary produced dramatic results. There was rapid accumulation of platelets both onto the original thrombus and to the neighboring vessel wall which perhaps was also somewhat damaged by the freezing procedure. In several experiments the thrombus grew sufficiently to block the vessel completely. However, such occluding thrombi were soon dislodged as fluid pressure built up, and alternating growth and fragmentation were seen. A representative example of such thrombus growth is shown in Fig. 1. When the ADP infusion was discontinued, there was progressive shrinkage of the thrombi as bits embolized off; after an hour the thrombi were reduced to virtually their original size. Concentrations of ADP in the perfusate ranging from 1 to 100 μg/ml gave similar results. Simultaneous administration of thrombokinase via the inferior vena cava and of ADP via the regional tributary failed to produce greater growth or more prolonged survival of thrombi than did regional ADP alone.

The data from these experiments are summarized in Table I.

Discussion. In the literature, several different phenomena have been described with the designation of "thrombus." These include diffuse intravascular clotting with widespread formation of fibrin and platelet emboli, as

TABLE I. Total Number of Responses in Each Category of Preformed Thrombi to Various Maneuvers.

Agent given	Type of growth					
	0	±	1+	2+	3+	4+
Regional saline	8	1	1	0	0	0
Thrombokinase into inferior vena cava	9	1	0	0	0	0
ADP into inferior vena cava	9	0	0	1	0	0
Regional ADP	0	1	0	3	4	2
Regional ADP + thrombokinase into inferior vena cava	0	1	1	3	5	0

seen in the generalized Schwartzman reaction(10); massive whole blood clots produced by vascular stasis during an induced hypercoagulable state(1); and the structured thrombi which grow in a defined and orderly manner *in situ,* as illustrated by the pathological material of the earlier investigators (2). These 3 phenomena represent distinctly different reactions, and each probably has its own unique pathogenesis. The present study is concerned only with the structured thrombus, which can be assumed to arise initially on a site of vascular injury with formation of a platelet "head"(2), and which grows in a manner paralleling that which occurs in normal hemostasis(1).

The above data do not support the hypothesis that a hypercoagulable state can produce or influence the growth of structured thrombi. Not only did already established thrombi fail to grow during the period of hypercoagulability, but in many cases there was fragmentation and loss of thrombus mass. These observations are of particular interest, since the thrombi were studied in the venous circulation where conditions of low pressure and relatively slow blood flow should have been most favorable to thrombus growth. Similar findings were obtained by Honour and Russell(3) with hypercoagulability produced by parenteral Stypven.

Adenosine diphosphate (ADP) is a physiological compound known to be a potent platelet agglutinating agent(12); it is probably an essential factor in mediating the growth of platelet hemostatic plugs(11). ADP in the general circulation was without effect on established thrombi even in the presence of circulating platelet clumps. Perhaps significant local ADP concentrations were not achieved owing to rapid ADP inactivation by plasma(13). Regionally administered ADP which directly bathed the thrombi gave rapid thrombus growth, but even these effects were transient, persisting only as long as the ADP perfusion was continued.

Numerous past experiments have failed to produce structured thrombi by altering only the state of the blood. It now appears that even established thrombi are resistant to influence by such measures. We occasionally found occluding thrombi when a sufficiently large area of vascular damage was produced by dry ice injury. This reaction was studied only in a preliminary manner, and a more systematic project along these lines is to be undertaken. However, the hypothesis now tentatively proposed is that structured thrombi develop primarily as a result of vascular injury, and that obstruction occurs when the lesions are of sufficient extent and strategically so located that there is confluence.

Summary. Thrombi were produced in rabbit mesenteric veins by dry ice trauma. Intravenous infusion of thrombokinase to produce hypercoagulability or ADP to increase platelet agglutinability failed to augment thrombus growth. Regional perfusion of ADP caused dramatic increase in the size of thrombi, but the effects were transient and persisted only during the period of infusion. It is concluded that structured thrombi are primarily vascular in origin.

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