doses of PGE_1 into unanesthetized dogs caused marked decreases in plasma FFA levels lasting up to 40 minutes. On the other hand, vasodepression produced by injection of similar doses of PGE_1-217 or by injection of nitroglycerin produced no fall in FFA levels, but instead a slight rise. This is interpreted as a response to sympathetic discharge induced by vasodepressor agents that do not share the ability of PGE_1 to block the lipolytic action of epinephrine on adipose tissue. Simultaneous intravenous injection of PGE_1 and epinephrine diminished or abolished the lipid-mobilizing action of the latter, but glucose response was unaffected.

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Activation of Intravascular Coagulation by Collagen.* (31440)

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It has been suggested that the activation of Factor XII (Hageman Factor) triggers the so-called "waterfall sequence" of intrinsic blood coagulation(1,2). It has recently been found that Factor XII is almost selectively adsorbed from human plasma by collagen fibers(3) and that this factor is activated following its adsorption(4). The purpose of the present study was to evaluate the effectiveness of collagen in activating blood coagulation *in vitro* and *in vivo*, and to study further the mechanism by which this occurs. The effect of collagen and elastin on blood coagulation *in vitro* was compared with that of inorganic surface active agents. Eluates from collagen previously exposed to plasma were also injected into rabbits, and tested for their ability to produce stasis thrombi in veins.

Materials. Undenatured collagen and elastin were obtained from Calbiochem, Los Angeles, Calif. Kaolin was obtained from the Fisher Scientific Co., Fair Lawn, N. J., and dicalite from the Great Lakes Carbon Corp., Los Angeles, Calif. Plastic test tubes, made of clear polystyrene, were obtained from Falcon Plastics Co., Los Angeles, Calif. New Zealand white rabbits, weighing approximately 3 kg, were used for obtaining blood and for

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testing the thrombogenicity of collagen eluates.

Methods. Intact rabbit blood was obtained from the carotid artery by means of a polyethylene catheter (PE 90), and immediately mixed in plastic test tubes with 10% by volume of 3.8% sodium citrate. Human blood was obtained by venipuncture of the antecubital vein. Platelet-rich plasma was obtained by centrifugation at 120 *g* for 10 minutes at room temperature, and platelet poor plasma by centrifugation at 2500 *g* for 20 minutes at 4°C. Exhausted plasma (plasma artificially deprived of Factors XI and XII), was obtained according to the technique of Waaler(5). Plasma samples were obtained from patients with congenital Factor XII, XI, IX and VIII deficiencies.

Eluates of kaolin and collagen, which had been shaken previously with platelet-poor rabbit or human plasma, were prepared by a modification of techniques previously described(4,6): 10 ml of intact platelet-poor plasma was shaken with 40 mg of kaolin or with 200 mg of collagen for 10 minutes. After centrifugation, the precipitate was washed twice with 10 ml of 0.9% NaCl and then eluted with 2.5 ml of 0.9% NaCl at pH 10. Alkaline eluates were neutralized with 0.01 N hydrochloric acid to pH 7.4 before use. The protein content of kaolin eluates was determined using the biuret method and was found to be approximately 0.3 mg per ml, and that of collagen eluates 1.7-2.0 mg per ml.

The clotting time of whole blood obtained from the carotid artery was tested in plastic test tubes, according to a technique previously described(7). The influence of dicalite, collagen and elastin on clotting time of intact rabbit blood was investigated. These substances were weighed and placed directly into test tubes. Two ml of blood were then dripped directly into the test tube from a carotid artery catheter, and the clotting times recorded at room temperature.

Estimation of collagen and kaolin eluate clotting activity was performed in the following system at 37°C: 0.2 ml of eluate (differing dilutions), 0.2 ml of exhausted plasma and 0.2 ml of 0.025 M calcium chloride. A reference dilution curve of kaolin eluate was prepared, against which the collagen eluate was compared. Recalcification times were performed at 37°C as follows: 0.1 ml collagen or kaolin eluate, 0.1 ml of intact or deficient plasma and 0.1 ml of 0.025 M calcium chloride.

Estimation of Factor V levels was performed using human aged plasma as a substrate(8). The level of Factors VII and X was measured using bovine, Seitz-filtered plasma as a substrate(9). Estimation of Factor VIII levels in collagen eluates was performed using the one-stage method of Hardisty and MacPherson(10). Factor IX levels in the eluates were determined by the same technique, using Factor IX deficient plasma as a substrate.

The formation of stasis thrombi in isolated rabbit jugular vein segments was determined according to a modification of techniques previously described(7,11). Ten ml of collagen eluate were injected over a 30-second interval into the ear vein of each animal. Fifteen seconds after the end of the infusion, 2 contralateral jugular vein segments were isolated from the circulation by ligatures. The segments were removed after 15 and 30 minutes respectively, and examined for the presence of thrombi. A third segment of jugular vein was isolated before the collagen eluate was infused, and removed after 30 minutes. Clotting times were obtained immediately before and after the eluate infusion.

Results. 1. In vitro *activation of clotting of whole blood by collagen and elastin.* The results are presented in Fig. 1. The mean control clotting times in these experiments ranged from 37 to 41 minutes. Both collagen and dicalite considerably shortened the clotting time, although it can be seen that dicalite was much more active than collagen. On the other hand, elastin was considerably less active than collagen. Two mg of collagen produced approximately the same degree of shortening of the clotting time as 20 mg of elastin.

2. *Influence of kaolin and collagen eluates on clotting time of Factor XII and XI deficient plasma, and human intact plasma.* Alkaline kaolin and collagen eluates were both found to accelerate markedly the clotting of

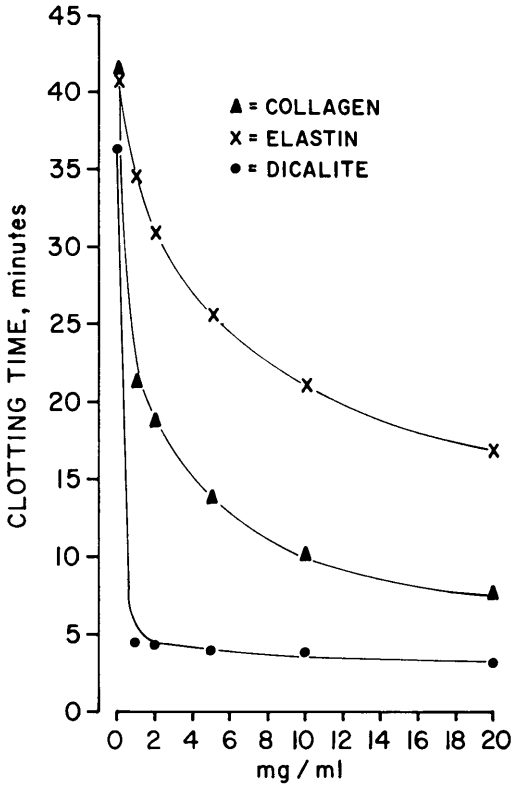

FIG. 1. Effect of varying concentrations of collagen, elastin and dicalite on whole blood clotting time of intact rabbit blood. Mean data from 3 rabbits in each group.

Factor XII deficient plasma, Factor XI deficient plasma, and human intact plasma (Table I). The undiluted collagen eluate was found to have an effect in accelerating clotting equivalent to a 1:5 dilution of the kaolin eluate (Table I). These experiments suggest that collagen eluate has an effect similar to but weaker than kaolin eluate in promoting clotting.

The clotting activity of undiluted collagen eluates was also determined by comparison with varying dilutions of an alkaline kaolin eluate. In 6 experiments using rabbit plasma the activity of collagen eluate, as calculated per volume, varied between 14 and 20% of the kaolin eluate.

3. *Factors V, VII + X, VIII, and IX levels in collagen eluates.* The level of Factor V in alkaline eluates of collagen previously shaken with human platelet-poor plasma was found to be 1.2% (average of 3 samples). On the same samples, the level of Factor VII + X was found on average to be 0.6% of the level in platelet-poor plasma. Factors VIII and IX levels were measured in eluates from collagen exposed to human and rabbit plasma. In 3 human plasma eluates the level of Factor VIII was found on average to be less than 2%. In 6 rabbit plasma samples, the average level was 8%. On the same samples, the average level of Factor IX was 4% (human) and 8% (rabbit), respectively.

When 0.1 ml samples of collagen eluate were added to 0.1 ml of a solution of 400 mg % fibrinogen (Armour bovine and Kekwick) in veronal buffer in saline, no clotting occurred over a 3-hour observation period.

4. *Production of thrombosis by eluates from collagen exposed to human and rabbit plasma.* Following the injection of 10 ml of eluate from collagen exposed to rabbit plasma, thrombi developed in 8 of 10 rabbits after 30 minutes of stasis (Table II). No thrombi developed in the control segments, isolated before the injection of collagen. It can also be seen from Fig. 2 that the plastic clotting times in rabbits injected with collagen eluate were considerably shortened following the

TABLE I. Influence of Kaolin and Collagen Eluates on Recalcification Time of Intact, Hageman (Factor XII), and PTA (Factor XI) Deficient Plasma.

System	Clotting time (sec)		
	Dilutions of eluate		
	Undiluted	1:5	Control (saline)
Kaolin eluate + intact human plasma	105	170	550
Collagen eluate + intact human plasma	185	225	550
Kaolin eluate + HF plasma	95	145	770
Collagen eluate + HF plasma	180	270	770
Kaolin eluate + PTA plasma	100	200	1750
Collagen eluate + PTA plasma	220	380	1750

TABLE II. Stasis Thrombus Formation in 15 Rabbits Following Infusion of Eluates from Collagen Exposed to Rabbit and Human Plasma.

	No. exp	% Thrombosis 15 min stasis	% Thrombosis 30 min stasis
Collagen eluate (rabbit plasma)	10	20	80
Collagen eluate (human plasma)	5	80	100
Control	15	0	0

eluate injection.

Collagen exposed to human plasma produced a more active eluate. Ten ml of such eluate produced stasis thrombi in 15 minutes in 4 out of 5 rabbits that were tested.

Discussion. The above experiments confirm the previous finding that Hageman Factor (Factor XII) is activated by collagen(4). Collagen appears to act in a manner quite similar to silica particles; such particles are known to adsorb and activate Factor XII, which is then followed by activation of Factor XI(5,12,13). We found that both collagen and kaolin eluates greatly shorten the clotting times of intact human plasma, and Factor XII and XI deficient plasma. The rate and extent of the activation of Factor XII by collagen is, however, considerably less than by kaolin or dicalite.

Collagen eluates can be shown to trigger sufficient intravascular coagulation to produce thrombosis in areas of vascular stasis. On the other hand, saline washings of collagen were found to be inactive in producing thrombosis in rabbits. Collagen eluates were incapable of clotting fibrinogen, and contained only negligible amounts of Factors V and VII + X. Assays of Factors VIII and IX levels in collagen eluates also revealed very low levels of both these factors, especially in eluates from collagen exposed to human plasma. These findings are in agreement with previously reported studies(4) where it was found that small decreases of Factors VIII and IX occurred in human plasma after it had been exposed to collagen. Collagen apparently behaves similarly to inorganic surface active agents in this respect, for eluates from celite exposed to plasma have been reported to contain around 1% of Factors VIII and IX(14). While the small amounts of Factors VIII and IX present in the eluates may contribute to the effects that we have observed, it seems reasonable to conclude that the thrombogenicity of collagen eluates is primarily related to the presence of activated Factors XII and XI.

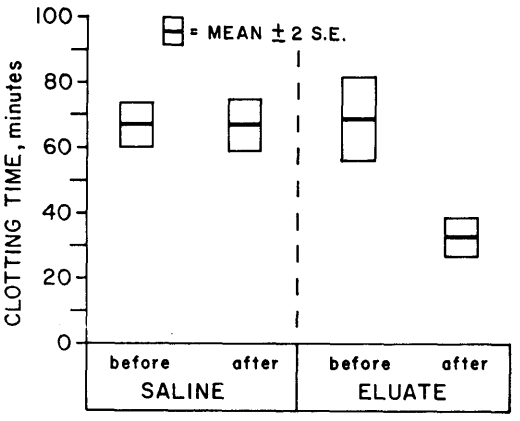

FIG. 2. Effect of infusions of 10 ml of saline or collagen eluate on whole blood intact clotting time of rabbits. Blood withdrawn for clotting times immediately before and after infusion. Mean data from 6 rabbits in each group.

The present experiments are analogous to those reported previously in which stasis thrombi were produced by infusion of activated Factor XI(14-16). Ellagic acid, a specific activator of Factor XII, has also been reported to produce stasis thrombi(17). It is possible that collagen is capable of specifically activating Factor XII *in vivo,* acting as a trigger for the chain-reactions leading to fibrin formation. It is of interest to note that elastin, a related protein of connective tissue, is nevertheless much less effective in Factor XII activation than collagen. In previously reported experiments(4) we were unable to elute significant amounts of activated Factor XI from elastin.

Platelets are known to adhere to collagen fibers(18,19), and Factors XII and XI have been shown to be adsorbed on the platelet surface(20,21). It is possible that activation of Factor XII is occurring in the early stages of hemostasis, at the same time as platelets are aggregated by exposed collagen fibers. Factor XII activation, amplified by a "cascade of proenzyme-enzyme transformations" (1), may result in the relatively rapid ap-

pearance of thrombin on the platelet surface. Thrombin is known to be involved in platelet aggregation, fibrin formation, and in making the platelet plug impermeable(22). According to the work of Johnson and her associates(23), thrombin is formed within a few seconds at the site of tissue injury. This phenomenon could be explained by assuming a simultaneous action of collagen in promoting platelet aggregation and also triggering the chain-reaction of the clotting sequence. Recent electron microscope observations by Hovig *et al*(24) have demonstrated a fibrin layer in hemostatic plugs localized between collagen fibers and platelets. The dual action of collagen in aggregating platelets and activating Factor XII may therefore be of considerable physiological importance in hemostasis and thrombosis.

Summary. The activation of Factor XII (Hageman Factor) by bovine collagen has been studied *in vitro* and *in vivo*. Alkaline eluates obtained from collagen exposed to human and rabbit plasma contained Factors XII and XI, and were found to shorten markedly the whole blood clotting time of rabbits *in vivo* and *in vitro*. Formation of stasis thrombi could also be demonstrated in rabbits following injection of collagen eluates. The possible significance of collagen-induced Factor XII activation in the mechanism of hemostasis and thrombosis is discussed.

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