entrance of barbital into the cerebrospinal fluid cannot be stated now.

In contrast to the slow passage of barbital into brain and cerebrospinal fluid, the more lipid soluble thiopental passes rapidly into the central nervous system: maximal concentrations appear in the brain almost immediately after intravenous injection(6) and in the cerebrospinal fluid within 7 minutes(7).

Summary. Experiments in dogs have shown that barbital passes slowly into the brain and even more slowly into cerebrospinal fluid. It is possible that the blood cerebrospinal fluid barrier and blood brain barrier are similar for this drug.

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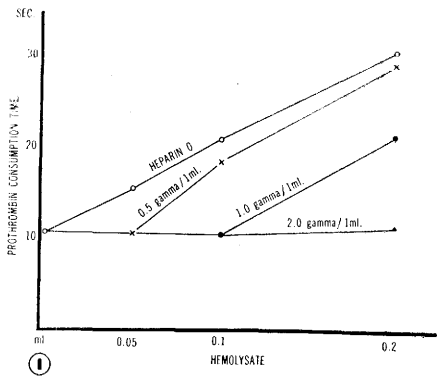
Erythrocyte Extract (Hemolysate) and Tissue Thromboplastin: Their Action on Heparin Activity.* (25611)

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Heparin with its co-factor, which originally was designated albumin X, is a powerful antithrombin(1). By means of certain agents such as protamine(2), toluidine blue(3) and polybrene(4) (a poly 1,5 dimethyl-1,5-diazoundecamethylene methobromide), heparin is inactivated directly and, presumably, stoichiometrically. Tissue thromboplastin also inactivates heparin activity, but from results of earlier studies(5), it was concluded that the anti-heparin action of thromboplastin is indirect, brought about by increase in rate and amount of thrombin produced, thereby exceeding the antithrombin activity of heparin. In the present study an attempt is made to determine the nature of the antiheparin action of erythrocyte extract (hemolysate)(6,7,8). For comparison the antiheparin activity of tissue thromboplastin was restudied.

Materials and methods. Preparation of platelet-poor human native plasma. Blood collected with silicone-coated syringe and needle was transferred to heavy walled test tubes coated with silicone. After chilling in ice bath for 20 minutes, blood was centrifuged for 30 minutes in a Servall Superspeed centrifuge kept in cold room (4-6°C) and the plasma carefully removed with a silicone-coated pipette. *Erythrocyte extract*(9). Human erythrocytes freed from platelets and leucocytes by repeated washing and differential centrifugation were suspended in a volume of physiological saline equal to that of blood from which they were obtained. Complete hemolysis was obtained by freezing at –20°C and then thawing. *Heparin.* One hundred mg sodium salt in 10 ml (Lederle). *Protamine sulfate.* Nutritional Biochemicals Corp. product was dissolved in distilled water. *Prothrombin consumption time with added hemolysate.* In the basic test as previously described(1), 1 ml of native platelet-poor plasma is mixed with a fixed amount of hemolysate and incubated at 37°C. Fifteen minutes after solid clot has formed, it is removed by wrapping it on to a wooden applicator. After 45 minutes of further incubation at 37°C, prothrombin of the serum is determined by the one-stage technic using rabbit plasma adsorbed with $Ca_3(PO_4)_2$ as source of fibrinogen

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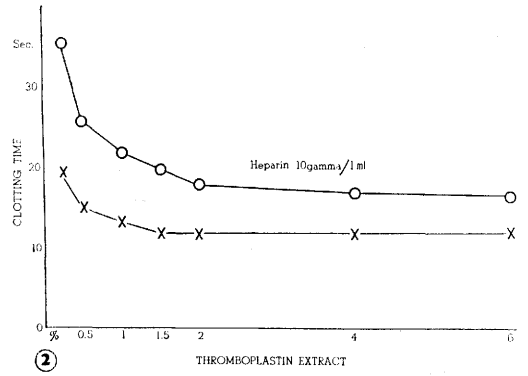


FIG. 1. Effect of increasing amounts of hemolysate on heparin activity as measured by prothrombin consumption test.

FIG. 2. Influence of heparin on prothrombin time of human plasma with varying concentrations of tissue thromboplastin (rabbit brain extract).

and labile factor. In the present investigation, the test was carried out in silicone-coated test tubes. The agents, heparin and protamine sulfate, which were studied were mixed with native plasma before hemolysate was added. *Prothrombin time.* The one-stage technic as previously described was employed (10). Thromboplastin was prepared from rabbit brain by the acetone dehydration method. Suspensions of thromboplastin varying from 0.25 to 6% in saline were incubated at 50°C for 20 minutes.

Results. Effect of heparin on prothrombin consumption time of native platelet-poor plasma with added hemolysate. Native platelet-poor human plasma fails to clot in silicone at 37°C. On adding hemolysate, clotting occurs and prothrombin consumption time increases in direct proportion to amount added (Fig. 1). As little as 0.5 μg of heparin/ml of plasma usually inhibited completely consumption of prothrombin resulting from addition of 0.05 ml of hemolysate. On increasing the quantity of hemolysate, consumption of prothrombin is resumed and when prothrombin consumption is plotted against amounts of added hemolysate, a straight line, fairly parallel to the control curve without heparin, is obtained. When concentration of heparin is 2 μg/ml of plasma, little or no consumption of prothrombin occurs, even when 0.2 ml of hemolysate is added.

Effect of protamine sulfate on heparin action in prothrombin consumption test with added hemolysate. When plasma contains 1 μg of heparin/ml, no consumption of prothrombin occurs on addition of 0.05 ml of hemolysate until concentration of protamine sulfate is 2-3 μg/ml of plasma (Table I). In the absence of added heparin, protamine sulfate does not influence the effect of hemolysate on consumption of prothrombin.

Effect of heparin on prothrombin time with varying amounts of thromboplastin. Prothrombin time of normal plasma is progressively decreased by increasing quantities of tissue thromboplastin (rabbit brain) until a minimal constant value of 12 seconds is obtained. Concentrations of thromboplastin greater than 6% may have a slightly inhibitory effect.

When 10 μg of heparin are added to 1 ml of oxalated plasma, and a series of prothrombin time determinations made using same concentrations of thromboplastin as in the control, a similar curve but with longer clotting time values is obtained (Fig. 2). As in the control, a constant value is obtained which is not shortened by excess thromboplastin.

Discussion. The clotting factor in erythrocytes corresponds chemically to a phospholipid(11). It possesses the unique property of causing a high consumption of prothrombin when added to native platelet-poor human plasma in a silicone-coated test tube(12), whereas a platelet extract causes no measurable consumption of prothrombin under similar conditions(13). This suggests that the clotting factor, erythrocytin, is not identical with the clotting factor in platelets which functions in the generation of thromboplastin

TABLE I. Inactivation of Heparin by Protamine Sulfate.

Heparin,* μg	Protamine sulfate, μg	Hemolysate, ml	Prothrombin consumption, sec.
			12
1		.05	12.6
1	1	"	12.6
1	2	"	17.2
1	3	"	26
1	5	"	26
	5	"	24
		.05	26
		.1	31
		.2	40
.5		.05	12
"		.1	12
"		.2	31
"	10	.05	26
"	"	.1	31
"	"	.2	46

* Heparin was mixed with 1 ml of platelet-poor normal native human plasma in a silicone-coated test tube. Protamine sulfate was then added, followed by the hemolysate.

even though both can participate in production of intrinsic thromboplastin.

Heparin and hemolysate have a mutually antagonistic action on prothrombin consumption. As little as 0.5 μg of heparin/ml of plasma completely inhibits action of 0.05 ml of hemolysate. On increasing the quantity of hemolysate, inhibitory activity of heparin is sufficiently overcome to allow consumption of prothrombin and a proportionality between amount of hemolysate and the prothrombin consumption occurs. However, even when the amount of hemolysate is quadrupled, heparin activity is not completely abolished as it is when heparin is neutralized by protamine sulfate (Table I). When concentration of heparin is increased above 2 μg/ml of plasma, complete depression of prothrombin consumption results.

Two explanations for the antiheparin action of hemolysate seem probable: either hemolysate neutralizes heparin directly as does protamine sulfate, or hemolysate acts by participating in production of thromboplastin which brings about generation of sufficient thrombin to overcome antithrombic activity of heparin. Since hemolysate does not completely overcome the heparin effect and fails to cause consumption of prothrombin when concentration of heparin is above 2 μg/ml of plasma, the second explanation appears to be the more likely.

Generation of intrinsic thromboplastin is a complex reaction. It not only requires adequate quantities of a platelet or erythrocyte factor, thromboplastinogen (factor VIII), PTC (factor IX) and perhaps other primary agents, but it also depends on an autocatalytic reaction which is probably mediated through thrombin(13). Presumably, prompt neutralization of thrombin as it is formed effectively blocks the clotting reaction. By carrying out the reaction in a silicone-coated test tube, the catalytic action of a glass surface is eliminated and sensitivity of the test to minute amounts of heparin is greatly increased.

Protamine sulfate neutralizes heparin completely. Approximately 2-3 μg are required to inactivate 1 μg of heparin. Significantly, addition of protamine sulfate to normal native plasma does not alter prothrombin consumption resulting from a fixed amount of hemolysate. Since the method is sensitive to amounts of heparin less than 0.5 μg/ml, it appears unlikely that normal plasma contains a significant quantity of free or active heparin.

The prothrombin time curves in Fig. 2 suggest the nature of antiheparin action of tissue thromboplastin. If a direct or true inactivation of heparin occurred, prothrombin time of plasma containing heparin should become 12 seconds at high concentrations of thromboplastin. A more likely explanation is an indirect inactivation. The thrombin produced exceeds the antithrombin capacity of heparin.

If the antiheparin action of hemolysate and of tissue thromboplastin is indirect and depends on generation of thrombin, it can be concluded that this is not a specific property but a general one possessed by any material which has thromboplastic activity or takes part in the generation of intrinsic thromboplastin. Since it has not been established that heparin has an essential role in maintaining fluidity of blood, and since clinical evidence has been found that even marked hyperheparinemia may produce only a mild bleeding state (14), antiheparin activity may perhaps be merely incidental and have little physiological significance.

Summary. The inhibitory action of heparin on prothrombin consumption of normal platelet-poor human plasma in a silicone-coated test tube can be partially overcome by increasing amounts of hemolysate. Likewise, prothrombin time of human plasma to which a fixed amount of heparin has been added can be shortened and brought to a constant but higher value than normal by adding increasing amounts of tissue thromboplastin.

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Quantitative Relationship of Osteoclasts to Parathyroid Function.* (25612)

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Problems concerning the role of osteoclasts in bone dissolution have long been investigated. Numerous studies have attempted to clarify this basic relationship, particularly in reference to the part played by osteoclasts in parathyroid hormone effect on bone resorption. These earlier studies have been reviewed by Hancox(1,2). Of particular interest are reports of Selye(3), which referred to increases in numbers of osteoclasts after parathyroid extract administration, and of Bloom *et al.*(4), which demonstrated qualitative increases in osteoclasts coupled with decreases in numbers of osteoblasts during laying cycle of pigeons. More recently Gaillard(5) noted significant increases both in number and activity of osteoclasts in incubated foetal bone, under the influence of a substance elaborated from concurrently incubated parathyroid tissue, or from parathyroid extract added to the media. Talmage *et al.*(6) were able to show qualitative increases in numbers of osteoclasts in long bones of rats undergoing continuous peritoneal lavage with calcium- and phosphate-free rinse. The only attempt to use quantitative determination of osteoclast concentrations as an index of metabolic activity has been the recent work by Myers *et al.*(7) on the mandibular condyle. The purpose of our study was to develop a method for quantitation of osteoclastic material which could be used as index of bone dissolution and of parathyroid activity.

Materials and methods. These experiments utilized approximately 75 male Holtzman rats with weight range restricted to between 225 and 250 g. All were placed on a calcium- and phosphate-free diet for 24 hr before start of each experiment. Bilateral nephrectomies and parathyroidectomies were performed under ether anesthesia. Continuous peritoneal lavage was carried out by introducing into the peritoneal cavity, through a stainless steel indwelling plug(8), 30 ml of a solution containing 0.8% NaCl and 0.5% glucose at pH 7.4. After equilibration for 1 hour, this fluid was removed and immediately replaced with another 30 ml of lavage solution. Animals were

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