parable with fed animals at atmospheric pressure, 6 of 6 pertussis-inoculated mice did not survive 24 hours at 278 mm Hg, the majority of animals succumbing within a few hours after exposure.

In 2 succeeding experiments in which equal numbers of pertussis-inoculated and uninoculated mice were placed in the low pressure chamber at 278 mm Hg for 24 hours similar results were obtained. It was found that 17 of 18 uninoculated mice survived a pressure of 278 mm Hg for 24 hours. Only 3 of 18 pertussis-injected animals remained alive under these conditions. The difference between these groups is a significant one ($p = <.01$).

Discussion. It is apparent from these experiments that under moderate anoxic conditions (380 mm Hg) the adrenals of pertussis-injected, fasted mice function as well as those of uninoculated fasted animals with respect to the maintenance of liver glycogen above the fasting level of control mice. However, under more severe anoxic conditions (278 mm Hg) the survival ability of the pertussis-inoculated mouse, like that of an adrenalectomized animal(1), is low.

Although cortical extract will restore the lowered susceptibility of the adrenalectomized animal to the lethal effects of reduced oxygen tension(1), experiments employing ACTH, cortisone and aqueous adrenal extracts in order to increase the resistance of pertussis-inoculated mice to anoxia have thus far been unsuccessful.

Summary. An increase in liver glycogen occurred in both fasting uninoculated and fasting pertussis-inoculated mice when exposed to ½ atmosphere pressure for 24 hours. Exposure at ⅜ atmosphere for 24 hours killed 17 of 18 pertussis-inoculated mice and only 3 of 18 uninoculated animals.

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Prevention of Toxic Effects of Injected Thromboplastin by "Thromboplastinase".* (20650)

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Intravenous injection of thromboplastic suspensions in experimental animals has been reported to cause ataxia, convulsions and death(1,2). These effects have been prevented by prior injection of several agents such as rabbit serum, heparin, and congo red(4), soybean trypsin inhibitor(5), monosodium alpha tocopherol phosphate, and inositol phosphatide(6). The morbid or lethal effects of injected thromboplastin have been attributed to thromboembolism(1-3).

Our laboratory recently reported on a new bacterial enzyme, "thromboplastinase" which caused a marked reduction of thromboplastic potency of tissue suspensions *in vitro.* Its preparation, properties, and mode of action have been described(7). In this paper we wish to report the effect of "thromboplastinase" on the toxicity of injected thromboplastic suspensions.

Materials and methods. Active, stable, reproducible rabbit brain or human brain thromboplastic suspensions(8) were used except when indicated. The saline extract of acetone-dried tissue contained finely divided, suspended material. Such suspensions were variously treated before injection into animals:

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1) Boiled thromboplastic preparations were made by keeping the above suspensions in a 100°C water bath for 30 minutes, which reduced thromboplastic potency from 13 seconds to 40 seconds (one-stage prothrombin time). 2) An enzyme-inactivated thromboplastic preparation ($\triangle$TP) was made by incubating the original suspensions with "thromboplastinase" at 37°C for various periods. Such treatment progressively rendered the thromboplastic suspensions less potent. 3) Active thromboplastic preparation received no treatment other than refrigeration. These 3 types of suspensions were finally centrifuged at high speed (R.C.F. 20,000 G) for 3 hours, and the sediment resuspended to its original volume with 0.85% NaCl. All suspensions were subjected to the Potter homogenizer in an attempt to reduce and control particle size. There was no $CaCl_2$ in the suspensions used for injection. Potency of the thromboplastic suspensions was tested using a modification(9) of the Quick one-stage method, with normal human oxalated plasma. Concentrations of "100%" (*i.e.* undiluted) thromboplastin clotted such plasma in 12-13 seconds, while clotting times of 40 seconds were obtained with suspensions diluted to 1% with normal saline. Experimental animals employed in this study were male albino rabbits weighing about 1½ kg. The injected materials were pre-warmed to 37°C. Rapid injections of "thromboplastinase" were made in one marginal ear vein. Injections of the thromboplastins were made in the marginal vein of the opposite ear. A positive reaction to injected materials usually occurred within 1-2 minutes after injection. All animals were observed for a maximum of 10 minutes, at the end of which time the reaction of those failing to exhibit ataxia and convulsions was considered negative. Two general procedures were employed whereby "thromboplastinase" could effect the toxicity of injected thromboplastic suspensions: 1) Incubation of thromboplastin with "thromboplastinase" (formation of $\triangle$TP) prior to injection. 2) Injection of "thromboplastinase" into experimental animals prior to injection of active thromboplastin.

Results. Injection of active thromboplastic suspensions was observed to cause ataxia, convulsions and death as others have previously reported. The approximate least toxic dose of the thromboplastic suspensions was determined prior to each experiment. Table I shows a representative toxicity chart for untreated rabbit brain, human brain and boiled human brain thromboplastin. The least lethal dose in all cases was found to vary between ½ cc/kg body wt and 1 cc/kg body wt, depending on the materials used.

In Table II it can be seen that prior incubation of thromboplastin with "thromboplastinase" resulted in a $\triangle$TP which was nontoxic when subsequently injected. The toxicity

TABLE I. Showing Toxicity of Rabbit Brain, Human Brain and Boiled Human Brain Thromboplastin; note that boiled human brain thromboplastin with very low clotting potency was approximately as toxic as untreated thromboplastin.

Material	Clotting time, sec.	Dose, cc/kg	# animals	Deaths
Rabbit*	12	.25	2	0
		.5	8	0
		.75	4	2
		1.0	11	10
		1.25	4	4
		1.5	2	2
		2.0	4	4
		3.0	4	4
Human*	12	.25	2	0
		.5	6	4
		1.0	4	4
		1.5	2	2
Boiled human*	40	.25	2	0
		.5	2	0
		1.0	2	2

* Brain thromboplastin.

TABLE II. Comparison of the Toxicity of Fresh and Enzyme-Inactivated Thromboplastin at Various Doses.

	Materials injected			
	Fresh rabbit brain thromboplastin CT* 12″		Enzyme inactivated thromboplastin CT 40″	
Dose, cc/kg	No. rabbits	Deaths	No. rabbits	Deaths
½	4	0	—	—
¾	4	2	2	0
1	4	3	2	0
1¼	4	4	—	—
2	4	4	2	0
2½	—	—	2	0
3	4	4	2	0
4	—	—	2	0
5	—	—	2	0

* CT = Clotting time.

TABLE III. Results of Typical Experiment in Which Thromboplastin, 1 cc/kg Body Weight, Was Injected Intravenously 5 Min. after Administration of the Enzyme.

Inj.	Conc. thromboplastinase, mg/kg	Rabbits	No. of fatalities
Intrav.	5 mg/kg: heat inactivated	3	3
	0.85% NaCl	2	2
	1 mg/kg	2	2
	2	2	2
	3	2	2
	5	5	0
	10	2	0
Intramusc.	5	2	2
Subcut.	5	2	2

of "thromboplastinase" was very low. It was tolerated in doses up to 10 times that required to exert a protective effect. In such doses, its effect on blood clotting (One-stage), blood pressure, and smooth muscle tone of the experimental animal was negligible. When active "thromboplastinase" was administered before the injection of human brain thromboplastin, no deaths occurred after an effective "thromboplastinase" dose was reached. Table III shows a typical experiment where the injection of "thromboplastinase" was followed in 5 minutes by an injection of 1 cc/kg body wt rabbit brain thromboplastin. It can be seen that 5 mg/kg of the crude enzyme was sufficient to prevent toxic effects. Similar results were noted using human brain thromboplastin and boiled human brain thromboplastin as toxic agents.

Intramuscular and subcutaneous injections of "thromboplastinase" exerted no protective action in the 5 mg/kg concentration. Heat-inactivated "thromboplastinase" exerted no protective effect.

The interval between the injection of "thromboplastinase" and the subsequent injection of thromboplastin necessary for effective protection from the lethal effects of thromboplastin varied with the enzyme preparation. In Table IV the effective time of one "thromboplastinase" preparation is seen to be 90 seconds. Other "thromboplastinase" preparations have exhibited the protective effect when only 15 seconds were allowed to elapse between injections of the enzyme and thromboplastin.

Discussion. The observation that injection of thromboplastically active tissue suspensions are toxic to experimental animals has been confirmed on rabbits using tissue from the same species and from other species. No gross difference between species was observed when their thromboplastic potency was approximately equal. It was striking, however, to note that boiled human brain thromboplastic suspension with almost all thromboplastic activity lost still retained its full toxicity as an injected suspension. Chargaff has presented evidence that thromboplastin is a phospholipoprotein(10). One interpretation of the effect of boiling such thromboplastic suspensions is that although the protein moiety was probably denatured, the lipid moiety remained effective. If this were so, one might be led to conclude that the lipid moiety played an essential role in the toxic reactions initiated by injection of thromboplastic suspensions, while the protein portion of the phospholipoprotein was either non-essential or could be replaced by some material *in vivo*. Unless some reservation such as the latter were true, it would appear that there is no necessary correlation between thromboplastic potency and toxicity, since it has been

TABLE IV. Effect of Time Interval between Intravenous Injection of Thromboplastinase (5 mg/kg) and Intravenous Injection of Human Brain Thromboplastin (Clotting Time 13 Seconds) on Toxic Reaction to Thromboplastin.

Interval between "thromboplastinase" and thromboplastin inj., sec.	Result
0	Both developed ataxia, convulsions and death
30	*Idem*
45	1 showed no reaction 1 developed ataxia, convulsions, paralysis, death
60	1 showed no reaction 1 developed ataxia, paralysis, but recovered
90	Both no reaction
120	"
150	"
180	"

Injections of 5 mg/kg crude "thromboplastinase" followed at intervals by least toxic dose of human brain thromboplastin (clotting time 13 sec). 2 rabbits in each exp.

shown that poorly potent thromboplastic suspensions (boiled thromboplastin) can have the same approximate minimal lethal dose as potent thromboplastic suspensions. On the other hand, 2 tissue suspensions of equally poor thromboplastic potency have been shown to have widely divergent least toxic doses: boiled thromboplastin, clotting time = 40″ or more which was highly toxic: △ thromboplastin, clotting time = 40″, which was nontoxic.

The fact that thromboplastic suspensions incubated with "thromboplastinase" lost both their thromboplastic potency as well as their toxicity would seem to support the interpretation that these two properties are functionally correlated. It has been shown elsewhere(11) that "thromboplastinase" probably exerts its action on the lipid moiety of thromboplastin which seems to be associated with the Folch inositol phosphatide fraction. It has been further demonstrated that as a result of "thromboplastinase" action, an organic phosphorus moiety is split from thromboplastin (7).

It is noteworthy that boiling thromboplastin does not lead to a diminution of the amount of phosphorus splittable by "thromboplastinase"(11). This observation would seem to support our interpretation (above) that the lipid moiety of thromboplastin is essential for the production of toxic reactions.

The manner in which pre-injected "thromboplastinase" protects animals against the toxic effects of injected thromboplastin is difficult to explain at present, in view of the rapidity with which the "thromboplastinase" afforded protection. Thromboplastinase *in vivo* seems to exert its protective action almost immediately. *In vitro,* however, destruction of thromboplastin by "thromboplastinase" in the concentrations used followed a much slower course.

Although we cannot interpret this effect, it seems clear that "thromboplastinase" is effective in preventing in rabbits the lethal effect of injected thromboplastin by a mechanism that awaits clarification.

Summary. 1. Loss of potency of tissue thromboplastic suspensions due to "thromboplastinase" action results in a concomitant loss of toxicity. Loss of potency by boiling does not alter the toxicity of thromboplastin. It would appear that the lipid moiety of thromboplastin may be essential for the production of toxic reactions. 2. Prior intravenous injection of "thromboplastinase" into rabbits protected the animals against the otherwise morbid or lethal effects of subsequent injections of a minimal toxic dose of thromboplastin. Heat-inactivated "thromboplastinase" did not induce this protection. 3. Active "thromboplastinase", in doses up to 10 times that needed for protection, was observed to cause no untoward reactions in the animals. 4. The mechanism of the protective effect *in vivo,* seems to be different from the *in vitro* destruction of thromboplastin by "thromboplastinase". The nature of this effect is presently under investigation.

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