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Effect of Ultrasonics on Thromboplastinase-Labile Component and Toxicity of Injected Thromboplastin. (23055)

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The effects of heat on the thromboplastinase-labile component of tissue thromboplastins have been previously reported(1). Loss of thromboplastic potency due to heating of tissue suspensions and loss due to thromboplastinase are apparently distinct phenomena. Further, heat inactivated thromboplastin retained all of its *in vivo* toxicity for rabbits while thromboplastinase inactivated suspensions were non-toxic(2). Axelrod (3) recently reported on loss of potency of commercial thromboplastin following ultrasonic treatment. It was of interest to determine what effect ultrasonics would have both on the TPase-labile component of human brain thromboplastin and on the lethal effect upon intravenous injection of such thromboplastic suspensions into experimental animals.

Materials and methods. Thromboplastinase (TPase) was prepared as previously described(4). 2 mg crude TPase powder/ml water was employed as standard enzyme concentration. Heat inactivated enzyme (100° for 10 min) served as a control. *Thromboplastin (TP).* Thromboplastic suspensions were freshly prepared 1 hr before use by extracting 0.5 g acetone dehydrated human brain powder with 10 ml 0.9% saline at 50°C for 15 min and then centrifuging at 3000 rpm for 5 min. The supernatant was decanted and refrigerated prior to use. *Clotting time determinations (CT).* A modification(4) of the Quick one-stage test was used for assay of clotting potency. Duplicate determinations were made. *Thromboplastin dilution curves* were constructed as previously described(4). *Ultrasonic treatment.* Raytheon Model DF-101 200 watt sonic oscillator was used with a frequency of 10 kc. 50 ml samples of thromboplastic suspensions were treated at one time and aliquots were removed at intervals for assay of potency, preparation of dilution curves, treatment with TPase and injection into animals. *TPase assay.* The activity of TPase was followed by change in clotting potency and liberation of organic phosphorus after incubation at 37°C for 60 min(1). 10 ml of thromboplastic suspension and 1.0 ml TPase solution (2 mg) was used as a standard incubation mixture. *Experimental animals* employed were male albino rabbits weighing 2-2½ kg. Preparation of suspensions for intravenous injection and technics used have been described(2).

Results. The loss in clotting potency of human brain thromboplastic suspensions due to ultrasonic treatment is shown in Fig. 1. It can be seen that there was a progressive loss in potency over a time course. When dilution curves were plotted for aliquots removed at intervals (Fig. 2), it was evident that most of the activity had been destroyed quite rapidly. After 30 min of ultrasonic treatment clotting time was 19 sec. On the

TABLE I. Loss in Clotting Potency and Liberation of Organic Phosphorus Due to TPase Action on Ultrasonically Treated Thromboplastin.

Ultrasonic time (min.)	CT (sec.) after sonic treatment	CT (sec.) after TPase action for 60′ at 37°C	Phosphorus found (μg P/mg dry wt TP) Before TPase	After TPase
0	11.3	36.0	2.5	30.9
30	20.2	42.5	2.4	31.0
90	40.7	60.7	2.4	31.6

dilution curve of untreated thromboplastin, 19 sec represents approximately 5% thromboplastin. Similar calculations have been described previously(4).

Aliquots removed at 0, 30, and 90 min were subsequently treated with TPase. Table I summarizes the data obtained in one series of experiments on liberation of organic phosphorus after TPase action on these aliquots.

Although there was a definite loss in clotting potency due to ultrasonic treatment (11.3 to 40.7 sec), there was no destruction of the TPase-labile moiety of thromboplastin inasmuch as the same amount of organic phosphorus was liberated from each aliquot due to TPase action. The maximum phosphorus liberated by TPase in 180 min was 33.6 μg P/mg dry wt TP. Heat inactivated TPase caused no loss in clotting potency and no phosphorus was liberated.

These suspensions were then prepared for intravenous injection into rabbits in order to assay for lethal potency. Table II summarizes the findings. Loss of clotting potency due to ultrasonic treatment (11.3-40.7 sec)

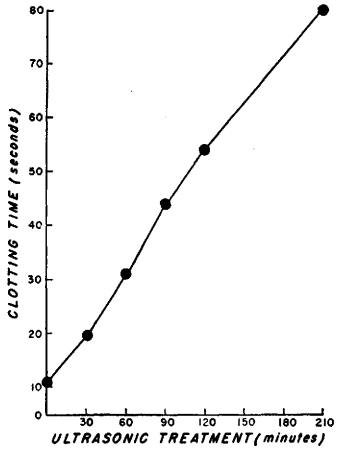

FIG. 1. Loss in clotting potency of human brain thromboplastin due to ultrasonic treatment.

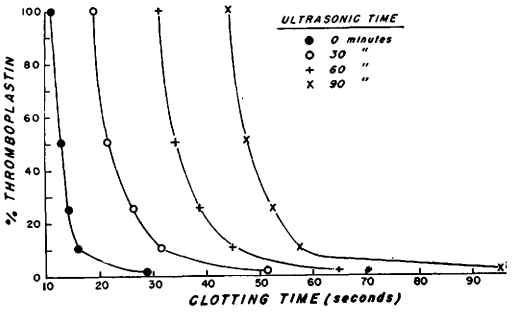

FIG. 2. Dilution curves of ultrasonically treated human brain thromboplastin.

did not result in a decrease in lethal potency of the thromboplastic suspensions. However, loss due to TPase action to approximately the same level (11.3-36.5 sec) resulted in a thromboplastic suspension no longer lethal in as high as 3x the normal lethal dose. Ultra-

TABLE II. Effects of Intravenous Injection of Variously Treated Brain Thromboplastic Suspensions in Rabbits.

Treatment	CT, sec.	No. animals	Dose, ml/kg body wt	Effects
None	11.3	4	1.5	4 died
Idem, with subsequent TPase treatment	36.5	4	1.5	4 no reaction
		2	3.0	2 *Idem*
		1	4.5	1 "
30′ at 10 kc	20.2	4	1.5	4 died
90′ " "	40.7	4	1.5	4 died
Idem, with subsequent TPase treatment	65.0	2	1.5	2 no reaction
		2	3.0	2 *Idem*
		1	4.5	1 "

sonically treated thromboplastin subsequently incubated with TPase lost its lethal potency for rabbits.

Discussion. The results of this study lend further support to the assumption(2) that TPase-labile moiety of thromboplastin and its *in vivo* toxicity are related. As in the case of heat inactivated thromboplastic suspensions, loss in clotting potency after ultrasonic treatment is not reflected by a concomitant loss in lethal potency for rabbits. In addition, the data indicate that there are at least 2 active thromboplastic moieties in tissue suspensions. By clotting measurements, the TPase-treated thromboplastin used in these studies was almost identical to that of the ultrasonically treated suspension yet the former was no longer toxic to rabbits while the latter had lost none of its toxicity. Quick (5,6) has presented evidence suggesting that brain thromboplastic extracts have a heat labile factor which acts directly with prothrombin, and a heat stable factor requiring a plasma factor to produce active thromboplastin. Whether the TPase labile factor is the same as, or distinct from, the heat stable factor is not known. However, the former appears to be important in producing the lethal effects seen upon injection of thromboplastin into experimental animals. In addition, it is apparent that clotting potency *per se,* as measured by *in vitro* clot acceleration, gives no indication of the physiological activity of tissue thromboplastins. The amount of phosphorus liberated by TPase under standard conditions appears to be a much better criterion.

Summary. The effects of ultrasonic treatment on human brain thromboplastic suspensions have been presented. Although causing a loss in clotting potency, ultrasonic treatment does not affect the toxicity of intravenous tissue thromboplastin nor has it destroyed the TPase-labile component. The results are discussed and possible implications presented.

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Method of Chorioallantoic Membrane Inoculation which Decreases Nonspecific Lesions. (23056)

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Since the original method of chorioallantoic membrane (CAM) inoculation was introduced by Goodpasture(1), numerous variations for preparing eggs for this route of inoculation have followed(2-5). The preparation of embryonated eggs for CAM inoculation with pock inducing viruses is of considerable importance if accurate counts of the pocks are to be made(5-7). Nonspecific lesions due to trauma invariably appear when eggs are prepared for CAM inoculation. They may be large enough to obscure the field to be counted or small enough to be mistaken for specific lesions. Their presence can be a source of variation in pock enumeration and differentiation and can influence the accuracy of pock titrations.

The purpose of this paper is to present a modification of the technic of CAM inoculation which greatly reduces nonspecific lesions. A statistical assessment of the accuracy of this method is reported.

Materials and methods. Virus and seed preparations. The virus used in this study