Antithromboplastic Activity of Monosodium Alpha Tocopherol Phosphate and Inositol Phosphatide.* (17714)

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Thomas(1) showed that intravenous injections of thromboplastin, a sedimentable tissue component, into mice caused a characteristic response consisting of ataxia, convulsions and coma. The easy reproduction of these symptoms with small, relatively constant quantities of thromboplastin provided a more or less quantitative method for titrating the thromboplastic activity of these suspensions. Thomas further showed that this response could be prevented by both heparin and congo red. This paper deals with the inhibition of thromboplastin by two other substances, monosodium alpha tocopherol phosphate† and inositol phosphatide‡. Overman(2) reported the antithromboplastic action of inositol phosphatide on the basis of *in vitro* work. No similar studies seem to have been carried out with the alpha tocopherol preparation.

Method. These experiments were carried out on mice, each weighing from 12 to 14 g. Mixed brain and lung thromboplastin§ was standardized by the method of Thomas(1) as follows: It was diluted with distilled water to make up concentrations varying from 1:10 to 1:50. Each solution was incubated for 60 minutes at 37.5°F and was injected into the tail vein of a mouse, which was observed for the characteristic response of ataxia, convulsions and coma. The minimum effective dose which produced this response was 0.2 ml of thromboplastin in 1:30 dilution. Alpha tocopherol was then added to a 1:20 thromboplastin solution in amounts varying from 7.5 to 100 γ. Immediate injection of the solution into the tail veins of mice in 0.2 ml aliquots was ineffective in inhibiting the characteristic response to thromboplastin. When, however, the solution was incubated for 60 minutes at 37.5°C and was similarly injected, it was found that 15 γ of alpha tocopherol phosphate was effective for this purpose, while 7.5 γ per 0.2 ml aliquot lacked sufficient antithromboplastic activity to prevent the reaction.

Results. Similar experiments with inositol phosphatide showed (1) that without incubation the test solution had no inhibitive effect and (2) that 36 γ of the substance per 2 ml aliquot was capable of inhibiting the response of a mouse to a 1:20 dilution of thromboplastin when the same aliquot was used. At 18 γ no antithromboplastic activity was observed. Since it had been shown that both monosodium alpha tocopherol phosphate and inositol phosphatide were antithromboplastic in the concentrations used in this experiment after the solutions had been incubated, another experiment was done to determine whether the antithromboplastic action would occur if the test solutions were injected into the mouse prior to the injection of a 0.2 ml aliquot of the minimum effective dose of thromboplastin. After 15 to 100 γ of monosodium alpha tocopherol phosphate per 0.2 ml aliquot had been injected intravenously into the mouse, either 5 or 30 minutes were permitted to elapse before 0.2 ml of the 1:20 thromboplastin dilution was injected into the same mouse. All of the mice showed the characteristic response to thromboplastin, no antithromboplastic activity being demonstrated in any of them. At 1,000 γ inhibition of thromboplastin occurred after a 5 and a 30 minute interval.

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1. Thomas, L., *Bull. Johns Hopkins Hosp.*, 1947, v81, 1.

† Supplied by Mr. Rudolph Frundt of the Warren-Teed Products Co., Inc.

‡ Supplied by Dr. George Mast of C.S.C. Pharmaceuticals.

2. Overman, A. S., and Wright, I. S., *J. Biol. Chem.*, 1948, v174.

§ Sol U Plastin supplied by Mr. Blanchard of Schafflin and Co.

TABLE I.
Effect of Alpha Tocopherol Phosphate and Inositol Phosphatide on the Reaction to Thromboplastin in Mice.

Thromboplastin dilution	Diluent							
	Distilled H_2O	$aTPO_4$ 7.5 γ per 0.2 ml	$aTPO_4$ 15 γ per 0.2 ml	$aTPO_4$ 15 γ/0.2 ml Fibrinogen 50 mg/0.2 ml	Inositol phosphatide 18 γ/0.2 ml	Inositol phosphatide 36 γ/0.2 ml	Inositol phosphatide 36 γ/0.2 ml Fibrinogen 50 mg/0.2 ml
1/40	0 0 0 0 0 0						
1/30	+ + + + + +						
1/20	+ + + + + +	+ + +	0 0 0	+ + +	+ + +	0 0 0	+ + +

* 0, no reaction. +, positive reaction. Symbols indicate number of mice used in each test.

Similar studies were carried out with inositol phosphatide in 36 γ amounts per 0.2 ml aliquot. No antithromboplastic activity could be observed. When, however, the same experiment was repeated with injections of 72 γ, antithromboplastic activity was observed at both the 5- and the 30-minute interval following the injection of the inositol phosphatide. The clotting time of the blood, which had not been affected when monosodium alpha tocopherol phosphate was used, was raised to 9 minutes or higher in the mice tested. Finally since fibrinogen‖ is known to block the antithrombic action of monosodium alpha tocopherol phosphate, its effect on the antithromboplastic activity of this substance and of inositol phosphatide was tested.

The control group of mice used in this experiment were tested with injections of 100 γ of alpha tocopherol per 0.2 ml incubated with thromboplastin in 1:20 dilution. None of the animals showed the typical reaction to thromboplastin. When, however, the same solution was tested in mice with the addition of 50 mg of fibrinogen per 0.2 ml aliquot, the typical reaction did occur. The same experiment was carried out with inositol phosphatide in concentration of 36 and 72 γ, respectively. With each concentration the addition of fibrinogen blocked the protective effect of the inositol phosphatide solution. The end points in these experiments were checked, using at least 3 mice for each test. A 100% effect was noted with each concentration of solution, as recommended by Thomas (Table I).

Discussion. Since it has been suggested that the activity of thromboplastin may be the result of its effect on surface tension, it was postulated that the antithromboplastic effect of the substances tested in these experiments might result from their ability to reduce surface tension. Studies done by Mr. Rudolph Frundt of Warren-Teed Products Co. showed a value of 33 dynes cm^2 for a 10 mg/ml solution of monosodium salt of alpha tocopherol phosphate and a value of 45.4 dynes cm^2 for a 3.571 mg/cc solution

‖ Fraction I from bovine plasma, purchased from Armour & Co., was used.

of inositol phosphatide, compared with a value of 72.8 dynes cm^2 for distilled water.

These observations suggest the possibility (1) that thromboplastin presents a surface which can adsorb monosodium alpha tocopherol phosphate and inositol phosphatide, (2) that the utilization of this surface by these surface-acting agents explains why their injection into mice in a solution of thromboplastin prevents the typical reaction which normally occurs in these animals following the injection of thromboplastin alone, and (3) that the addition of fibrinogen to the solution interferes with this protective action because of the surface presented by fibrinogen molecules.

Summary. Experimental studies on mice showed that the typical reaction of these animals to the injection of thromboplastin can be prevented if monosodium alpha tocopherol phosphate or inositol phosphatide is incubated with the thromboplastin before it is injected. When fibrinogen is added to the test solution, the inhibitory effect of each of these substances is lost. It is possible that alterations in surface tension may explain these phenomena.

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Use of Maxted's Method for Group Classification of Hemolytic Streptococci.*† (17715)

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Maxted(1) has recently described a method for grouping beta-hemolytic streptococci in which the streptococci are lysed by a substance, presumably an enzyme, produced by a strain of *Streptomyces albus*. Such lysates contain the streptococcal C substance and can be used as antigens in the precipitin reaction for group classification. The simplicity, reliability and usefulness of Maxted's technique have been confirmed in a study of 1,010 consecutive strains of streptococci.

Methods. The strain of *Streptomyces albus*, obtained from Maxted, was grown on the following medium which is a modification of that described previously(1).

Bacto-peptone (Difco)	0.5 g
Yeast extract, desiccated	0.6 "
Dibasic sodium phosphate, hydrous	0.2 "
Glucose	0.2 "
Fildes' extract(2)	2.5 ml
Agar	1.25 g
Distilled water	100.00 ml

These constituents, with the exception of the Fildes' extract, were mixed and dispensed into a 1000 ml Roux bottle. After sterilization at 120°C for 20 minutes, the bottle was cooled to about 40°C, the Fildes' extract added, and the agar medium allowed to gel with the bottle in the horizontal position. The surface was inoculated evenly with 1 ml of a heavy suspension of *Streptomyces albus* spores in 0.85% sodium chloride solution. After incubation at 35°C for 5 days, the culture was frozen at −10°C for 12 hours and then allowed to thaw at room temperature. This procedure separated the agar gel of the

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1. Maxted, W. R., *Lancet*, 1948, v255, 255.

2. Fildes, P., *Brit. J. Exp. Path.*, 1920, v1, 129.