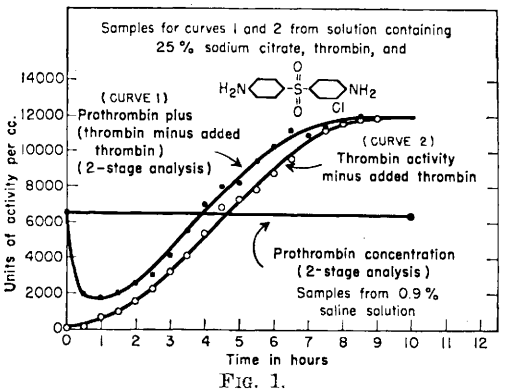

FIG. 1.

The purified prothrombin had lost 50% of its activity. A sample was dissolved in 25% sodium citrate solution. To this was added 400 units of thrombin per cc of solution and enough 3-chloro-4,4'-diaminodiphenyl sulfone to saturate the solution with respect to the sulfone. From this solution samples were taken for 2-stage prothrombin analysis and also for thrombin analysis.

and the other half in the sodium citrate solution within 30 minutes. Presumably the manner in which the prothrombin became inert did not influence the rate of transformation to thrombin in sodium citrate solutions. The question arises whether there were two kinds of altered prothrombin being activated simultaneously; however, it cannot be answered with the data now available.

Discussion. The prothrombin derivative produced by mixing prothrombin and thrombin in aqueous solutions has a lower electrophoretic mobility than the original prothrombin(1). The altered prothrombin produced after freeze drying has electrophoretic properties identical with fully active prothrombin. It must, therefore, be concluded that this paper describes a new derivative of prothrombin which is different from the one produced through the action of thrombin. Both can, however, be activated to thrombin by autocatalysis in sodium citrate solution.

It is evident that one cannot expect to preserve prothrombin samples after they have been dried from the frozen state unless conditions can be found under which the activity will be retained. This is a major practical handicap because the freeze drying technic is convenient not only in the research laboratory but also in industry. Perhaps it is possible to dry prothrombin by using cold acetone. In quite a number of experiments it has been possible to do so without loss of activity. Furthermore, the acetone dried material seems to be stable; however, our experience covers a period of only one half year. It is, therefore, possible to say only that this procedure promises to be satisfactory.

Summary. Purified prothrombin can be dried from the frozen state without immediate loss of activity. Thereafter progressive loss of prothrombin activity occurs, characterized by refractivity to the action of calcium plus thromboplastin plus Ac-globulin. In about one year most of the prothrombin is altered and in addition some is insoluble in aqueous solution. Prothrombin altered as the result of the freeze drying technic can be activated autocatalytically in 25% sodium citrate solution. The altered prothrombin has essentially the same electrophoretic properties as purified prothrombin and represents a derivative of prothrombin not previously described.

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Effect of Dibenamin on the Vascular Response of Rabbits to Typhoid Endotoxin. (18163)

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Menten and Manning(1) observed that injections of *E. coli* and *S. paratyphi* induced hyperglycemia in animals. Zeckwer and Goodell(2) and later Evans and Zeckwer(3) confirmed these results and stated that hyperglycemia was due to the action of adrenalin.

Similar observations were reported by Boivin and Mesrobeanu(4).

We have shown(5) that intraperitoneal or intravenous injections of 1.5 to 3.0 ml of typhoid endotoxin, obtained by the Boivin method, killed rabbits weighing 2,000 to 2,500 g within a few hours, and that the initial symptom of intoxication was an intense peripheral vasoconstriction. This reaction commenced 5 to 15 minutes after the injection; it was followed by dyspnea, intense diarrhea, elevation of internal temperature, which lasted until the preagonal stage. If this phenomenon is due to the action of adrenalin or adrenaline-like substances, then it should be possible to modify or inhibit this vascular response by adrenolytic substances. Dibenamin (N-N dibenzyl-beta-chlorethylamine) was therefore selected for our study.†

Materials and methods. Albino rabbits weighing 2,000 to 2,500 g were used. The endotoxin was obtained from *Salmonella typhosa* by extraction of the organisms with trichloracetic acid (Boivin and Mesrobeanu)(6). Five- to eight-tenths ml of this preparation, per kg of body weight, was injected intravenously. Six to 10 mg of Dibenamin per kg of weight was diluted to 10.0 ml in physiological saline, injected slowly (2 to 3 minutes) intravenously. These injections were made from 30 to 180 minutes following the administration of the endotoxin.

The cutaneous circulation was observed by 3 different technics: (1) observation of the small vessels in the ear by means of Clark's "preformed tissue" chamber; (2) direct observation of these vessels under the microscope(7); (3) an indirect method in which the temperature variations of the skin of the ear were taken with the aid of thermocouples. It has been established that these temperature variations are proportinal to the blood supply in the skin and therefore to the intensity of the vasoconstriction. This method permits a graphic recording of the results and serves as a comparison with the other two technics.

Results. Intravenous injection of 1 m.l.d. of endotoxin changed markedly the rhythm of contraction and distension of small arteries. A long phase of contraction ensued so the arterioles and metarterioles were completely obliterated for several minutes. This was followed by short periods of relaxation. The process continued until the terminal stage of the intoxications (Curve 1). The final outcome was not changed by the administration of Dibenamin, although the intensity of diarrhea was markedly decreased. On the other hand, if the injection of endotoxin was preceded by intravenous administration of Dibenamin, the vasoconstriction was greatly modified (Curve 2). After a short period of contraction, the normal rhythm was temporarily re-established.

When administered after the injection of endotoxin, Dibenamin rapidly suppressed the contraction (Curve 3). It has been shown that dienervated peripheral vessels are more sensitive to adrenalin or sympathin than the normal vessels(8); similarly we have demonstrated that dienervated vessels of the rabbit's ear reacted to the endotoxin with greater intensity than the normal vessels(9). This intense contraction of the dienervated vessels

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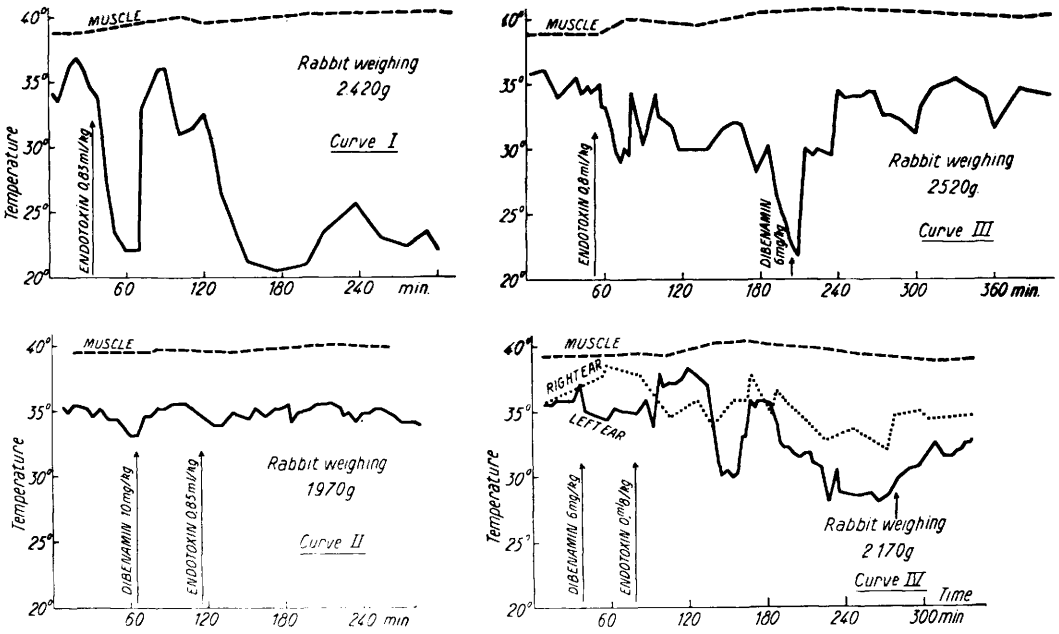

FIG. 1.

Effects of injections of dibenamin before and after the administration of typhoid endotoxin on the skin temperature of the rabbit's ear.

Curve 1—Skin temperature after an intravenous injection of typhoid endotoxin (1 m.l.d.).

Curve 2—Effect of an injection of dibenamin before the administration of the toxin.

Curve 3—Effect of an injection of dibenamin after the administration of the toxin.

Curve 4—Effect of 2 injections of dibenamin, one before and one after the administration of the toxin (the left ear has been dienervated four days before the experiment).

In the 4 curves, the internal temperature is indicated by a dotted line.

can be decreased by previous administration of Dibenamin. However, to maintain this effect, it is necessary to follow with another injection of Dibenamin (3 mg per kg) (Curve 4).

Summary and conclusions. The injection *S. typhosa* endotoxin appears to stimulate the liberation of adrenalin or adrenalin-like substances, bringing about peripheral vasoconstriction. Dibenamin, a sympatho-adrenal blocking substance, antagonizes this effect.

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Studies on the Effect of Different Carbohydrates on Chick Growth.* (18164)

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Nutritional differences among sucrose, dextrin, lactose, and cerelose have been reported by several workers. Elvehjem(1,2) has reviewed much of the work done on carbo-

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