tion in mast cells by cortisone and ACTH injections has been reported for other species (8).

The results of experiments reported here show that the extractable heparin level in rat skin is decreased by the injection of cortisone. Presumably this decreased level is due to an inhibition of heparin formation, since Layton (9) showed that cortisone inhibited mucopolysaccharide synthesis in the intact rat and Böström and Odeblad(10) have shown that cortisone decreases the rate of sulphate exchange in chondroitinsulphuric acid.

The chance finding that carbutamide augments the depressing action of cortisone on heparin level is of immediate interest. It is worth noting that Dr. D. W. Clarke of this department found, that compared to controls, the cortisone treated animals had significantly higher per cent liver fat and, that the animals treated with both cortisone and carbutamide had a higher per cent liver fat than those given cortisone only. An increase in liver fat is thus found under conditions which also produce a decreased heparin level in the skin. Further experiments will be required to determine if these are merely coincidental happenings or if they are indeed related.

Summary. The extractable heparin level in different rat tissues has been measured. The skin of the rat is relatively high in heparin but the content of the other tissues measured is low. The heparin extracted has a lower specific anticoagulant activity than either beef or dog heparin. The skin of rats receiving daily cortisone injections has a significantly lower heparin level than that of controls. Injection of Carbutamide (BZ-55) alone into rats does not affect the extractable heparin level but it greatly augments the depressing action of cortisone.

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Received May 2, 1957. P.S.E.B.M., 1957, v95.

Effect of Intravenous Soy Bean Phosphatides on Blood Coagulation in Rabbits.* (23263)

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Inosithin® is a commercial preparation of alcohol-insoluble phosphatides derived from soy beans. This material has coagulant properties resembling those of platelets, and it functions as an effective platelet substitute in the thromboplastin generation test and in the partial thromboplastin time.† Moreover, like platelets, Inosithin® is anticoagulant *in vitro* when concentrations above the optimal range are used(1,2). The following study was performed to determine the *in vivo* effects of Inosithin® on blood coagulation.

Methods and materials. Experimental subjects were male rabbits weighing between 2.5 and 3.5 kg. Inosithin® was administered as a 5% emulsion suspended in 5% glucose solution.‡ This emulsion gave optimal activity

* This study was supported by USPHS grant of Natl. Heart Inst.

† Partial thromboplastin studies were performed by Dr. John Graham of Univ. of North Carolina.

‡ Inosithin was kindly provided by Mr. J. Eichberg of Associated Concentrates, Woodside, L. I., and prepared as a sterile emulsion by Don Baxter, Inc., Glendale, Calif.

in the thromboplastin generation test when diluted 1:100. Infusions were given into the marginal ear vein at a rate of about 5 cc per minute. Blood was collected from the central artery of the ear using 20 gauge needles, and into untreated glassware. When plasma was

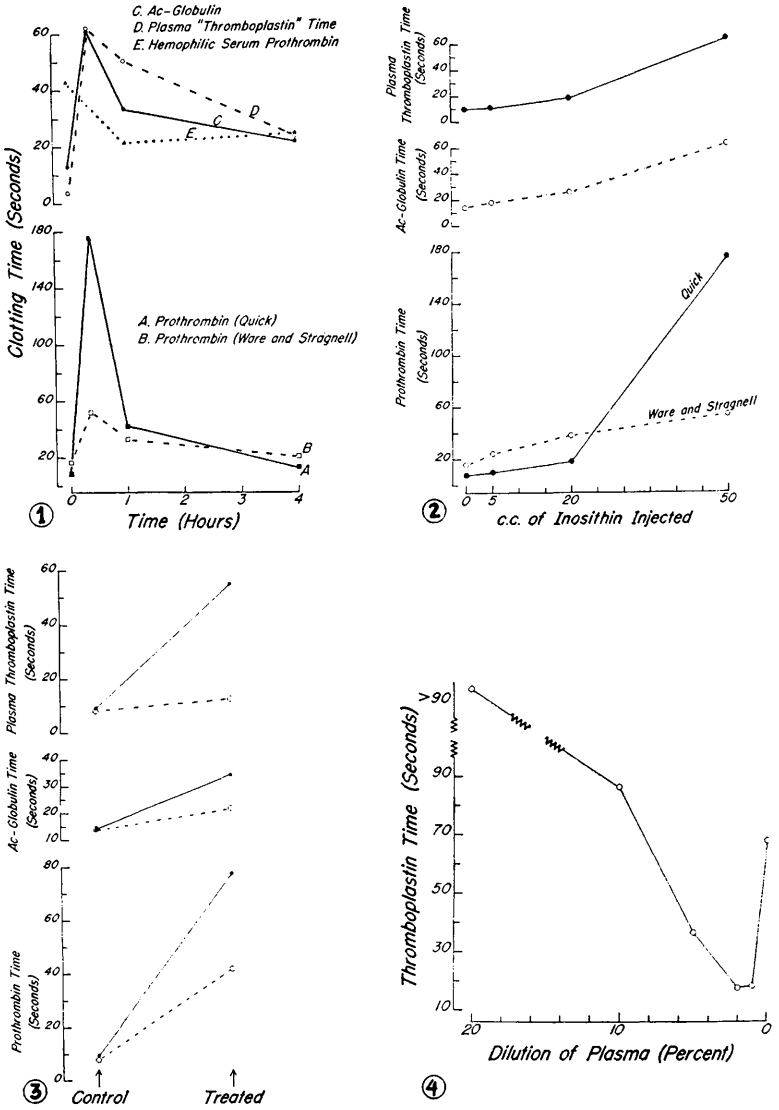

FIG. 1. Serial clotting changes in a 3 kg rabbit following 50 cc of Inosithin® given intrav. Curves A, B, and C are self-explanatory. Curve D represents clotting time in thromboplastin generation test after 6 min. of incubation, with animal's plasma used as source of AHF. Curve E is serum prothrombin time of hemophilic blood to which had been added test plasma in final conc. of 1%.

FIG. 2. Coagulation changes in rabbits given varying doses of Inosithin® intrav. See legend of Fig. 1 for explanation of terms.

FIG. 3. Comparison between *in vitro* and *in vivo* effects of Inosithin® on coagulation in rabbits. Solid line shows effect of 50 cc dose into a 3.5 kg rabbit 15 min. after completion of inj. Broken line shows effect of mixing normal rabbit plasma with ¼ volume of Inosithin® emulsion.

FIG. 4. Use of post-inj. plasma as platelet reagent in thromboplastin generation test. Plasma was taken from blood drawn 15 min. after a 50 cc dose of intrav. Inosithin®. The times shown are those obtained with normal plasma and serum reagents, and following 6 min. of incubation of the generating mixture.

required, a syringe was used containing anticoagulant; blood for serum was allowed to drip directly into test tubes. Plasma was obtained from blood anticoagulated with one-tenth volume of 0.1 molar potassium oxalate. Coagulation procedures were performed according to methods previously described(3,4).

Results. When Inosithin® was given to the rabbits intravenously in doses up to 50 cc. no visible toxic effects were evident. Blood specimens drawn within 2 hours after injection of the maximal dose showed greatly prolonged whole blood clotting times in glass, and in lusteroid tubes clotting failed to occur even after 8 hours in some samples. In the many animals tested the coagulation response was qualitatively uniform, although there was a moderate variability in degree of coagulation changes.

Fig. 1 shows a representative study of serial changes following a 50 cc dose of Inosithin®. There was rapid and profound depression of prothrombin, Ac globulin, and AHF. These changes were most pronounced in blood samples drawn shortly after completion of the injection, and gradual restoration toward normal occurred in the subsequent 4 hours. No marked or consistent abnormality was encountered when serum from injected animals was used in the thromboplastin generation test, indicating no significant depression of plasma thromboplastin component (PTC), Factor X, or Stuart Factor. Likewise, there was no change in the plasma fibrinogen.

The degree of clotting abnormality developing in injected animals was dose-dependent. Fig. 2 shows the effect of varying the volume of injected Inosithin® from 5 to 50 cc. In each case blood specimens were collected 15 minutes after completion of the injection. Changes were relatively minor until a dose of 20 cc was reached despite the enormous amount of coagulation-active lipid transfused. Of additional interest is the greater prolongation of the Quick prothrombin as compared to the Ware and Stragnell(5) test, at maximal dose levels. This discrepancy is partly due to the provision in the Ware and Stragnell reagent of excess Ac globulin. However, as will be shown, this is not the entire explanation.

At least part of the Inosithin® effect in the animals was independent of its direct action on the blood, for the changes induced *in vivo* differed from those resulting from *in vitro* mixing. As shown in Fig. 3, the addition of ¼ volume of Inosithin® emulsion to pre-injection rabbit plasma had a significantly lesser effect on the Quick prothrombin and Ac globulin than an *in vivo* dose of 50 cc. Moreover, the *in vitro* Inosithin® had little effect on activity of plasma in the thromboplastin generation test, indicating no direct depression of AHF. The blood Inosithin® level of the injected rabbit could have been no greater than that achieved by *in vitro* mixing, for the plasma volume was approximately 180 cc. Actually, because of some lipid clearing from the blood of the injected animal during injection and prior to collection of specimens, the Inosithin® level was probably appreciably lower than in the specimen mixed *in vitro*.

Anticoagulant activity was present in the blood of rabbits injected with the 50 cc dose of Inosithin®. Table I shows that plasma drawn 15 minutes following this dose of the lipid prolonged the prothrombin time of pre-injection plasma when these plasmas were mixed in equal amounts. This prolongation was considerably greater than that produced by simple dilution of the pre-injection plasma with isotonic saline. The anticoagulant effect was not the result of antithrombin, for the thrombin times of plasma from injected animals were identical to that of their pre-injection sample.

Coagulant activity resembling that of Inosithin® could be demonstrated in the plasma of injected rabbits. The plasma of blood drawn 15 minutes after completion of a 50 cc dose was an effective platelet substitute in the thromboplastin generation test. As shown in

TABLE I. Anticoagulant Action of Post-Injection Rabbit Plasma.

	Quick prothrombin time (sec.)
Control plasma	9.0
Idem + saline	11.4
Post-injection plasma	360
Idem + control plasma	42.4

Fig. 4, such plasma after barium sulfate adsorption effected normal thromboplastin generation when appropriately diluted, but was anticoagulant in excessive concentration. Plasma from untreated controls had no such platelet-like activity.

Discussion. The data presented above show that soy bean phosphatides with platelet-like coagulant activity have profound effects on blood coagulation when given intravenously. The findings indicate that clotting is depressed as a result of two mechanisms. The first of these is a direct anticoagulant action of highly concentrated phosphatides, shown by the ability of post-injection plasma to interfere with clotting in normal plasma. However, the greater effect of *in vivo* phosphatides over that produced by comparable concentrations added *in vitro* suggests a second mechanism: a partial conversion of prothrombin into thrombin. Evidence in favor of this view is seen in the combination of prothrombin, Ac globulin, and AHF depression together with intact levels of those clotting factors not consumed in the course of clotting. In other words, the blood of injected animals showed many properties of serum.

It is of considerable interest that the above effects were produced only by massive doses of phosphatides, and that the "small" doses had little demonstrable action. Even the 5 cc dose of Inositbin® possessed activity equalling that of platelets from an entire blood volume of the experimental subjects. Despite the intimate mixture of all elements necessary for clotting, generalized coagulation was not precipitated nor was there a demonstrable change in fibrinogen even following the largest doses. Such findings with injected platelet products have been interpreted to mean that platelets do not initiate blood clotting under physiological conditions(5). However, an alternative hypothesis might be that intermediate products of blood coagulation are cleared from the blood as it circulates, possibly by some cellular mechanism. This would account for our observed prothrombin conversion without fibrinogen depression, and for the apparent inability of smaller amounts of Inositbin® to cause extensive blood thromboplastin formation.

Summary. Emulsions of soy bean phosphatides with platelet-like coagulant activity were given intravenously to rabbits. Small doses were followed by little change in blood coagulation, but larger doses produced depression of prothrombin, Ac globulin, and AHF. Fibrinogen and the "serum factors" were unaffected. The observed changes resulted from direct anticoagulant activity, and probably also from partial conversion of prothrombin.

The authors wish to thank Dr. Bernard A. Sachs and Miss Ethel Danielson whose valuable suggestions contributed to this study.

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