TABLE III. Insulin Requirement of Depancreatized Cats.

Group*	Dietary supplement	Avg values: Meat per day, g	Insulin/day, units	Glycosuria/day, g	Initial wt, kg
A	Raw pancreas	182	9.5	3	2.3
B	Viokase	180	15.4	8.7	2.7

* A—based on 6 metabolic periods of 3 to 5 days each in 5 cats; B—based on 7 metabolic periods in 4 cats.

cited(2,3) no mention is made of the effect of any replacement treatment on the insulin requirement of depancreatized dogs. One would expect that improved digestion might influence the need for insulin and the data in Table III suggests that this is so. In Table III, group A consists of animals prepared 10 years ago when raw pancreas (50 g daily) was part of the diet. Group B is composed of animals on Viokase, some of which were not studied for fat excretion and received diets of 200 g. The average diet and body weight for each group is similar. The cats given Viokase received almost twice the amount of insulin used in group A, but in spite of this excreted nearly 3 times as much glucose in the urine. The dose of insulin varied widely in different cats (6 to 24 units per cat in the past few years in this laboratory). For this reason one must be guarded in the interpretation of such averages. Nevertheless, the number of animals and of metabolic periods suggest that there is a true and possibly striking effect of Viokase on the insulin requirement. When one surveys the reports on impaired pancreatic digestion and the relative inadequacy of available substitutes, it is not surprising that the depancreatized animal(4) and man(5) require less insulin than their counterparts (pituitary or alloxan diabetes(6,4) and clinical diabetes) in which an essentially normal external secretion is present.

The general usefulness of Viokase in depancreatized cats may be summarized. It is more convenient, and probably cheaper, to add a stable powder to the diet than to procure fresh pancreas. The steatorrhea and loss of body weight which follow pancreatectomy are perhaps better controlled than with raw pancreas. In general, Viokase has kept depancreatized cats in satisfactory condition. The effect on insulin requirement, which may be regarded as an index of improved digestion, suggests that this pancreatic preparation deserves clinical trial, especially when faulty absorption of protein is present.

Summary. A pancreatic preparation (Viokase) has been used to substitute for the external secretion in a series of depancreatized cats. It had no effect on fat absorption but diminished the bulk of the stools, probably by improving the digestion of protein. The improved digestion was accompanied by an increased requirement for insulin.

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Detoxification of Alloxan by Borate.* (18844)

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It has recently been reported(1) that normal subjects excrete an alloxanic acid-like compound which on hydrolysis yields oxo-

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1. Seligson, D., Seligson, H., Shapiro, B., Paley, R. G., Riaboff, T. and Lukens, F. D. W., *Fed. Proc.*, 1951, v10, 124.

malonic acid. This in turn suggests that alloxan may be an intermediate metabolic product in man. If calculated as alloxan, the oxomalonic acid which was isolated would represent a urinary excretion of about 100 mg per day. If urinary oxomalonate arises from alloxan, which is a likely possibility, then it is desirable to learn more of the biochemical reactions of alloxan. It is the purpose of this report to show that borate detoxifies alloxan in a hitherto unrecognized manner. Rose and Gyorgy(2) have reported that boric acid injected into rats prior to alloxan prevents the development of alloxan diabetes, but they did not study the chemical reactions involved.

Seligson and Seligson(3) found that alloxan in neutral buffers is converted to alloxanic acid and that the reaction was catalyzed

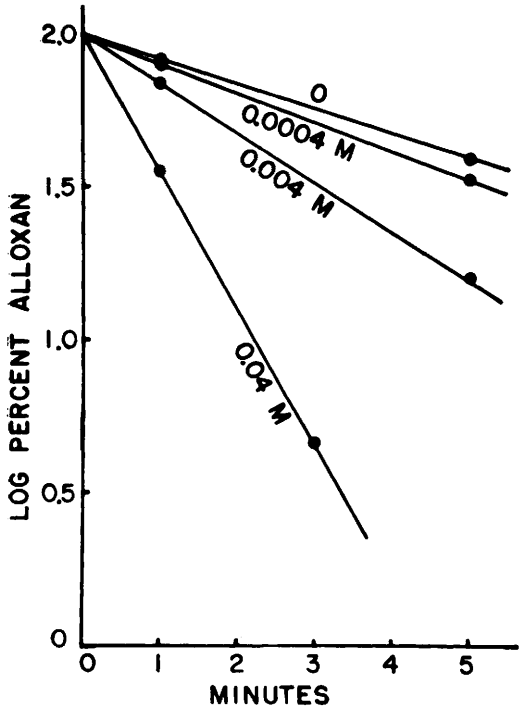

FIG. 1. Curves represent the log of the per cent of added alloxan remaining in a 0.1 M phosphate reaction mixture during the course of the reaction. Figures adjacent to each curve show the concentration of added borate in the reaction mixture. The pH at 5 min was 7.1 for each mixture.

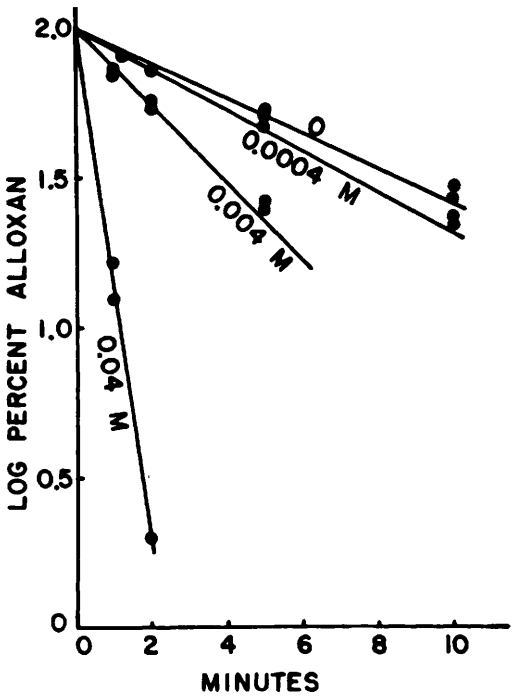

FIG. 2. Curves represent the log of the per cent of added alloxan remaining in a reaction mixture containing plasma and varying amounts of borate. Reaction mixtures at 5 min were pH 7.4.

by hydroxyl ions. The conversion was a rapid first order reaction with alloxan being half decomposed in 2 to 4 minutes depending upon temperature and pH (Fig. 1, curve O). Alloxan in plasma behaved similarly, half the alloxan having disappeared in 5 minutes (Fig. 2, curve O).

Alloxanic acid contains a free carboxyl group which is readily and stoichiometrically oxidized by ceric sulfate yielding parabanic acid and 1 molecule of carbon dioxide. Alloxan is not so oxidized. Thus, measurement of the ceric sulfate consumed or CO_2 liberated provided a method for following the kinetics of the conversion of alloxan to alloxanic acid. A microdiffusion method(4) was used for measuring the liberated carbon dioxide.

Procedure. Phosphate buffer‡ 5 parts water 4 parts and alloxan‡ 1 part, were rapidly mixed

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‡ The phosphate buffer solution was 0.2 M and its pH was 7.49. The alloxan solution was 0.01 M in 0.001 N HCl.

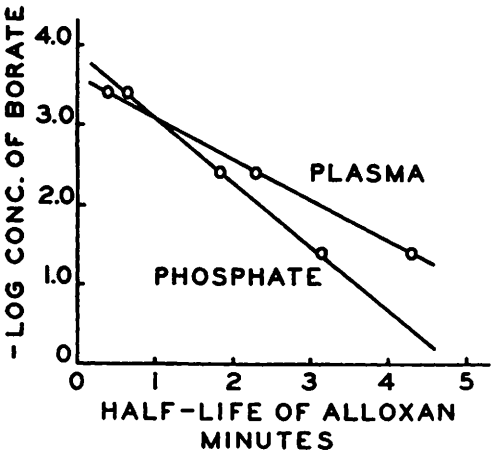


FIG. 3. Log of the concentration of borate plotted against the half life of alloxan added to a phosphate buffer or plasma follows a straight line function.

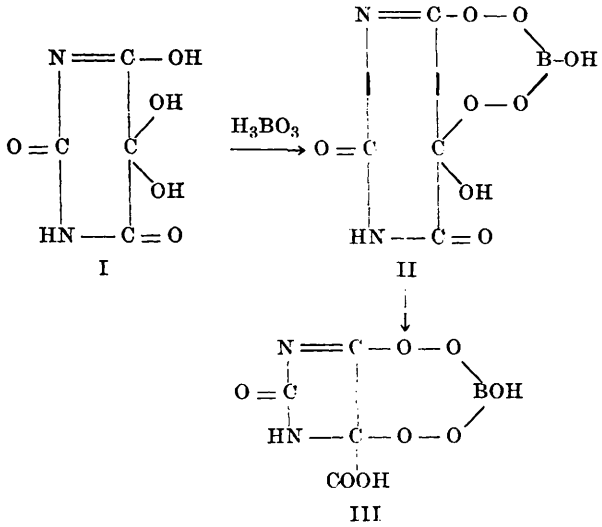
and periodically 2 ml of reaction mixture was rapidly transferred to 3 ml of 6 N H_2SO_4. 0.5 ml was treated with 1 drop of ceric sulfate reagent (ceric sulfate, 0.3 N in 3 N H_2SO_4 containing sufficient osmium tetroxide to make the solution 0.00001 M) and the liberated carbon dioxide was measured by a microdiffusion procedure(4). A reagent blank was run for the CO_2 content of the system, using water instead of alloxan. In this manner the values for alloxan, shown in Fig. 1, were obtained. The effect of borate was measured by substituting dilutions of the borate buffer (0.1 M and pH 7.40) for the 4 ml of water above and thus making reaction mixtures 0.0004 M, 0.004 M, and 0.04 M with respect to borate.

Plasma was studied in the above systems by replacing the 5.00 ml of phosphate buffer with plasma diluted 1:2.

Results. The data shown in Fig. 1 and 2 show the remarkable accelerating effect of borate on the conversion of alloxan to alloxanic acid. Increasing concentrations of borate accelerate the reaction in a regular manner as shown in Fig. 3, where the log of the concentrations of borate plotted against the half life of alloxan gives straight lines in phosphate buffer and in plasma.

Discussion. The effect of borate in catalyzing the reaction may be through a Boeseken type ester as follows: At pH 7.4 alloxan forms an enol (I) which results in a cis-dehydroxy form and this is assumed to form a Boeseken type of ester (II) which quickly goes to the more stable alloxanic acid borate complex (III). Evidence for the latter is obtained by the greater difficulty in oxidizing alloxanic acid in the presence of borate and also the greater acidity of the borate alloxanate complex.

An explanation on the protective effect of boric acid(2) against the diabetogenic effect of alloxan in rats is supplied by the accelerating effect of borate on the conversion of toxic alloxan to non-toxic alloxanic acid(5). This



constitutes a previously unrecognized mechanism for the destruction of alloxan differing from those based on reduction as by glutathione(5), or condensation(5).

Conclusion. Borate greatly accelerates the conversion of alloxan to alloxanic acid. This observation provides an explanation for its protective effect against alloxan diabetes.

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Use of Desoxycorticosterone Acetate in Treatment of "Hyperheparinemia".* (18845)

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It has long been assumed that mast cells contain heparin in an active or inactive form(1). Recently, evidence has been presented(2) to show that, in response to certain hormonal products, chiefly of the stress-producing type, the morphologic pattern of the mast cells of mice and other animals are altered and, in the animals studied, the heparin content of the blood may increase. Of the hormones studied, only desoxycorticosterone acetate (DCA) was found to preserve the morphology intact and, in larger doses, prevented its alteration by subsequent administration of the stress-producing hormones(2).

Recently, an opportunity was afforded to study a case of idiopathic "hyperheparinemia"(3). Since this report, we have studied the effect of protamine sulphate and DCA on this patient's coagulation defect. The response to protamine therapy was excellent but, after a month, evidence of resistance to protamine appeared and he relapsed. Because of his subsequent failure to respond to protamine sulphate, it was decided to try the effect of large doses of DCA on this patient. The administration of 25 mg of DCA in oil daily by the intramuscular route resulted in a drop of the endogenous heparin from 23 γ per ml of plasma to 0 γ in 4 days (Table I).

During the course of observation, this patient, under control conditions, showed a clotting time (Lee-White) of 110-130 minutes, a prolonged one stage prothrombin time (Quick) of 75-90 seconds (normal 13.2 seconds), despite a normal prothrombin concentration by the modified 2-stage procedure of Ware and Seegers(4), and a prolonged recalcification time of oxalated plasma. Both the protamine titration test(5) and the protamine-heparin titration method(6) gave consistently abnormal values under control conditions. During therapy with DCA, the three other abnormal tests improved along with the drop in the protamine titration. A complete reversion to normal values occurred when the endogenous heparin had fallen to an insignificant amount in the plasma.

To rule out the possibility of a spontaneous remission, treatment was discontinued for 72 hours following which hyperheparinemia recurred. On the readministration of 90 mg of DCA in a period of 4 days, the heparin level of the blood again fell from 23 to 2 γ per ml of plasma. This observation suggests the possible use of this therapy in the hemorrhagic

* Aided in part by a grant from Eli Lilly and Co., Indianapolis.

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