fall of blood pressure in each instance, being only temporary in the first 3 instances, while continuing until death of the animal in the last instance. In 2 other animals a portion of the liver was excised (using an electro-surgical knife to obviate bleeding*) before and after the trypsin injection. The liver samples were assayed for their histamine content by the method of Best and McHenry. In one experiment the liver content of histamine decreased from a normal value of 66 γ histamine base per gram to a value of 33 indicating a liberation of 10 mg for the whole liver, and in the other experiment there was a decrease from 50 γ per gram to 17 indicating a liberation of 6 mg from the entire liver.

It is thus clear that trypsin, like proteoses and certain snake venoms has the ability to liberate histamine from the tissues of the intact animal. Enough experiments have not as yet been done to determine whether the toxicity of trypsin can be exclusively related to this property.

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Possible Physiologic Significance of the Zinc Content of Insulin.

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It is known that the effectiveness of insulin in lowering the blood sugar level is dependent upon the integrity of the S-S groups which form part of its protein structure.[1] This suggested the possibility that insulin might exert its physiologic action through the influence of its S-S groups upon such tissue enzymes as are dependent upon S-H groups for their activity.[2-5] We therefore studied the *in vitro* effect of insulin upon succino-oxidase activity. A significant effect was found. But, it soon became apparent that this *in vitro* action

* We are indebted to Dr. Robert S. Hardwick for the surgical operations in these experiments.

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TABLE I.
Effect of Crystalline Insulin on Succino-Oxidase Activity *in vitro*.

Amount of insulin added per vessel	μl O_2 uptake in			% inhibition in 60 min
	20 min	40 min	60 min	
0	131	218	276	—
0.27 mg (6 units)	99	182	220	20.3
0.54 mg (12 units)	77	124	165	40.0

All vessels contained: 1 ml enzyme solution, 0.4 ml M/10 succinate and M/15 phosphate buffer (pH 7.4) to make a total volume of 3 ml. Gas phase; air.

of insulin did not depend upon its S-S groups, but rather upon its zinc content.

Methods and Results. An active succino-oxidase preparation was obtained from minced, fresh beef heart. Its activity was determined by measuring the oxygen consumption in the presence of excess succinate in the Warburg apparatus (38°C; gas phase—air; pH 7.4).

Table I presents a typical experiment of a large number which consistently showed a significant inhibition of the succino-oxidase system by crystalline insulin.†

To test whether this action of insulin ran parallel with its hypoglycemic effects *in vivo*, experiments were repeated using a sample of crystalline insulin which had been previously inactivated as regards its *in vivo* effects by boiling in dilute alkaline solution. Table II shows that this "inactivated" insulin still retained its full inhibitory action on the succino-oxidase system.

In view of these results further experiments were performed to see whether the *in vitro* activity of insulin depended upon its content of zinc. Accordingly we compared the inhibitory effects of our crystalline insulin (zinc content 0.47%) with that of amorphous insulin† of equal *in vivo* potency (zinc content 0.18%). It may be seen from Table II that the amorphous insulin containing less zinc, exerted no significant inhibitory influence. Furthermore, zinc sul-

TABLE II.
Effect of Zinc, in Insulin and Alone, on Succino-Oxidase Activity *in vitro*.

Additions	μl O_2 uptake in 60 min	% inhibition
0	258	—
0.54 mg crystalline insulin (2.5 γ Zn)	200	24
0.54 mg crystalline insulin "inactivated"	188	27
0.54 mg amorphous insulin (0.97 γ Zn)	255	1
Zinc sulphate solution containiing 2.5 γ Zn	180	30

Contents of vessels before additions as in Table I.

† We are indebted to Dr. Rhodehamel and the Eli Lilly Company, for supplies of pure crystalline and amorphous insulin.

phate in a concentration equivalent to the zinc content of the amount of crystalline insulin which we employed (*circa* 1×10^{-5} M) caused a similar inhibition in the absence of insulin.

Discussion. The *in vitro* inhibition of succino-oxidase activity by insulin is due solely to the zinc content of the insulin sample, and can be duplicated by equivalent amounts of the zinc ion. The possible physiologic significance of these results depends upon whether zinc is an integral part of the insulin molecule as secreted by the pancreas and, if so, whether the zinc moiety exerts a similar effect *in vivo*. The work of Scott and Fischer[6] and of Cohn, *et al.*,[7] indicates that zinc is very closely associated with the insulin molecule. Crystalline insulin has a constant percentage content of zinc, which is not decreased by repeated recrystallizations, and which cannot be separated from the protein by electrodialysis. However, even if it may be assumed, for the time being, that zinc is an integral part of the insulin molecule as secreted, it still remains to be shown that it is effective *in vivo*. Recent work has shown that a number of other enzyme systems may be influenced by very small concentrations of zinc *in vitro*.[8, 9] But evidence is lacking as regards similar *in vivo* actions of zinc, either when associated with insulin or by itself.

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Effect of NaCl and Desoxycorticosterone on Body Weight and Carbohydrate Stores of Adrenalectomized Rats.*

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It is well known that additional amounts of sodium chloride will enable adrenalectomized animals to survive a considerable length of time beyond the expected survival period following adrenalectomy. It has been found that adrenalectomized rats given 1% solution of

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