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The effect of ATP and certain trace elements on the induction of experimental diabetes

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With 4 figures and 3 tables

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Alloxan selectively destroys the B-cells of the pancreas (1). It has been shown by radio-autography that in the adult mouse the B-cells differ from other cells in being able to concentrate alloxan. By contrast the B-cells of young alloxan-resistant mice do not perform such a concentration (2).

Diabetes similar to that produced by alloxan was produced by intravenous administration of dithizone to rabbits (3). Histological examination of the pancreas showed that the extent of the glycaemic reaction induced by dithizone was dependent on the degree of damage of the islet tissues (4).

The diabetogenic effect of either alloxan or dithizone can be avoided in some species, if certain compounds are administered immediately before its administration (5, 6).

Weanling rats on a sodium-deficient diet are more susceptible to alloxan-induced diabetes than on a diet adequate in sodium. Desoxycorticosterone which increases the body sodium exerted an indirect protective effect against alloxan diabetes (7).

The protective effect of ATP on various organs has also been demonstrated by many authors (8, 9).

An attempt has been made in this work to study the protective effect of ATP and certain trace elements such as zinc, manganese, chromium, and cobalt on the diabetes induced by alloxan or dithizone.

Materials and methods

Sprague Dawley rats bred in the laboratory, weighing 200–250 g and fed stock diet ad libitum, were used throughout.

The experiments were performed on two sets of animals comprising alloxan- and dithizone-diabetic rats.

1. Alloxan-diabetic rats

In these experiments the rats were made diabetic by intraperitoneal injection of freshly prepared 5% aqueous solution of alloxan following a twenty four hours fast. The dose used was 150 mg/kg.

2. Dithizone-diabetic rats

Rats were rendered diabetic by the slow intravenous injection of freshly prepared dithizone solution in a dose of 200 mg/kg body weight. Dithizone was

dissolved in ammoniated absolute ethanol with constant stirring and gentle warming and then diluted fourfold with distilled water (10). The selected rats were fasted for 24 hours before injection. The rats became diabetic within 24 hours. Confirmation was obtained by removal of the pancreas, sectioning, and staining.

The rats in this study were divided into seven groups, control group comprised 15 normal rats. Diabetic group comprised 10 alloxanized and 10 dithizonized rats. Group 3, 4, 5, and 6 comprised each 20 rats injected intravenously with either zinc, manganese, chromium or cobaltchloride solution to a dose 1 mg of the salt/kg body weight immediately before and 1 mg fifteen minutes after alloxan and dithizone.

Group 7 in this group the effect of ATP on the blood glucose level and on the glycogen and fat contents of the liver in ten alloxan-diabetic female rats were investigated. ATP *(Richter)* was given by the intramuscular route of administration one hour before alloxan in the dose of 50 mg/kg body weight. Ten days after injection, the animals were killed by decapitation, and the liver quickly removed.

Before sacrificing the animals blood samples were aspirated from the apex of the heart. The blood was used for analysis of glucose. Plasma glucose was measured by *Nelson's* modification of *Somogyi's* procedure (11). Liver glycogen was hydrolysed to glucose according to the method of *Good, Kramer,* and *Somogyi* (12) and the resulting glucose was estimated by the previous method. The fat content of the liver was estimated by the method of *Folch* et al. (13).

Results and discussion

Several workers (3, 14, 15) have demonstrated that dithizone is a diabetogenic substance, and most of them explained its action by making the assumption that dithizone has a strong affinity for zinc and chelates it, even when present within living cells like the beta cells of the islets, and secondly that the zinc present in the beta cell is an integral part of its structure and functional activity, so without it the cell cannot live or at least cannot secrete insulin.

However alloxan effect has been stated to be in preventing the enzymatic synthesis of insulin as well as inhibition of its release (16).

In the present work it was found that the intravenous injection of zinc chloride (1 mg zinc chloride/kg body wt.) immediately before and fifteen minutes after dithizone or alloxan prevented the usual hyperglycaemia observed 24 hours after induction of diabetes.

There are two possible explanations, the first is that we are dealing with the resultant of two opposing changes hyperglycaemia induced by dithizone or alloxan on the one hand, and a hypoglycaemia induced by zinc salt on the other. In support of this possibility is the fact that *Prasad* (17) suggested that zinc seems to be essential for the utilization of glucose by various tissues, as well as the enhanced hypoglycaemic action of insulin when zinc is given with it (18). *Quarterman* (19) confirmed this statement by his observation that glucose uptake by rat epididymal fat pads was increased proportionally with the increase of zinc concentration.

The other possibility to explain the lack of subsequent hyperglycaemia 24 hours after dithizone or alloxan plus zinc, is that genuine protection of the islets occurred.

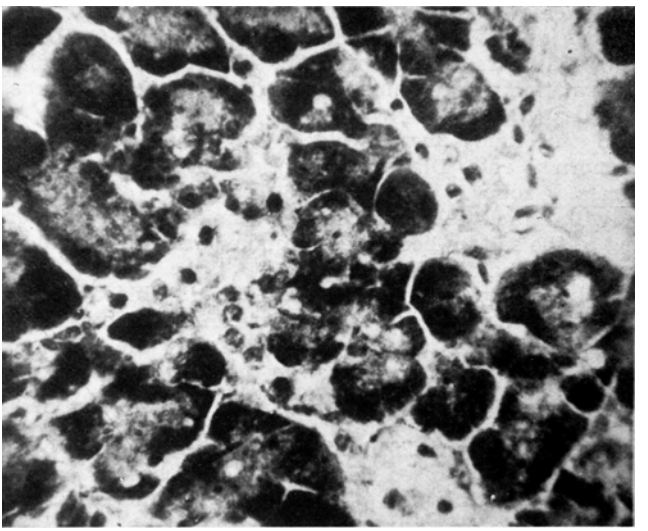

Fig. 1. Pancreatic islets of the rat, 48 hours after alloxan.

Histological examination of the pancreas verified the protective effect of zinc since on histological examination, the islets of those animals injected with zinc were intact and their beta-cells stained normally (fig. 1, 2, 3, and 4).

Intravenous injection of manganese chloride before and after dithizone or alloxan prevented also the expected rise of blood glucose, 24 hours

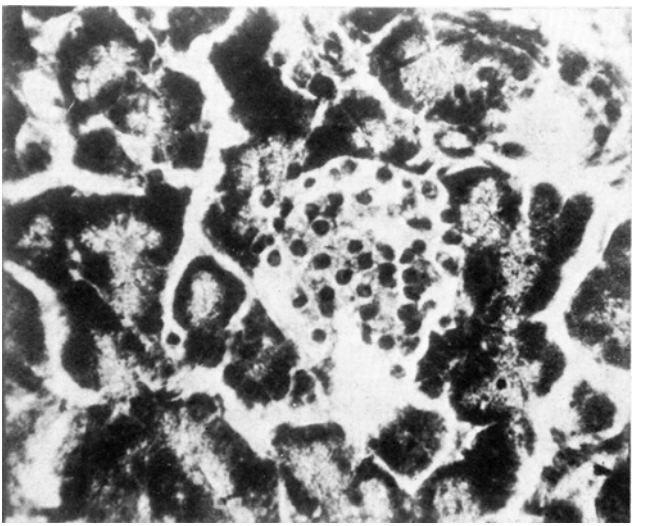

Fig. 2. Pancreatic islets of the rat, 24 hours after dithizone.

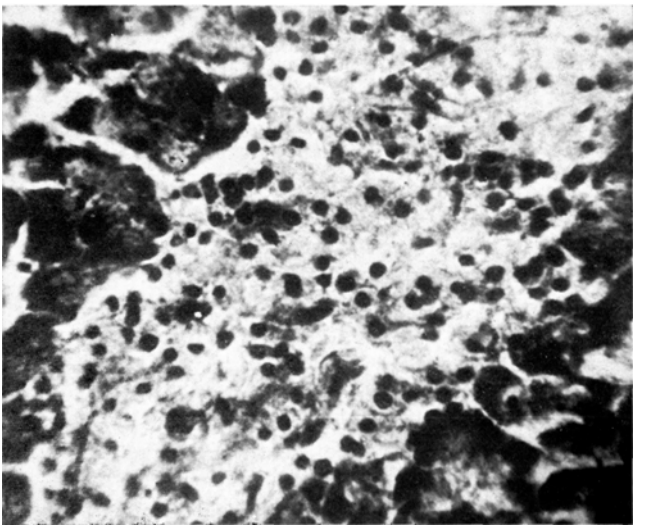

Fig. 3. Pancreatic islets of the rat, injected with zinc chloride immediately before and fifteen minutes after alloxan.

later. However histological examination of the islets, demonstrated that the injected manganese had not protected the islets from the toxic effects of dithizone or alloxan.

Thus in the case of manganese, unlike zinc, the explanation is probably to be found in the hypoglycaemic effect of manganese counteracting the hyperglycaemic effect of the destructed beta cells.

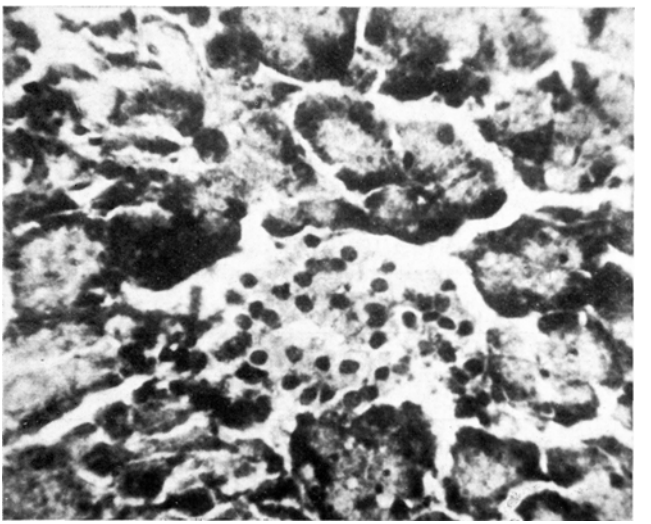

Fig. 4. Pancreatic islets of the rat, injected with zinc chloride immediately before and fifteen minutes after dithizone.

The hypoglycaemic effect of manganese has been reported by many workers (19, 20, 21). Oral manganese chloride was given to cases of diabetes mellitus, the drug produced a statistically significant fall in blood sugar. Increasing the dose, however, did not produce any further drop, while a single oral dose was not adequate to control the hyperglycaemia (22).

Concerning the possible mechanism of the hypoglycaemic effect, *Helmi* et al. (21) suggested that this effect is not totally dependent on the presence of the beta cells; since it occurred in both normal and alloxan diabetic rabbits, and in all types of diabetic patients.

Manganese may act peripherally on utilization of glucose, this is in agreement with its known role as a coenzyme for many reactions involved in carbohydrate metabolism (21).

It may act, however, through glucagon, inhibiting its release or interfering with its glycogenolytic action. Finally, it may accelerate the release of stored insulin from the beta cells. Perhaps all these mechanisms are at work (20).

It can also be postulated that manganese ion may be required as the other cations for insulin secretion (23, 30).

In case of intravenous injection of chromium chloride into rats, the blood sugar, 24 hours later, attained a mean level of 179.7 ± 27.3 mg% instead of 231.8 ± 79.9 mg% in case of dithizone diabetes and 269.5 ± 48.6 mg/100 ml instead of 451.9 ± 133.5 mg/100 ml in case of alloxan diabetes.

Histological examination revealed degeneration of the beta cells in the islets. Thus chromium unlike zinc did not protect the rat against the diabetogenic effect of dithizone or alloxan.

Mertz et al. (24) found that trace amounts of trivalent chromium are required for the maintenance of normal glucose tolerance. The amount of chromium needed by the tissues to prevent any impairment in physiological function is about 20 μg of Cr^{+3}/100 g of body wt. Such an amount is normally supplied by dietary constituents (25).

Chromium may be a cofactor for the insulin-cell membrane interaction. The membrane, presumably, can only fulfill its normal function when chromium is present at the reactive sites (26).

Table 1. Fasting blood glucose (mg/100 ml) in rats injected intravenously with zinc, manganese, chromium, and cobalt chloride immediately before and fifteen minutes after alloxan.

	Control	alloxan	Effect of intravenous injection of			
			zinc	manganese	chromium	cobalt
Mean	116.7	451.9	110.5	114.0	269.5	196.5
SD ±	18.8	133.4	11.8	15.6	48.6	33.9
n	(15)	(15)	(10)	(10)	(10)	(10)
P			.0005	.0005	.0025	.0005

Figures between parenthesis indicate number of animals.

Table 2. Fasting blood glucose (mg/100 ml) in rats injected intravenously with zinc, manganese, chromium, and cobalt chloride immediately before and fifteen minutes after dithizone.

	Control	dithizone	Effect of intravenous injection of			
			zinc	manganese	chromium	cobalt
Mean	116.7	231.8	111.7	120.0	179.7	174.8
SD ±	18.8	79.9	19.7	27.6	27.3	32.2
n	(15)	(15)	(10)	(10)	(10)	(10)
P			.0005	.0005	.05	.05

Figures between parenthesis indicate number of animals.

With regard to the intravenous injection of Cobalt chloride, the mean blood sugar level 24 hours later was 174.80 ± 32.29 mg% instead of 231.87 ± 29.92 mg/100 ml in case of dithizone diabetes and 196.5 ± 33.9 mg/100 ml instead of 451.9 ± 133.5 mg/100 ml in case of alloxan diabetes. Histological examination of the islets revealed destruction of the beta cells, indicating that cobalt did not protect the beta cells against toxic agents. The intravenous injection of cobalt chloride also causes a rapid selective injury to the alpha cells of the pancreatic islets (20, 21). It was found that intravenous injection of 25–50 mg/kg body weight caused a transitory hyperglycaemia. This may be due to liberation of pre-formed glucagon from the alpha cells destroyed by cobalt.

On the other hand, repeated subcutaneous injections tend to lower the blood sugar slightly (28). *Padmaker* et al. (29) suggested that cobalt ions enhance glucose uptake, its oxidation to CO_2, and its incorporation into fat pad lipids, thus stimulating the action of insulin.

Moreover, the ionic movements across the beta cell membrane are important in setting off insulin secretion by the beta cell and in maintaining it. It is possible that certain of these ions are more closely related to the different stages of the insulin-secretion process in the beta cell (23, 30).

In case of ATP, its administration one hour before alloxan in female rats prevented any marked rise in the blood glucose level in those animals rendered moderately diabetic by the injection of 150 mg alloxan/kg body wt. (table 3).

Table 3.

Experimental procedure	Time of sacrifice	Blood sugar mg/100 ml	g% of wet liver wt.	
			Fat	Glycogen
Controls (5)	—	105 ± 12	3.2 ± 0.6	1.45 ± 0.42
alloxan 150 mg/kg (5)	10 days after alloxan	215 ± 69	8.3 ± 3.8	0.48 ± 0.30
ATP 50 mg/kg (5)	10 days after alloxan and ATP	125 ± 14	2.6 ± 0.8	1.64 ± 0.14
P		<.01	<.01	<.01

As can be seen from the table, alloxan resulted in significant increase in blood glucose and liver fat and significant decrease in liver glycogen. ATP resulted also in a significant reduction in the fat and significant increase in the glycogen content of the liver.

It is likely that the protective effect of ATP is mediated by enhanced insulin release from the still functioning beta cells. In a previous work (31), we found that ATP promoted insulin secretion in normal and in the diabetic rats after glucose stimulation.

Although what is generally accepted is that the metabolic processes in which ATP takes part are intracellular. *Hyams* et al. (32) found that the administration of exogenous ATP served to elevate intracellular ATP concentration. ATP, under the influence of adenylcyclase, is converted to cyclic AMP which has been shown to stimulate insulin secretion (33).

Studies of cyclic AMP indicated that it exerts two insulin-like effects, increased glucose uptake and decreased cellular space (34). We have found that ATP increased glucose uptake of normal rats (31).

Nevertheless the administration of ATP before dithizone had a slight effect (35). The toxic effect of dithizone can be explained that through its chelation of zinc, it blocks enzyme action, especially those enzymes related to the sulfhydryl groups (36). Since the insulin molecule contains SH groups, it is therefore expected that anything which interferes with SH reaction will necessarily interfere with the formation, storage, or release of insulin (36).

It is concluded, therefore, that although zinc chloride prevented the destruction of the beta cells in both alloxan and dithizone diabetic rats, yet it is suggested that ATP as well as zinc, manganese, chromium, and cobalt ions seem to be essential for both insulin secretion as well as the utilization of glucose by various tissues.

Summary

The intravenous injection of zinc chloride immediately before and fifteen minutes after alloxan or dithizone prevented the usual hyperglycaemia observed 24 hours after induction of diabetes. This is supported by histological examination which showed that the itslets of those animals which were injected with zinc were intact and their beta cells stained normally.

The intravenous injection of manganese chloride prevented any marked rise of blood glucose, without protecting the islets. Chromium and cobalt chloride lowered the blood-glucose level to a certain extent.

ATP given before alloxan could prevent any marked rise in blood sugar. ATP resulted also in a significant reduction in the fat and significant increase in the glycogen content of the liver in female rats examined on the 10th day after induction of alloxan diabetes.

It is suggested that ATP as well as zinc, manganese, chromium, and cobalt ions seem to be essential for both insulin secretion as well as glucose utilization by various tissues.

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