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Serum enzyme changes due to trace amounts of some transition metal ions on the induction of experimental diabetes

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With 3 tables

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Experimental diabetes was produced by intravenous administration of dithizone to rats (1, 14, 22), or intraperitoneal injection of alloxan (21). On one hand, the beta cells of the pancreas are selectively destroyed by alloxan (18). On the other hand, histological examination of the pancreas showed that the extent of the glycaemic reaction induced by dithizone was dependent on the degree of damage of islet tissues (35).

Awadallah et al. (2) showed that in alloxan-induced diabetes, serum GOT, GPT and coeruloplasmin were significantly increased compared to normal rats, while the level of serum alkaline phosphatase was decreased. In dithizone-induced diabetes, the levels of serum GOT, GPT and alkaline phosphatase were found to be higher than normal, while coeruloplasmin levels were unchanged (2).

We have also reported that the intravenous injection of zinc^{II} chloride (1 mg/1 kg body weight) immediately before and 15 minutes after alloxan or dithizone prevented the usual hyperglycaemia observed 24 hours after induction of diabetes. The intravenous injection of manganese^{II} chloride prevented any marked rise of blood glucose, with protecting the islets. Chromium^{III} and cobalt^{II} chloride lowered the blood glucose level to a certain extent (34).

The present investigation was carried out to study the metabolic changes in activities of certain enzymes such as glutamic oxalic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (AP) and coeruloplasmin oxidase in alloxan and dithizone-induced diabetes in presence of those elements.

Material 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 24-hour fast. The used dose was 150 mg/kg of body weight.

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 of body weight following a 24-hour fast (33).

The rats in this study were divided into six groups: Group 1 is a control group comprising 15 normal rats, and group 2 is a diabetic group comprising 10 alloxanized and 10 dithizonized rats. Groups 3, 4, 5 and 6 comprised each 20 rats injected intravenously with either Zn^{II}, Mn^{II}, Cr^{III} or Co^{II} chloride solution. The dose was 1 mg of the salt/kg of body weight immediately before and 15 minutes after alloxan and dithizone.

Before sacrificing the animals, blood samples were asperated from the apex of the heart, and were used for analysis of glucose. Plasma glucose was measured by *Nelson's* modification of *Somogyi's* procedure (32). Serum GOT and GPT were determined by the method of *Reitman* and *Frankel* (27). Serum alkaline phosphatase was estimated by the method of *King* and *Armstrung* (17). The ceruloplasmin-oxidase activity was determined by the method of *Henry* et al. (11).

Results and discussion

Several workers (15, 22, 20) have demonstrated that dithizone is a diabetogenic substance, and most of them explained its action by making the assumption that dithizone chelates Zn^{II} which is an integral part of the structure and functional activity of the beta cells (insulin producers). However, alloxan effect has been stated to be in preventing the enzymatic synthesis and release of insulin (28).

In our study, it was found that the intravenous injection of Zn^{II} chloride in dithizone, or alloxan, prevented the usual hyperglycaemia observed 24 hours after induction of diabetes (34, 3). For this finding, there are two possible explanations, the first is that we are dealing with the resultant of two opposing changes, hypoglycaemia induced by Zn^{II} on one hand, and hyperglycaemia induced by alloxan, or dithizone on the other hand. *Prasad* (25) suggested that Zn^{II} seems to be essential for the utilization of glucose by various tissues, as well as the enhanced hypoglycaemic action of insulin (24). The second explanation is that genuine protection of the islets occurred as has been verified by histological examination of the pancreas (34).

Intravenous injection of Mn^{II} chloride prevented also the expected rise of blood glucose. Manganese may act peripherally on utilization of glucose which is in agreement with its role as a coenzyme for many reactions involved in carbohydrate metabolism (10).

In case of intravenous injection of Cr^{III} chloride, the blood sugar attained a lower level than that of alloxan or dithizone, but a higher level than the control value. Thus Cr^{III} unlike Zn^{II} did not protect the rat against the diabetogenic effect of dithizone or alloxan.

With regard to intravenous injection of Co^{II} chloride, the blood sugar attained a lower level then alloxan and dithizone diabetes. It also causes a rapid selective injury to the alpha cells (30, 10). *Padmaker* et al. (23) suggested that Co^{II} ions enhance glucose uptake, its oxidation to carbon dioxide, and its incorporation into fat lipids, thus stimulating the action of insulin. Moreover, the ionic movements across the beta cell membrane are important in setting off and maintaining insulin secretion by the beta cells.

Table 1. Fasting blood glucose and serum glutamic oxalic transaminase (GOT) glutamic pyruvic transminase (GPT), alkaline phosphatase and coeruloplasmin in control and other treated groups of alloxanized rats.

Item	Control	Alloxan	Zn	Mn	Cr	Co	Direction of trend		
Glucose mg'/100 ml	116.7	451.9	110.5	114.0	269.5	196.5	control →	alloxan	+ 335.2 +
							alloxan →	Zn	− 341.4 −
S. D. ±	18.8	33.4	11.8	15.6	48.6	33.9	alloxan →	Mn	− 337.9 −
		(a)					alloxan →	Cr	− 182.4 −
	(100)	(387) (+)	(95) (−)	(98) (−)	(231) (+)	(168) (+)	alloxan →	Co	− 225.4 −
							control →	alloxan →	+ 78.5 +
GOT	64.0	142.5	120.3	110.8	150.6	148.9	alloxan	Zn	− 22.2 −
I. U./L							alloxan →	Mn	− 31.7 −
S. D. ±	8.7	10.8	20.6	19.6	22.4	16.4	alloxan →	Cr	+ 8.1 +
	(100)	(222) (+)	(188) (+)	(173) (+)	(235) (+)	(233) (+)	alloxan →	Co	+ 6.4 +
							control →	alloxan	+ 82.8 +
GPT	26.2	136.4	82.8	68.4	125.6	140.6	alloxan →	Zn	− 53.6 −
I. U/L							alloxan →	Mn	− 68.0 −
S. D. ±	5.3	10.3	8.7	6.2	21.0	18.2	alloxan →	Cr	− 10.8 −
	(100)	(521) (+)	(316) (+)	(261) (+)	(479) (+)	(537) (+)	alloxan →	Co	+ 4.2 +
							control →	alloxan	− 9.2 −
Alkaline phosphatase	17.4	8.2	10.2	8.9	7.8	12.6	alloxan →	Zn	+ 2.0 +
K. A. Unit							alloxan →	Mn	+ 0.7 +
S. D. ±	2.8	2.3	4.6	1.3	3.1	2.6	alloxan →	Cr	− 0.4 −
	(100)	(47) (−)	(59) (−)	(51) (−)	(45) (−)	(72) (−)	alloxan →	Co	+ 4.4 +
							control →	alloxan	+ 154.0 +
coeruloplasmin units	520	674	665	570	670	680	alloxan →	Zn	− 9 −
							alloxan →	Mn	− 104 −
S. D. ±	22.4	83.2	22.3	53.4	60.3	45.4	alloxan →	Cr	− 4 −
	(100)	(130) (+)	(128) (+)	(110) (+)	(129) (+)	(131) (+)	alloxan →	Co	− 6 +
Number of animals	15	15	10	10	10	10			

(a) $\frac{\text{Tabulated value}}{\text{Control value}} \times 100$ (b) Control → alloxan

Table 2. Fasting blood glucose and serum glutamic oxalic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase and coeruloplasmin in control and other treated groups of dithizonized rats.

Item	Control	Dithizone	Zn	Mn	Cr	Co	Direction of trend		
									(b)
Glucose	116.7	231.8	111.7	120.0	179.7	174.8	control →	dithizone	+ 115.1 +
mg/100 ml							dithizone →	Zn	− 120.1 −
S. D. ±	18.8	79.9	19.7	27.6	27.3	32.2	dithizone →	Mn	− 111.8 −
		(a)					dithizone →	Cr	− 52.1
	(100)	(199) (+)	(96) (−)	(103) (+)	(154) (+)	(150) (+)	dithizone →	Co	− 57.0 −
							control →	dithizone	+ 17.1 +
GOT	64.0	81.1	79.4	75.6	90.6	100.8	dithizone →	Zn	− 1.7 −
I. U/L							dithizone →	Mn	− 5.5 −
S. D. ±	8.7	7.7	22.2	16.4	26.8	24.5	dithizone →	Cr	+ 9.5
	(100)	(127) (+)	(124) (+)	(118) (+)	(142) (+)	(158) (+)	dithizone →	Co	+ 19.7 +
							control →	dithizone	+ 11.7 +
GPT	26.2	37.9	39.6	30.4	40.2	55.6	control →	dithizone	+ 11.7 +
GPT	26.2	37.9	39.6	30.4	40.2	55.6	dithizone →	Zn	+ 1.7 +
I.U./L							dithizone →	Mn	− 7.5 −
S. D. ±	5.3	4.7	25.3	12.6	18.9	29.4	dithizone →	Cr	+ 2.3 +
	(100)	(145) (+)	(151) (+)	(116) (+)	(153) (+)	(212) (+)	dithizone →	Co	+ 7.7 +

Item	Control	Dithizone	Zn	Mn	Cr	Co	Direction of trend			
Alkaline phosphatase K. A. Unit	17.4	25.1	30.1	26.6	32.4	29.4	dithizone →	Zn	+	5.0 +
							dithizone →	Mn	+	1.5 +
S. D. ±	2.8	3.6	9.1	2.6	8.4	6.1	dithizone →	Cr	+	7.3 +
	(100)	(144) (+)	(173) (+)	(153) (+)	(186) (+)	(169) (+)	dithizone →	Co	+	4.3 +
							control →	dithizone	−	4.0 −
Coeruloplasmin units	520	516	520	515	512	540	dithizone →	Zn	+	4.0 +
							dithizone →	Mn	−	1.0 +
S. D. ±	22.4	25.2	26.4	20.1	18.6	12.6	dithizone →	Cr	−	4.0 −
	(100)	(99) (−)	(100)	(99) (−)	(98) (−)	(104) (+)	dithizone →	Co	+	24.0 +
Number of animals	15	15	10	10	10	10				

(a) $\frac{\text{Tabulated value}}{\text{Control value}} \times 100$

(b) Control → dithizone

$$(199) = \frac{231.8}{116.7} \times 100$$

116.7 → 231.8 = 231.8 − 116.7 = 115.1 (+)

It is possible that some of these ions are more closely related to the different stages of the insulin secretion process in the beta cell (6, 7).

In alloxan diabetic rats, serum GOT and GPT levels were significantly higher than normal (table 1). The serum GOT levels were higher in animals injected with Cr^{III} than Co^{II}, Zn^{II} and Mn^{II}, while serum GPT levels were higher in Co^{II} than in Cr^{III}, Zn^{II} and Mn^{II}.

Cruickshank (5) found that the liver was necrotized in alloxan diabetes. This supports the view that the hypoglycaemic phase of alloxan poisoning may be the result of liver damage. In tissue damage, cellular enzyme activity actually increases, simultaneously with serum enzyme activity increase (4). *Ingmar* and *Claus* (3) observed a considerable depletion of hepatic and muscle glycogen in alloxan diabetic rats. During alloxan diabetogenesis, the liver glycogen tends to move contrary to the direction of blood sugar changes (29). The gluconeogenetic action of GOT and GPT could represent a compensatory response by providing new supplies of glucose precursors. Therefore, the high levels of transaminase enzyme may be due to hepatotoxic effect of alloxan. On the other hand, *Kalk* (16) and *Leey* (19) suggested that approximately 40% of the diabetic animals have moderate fatty infiltration with or without cirrhosis.

Diabetic rats, 24 hours after intravenous injection of dithizone, had a slight elevation in serum GOT and GPT (table 2). This may be due to the fact that dithizone has no toxic effect on the liver, and hence no highly elevation in serum enzymes was observed as in alloxan. Serum GOT and GPT levels were increased in animals injected with Co^{II} than Cr^{III}, Zn^{II} and Mn^{II}.

Data for serum GOT shows that there are no statistically significant differences between the emans of Zn^{II}-Mn^{II} and Cr^{III}-Co^{II}, and there are statistically significant differences between the means of Zn^{II}-Cr^{III}, Zn^{II}-Co^{II}, Mn^{II}-Cr^{II} and Mn^{II}-Co^{II} for alloxan. In case of dithizone, there are statistically significant differences in all couples except the Zn-Mn couple (table 3).

Table 3.

	Zn–Mn	Zn–Cr	Zn–Co	Mn–Cr	Mn–Co	Cr–Co
Alloxan	n. s.	s.	s.	s.	s.	n. s.
GOT						
Dithizone	n. s.	s.	s.	s.	s.	s.
Alloxan	s.	s.	s.	s.	s.	n. s.
GPT						
Dithizone	n. s.	n. s.	n. s.	n. s.	s.	n. s.
Alloxan	n. s.	n. s.	n. s.	n. s.	s.	s.
Alloxan	n. s.	n. s.	n. s.	n. s.	s.	s.
Alkaline phosphate						
Dithizone	s.	n. s.	n. s.	s.	s.	s.
Alloxan	s.	n. s.	n. s.	s.	s.	n. s.
Coeruloplasmin						
Dithizose	n. s.	n. s.	s.	n. s.	s.	s.

A similar comparison of serum GPT values showed that there are statistically significant differences for all couples except the Cr^{III}-alloxan. For dithizone, there are no statistically significant differences in all cases except Mn^{II}-Cr^{III} case.

The increase of serum transaminase may be due to either fatty infiltration, hepatotoxic effect, or both.

Our alloxan diabetic rats showed lower serum alkaline phosphatase levels and higher in animals injected with Co^{II} than Cr^{III}, Zn^{II} and Mn^{II} (table 1). The initial decrease of serum alkaline phosphatase in our diabetic rats could be attributed to the increase call for energy through glycolytic and oxidative pathways of glucose 6-phosphate. However, the glycogen depletion due to insulin deficiency and transaminase increment observed later could be the cause of the increased alkaline phosphatase activity.

On the other hand, there are no statistically significant differences in serum alkaline phosphatase for the couples Zn^{II}-Mn^{III}, Zn^{II}-Cr^{III}, Mn^{II}-Cr^{III}, and there are statistically significant differences for the couples Zn^{II}-Co^{II}, Mn^{II}-Co^{II} and Cr^{III}-Co^{II} for alloxan (table 3). For dithizone, there are statistically significant differences in all cases except Zn^{II}-Cr^{III} and Zn^{II}-Co^{II}. The increase in the enzyme could be attributed to an increase of serum Zn^{II}. Zinc is present in several metaloenzymes and is important in their activities (26). *Halim* et al. (8, 9) recorded high serum zinc levels in alloxan and dithizone diabetes due to release of zinc from the islet cells as a result of its destruction by alloxan or dithizone.

In alloxan-diabetic rats, ceruloplasmin was higher than normal, while intravenous injection of dithizone was without effect on serum coeruloplasmin (tables 1, 2).

Data for coeruloplasmin show that there are no statistically significant differences between the increase of Zn^{II}-Co^{II}, Zn^{II}-Cr^{III} and Cr^{III}-Co^{II} and there are statistically significant differences for the other couples. For dithizone, there are statistically significant differences for the couples Zn^{II}-Co^{II}, Mn^{II}-Co^{II} and Cr^{III}-Co^{II}, there are no statistically significant differences for the couples Zn^{II}-Mn^{II}, Zn^{II}-Cr^{III} and Mn^{II}-Cr^{III}.

Coeruloplasmin is an ascorbic acid oxidase (12), and its activity is a function of free copper (II) ions in solution (26), hence an increase in serum copper level may be mainly due to the increase in coeruloplasmin. *Halim* et al. found that serum copper was higher in alloxan diabetes probably due to its hepatotoxic effect (9).

Summary

The intravenous injection of zinc chloride immediately before and 15 minutes after alloxan or dithizone prevented the usual hyperglycaemia observed 24 hours after induction of diabetes. The intravenous injection of manganese chloride prevented any marked rise of blood glucose, while chromium and cobalt chlorides lowered the blood glucose level to a certain extent.

In alloxan diabetic rats, serum GOT and GPT levels were significantly higher than normal. The serum GOT levels were higher in animals injected with chromium than cobalt, zinc and manganese; while serum GPT levels were higher in cobalt than in chromium, zinc and manganese. In dithizone diabetes, serum GOT and GPT were increased in animals injected with cobalt than chromium, zinc and manganese.

Alloxan diabetic rats showed lower serum alkaline phosphatase levels and higher in animals injected with cobalt than chromium, zinc and manganese. For dithizone, there are statistically significant differences in all cases.

In alloxan diabetes, coeruloplasmin was higher than normal, while intravenous injection of dithizone was without effect on serum coeruloplasmin.

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