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Serum mineral changes in dithizone-induced diabetes before and after insulin treatment

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With 1 table

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Experimental diabetes was produced by intravenous administration of dithizone to rabbits (2, 8, 14). The duration and severity of the diabetes were proportional to the dose of dithizone injected (3). *Stampfl* (18) mentioned that a dose of 125 mg/kg body weight was the minimal dose to produce permanent diabetes and that 250 mg/kg body weight was too toxic.

Histological examination of the pancreas showed that the extent of the glycemic reaction induced by dithizone was dependent on the degree of damage of the islet tissues (15, 20).

The toxic effect of dithizone can be possibly explained by the assumption that through its chelation of zinc, it blocks enzyme action, especially those enzymes related to the sulphydryl groups (6). Since the insulin molecule which interferes with SH reaction will be necessarily important in the formation, storage or release of insulin. Once a zinc-dithizone complex is formed, zinc ion is no longer available as a catalyst or cofactor for metabolic processes within the beta cells (6). Thus, insulin deficiency, with possible destruction of the beta cells, may issue (9).

As in alloxan diabetes, after administration of dithizone, there is a triphasic mode of the blood sugar levels: initial hyperglycemia, hypoglycemia, and finally permanent hyperglycemia (12). A typical diabetic condition developed within 24–48 hours after the injection of dithizone.

Wolff et al. (22) reported an increase in serum zinc after dithizone. This was followed by a greater urinary output of zinc, which reached its maximum about 10 hours after the injection of dithizone. The iron content of the serum and urine showed the same pattern as found for zinc, but no significant change was found in copper, cobalt, and magnesium (22). Serum chloride, sodium, and calcium were reduced, while potassium was raised (1).

This was carried out to study the metabolic changes of certain inorganic elements such as zinc, copper, iron, calcium, magnesium, potassium, and sodium as a result of dithizone diabetes before and after treatment with insulin.

Table 1. Blood glucose levels and serum inorganic elements in control and dithizonised rats before and after insulin treatment.

Item		Control rats	Diabetic rats		Diabetic rats after insulin treatment	
		Mean ± SE	Mean ± SE	P>	Mean ± SE	P>
1. Glucose	mg %	116.70 ± 18.89	231.81 ± 79.92	0.005	104.00 ± 14.3	0.005
2. Zinc	μg %	131.65 ± 15.85	214.76 ± 39.77	0.005	169.71 ± 16.55	0.005
3. Copper	μg %	116.95 ± 20.05	105.26 ± 18.26	n. s.	105.87 ± 16.23	n. s.
4. Iron	μg %	132.11 ± 19.75	184.84 ± 31.63	0.005	127.30 ± 35.87	0.005
5. Calcium	mg %	7.55 ± 0.78	6.02 ± 1.20	0.005	7.03 ± 0.83	0.05
6. Magnesium	mg %	3.92 ± 0.44	3.49 ± 0.64	n. s.	2.81 ± 0.24	0.01
7. Potassium	mg %	23.28 ± 2.19	25.58 ± 2.37	0.05	24.54 ± 1.93	n. s.
8. Sodium	mg %	225.34 ± 10.84	195.31 ± 15.87	0.005	211.86 ± 11.02	0.025

n. s. = not significant

Materials and methods

The present study was carried out on 80 normal albino rats of body weight ranging from 200–250 g. The rats were maintained on the laboratory diet. 50 rats were rendered diabetic after fasting for 24 hours by slow intravenous injection of freshly prepared dithizone solution in a dose of 200 mg/kg body weight. Dithizone was prepared by dissolving a known weight in ammoniated absolute ethanol following the method of *Stampfl* (19).

The rats were categorised into 3 groups. 30 normal rats used as controls, 30 rats treated with dithizone only and 20 dithizonised rats treated with insulin, injected subcutaneously in a dose of 1 unit/kg body weight twice a day for 10 days.

Blood samples were collected from the heart by means of disposal needles and syringes. Each 2 blood samples were pooled together. A portion of the blood was used for analysis of glucose and the remaining portion was allowed to clot. The serum was separated for analysis of zinc, copper, iron, calcium, magnesium, sodium, and potassium.

The method of *Nelson's* modification of *Somogyi's* (17) was used for determination of blood sugar. Serum zinc and copper were determined by the method of *Sinaha* and *Gabrieli* (16). Serum iron, potassium, and sodium were estimated by the method published in *Beckman*, analytical method by Atomic Absorption Spectrophotometer. Serum calcium and magnesium were determined using the method of *Willis* (21).

Results and discussion

Dithizone is known to be a chelator of zinc and since the beta cells are known to contain zinc in considerable quantities, it must be presumed that the action of dithizone on the islets is through chelation of zinc. This is supported by the histochemical findings after induction of diabetes by dithizone. The beta cells assume a purplish red colour due to the dithizone-zinc complex formed within the beta cells (10, 13).

When we attempted to treat our dithizone-diabetic rats with various hypoglycemic agents, we found only insulin, but not the oral hypoglycemic drugs, such as daonil and lycanol, was effective. The explanation for the lack of response of dithizone animals may lie in the fact that daonil and lycanol are believed to excert their therapeutic action by promoting the release of insulin from the beta cells that remained intact. It has been suggested recently that zinc is necessary for the functioning of the insulin release mechanism within the cell. If zinc is chelated by dithizone, the release mechanism may not operate and the oral hypoglycemic agents will have no therapeutic effect (10).

Diabetic rats, 24 hours after an intravenous injection of dithizone, had a serum zinc level of 217.76 ± 39.77 $\mu g\%$ compared to 131.65 ± 15.85 $\mu g\%$ in control rats. Our findings are in agreement with those obtained by *Wolff* et al. (22) and *Lazaris & Balvlsku* (11), who reported that serum zinc was markedly increased after the injection of 100–150 mg dithizone/kg body weight. It is possible that when dithizone chelates the intra-beta cell zinc, while other cells are destroyed releasing their zinc-dithizone chelates into the blood stream. Thereby contributing to the higher level of serum zinc (22). In addition, dithizone probably causes hemolysis of some red

cells, releasing their zinc content into the serum. Since red cells contain about 85 % of all blood zinc, an appreciable hemolysis will no doubt cause a considerable increase in the serum-zinc level.

In our study, the intravenous injection of dithizone was without effect on the serum copper. This may be due to the fact that dithizone has no toxic effect on the liver, which is the source of copper (7), and hence no change in serum copper was observed.

Dithizone diabetic rats showed an increase in the serum iron. This increase was reported by *Wolff* et al. (22) to be due to hemolysis of the erythrocytes. Also, the level of serum potassium was higher than normal, while those of serum calcium and sodium were lower. Magnesium level was unchanged. The rise in serum potassium may be due also to red cells hemolysis. The lower sodium levels are probably brought about by the diabetic polyuria, but no explanation is available for the changes in serum calcium.

After 10 days treatment with insulin (1 unit/kg body weight), subcutaneously injected twice a day, most of the serum levels approached their normal values, except for serum potassium and magnesium. The serum magnesium showed a statistically significant drop. This was supported by clinical findings of *Berthaux* and *Maurat* (4), and *Binet* (5), who reported that a slow simultaneous intravenous injection of insulin and glucose caused a decrease in the magnesium content of blood plasma. *Binet* (5) suggested that in diabetic animals insulin acts on the cell membrane promoting the entry of magnesium from the surrounding tissue fluids, thereby creating optimum conditions within the cell for the metabolism of glucose and adenosine triphosphate, and hence its decrease in the serum after insulin injection.

Summary

In this study, diabetes was induced by intravenous injection of dithizone. In dithizonised diabetic animals, the levels of serum zinc, iron, and potassium were found to be higher than normal, while those of serum calcium and sodium were lower. Copper and magnesium levels were unchanged.

After treatment with insulin, most of these serum levels approached the normal, except for serum potassium and magnesium.

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