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Serum enzyme changes in experimental diabetes before and after treatment with some hypoglycaemic drugs

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With 2 tables

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Experimental diabetes was produced by intravenous administration of dithizone to animals (1, 14, 25), or intraperitoneal injection of alloxan (23). The experimental animals show a triphasic change in the blood sugar levels, initial hyperglycaemia, hypoglycaemia and finally hyperglycaemia. The development of these phases of alloxan diabetes are mainly due to insulin deficiency, insulin surplus and then insulin lack, respectively (13).

Many workers reported a significant elevation in glutamic oxalic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities in heart diseases, extrahepatic biliary obstructive jaundice and biliary cirrhoses (7, 22). In most instances the quantitative and serial changes in these two enzymes are an index of various types of liver disease to assist in diagnostic differentiation (4).

Raised level of alkaline phosphatase was recorded in *Paget's* disease, hyperparathyroidism, rickets, osteomalacia intra and extrahepatic obstruction, and in hepatic lesion such as metastasis and abcess (18, 19). Low enzyme level was found in achondroplasia and cretinism, also in scurvy and after exposure to radio active substances which are deposited in the bone (2).

Ceruloplasmin activity is known to be high during pregnancy, it is increased in response to estrogen and contraceptives administration (16, 24, 11). Increased levels in thyrotoxicosis has been suggested to be related to peripheral transformation of gonadal steroid (6). No change in ceruloplasmin level in juvenile diabetes compared to normal subjects with or without insulin treatment (16).

This work was carried out to study the metabolic changes of certain enzymes such as glutamic oxalic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase and ceruloplasmin oxidase activity in alloxan diabetes before and after treatment with different hypoglycaemic drugs and in dithizone diabetes before and after treatment with insulin.

Materials and methods

The present study was carried out on 115 normal albino rats of body weight ranging from 200–250 g. The rates were maintained on the laboratory diet and allowed to eat ad libitum.

Fasted rats were rendered diabetic by alloxan by intraperitoneal injection of freshly prepared alloxan monohydrate solution in a dose of 150 mg/kg of body weight. In case of dithizone diabetes, rats were rendered diabetic after 24 hours by slow intravenous injection of freshly prepared dithizone solution in a dose of 200 mg/kg of body weight (34).

The rats were divided into 10 groups; 15 untreated rats served as control group, 15 rats treated with alloxan only. Six groups of alloxan diabetic rats containing 10 rats each, a group of these was given glibenclamide (daonil) in a dose of 0.1 mg/kg body weight i.v./day for 7 days. The second group was continued the same treatment for 14 days. The third group was given glucodiazine (lycanol) in a dose of 20 mg/kg body weight i.v./day for 7 days and the fourth group for 14 day. The fifth and sixth groups were treated with insulin (1 unit/kg of body weight s.c.) twice a day for 7 and 14 days respectively. The remaining two groups, 15 rats treated with dithizone only and 10 dithizonised rats were treated with insulin, injection subcutaneously in a dose of 1 unit/kg of body weight twice a day for 10 days.

The method of *Nelson's* modification of *Somogyi's* (33) was used for determination of blood sugar. Serum (GOT) and (GPT) by the method of *Reitman* and *Frankel* (27). Serum alkaline phosphatase was estimated by the method of *King* and *Armstrong* (17). The ceruloplasmin-oxidase activity was determined by the method of *Henry* et al. (10).

Results and discussion

In alloxan diabetic rats, serum (GOT) and (GPT) levels were significantly higher than normal (table 1). *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 enzymes activity actually increases, simultaneously with serum enzyme increase (4). There is reciprocal relationship between serum enzymes and liver activity. An increase in cellular and serum enzyme could represent a homeostatic rather than retrograde response.

Ingmar and *Claus* (13) observed that 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 (28). The liver glycogen concentration is low during the hyperglycaemic phase.

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 enzymes may be due to hepatotoxic effect of alloxan.

On the other hand, *Kalk* (15) and *Leevy* (21) suggested that approximately 40 % of diabetic animals have moderate fatty infiltration with or without cirrhosis. Therefore, the increase of serum transaminase may be due to fatty infiltration.

In our study, the treatment of alloxan-diabetes rats with insulin led to lowering of serum GOT and GPT. On the other hand, when lycanol was administered to diabetic rats, they showed non significant effect on serum GOT and GPT neither after 7 days nor after 14 days. Similarly, there was no return of serum enzymes to normal levels when daonil was used.

Table 1. Fasting blood glucose and serum GOT, GPT, alkaline phosphatase, and ceruloplasmin in control and other treated groups of alloxanized rats.

Item	Control	Alloxan diabetes	Daonil		Lycanol		Insulin	
			7 days	14 days	7 days	14 days	7 days	14 days
Glucose								
mg/100 ml	116.7	451.9	303.8	147.7	216.6	221.9	137.6	110.5
S.D. ±	18.9	33.4	31.7	23.9	42.7	26.2	39.3	11.3
P		0.005	0.005	0.05	0.005	0.005	ins.	ins.
GOT								
I.U./L.	64.0	142.5	94.4	80.0	118.5	97.6	77.9	66.8
S.D. ±	8.7	10.8	13.8	17.8	12.6	11.4	6.9	5.7
P		0.005	0.025	0.05	0.005	0.025	ins.	ins.
GPT								
I.U./L.	26.2	136.4	62.4	50.3	70.2	52.6	36.8	27.0
S.D. ±	5.3	10.3	10.5	7.4	9.2	9.9	4.4	3.6
P		0.005	0.005	0.025	0.005	0.025	ins.	ins.
Alkaline phosphatase								
K.A. unit	17.4	8.2	27.9	26.6	30.2	27.7	11.5	10.6
S.D. ±	2.8	2.3	4.4	5.5	3.7	3.3	2.3	1.9
P		0.005	0.025	0.025	0.025	0.025	0.05	0.05
Ceruloplasmin								
unit	520	674	609	542	508	515	593	514
S.D. ±	22.4	83.2	39.0	40.6	42.6	40.3	28.7	27.9
P		0.05	0.05	ins.	ins.	ins.	0.05	ins.
n	(15)	(15)	(10)	(10)	(10)	(10)	(10)	(10)

Figures between parentheses indicate number of animals.
ins.: insignificant.

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 occurred in alloxan.

After 10 days treatment with insulin (1 unit/kg body weight), subcutaneously injected twice a day, serum enzymes return to the normal with disappearance of hyperglycaemia occurred by dithizone.

Our alloxan-diabetic rats showed low serum alkaline phosphatase level compared to normal control, while increase after 7 and 14 days from treatment with oral hypoglycaemic drugs such as daonil and lycanol (table 1). These findings agree with those obtained by *Cantor* et al. (3) who found that an initial decrease in serum alkaline phosphatase activity in alloxan diabetes followed by a gradual rise more than three times the original values in 14 days. *Sen* (32) found that low concentration of alloxan exerted some retarding or assisting influence on succinic dehydrogenase, pyruvic acid oxidase, and alkaline phosphatase.

The initial decrease of serum alkaline phosphatase in our diabetic rat could be attributed to the increased call of energy through glycolytic and oxidative pathways of glucose 6-phosphate, however, the glycogen depletion due to insulin deficiency and transaminase increment observed later on could be the cause of the increased alkaline phpsphatase activity observed later on. On the other hand, the elevation occurred after treatment with hypoglycaemic drugs may be attributed from leakage out of necrotic or damage cells of the liver.

On the other hand, when insulin was administered to diabetic rats, the serum-alkaline phosphate remained low after 7 and 14 days, since insulin administration reduced sugar and alkaline phosphate in alloxan-diabetic rats (3).

In dithizone-diabetic rats, serum alkaline phosphatase was higher than normal. After 10 days treatment with insulin the serum alkaline phosphatase approached to normal value (table 2).

The increase of serum alkaline phosphatase in alloxan and dithizone diabetes could be attributed to increase of serum zinc. *Prasad* (26) showed that zinc is present in several metaloenzymes and is important in their activities, such as alkaline phosphatase. *Halim* et al. (8, 9) recorded high serum zinc level 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. Seven days after the treatment of alloxan-diabetic rats with daonil, the serum ceruloplasmin decreased to some extent till the 14th day of treatment, then returned to the normal level. When lycanol or insulin were administered to alloxan-diabetic rats, serum ceruloplasmin was normalized on the 14th day (table 1).

Intravenous injection of dithizone was without effect on the serum ceruloplasmin. After 10 days treatment with insulin, serum ceruloplasmin remained unchanged (table 2).

Ceruloplasmin is an ascorbic acid oxidase (12). This may indicate that, in the process of delivering it from ionic copper, the ceruloplasmin molecule was denatured, or that the oxidase activity is a function of the

Table 2. Fasting blood glucose and serum GOT, GPT, alkaline phosphatase and ceruloplasmin in control, dithizonized rats and after 10 days from treatment with insulin.

Item	Control	Dithizone diabetes	After 10 days of treatment with insulin
Glucose			
mg/100 ml	116.7	231.9	104.0
S.D. ±	18.9	79.9	14.3
P	–	0.005	ins.
GOT			
I.U./L.	64.0	81.1	66.8
S.D. ±	8.7	7.7	3.3
P	–	0.05	ins.
GPT			
I.U./L.	26.2	37.9	26.9
S.D. ±	5.3	4.7	2.4
P	–	0.05	ins.
Alkaline phosphatase			
K.I.U.	17.4	25.1	18.1
S.D. ±	2.8	3.6	1.9
P	–	0.05	ins.
Ceruloplasmin			
unit	520	516	513
S.D. ±	22.4	25.2	25.2
P		ins.	ins.
n	(15)	(15)	(10)

Figures between parentheses indicate number of animals.
ins.: insignificant.

presence of the free cupric ions in its solutions (30). The copper of ceruloplasmin is normally very tightly bound to the protein (31). Ceruloplasmin may transport copper such as transferin transports iron (20). It seems that the copper is incorporated into the ceruloplasmin molecule in vivo only at the time of synthesis of the protein and the amount of copper incorporated daily into ceruloplasmin in a normal control corresponded closely to the amount absorbed from the diet (35). The increase in serum copper level may be mainly due to the increase in ceruloplasmin (29).

Halim et al. found that serum copper was high in alloxan diabetes (8) and was normal in dithizone diabetes (9). The higher level may be due to the hepatotoxic effect of alloxan. Therefore, the changes of ceruloplasmin levels in our experimental rats was related to serum copper.

Summary

In alloxan diabetes, serum GOT, GPT, and ceruloplasmin were significantly increased compared to normal rats, while the level of serum alkaline phosphatale was decreased.

Treatment with insulin led to lowering of serum GOT, GPT, and ceruloplasmin while serum alkaline phosphatase remained low. Then lycanol or daonil were used for treatment, serum GOT, GPT, and ceruloplasmin were changes towards normalization, while ceruloplasmin returned to normal values. Serum-alkaline phosphatase increased after 7 and 14 days from treatment with oral hypolgylcaemic drugs.

In dithizonized diabetic animals, the levels of serum GOT, GPT, and alkaline phosphatase were found to be higher than normal, while ceruloplasmin levels were unchanged.

After treatment with insulin all serum enzyme activities were normalized.

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