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Effect of repeated doses of ATP on serum protein pattern and fat content of the liver in experimental diabetes

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With 3 tables

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Changes in protein metabolism noted in diabetes mellitus are grossly reflected in a negative nitrogen balance (1). This may be due to depressed protein synthesis or to increased protein catabolism.

Turnover studies with N^{15} glycine are in favour with the depressed effect on protein synthesis (2). *Krahl* (3) has demonstrated that liver slices from diabetic rats have a decreased capacity to incorporate glycine-1-C^{14} into glutathione and into liver proteins.

The increased urea-nitrogen production in alloxan diabetes may be accounted for by enhanced catabolism of both liver and plasma proteins (4). These results are compatible with the view that diabetic liver has a major disturbance in both synthesis and catabolism of proteins.

A correlation exists between the disturbance in protein metabolism and the accumulation of liver lipid (5). The interrelationships between these two lesions and their dependence on a more basic alteration in ATP metabolism has been clarified by *Farber* et al. (6).

The aim of the present work is to study the effect of exogenous ATP on the serum protein pattern in alloxan diabetes and correlate them with the amount of fat in the liver as well as with the duration and degree of diabetes.

Material and methods

All experiments were made on albino rats of both sexes (Sprague Dawley strain). The diet was an adequate one and was supplied in plenty.

The animals are classified into two main groups:

Group I: was made to study the effect of mild and severe alloxan diabetes on the blood glucose, the fat content of the liver, total serum proteins, and electrophoretic pattern.

Group II: was made to study the effect of intramuscular injection of ATP on normal and mild alloxan diabetic rats. The same investigations were performed.

Alloxan (B. D. H.) was freshly prepared as a 5 % aqueous solution and was injected intraperitoneally after a 24 hour fast. A mild state of diabetes was made by the intraperitoneal injection of 150 mg alloxan per kg rat weight and the estimations were made 10 days later. In these rats a 48 hour blood glucose sample was withdrawn from the tail to assess the degree of diabetes.

Severe diabetes was made by the administration of 200 mg alloxan per kg rat weight and the experiment was started 2 days later, since the animals usually died within 3 to 5 days.

Administration of ATP sodium salt (Richter) was made intramuscularly, each animal received two injections separated by one day. The dose of ATP was either two injections of 2.5 mg or 5 mg per rat and the estimations were made one day after the second injection. Ten days after alloxan administration the animals were killed by decapitation. The serum was kept frozen for further analysis.

Total serum proteins were determined by the biuret method and electrophoretic separation of serum proteins by the method of *King* and *Wootton* (7). Blood glucose was estimated by the iodometric titration method described in *King* and *Wootton* (7). The fat content of the liver was estimated according to *Folch* (8).

Results

In the analysis of the serum proteins of rats by paper electrophoresis five fractions were apparent: albumin and the four globulin fractions designated as α_1, α_2, B and γ.

The electrophoretic pattern of control animals is shown in table 1 and comparison of both sexes is given. There is no statistically significant difference in the percentage of the different protein fractions nor in the A/G ratio.

Table 2 represents the changes in the total proteins and their fractions in alloxan diabetic rats. The level of the blood glucose and liver fat are included to assess the severity of the diabetic state. In these experiments the estimations in severely diabetic animals were made 48 hours after the administration of alloxan, while in mild diabetes they were made 10 days after alloxan.

In 48 hrs. severe diabetic animals neither the total protein content nor the serum electrophoretic pattern showed any significant difference from the results of the control animals, though there was a tendency for the total protein and the serum albumin to decrease.

On the other hand, in the case of 10 days mild diabetes there was a significant decrease in total protein and in serum albumin, while the globulin fractions remained virtually unaltered. The A/G ratio was therefore significantly decreased.

Effect of exogenous ATP on serum total protein and electrophoretic pattern of normal rats. The results are illustrated in table 1.

On comparison with the control experiments that received no ATP, it was found that under the influence of both doses of ATP there was no statistically significant difference in the total percentage of serum proteins nor any of its fractions that are seen in the electrophoretic pattern.

Effect of ATP in alloxan diabetic rats

The effect of ATP was investigated in mild diabetics (table 3). Total protein under the influence of both the high and low dosage of ATP treatment, of mild diabetic rats showed no significant change from the results observed without ATP. It is recalled that in mild induced diabetes the total serum protein showed a significant decrease when compared with the control animals.

Table 1.

	Total proteins g%	Albumin	Grams % Globulins α_1	α_2	β	γ	Total Globulins	A/G
Control Males (11)	7.52 ±0.38	3.11 ±0.60	1.12 ±0.26	0.68 ±0.19	1.20 ±0.21	1.16 ±0.50	4.18 ±0.97	0.74 ±0.13
Control Females (13)	7.90 ±0.87	3.27 ±0.51	1.24 ±0.19	0.57 ±0.17	1.35 ±0.20	1.42 ±0.38	4.61 ±0.59	0.73 ±0.13
P	> .05	> .05	> .05	> .05	> .05	> .05	> .05	> .05
Control + ATP 2 × 2.5 mg (12)	7.54 ±0.79	2.99 ±0.37	1.12 ±0.33	0.69 ±0.13	1.22 ±0.18	1.26 ±0.23	4.31 ±0.67	0.71 ±0.09
Control + ATP 2 × 5 mg (12)	7.62 ±1.13	3.09 ±0.50	1.19 ±0.42	0.67 ±0.18	1.20 ±0.30	1.30 ±0.46	4.40 ±1.20	0.73 ±0.18

Figures between parantheses indicate number of animals.

Table 2.

	Bl Glucose mg/100ml	Fat gm % of wt. Liver wt.	Total protein g/100ml	Albumin	Grams % Globulins α_1	α_2	β	γ	Total Globulins	A/G
Control (11)	91.3	4.01	7.52	3.11	1.12	0.68	1.20	1.16	4.18	0.74
Rats	±10.4	±1.01	±0.38	±0.60	±0.26	±0.19	±0.21	±0.90	±0.97	±0.13
Severe diabetes (5)	530	11.30	7.1	2.82	1.19	0.97	1.13	1.02	4.14	0.65
48 hrs.	±136	±2.40	±1.0	±0.65	±0.22	±0.16	±0.21	±0.16	±0.34	±0.12
P	<.05	<.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05
Mild diabetes (6)	249	6.40	6.55	2.21	1.15	0.96	1.22	0.99	4.39	0.51
10 days	±125	±0.90	±0.47	±0.18	±0.17	±0.43	±0.18	±0.32	±0.38	±0.08
P	<.05	<.05	<.05	<.05	>.05	>.05	>.05	>.05	>.05	<.05

Figures between parantheses indicate number of animals.

Table 3.

	Bl Glucose mg/100 ml		Fat gm % of wet liver wt.	Total protein gm/100ml	Albumin	α_1	Grams % Globulins α^2	β	γ	Total Globulins	A/G
Mild diabetes	214	238	6.88	6.55	2.21	1.15	0.96	1.22	0.99	4.39	0.51
10 days (6)	± 99	±110	±1.12	±0.47	±0.18	±0.17	±0.43	±0.18	±0.32	±0.38	±0.08
Diabetic	203	233	3.55	6.71	2.16	1.25	1.01	1.26	0.97	4.53	0.47
+ ATP	± 97	±159	±0.17	±0.79	±0.41	±0.16	±0.14	±0.28	±0.25	±1.18	±0.09
2 × 2.5 mg (6)											
P	>.05		<.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05	>.05
Diabetic	296	198	4.26	6.48	2.62	1.16	0.84	0.91	0.81	3.75	0.69
+ ATP	±127	± 91	±0.31	±0.50	±0.42	±0.27	±0.10	±0.08	±0.05	±0.44	±0.11
2 × 5 mg (6)											
P	<.05		<.05	>.05	<.05	>.05	>.05	<.05	>.05	<.05	<.05

Figures between parantheses indicate number of animals.

ATP in the larger doses resulted in a significant increase in serum albumin and a decrease in β-globulin and total globulins, with consequent increase in A/G ratio. Under the influence of ATP in the diabetic rats, there was a statistically significant decrease in the level of blood glucose when the dose of ATP was 2×5 mg. However, under the influence of the smaller dosage of ATP, there occurred no significant change in the level of blood glucose. The fat content of the liver showed a significant reduction under the influence of each of the small and high doses of ATP.

Discussion

Numerous investigators have reported abnormalities in serum proteins, lipoproteins, and protein-bound carbohydrate in diabetes mellitus (9, 10). In diabetic rats there are profound changes in protein metabolism (4).

A comparison of serum-protein fractions between female control and alloxan-diabetic rats indicates a decrease in percentage albumin and an increase in percentage α_2, B and γ globulins. Total protein also decreased [*Peterson* (11)].

However, *Hendley* (12) found little or no change in total protein or in serum-protein fractions in male alloxan-diabetic rats as compared to normal rats 3 days after alloxan administration. *Peterson* (11) found that both male and female rats show the same electrophoretic changes following the production of alloxan diabetes. Nevertheless, in their work serum was obtained from alloxan-diabetic rats 3 weeks or more after injection of alloxan.

In the present work, in severe diabetic animals examined 48 hours after the administration of alloxan neither the total protein content nor the serum electrophoretic pattern showed any significant difference from the results of the control animals, though the blood glucose amounted to 530 ± 136 mg/100 ml. On the other hand, in the case of mild diabetes examined 10 days after alloxan and in which the blood glucose was 249 ± 125 mg/100 ml, there was a significant decrease in total protein and in serum albumin, while the globulin fractions remained virtually unaltered. The A/G ratio was therefore significantly decreased.

The difference in the changes in the serum protein and serum albumin in animals with severe and in those with mild diabetes are most probably not the result of the degree of the diabetic state only. But they are rather explained by the longer time interval of the uncontrolled diabetic state in the case of mild diabetes. The changes that might have occurred in severe diabetic rats after 10 days would have been higher.

In human beings, *Khattab* and *Danasoury* (1) found that many diabetic patients of long duration who were badly controlled, showed diminution of serum proteins, mostly hypo-albuminemia, and there was hyper α_2 globulinemia. They found also that no significant deviation from normal values of serum proteins was observed in cases below 4 years duration and also in mild controlled cases.

Interference with hepatic protein synthesis could readily lead to the development of a fatty liver (13). *Farber* (5) suggested that protein changes precede the fatty liver by a few hours.

In the present work the liver fat was significantly increased in case of severe diabetes, but not in mild diabetes. However, neither the total protein content nor the serum electrophoretic pattern showed any significant difference in severe diabetic animals, while in case of mild diabetes there was a significant decrease in total protein and in serum albumin. Thus in case of severe diabetes there is no direct evidence implicating impairment of protein synthesis as the major mechanism for the fatty liver produced by alloxan.

Also *Pottenger* (14) found that fatty liver developed in rats fed a diet containing 1% orotic acid without altering general liver protein synthesis.

Insulin lack may explain the marked increase in liver fat in severe diabetic rats in the present work. This is in agreement with the well-known observation of *Gibbs* and *Chaikoff* (15, 16) that the complete removal of insulin from an animal such as the dogs produces an intense fatty liver.

In the present work under the influence of two doses of 5 mg ATP to control and mild diabetic rats, the blood glucose was decreased. In a previous work in in-vivo experiments, we found that ATP increased the immunologically reactive insulin (IRI) after glucose stimulation (17). ATP increased also insulin secretion from the still functioning B-cells in the partially alloxan-diabetic rats.

The decrease in blood-glucose level in mild diabetic rats under the influence of ATP was associated with a significant reduction in the fat content of the liver. It has been shown that injections of ATP are able to prevent the fatty liver produced by other hepatotoxic drugs such as ethionine, ethanol, carbontetrachloride, and the antibiotic azaserine (18).

Total protein under the influence of both the high and low dosage of ATP treatment of mild diabetic rats showed no significant change from the control. Although in mild diabetes the total serum protein showed a significant decrease when compared with the control animals (table 2).

ATP in the larger doses resulted in a significant increase in serum albumin and a decrease in β-globulin and total globulins with consequent increase in A/G ratio (table 3). Thus in the case of serum albumin, ATP resulted in a partial recovery of the effect of alloxan. During the short treatment with ATP the serum albumin increased, but it did no attain the normal level of the control. Insulin treatment of diabetic patients raised the albumin to normal levels (1). Insulin also promotes protein synthesis causing reversal of the negative nitrogen balance (19).

Sacks (20) reported a decrease in the levels of ATP in the livers of alloxan-diabetic rats. A variety of agents, which produce a fatty liver, lead to decreased ATP concentrations in the liver and that injections of ATP are able to prevent both the fatty liver and the drop in ATP (18, 21).

One may suggest that intracellular depletion of ATP as a result of alloxan administration is the cause of the lack of insulin secretion from the pancreas, which lead to fatty liver as well as the impaired protein synthesis. Injection of ATP prevents the cellular depletion of ATP and promote insulin secretion.

Summary

A comparison of serum protein fractions (electrophoretic separation) between control and mild alloxan-diabetic rats examined 10 days after alloxan indicates a decrease in total protein, a decrease in percentage albumin accompanied by a decrease in A/G ratio.

In servere diabetic rats examined 48 hours after the administration of alloxan, there were no changes in total protein or in serum-protein fractions. The changes in the serum protein and serum albumin in mild diabetic cases are not the result of the degree of diabetes only. But they are rather explained by the longer time interval of the uncontrolled diabetic state.

ATP administered to mild diabetic rats produced the following changes: two injections of 5 mg per rat exhibit a lowering effect on the blood glucose, with a decrease in liver fat. ATP resulted also in a significant increase in serum albumin and a decrease in β-globulin with a consequent increase in the A/G ratio.

Comparison of the different protein fractions of male and female control rats did not show any significant difference. ATP administered to control animals did not alter the normal electrophoretic pattern.

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