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Effect of ATP on liver function tests in experimental diabetes

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With 3 figures and 4 tables

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Liver dysfunction may occasionally produce the diabetic syndrome or may be secondary to it.

A battery of hepatic tests was performed by *Leevy* et al. (17) in 380 patients with diabetes to determine the incidence of liver dysfunction and its relationship to etiology of manifest hyperglycemia. 38.9 per cent of those studied were found to have evidence of liver dysfunction. Gross dietary and insulin insufficiency were accompanied by liver dysfunction more frequently.

ATP could ameliorate insulin insufficiency in diabetes. In a previous work we found that ATP did increase insulin secretion in normal and alloxan diabetic rats. The values for the insulinogenic index or glucose: insulin ratio (G/I) suggested an insufficient insulin secretion in the diabetic group which became normal by ATP (28).

It is well established also that ATP plays a very important role in protein synthesis in lipoprotein synthesis and in phospholipid synthesis as well (22).

In alloxan diabetes, the increased protein catabolism is supposed to be met with by a decrease in plasma protein particularly of serum albumin (12). The liver is the only site of albumin synthesis.

However, albumin synthesis is not necessarily a sensitive measure of decreased liver function because the liver cells have some reserve capacity for albumin formation. Thus the normal liver may increase its albumin production up to 100 % when serum albumin is depressed due to external protein loss (14).

Thus the effect of ATP in case of alloxan diabetes on commonly used liver function tests besides serum albumin were studied in the present work.

Material and methods

All experiments were made on male albino rats (Sprague Dawley strain). The diet was an adequate one and was supplied ad libitum.

The animals were classified into three main groups.

Group I was made to study the effect of ATP on liver function tests of control rats.

Group II was made to study liver function in mild and severe alloxan diabetes.

Group III was made to study the effect of intramuscular injection of ATP in mild alloxan diabetic rats on liver function tests.

The liver function was investigated by determination of blood glucose, serum proteins, serum total cholesterol, serum bilirubin, serum alkaline phosphatase and thymol turbidity tests.

Alloxan (B.D.H.) was freshly prepared as a 5 % aqueous solution and was injected intraperitoneally after a 24 hour fasting. 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. 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. The experiments were made one day after the second injection of ATP and ten days after alloxan administration. The animals were killed by decapitation and their blood was collected. The separated serum was kept frozen for further analysis. In the meantime samples of the livers were taken for estimation of the fat content or for pathological examination.

Blood glucose was estimated by the iodometric titration method of *King* and *Wootton* (16). The fat content of the liver was estimated by the method of *Folch* (9), serum total cholesterol by a modification of the method of *Bloor* et al. (1), serum bilirubin by the method of *Malloy* and *Evelyn* (19). Total proteins and serum albumin, serum alkaline phosphatase and thymol turbidity were determined according to the methods described in *King* and *Wootton* (16).

Results

In table 1 the data obtained for the liver function tests viz. total protein, serum albumin, serum total cholesterol, serum alkaline phosphatase serum bilirubin and thymol turbidity of normal male rats are given.

The effect of exogenous ATP on normal rats is also given. Comparing it with the control experiments that received no ATP, it was found that none of the liver function tests of normal adult rats did undergo any significant change under the influence of both the small and high dose of ATP.

Effect of alloxan diabetes on liver function tests is shown in table 2. The level of the blood glucose and liver fat are included to assess the severity of the diabetic state.

In 48 hrs severe diabetic animals neither the total protein nor the serum albumin showed any significant difference from the results of the control animals. On the other hand, in the case of 10 days mild diabetes there was a significant decrease in total protein and in serum albumin. In mild diabetics the serum cholesterol was significantly increased. On the other hand, in severe diabetics the serum cholesterol showed an insignificant decrease compared to control rats. But it was significantly lower than in mild diabetes. The serum alkaline phosphatase and serum bilirubin were significantly increased in both the severe and mild diabetic states. While the thymol turbidity test was insignificantly changed in both states of diabetes.

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. ATP in the larger doses resulted in a significant increase in serum albumin, and decrease in total globulins with the resultant increase in A/G ratio (table 4).

The serum alkaline phosphatase exhibited a significant reduction under the influence of both the high and low dosage of ATP treatment, but it still

remained higher than that of the control. ATP in both doses showed no significant effect on the elevated serum bilirubin and cholesterol in alloxan diabetic rats, there was also no change in the thymol turbidity test.

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 only. The fat content of the liver showed a significant reduction under the influence of each of the small and high dose of ATP.

Histopathological examination of the liver revealed that alloxan diabetes produced structural changes which consisted of the presence of fat in the form of droplets scattered through the cytoplasm.

The administration of ATP resulted in regression of the microscopic structure towards the normal.

Discussion

Experimental diabetes has been very valuable in the study of the pathogenesis and prevention of diabetes in man. The morphological and functional changes in various organs that occur in diabetes or in the prediabetic state could be investigated in experimental animals.

Approximately 40 % of diabetics have moderate fatty infiltration with or without cirrhosis (15,17). With the development of fatty liver, hepatic function is impaired (26).

In the present work the hyperglycemia in case of alloxan diabetes was associated with a significant increase in liver fat, which liver changes were naturally more evident with severe diabetes than mild diabetes (table 2). Insulin lack may explain the marked increase in liver fat in severe diabetic animals (10).

The basic biochemical mechanism in the development of several types of fatty liver has been recognized in changes of nucleotide metabolism and in particular of ATP (8, 13, 20, 24, 32).

The shift in the oxidation reduction state of the mitochondria to a more reduced level and the decreased synthesis of ATP in the liver of these animals exhibited a fatty liver (11).

Talaat et al. (29) showed that ATP could protect the liver against ischaemic necrosis which would result after hepatic artery ligature in experimental animals. It is also reported that ATP diminished the fibrosis and favours regeneration of hepatic parenchyma in CCl_4 hepatotoxicity (23).

In the present work, ATP lowered the fat content of the liver of alloxan diabetic rats. The reduction of liver fat is due to its more rapid mobilisation from the liver which is a beneficial effect in the case of diabetes.
In this regard the basic biochemical mechanism has been recognized in a decreased lipoprotein synthesis by the liver, secondary to a disturbance in ATP metabolism (25).

Under the influence of ATP in mild diabetic rats there is a statistically significant decrease in the level of blood glucose when the dose of ATP was 2 × 5 mg (table 3).

The lowering effect of ATP on blood sugar may be due to increased insulin secretion. In a previous publication, the effect of ATP on i.v. glucose tolerance in normal and alloxan diabetic rats was investigated. We found

Table 1.

	Total proteins g%	Albumin g%	Serum cholesterol mg%	Alkaline phosphatase KAU	Serum bilirubin mg%	Thymol turbidity units
Control rats (9)	7.52 ± 0.38	3.11 ± 0.60	59.7 ± 11.3	18.5 ± 5.8	0.66 ± 0.21	1.52 ± 0.70
Control + ATP (6) 2 × 2.5 mg	7.54 ± 0.79	2.99 ± 0.37	64.0 ± 12.9	21.5 ± 5.9	0.75 ± 0.24	1.36 ± 0.43
P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
Control + ATP (6) 2 × 5 mg	7.62 ± 1.13	3.09 ± 0.50	62.0 ± 11.9	16.5 ± 6.4	0.71 ± 0.25	1.38 ± 0.42
P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Figures between paranthesis indicate number of animals.

Table 2.

	Bl Glucose mg/100 ml	Fat g% of wet liver wt.	Total protein g/100 ml	Albumin g/100 ml	Cholesterol mg/100 ml	Alkaline phosphatase KAU	Bilirubin mg/100 ml	Thymol turbidity units
Control rats (9)	91.3 ±10.4	4.01 ±1.01	7.52 ±0.38	3.11 ±0.60	59.7 ±11.3	18.5 ±5.8	0.66 ±0.21	1.52 ±0.70
Severe diabetics 48 hrs (6)	530 ±136	11.30 ±2.40	7.1 ±1.0	2.82 ±0.65	46.8 ±11.3	71.1 ±17.5	2.76 ±1.74	2.05 ±0.51
P	<0.05	<0.05	>0.05	>0.05	>0.05	<0.05	<0.05	>0.05
Mild diabetics 10 days (9)	249 ±125	6.40 ±0.90	6.55 ±0.47	2.21 ±0.18	82.5 ±15.1	57.1 ±10.4	1.19 ±0.37	1.31 ±0.42
P	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	>0.05

Table. 3.

	Bl Glucose mg/100 ml		Fat g% of wet liver wt.	Total protein g/100 ml	Albumin g/100 ml	Serum cholesterol mg/100 ml	Alkaline phosphatase KAU	Serum bilirubin mg/100 ml	Thymol turbidity units
	24 hrs	10 days							
Mild diabetes	214	238	6.88	6.55	2.21	82.5	57.1	1.19	1.31
10 days (9)	± 99	±110	±1.12	±0.47	±0.18	±15.1	±10.4	±0.37	±0.42
Diabetics	203	233	3.55	6.71	2.16	76.6	38.5	1.05	1.32
+ ATP (10)	± 97	±159	±0.17	±0.79	±0.41	±17.9	± 7.6	±0.39	±0.27
2 × 2.5 mg									
P	>0.05		<0.05	>0.05	>0.05	>0.05	<0.01	>0.05	>0.05
Diabetics	296	198	4.26	6.48	2.62	78.3	36.0	1.09	1.30
+ ATP (11)	±127	± 91	±0.31	±0.50	±0.42	±15.3	±6.5	±0.84	±0.36
2 × 5 mg									
P	<0.05		<0.05	>0.05	<0.05	>0.05	<0.01	>0.05	>0.05

Table 4.

	Total protein gm/100 ml	Albumin gm/100 ml	Total Globulins g%	A/G ratio
Control Rats (9)	7.52 ± 0.38	3.11 ± 0.60	4.18 ± 0.97	0.74 ± 0.13
Mild diabetes 10 days (9)	6.55 ± 0.47	2.21 ± 0.18	4.39 ± 0.38	0.51 ± 0.08
P	<0.05	<0.05	>0.05	<0.05
Diabetic+ATP (11) 2 × 5 mg	6.48 ± 0.50	2.62 ± 0.42	3.75 ± 0.44	0.69 ± 0.11
P	>0.05	<0.05	<0.05	<0.05

that the much faster clearance of blood sugar under the influence of ATP was associated with significant increase in insulin secretion (28).

None of the liver function tests, namely serum albumin, serum cholesterol, alkaline phosphatase, serum bilirubin and thymol turbidity test, of normal adult rats did undergo any significant change under the influence of both the small and high doses of ATP (table 1).

In severe diabetic animals neither the total protein nor the serum albumin showed any significant difference from the results of the control animals. On the other hand, in the case of mild diabetes there was a significant decrease in total protein and in serum albumin. The A/G ratio was therefore significantly decreased (table 4).

The differences in the changes in 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 diabetes. But they are rather explained by the longer time interval of the diabetic state in the case of mild diabetes, since the severely diabetic animals were sacrificed 48 hrs after the administration of alloxan while those of mild diabetes were sacrificed 10 days after alloxan. The albumin synthesis in case of 48 hrs severe diabetes was not a sensitive measure of decreased liver function, since as mentioned before the liver cells have some reserve capacity for albumin formation which may increase its albumin production up to 100 % when serum albumin is depressed due to external protein loss (14).

However, general agreement exists that the albumin level in the serum is inversely related to the severity of the liver functional impairment (21).

Total protein under the influence of both the high and low dosage of ATP treatment in mild diabetic rats showed no significant change from the results observed without ATP.

ATP in the larger doses resulted in a significant increase in serum albumin and a decrease in total globulin, with consequent increase in A/G ratio. Thus in the case of serum albumin in mild diabetes ATP resulted in a partial recovery of the effect of alloxan.

Liver function tests other than the glucostatic function and the serum albumin which were studied in alloxan diabetes are the levels of serum cholesterol, bilirubin, alkaline phosphatase and thymol turbidity.

In mild diabetes the serum cholesterol was significantly increased. On the other hand, in severe diabetics the serum cholesterol was significantly lower than in mild diabetes.

Vladimir et al. (31) studied the changes in content of cholesterol in mice turned diabetic by alloxan. They found that free cholesterol reaches its maximum increase 18 hours after alloxan administration. However *Chiape* and *Brenner* (5) found that total plasma cholesterol in diabetic animals deficient in essential fatty acids remained very near to those of the control. And in diabetics non-deficient in essential fatty acids, an increase in total plasma cholesterol level was observed.

The data of *Van Bruggen* et al (30) suggest that cholesterol synthesis remained unaltered in the alloxan diabetic preparations. However, in severe diabetes decrease in cholesterol occurs. Also *Brady* and *Gurin* (2) reported that the conversion of acetate to cholesterol by livers of alloxan diabetic rats and pancreatectomized cats is unimpaired. Although this process is inhibited when the diabetes is sufficiently prolonged and severe.

Under the influence of ATP, whether small or large doses used, the elevated cholesterol of mild diabetes remained significantly elevated and was not reduced. Although the fat content of the liver showed a significant reduction under the influence of each of the small and high dose of ATP (table 3). This may be due to more rapid mobilisation of fat from the liver under the influence of ATP.

It has been shown that in fatty liver produced in rats by ethionine administration, the injections of ATP are able to prevent the fatty liver (8). The administration of ATP also prevents the decrease in serum lipid levels which is so characteristic a concomitant of several forms of fatty liver (18).

This may explain the changes in serum cholesterol in severe and mild diabetes in the present work. Thus in severe diabetes the decreased serum cholesterol may be an indication for the development of fatty liver.

The serum alkaline phosphatase was significantly increased in both the severe and mild diabetic states which indicates that the changes in serum alkaline phosphatase occur much more rapidly than in serum protein.

Serum bilirubin was significantly increased in both the severe and mild diabetics which indicates that, like alkaline phosphatase, serum bilirubin has got a more rapid rate of turnover. The thymol turbidity test was insignificantly changed in both states of diabetes, indicating that it is a less sensitive test for the diabetic state.

Cantor et al. (3) found that adult rats injected subcutaneously with alloxan showed an initial decrease in serum alkaline phosphatase activity of about 50% followed by a gradual rise to more than three times the original value in 14 days. Insulin administration reduced sugar and alkaline phosphatase activity in the serum of alloxan diabetes.

In the present work, the serum alkaline phosphatase exhibited a significant reduction under the influence of both the high and low dosage of ATP treatment, but it still remained higher than that of the control.

ATP in both doses showed no significant effect on the elevated serum bilirubin in alloxan diabetic rats, there was also no change in the thymol turbidity test.

ATP can form complexes with ions as copper and magnesium (6). The chelation of metal ions by adenosine triphosphate could effect enzyme sys-

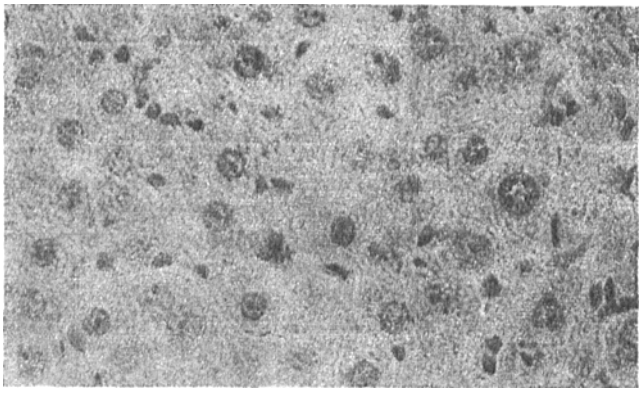

Fig. 1. Paraffin section stained by haematoxylin and eosin showing normal liver cells (× 450).

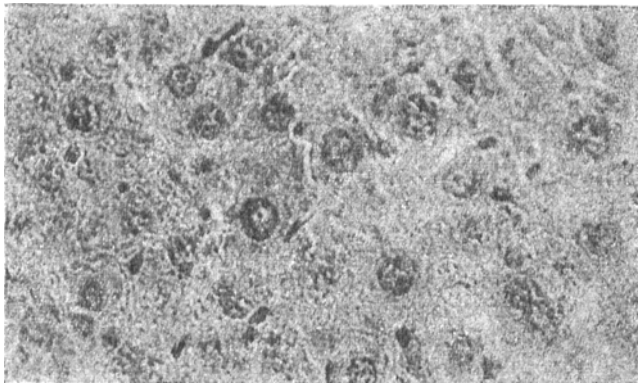

Fig. 3. Paraffin section stained by haematoxylin and eosin showing the return of liver cells of alloxan diabetics towards normal under the influence of ATP (× 450).

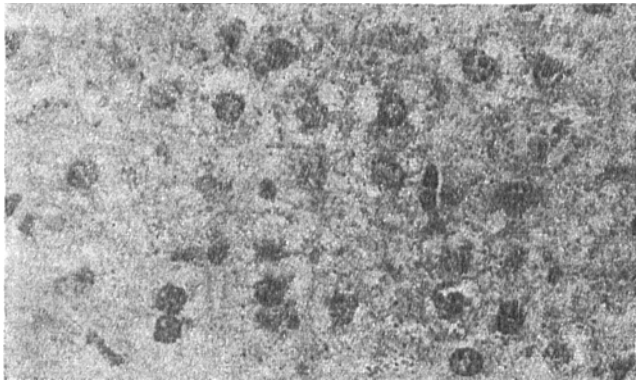

Fig. 2. Paraffin section stained by haematoxylin and eosin showing liver cell of alloxan diabetic rats containing minute and moderate sized globules of fat in the cytoplasm (× 450).

tems requiring metal ions as activators. Alkaline phosphatase is an enzyme systems of this type in which zinc and magnesium ions under suitable conditions have been shown to be activators (7).

Also there is a relationship between diet and alkaline phosphatase *Sukumaran* et al. (27) found that alkaline phosphatase in rats and man was lowered on fasting and increased by ingestion fat. According to *Cantor* et al. (4), the increase in serum alkaline phosphatase in alloxan diabetes, although nearly parallel to the increase in blood sugar, was not due to the hyperglycemia, but was related to the protein and especially the fat metabolism. Diets high in proteins and especially fats rapidly increased the phosphatase activity, and at the same time reduced the hyperglycemia in diabetic rats (4).

It is clear that some liver function tests as serum cholesterol (3) and alkaline phosphatase (4, 7, 27) depend on the nutritional status of the animal. For clinical purposes, repetition of the same test is indicated to inform about the progress of the disease.

The increase in serum alkaline phosphatase in case of alloxan diabetes in the present work may result from leakage out of damaged cells of the liver.

The decreased alkaline phosphatase under the influence of ATP may be due to the regenerative ability of ATP as proved from the pathological examination fig. 1, 2 and 3. The microscopical examination of the liver revealed that administration of ATP to alloxan diabetic rats had a beneficial effect. It resulted in disappearance of the fat globules from the liver cells with consequent amelioration of hepatic function.

Summary

Liver function tests were performed in severe and mild diabetic rats and under the influence of ATP.

In mild diabetics the serum cholesterol was significantly increased, while in severe diabetes the serum cholesterol was significantly lower than in mild diabetes.

The decreased serum cholesterol in severe diabetes may be an indication for the development of fatty liver.

The serum alkaline phosphatase and serum bilirubin were significantly increased in both the severe and mild diabetic states, while the thymol turbidity test was insignificantly changed in both states of diabetes.

Serum albumin was significantly decreased in 10 days mild diabetes, while it was insignificantly changed in 48 hrs severe diabetic animals.

The effect of ATP was investigated in mild diabetes. ATP resulted in a significant increase in serum albumin and a decrease in total globulins with the resultant increase in A/G ratio.

The serum alkaline phosphatase exhibited a significant reduction under the influence of ATP. The elevated cholesterol of mild diabetic rats remained significantly evelated and was not reduced by ATP, though the fat content of the liver showed a significant reduction. This may be due to more rapid mobilisation of fat from the liver under the influence of ATP.

ATP showed no significant effect on serum bilirubin and thymol turbidity test.

The histopathological examination of the liver revealed that administration of ATP to alloxan diabetic rats had a beneficial effect. It resulted in disappearrance of the fat globules from the liver cells.

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