Studies on Liver Function in Alloxan Diabetes

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It is an old recognized fact that diabetes mellitus has a close bearing on the metabolic function of the liver, often causing its disturbance. Recently minute investigations have been done on latent disturbance by various liver function tests, but the results arrived at by the researchers are not always in agreement. Reports in which influences of dietary treatment on liver function are pursued are very few.

On the other hand, many researchers acknowledged the pathohistological changes of liver in case of alloxan diabetes, but as shall be stated later, we have had no report giving satisfactory observations on liver function.

I made alloxan diabetes induced in rabbits and tried to inquire into the functioning of liver in its natural process and at the same time the effect upon the liver function of the standard diet, which had been given to some of the stricken rabbits.

EXPERIMENTAL

Method

Animals employed were adult male rabbits weighing 1.8-2.7 kg. when the experiment was begun. These animals were fed as much as they desired all through the period of experiment except for a short time of "standard diet." In good health they ate 130-150 g. of *tofukara* (bean-curd husk) and 80-100 g. of vegetables or greens per kg. of body weight per day, but 1.5-2.5 times as much when they suffered from diabetes. By the way the "standard diet" consists of 120 g. of *tofukara* and 60 g. of vegetables or greens per kg. per day.

Tests applied for liver function were sodium santonate test (S test) and azorubin S test (A-S test), the former being used as detoxicating test and the latter as excretory function test.

S test was applied after Takasugi-Miyamoto's method¹⁾; only to one same animal the same dosage of santonate as that administered in

the first test was always given regardless of the loss of its weight.

In A-S test always 2 cc. of 1% A-S solution was intravenously injected. The amount of this dye excreted in the urine after an hour was determined by using Dubosq's colorimeter and the percentage of the amount excreted to that injected was obtained.

After more than a week's acclimatization liver function tests were put to each animal to know its normal value. I obtained the average value of more than three successive tests where almost equal readings were got; then alloxan was administered.

To most of the animals a large dosage (190–200 mg. per kg. of body weight) and to a part a small dosage (100 mg. per kg.) of alloxan were intravenously injected each at a single time.

Blood sugar was determined through Fujita-Iwatake's method²⁾ in the morning fasting time of the subject.

For renal injury after injection examination was made by albuminuria and phenolsulfonphthalein test (PSP test) and when there was found any injury liver function tests were not carried out.

Results

1) Appearance of alloxan diabetes and their classification

Among the 32 rabbits to which alloxan had been administered, diabetes was observed to have appeared in 26 cases out of 28 with a large dose injected and 2 cases out of 4 given a small amount, the 4 others not responding.

It was 21 among these 32 that were with satisfaction employed for my observation of liver function. They are classified under the following 4 types for the convenience of consideration.

Type I (Cases 1–4): To this type belong those animals that suffered from serious diabetes, rapidly grew weak, and died in the early stage of the disease. In all the cases alloxan was administered in large amount.

Type II (Cases 5–12): In these cases permanent diabetes was induced. All the rabbits of this type were given a large dose except Case 11 into which a small dose was injected.

Type III (Cases 13–17): Under this type those animals fall which presented transitory diabetes. Glycosuria, which had been found in all the cases of this type, disappeared within 3 weeks. In 4 cases a large, and in 1 case a small quantity of alloxan had been administered.

Type IV (Cases 18–21): This type consists of 4 cases in which alloxan found no response. Two were given a large amount and the other two a small amount.

2) Duration of renal injury after alloxan administration

In the group injected with small amount renal injury was detected in neither of the 4 cases including Case 11 of Type II, but in the other with large amount 13 cases out of 17 (76.5%), the 2 cases of Type IV included, gave albuminuria. Albuminuria was most remarkably found about 24 hours after alloxan administration, occasionally accompanied by macroscopic hematuria; then speedily better on towards recovery, and disappeared in 2-10 days (in most cases 5 days).

According to PSP test the duration of excretory dysfunction of the kidney was in all cases corresponding to that of presence of albuminuria; only three cases required 1-3 days more to be recovered after the disappearance of albuminuria.

To say in addition, incidental injury in the kidney seemed to have hardly anything to do with the diabetes or its severity.

3) Liver function

a) *S test*

Type I. In all cases the output of the dye (santogenin) decreased remarkably (i.e. lower than 16% of normal value) on the occurrence of diabetes, decreasing more and more towards 0 as time went and in the 3rd or the 4th week after injection the diabetic animals died.

Type II. As shown in Table I and Fig. 1, the curves of output fell sharply simultaneously with the appearance of diabetes in all cases except Case 12 which escaped satisfactory observation in its early stage, arriving at their lowest values (8.3-31.1% of normal value) in the 1st or 2nd week. The curves thereafter rose again and culminated about 4 weeks after the administration. The readings at their uttermost points, however, are considerably lower than normal values except in Case 6.

In chronic diabetes extending beyond the 5th week, the curves, in so far as some special controlling treatment was not given, ran, generally in the sphere lower than normal level, almost in response to the severity of diabetes. In other words, the output increased towards normal level as diabetes improved (Cases 7, 8, 10 and 12), decreased off the level as it deteriorated (Cases 5, 6, 9 and 10), and further decreased towards 0 when the disease was critically serious (Cases 5, 6 and 9).

Type III. In all cases the output decreases and increases in a curve similar to that described by Type II. But in this type the lowest value is generally higher (18.3-61.1% of normal value) than in Type II, the curve not stopping rising about the 4th week till it reaches the normal level before the diabetes has been recovered.

Type IV. The curve of output of this type resembles that of Type III, but the lowest value reached in the 1st or 2nd week was generally far higher (32.9-77.1% of normal value) than that in Type III and re-

covery since then was expedite. Normal value was reached in all cases in the 2nd to 4th week.

TABLE I
Liver Function in Alloxan Diabetes

No. of cases	Type	Test*	Before alloxan (normal value)	After alloxan (weeks)									
				1	2	3	4	5	7	10	15	20	
5	II	B S A-S S	103 26.1 24	460↓ ×	352 28.3 × 4	292 28.2 4	295↓ 26.5 16	270↓ 26.0 20	320 25.9 11	418 25.6 15	425 29.1 11	186† 19.0 1	
6	II	B S A-S S	100 24.2 81	386↓ 23.0 × 65	342 25.5 21	292 25.6 51	352 24.6 88	352 24.9 64	331 23.8 19	205 3	†		
7	II	B S A-S S	106 28.5 96	324↓ 29.3 × 32	292 24.5 25	338 30.4 41	312 31.3 15	285	266 30.2 30	205 29.1 32	174 29.4 40	164 30.2 64	
8	II	B S A-S S	102 27.6 46	330↓ 29.3 × 10	344 32.5 29	334 30.3 20	244 25.4 22	202 26.7 28	216 30.2 36	184 30.6 53	178 32.5 29	150 32.2 29	
9	II	B S A-S S	112 28.7 253	414 29.7 × 65	386 29.7 21	291 27.1 28	284 23.8 25	23.7 2	265† 26.7 0				
10	II	B S A-S S	107 27.8 252	344 27.8 × 49	292 27.8 × 26	263 31.9 68	249 25.8 124	205 25.5 158	166 26.5 167	141 28.2 217	277† 35.2 99		
11	II	B S A-S S	110 32.1 45	341 40.6 16	282 36.0 30	237 26.8 35	277 25.2 21	246 25.8 34	278 32.5 38	280 41	284 32.6 40		
12	II	B S A-S S	104 27.7	365 31.5 ×	372 34.7 5	284 29.8	153 27.5 36			191 27.2 37	261 27.8 32	219 62	
13	III	B S A-S S	107 30.2 82	310 31.7 × 15	230 31.6 22	216 30.2 31	146 30.8 43	144 30.5 57	131 30.4 81				
19	IV	B S A-S S	109 27.5 70	146 27.8	108 27.2 23	112 27.6 54	103 27.6 72						

* BS blood sugar (mg.%), A-S azorubin-S test (%), S sodium santionate test.

↓ insulin administration. × renal injury. † died of serious disease. ‡ died by accident.

b) A-S test

Type I. The percentage of output in the 1st or 2nd week increased

in all cases over the normal value by 2-16%.

Type II. On the occurrence of diabetes reading of the percentage increased, as is shown in Table I and Fig. 1, except in Case 7, reaching the highest value (1-8.5% higher than normal) in the 1st or 2nd week, then decreased till it came to the lowest value almost in the 4th week. Here, the readings of Cases 5, 6 and 12 were directly restored to normal, while those of Cases 8, 9, 10 and 11 decreased to lower than the normal value, then gradually increasing again to normal by the 7th week. In other words, the lowest values of the former three were their respective normal values, while those the latter four arrived at were lower than normal. Case 7 alone, taking its own course, culminated in the 4th week and then decreased, still showing higher values than normal.

In the chronic diabetic stage Cases 5 and 10 showed a significant increase of reading when the diabetes was aggravated, but generally speaking, other cases, in spite of rather manifest diabetes persisting, presented most often normal values except for Cases 7 and 8 which still had readings higher than normal in their improving stage when glycosuria had disappeared. Further to say, Case 5 showed remarkably low reading, by far lower than normal, when the disease was serious.

Type III. When diabetes was produced, in one case (Case 13, Table I) a slight and in 2 other cases a mild decrease were exhibited of their readings; they returned to normal in the 3rd to 5th week when the diabetes improved. In another case no change took place. The other one continued to increase mildly from the beginning of the disease till after recovery.

Type IV. In 2 cases the reading showed no change. In another it decreased mildly till the 2nd week, afterwards became normal. In the other one an increase in the reading of 8.5% was shown in the 1st week, after the 2nd week a mild increase followed.

c) Effect of standard diet upon liver function

As experimental materials were employed 4 diabetic rabbits of Type II of 50 to 150 days after alloxan administration and 3 uninjected rabbits for control.

TABLE II
Influence of Standard Diet upon Liver Function

No. of cases	Duration of standard diet	S test			A-S test			Blood sugar mg.%	
		before alloxan	Standard diet		before alloxan	Standard diet		Standard diet	
			before	after		before	after	before	after
5	4	24	11	43	26.1	29.1	26.9	422	428
	9			71			21.2		329
6	4	81	8	31	24.2	23.8	18.0	302	268
	9			94					268
7a*	5	96	22	56	28.5	30.0	26.5	268	205
	15			83			26.5		205
7b*	3	96	64	64	28.5	28.4	28.0	174	93
	10			94			24.1		93
11a*	8	45	38	107	32.1	32.5	22.1	278	188
11b*	4	45	41	56	32.1	32.5	24.6	280	181
	11			108			23.4		181
22 †	10		70	76		23.5	23.7		
23 †	10		44	39		26.5	26.8		

* a and b signify the 1st and 2nd experiments respectively of the same animal.

† control case.

Effect of the standard diet on liver function was without any exceptions observed remarkably in diabetic cases, though not in control cases at all (Table II).

S test. Before putting them upon the standard diet their output had in all cases decreased, though in different degrees, but about the 4th day of the diet it obviously increased except the mild Case 7b, and between the 8th and 15th day the increase was so great as to exceed the normal value except for Case 7.

A-S test. The reading before the standard diet was begun was normal or increased to a mild degree. After the diet was given for 8-15 days it conspicuously decreased to show a value lower than normal in all cases.

SUMMARY AND DISCUSSION

In respect to the liver lesions in the early stage of alloxan diabetes there have been a number of experimental results published by various researchers.

In several kinds of animals hepatic injury is often observed after alloxan administration. A report³⁾ is made that in this case the severity is very different according to different species. As to the relation between hepatic injury and dosage of alloxan Bailey⁴⁾ emphasizes that the larger the dosage that has been administered, the greater the toxic effect in all species. On the other hand, Lukens⁵⁾ reports "the incidence and severity of hepatic involvement could not be related to the dosage of alloxan."

To turn our eyes on the reports on hepatic injury accompanying the diabetes in rabbits, Hard et al.⁶⁾ observed no histological change after administering 50-100 mg. of alloxan per kg. of body weight. Bailey et al.³⁾ and Okamoto⁷⁾ observed slight degeneration with 200 mg. injected; Kendell et al.⁸⁾ reported fairly serious lesion caused by 125 mg. of alloxan per kg. In my observation of alloxan diabetes dysfunction of liver was noticed in all cases regardless of the dosage of alloxan. It was characteristic especially in Type II. The disturbance of liver in Type II was detected by both S test and A-S test, though slightly by the latter, simultaneously with the appearance of diabetes. This injury reached its maximum in the 1st or 2nd week, then gradually grew improved and about in the 4th week was at its minimum. In this stage A-S test proved the recovery of the excretory function of the liver to normal or inferred its hyperfunction, but S test showed the disturbance of detoxicating function was still somewhat serious.

On the other hand, in Type IV disturbance was discovered in all cases by S test and in half of the cases by A-S test. It generally dis-

appeared by the 4th week. This disturbance in Type IV was caused by alloxan alone, as there was no diabetes induced.

In respect to liver disturbance Type II and Type IV are found to hold parallel course in decreasing or increasing, especially through S test. But the disturbance in the former type is much severer and in the 4th week is cleanly told by S test to still persist, though by this time that in the latter has completely disappeared, and afterwards shows no great change. This comparison leads to conclude that the remarkable disturbance observed in Type II in the 1st or 2nd week had not been produced by alloxan alone, but with collaboration from diabetes and that the disturbance in the same type noticed after the 4th week was that caused by diabetes which was made exposed in consequence of disappearance of alloxan influences.

As for other types, Type I is the extreme case of Type II, where the serious diabetes made the liver dysfunction severe enough to cause the death of the stricken animals. Type III is, as it was, the abortive Type II. In this type disturbance shown by S test ran an intermediate course of Type II and Type IV to recovery, not stopping in the 4th week as in Type II, because there was improvement in diabetes in the preceding 2nd or 3rd week, and spending more time in returning to normal than in Type IV.

As for the liver function in chronic diabetes, influences of alloxan were, as stated before, gone out by about the 4th week. Therefore, the dysfunction of liver observed after the 5th week, was, unless complicated, caused by diabetes.

On the histological examination of rabbits Bailey et al.,⁸⁾ Duff et al.⁹⁾ and Okamoto⁷⁾ recognized no abnormality of liver, and Lewis et al.¹⁰⁾ told of the same plasma protein changes (i.e. a marked increase in the β -globulin and decrease in albumin levels) in rabbits as in human diabetes.

According to the results of my observation on uncontrolled alloxan diabetes, S test proved there almost always to be disturbance in all cases and there to be a certain relation between the severity of disturbance and that of diabetes. But in A-S test disturbance was detected only in half of the cases, the disturbance not always being related to the severity of diabetes.

As regards liver dysfunction of human diabetes Kaito¹¹⁾ found positive result in 70% of 45 cases (54% of mild and 89% of serious cases) by S test and in 65% of 43 cases (there is no statistically significant difference between the mild and serious cases) by A-S test. Kōga¹²⁾ reports positive S test in 45.5% of 22 cases, Nakagawa et al.¹³⁾ positive A-S test in 2 cases out of 4.

It gives us a deep interest to know that my experiment of alloxan diabetes has reached substantially the same result as of human diabetes.

Now for the effect of "standard diet" Kaito¹¹⁾ examined by S test the influences of dietary treatment on liver function of human diabetics and observed improvement in 6 out of 13 mild and 6 out of 10 serious cases. I made researches as to the effect of standard diet upon liver function in alloxan diabetes by both S test and A-S test and not only found improvement in all cases, but obtained such results as to suggest hyperfunction of liver in most cases. This effect gradually strengthened till about 8 days from the beginning of the treatment, but thereafter seemed not to make any remarkable changes.

When the standard diet was followed by free diet, the effect was gone off almost in a week and the liver function resumed the level before the standard diet.

CONCLUSIONS

1. In the early stage of alloxan diabetes there was remarkable dysfunction of liver observed which was caused by diabetes and, further, strongly influenced by alloxan, influence of the latter acutely passing away.

2. Dysfunction of the liver in chronic alloxan diabetes was detected in all cases by santonin test and in half of the cases by azorubin S test.

3. Liver function in alloxan diabetes was remarkably improved by the treatment of standard diet.

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