GLUCOSE TOLERANCE IN RABBITS FOLLOWING REPEATED SMALL DOSES OF ALLOXAN*

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Alloxan has generally been acknowledged to have strong diabetogenic action on many species of experimental animals when administered by an intravenous, intraperitoneal or subcutaneous route in a single sufficiently large dose. It has been reported that a single intravenous injection of 100~125 mg per kg of alloxan induced severe and permanent diabetes in rabbits by causing necrosis of beta cell in pancreatic islets (Kendell *et al.*, 1945; Kobayashi *et al.*, 1951; Kosuge, 1958). Furthermore, the effect of small repeated doses of alloxan on the function capacity of the islets tissue was generally believed to be depressive (Thorogood, 1944; Kennedy and Lukens, 1944; Shipley and Rannefeld, 1945). However, almost all the investigators have used, for the diagnosis of diabetic state, fasting blood sugar level, amount of urinary sugar, or, occasionally, oral or intraperitoneal glucose tolerance test. It does not seem to be sufficient to determine the phase of carbohydrate metabolism in animal only by these tests. Recently, some investigators (Goldberg and Luft, 1948; Moyer and Womack, 1950) have illustrated the excellency of intravenous glucose tolerance test in diagnostic value on diabetes mellitus. The author and his co-workers investigated blood sugar curves from animals which were intravenously injected with 1 g per kg of glucose, and reported that the curve which was considered to represent the composite result of two different factors, the tolerance and the humping curves, seemed to express relatively well glucose assimilating ability when it could be analysed with regard to the tolerance curve (Takeuchi *et al.*, 1957; Fukushima, 1957 a and b; Tobe, 1957). In this viewpoint, it seems to be necessary to determine whether there is the functional response of the pancreatic islets to the intravenous administration of small doses of alloxan. The present communication describes the experiments in rabbits treated daily with 1~20 mg per kg of alloxan.

MATERIALS AND METHODS

Experimental animals: Twenty male and female rabbits weighing 2.0~2.5 kg were used. They were housed in separated cages and were maintained on a constant daily ration consisting of 300 g of bean curd residue and 100 g carrot. After the first intravenous glucose tolerance test, they were divided into the three groups of 6 rabbits each (group I, II and III) and other group (group IV) consisted of 4 animals. Group I were non-treated controls, and groups II and

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III were treated daily with 1 mg and 10 mg per kg of alloxan, respectively. The 2nd test was carried out at 5 days, and the 3rd at 10 days after the start of repeated administrations. Group IV consisting of two animals selected from group II and other two treated daily with 20 mg per kg was retested at the 10th and the 30th days.

Alloxan was injected in left ear vein as 100-fold dilution in physiological saline at 1 hr. before meal. Glucose tolerance test, based on Fukushima's method (1957 a) was carried out at 20 hrs. after the last alloxan administration. Examination for sugar in 24 hrs. urine was made by Nylander's test.

RESULTS

In the first place, the effect of alloxan administration in a small dose on blood sugar level was investigated with six normal rabbits. It has been reported that blood sugar level of animal treated with a sufficiently large dose of alloxan altered through three phases; initial hyperglycemic, hypoglycemic, and continuous hyperglycemic phases (Goldner and Gomori, 1946), but in the present experiments it did not fluctuate over the limits of physiological pattern following a single injection of 1~10 mg per kg of alloxan (Fig. 1).

Fig. 1. Mean blood sugar values and standard errors of means from each group following a single injection of alloxan in small dose. ↓ : Injection of alloxan

In the next place, the fasting blood sugar level, that is, the level before glucose injection, was found scarcely increased in rabbits treated with repeated administrations even after thirty days, ranging 116 ± 21 mg %. In some animals of group III it was slightly increased to about 140 mg % after 5 days (Table 1). But there was not any significant difference, and in all cases sugar was never detected in urine. On the other hand, some noticeable alterations were found in the results of glucose tolerance tests.

Table 1. Effect of repeated administrations of alloxan for 10 days on blood sugar values following intravenous glucose injection

Treatment (No. of Rabbits)	Time of bleeding (mins.)	Mean blood sugar level (mg % $\pm$ S.E.)		
		Before alloxan administration	5th day	10th day
Group I non-treated (6)	Pre-inj.	97 ± 14		104 ± 8
	After			
	3	392 ± 52		401 ± 62
	23	321 ± 42		325 ± 55
	43	262 ± 51		281 ± 48
	63	221 ± 42		230 ± 35
	83	185 ± 23		182 ± 28
	103	149 ± 18		143 ± 25
	123	129 ± 19		121 ± 22
Group II 1 mg per kg per day of alloxan (6)	143	112 ± 8		118 ± 17
	Pre-inj.	107 ± 16	101 ± 11	107 ± 12
	After			
	3	342 ± 10	341 ± 8	352 ± 11
	23	294 ± 48	279 ± 21	251 ± 16
	43	252 ± 36	211 ± 15	184 ± 13
	63	194 ± 35	189 ± 26	136 ± 15
	83	172 ± 29	163 ± 24	110 ± 6
	103	143 ± 22	123 ± 24	109 ± 9
Group III 10 mg per kg per day of alloxan (6)	124	120 ± 21	109 ± 26	104 ± 13
	143	102 ± 19	104 ± 18	99 ± 8
	Pre-inj.	102 ± 14	148 ± 39	113 ± 11
	After			
	3	386 ± 27	401 ± 31	422 ± 50
	23	274 ± 45	279 ± 41	354 ± 48
	43	242 ± 48	257 ± 38	291 ± 25
	63	182 ± 46	220 ± 42	255 ± 18
	83	152 ± 51	171 ± 35	216 ± 20
	103	130 ± 32	142 ± 37	189 ± 28
	123	117 ± 28	121 ± 17	154 ± 17
	143	110 ± 21	119 ± 19	145 ± 7

Group II (treated daily with 1 mg per kg): The slope of intravenous glucose tolerance curve calculated from mean blood sugar values from this group was generally steeper after repeated administrations than before. This tendency was more remarkable at the 10th day than at the 5th. That is to say, times of recovery to the pre-injection level at the 5th and the 10th days, were 103 and 83 mins., 20 or 40 mins. shorter, respectively, than before the treatment or in non-treated rabbits. Since the variances of mean were relatively large, as shown in Table 1, there were not significant differences between the regression lines from

mean blood sugar values. But since the slope for animal was stable (Fukushima, 1957) and time of recovery for group I was always 123 mins. in the present experiments, it can be considered to have shortened by about 40 mins.

It was worthy of notice not only that the descending rate was high, but also that the standard errors of means were small. While standard errors in control groups were ± 30 mg per cent or more, those in group II at the 10th day were only $\pm 10 \sim \pm 20$ mg per cent as shown in Table 1. For the purpose of study on a cause of these standard errors some investigations of the curve-type from each individual were made. Figure 2 illustrates that the administrations of 1 mg per kg per day

Fig. 2. Intravenous glucose tolerance curves. Results of treating rabbits daily with 1 mg per kg of alloxan for 5 days. $\downarrow$: Injection of 1 g per kg of glucose

of alloxan for 5 days, transformed curve-types of three cases: the exponential curve (A) into the humped curve, the humped curve (B) into the sigmoid and the sigmoid (C) into the exponential. On the other hand, Figure 3 shows that all the curves from rabbits treated for 10 days are transformed into the exponential. Some cases were found to belong to the exponential. Some cases were found to belong to the exponential type both in the 1st and the 2nd tests, but the descending rate always higher in the latter than in the former.

Fig. 3. Intravenous glucose tolerance curves. Results treating rabbits daily with 1 mg per kg of alloxan for 10 days. ↓ : Injection of 1 mg per kg glucose

Group III (treated daily with 10 mg per kg): It was of interest that the slope of intravenous glucose tolerance curves from this group was generally slower contrary to group II. Table 1 also shows that it takes over 143 mins. to return to the pre-injection level after 10 days, though difference of the recovery times between after 5 days and in the control group is not clear. As shown in Figure 4, the curve-type from this group seems to be not evidently transformed, two curves in (A) belong to the humped curve type and each two curves in (B) and in (C) to the sigmoid and exponential one, respectively. Accordingly, the values of standard error were invariable all through the experimental period. All these alterations of glucose tolerance curves are considered to be similar to those in diabetic state. However, the rise of fasting blood sugar level or sugar in urine was not found in these animals.

To eliminate any transitory variation in glucose tolerance, two rabbits selected from Group 1 and other two treated daily with 20 mg per kg, were tested again at 30 days after. The data in Table 2 clearly indicates that the alterations of glucose tolerance were not transitory and not progressive.

Fig. 4. Intravenous glucose tolerance curves. Results of treating rabbits daily with 10 mg per kg of alloxan for 10 days. ↓ : Injection of 1 g per kg of glucose

Table 2. Effect of repeated administrations of alloxan for 30 days on blood sugar values following intravenous glucose injection

Treatment (No. of Rabbits)	Time of bleeding (mins.)	Mean blood sugar level (mg % $\pm$ S.E.)		
		Before alloxan administration	10th day	30th day
1 mg per kg per day of alloxan (2)	Pre-inj.	98 $\pm$ 5	99 $\pm$ 2	95 $\pm$ 3
	After			
	3	352 $\pm$ 22	367 $\pm$ 11	367 $\pm$ 14
	23	287 $\pm$ 15	262 $\pm$ 8	252 $\pm$ 7
	43	266 $\pm$ 8	195 $\pm$ 8	188 $\pm$ 8
	63	215 $\pm$ 11	142 $\pm$ 5	134 $\pm$ 3
	83	157 $\pm$ 3	97 $\pm$ 4	74 $\pm$ 8
	103	122 $\pm$ 7	70 $\pm$ 5	87 $\pm$ 10
	123	99 $\pm$ 7	83 $\pm$ 7	103 $\pm$ 16
	143	73 $\pm$ 12	110 $\pm$ 2	105 $\pm$ 13
20 mg per kg per day of alloxan (2)	Pre-inj.	93 $\pm$ 2	115 $\pm$ 8	112 $\pm$ 10
	After			
	3	365 $\pm$ 18	370 $\pm$ 21	388 $\pm$ 14
	23	268 $\pm$ 5	352 $\pm$ 18	342 $\pm$ 11
	43	201 $\pm$ 22	306 $\pm$ 8	306 $\pm$ 8
	63	161 $\pm$ 13	268 $\pm$ 7	259 $\pm$ 10
	83	146 $\pm$ 15	196 $\pm$ 12	219 $\pm$ 8
	103	134 $\pm$ 7	188 $\pm$ 10	174 $\pm$ 9
	123	117 $\pm$ 6	160 $\pm$ 7	151 $\pm$ 11
	143	112 $\pm$ 2	148 $\pm$ 6	142 $\pm$ 10
			148 $\pm$ 5	141 $\pm$ 4

DISCUSSION

It has been shown conclusively that a single intravenous injection of a large dose of alloxan (over 100 mg per kg) produced severe diabetes in rabbits (Kosuge, 1958). However, the effect of small repeated doses of alloxan is not so certain. The difficulties to ascertain pharmacological action of alloxan seem to be due to two important obstructions, chemical instability of alloxan, and the low sensitivity of test to detect abnormality of carbohydrate metabolism. To overcome the former an animal must be given a pure sample by intravenous injection without damage to the surrounding subcutaneous tissue. And to remove the latter the intravenous glucose tolerance test must be used. Comparison of results by many investigators shows that effect of intravenous administration of alloxan is the most stable and the intravenous glucose tolerance test the most suitable of all. Namely, while Torogood (1944) found that only four out of six rats developed glycosuria when subcutaneously injected with alloxan in a daily dose of 50 mg per kg, Shipley and Rannefeld (1945) reported that all of 14 rats produced diabetic tolerance to glucose in oral glucose administration test when intravenously injected with 25 mg per kg. In the present experiments the intravenous tolerance curves from all of six rabbits treated with 10 mg per kg per day of alloxan had diabetic nature. Fifty mg per kg per day intravenously given to rabbits by Kennedy and Lukens (1944) produced glycosuria, but 25 mg per kg per day did not. Of course, there are some investigators who have doubted the usefulness of intravenous glucose tolerance test in which the glucose has been given in a single rapid injection. Jokipii and Turpeinen (1954) concluded that such a method can hardly be regarded as a quite physiological one. On the other hand, Takeuchi *et al.* (1957) doubted the possibility to compare each individual, since the tolerance curves from dogs and rabbits conditioned by them were not uniform in shape. However, Fukushima (1957 b) introduced a tentative formula to analyse atypical curve from normal rabbits. At any rate it may be possible to compare each other if curves can be analysed into two components, namely, the tolerance and the humping curves.

The fact that the intravenous glucose tolerance curves from rabbits treated daily with 1 mg per kg of alloxan for 10 days descended more rapidly was very interesting for pharmacological study on its action, since it had never been reported to have stimulating effect on glucose assimilating ability in animals. In group II in the present experiment, time for recovery to pre-injection level was shortened by 40 mins. and all the curves were transformed into exponential type after alloxan administrations. Furthermore, there were some cases, which gave exponential curves in control test and gave again steeper exponential one in the 2nd or the 3rd test. According to Takeuchi *et al.* (1957), Fukushima (1957) and Tobe (1957), these alterations might be led by hyperinsulinism or hypoadrenalism. Hughes and Hughes (1944) supposed from their studies that prolonged repeated administrations of small doses of alloxan led to the destruction of only older beta cells and accelerated maturation of the younger cells. In that case, it seems to be possible that hyperinsulinemia is induced by over-compensation in maturation process of beta cells. Recently, Ikeda *et al.* (1957) reported an interesting view. They studied on the histological findings in pancreatic islets of rabbits treated

daily with 1~10 mg per kg for 10 days, and found that proliferation of islets was characteristic under those conditions. Namely, when 1 mg per kg doses were administered, the number of beta cells increased and when 10 mg per kg doses were administered, increase of alpha cells was remarkable. If alloxan in an adequate dose stimulates the function of beta cells, the increase in glucose tolerance of rabbits in group II in the present experiments is quite reasonable. But why was observed decrease in glucose tolerance in group III? Is proliferation of alpha cells induced by direct stimulation of alloxan or not? How does hyperfunction of alpha cells influence on general carbohydrate metabolism? Naturally, the complete answers to these problems can not be given from the present data. The fact that the descending curve of blood sugar in group III became slow without transformation of curve-type might suggest a possibility that the low descending rate can be led by hyperfunction of alpha cells, since the curve-types in diabetic state induced by destruction of beta cells are different from them. There are always evident humped curves as reported in previous paper (Kosuge, 1958). But Abe (1956) described that glucagon, which is believed to be a hyperglycemic factor in alpha cells, does not lower the glucose tolerance in animals. In either event, the author believes that the difference between diabetic states induced by a single administration of a sufficiently large dose of alloxan and repeated administrations of a small dose is not due to the specific action of alloxan on pancreatic islets, but to its toxic effect on general parenchymatous organs. Perhaps, effect of alloxan administration in a large dose would be so strong that not only severe diabetes would develop, but also non-specific response, as it were *stress*, would be manifested. As a consequence, evident humping is considered to be found in glucose tolerance curve. It is concluded from these that, in spite of absence of the evident humping or the over-humping, the slow descending of glucose tolerance curve from rabbits treated daily with 10 mg per kg or more indicates decrease of tolerance to glucose caused by hypofunction of beta cells.

SUMMARY

The effect of small repeated intravenous administrations of alloxan (1~20 mg per kg per day) on glucose tolerance was examined in 20 rabbits.

The intravenous glucose tolerance curves from 6 rabbits intravenously given 1 mg per kg per day of alloxan for 5 or 10 days, were steeper than those from controls. And those from 3 rabbits treated daily with 10~20 mg per kg of alloxan for 10 days or more showed lower slope, resembling the diabetic one. These tendencies were neither transitory nor progressive.

Mechanism of action of alloxan was discussed briefly. It was concluded that 1 mg per kg per day doses of alloxan given intravenously, stimulated the function of beta cells and 10 mg per kg per day doses or more produced a damage to beta cells in pancreatic islets of rabbits.

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