STUDIES ON GLYCOGEN NEPHROSIS IN ALLOXAN-TREATED DIABETIC RATS

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Since 1883 (1) when Ehrlich first demonstrated the renal tubular changes in diabetic patients to be glycogen nephrosis, this lesion has been accepted as the principal anatomic evidence of diabetes mellitus at postmortem examination. However, at present there is some disagreement as to whether glycogen nephrosis is a specific lesion of diabetes mellitus (2) or merely an anatomic reflection of glycosuria (3).

With the advent of insulin therapy a definite decrease in both the incidence and severity of glycogen nephrosis in diabetic patients was noted at autopsy (4). It has been assumed therefore that the appearance of this substance in the renal tubular epithelium of diabetic patients must be related to the severity of glycosuria. This impression has been borne out as progressive improvement in the control of diabetic patients has made glycogen nephrosis an increasingly uncommon finding. In a series of postmortem examinations on diabetic patients performed from 1932 to 1942 (2) only a 42 per cent incidence of glycogen nephrosis was found, in contrast to the pre-insulin finding of glycogen nephrosis in practically every kidney of diabetics. Save for these morphologic observations little is known about the cause of this lesion.

With the introduction of the drug, alloxan, as a medium of reproducing in animals diabetic lesions comparable to those of humans with diabetes mellitus, it became possible to study the development of glycogen nephrosis. Specifically, it was our desire to investigate the following aspects of glycogen nephrosis in experimentally induced diabetes: (*a*) The frequency of its occurrence in a series of diabetic rats. (*b*) The rapidity with which it develops following the onset of diabetes. (*c*) The existence of any correlation between the occurrence of glycogen nephrosis and the initial postinjection levels of hyperglycemia. (*d*) The relationship of the incidence of glycogen nephrosis to the maximum blood sugar levels attained by individual rats. (*e*) The relationship of the occurrence of glycogen nephrosis to the terminal blood sugar levels. (*f*) Whether glycogen nephrosis as it occurs in diabetic rats is a reversible lesion.

Material and Methods

All studies were performed on young, 180 to 220 gm. albino male and female rats. The method used for the production of the diabetes in these animals varied from the administration of one massive dose of alloxan monohydrate to the administration of repeated small doses

over a period of time. All injections were given subcutaneously. These technics were employed in an effort to obtain many different levels of hyperglycemia. The largest single dose used was 200 mg. of alloxan per kilo of body weight. The smallest was 140 mg. per kilo of body weight. Using these smaller doses it was possible by the administration of multiple injections to raise repeatedly the level of the blood sugar. If the individual rat responded at all to the initial injection of alloxan, subsequent injections would produce successive increases in the hyperglycemia until the desired level was attained.

The rats were fed a diet of commercial rat pellets and water *ad libitum*. Fifteen control animals receiving no alloxan were maintained under identical conditions.

The animals were sacrificed at times of election by a blow on the head, and sections of kidney, liver, and pancreas were fixed as routine in Zenker's solution, 10 per cent formalin, and absolute alcohol. Slides of all tissues were prepared with phloxine methylene blue stains. Best's carmine stain was used on the kidney sections to demonstrate the presence of glycogen (5). The severity of the diabetes was followed by repeated blood and urine sugar determinations, with occasional acetone determinations. The Folin-Malmros blood sugar micromethod (6) was used on the tail blood samples, and a routine Benedict's qualitative test was used on the urine for the demonstration of glycosuria.

RESULTS

A total of two hundred and seven rats were injected with alloxan, of which seventy-six, or 37 per cent, survived the immediate postinjection period. Only three of these seventy-six surviving rats failed to develop hyperglycemia, that is, levels of blood sugar over 150 mg. per cent. With regard to the three alloxan-resistant rats, it was found that if the animals failed to react to the initial dose of alloxan, repeated larger doses would also have no diabetogenic effect. Some of the diabetic rats were not autopsied soon enough after death to obtain satisfactorily fixed tissues and hence only sixty-two animals are reported upon in this study, of which three are the previously mentioned alloxan-resistant, non-diabetic rats.

Using the previously described technics, blood sugar values from the minimum hyperglycemic level of 150 mg. per cent up to a maximum level of 1200 mg. per cent were achieved. This last value was so unexpected that blood samples were sent to an independent laboratory for corroboration and were found to be correct.

The periods of observation of these diabetic rats, beginning at the time of the initial injection of alloxan, varied from 5 days to 32 weeks. Table I indicates the distribution and height of the terminal blood sugar levels as they related to to the length of the diabetic period in individual rats. It can be seen from this table that most of the diabetic rats died with terminal sugar levels between 250 to 499 mg. per cent, with the highest level 1200 mg. per cent. For the most part the animals were sacrificed at the end of a 6 week period of observation. However, a few rats were maintained for as long as 32 weeks.

Glycogen stains on the kidneys of the fifty-nine diabetic animals revealed nephrosis in twenty-six, or 44.0 per cent. The glycogen was found in the loop of Henle and to a lesser degree in the convoluted tubules. No glycogen was

demonstrated within the glomeruli. No lesions suggestive of intercapillary glomerulosclerosis were noted.

In an effort to determine how rapidly glycogen nephrosis becomes anatomically evident after the onset of diabetes, a group of eight rats were sacrificed between 5 to 9 days after the administration of a single dose of alloxan. Table

TABLE I

Distribution of Rats in Relation to Their Terminal Blood Sugar Levels and Duration of Diabetes

Terminal blood sugar levels	Time of experimental diabetes, *wks.*									
	1–2	2–4	4–6	6–8	8–10	10–12	13–14	—	32	Total
mg. per cent										
50–149		1	1	1						3
150–249	2	1	2	2	5					12
250–349	3	5	1	2	6					17
350–449	6	8	2		1					17
450–549	2	4	1	1						8
550–649		1	1				1		1	4
1200	1									1
Total.....	14	20	8	6	12		1		1	

TABLE II

Speed of Development of Glycogen Nephrosis

Rat No.	Duration of diabetes	Blood sugar	Glycogen in kidneys
	days	*mg. per 100 cc. blood*	
19	5	364	0
21	5	320	0
20	5	400	±
54	6	1200	+++
22	8	420	0
23	8	302	0
42	9	254	0
33	9	527	+++

II demonstrates the development of the glycogen nephrosis within these time intervals in relation to the terminal blood sugar levels of the individual rat. In a general way, it can be observed that the development of glycogen nephrosis, as indicated by histologic methods, is a function of the severity of the hyperglycemia and its duration.

During the period of observation of the diabetic rats it was noted that some of the animals receiving an initial single injection of alloxan continued to become more severely diabetic with blood sugar levels rising progressively throughout the experimental period, while other rats also receiving a single injection after

an initial response showed progressively falling sugar levels, some becoming apparently non-diabetic. This tendency of the blood sugar to return to normal levels was not limited to the single injection group, but was also observed at the termination of the course of alloxan in rats receiving several injections. This variability in course of the disease appeared to be a function of the individual rat.

It was also apparent that the rats with falling blood sugar levels frequently showed no glycogen deposits in their kidneys at the time of sacrifice. To determine whether the glycogen nephrosis in any individual rat was most closely correlated with the initial postinjection hyperglycemia, the maximum hyperglycemia attained by the animal at any time, or the terminal hyperglycemia, the blood sugar trends of each of twenty-six rats, diabetic for periods of 2 weeks or longer, were charted, indicating at the time of sacrifice whether glycogen nephrosis was present or absent (Chart 1). This chart demonstrates that these twenty-six animals could be divided into three groups.

Group 1: These rats started with initial low levels of blood sugar and showed progressively rising hyperglycemia. If the levels of blood sugar at the time of sacrifice were above 350 mg. per cent, glycogen was found in the renal epithelium.

Group 2: These rats started with high levels of blood sugar (above 500 mg. per cent) and showed progressively falling levels. If these animals were sacrificed with terminal levels above 350 mg. per cent, glycogen nephrosis was present, whereas if the terminal levels were below 300 mg. per cent, no glycogen could be demonstrated.

Group 3: This group of rats maintained fairly constant levels of hyperglycemia throughout their diabetic courses. As noted above, those rats with sugar levels above 350 mg. per cent showed glycogen nephrosis while those with terminal sugar levels below 300 mg. per cent showed no glycogen deposits.

DISCUSSION

The 37 per cent survival rate of rats after injection of alloxan monohydrate for the production of experimental diabetes agrees well with other authors in this field. The causes of the immediate postinjection mortality are twofold: the one being diabetic reactions resulting from too great destruction of the islets, and the other being uremia, which frequently supervenes on the basis of widespread renal tubular necrosis (7). The single large dose of alloxan produced most of these fatalities in the present study.

The response of any individual rat to the alloxan was completely unpredictable. Some animals, three, proved to be alloxan-resistant; others gave widely differing responses, with all levels of hyperglycemia up to the level of 1200 mg. per cent. These reactions had the character of individual variations and could not be correlated with the age or size of the animal, or the size of the diabetogenic dose.

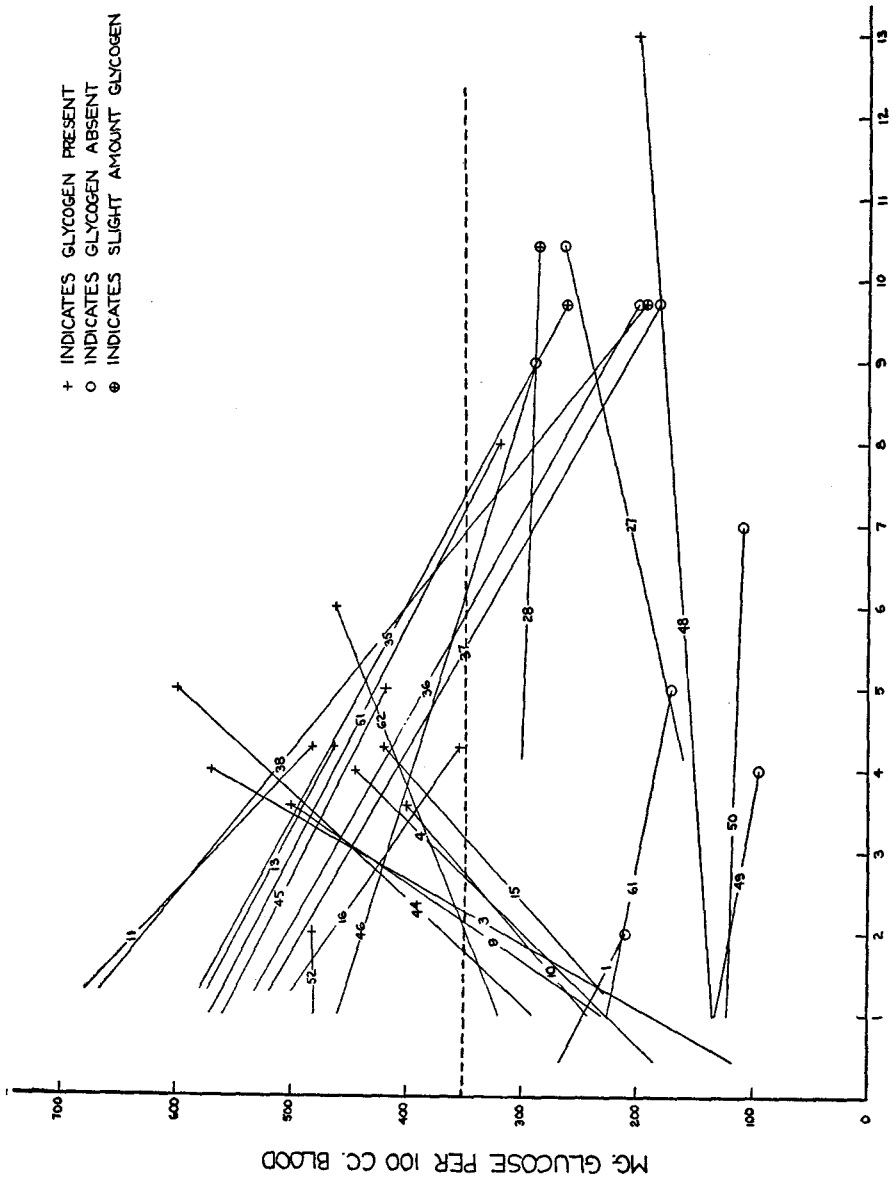

CHART 1. Blood sugar levels of individual rats.

The glycogen nephrosis produced in these experimental rats, which occurred in 44.0 per cent of the animals, exactly resembled the lesions found in patients dying with diabetes mellitus (8). It was limited to the tubular epithelium, chiefly in Henle's loops and in the convoluted tubules. Glycogen deposits in the glomeruli, described as occurring occasionally in human beings, were not found in these experimental lesions. In those animals proving refractory to alloxan and failing to develop diabetes, despite repeated administrations of the drug, no glycogen deposits were found, ruling out the possibility that these deposits were in any way related to any toxic effects of alloxan. Moreover, microscopic sections of the kidneys of the control animals revealed no glycogen deposits.

It is of interest to note that no intercapillary glomerulosclerosis was observed within the time limits of this experiment.

In no instance was it possible to detect glycogen nephrosis in animals which had been diabetic for less than 5 days. This observation is in agreement with those of others who report its first appearance after 4 days of experimental diabetes. Beyond the initial 5 day period the severity and rapidity with which glycogen nephrosis developed in rats which received a single injection of alloxan were correlated with both the level and the duration of hyperglycemia.

It seems safe to assume that an increase in blood sugar above a certain level is required before the time factor becomes effective in determining the appearance of renal glycogen. For example, no glycogen was observed in animals diabetic for long periods of time (74 days) with levels of blood sugar below 300 mg. per cent.

In animals diabetic for more than 9 days, the single factor with which the postmortem finding of glycogen nephrosis could be correlated, was the terminal blood sugar level. In all animals, with rare exception, glycogen nephrosis was observed associated with terminal blood sugar levels of 350 mg. per cent or above (Chart 1). This correlation was observed regardless of whether the trend of the hyperglycemia was rising or falling at the time of sacrifice. Moreover, this relationship between glycogen nephrosis and terminal blood sugar level was observed irrespective of the initial blood sugar level or the height which the blood sugar may have attained at any time in the course of experimental diabetes in individual animals. The fact that glycogen nephrosis apparently requires a terminal blood sugar level considerably above the normal indicates that the deposition of glycogen within the renal tubular epithelium is not merely an anatomic reflection of the existence of any degree of hyperglycemia. If these observations can be applied to diabetes as it occurs in human beings, then one may infer that the autopsy finding of glycogen within the renal tubular epithelium depends chiefly upon a pronounced terminal diabetic state rather than the antecedent diabetic course.

The findings in this experiment further suggest that glycogen nephrosis is a reversible lesion. As has been pointed out above, a terminal blood sugar level

of over 350 mg. per cent was invariably associated with a postmortem finding of glycogen nephrosis. Some animals started with initial blood sugar levels of over 500 mg. per cent and showed progressively falling hyperglycemia. Glycogen nephrosis was not found at autopsy when the terminal blood sugar level fell below 300 mg. per cent. The inference seems warranted that if these animals had been sacrificed at some time during the course of the diabetic period when the blood sugar level was over 350 mg. per cent, glycogen nephrosis would have been encountered. The findings strongly suggest the reversibility of glycogen deposition within the kidneys. Further study of this point is now in progress.

SUMMARY

Two hundred and seven albino rats were injected subcutaneously with alloxan in doses varying from 140 to 200 mg. per cent per kilo of body weight. Fifty-nine animals which developed hyperglycemia (blood sugar levels above 150 mg. per cent) were observed for periods from 5 days to 32 weeks.

Postmortem examination of the kidneys of these diabetic animals revealed glycogen deposition in the loops of Henle and convoluted tubules in 26 rats or 44 per cent. Glycogen could not be demonstrated in the glomeruli. Within the time limits of this experiment (32 weeks) no intercapillary glomerulosclerosis was observed.

The following facts were revealed regarding glycogen nephrosis in alloxan diabetes:

(*a*) Its appearance in the kidneys of the diabetic rats depended solely upon the terminal blood sugar levels of these animals. A value of 350 mg. per cent was the critical level, above which glycogen nephrosis was almost invariably demonstrable. With terminal levels below 300 mg. per cent no glycogen nephrosis was found.

(*b*) No relationship existed between the postmortem finding of glycogen nephrosis and the initial blood sugar level, or the maximum height of the hyperglycemia attained by individual rats.

(*c*) The results suggest that glycogen nephrosis is a reversible lesion.

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