REGENERATION OF THE LIVER IN ALLOXAN DIABETES

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Regeneration hypertrophy of the liver in rats is intensified in the presence of alloxan diabetes. The glycogen content in the regenerating liver is much lower in diabetic animals than in controls. The number of nuclei and the number of binuclear cells are increased.

In alloxan diabetes the liver glycogen content is reduced [4, 6] in the same way as during the first few days after partial hepatectomy. The object of the present investigation was to study the course of regeneration of the liver under these conditions.

EXPERIMENTAL METHOD

Male albino rats weighing 300 g or over were used. Previous observations showed that the course of alloxan diabetes in rats of this weight is milder than in young animals. The animals consisted of four groups: intact, partially hepatectomized, diabetic animals with an intact liver, and diabetic partially hepatectomized rats. About two-thirds of the liver was removed. The course of regeneration hypertrophy of the liver was studied on the seventh day after resection. At this time the relative weight of the regenerating liver of the rats of each group was expressed as a percentage of the weight of the intact liver of the animals in the corresponding control groups. Diabetes was induced by subcutaneous injection of a freshly prepared aqueous solution of alloxan in a dose of 18 mg/100 g body weight. Partial hepatectomy was carried out 2 weeks after the development of diabetes, as indicated by increased diuresis and glucosuria. Only rats with a high sugar concentration in the urine were included in the experiments. The weight of the liver and adrenals, the glycogen concentration in the liver, and the blood sugar were determined. Half-gram samples of liver were dehydrated in absolute alcohol and defatted in anhydrous acetone. The DNA content in 50 mg of tissue treated in this way was determined by the diphenylamine method [5]. Pieces of liver were fixed in Bouin's fluid, embedded in paraffin wax, sections were cut to a thickness of 6 μ, and these were stained with hematoxylin. Under the immersion objective the number of nuclei in 30-50 fields of vision of the microscope and the number of binuclear cells in 100-200 fields of vision of the microscope were determined for each animal and the volume of the nuclei was calculated. The structure of the experiments and their results are reflected in Tables 1 and 2.

EXPERIMENTAL RESULTS

As Table 1 shows, alloxan diabetes led to a marked decrease in the glycogen content in both the intact and the regenerating liver, an increase in the blood sugar concentration, and an increase in size of the adrenals. The relative weight of the regenerating liver of the diabetic rats reached 98.36-99.04% of the weight of the intact liver of the diabetic animals (compare group 3 and groups 4 and 5; $P > 0.05$). In normal rats the weight of the regenerating liver did not reach the weight of the liver in the rats of the control group, the difference being statistically significant (compare groups 1 and 2; $P < 0.05$). Restoration of the weight of the liver after resection thus took place distinctly faster in diabetic than in nondiabetic rats.

As Table 2 shows the number of nuclei in the regenerating liver of the control rats was a little lower than in the intact liver. In the diabetic rats, on the other hand, the number was increased. The number of

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TABLE 1. Effect of Alloxan Diabetes on Regeneration Hypertrophy of the Liver in Rats (M ± m)

Experimental conditions	No. of animals	Final body weight (g)	Mean weight of liver			Glycogen content (mg/g)	Blood sugar (mg/100 ml)	Weight of adrenals (mg/100 g body weight
			mg	mg/100 g body weight	in %			
1. Intact	9	297	10450±458	3518±156	100	62±3,1	97,5±1,5	13,3±0,7
2. Partial hepatectomy	9	314	9611±452	3061±118 $P_{1,2}<0,05$	87,01	27±2,1	98,5±2,0	17,2±1,5
3. Diabetes, intact	9	281	11372±315	4047±165	100	21,7±4,6 $P_{1,3}<0,001$	279±16,0 $P_{1,3}<0,001$	27,1±2,2 $P_{1,3}<0,001$
4. Diabetes, partial hepatectomy	9	260	10422±337	4008±117 $P_{3,4}>0,05$	99,04	5,6±0,2 $P_{2,4}<0,001$	284±25,7 $P_{2,4}<0,001$	32,2±1,9 $P_{2,4}<0,001$
5. Diabetes, partial hepatectomy	7	276	10986±696	3981±97 $P_{3,5}>0,05$	98,36	13±0,2 $P_{2,4}<0,001$	223±13 $P_{2,5}<0,001$	22,8±1,9 $P_{2,5}<0,05$

TABLE 2. Effect of Alloxan Diabetes on Number of Nuclei, Binuclear Cells, Volume of Nuclei, and DNA Content in Liver of Rats (M ± m)

Experimental conditions	No. of nuclei	No. of binuclear cells	Volume of nuclei (μ^3)	DNA content (in extinction units)
1. Intact	25,8±0,4	75,2±4,1	327,9±17,2	0,441±0,01
2. Partial hepatectomy	21,8±0,7 $P_{1,2}<0,001$	25,4±1,2 $P_{1,2}<0,001$	323,8±15,2	0,515±0,01 $P_{1,2}<0,01$
3. Diabetes, intact	27,0±0,3	83,0±3,9	287,2±14,3	0,405±0,01
4. Diabetes, partial hepatectomy	29,4±1,07 $P_{2,4}<0,001$ $P_{3,4}<0,05$	32,2±1,9 $P_{2,4}<0,001$ $P_{3,4}<0,001$	234,2±14,4 $P_{2,4}<0,05$ $P_{3,4}<0,05$	0,553±0,02 $P_{3,4}<0,001$

binuclear cells in the regenerating liver was sharply reduced in both the control and the diabetic animals. In the liver of the diabetic rats, especially in the regenerating liver, the volume of the hepatocyte nuclei was reduced. The DNA content in 50 mg of dehydrated and defatted tissue was increased in the regenerating liver in both the control and the diabetic rats, a little more in the latter.

The effect of diabetes on the regenerating liver is thus complex in character: an increase in the number of nuclei is accompanied by a decrease in their volume, while regeneration is accelerated.

How can this process of stimulation of regeneration of the liver in diabetic animals be explained? Presumably when insulin production is reduced in intensity the effects of adrenalin and glucagon on the liver begin to predominate. As the writer has shown, these glycogenolytic hormones, especially in conjunction with theophylline, can stimulate regeneration of the liver [1]. In human diabetes the excretion of adrenalin is increased [2]. Presumably in rats with alloxan diabetes the production of adrenalin is also increased, as the increase in weight of the adrenals suggests. In rats with alloxan diabetes the activity of the adrenalin-sensitive component of adenyl kinase is also increased [3]. All these changes must lead to an increase in the intensity of synthesis of cyclic AMP which, in turn, leads to more intensive glycogenolysis and, perhaps, to direct activation of the hepatocytes. If this explanation is correct, the action of theophylline (a drug conserving cyclic AMP) would be expected to accelerate regeneration of the liver even more in diabetic rats.

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