ACTIVATION OF CELLS DURING REGENERATION OF THE LIVER OF WHITE MICE UNDER CONDITIONS OF SEVERE LOAD

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It has been shown that regeneration of various viscera is associated with an enhanced mitotic activity, an increased content of nucleic acids in the cells, and cellular hypertrophy [1, 2, 4, 5, 7, 8, 14-17]. However, the mechanism of the activation of cell division and growth during regeneration has been little studied. Available indications are that stimulation of mitotic activity during the regeneration of damaged organs is related to the action of protein disintegration products [1, 2, 4, 5, 8, 13]. A study of renal regeneration has shown that an increased function on the part of the damaged organ by way of compensation may be another important factor. The reaction of activation of cell growth and division may occur when the action of the protein tissue disintegration products has been eliminated, for example after unilateral nephrectomy [8]. Regeneration of a portion of a kidney took place more rapidly when the opposite kidney has been extirpated [6, 9, 10].

Similar results were obtained on the rat liver. Here also regenerative processes were enhanced when functional activity was increased. When glycogen was given together with a diet which enhanced biliary secretion regeneration of the remaining portion of liver was accelerated. On the other hand a glucose load exerted no influence on hepatic regeneration [11, 12].

We have set out to determine the influence of the increased function of an organ on the mitotic activity of cells during hepatic regeneration in mice given carbohydrate, and under conditions in which biliary secretion was stimulated.

METHOD

The experiments were carried out on 68 mice aged $2\frac{1}{2}$-3 months; they were arranged in 2 series.

In series I we studied the cellular changes in the one third of the liver which remained after partial hepatectomy, under conditions of a carbohydrate load. Each animal received a subcutaneous injection of 26 mg glucose in a 5% solution. At the same time we injected 0.01 milli-equivalents of Zn-insulin per mouse. Preliminary histochemical investigations showed that when glucose and Zn-insulin are injected together in the amounts just mentioned, a maximum amount of glycogen accumulates in the liver. To establish a prolonged functional load the Zn-insulin and glucose were injected once per day for the 10 days before and 2 days after the operation.

From the control mice we also removed one third of the liver, but no glucose or zinc insulin was given. The mice were killed 2 days after the operation, and at the same time (4 p.m.).

In the second series of experiments we investigated the changes in mitotic activity in a liver regenerating under conditions evoking biliary secretion. One third of the liver was removed. Biliary secretion was stimulated in the experimental group by means of a special diet. Starting 4 days before the operation and continuing until 3 days after it we gave each mouse weighing 17-18 g 0.05 ml of fresh ox bile by mouth. Before it was given the bile it was diluted with milk to a volume of 0.1 ml. In addition to their normal ration the mice were given milk and boiled meat.

TABLE 1. Changes in the Mitotic Activity in the Liver after Resection and Injection of Glucose with Insulin

Group of animals	No. of animals	No. of mitoses	Phase coef-ficient	Area (in μ^2)	
				Of cells	Of nuclei
Control	9	10	3.4	169.6±1.4	23.3±0.2
Experimental	10	33.6	2.7	260.0±2.5	29.8±0.2
P		0.004		<0.0001	<0.0001

TABLE 2. Changes in Mitotic Activity in the Liver of Animals Fed on a Diet Stimulating Biliary Secretion, after Resection of a Portion of Liver

Time of killing	Groups of animals	No. of animals	No. of mitoses	Phase coef-ficient	Area (in μ^2)	
					Of cells	Of nuclei
8 a.m.	Control	12	9.6	4.6	186.9±1.4	22.4±0.2
	Experimental	13	48.5	2.3	294.4±2.8	28.2±0.3
P			0.001		<0.0001	<0.0001
4 p.m.	Control	10	4.3	2.8	216.2±2.1	27.9±0.2
	Experimental	14	13.5	2.4	288.6±3.3	30.7±0.3
P			0.008		<0.0001	<0.0001

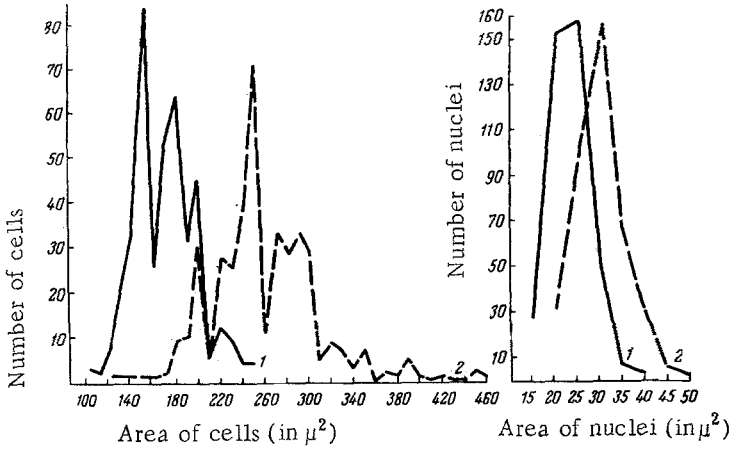

Fig. 1. Curves showing variation in size of cells and of nuclei in a regenerating liver of an animal injected with glucose.

Part of the liver was also removed from the control mice. Unlike the experimental group this group received no bile in the diet. The mice were killed 3 days after the operation. The first group of animals of series II were killed at 8 a.m. during the period of maximum mitotic activity, and the second group was killed in the evening at 4 p.m., at the period of minimum mitosis.

The mitotic activity of the cells was measured on liver sections by counting the number of mitotically dividing cells in a given area (2.85 mm^3).

We also calculated the coefficient of the mitotic phase (ratio of first 2 to the next 2 phases). At the same time we measured the size of the cells and of their nuclei, the amount of glycogen in the cells during a carbohydrate load, and the RNA and DNA contents in the cells during a biliary load. The area of the cells and of the nuclei was determined by drawing the image projected onto a screen; drawings were then made and measured by

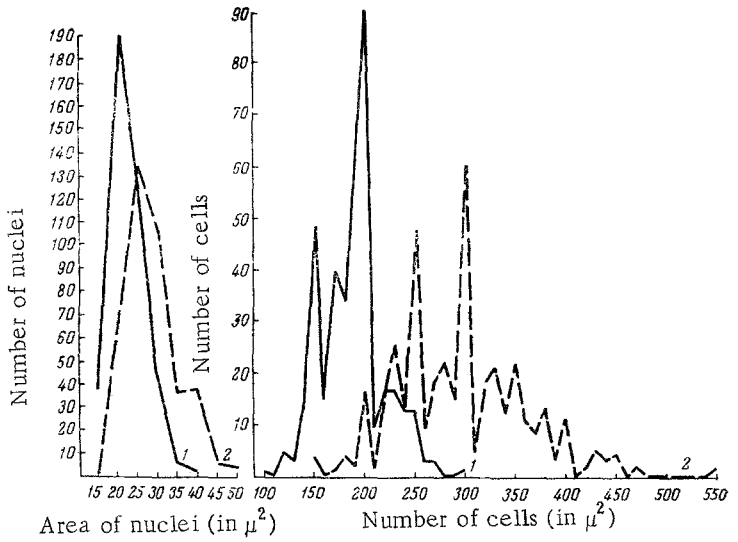

Fig. 2. Curves showing variation in size of cells and of nuclei in a regenerating liver of an animal injected with glucose. Indications as in Fig. 1.

means of a planimeter. In each group measurements were made on sections of livers taken from 2 animals. We measured 200 cells in each section. Glycogen was revealed by Bauer's method, and the material was fixed in Shabadash. To determine the nucleic acids the livers were fixed in Helli. RNA was demonstrated by methylene green and pyronine, and DNA by Feulgen's method.

RESULTS

In the adult mice only occasional mitoses can be observed in the intact liver. However, in a liver regenerating after partial resection the number of mitoses shows a considerable increase, and the mitotic rate depends upon the functional condition of the organ.

In the first set of experiments a change in the rate at which glycogen was produced in the liver was induced by the administration of glucose with Zn-insulin. In the histological determination the amount of glycogen showed considerable differences as between the control and experimental groups. In the experimental animals there were many large clumps, many of them merging into each other. On the other hand, in the control group the clumps were smaller and fewer. Therefore the prolonged administration of glucose with Zn-insulin led to a considerable increase in the amount of glycogen formed by the regenerating liver.

An increase in the functional activity of the organ associated with the injection of glucose influenced mitotic rate. Under these conditions the number of mitoses in the experimental group exceeded by more than 3 times the number in the control group (Table 1).

Not only was mitotic activity enhanced but the cells and their nuclei were hypertrophied. There was an increase in the number of large cells and large nuclei and a reduction in the number of small cells and nuclei. On the graph this change is shown by a shift of the curves to the right (Fig. 1).

The other kind of functional load on the liver—the stimulation of biliary secretion—also influenced mitotic activity. The number of mitoses in the experimental group was 3-5 times greater than the number in the controls. This effect was present in the animals killed either in the morning or in the evening (Table 2).

Measurements showed an increase in the area of cells and of nuclei in the experimental group (Fig. 2). The curves obtained for the two groups of animals were identical, and the time at which the animals were killed was without any effect. We may note the appearance of quite a large number of large cells and nuclei and the reduction in the number of small cells and nuclei. In the experimental group the curves were shifted to the right.

When the animals were fed on a diet stimulating biliary secretion regeneration was accompanied by an enhanced reaction for RNA and DNA (regeneration without stimulation of function).

This investigation has given us reason to suppose that in addition to the action of proteins and their disintegration products a further factor causing activation of the cells is the condition of long-maintained enhanced function. On the other hand our data confirm the view [3] that there is a relationship between cellular division and the work of the cell, a relationship which is not limited merely to the interdependents of these two processes. During brief functional loads there is a reduction of mitotic activity, but with prolonged functional stimulation the rate of cellular proliferation is increased.

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