ACTION OF HUMORAL FACTORS CONTROLLING CELL DIVISION DURING THE FIRST HOURS OF LIVER REGENERATION

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Blood plasma taken from rats 4 and 20 h after partial hepatectomy, if injected into intact rats, increases the mitotic activity of their liver cells. The stimulant action is less marked if the plasma is obtained 12 h after the operation, and is absent if the plasma is taken 28 h after hepatectomy.

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Despite numerous investigations into mechanisms of liver regeneration, the question of the possible presence of humoral factors in the blood of hepatectomized animals causing increased proliferation of the parenchymatous cells of the liver has not been finally settled [1-4, 8-12]. Bearing in mind that biochemical changes characteristic of regeneration take place within a few hours after hepatectomy [7], it may be expected that humoral factors accumulate in the blood and influence proliferation of the parenchyma even at this stage. However, in the work cited above attempts were made to demonstrate the effect of these factors at later periods (24-72 h) after hepatectomy, when a distinct increase in mitotic activity is observed in the liver.

For the reasons given above, in the present investigation the object was to study the action of blood plasma taken soon (4 and 12 h) after partial hepatectomy on the donors, and also at later periods, characterized by maximal DNA synthesis (after 20 h) and maximal mitotic activity (after 28 h) [5], on mitoses in the liver of intact rats.

EXPERIMENTAL METHOD

Two series of experiments were carried out on noninbred male albino rats weighing 100-130 g. The donor rats were divided into four groups, in each of which the animals underwent partial hepatectomy (about 65%) [6] and blood was taken from them 4, 12, 20, and 28 h after the operation.

In addition to the four groups of hepatectomized rats, a group of intact donors was taken. The animals of all groups were bred at the same time (from 1 to 2 A.M.), and the time at which the operations were performed varied with these times. Plasma was prepared from the blood obtained, and injected in a dose of 1 ml intraperitoneally into intact recipients.

In the experiments of series I, injections were given to the recipients immediately after obtaining the plasma at 3 A.M., and the animals were sacrificed at 7 P.M. next day, after an interval of 28 h.

In the experiments of series II plasma was injected at 7 A.M. and the rats were sacrificed 48 h later, also at 7 A.M. The control consisted of a group of intact animals sacrificed at the same time.

The action of the injected plasma was assessed by the relative weight of the liver and from counting the number of mitoses in 15,000 parenchymatous cells of the liver in each animal (thickness of sections 7 μ, stained with Mayer's hematoxylin and eosin). The mitotic index (MI) was calculated in pro mille.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that injection of the plasma of intact donors caused no changes in mitotic activity of the liver cells of normal rats compared with intact animals in the experiments either of

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TABLE 1. Effect of Blood Plasma Obtained at Different Times After Partial Hepatectomy on Donor Rats on Mitotic Activity and Relative Weight of the Liver of Intact Recipients

Experimental condition	Time from operation to taking plasma (in h)	Series I (animals sacrificed 28 h after injection)			Series II (animals sacrificed 48 h after injection)		
		number of rats in group	MI (in parts per thousand)	relative weight (in parts per thousand)	number of rats in group	MI (in parts per thousand)	relative weight (in parts per thousand)
Control, without injection of plasma	—	16	0,39± 0,10	4,96± 0,14	16	0,39± 0,10	4,96± 0,14
Control (with injection of plasma of intact donors)	—	14	0,58± 0,11	5,08± 0,13	16	0,29± 0,07	4,86± 0,15
Injection of plasma of hepatectomized donors	4	16	2,59± 0,61	4,49± 0,06	15	1,91± 0,57	4,80± 0,16
	12	18	1,23± 0,28	4,58± 0,12	16	0,51± 0,13	4,64± 0,15
	20	12	2,63± 0,70	5,39± 0,24	12	2,21± 0,74	5,28± 0,12
	28	12	0,71± 0,22	5,13± 0,15	11	0,43± 0,11	4,87± 0,17

series I or of series II. On the other hand, plasma obtained 4 h after hepatectomy on the donors increased the mitotic activity of normal liver cells by 5-6 times compared with the liver of intact rats, both 28 and 48 h after injection (P 0.0003 and 0.009 respectively). Injections of this plasma led to an increase in the value of MI in the liver of the recipients also when compared with injection of normal plasma, in both series I and series II (P 0.001 and 0.005 respectively). Differences were found between the action of plasma obtained 12 h after the operation in the experiments of series I and II. For instance, 28 h after injection of this plasma into normal recipients, the mitotic activity of their liver cells was increased compared with the liver of intact animals (P = 0.005), although no significant differences were found by comparison with injection of normal plasma (P = 0.04). No change was found in the value of MI in the liver of normal recipients 48 h after injection of plasma taken 12 h after hepatectomy on the donors by comparison with this index in intact animals and in recipients receiving normal plasma (P 0.5 and 0.1 respectively). Injection of plasma obtained 20 h after the operation caused an increase in the number of mitoses in the liver of normal rats by about 6 times compared with their number in the liver of intact rats both 28 and 48 h after injection (P 0.001 and 0.01 respectively). Significant stimulation was also observed by comparison with injection of normal plasma (P = 0.005 in series I, P = 0.009 in series II). On the other hand, plasma taken 28 h after the operation had no effect on the number of mitoses in the liver of the intact recipients, compared with the action of normal plasma in series I or series II (P 0.5 and 0.3 respectively). The value of MI in the liver of animals receiving this plasma was indistinguishable from that in the liver of intact rats (in series I P = 0.3, in series II P = 0.8).

Comparison of MI for the liver of the three groups of recipients of plasma taken 4, 12, and 20 h after hepatectomy in the experiments of series I shows that the differences between them are not significant (P 0.04 and 0.07, and 1 respectively). All these values of MI differ significantly from MI for normal liver. However, in contrast to the other two groups of recipients, receiving plasma 12 h after the operation, there was no significant difference between the value of MI in their liver and in that of the group of animals receiving normal plasma.

The impression was obtained that the stimulant property of plasma taken 12 h after the operation was slightly depressed. This phenomenon was still more marked in the experiments of series II, where MI for the liver of recipients of plasma taken 12 h after the operation was indistinguishable from MI in the liver of intact rats and of rats receiving normal plasma. A tendency also was observed for the value of MI to decrease in the liver of animals receiving plasma taken 12 h after the operation, by comparison with MI for animals receiving plasma taken 4 and 20 h after the operation (P 0.01 and 0.02 respectively).

With respect to the relative weight of the liver, no significant difference was found between the animals of all groups receiving plasma of hepatectomized donors in the experiments of series II, normal rats,

and rats receiving normal plasma. However, injection of plasma obtained 4 h after hepatectomy caused a decrease in the relative weight of the recipients' liver compared with intact animals and rats receiving plasma of intact donor (P 0.002 and 0.0001 respectively).

It may thus be concluded from the results described that the action of humoral factors controlling regeneration of the liver is manifested during the first few hours after hepatectomy, long before the appearance of mitoses in the regenerating liver.

The conflicting results concerning the existence of humoral factors causing increased proliferation of the liver cells may evidently be explained by the fact that in the period of increased mitotic activity in the regenerating organ, the concentration of active factors in the blood has now fallen so low that, depending on the experimental conditions (the proportion of the donor's liver removed, the time of taking the blood, the dose injected into the recipient), their action is not manifested in some cases, while in others it produces the expected effect.

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