EXPERIMENTAL BIOLOGY

THE EFFECT OF FUNCTIONAL LOADING ON LIVER REGENERATION IN RATS

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Although the morphological changes which occur during mamalian liver regeneration have been well studied, the functional condition of the liver during the regeneration process and the influence of the liver function on liver regeneration have not yet been sufficiently investigated. As well as being of general biological interest, these questions are extremely important to medicine, especially surgery.

With the question of how the functional condition of the liver influences the intensity of regeneration, we started from a proposition expressed by M. A. Vorontsova and L. D. Liozner [1], according to which, there is a close connection between physiological and reparative regeneration. Processes restoring the structural elements of each organ, i.e., physiological regeneration, occur as a result of the organ's functional operation. These regenerative processes, which occur in the normal conditions of the animal's life, constitute the original background on which the processes of reparative regeneration occur. One can therefore assume that intensification of the organic function, which causes intensification of physiological regeneration, would also affect reparative regeneration. The almost complete lack of data covering this important aspect of research caused us to conduct experiments which would throw some light on this relationship. How different degrees of functional loading affected liver regeneration was a question that interested us particularly. For this purpose, we made experimental tests to find whether the bile-secreting function of the liver increased or decreased by using choleretic substances and selecting a corresponding diet. Although there are a series of works in the literature studying the influence of different diets on liver regeneration in mammals (Opie and Alford [6], Davis and Whipple [4], Moise and Smith [5], Brues, Drury and Brues [3], and Podzolkov [2]), the data in these works is rather contradictory. Moreover, these studies did not treat the question of how liver function influences liver regeneration and thus did not have direct bearing on the problem which interested us.

Our experiments were conducted on white rats.

The plan of the experimental setup is illustrated in Table 1.

The experimental animals were divided into three groups. The first group of rats (30 animals) were kept on a choleretic diet. The rats were given fresh dog's bile daily as the choleretic substance. In addition, the diet consisted principally of food substances causing strong bilification.

The second group of rats (30 animals) received a sparing bilification diet, which principally consisted of food substances causing weak bilification.

The third group (30 animals) was fed a normal diet.

After being fed the indicated diets for 3 days, the animals of all three groups were operated upon to remove the large, left liver lobe. The average weight of the extirpated lobe was 2 g. Since the average weight of the

liver was 9 g, approximately $^1/_5$–$^1/_4$ of the liver was removed.

After the operation, the animals continued on their respective diets (choleretic, sparing and normal).

TABLE 1

Time of fixation	Experimental rats			Control rats		
	Diet					
	Choleretic	Sparing	Normal	Choleretic	Sparing	Normal
Day of operation						10
1 day after operation	10	10	10			
7 days after operation	10	10	10			
14 days after operation	10	10	10	10	10	10
Total number of rats	30	30	30	10	10	20

The animals were killed 1 hour, 1 week and 2 weeks after the operation (10 rats from each group were killed at each of these times).

The material was fixed in Carnoy's, Helly's and Zenker's fluids and in formalin. The material was processed for glycogen and fat, and the preparation was stained with hematoxylin-eosin, by Mallory's method and by Van Gieson's method.

The group of rats which had been fed a normal diet, but had also had the liver partially removed, served as the control in the examination of the effect of the choleretic and sparing diets on liver regeneration.

In addition, 10 rats were killed before the operation (initial control) and 10 rats at the end of the experiment (final control).

Supplementary controls were used in order to distinguish the effects of the diets on the liver condition and on the course of the regeneration process in the liver; rats which had not had the operation but had been fed the choleretic diet (10 animals) served as the control for the choleretic diet, and rats which had not had the operation but had been fed the sparing diet (10 animals) served as the control for the sparing diet. The animals of these two controls groups were killed at the same time as the experimental rats, 2 weeks after the operation.

We determined the weight of the rats before the operation, the weight of the extirpated portion of the liver, the weight of the rat when killed and the weight of the liver at the time the rat was killed. The relative weight of the extirpated portion of the liver to the weight of the body and the relative weight of the liver to the weight of the body were also computed. The material was processed statistically. The data obtained is summarized in Table 2 and graphically presented in Figures 1-3.

As Table 2 shows, the weight of the remaining part of the liver sharply increased in the rats of all groups as early as a day after the liver lobe had been resected. Although a rather large number of mitoses was observed in the hepatic cells at this time, it did not seem possible after such a short time that the liver weight had increased only because of the increased number of hepatic cells. It seemed more likely that this liver weight increase was connected with the reaction processes. At this time, histological study showed, along with the necrotic changes in the injured surface region of the liver, a profuse infiltration of large drops of fat, most clearly expressed around the periphery of the small lobes and change in glycogen distribution, which was shown by the fact that while some of the hepatic cells had lost their glycogen, others, which did contain it, were almost completely clogged with it.

One week after the operation, the weight of the regenerating liver was substantially different in the different groups of experimental rats. For example, in the rats kept on a choleretic diet, the weight of the regenerating liver exceeded the weight of the normal liver, while, in the animals fed the control diet, the weight of the liver

was less than the weight of the liver a day after the operation. The liver weight in the rats fed the sparing diet was about the same as it had been one day after the operation. The decrease in the liver weight 1 week after the operation, as compared with the first days after the operation, which was observed in the rats kept on the control diet confirmed our proposition that the increase in the liver weight the first day after the operation was due to a reaction. That liver regeneration continued during this period was indicated by the still rather large number of mitoses, while the inflammatory phenomena connected with the trauma were already abating. The drops of fat infiltrating the hepatic cells became small and the infiltration less marked. Staining for glycogen showed a picture approximating that in the control animals.

TABLE 2

Liver Regeneration in Experimental and Control Rats

No. of test	Series	Average postop. wt. of rats	Average wt. of rats when killed (in g)	Average wt. liver when rat killed (in g)	Average rel. wt. liver to wt. body when killed (in %)
1	Initial control		201	9 4	4 6
2	Experiment, normal diet				
	1 day after operation . . .	197	193	8.0	4.5
	1 week after operation . .	197	193	7.2	3.6
	2 weeks after operation . .	196	189	9.5	5.0
3	Experiment, sparing diet				
	1 day after operation . . .	203	197	8.3	4.12
	1 week after operation . . .	204	205	8.3	4.0
	2 weeks after operation	194	202	9.8	4.8
4	Control. Sparing diet		195	8.8	4.5
5	Experiment, chloretic diet				
	1 day after operation . . .	195	186	7.8	4.1
	1 week after operation . . .	202	173	9.2	5.2
	2 weeks after operation . . .	203	206	10.4	5.0
6	Control, chloretic diet . . .		206	9.3	4 5
7	Final control		204	8.6	4.2

Two weeks after the operation, the weight of the regenerating liver in the rats fed the control and sparing diets became slightly greater than the liver weight in the rats of the intial and final controls. After the same period, only the absolute weight of the liver had increased in the rats fed the choleretic diet. This is explained by the fact that the whole body of the rat had grown rather considerably by the second week after the operation.

Two weeks after the operation, the weight of the regenerating liver in all three groups of the rats exceeded the original weight.

During the period of the greatest increase in the weight of the regenerating liver, the body weight of the rats decreased; this was especially evident in the rats fed the choleretic diet.

One can assume from the results of the histological examinations made of the regenerating liver of the rats that the restoration is realized by regenerative hypertrophy.

The intense regeneration in the liver of rats fed the choleretic diet is evidently connected with the more rapid divisions of the cells; we cannot, however, prove this completely satisfactorily since there was no detailed study of hepatic cell mitotic activity in our work.

Our data, then, showed that the functional condition of the liver does affect liver regeneration. Enhanced functional load created by feeding the rats the choleretic diet caused the weight of the regenerating liver to exceed the weight of the normal liver as soon as the end of the first week after the operation. Therefore, liver regeneration is intensified by an enhanced functional load. That intense, regenerative hypertrophy did in fact occur was shown by the increase in the liver weight and by the fact that the regenerated liver had a normal structure.

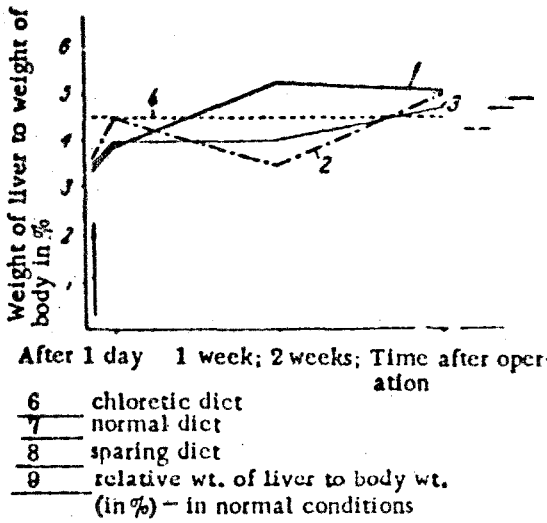

Fig. 1. Change in absolute liver weight of rats during the regeneration process. 1) choleretic diet; 2) normal diet; 3) sparing diet; 4) average weight of liver in the control rats.

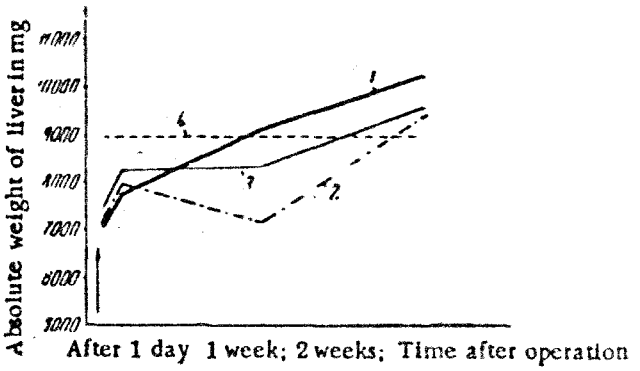

Fig. 2. Change in relative weight of rat's liver during regeneration. 1) choleretic diet; 2) normal diet; 3) sparing diet; 4) relative weight of liver to body weight in the norm (in %).

The latter proves that the liver weight increase observed was not caused by any pathological processes. Since in no case did we find abnormal growths of hepatic cells, in our experiments, liver regeneration was apparently caused both by hepatic cell divisions and by organization in the lobules. The cells of the bile ducts did not take any important part in the regeneration process. Only in two cases did we observe abundant growth, similar to adenomatous nodes, in the bile ducts.

SUMMARY

Ninety albino rats with the large left liver lobe extirpated were sustained on various diets (control, sparing and choleretic). In rats fed a choleretic diet regeneration of the liver was most complete. In one week after the operation the initial relative weight of the liver was already restored, while in the other groups restoration took place only after two weeks.

Restoration of the weight of the liver was due to an increase in the number of liver cells.

Thus, an enhanced functional load of the liver stimulates its regeneration.

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* In Russian.