Mitotic Response to Various Dietary Conditions in the Normal and Regenerating Rat Liver (35269)

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Brues *et al.* (1) have shown that the regenerating rat liver is affected by various dietary conditions. They found that when the weight of the liver was measured after partial hepatectomy, liver regeneration decreased in rats fed diets high in carbohydrate or protein and in starved rats but not in rats fed a high fat diet. However, when the number of nuclei in the regenerating liver was measured, only a slight decrease was noted in the rats fed a high carbohydrate or high protein diet, while a significant decrease was found in the rats fed a high fat diet. Rats that were starved up to 4 days showed no differences in nuclei number when compared to fed animals.

The above data were collected from rats 2 to 4 days after partial hepatectomy. In our laboratory, we have been concerned more with the earlier time periods following partial hepatectomy, namely, 20–36 hr, during which mitosis is initiated and reaches a maximum value. The present experiment was designed to investigate whether early mitotic activity in the liver following partial hepatectomy is affected when rats are fed diets high in carbohydrate, protein, or fat for 7 days and when starved 24 hr. Also studied was the effect of these dietary conditions on the mitotic activity of the intact liver from unoperated rats.

Methods. Four-week-old male Sprague-Dawley rats weighing 85-90 g were fed various diets for 7 days. The diets, purchased from Nutritional Biochemicals Corp., varied only in percentage composition of protein, fat, and carbohydrate and were supplemented with vitamin and salt mixtures. The four test diets, fed *ad libitum* to 4 different groups of rats, were normal (27% casein, 59% starch, 10% vegetable oil), high protein (64% casein, 22% sucrose, 8% vegetable oil), high carbohydrate (18% casein, 68% sucrose, 8% vegetable oil), and high fat (18% casein, 29% sucrose, 45% vegetable oil). A fifth group of rats was fasted for 24 hr after being fed the normal diet *ad libitum* for 7 days.

Because of the low caloric intake of rats fed the high protein and high fat diets, the normal diet was given to a sixth group of rats, whose caloric intake was restricted so that it was comparable to that of the rats on the high protein and high fat diets. The caloric intake of the rats maintained on the latter two diets was 65% and 75%, respectively, that of the rats fed the normal diet *ad libitum*. The caloric intake of the rats fed the high carbohydrate diet was less but did not differ significantly from that of the rats maintained on the normal diet *ad libitum*.

The rats were kept in individual metabolic cages in an air conditioned room with fluorescent lighting automatically set for on–off cycling at 6:00 a.m. and 6:00 p.m., respectively. Food and water were given *ad libitum* to all rats except the calorically restricted control rats, which received only water *ad libitum*. The calorically restricted rats were fed in the late afternoon in order to minimize any differences in the eating patterns between these rats and the rats fed *ad libitum*.

After the 7 day dietary period and the additional 24 hr for the fasted group, all rats were either sacrificed or partially hepatectomized (2). The partially hepatectomized rats were sacrificed either 20 or 24 hr postoperatively, the time period when mitosis rapidly increases. At the time of sacrifice, liver samples were removed from all animals and prepared for mitotic analysis, which has been described in an earlier paper (3).

During the postoperative period, the calorically restricted group continued to serve as

TABLE I. Average Daily Caloric Intake and Percentage Body Weight Gain of Rats Fed Various Diets for 7 Days.

	Group I		Group II		
	Control:	Experimental:	Control:	Experimental	
	Normal diet; fed *ad libitum*	High carbohydrate diet	Normal diet; restricted intake	High protein diet	High fat diet
Av daily caloric intake (kcal)	61.1	55.3	40.1	39.6	46.1
Body wt gain (%)	59	43	26	34	13

TABLE II. Effect of Feeding Various Diets for 7 Days on the Mitotic Indices[a] of Normal and Regenerating Rat Livers.

	Group I			Group II		
	Control:	Experimental		Control:	Experimental	
Postoperative time (hr)	Normal diet; fed *ad libitum*	High carbohydrate diet	Normal diet; 24 hr fast[b]	Normal diet; restricted intake	High protein diet	High fat diet
Unoperated	0.74	0.47	0.04[f]	0.11[g]	0.47[e]	0.02[e]
	±0.12[c]	±0.17	±0.01	±0.03	±0.10	±0.005
	(8)[d]	(8)	(8)	(8)	(8)	(8)
20	2.42	2.61	0.05[e]	3.30	0.10[f]	0.38[e]
	±0.81	±0.79	±0.01	±0.72	±0.05	±0.22
	(8)	(8)	(8)	(8)	(8)	(7)
24	7.21	9.37	1.60[f]	6.38	3.56[e]	2.71[e]
	±1.18	±1.41	±0.58	±0.52	±1.05	±1.06
	(8)	(8)	(8)	(8)	(8)	(7)

[a] Mitoses/100 nuclei.
[b] 24-hr fast following 7 day normal diet feeding.
[c] Mean ± SE.
[d] Number of animals.
[e] Experimental significantly different from control, $p < 0.05$; [f] $p < 0.001$.
[g] Group II control significantly different from Group I control, $p < 0.001$.

the control group for the rats on the high protein and high fat diets; no food was given to the rats that were fasted for 24 hr; and the remaining groups of rats continued to receive their respective diets and water *ad libitum*. All animals were sacrificed by decapitation between 8:30 a.m. and 10:30 a.m. to minimize the effects of diurnal variation in metabolism.

Results. Tables I and II show two different groups because of the two different controls used. Group I consists of the experimental rats fed the high carbohydrate diet and those fasted for 24 hr and their corresponding common control (rats fed the normal diet *ad libitum*). Group II consists of the experimental rats fed the high protein and high fat diets and their corresponding common control (rats fed the normal diet with restricted caloric intake).

Table I shows the average daily caloric intake and percentage body weight gain of the rats fed the various diets for 7 days. Since the *ad libitum* control rats and the rats fasted for 24 hr were identically treated for the first 7 days, their data are included together as the *ad libitum* fed control group. It is apparent that even though the rats in Group II consumed approximately the same number of calories, the differences in the composition of the three diets produced differences in percentage body weight gain. The rats fed the high protein diet and the high fat diet gained 34 and 13%, respectively, while the calorically restricted rats fed the normal diet gained 26%.

The mitotic indices of normal (unoperated) and regenerating livers from rats fed various diets for 7 days are shown in Table II. In the unoperated rats, the calorically restricted control group subjected to a 34% caloric restriction showed a mitotic index of 0.11, which was significantly lower than that of the *ad libitum* fed control group (0.74). When the rats were fasted for 24 hr or fed the high fat diet, the mitotic index was significantly reduced to 0.04 and 0.02, respectively. However, the rats fed the high protein diet showed a significantly higher mitotic index (0.47) than the calorically restricted control group (0.11).

In the partially hepatectomized rats, there were no significant differences between the mitotic indices of the controls of Groups I and II at either postoperative time periods despite the caloric restriction imposed on the latter group. The rats fed the high carbohydrate diet did not show any significant changes in mitotic activity at either the 20 or 24-hr postoperative time period when compared to their corresponding control, while the rats fasted for 24 hr and the rats fed the high protein and high fat diets showed a significantly lower mitotic activity at both postoperative time periods. During the postoperative period, the degree of weight loss and reduction in caloric intake due to the surgery was similar in all groups of rats.

Discussion. The mitotic activity of the intact liver is reduced in rats placed on a restricted diet (Group II control) or completely deprived of food (24-hr fast). When the caloric intake remains constant but the composition of carbohydrate, protein, and fat in the diet is changed (Group II rats), changes in mitotic activity appear to be directly related to changes in body weight gain. The unoperated rats fed the high protein diet had the highest mitotic activity and weight gain, the rats fed the high fat diet had the lowest, and the calorically restricted rats fed the normal diet showed values in between these two groups. This relationship between body weight gain and resting mitotic activity also applies to the rats fed the normal diet *ad libitum* and the high carbohydrate diet. These two groups showed the highest weight gain and also the highest resting mitotic activity.

In this study, the caloric intake alone does not determine the resting mitotic activity level. The change in the percentage of protein in the diet of the rats in Group II also appears to affect the resting mitotic activity since this is the only component of the Group II diets that, when increased or decreased, produces a like change in mitotic activity. The relation between mitotic activity and body weight gain is possibly due to the efficiency of the assimilation of the diet. The rat may more efficiently assimilate a diet higher in protein and therefore show a greater increase in body weight. This observation may only be true in conditions where caloric intake is restricted.

In the regenerating liver, food deprivation and the high fat diet had the same reducing effect on the mitotic activity as in the intact liver. The feeding of the high carbohydrate diet again produced no change in the pattern of mitotic activity. However, caloric restriction (Group II control) did not affect the mitotic activity in the regenerating liver, whereas the high protein diet reduced it, these results being contrary to those found in the intact liver.

Brues *et al.* (1) and others (4) have suggested that the decrease in the rate of liver regeneration in starved animals could be the consequence of the high fat infiltration of the liver prior to partial hepatectomy rather than to the lack of food. We found that the rats showing a reduction in the mitotic activity of the regenerating liver, namely, those fed the high protein, high fat diets or starved for 24 hrs, displayed significantly high plasma free fatty acid (FFA) levels, whereas the rats fed the high carbohydrate diet and the calorically restricted rats fed the normal diet had normal plasma FFA levels (data not shown). Weber *et al.* (5) have shown that FFA selectively inhibits the key enzymes of glucose catabolism. Therefore, immediately following partial hepatectomy, glucose may not be available in sufficient quantities as an energy source to the liver, causing a retardation in mitotic activity.

Summary. Rats were subjected to diets high in carbohydrate, protein, or fat for 7 days or to a 24-hr fast. Some of these rats were partially hepatectomized and the mitotic activity of the regenerating liver was measured either 20 or 24 hr postoperatively. The level of mitotic activity of the intact liver from the unoperated rats were also determined. The unoperated rats fed the high protein diet showed an increase in the resting mitotic activity, while those fed the high fat diet showed a decrease when these rats were compared to control rats fed a normal diet. Caloric restriction and food deprivation (24-hr fast) also caused a decrease in the resting mitotic activity. The partially hepatectomized rats fed the high protein and high fat diets or fasted for 24 hr showed a reduced mitotic activity during the periods studied, whereas caloric restriction had no effect. The high carbohydrate diet had no effect on the mitotic activity of either the intact or regenerating liver.

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