gested that the Cd^{2+} effect results from its interaction with some metal-binding ligands, other than sulfhydryl groups, which are presumably present in the carbohydrate-containing components at the brush border membranes.

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2 Present address: Department of Dermatology, Faculty of Medicine, Kyoto University, Kyoto 606, Japan.
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The effect of aging on rat liver regeneration[1]

H. Schapiro, S.S. Hotta, W.E. Outten and A.W. Klein[2]

Departments of Anatomy and Biochemistry, Eastern Virginia Medical School, P.O. Box 1980, Norfolk (Virginia 23501, USA), 11 December 1981

Summary. The effect of age on hepatocyte mensuration and mitotic activity 48 h after partial hepatectomy was investigated in rats. Both age and partial hepatectomy had significant effects upon hepatocyte counts per microscopic field. The number of hepatocytes per microscopic field declined with age in the control groups of different advancing ages and in the experimental groups of advancing ages. There was essentially no mitotic activity in the livers of the control groups. However, mitotic counts were greatly increased in livers from those animals that were partially hepatectomized; the increase in mitotic activity in the 13-month-old animals was double over that observed in both the very young and the very old.

It has been reported that the rate of liver regeneration in mice at 7 days decreased with age and was slowest in 18-month-old mice[3]. In partial hepatectomy studies of rats, the peak rate of DNA synthesis was delayed 5–9 h in 12–15-month-old rats when compared to 4-month-old rats[4]. This delay in peak rate of labeling occurred after the removal of only 9% of the liver. At least 68% of the liver needed to be excised in the younger rats to stimulate liver regeneration[5]. 14 days after partial hepatectomy, 1–2-month-old rats had a greater percentage of hepatic and binucleate cells[6] and a higher percentage of liver restored than 21–30-month-old rats[6,7]. Fine structural analysis of the organelles of the regenerated hepatocytes in the older rats showed that the cell components were changed in regenerated liver cells in the same manner and degree as in unoperated aged livers[8]. Our experiment used hepatocyte counts and mitotic activity to test the effect of partial hepatectomy in a more acute phase (at 48 h post surgery) of liver regeneration in rats of 3 different ages.

Methods. 3 groups of female Wistar rats were obtained from the Gerontology Research Center (NIA), Baltimore, Maryland. The 3 groups were 3 months old, 13 months old and 24 months old. 6 rats from each group were weighed and then partially hepatectomized (70%) under ether anesthesia[9]. The left lateral and median lobes of the liver were removed[9] and weighed. 48 h after surgery the rats were weighed, lightly anesthetized with ether and killed by decapitation. The remaining liver was weighed, and tissue samples were similarly prepared for microscopy. Mitotic and hepatocyte counts were performed on coded samples of liver at a magnification of $\times 450$. The results were expressed as the number of mitotic figures per 60 high power fields (HPF) and number of hepatocytes per high power field. Alternating fields equivalent to 2000–3000 nuclei were searched for mitotic figures. 3 additional rats from each age group served as controls. Significant differences were determined by Student's t-test.

Results. Liver weights. The average amounts of liver to be removed at partial hepatectomy increased with age; 4.9 g of liver in 3-month-old rats, 6.5 g of liver in 13-month-old rats, and 7.6 g of liver in 24-month-old rats (table 1). As expected, at autopsy, 48 h after partial hepatectomy, the amount of liver remaining increased with age (see table 1).

Hepatocyte mensuration and mitotic activity. Light microscopic examination of the liver tissue revealed hepatocyte changes and mitotic activity that were dependent upon both the partial hepatectomy and upon the age of the rats. Livers from the 3-month-old control rats showed 71.1 hepatocytes per HPF. Livers from the 13-month-old control rats had 58.3 hepatocytes per HPF, an 18% ($p < 0.01$) decrease in number, while the livers of the 24-month-old control rats had 49.2 hepatocytes per HPF, a 16% ($p < 0.01$) decrease (table 2). This steady age-dependent decrease of hepatocytes in control rats was paralleled in regenerating livers, but at a considerably lower hepatocyte count. The regenerating liver in the 3-month-old rat had 49.5 hepatocytes per HPF, while that of the 13-month-old rat had 43.5 hepatocytes per HPF, a 12% ($p < 0.01$) decrease, and that of the 24-month-rat had 35.5 hepatocytes per HPF, an 18% ($p < 0.01$) decrease (table 2). There were fewer hepatocytes

Table 1. Liver to body weight ratios of normal and partially hepatectomized rats of different ages

Treatment and measurements	No. per each age group	Ages 3 months	13 months	24 months	Significant difference from other ages
Control (body weights; g) ± SD	3	199.3 ± 3.9	320.0 ± 5.9	394.7 ± 5.9	$p < 0.01$
Liver removed (g); body weight (partial hepatectomy)	6	$\frac{4.97}{196.7} = 0.0254$	$\frac{6.52}{318.8} = 0.0204$	$\frac{7.61}{392.7} = 0.0194$	$p < 0.02$
Liver remaining (g): body weight (48 h post partial hepatectomy)	6	$\frac{3.60}{181.3} = 0.0199$	$\frac{4.61}{298.3} = 0.0155$	$\frac{5.32}{376.2} = 0.0141$	$p < 0.02$

Table 2. Relation of age to hepatocytes and mitotic counts

Treatment	Measurement	Age 3 months	13 months	24 months
Control ± SD	Hepatocytes/HPF[a]	71.1 ± 2.4	58.3 ± 2.3 $p < 0.01$[b]	49.2 ± 5.3 $p < 0.01$[b]
Control ± SD	Mitoses/60 HPF	0	1.7 ± 0.5	0
Partial hepatectomy ± SD	Hepatocytes/HPF	49.5 ± 2.2	43.5 ± 1.6 $p < 0.01$[b]	35.5 ± 2.1 $p < 0.01$[b,c]
Partial hepatectomy ± SD	Mitoses/60 HPF	52.6 ± 9.8	105.0 ± 35.2 $p < 0.02$[b]	54.0 ± 8.2 $p < 0.02$[c]

[a] HPF = High powered microscopic field; [b] significance from 3-month measures; [c] significance from 13-month measures.

per HPF in the regenerating livers of the rats of all age groups when compared to liver cell counts in control rats of comparable ages (table 2).

There were essentially no mitotic counts in the livers of the control rats (table 2). However, mitosis was induced in the partial hepatectomized rats and the activities were modified by age. The regenerating livers of the 3-month-old rats had 52.6 mitoses per 60 HPF, while the regenerating livers in the 13-month-old rats showed 105.0 mitoses per 60 HPF, an increase of 50% ($p < 0.02$). The mitotic activity in the regenerating livers of 24-month-old rats was similar to that in 3-month-old rats (table 2). Thus, the rates of mitotic change were highest in middle aged animals.

Discussion. These data support certain previous observations and supply missing information at the acute 48 h liver regeneration stage. First, up to 24 months there was an increase in the rat body weights. It would be expected that animals of this age would have already begun (or would soon begin) to lose weight[11,12]. Second, as observed from the amounts of liver removed plus that remaining there was an increase in the rat liver weights with age. This increase in rat liver weight with increasing age should be considered against the data published by Schmucker et al.[13] and Pieri et al.[14] where they both demonstrated specific reductions in the amount of protein synthesizing apparatus within aging hepatocytes. Their studies used fine-structural stereologic methods and demonstrated age reductions in the surface area of rough endoplasmic reticulum. Third, with advancing age there was a decrease in the hepatocyte count in both the partially hepatectomized rats and in the unoperated controls. In addition, there was a difference in the hepatocyte count between the experimental and the control rats for each age group with the hepatocyte count in the partially hepatectomized rats lower than the hepatocyte count in the control rats. This decreased hepatocyte count in the regenerating livers might be explained by an augmented hepatocyte volume[10]. Fourth, while essentially no hepatic mitosis was observed in control rats the partially hepatectomized rats exhibited a significant amount of hepatic mitotic activity. When the livers of the 13-month-old rats were compared to that of the 3-month-old rats, the livers of the older rats exhibited marked increases in the mitotic activities and decreases in the hepatocyte count. This showed that the number of mitoses per number of hepatocytes were elevated. However, when the livers of the 24-month-old rats were compared to that of the 13-month-old rats, the livers of the older rats exhibited sharp decreases in the mitotic activities. It may be hypothesized that hepatocytes in the older animals are fewer in number, possibly replaced by fat cells or connective tissue, or that the hepatocytes are larger and therefore appearing in fewer number per microscopic field area. The specific mechanism of decrease in mitotic activity recorded for the older rats is unknown and subject to all the theories of aging. Therefore, in partially hepatectomized rats, advancing age reduced the relative number of hepatocytes and the relative amount of mitotic activity.

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2 To whom reprint requests should be addressed.

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