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Liver Regeneration in Dogs: Morphologic and Chemical Changes[1]

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Forty-four percent and 72% hepatectomy were carried out in dogs and the animals were sacrificed for biochemical and pathologic studies from 0.5 to 6 days later. Compensatory hypertrophy and hyperplasia ("regeneration") were evident within 1 day, reached a maximum in 3 days, and were almost complete by 6 days. Coincident with the histologic events of regeneration were decreases in responsiveness of receptor adenyl cyclase to glucagon stimulation, increases of cyclic AMP, inconsistent changes in plasma insulin, and increases in plasma glucagon. These studies have standardized hepatic resection in dogs and they have focused attention upon some possible mechanisms that will require further study.

INTRODUCTION

The timing and events of hepatic regeneration in rats have been well studied, both in the past [28] and more recently [1, 2, 15, 18, 21, 30]. There is little analogous information about hepatic regeneration in dogs. The purpose of this paper is to provide this information and to focus attention upon some hitherto undescribed biochemical changes that occur in regenerating livers after both large and small resections.

METHODS

Mongrel dogs weighing 14.5 to 23.5 kg were used. Anesthesia for the operation was with sodium pentobarbital supplemented with phencyclidine hydrochloride and succinyl choline chloride. At the postoperative times indicated in Table 1 the animals were sacrificed under the same anesthesia to obtain tissues for biochemical and pathologic analysis. About 2 hr before killing, 0.2 mCi/kg body weight [*methyl*-^{3}H]thymidine was given intravenously. The specific activity was 47 Ci/mmol. For the biochemical studies, some of the liver tissues were kept in cold saline for fresh use and some were frozen and stored in liquid nitrogen.

The following biochemical techniques were used. Tissue protein was measured by the method of Lowry *et al.* [17]. Deoxyribonucleic acid (DNA) was purified by the method of Schneider and Greco [23], and its content was measured by Giles and Meyers' modification [8] of Burton's [3] diphenylamine method. [*Methyl*-^{3}H]-thymidine incorporation into DNA was expressed as disintegrations per minute per 100 μg of purified DNA. Adenylate cyclase was determined by the methods of White and Zenser [29], Krishna *et al.* [13], and Salomon *et al.* [22] after homogenation and incubation of the liver by the method of Makman and Sutherland [19] which was modified by freezing the homogenate in liquid nitrogen. Cyclic AMP was analyzed by the radioimmunoassay of Harper and

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TABLE 1

EXPERIMENTAL GROUPS

Group	Number of dogs	Duration of experiment (days)
72% Resection		
1	4	0.5
2	4	1
3	6	2
4	3	3
5	2	4
6	2	5
7	2	6
44% Resection		
8	3	1
9	3	2
10	3	3
11	3	4

Brooker [10] from a trichloroacetic acid extract that had been passed through a Dowex 50W-X8 column of 200 to 400 mesh.

For the pathologic studies, parts of the liver tissues were fixed in buffered formaldehyde and parts in glutaraldehyde.

Frozen and paraffin sections were prepared from the formalin-fixed material. The frozen sections were stained for fat and some of the paraffin sections were stained with hematoxylin and eosin and other special stains. The size of hepatocytes in the middle zones of the lobules was measured on the stained sections by a method previously described [25] and the results are expressed in arbitrary size units. Other paraffin sections were dewaxed, dipped in Ilford K2 nuclear emulsion, and used for autoradiography. They were exposed to the photographic emulsion for 3 to 6 weeks until counts of the labeled nuclei stopped increasing.

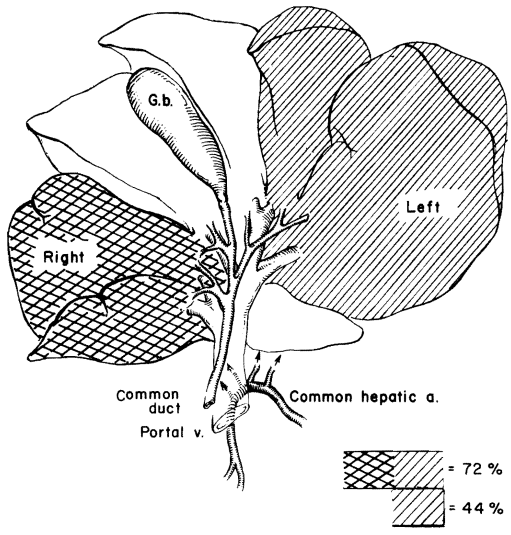

FIG. 1. Extent of hepatectomies. For 72% resection the two far right and two far left lobes were removed. For 44% resection only the two far left lobes were excised.

The liver tissues that had been immersed in glutaraldehyde were trimmed and the outer overfixed and the inner underfixed portions discarded. The well-fixed middle layer of tissue was then cut into small blocks, postfixed in osmium tetroxide, and embedded in epoxy resin. Sections 0.5 μm thick were cut and stained with Azure II for light microscopy. The middle zone of the liver lobules was identified and the blocks were retrimmed to contain only this area. Ultrathin sections were then cut from random blocks and examined under a Phillips EM 300 electron microscope. The volume of the cytoplasm of a large number of hepatocytes, the area of rough and smooth endoplasmic reticulum within these cells, and the dimensions of many mitochondria, microbodies, lysosomes, lipid inclusions, and clumps of glycogen were determined by the methods and formulas of Loud [16]. These measurements were then expressed in micrometers as volumes and areas for the average-size midzonal hepatocyte.

Two kinds of hepatectomy were studied. One was designed to remove more than two-thirds of the canine liver. The two leftmost and the two rightmost lobes were excised (Fig. 1). The other resection which was designed to remove less than half of the dog liver involved resection of the two leftmost lobes (Fig. 1). Removal of the two left lobes was most effectively done by a mass ligature which encompassed the hilar structures of these lobes as well as the

TABLE 2

AUTORADIOGRAPHY, CELL SIZE, AND MITOTIC ACTIVITY AFTER 44% HEPATIC RESECTION

Group	Number of dogs	Time delay (days)	Hepatocyte size (size units)	P^a	Number of labeled hepatocytes per 1000 hepatocytes	P^a	Number of mitoses per 1000 hepatocytes	P^a
Normal	6	0	0.170 ± 0.020	—	1.57 ± 0.27	—	0.078 ± 0.036	—
8	3	1	0.188 ± 0.037	NS	3.2 ± 0.37	<0.001	0.26 ± 0.038	<0.001
9	3	2	0.163 ± 0.022	NS	6.70 ± 1.37	<0.01	0.65 ± 0.17	<0.01
10	3	3	0.188 ± 0.047	NS	9.10 ± 1.59	<0.001	0.84 ± 0.17	<0.001
11	3	4	0.194 ± 0.029	NS	7.27 ± 0.66	<0.001	0.77 ± 0.10	<0.001

[a] *P* values compared to normal by Student's *t* test.

hepatic veins leaving the specimen. When the large ligature was tied down it amputated the liver down to the main structures. The two right lobes were best removed by ligation of the hilar structures of each and by individual ligation of the hepatic veins. Postoperatively, intravenous or subcutaneous glucose/electrolyte solutions were given for 1 day. Diet was then resumed and no further special care was required.

The times of sacrifice and the extent of resection which determined the experimental groups are summarized in Table 1. Just before performing the 72% hepatic resections, venous samples were drawn from the portal vein and the suprarenal inferior vena cava. Samples were drawn again when the abdomen was reopened for sacrifice 0.5 to 6 days later. Plasma hormone concentrations were measured in the laboratory of Dr. R. H. Unger, Dallas, Texas. Insulin was analyzed using the immunoassay of Herbert and his associates [11]. Glucagon and glucagonlike immunoreactivity were determined by the radioimmunoassay methods of Faloona and Unger [5]. The primary pancreatic glucagon measured with this technique has a molecular weight of 3500 although other larger moieties have some activity. Glucagonlike immunoreactivity arising from a variety of sources including gastrointestinal tract and salivary glands consists of at least two different molecular weight fractions as demonstrated by Moody [20]. The cross-reactivity of glucagon and glucagonlike immunoreactivity was about 2% in the Dallas laboratory.

RESULTS

Extent of Resection

Ten normal dogs weighing 8.8 to 35.9 kg were sacrificed. The liver weight was 2.43 ± 0.66% (SD) of the body weight. The two leftmost lobes plus the two rightmost lobes (Fig. 1), as removed in Groups 1 through 7, constituted 71.6 ± 1.6% (SD) of the total liver. The two leftmost lobes (Fig. 1), as removed in Groups 8 through 11, constituted 44.1 ± 3.9% (SD) of the liver.

44% Hepatic Resection

Pathologic observations. The animals killed 1, 2, 3, and 4 days after 44% hepatic resection all showed enlargement of the hepatocytes and the lobules in the remaining liver (Table 2). The enlargement of the hepatocytes included the nucleus and nucleolus and was accompanied by accumulation of large numbers of fat globules within the cytoplasm (Fig. 2). At 1 day there was very little glycogen in the liver cells; subsequently, the amount of glycogen slowly increased, but was still low at 4 days. The swollen hepatocytes caused narrowing of the sinusoidal spaces. The amount of both

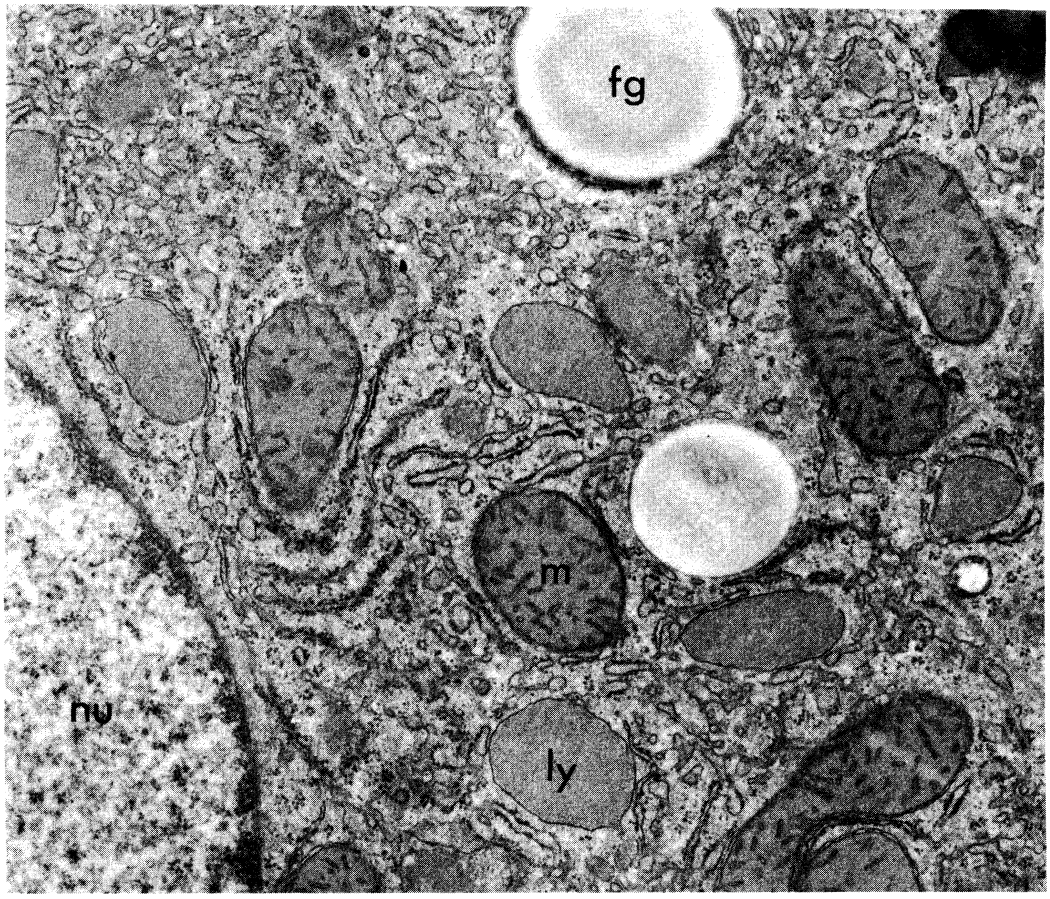

FIG. 2. Part of an enlarged hepatocyte from a dog subjected to 44% hepatectomy 24 hr earlier. Both rough and smooth endoplasmic reticulum are prominent. Fat globules (fg) are present. The number of lysosomes (ly) is increased. Glycogen is depleted. The mitochondria (m) appear normal. nu, Nucleus. Electron micrograph; ×20,250.

rough and smooth endoplasmic reticulum in each enlarged hepatocyte was increased. This increase was greatest 3 days after hepatic resection. Lysosomes were larger and more numerous; some formed large autophagosomes containing cell fragments. Microbodies also increased in numbers. Mitochondria remained normal and there was no evidence of loosening of the attachments between adjacent hepatocytes. The number of free ribosomes in the hepatocytes was not increased.

Autoradiography demonstrated increased uptake of tritiated thymidine into DNA (Table 2). This process had started the day after partial hepatectomy and reached a peak in 3 days. The labeled hepatocytes were most common in the peripheral part of the liver lobule. The number of hepatocytes in mitosis also increased after hepatectomy (Table 2).

The Kupffer cells increased in size and number and their phagosomes enlarged. The appearances of the other littoral, ductular, and connective tissue cells were not altered. These cells did show an increase in thymidine uptake and in the number of mitoses, but these changes lagged behind those in the hepatocytes and were randomly distributed in the lobule.

DNA synthesis. The increase in DNA synthesis had started by 24 hr reached a

TABLE 3

DNA SYNTHESIS AFTER 44% HEPATECTOMY

Group	Number of dogs	Duration of experiment (days)	DNA concentration (μg/g of wet liver)	DNA synthesis (dpm/100 μg of DNA)
Normal	6	Control	2691 ± 291	461 ± 98
8	3	1	1825 ± 188	741 ± 120
P[a]			<0.01	<0.05
9	3	2	2131 ± 97	1299 ± 229
P			<0.01	<0.001
10	3	3	2892 ± 357	1750 ± 235
P			NS	<0.001
11	3	4	2548 ± 411	1499 ± 81
P			NS	<0.001

[a] Statistical comparisons with the six normals by Student's *t* test.

maximum at 3 days, and began to decline in 4 days (Table 3).

Adenyl cyclase. The basal adenyl cyclase activity was unchanged at all time periods after 44% resection. However, there were highly significant changes in the receptor or glucagon-stimulated component of adenyl cyclase beginning the first day and continuing through the third postoperative day. At all the glucagon concentrations there was a marked decrease of responsiveness (Table 4). The total or sodium fluoride-stimulated adenyl cyclase activity was not significantly changed.

Cyclic AMP. The most dramatic change occurred 1 day after hepatectomy. Cyclic AMP concentration tripled, then started to return to normal, but remained significantly elevated even after 2, 3, and 4 days (Table 5).

72% Hepatic Resection

Pathologic observations. The changes in the liver remnants of the animals killed 0.5 to 6 days after 72% hepatectomy were the same as those after 44% resection but were more pronounced (Tables 6 and 7).

Incorporation of labeled thymidine into the nuclei had commenced in the hepatocytes at the periphery of the lobules by 12 hr after partial hepatectomy. Later the loca-

TABLE 4

ADENYLATE CYCLASE ACTIVITY AFTER 44% HEPATECTOMY

Group	Number of dogs	Duration of experiment (days)	Adenylate cyclase activity (nmol of cAMP/mg of protein homogenate/15 min)						
			Basal[a] (H_2O)	Glucagon stimulated					NaF stimulated (10 m*M*)
				10^{-9} *M*	10^{-8} *M*	10^{-7} *M*	10^{-6} *M*	10^{-5} *M*	
Normal	19		19 ± 5	40 ± 13	59 ± 13	77 ± 17	93 ± 22	97 ± 24	126 ± 40
8	3	1	19 ± 3	26 ± 3	38 ± 7	72 ± 11	78 ± 8	92 ± 21	143 ± 19
P[b]			NS	<0.1 > 0.05	<0.05	NS	NS	NS	NS
9	3	2	16 ± 9	21 ± 13	31 ± 19	43 ± 26	50 ± 31	54 ± 37	106 ± 65
P[b]			NS	<0.05	<0.02	<0.05	<0.02	<0.02	NS
10	3	3	17 ± 5	18 ± 5	27 ± 3	31 ± 4	38 ± 3	41 ± 3	81 ± 8
P[b]			NS	<0.01	<0.001	<0.001	<0.001	<0.001	NS
11	3	4	25 ± 6	42 ± 14	49 ± 14	61 ± 10	74 ± 21	79 ± 14	186 ± 22
P[b]			NS	NS	NS	NS	NS	NS	NS

[a] Values under basal activity reflect receptor and catalytic components of adenyl cyclase activity. Values under glucagon stimulated reflect receptor activity at different molar concentrations of glucagon. The sodium fluoride (NaF) stimulation reflects the full (catalytic) activity of adenyl cyclase.

[b] Statistical comparisons with the normals by Student's *t* test.

TABLE 5

CYCLIC AMP AFTER 44% HEPATECTOMY

Group	Number of dogs	Duration of experiment (days)	Cyclic AMP (pmol/g of wet liver)	P^a
Normal	12	Control	1077 ± 204	
8	3	1	3138 ± 104	<0.001
9	3	2	1629 ± 206	<0.01
10	3	3	2016 ± 244	<0.001
11	3	4	1600 ± 81	<0.001

[a] Statistical comparisons with the 12 normals by Student's *t* test.

tion of labeled hepatocytes became random. The peak response was at 3 days. The increase in the number of hepatocytes in mitosis followed the same pattern. Enlargement of the hepatocytes, accumulation of lipid within their cytoplasm, and increase in the amount of both rough and smooth endoplasmic reticulum also reached a peak at about 3 days after hepatectomy. The growth in numbers of microbodies and the multiplication and increase in size of lysosomes reached a maximum a day earlier. The glycogen content of the cells was at its lowest 24 hr after hepatectomy; thereafter there was a slow recovery. The size and structure of the mitochondria were unaltered at all time periods following liver resection.

The wave of increased incorporation of labeled thymidine and mitoses in the littoral, ductular, and connective tissue cells reached its peak at 4 to 5 days after partial hepatectomy.

DNA synthesis. The timing of response after 72% hepatic resection was the same as with the 44% resection, but its magnitude was four times as great (Table 8). As with 44% resection the first significant increase was at 1 day, the peak response was at 3 days, and by 4 days a downward trend was identifiable. However, even after 6 days, DNA synthesis was still higher than normal. DNA concentration after Days 2 and 3 was decreased to a significant extent (Table 8).

Adenyl cyclase. The basal adenyl cyclase was unchanged at all sampling times from 0.5 to 6 days postoperative. The same applied to the total catalytic adenyl cyclase (Table 9).

In contrast, the receptor, or glucagon-stimulated, component of adenyl cyclase was significantly depressed at low glucagon concentrations as early as 24 hr. At 1, 2, and 3 days this decrease of responsiveness to glucagon was evident at all the lower concentrations. By 4 days these adenyl cyclase

TABLE 6

AUTORADIOGRAPHY, CELL SIZE, AND MITOTIC ACTIVITY AFTER 72% HEPATIC RESECTION

Group	Number	Time delay (days)	Hepatocyte size (size units)	P^a	Number of labeled hepatocytes per 1000 hepatocytes	P^a	Number of mitoses per 1000 hepatocytes	P^a
Normal	6	—	0.170 ± 0.020	—	1.57 ± 0.27	—	0.078 ± 0.036	—
1	4	0.5	0.174 ± 0.016	NS	2.1 ± 0.5	NS	0.18 ± 0.083	NS
2	4	1	0.195 ± 0.028	NS	7.7 ± 2.0	<0.001	0.70 ± 0.136	<0.001
3	6	2	0.213 ± 0.026	<0.02	13.4 ± 1.2	<0.001	1.08 ± 0.136	<0.001
4	3	3	0.252 ± 0.056	NS	31.1 ± 3.5	<0.001	2.99 ± 0.389	<0.001
5	2	4	0.282 ± 0.000	<0.001	20.9 ± 5.1	<0.01	1.50 ± 0.485	<0.05
6	2	5	0.179 ± 0.027	NS	11.3 ± 3.1	<0.02	0.98 ± 0.234	<0.01
7	2	6	0.181 ± 0.019	NS	7.4 ± 1.1	<0.01	0.66 ± 0.065	<0.001

[a] *P* values compared to normal by Student's *t* test.

TABLE 7

ULTRASTRUCTURAL COMPOSITION OF AVERAGE MIDZONAL HEPATOCYTE IN THE DOG AT VARIOUS INTERVALS AFTER 72% HEPATECTOMY

Group	Days after partial hepatectomy	Number of dogs	Average volume (μm^3)[a] of hepatocyte occupied					Average area (μm^2)[a]	
			Mitochondria	Microbodies	Lysosomes	Lipid	Glycogen	Rough endoplasmic reticulum	Smooth endoplasmic reticulum
Normal	0	6	1,262 ± 235	85 ± 10	31 ± 5	19 ± 10	869 ± 31	32,243 ± 379	18,714 ± 430
1	0.5	4	1,180 ± 273	240 ± 19	107 ± 9	276 ± 16	117 ± 12	36,605 ± 467	19,800 ± 441
P[b]			NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01
2	1	4	1,259 ± 177	194 ± 5	327 ± 17	1,216 ± 458	103 ± 7	35,767 ± 626	20,081 ± 337
P			NS	<0.001	<0.001	<0.01	<0.001	<0.001	<0.01
3	2	6	1,251 ± 192	183 ± 19	245 ± 35	692 ± 284	188 ± 15	39,879 ± 689	25,172 ± 483
P			NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
4	3	3	1,197 ± 300	169 ± 13	217 ± 10	851 ± 63	219 ± 26	51,116 ± 110	32,700 ± 394
P			NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
5	4	2	1,134 ± 115	113 ± 4	190 ± 9	275 ± 35	439 ± 38	44,121 ± 2,070	29,516 ± 3,719
P			NS	<0.01	<0.001	<0.001	<0.001	<0.01	<0.05
6	5	2	1,214 ± 97	107 ± 9	132 ± 5	333 ± 301	585 ± 78	37,000 ± 3,153	27,871 ± 864
P			NS	NS	<0.001	NS	<0.02	NS	<0.001
7	6	2	1,242 ± 15	91 ± 2	90 ± 15	514 ± 167	792 ± 23	33,613 ± 605	20,018 ± 1,120
P			NS	NS	<0.01	<0.05	<0.05	NS	NS

[a] Measured and calculated by the methods of Loud [16].
[b] Compared to normal by Student's *t* test.

changes had reverted to normal (Table 9) in spite of the fact that active regeneration was still proceeding by parameters of autoradiography and DNA synthesis (see below).

Cyclic AMP. As with the 44% resection there was an early increase in hepatic cyclic AMP. This change was maximum after 1 day, but was already fully evident within 12 hr. By 3 days the cyclic AMP had returned to normal and on Days 4 and 5, the cyclic AMP was depressed below normal to a statistically significant degree. The cyclic AMP response after 72% resection was distinctly more transient than after the 44% resection. With the smaller hepatectomy the cyclic AMP increase lasted for 3 days instead of 1 day with the more extensive operation (Table 10).

Hormone Studies

The insulin and glucagon levels after 72% hepatectomy are summarized in Tables 11 and 12. No consistent change in either portal or vena caval insulin concentration could be detected. In contrast, portal and vena caval glucagon concentrations tended to be higher than control up to 4 days after resection.

TABLE 8

DNA SYNTHESIS AFTER 72% HEPATECTOMY

	Group	Number of dogs	Duration of experiment (days)	DNA concentration (μg/g of wet liver)	DNA synthesis (dpm/100 μg of DNA)
	Normal	6		2691 ± 291	461 ± 98
	1	4	0.5	2708 ± 516	570 ± 128
P[a]				NS	NS
	2	4	1	2278 ± 245	870 ± 93
P				NS	<0.001
	3	6	2	2057 ± 237	2934 ± 697
P				<0.01	<0.001
	4	3	3	2158 ± 87	7162 ± 1196
P				<0.01	<0.001
	5	2	4	2529 ± 2	3303 ± 515
P				NS	<0.01
	6	2	5	2431 ± 450	1169 ± 66
P				NS	<0.001
	7	2	6	1952 ± 111	887 ± 63
P				<0.01	<0.01

[a] Statistical comparisons with the normals by Student's *t* test.

TABLE 9

ADENYLATE CYCLASE ACTIVITY AFTER 72% HEPATECTOMY[a]

Group	Number of dogs	Duration of experiment (days)	Adenylate cyclase activity (nmol of cAMP/mg of protein homogenate/15 min)						
			Basal[a] (H_2O)	Glucagon stimulated[b]					NaF stimulated[b] (10 m*M*)
				10^{-9} *M*	10^{-8} *M*	10^{-7} *M*	10^{-6} *M*	10^{-5} *M*	
Normal	19	Control	19 ± 5	40 ± 13	59 ± 13	77 ± 17	93 ± 22	97 ± 24	126 ± 40
1	4	0.5	13 ± 2	27 ± 3	48 ± 8	62 ± 18	101 ± 13	103 ± 13	163 ± 27
P[b]			NS	NS	NS	NS	NS	NS	NS
2	4	1	16 ± 3	25 ± 6	38 ± 9	61 ± 17	85 ± 21	95 ± 30	135 ± 36
P			NS	<0.05	<0.01	NS	NS	NS	NS
3	6	2	23 ± 6	25 ± 7	38 ± 11	54 ± 18	75 ± 26	84 ± 29	151 ± 15
P			NS	<0.01	<0.01	<0.01	NS	NS	NS
4	3	3	24 ± 5	30 ± 6	37 ± 9	51 ± 10	73 ± 19	73 ± 14	153 ± 17
P			NS	NS	<0.02	<0.02	NS	NS	NS
5	2	4	29 ± 5	45 ± 9	58 ± 17	79 ± 10	97 ± 19	94 ± 11	175 ± 7
P			NS	NS	NS	NS	NS	NS	NS
6	2	5	25 ± 12	31 ± 21	44 ± 20	66 ± 24	73 ± 8	81 ± 9	176 ± 93
P			NS	NS	NS	NS	NS	NS	NS
7	2	6	31 ± 7	42 ± 9	54 ± 10	63 ± 4	73 ± 3	71 ± 7	172 ± 45
P			NS	NS	NS	NS	NS	NS	NS

[a] Explanation of terms in Table 4.
[b] Statistical comparison with normals by Student's *t* test.

DISCUSSION

These results have shown that regeneration of the dog liver is as fully predictable as hepatic regeneration in the rat, but with important differences in timing. The maximum response in the dog is seen at 3 days in contrast to the well-known 24-hr peak in the rat.

The technique of 72% hepatic resection in the dog proved to be a model of reproducibility. Excision of the two leftmost and the two rightmost lobes has a percentage ablation that in 10 dogs ranged only from 70 to 75%, with a very small standard deviation. The 44% resection was somewhat less reliable in that the removal of the two leftmost lobes alone was an ablation ranging from 40 to 51%.

After both the 44 and 72% canine liver resections all the phenomena described after rat hepatectomies [1, 28] were seen including hepatocyte enlargement, lipid

TABLE 10

CYCLIC AMP AFTER 72% HEPATECTOMY

Group	Number of dogs	Duration of experiment (days)	Cyclic AMP (pmol/g of wet liver)	*P*[a]
Normal	12		1077 ± 204	—
1	4	0.5	1628 ± 421	<0.05
2	4	1	1867 ± 160	<0.001
3	6	2	989 ± 210	NS
4	3	3	738 ± 65	<0.01
5	2	4	646 ± 8	<0.001
6	2	5	819 ± 87	<0.05
7	2	6	877 ± 3	<0.02

[a] Statistical comparisons were with 12 normals by Student's *t* test.

infiltration, glycogen depletion, and temporary increase in numbers of lysosomes, autophagosomes, and microbodies. We did not find any marked alterations in the mitochondria, nor did we find increased numbers of free ribosomes in the cytoplasm of the regenerating hepatocytes or any indications of loosening of the attachments of the hepatocytes to their neighbors. This is in agreement with Grisham's [9] findings in the rat. The observations with autoradiography were also similar to those seen in the rat. There was the same commencement of DNA synthesis and mitosis in the hepatocytes at the periphery of the lobules with later extension to the hepatocytes in the central zones. The only difference was the time of maximal activity.

It was of interest that when increased thymidine incorporation occurred in cells of the hepatic remnant other than the hepatocytes, the littoral, ductular, and connective tissue cells participated fully. Particularly because of this, it was interesting to compare the overall results of autoradiography with those of DNA synthesis. The conformity between the two techniques was close. Furthermore, the number of cells in mitoses was proportional to thymidine uptake even though the number of cells actually dividing was only about 10% of those imbibing thymidine autoradiographically.

In recent years there has been a great interest in the influence of portal blood constituents upon liver regeneration [2, 4, 7, 12, 14, 15, 25, 26]. Attention has been focused upon hormones such as insulin and glucagon as important factors for the normal expression of regeneration [6, 21, 26, 27]. Although the present study was not designed to test the hormone hypothesis, some of the observations are worth noting. After 72% hepatectomy, the portal and vena caval levels of plasma insulin underwent inconsistent changes, but glucagon rose first and later returned toward normal. Cyclic changes in hormone concentrations and in hepatocyte cell membrane receptivity to the hormones was noted earlier by Leffert *et al.* [15] after hepatectomy in rats. Thus, the measurement of plasma hormone concentrations gives a woefully incomplete picture of the dynamic events following hepatectomy.

The same can be said of the swiftly occurring changes in adenyl cyclase and cyclic AMP. Beginning within 12 hr after hepatic

TABLE 11

PLASMA INSULIN LEVELS IN PORTAL VEIN AND INFERIOR VENA CAVAL BLOOD

Time delay (days)	Dog name	Plasma insulin (μU/ml)			
		Portal venous blood		Inferior vena caval blood	
		Pre-operative	Sacrifice	Pre-operative	Sacrifice
0.5	REG 14	6	43	3.4	16.5
0.5	REG 15	55	>200	25	14
1	REG 18	>200	85	71	80
1	REG 20	70	>200	22	58
2	70HX 1	88	46	10	7
2	70HX 2	Not done	27	Not done	17
3	70HX 5	40	48	19	0
3	70HX 6	8	85	8	7
4	REG 21	0	39	8	0
4	REG 23	12	7	8	9
5	REG 19	80	39	7	12
5	REG 22	43	49	30	27
6	REG 16	10	66	18	49
6	REG 17	43	39	95	18

TABLE 12

PLASMA GLUCAGON LEVELS IN PORTAL VENOUS AND INFERIOR VENA CAVAL BLOOD

Time delay (days)	Dog name	Plasma glucagon (pg/ml)			
		Portal venous blood		Inferior vena caval blood	
		Pre-operative	Sacrifice	Pre-operative	Sacrifice
0.5	REG 14	130	120	65	1050
0.5	REG 15	86	480	160	175
1	REG 18	53	335	0	150
1	REG 20	98	410	30	160
2	70HX 1	140	1400	74	470
2	70HX 2	—	170	—	275
3	70HX 5	98	120	74	640
3	70HX 6	140	1150	86	470
4	REG 21	160	620	86	160
4	REG 23	100	98	64	110
5	REG 19	115	520	90	360
5	REG 22	86	53	110	64
6	REG 16	130	240	110	200
6	REG 17	150	90	115	290

resection the glucagon-stimulated adenyl cyclase activity became depressed and remained so for 1 to 3 days, the same general time period during which hyperglucagonemia was documented and cyclic AMP was elevated. Presently, however, we can only speculate how central these changes in adenyl cyclase and cyclic AMP are to the initiation and maintenance of an appropriate regeneration response. In this regard are the suggestions made by McManus [18] and by Short and his associates [24] that a very early elevation in cyclic AMP is an essential first step, if not a triggering step, for cell renewal.

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