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THE EFFECT OF SPLANCHNIC VISCERA REMOVAL UPON CANINE LIVER REGENERATION

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THERE has been growing interest in recent years about the role of portal blood factors in fostering and controlling the hepatic regeneration that follows partial hepatectomy. That portal blood might have a specific effect on regeneration was postulated from our early observations in dogs which showed that portal blood profoundly influenced the size, chemical composition, microscopic structure and spontaneous mitoses of intact isogeneic or allogenic livers (34, 35, 36, 55). The possibility of an effect on regeneration was purely speculative, since hepatectomies were not performed in any of these studies. However, Lee (29), Fisher (17) and Chandler (9) and their associates soon demonstrated a portal blood effect on regeneration after resection of the liver in rats, and there have been many subsequent confirmatory reports.

Why portal blood affects regeneration remains controversial with much conflicting evidence. In 1972, Price and co-authors (43) suggested that glucagon was the responsible factor. A year later, we (52) postulated from observations with livers which had not been partially resected that multiple splanchnic factors including, but by no means confined to, endogenous insulin probably contributed to the over-all hepatotrophic effects of which regeneration was presumed to be one. The multifactorial hypothesis was promptly tested in our laboratory (57) in actual hepatectomy experiments and found valid. Although an insulin effect was easily demonstrated, the evidence was equally convincing that there were additional portal factors significant to regeneration, including important unidentified substances from the intestine. However, reports by Bucher and Swaffield (7), Duguay and Orloff and their associate (11, 12, 51) seemingly assigned an overriding role in normal regeneration to the pancreas, and, particularly, to the synergistic action of endogenous insulin and glucagon.

In the investigation herein reported, the multiple factor hypothesis has been examined again and confirmed in dogs by performing a partial hepatectomy in combination with various eviscerations with, or without, hormone replacement therapy. An attempt was made to determine some of the mechanisms of regeneration with extensive biochemical studies of the regenerating livers.

METHODS

Our previously published data from normal mongrel dogs or dogs submitted to a 44 or 72 per cent hepatectomy (19) were used as controls for the experimental series herein reported. In all this work, the anesthesia used for operations and sacrifice was pentobarbital sodium supplemented with phencyclidine hydrochloride and succinylcholine chloride. About two hours before sacrifice, 0.2 millicurie per kilogram of body weight (CH_3-^3H) thymidine, with a specific activity of 47 curies per millimole, was given intravenously. Liver tissues for biochemical analyses were kept in cold saline solution for fresh use or were frozen and stored in liquid nitrogen. For pathologic studies, hepatic tissues were fixed in 10 per cent normal buffered Formalin, 37 per cent aqueous solution of formaldehyde. Other liver bits were fixed in glutaraldehyde solution and then postfixed in osmic acid and embedded in Epon, a synthetic embedding medium.

Our biochemical techniques have been more completely described elsewhere (19, 53). Tissue proteins were measured by the method of Lowry and colleagues (32), deoxyribonucleic acid and deoxyribonucleic acid synthesis by the method of Giles and Myers (22), adenyl cyclase by the

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TABLE I.—REGENERATION AFTER SPLANCHNIC ORGAN EXTIRPATION, EXPERIMENTAL GROUPS

Group	Total experiments	Completed experiments	Duration experiment, days	Per cent hepatectomy	Pancreas removed	Other nonhepatic splanchnic organs removed: All except colon	Other nonhepatic splanchnic organs removed: All	Hormone infusion, units or mgm./kgm./day
1	53	24	1, 2, 3, 4	44	Yes			
2	23	10	1, 2, 3	72	Yes			
3	25	9	1, 2, 3	44	No	X		
4	15	4	1, 2	72	No		X	
5	30	12	1, 2, 3	44	Yes		X	
6	14	4	2	72	Yes		X	
7	29	9	1, 2, 3	44	Yes	X		
8	32	4	2	44	Yes		X	0.183 to 0.32*
9	36	4	2	44	Yes		X	0.021 to 0.028†
10	13	5	2	44	Yes		X	0.17 to 0.24* 0.021 to 0.029†
11	10	3	2	72	Yes		X	0.57, 0.40, 0.53†
12	6	3	2	72	Yes		X	0.11, 0.11, 0.08†

Control experiments of 44 and 72 per cent hepatectomy without splanchnic organ ablation are reported elsewhere (19). The results of these pure regeneration experiments are summarized on all graphs of the present study.
*Insulin, units per kgm. per day.
†Glucagon, mgm. per kgm. per day.

TABLE II.—CELL SIZE, AUTORADIOGRAPHY AND MITOTIC ACTIVITY FOLLOWING A 44 PER CENT HEPATECTOMY AND VARIOUS EVISCERATIONS

Group	No.	Time, days	Hepatocyte size, size units	P*	ARG labeled hepatocytes per 1,000	P*	Mitoses per 1,000 hepatocytes	P*
Normal	6	0	0.170±0.020	—	1.57±0.27	—	0.078±0.036	—
Normal + 44 per cent Hx	3	1	0.188±0.037	—	3.20±0.37	—	0.260±0.038	—
	3	2	0.163±0.022	—	6.70±1.37	—	0.650±0.170	—
	3	3	0.188±0.047	—	9.10±1.59	—	0.840±0.170	—
	3	4	0.194±0.029	—	7.27±0.66	—	0.770±0.100	—
1	6	1	0.185±0.032	NS	1.55±0.21	<0.001	0.16±0.10	<0.05
	6	2	0.190±0.010	NS	2.52±1.84	<0.01	0.26±0.18	<0.05
	9	3	0.201±0.051	NS	5.75±3.92	NS	0.70±0.73	NS
	3	4	0.213±0.027	NS	5.80±0.33	NS	0.79±0.35	NS
3	3	1	0.121±0.018	NS	1.51±0.39	<0.02	0.437±0.385	NS
	3	2	0.140±0.034	NS	1.03±0.95	<0.01	0.090±0.078	<0.02
	3	3	0.130±0.011	NS	3.55±3.43	NS	0.333±0.318	NS
5	3	1	0.142±0.031	NS	0.77±0.01	<0.001	0.063±0.004	<0.01
	7	2	0.161±0.042	NS	1.57±0.93	<0.001	0.144±0.080	<0.01
	2	3	0.141±0.013	NS	1.18±1.12	<0.02	0.090±0.090	<0.02
7	3	1	0.182±0.016	NS	0.29±0.34	<0.01	0.030±0.042	<0.01
	3	2	0.171±0.018	NS	1.68±2.07	<0.05	0.187±0.229	NS
	3	3	0.196±0.029	NS	1.29±0.16	<0.01	0.140±0.045	<0.01

*Compared to normal + 44 per cent hepatectomy for same day by Student's t test.
ARG, Autoradiography.
Hx, Hepatectomy.
NS, Not significant.

method of Salomon and associates (46) and cyclic 3′, 5′-adenosine monophosphate by the method of Harper and Brooker (24).

Our techniques for pathologic study have also been fully described elsewhere (19, 52, 53). In essence, staining, autoradiographic, light and electron microscopic and analytic techniques were used that permitted quantitation of cell size, accurate determination of thymidine uptake, evaluation of the hepatocyte organelles and detection of other light microscopic or ultrastructural features.

The experimental groups are listed in Table I and were defined by the extent of hepatectomy; by extent of nonhepatic splanchnic organ ablation, and, in groups 8, 9, 10, 11 and 12, by infusion intraportally of insulin or glucagon, or both. In all the dogs, a 44 or 72 per cent hepatectomy was carried out. As described elsewhere (19), the 72 per cent hepatectomy required excision of the two most left and two most right lobes of the liver, whereas the 44 per cent hepatectomy required removal only of the two most left lobes.

The types of organ removal used are shown schematically in Figure 1 and ranged from total pancreatectomy (Fig. 1A) through removal of all nonhepatic splanchnic viscera with (Fig. 1B) or without retention of the colon and rectum (Fig. 1C). The total pancreatectomies of group 1 were performed at the same time as the 44 per cent hepatectomy in nine completed experiments and one day before hepatectomy in the other 15 completed experiments. Finally, operations were performed in which the isolated pancreas was re-

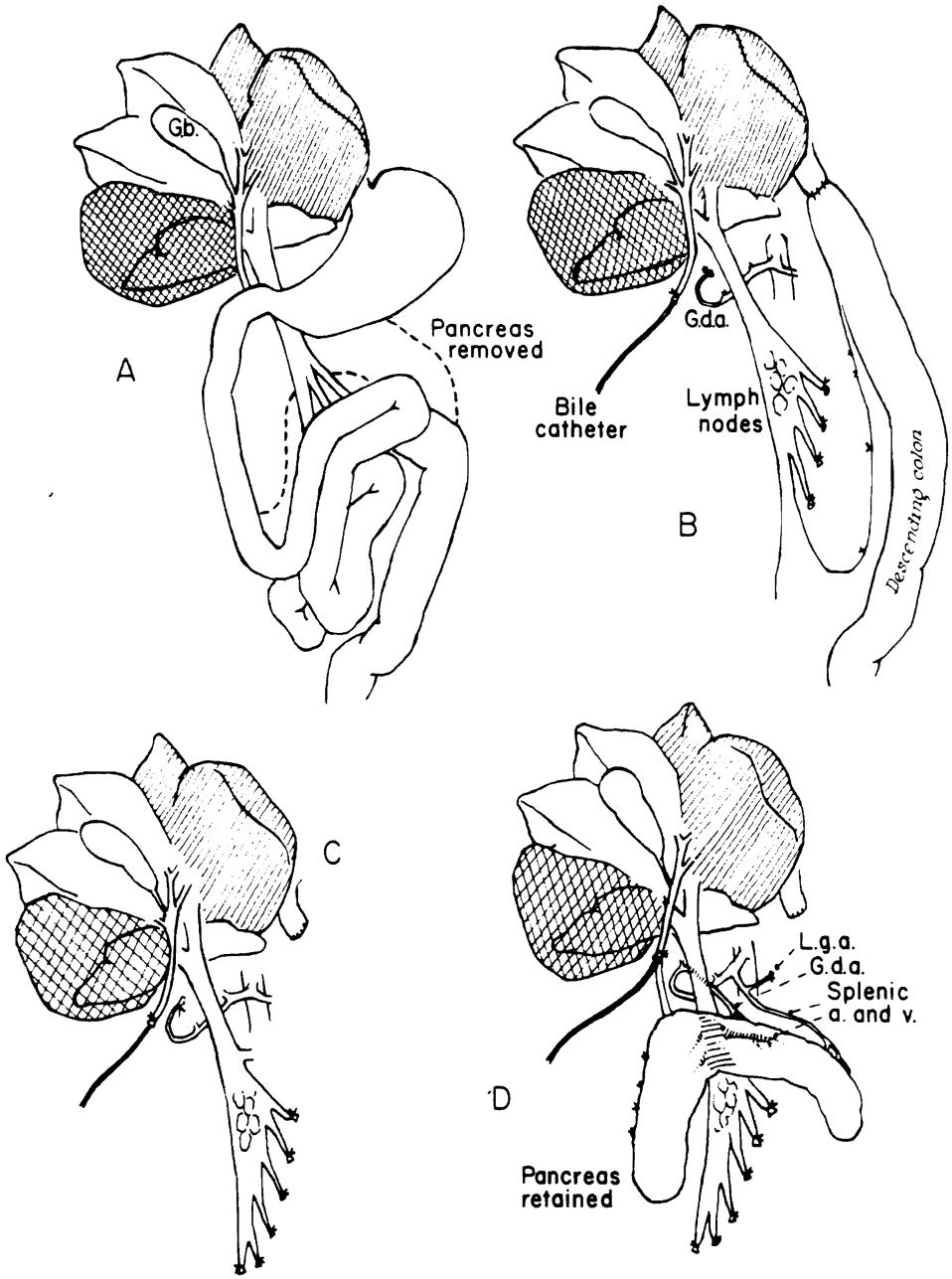

FIG. 1. Surgical procedures used in animals submitted to concomitant partial hepatectomy. A, Total pancreatectomy; B, nonhepatic splanchnic evisceration with retained distal colon; C, total nonhepatic splanchnic evisceration, and D, nonhepatic splanchnic evisceration with retention of isolated pancreas. *G.d.a.*, Gastroduodenal artery; *L.g.a.*, left gastric artery.

tained (Fig. 1D). A more complete description of our evisceration techniques has been published elsewhere (53). In all preparations except total pancreatectomy in groups 1 and 2, bile duct catheters were left in place to decompress the liver.

Plasma hormone concentrations were determined in the laboratory of Doctor R. H. Unger of Dallas. Postoperative plasma insulin concentrations were measured by the immunoassay of Herbert and associates (25) in four dogs submitted to the kind of complete evisceration shown in Figure 1C but without hepatectomy and in three more dogs without hepatectomy in which the distal parts of the colons were retained (Fig. 1B).

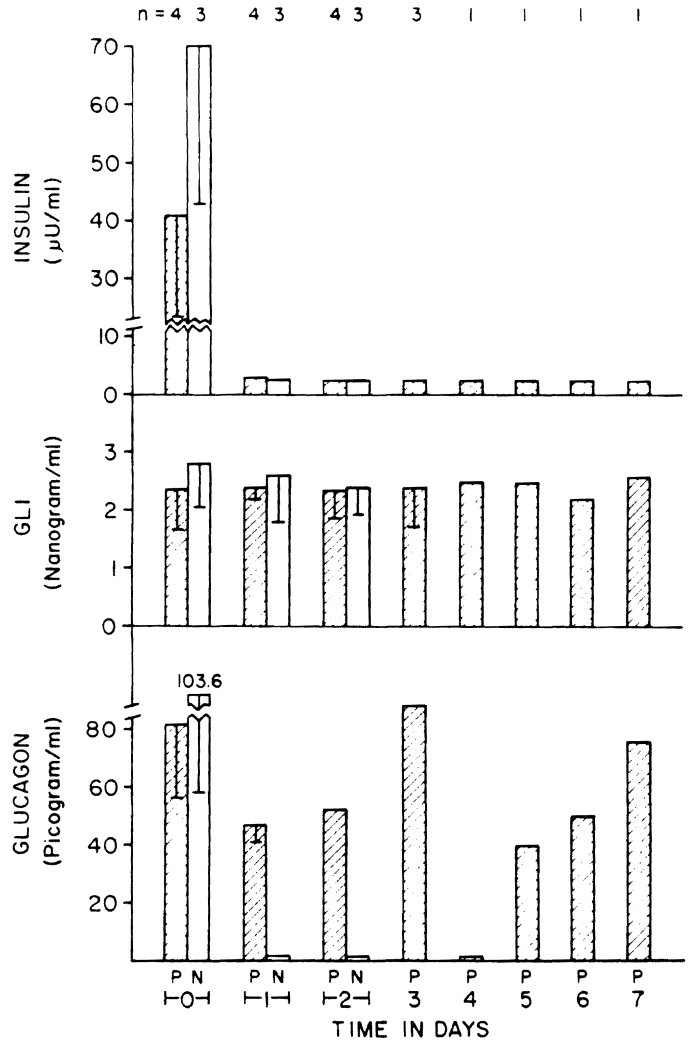

FIG. 2. Insulin, glucagon-like immunoreactivity, *GLI*, glucagon—mean ± S.D.—in dogs submitted to complete nonhepatic splanchnic evisceration, open bars, *N*, and to nonhepatic splanchnic evisceration with retention of distal part of colon, shaded bars, *P*. These dogs did not undergo partial hepatectomy. Statistical comparison by Student's t test is to intact fasted dogs.

In the same dogs, glucagon and glucagon-like immunoreactivity were determined by the radioimmunoassay methods of Faloona and Unger (14). The pancreatic glucagon measured with this technique has a predominant molecular weight of 3,500, whereas the glucagon-like immunoactivity is more heterogenous. The cross reactivity of glucagon and glucagon-like activity in the Dallas laboratory is about 2 per cent. In 15 more dogs of group 1, plasma insulin concentrations were measured one day after total pancreatectomy and just before performance of a 44 per cent hepatectomy.

When hormone infusions were given, a catheter was inserted into a side branch of the portal vein and connected to a battery driven constant infusion pump, as we have described before (59). To obtain the 91 finished experiments in Table I, 286 experiments were done. The heavy mortality occurred in spite of intensive fluid and electrolyte therapy and antibiotic treatment.

RESULTS

Hormone Studies

Within 24 hours after near complete (Fig. 1B) or complete (Fig. 1C) evisceration, plasma insulin concentrations fell to approximately 5 microunits per milliliter (Fig. 2). These trace

levels which are considered significant in the laboratories in which the analyses were done persisted for as long as a week. Comparably decreased, but still significant, levels persisted one day after total pancreatectomy (Fig. 3).

Circulating glucagon was not detectable 24 hours after complete evisceration (Fig. 2) but remained present in significant amounts if the distal part of the colon was retained (Fig. 2). The colon has been shown by Morita (37) and Samols (47) and their associates to produce glucagon. Glucagon-like immunoreactivity was unaffected by evisceration (Fig. 2) which was not surprising, since this hormone has been demonstrated by Lawrence and co-authors (28) to have extra-alimentary origins, including the salivary glands.

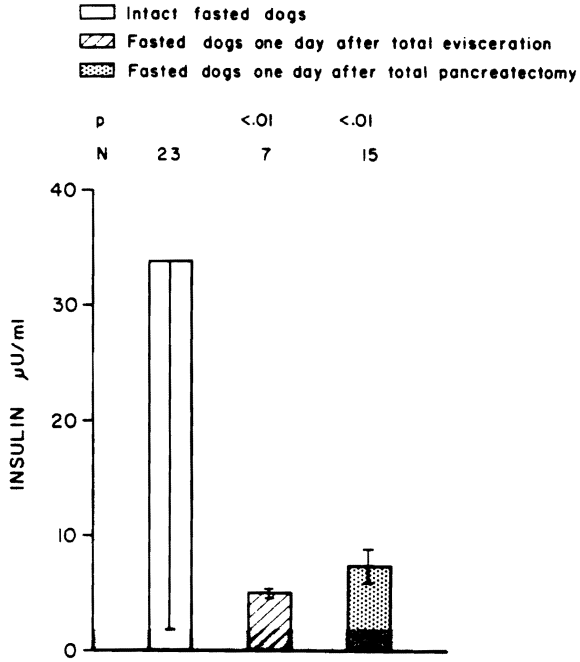

FIG. 3. Plasma insulin concentrations in normal dogs is shown at left. The center bar shows insulin one day after the evisceration shown in Figure 1B, four dogs, or Figure 1C, three dogs. The right bar shows plasma insulin concentration one day after total pancreatectomy. All values are mean ± S.D. The statistical comparison with Student's t test are to the intact dogs. The shaded area represents the minimum concentration detectable for this laboratory.

Pathologic Studies

Autoradiographic findings. After a 44 per cent hepatic resection in normal dogs, an increase in thymidine incorporation was evident by one day, which reached a maximum in three days. This was reflected in an elevenfold rise in number of actual mitoses (Table II).

The regeneration response during the three days of study was almost totally eliminated in groups 5 and 7 by complete, or nearly complete, evisceration (Table II). A significant, but less severe, reduction of response was caused in group 1 by pancreatectomy alone and, in group 3, by excision of all the viscera except the pancreas (Table II). In the completely eviscerated dogs of groups 8, 9 and 10, a normal pattern of regeneration was not restored at two days by the intraportal infusion of insulin or glucagon, or insulin and glucagon in combination (Table III).

The findings were similar after a 72 per cent hepatectomy in that complete evisceration in the dogs of group 6 completely eliminated regeneration at two days (Table IV). Evisceration but with retention of the pancreas in the dogs of group 4 yielded similar results. After pancreatectomy alone, there was an incongruously high incidence of thymidine incorporation and actual mitoses at two days but not at other times. The intraportal infusion of high and low dosages of glucagon in the dogs of groups 11 and 12 had no effect upon hepatocyte renewal two days after a 72 per cent hepatectomy and complete evisceration (Table III).

Other pathologic observations. The effect of different procedures upon hepatocyte size is summarized in Tables II, III and IV. All the dogs that underwent resection of the liver showed enlargement of the nucleus and cytoplasm of some,

TABLE III.—CELL SIZE, AUTORADIOGRAPHY AND MITOTIC ACTIVITY FOLLOWING PARTIAL HEPATECTOMY, TOTAL NONHEPATIC SPLANCHNIC EVISCERATION AND HORMONE INFUSION

Group	*No.*	*Time, days*	*Hepatocyte size, size units*	*P**	*ARG labeled hepatocytes per 1,000*	*P**	*Mitosis per 1,000 hepatocytes*	*P**
8	5	2	0.169±0.051	NS	2.15±1.44	NS	0.228±0.163	NS
9	4	2	0.170±0.033	NS	1.56±1.05	NS	0.650±0.095	NS
10	5	2	0.169±0.024	NS	2.63±4.11	NS	0.428±0.706	NS
11	3	2	0.203±0.038	NS	0.42±0.48	NS	0.047±0.052	NS
12	3	2	0.226±0.033	NS	0.37±0.50	NS	0.030±0.042	NS

*Compared to animals with total nonhepatic splanchnic evisceration, comparable hepatectomy and no hormone infusion on same day after hepatectomy by Student's t test.
ARG, Autoradiography
NS, Not significant

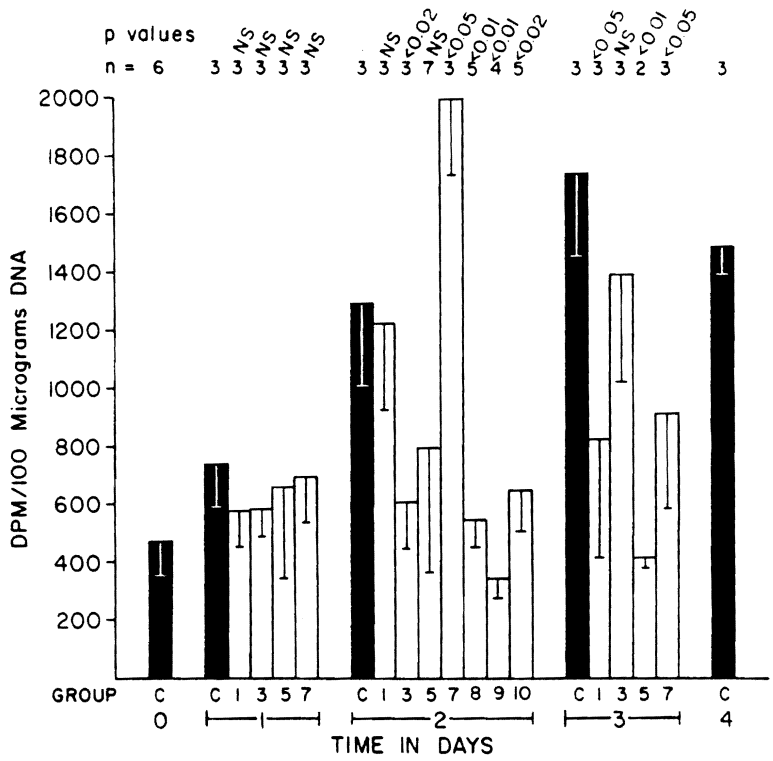

FIG. 4. Hepatic deoxyribonucleic acid, *D.N.A*, synthesis following 44 per cent hepatectomy and various eviscerations, mean ± S.D. Comparison using Student's t test is to intact dogs on same day after 44 per cent hepatectomy. *DPM*, Disintegrations per minute.

if not most, of the remaining hepatocytes. This change was least obvious in the dogs that had been completely eviscerated. The enlarged hepatocytes contained more fat globules and less glycogen than normal. The lysosomes were also enlarged, and the amount of rough and smooth endoplasmic reticulum was increased. The accumulation of lipid was most marked and the increase in rough endoplasmic reticulum least in those dogs that had a pancreatectomy.

In these dogs with diabetes, the mitochondria were enlarged, and the rough endoplasmic reticulum was disrupted and showed distortion of some of the cisternae. The fatty infiltration was lessened, and the mitochondrial and rough endoplasmic reticulum changes were reversed by the administration of insulin but not glucagon. Evisceration without pancreatectomy did not seem to produce any specific ultrastructural alterations in the hepatocytes after hepatic resection.

TABLE IV.—CELL SIZE, AUTORADIOGRAPHY AND MITOTIC ACTIVITY FOLLOWING 72 PER CENT HEPATECTOMY AND VARIOUS EVISCERATIONS

Group	*No.*	*Time, days*	*Hepatocyte size, size units*	*P**	*ARG labeled hepatocytes per 1,000*	*P**	*Mitoses per 1,000 hepatocytes*	*P**
Normal	6	0	0.170±0.020	—	1.57±0.27	—	0.078±0.036	—
Normal + 72 per cent Hx	4	1	0.195±0.028	—	7.70±2.00	—	0.700±0.136	—
	6	2	0.213±0.026	—	13.40±1.20	—	1.080±0.136	—
	3	3	0.252±0.056	—	31.10±3.50	—	2.990±0.389	—
2	3	1	0.210±0.042	NS	1.80±1.45	<0.02	0.180±0.163	<0.02
	4	2	0.222±0.018	NS	27.50±18.30	NS	2.400±1.530	NS
	3	3	0.209±0.004	NS	7.69±1.09	<0.001	0.853±0.111	<0.01
4	3	1	0.183±0.050	NS	1.56±1.05	<0.01	0.193±0.135	<0.01
	1	2	0.230	—	1.54	—	0.170	—
6	4	2	0.243±0.038	NS	0.253±0.299	<0.001	0.028±0.036	<0.001

*Compared to normal + 72 per cent hepatectomy for same day by Student's t test.
Hx, Hepatectomy.
ARG, Autoradiography.
NS, Not significant.

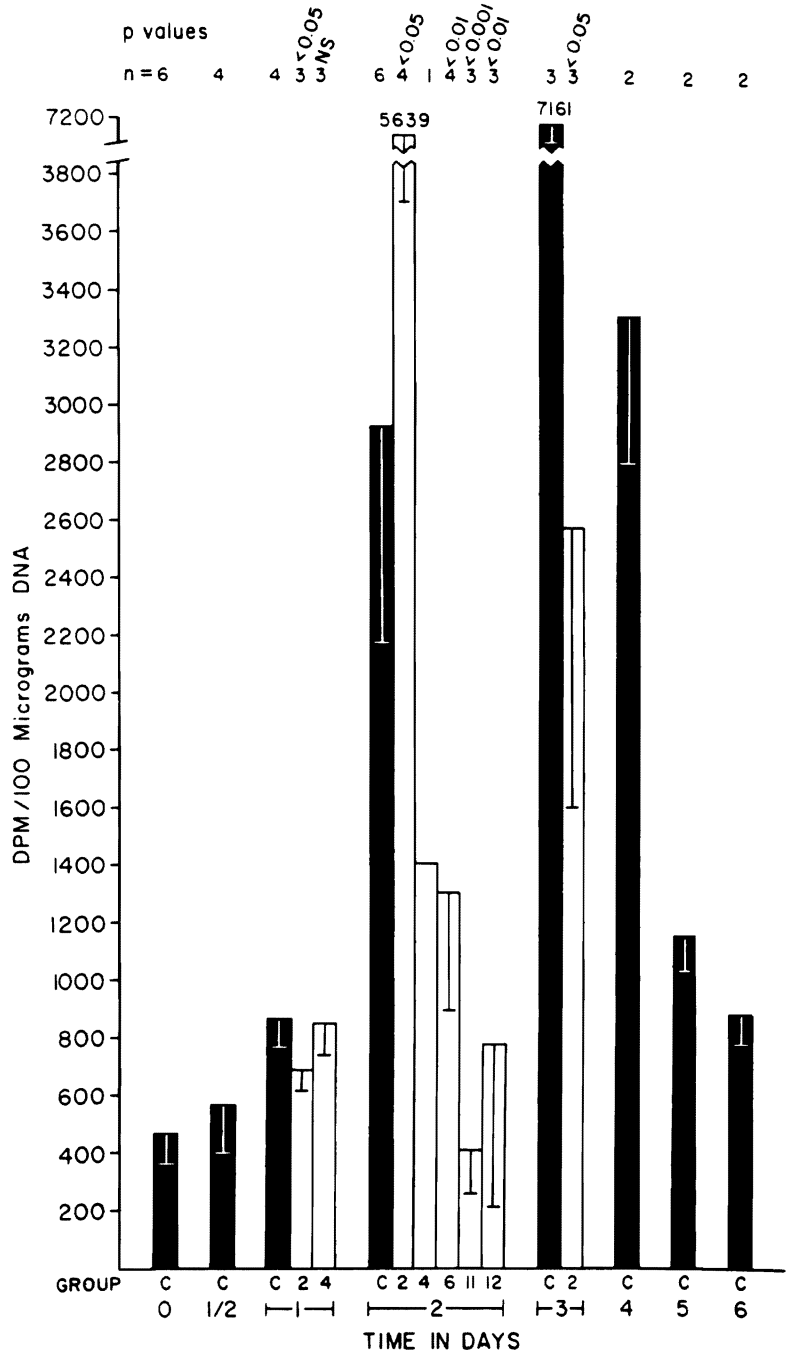

FIG. 5. Hepatic deoxyribonucleic acid, *D.N.A.* synthesis following 72 per cent hepatectomy and various eviscerations, mean ± S.D. Comparison using Student's t test is to intact dogs on same day after 72 per cent hepatectomy. *DPM*, Disintegrations per minute.

Chemical Findings

Deoxyribonucleic acid synthesis. The increase in deoxyribonucleic acid synthesis after a 44 per cent resection was usually decreased by different kinds of organ extirpation (Fig. 4), including pancreatectomy, group 1; excision of all nonhepatic splanchnic organs except the pancreas, group 3, and extirpation of all nonhepatic splanchnic organs, group 5. The only exception to the aforementioned generalization was that there

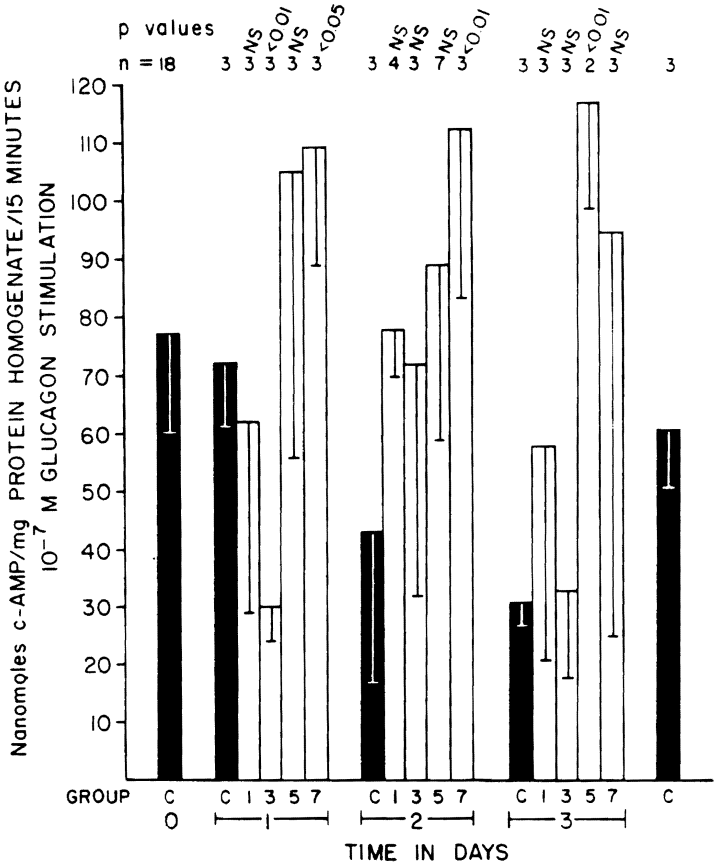

FIG. 6. Glucagon stimulated hepatic adenyl cyclase activity following 44 per cent hepatectomy and various eviscerations, mean ± S.D. Comparison using Student's t test is to intact dogs on same day after 44 per cent hepatectomy.

was a larger than normal increase in deoxyribonucleic acid synthesis at day 2 in dogs in which the colon was retained, group 7, but at one and three days, the deoxyribonucleic acid synthesis in the dogs of group 7 was subnormal, as seen in Figure 4.

Intraportal infusion of insulin, glucagon and insulin-glucagon in combination, groups 8, 9 and 10, did not restore a normal response to a 44 per cent hepatectomy at two days (Fig. 4). Although there was great variation in results, a number of the deviations from the normal regenerative response were statistically significant (Fig. 4).

The same reduction in deoxyribonucleic acid synthesis with the different kinds of organ extirpation was observed if the 72 per cent hepatectomy was performed, again without striking differences with visceral extirpation that included and excluded the pancreas (Fig. 5). The only exception was an incongruously high deoxyribonucleic acid synthesis in dogs of group 2 two days after hepatectomy and pancreatectomy, but at one and three days, this kind of result was not obtained. The regeneration response to a 72 per cent hepatectomy after complete evisceration was not augmented with either high or low dosages of glucagon, groups 11 and 12, and it may even have been inhibited (Fig. 5). After a 44 or 72 per cent hepatectomy, the measured deoxyribonucleic acid synthesis was in conformity with autoradiographic and mitosis counts.

Adenyl cyclase activity. After either a 44 or 72 per cent hepatectomy in otherwise unaltered dogs, it has been established (18) that there is a reduction of cell membrane glucagon stimulated adenyl cyclase activity, particularly after two or three days (Figs. 6 and 7). The various organ extirpations with or without pancreatectomy tended to prevent this decline both after a 44 and 72 per cent resection. However, the variation in results was so great that statistically significant deviations from either normal livers or normally

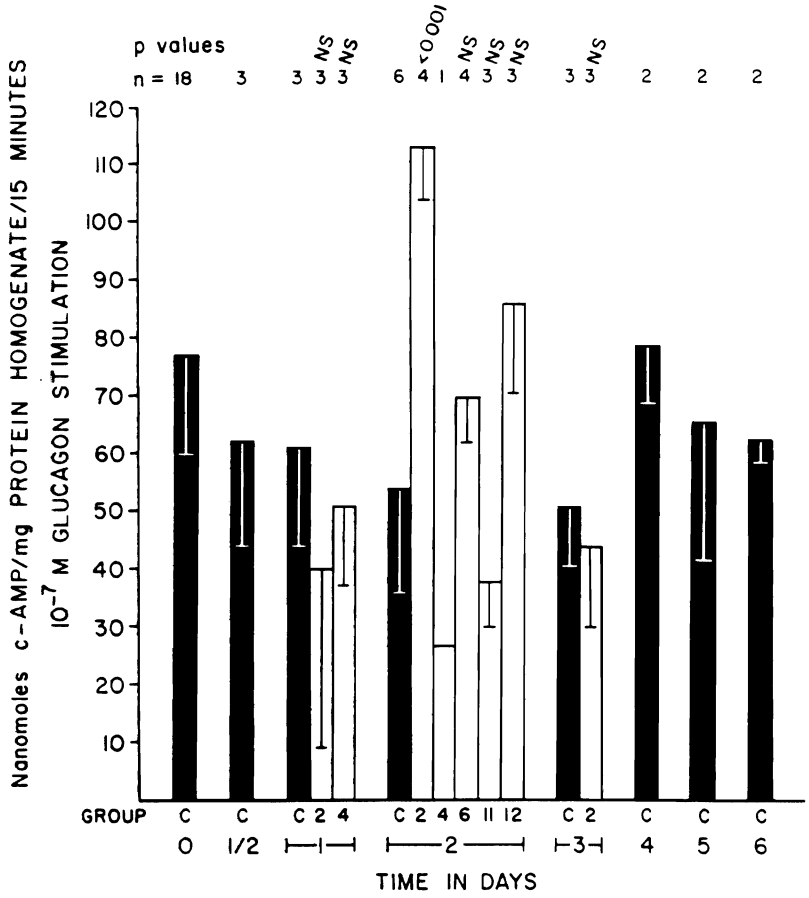

FIG. 7. Glucagon stimulated hepatic adenyl cyclase activity following 72 per cent hepatectomy and various eviscerations, mean ± S.D. Comparison using Student's t test is to intact dogs on same day after 72 per cent hepatectomy.

regenerating livers usually could not be demonstrated at molar glucagon concentrations, ranging from 10^{-5} to 10^{-9}. The most consistent results were obtained at a molar glucagon concentration of 10^{-7} (Figs. 6 and 7), but even under this condition of testing, the deviations from the normal regeneration response were erratic and abnormally low as well as abnormally high.

Cyclic 3′, 5′-adenosine monophosphate. The biphasic initial rise and secondary fall of cyclic 3′, 5′-adenosine monophosphate which occurred after a 44 per cent (Fig. 8) or 72 per cent (Fig. 9) resection was much altered by the various organ extirpations. In general, the removal of nonhepatic splanchnic organs, including the pancreas, caused cyclic 3′, 5′-adenosine monophosphate to be higher than would have been expected in the intact dog (Figs. 8 and 9). These changes were more consistent after a 72 per cent hepatectomy (Fig. 9) than after a 44 per cent hepatectomy (Fig. 8).

DISCUSSION

In reviewing the work on hepatotrophic factors that has emerged in the last 15 years, a distinction needs to be made between the effects of portal blood substances on hepatocyte structure and function, as opposed to their influence on hepatic regeneration. The importance of portal blood in maintaining healthy liver cells seems beyond dispute as we have reviewed on several occasions based upon our own work (52, 54, 56, 57, 58, 59) and that of others. A recent publication by Guest and colleagues (23) has reiterated the same theme.

Of the hepatotrophic factors, there has been no reason to doubt the central role of insulin in maintaining the integrity of the resting hepatocyte, although there is convincing evidence that other unknown portal constituents are contributory (52, 54, 56, 57, 58, 59). The crucial role of insulin in maintaining liver cells was demonstrated after removal of all the nonhepatic

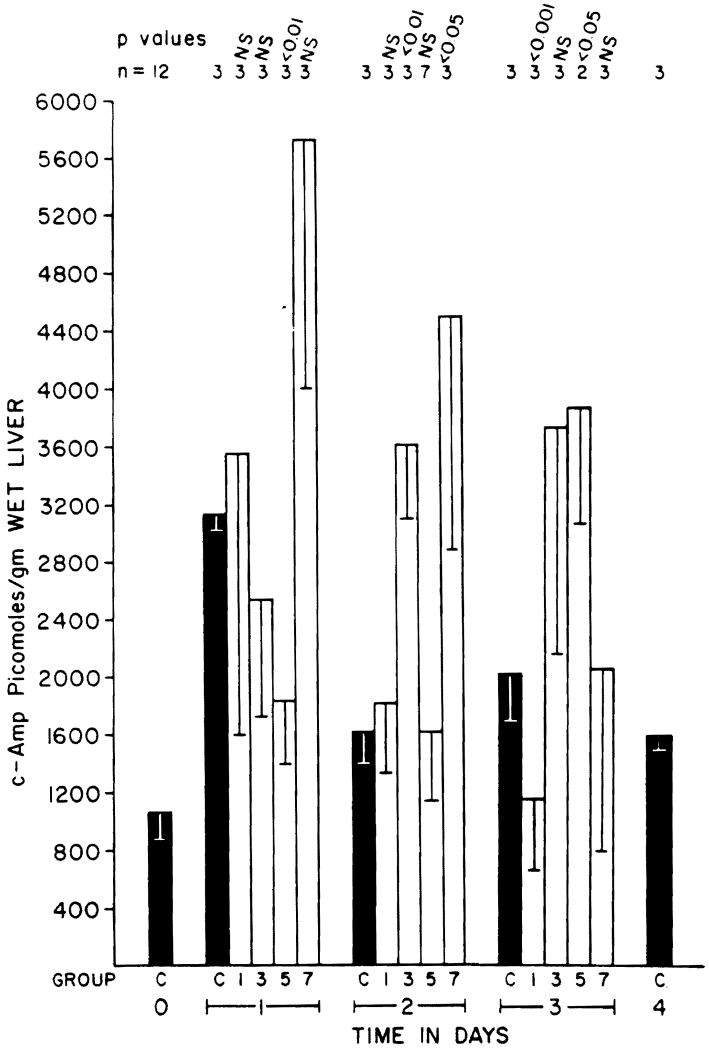

FIG. 8. Hepatic cyclic 3′, 5′-adenosine monophosphate following 44 per cent hepatectomy and various eviscerations, mean ± S.D. Comparison using Student's t test is to intact dogs on same day after 44 per cent hepatectomy.

splanchnic viscera, including the pancreas (53). The intraportal infusion of insulin alone prevented most of the atrophy and other structural deterioration of hepatocytes, and it preserved the rate of spontaneous liver cell renewal which was otherwise depressed. The hepatic protection in eviscerated dogs was almost identical to that observed with intraportal insulin therapy after portacaval shunt (58, 59) and was indistinguishable from the hepatotrophic effect of insulin described by Reaven and co-worker (44) in rats with diabetes. In hepatocyte tissue culture systems, Gerschenson and collaborators (21), Wagle and co-authors (61), Junge and Nagamori (27), Begnaert and associates (2) and many others have described analogous insulin effects. Ozawa and colleagues (39, 40, 64) have repeatedly emphasized the role of insulin in maintaining hepatocyte mitochondrial matabolism. No potentiating effect of glucagon has been demonstrated in any of these nonregeneration models.

In addition to the foregoing effects, it is equally clear that portal blood factors also profoundly influence hepatic regeneration. However, the nature of the regeneration promoting substances and their origin remain in dispute. Additional questions are: Do they initiate regeneration or merely permit the process to proceed and, in either case, how? The conflicting conclusions reached in various laboratories on the issues seem

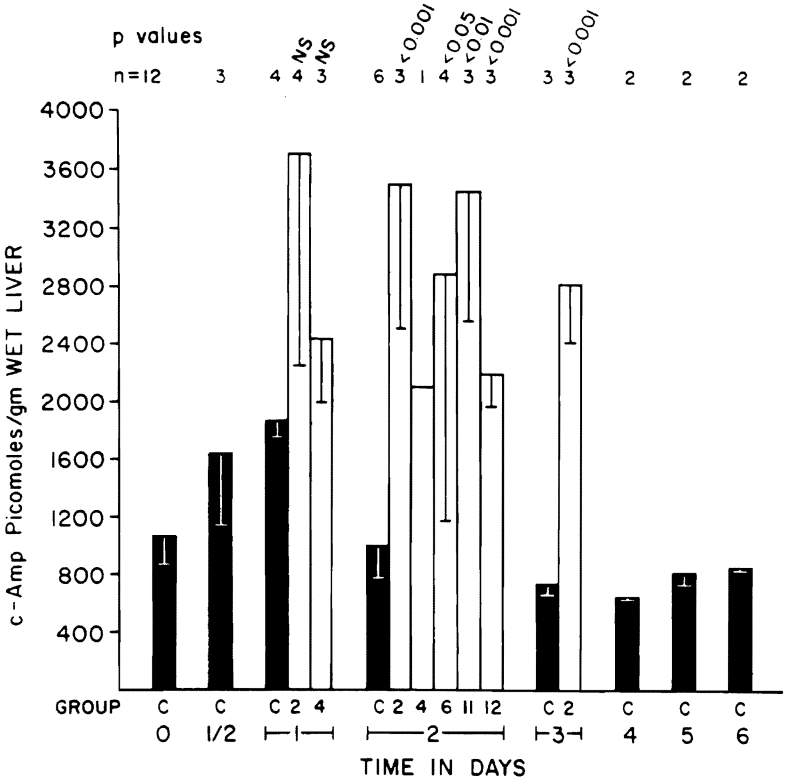

FIG. 9. Hepatic cyclic 3', 5'-adenosine monophosphate following 72 per cent hepatectomy and various eviscerations, mean ± S.D. Comparison using Student's t test is to intact dogs on same day after 72 per cent hepatectomy.

due, in part, to the use of different experimental models and, in part, to the way in which data have been interpreted.

Much information about the origin of regeneration promoting factors has come from evisceration procedures in conjunction with partial hepatectomy that were introduced in dogs by Price and co-workers (43) and adapted for rats by Bucher and Swaffield (6). An artifact existed in this early work in that exogenous insulin was incidentally administered as part of the postoperative parenteral fluid therapy. Results of later studies by Bucher and Swaffield (7), Price (42) and Whittemore and co-authors (62, 63) showed a striking depression and delay of regeneration after complete evisceration which could be restored toward, or even to, normal by treatment with a combination of insulin and glucagon in high dosages.

The crucial splanchnic factors did not seem to be from the intestine. Although Fisher and colleagues (18) found an obtunded regeneration response after intestinal resection, this could not be confirmed by Sgro and associates (48) or by Poirier and Cahow (41). In contrast, Sgro and collaborators (48) and Duguay and Orloff (11, 12) reported almost complete absence of liver regeneration after total pancreatectomy in rats and dogs, which Duguay and Orloff (12) observed in their latest publication could be restored to normal by treatment with insulin and glucagon. Duguay and Orloff (12) concluded that the crucial splanchnic organ for hepatic regeneration was the pancreas, that insulin and glucagon were the most critical elements in a pancreatic role and that the other nonhepatic splanchnic organs were of minor importance.

Evidence that this was an excessively simplified view was available from older work of Younger and co-workers (65), recently confirmed by Barra and Hall (1), that resection of the liver in rats with diabetes is followed by vigorous regeneration. In our own investigations with split liver preparations in dogs with and without diabetes, the importance of pancreatic blood in supporting regeneration after hepatectomy was emphasized, but important similar qualities in nonpancreatic splanchnic blood were also shown (57). Although their data were not so interpreted by them, Broelsch and co-authors

(3) demonstrated with clever isotransplantation experiments that venous effluent from the jejunum, ileum and duodenum supported hepatic regeneration albeit less well than blood from the pancreas.

The results in the present study have demonstrated again the complexity of control of regeneration by portal hepatotrophic factors and have strengthened the multifactorial hypothesis by clearly differentiating pancreatic influences from those originating in the rest of the intra-abdominal gastrointestinal tract. The removal of all of the nonhepatic splanchnic viscera resulted in quite a severe inhibition of deoxyribonucleic acid synthesis and, essentially, complete elimination of the histopathologic expression of liver regeneration. Leaving the distal part of the colon in place did not significantly improve the eviscerated dogs' response to hepatic resection as measured with autoradiography, in spite of the fact that plasma glucagon was thereby kept at a nearly normal concentration. Nor did the infusions of exogenous glucagon, insulin or glucagon and insulin in combination into the portal vein have a striking restorative effect upon regeneration.

In contrast, concomitant or prior removal of the pancreas alone reduced, but did not remotely abolish, the response to a 44 per cent hepatectomy. The response after one and three days to a 72 per cent hepatic resection was likewise dampened by pancreatectomy, although strangely at two days there were even more hepatocytes entering deoxyribonucleic acid synthesis than in normal dogs after a hepatectomy of this extent. Most importantly, it was shown that, with the pancreas left in place, extirpation of the rest of the nonhepatic splanchnic viscera, but with preservation of the pancreas, reduced the response to hepatic resection even more than did pancreatectomy alone. Since portal blood flow reduction is greater after removal of all the nonpancreatic viscera than after pancreatectomy alone, this nonspecific flow factor could result in overestimation of the role of nonpancreatic viscera compared with that of the pancreas itself. In spite of the possible distortion introduced by relative flow factors, the conclusion remains that removal of the pancreas and other viscera had a substractive effect upon regeneration.

Bucher and Malt (5) and, more recently, Leffert and Koch (31) and Bucher (4) have similarly written of regeneration as a complex series of events under multifactorial control. If hormones play an important regulatory role, precise delineation of their contributions may be difficult with any of the presently available experimental models, since a hormone-free environment is hard to achieve in intact animals, particularly dogs. The results herein reported showed that, in the dog, after pancreatectomy alone and even after evisceration, except for the distal part of the colon, normal or significant amounts of pancreatic-like glucagon remained circulating. After all the selective or complete evisceration procedures, trace quantities of an insulin-like substance were still detectable by a sensitive radioimmunoassay method. Small amounts of hormones could have major physiologic effects, since results of work by Bucher (4), Leffert and associates (30), Duguay and colleagues (13), Morley and co-workers (38) and Francavilla and collaborators (19) suggest the regenerating hepatocytes have changing sensitivity to insulin or glucagon, or both. It may be presumed that the same applies to other hormones.

The potential link between multiple hormone changes and regeneration is strengthened by the intriguing studies of MacManus and associates (33) who had previously shown with cultured thymus cells that increases in cyclic 3′, 5′-adenosine monophosphate levels induced with epinephrine, parathormone, prostaglandins and calcium immediately preceded the initiation of deoxyribonucleic acid synthesis and active cell proliferation. MacManus and colleagues (33) found the same early biphasic rises in cyclic 3′, 5′-adenosine monophosphate in rat livers two and one-half and 12 hours after partial hepatectomy with a return toward normal as deoxyribonucleic acid synthesis began. Thrower and Ord (60) and Byus and co-workers (8) have confirmed these findings in rats, and Francavilla and collaborators (19) have noted similar, but less well defined, changes in regenerating dog livers. In addition, Byus and associates (8) showed that increased cyclic 3′, 5′-adenosine monophosphate dependent protein kinases correlated perfectly in regenerating rat livers with the induction of ornithine decarboxylase.

Ornithine decarboxylase has been implicated by Cohen (10) and by Jänne and Raina (26) and Russell and Snyder (45) as the rate limiting enzyme in the polyamine biosynthetic pathways active in regeneration. Fischer and associates (16) demonstrated a blunted ornithine decarboxylase response to partial hepatectomy in animals with portacaval shunts. Short and coauthors (49) demonstrated that intravenously administered solutions containing triiodothyro-

nine, amino acids, glucagon and heparin could induce nuclear deoxyribonucleic acid formation and mitosis in the whole livers of unoperated upon rats without diabetes. Gaza and associates (20) demonstrated enhanced ornithine decarboxylase activity following treatment with this solution. Short and co-workers (50) later showed that the glucagon in this stimulatory solution could be completely replaced with a butyryl derivative of cyclic 3′, 5′-adenosine monophosphate, leading them to conclude that cyclic nucleotide plays a critical role in the induction of hepatic deoxyribonucleic acid synthesis and cell mitosis.

In our studies herein reported, all of the eviscerations which resulted in retarded regeneration caused severe pertubations as well in the liver cyclic 3′, 5′-adenosine monophosphate and adenyl cyclase changes that followed hepatectomy in normal dogs. Whether these deviations have a cause and effect relation to the retarded regeneration that was observed or are merely coincidental remains speculative.

When regeneration is more completely understood, major clinical advances should be possible in expediting recovery from liver injury, probably including hormone therapy as we (56) and others have suggested. Complicated solutions, such as those devised by Short and colleagues (49), could prove useful. In mice infected with hepatitis, Farivar and associates (15) have already shown a striking reduction in mortality if insulin and glucagon are given in doses from ten to 100 times larger on a weight basis than those used in the experiments on dogs herein reported. The necessity to give hormones beyond their physiologic dosage range would not be unacceptable if the same kind of benefit could be achieved in the treatment of human disease.

SUMMARY

The influence of portal blood factors on canine liver regeneration was studied with graded nonhepatic splanchnic evisceration, coupled with 44 and 72 per cent hepatectomies. In one type of experiment, the pancreas was retained while the rest of the intra-abdominal gastrointestinal tract was removed. In a second variety, total pancreatectomy was performed with preservation of the intra-abdominal organs. In a third kind of experiment, total nonhepatic splanchnic evisceration was performed.

Liver regeneration after hepatectomy was decreased by all three kinds of viscera removed as judged by deoxyribonucleic acid synthesis, autoradiography and mitotic index. Pancreatectomy and nonpancreatic splanchnic evisceration caused almost equal decreases in the regenerative response. Total nonhepatic splanchnic evisceration essentially halted regeneration during the first three postoperative days and intraportal infusions of insulin or glucagon, or both together, did not reverse this effect.

The decrease in liver membrane bound adenyl cyclase activity and biphasic change in liver cyclic 3′, 5′-adenosine monophosphate concentrations normally seen after partial hepatectomy were disrupted after the various eviscerations. Adenyl cyclase activity and cyclic 3′, 5′-adenosine monophosphate concentrations tended to be higher than normal in the eviscerated dogs.

These observations provide more support for our previously proposed hypothesis that control of liver regeneration is by multiple factors. Pancreatic hormones are important modifiers of this response but, by no means, exercise exclusive control. Other substances of gastrointestinal origin, presumably including hormones and nutrient supply apparently play important specific roles. The volume of portal flow is a secondary and nonspecific, but possibly significant, factor.

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