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Further Studies on Hepatic Stimulatory Substance (SS) after Partial Hepatectomy[1]

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The stimulatory substance (SS) that is found in the cytosol of regenerating dog livers and which promoted regeneration when injected into the portal vein after Eck fistula did not cause glomerular proliferation when active cytosol was injected into the renal artery. The finding was compatible with but did not prove organ specificity of hepatic SS. The development of SS was completely prevented by extirpation of all nonhepatic splanchnic organs at the same time as partial hepatectomy. This finding indicated that SS in the cytosol donors was a feature rather than an initiator of regeneration, and one that depended upon the collaboration of extrahepatic (including splanchnic hepatotrophic) factors.

INTRODUCTION

We recently described a growth-stimulating substance (SS) in the cytosol of regenerating dog livers [5, 6]. Its intraportal injection could provoke regeneration in the livers of test animals that had just been submitted to portacaval shunt (Eck fistula) [5]. Such injections in animals with an intact liver circulation amplified the regeneration following partial hepatectomy [6]. The SS had the same general features as a rat liver factor described earlier by La Brecque and Pesch [2].

Two unanswered questions about SS were examined in the present studies in dogs. One about organ specificity was whether the hepatic SS could stimulate proliferation in the kidney if injected into the renal arterial supply. The second question was if the SS represented an autonomously developing response of the hepatic fragment or alternatively if its appearance required collaboration with the nonhepatic splanchnic organs and the so-called hepatotrophic factors (3) which emanate from these organs.

METHODS

Cytosol Preparation

The cytosol was prepared from the intact liver or from liver fragments using the same ultracentrifugation techniques employed in earlier work by us [5, 6] and La Brecque and Pesch [2]. The cytosol from about 100 g liver tissue constituted the infusion for one test animal.

For kidney infusion. Previous canine studies had shown that the most potent SS appeared 3 days after 72% partial hepatec-

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tomy [5]. Consequently, cytosol donors were killed this long after 72% hepatectomy. The activity of this liver cytosol was compared to that of hepatic cytosol from normal animals.

For liver infusion. Cytosol was prepared from liver fragments 3 days after 44% hepatectomy in normal dogs. This cytosol was compared with that from the liver remnants of dogs which 3 days earlier had simultaneous 44% hepatectomy and evisceration of all nonhepatic splanchnic organs from the esophagogastric junction to the anus including the pancreas and spleen [3].

Cytosol Infusions

Into kidney. Laparotomy was performed in normal dogs under anesthesia with sodium pentobarbital and phencyclidine hydrochloride. The left renal artery and aorta were exposed. After placing an 18-gauge catheter into the left renal artery through the opposite aortic wall, infusion of the cytosol was carried out over 240 min. Killing was 3 days after injections of the cytosol obtained from normal or regenerating livers.

Into liver. The testing of liver responses was in an Eck fistula model [5]. Immediately after performing the portacaval shunt, the right portal vein was ligated. The left portal vein was also ligated and an infusion into it was carried out over 6 hr. The animals were killed 3 days after completion of the portacaval shunt and the beginning of the cytosol infusion.

Histopathologic Studies

All experimental endpoints were histopathological as previously described [5, 6]. Two hours before killing many of the cytosol donors and all of the test animals injected with the cytosol were given 0.2 mCi/kg [CH_3-^{3}H]thymidine (47 Ci/mmole) intravenously. Liver or kidney tissues were fixed in 10% buffered formaldehyde and ultimately examined by light and electron microscopy. In the midlobular zones, the size of the hepatocytes was measured and expressed in arbitrary size units [3, 5, 6] and the volume of endoplasmic reticulum per volume of cytoplasm was determined [3, 5, 6]. Hepatocytes and glomerular cells in mitosis were counted [5, 6].

Other paraffin sections of liver and kidney were dewaxed, dipped in Ilford K2 nuclear emulsion, and used for autoradiography. Exposure was for 4–8 weeks until counts of the labeled nuclei stopped increasing. With liver sections, only hepatocytes were counted, excluding stromal and other cells. In the kidney samples, labeled glomerular cells were recorded.

RESULTS

Renal Artery Infusions

Although cytosol from 72-hr regenerating liver has been shown to cause major stimulation of the Eck fistula liver [5], its intra-arterial infusion caused no glomerular proliferation in the injected or contralateral kidneys (Table 1). Cytosol from normal livers was also inactive (Table 1).

The Effect of Evisceration upon Regeneration in Cytosol Donors

The intact dogs submitted to 44% hepatectomy had a brisk regeneration at the time of killing 3 days later (Table 2). The numbers of hepatocytes incorporating thymidine or undergoing actual mitoses were several times baseline and these cells were slightly enlarged. The regeneration response to 44% hepatectomy was approximately the same as previously reported. [1].

After 44% hepatectomy plus evisceration, there was no regeneration whatever in the liver fragments that were harvested 3 days later as a cytosol source (Table 2). The absence of regeneration after evisceration has been noted before [3].

Cytosol Infusions after Eck Fistula

The cytosol obtained from normally regenerating livers after 44% hepatectomy

TABLE 1

EFFECT IN NORMAL DOGS 3 DAYS AFTER INJECTIONS INTO LEFT RENAL ARTERY OVER 240 MIN

		Number ± SD per 1000 glomerular cells			
		Labeled cells		Mitoses	
	No.	Left kidney	Right kidney	Left kidney	Right kidney
Uninjected controls	5	0.98 ± 0.129	—	0.17 ± 0.043	—
Cytosol from normal liver	10	0.99 ± 0.145	0.95 ± 0.139	0.20 ± 0.066	0.19 ± 0.051
Cytosol from 72-hr regenerating liver	10	0.97 ± 0.244	0.95 ± 0.164	0.17 ± 0.034	0.18 ± 0.047

caused a brisk regeneration in the infused left liver lobes of the Eck fistula test dogs, but not on the right side (Table 3). Furthermore, the atrophy of Eck fistula was almost completely prevented in the left lobes (Table 3), as was the depletion of both smooth and rough endoplasmic reticulum.

These effects were not caused by cytosol harvested from animals that had been submitted to evisceration plus 44% hepatectomy (Table 3).

DISCUSSION

One question asked in this investigation was if the stimulatory effect of hepatic cytosol was liver specific. Failure of active liver extracts given via the renal artery to stimulate proliferation of renal glomeruli could be consistent with such organ specificity. However, the experiments were not construed as proof of specificity. Even when active cytosol was injected directly into the portal circulation in dogs, a stimulatory effect was not clearly seen in normal livers [6]. Instead, cytosol activity was demonstrated after the procedures of minor hepatectomy [2, 6] and portacaval shunt [5] which evoke the common feature of heightened background proliferation prior to the intraportal cytosol infusion. It could be argued that the principal effect of cytosol is to amplify proliferative responses that are already in process, a condition that did not pertain to the kidney at the time of the renal artery infusions.

The second question was if SS could develop in liver fragments without the collaboration of the nonhepatic splanchnic organs. Within the 3-day time frame of our experiments, the answer was no. As has been reported before [3], removal of the

TABLE 2

REGENERATION RESPONSE 3 DAYS AFTER 44% HEPATECTOMY

	No.	Hepatocyte size units	Labeled hepatocytes/ 1000 hepatocytes	Mitoses/1000 hepatocytes
Normal controls	10[a]	0.173 ± 0.013[b]	1.61 ± 0.39	0.07 ± 0.03
44% Hepatectomy only	7	0.191 ± 0.013	9.20 ± 0.093*	0.87 ± 0.01*
44% Hepatectomy plus evisceration	3	0.173 ± 0.034	1.17 ± 0.35	0.11 ± 0.004

[a] Previously reported [5].

[b] Mean ± SD.

* Results significantly different ($P < 0.01$ by Student's t test) from normal controls and from 44% hepatectomy plus evisceration.

TABLE 3

LIVER FINDINGS 3 DAYS AFTER ECK FISTULA AND INFUSION OF LIVER EXTRACTS INTO LEFT PORTAL VEIN

Source of cytosol	No.	Cell size units		Labeled hepatocytes/1000 hepatocytes		Mitoses/1000 hepatocytes	
		Left	Right	Left	Right	Left	Right
No infusion	6[a]	0.123 ± 0.017	0.121 ± 0.017	3.20 ± 0.48	3.12 ± 0.73	0.14 ± 0.04	0.15 ± 0.04
3 days after 44% hepatectomy	7	0.164 ± 0.034*	0.128 ± 0.020	9.10 ± 1.61*	3.06 ± 0.51	0.53 ± 0.11*	0.13 ± 0.05
3 days after 44% hepatectomy plus evisceration	7	0.127 ± 0.021	0.127 ± 0.019	3.07 ± 0.47	3.01 ± 0.46	0.14 ± 0.04	0.14 ± 0.04

[a] Previously reported [5].

* Results significantly different ($P < 0.01$ or $P < 0.005$ by Student's t test) from untreated animals (no infusion), from the right liver side of the same animals, and from animals with hepatic cytosol from eviscerated dogs.

nonhepatic splanchnic organs completely prevented regeneration in the residual liver remnants of cytosol donors after partial hepatectomy. However, it was conceivable that SS of intrinsic hepatic origin was accumulating in these nonregenerating liver remnants under the conditions of evisceration but that the stimulatory effects of SS could not be expressed in the absence of the so-called splanchnic hepatotrophic factors. This criticism did not apply in the second stage of experiments herein reported in that the cytosols from eviscerated dogs were tested in other animals and under conditions previously shown [5] to reveal SS. The conclusion was that SS developed in normal cytosol donors contemporaneously with and was an *expression* of regeneration rather than being a forerunner or independent initiator of this process.

Possibly SS could be a synthesis product of the liver that results from the confluence of extrahepatic factors. Proof or disproof of this hypothesis will await isolation of the stimulatory substance(s) and testing of such moieties in more sensitive systems including tissue culture. For now, the best evidence is that SS is not itself a common hormone such as insulin or glucagon [5, 6].

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