Cellular Response to Partial Hepatectomy. (28037)

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The capacity of the mammalian liver to regenerate after partial hepatectomy is generally believed to depend on a compensatory proliferation of hepatic parenchymal cells (1,2). The stimulus initiating such proliferation is not known(3), but the effect is a prompt, clear-cut increase in hepatic DNA synthesis, mitotic activity and cellular multiplication(4).

Recent studies of compensatory red cell regeneration after partial removal of the red cell mass (hemorrhage) have indicated that this regeneration does not depend on a compensatory proliferation of existing nucleated red blood cells(5,6,7). It was shown that the stimulus to increased red cell production probably acts exclusively by differentiating stem cells to pronormoblasts. Rate of maturation and multiplication of individual erythroid cells was not changed but the presence of a greater number of differentiated pronormoblasts gave rise to an increase in DNA synthesis, mitotic activity and subsequently to an accelerated red cell production. In view of these findings in an organ which like the liver has the capacity for compensatory regeneration, the possibility must be raised that hepatic regeneration may depend on stem cell differentiation rather than on accelerated proliferation of mature parenchymal cells. The absence of recognizable stem cells in liver tissue should not be a major objection since even in bone marrow the exact histologic nature of the stem cells has not been established. They may be the so called Reticulum Cells (RE cells) or they may, as has been suggested by Yoffey(8), not be bone marrow cells at all but be multipotential, circulating lymphoid cells.

To test this possibility a population of rat liver parenchymal cells was labeled with H^3 thymidine by injecting the material during the phase of rapid DNA synthesis following partial hepatectomy. After sufficient time for the livers to regenerate the rats were again subjected to partial hepatectomy. During the subsequent wave of liver regeneration mitotic nuclei were examined for tritium labeling. If mature parenchymal cells respond to the stimulus of partial hepatectomy, the percentage of mitotic figures which were labeled should equal the percentage of total parenchymal nuclei labeled. If, however, stem cells rather than parenchymal cells respond to the stimulus to regenerate few, if any, of the mitotic figures should show tritium labeling.

Materials and methods. Fourteen Sprague-Dawley male rats 140-220 g were used. Partial hepatectomy was carried out under ether anaesthesia using the classical method of Higgins and Anderson in which 60-70% of the liver is removed(9). The animals received 0.7 microcurie of H^3 thymidine† per g s.c. at 20 and again at 24 hours after the operation. They were kept on a normal diet and 21 days later exhibited an average weight gain of 40%. They were then subjected to a second partial hepatectomy using the same method, but now it was only possible to remove from 40 to 70% of the regenerated liver. Forty-four hours after the second hepatectomy the animals were injected subcutaneously with 1 mg of colchicine per kilogram body weight. The animals were sacrificed 6 hours later (50 hours postoperatively) and the remaining liver was removed. Ten animals were used as controls to test the effects of colchicine on non-hepatectomized animals and to check the labeling of mitotic figures with H^3 thymidine after the first hepatectomy.

After removal, all tissues were placed in Davidson's fixative,‡ washed, dehydrated and imbedded in paraffin. Sections were cut to

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† Pure Methyl-labeled Thymidine (Schwarz Bio-Research, Inc.) with a specific activity of 3 curies per millimole.

‡ 2 parts formalin, 3 parts 95% ethyl alcohol, 1 part glacial acetic acid, 3 parts distilled water.

TABLE I. Tritium Labeled Cells and Mitotic Figures in Livers from Rats Exposed to 2 Hepatectomies.

Exp rat No.	Hepatic nuclei labeled with H^3 thymidine		Hepatic nuclei in mitosis		Mitotic figures labeled with H^3 thymidine	
	#/100,000 nuclei	%	#/100,000 nuclei	%	#/100,000 hepatic nuclei	% of mitotic figures
#11	30,400	30.4	10,800	10.8	3,120	28.9
#12	45,680	45.7	5,440	5.4	2,160	41.6
#16	6,640	6.6	13,200	13.2	880	6.7
#18	16,600	16.6	7,040	7.0	1,120	15.9

a thickness of 7-8 microns; the paraffin was removed with heat and xylene and the slides rehydrated.

Autoradiography was carried out by the emulsion dipping technic using Kodak Nuclear Track Bulk Emulsion N7B3(10) and stained with haematoxylin.

The slides were examined by two observers, 2000 cells were classified and the number of mitotic figures, labeled nuclei, and labeled mitotic figures were expressed per 100,000 hepatic nuclei.

Results. Examination of tissue obtained at the second hepatectomy revealed that there was a great deal of variation in the number of nuclei labeled by tritiated thymidine. The range in the 14 animals used was from 4,100 to 45,680 per 100,000 hepatic nuclei.

The number of mitotic figures in colchinized animals varied from 5,440 to 13,200 per 100,000 hepatic nuclei after the second hepatectomy and from 18,600 to 28,400 per 100,000 hepatic nuclei in controls examined after the first hepatectomy (an operative procedure in which it was possible to remove a larger percentage of the hepatic tissue). Other control animals showed that colchicine alone, without previous hepatectomy, did not increase the number of mitotic figures above the expected number in the quiescent rat liver.

The results in the 4 experimental animals are shown in Table I. While the experimental animals varied greatly as to number of labeled nuclei and number of mitotic figures the proportion of labeled mitotic figures to the total number of mitotic figures was, in each case, a close approximation of the proportion of total labeled nuclei to total nuclear count. It was also noted that the grain count of the mitotic figures was nearly the same as that of the other labeled nuclei although there was a great deal of variation among both populations.

Discussion. The remarkable regenerative capacity of the liver has made it possible to label the DNA of a large percentage of hepatic cells with tritiated thymidine. Following an initial labeling of a large number of cells it was possible to show that many mitotic figures appearing after the stimulus of a second partial hepatectomy were labeled. When quantitated it was found that the percentage of labeled mitotic figures was nearly the same as that of labeled total hepatic nuclei.

These findings suggest strongly that the cells responsible for the compensatory proliferation following hepatectomy are hepatic parenchymal cells and not undifferentiated stem cells. The possibility that a pool of stem cells labeled after the first hepatectomy was responsible for the labeled mitotic figures observed after the second hepatectomy seems remote. If stem cells actually were responsible for hepatic regeneration the label picked up 24 hours after the first hepatectomy should have been diluted materially during the subsequent period of intensive cellular regeneration. However, the mitotic figures observed after the second hepatectomy were labeled nearly as heavily as the surrounding cells.

In support of the above considerations were results from a separate experiment in which animals were allowed to live 24 days after the second hepatectomy. Comparison of the livers at that time with specimens taken at the second hepatectomy disclosed that although the grain count was slightly lower the percentage nuclei labeled was nearly

identical in the 2 specimens indicating the absence of a selective proliferation of non-labeled cells.

In conclusion it appears that liver regeneration in contradistinction to red cell regeneration is the result of multiplication of the general liver cell population rather than multiple divisions of a small segment of that population.

Summary. A study of hepatic regeneration in rats was carried out to see whether the stimulus of partial hepatectomy promotes proliferation of parenchymal liver cells or, in analogy with red cell regeneration, promotes the differentiation of stem cells to new hepatic parenchymal cells. By labeling a large proportion of hepatic cells with tritiated thymidine it was shown that the labeled cells rather than the presumably unlabeled stem cells were responsible for hepatic regeneration following partial hepatectomy.

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A Chemically Defined Medium for Growth of Animal Cells in Suspension. (28038)

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The growth of tissue cells (L cells) in suspension in a chemically defined medium, NCTC 109 with methylcellulose, has been reported by Bryant *et al.*(1). Bakken *et al.* (2) reported that this medium also supported the growth of suspension cultures of a line of human skin cells. Merchant and Hellman (3) have recently described the growth of L-M strain mouse cells in suspension culture using chemically defined 2X Eagle's medium. A serum-free medium containing lactalbumin hydrolyzate described by Higuchi(4) that supports the growth of several cell lines in monolayer cultures was modified for the growth of tissue cells in suspension culture by Tribble and Higuchi(5).

This report presents a chemically defined medium formulated as a result of experiments with a cat kidney cell line, and its successful application for the growth of 5 other cell lines.

Materials and methods. Preparation of the medium. Salts (except sodium bicarbonate), glucose, amino acids, and glutamine were dissolved together in hot distilled water. Vitamins, stored at –20°C as a 100X stock solution, were added to the cooled mixture. Sodium bicarbonate and phenol red dissolved together in a small amount of water were added and the resulting solution was sterilized by filtration through a 0.2 μ membrane filter and stored at 5°C as a 5X solution. Prior to inoculation, insulin, methylcellulose, streptomycin and penicillin were added aseptically and the final volume adjusted by addition of sterile distilled water. Methylcellulose was stored as a sterile 2% solution; antibiotics were stored at –20°C at 100X concentrations. The medium used for determining amino acid requirements was prepared by adding aseptically to the filtered salts-vitamin solution individual amino acids (100X