Liver Regeneration: Influence of Interval Postextirpation on *in Vitro* Growth of Cells from the Remnant Liver[1] (35962)

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During the first 48 hrs following subtotal hepatic resection in the rat, the principal indices of replication of cells in the remnant liver reach a peak and decline. These include: (a) RNA precursor uptake (1–2); (b) tissue protein synthesis (3); (c) DNA precursor uptake, in all types of liver cells (4); and (d) mitotic indices (5). The present study suggests that the increased *in vitro* growth potential of remnant (regenerating) liver *cells* is found only in tissue removed more than 48 hr following the hepatic extirpation.

Method. Excision of the right portion of the median and left hepatic lobes (approx 55% hepatic resection) was performed in 90 male Long Evans rats (complete diet); and excision of the right portion of median lobe (approx 25% hepatic resection) in 90 similar rats. Liver tissue was removed from the caudate lobe (not contiguous with either prior resection) of 12 of these rats (6 with prior 55% resection, and 6 with prior 25% resection) at intervals from 6 hr to 7 days following the original procedure.

The regenerating (caudate lobe) liver tissue from each group of 6 rats (30 groups) was pooled, minced, and trypsin-dispersed. Cells were counted and cultivated (1.8×10^6 cells/plate in 10 plates/group) in Eagles' medium supplemented with 10% calf serum at 37° in an incubator constantly flushed with 5% CO_2. Medium was replaced at 3 day intervals ($4\times$). The attached cells were removed with trypsin and counted in a hemacytometer after 16 days cultivation. The values in Table I are the mean cell count in each group of 10 plates. Rats were weighed immediately prior to the first extirpation (Table I). Experiments were started between 9 and 10 a.m. in Dec.–Jan. (Los Angeles).

The "growth curve" of liver cells from rats without prior resection employing the same technique was determined as follows. Pooled liver cells from 12 rats were cultivated (80 plates) in Eagle's medium with commercial calf serum (10%). Cells from 10 of these plates were counted at 2 day intervals from days 2 to 16 following planting. This was carried out in seven different series including 4 weight ranges, *i.e.*, 50–70 g (2 pools), 100–120 g (3 pools), 190–210 g (1 pool), and 300–330 g (1 pool). The initial phase of the growth curve as measured by these serial cultures was the same irrespective of animal weight. Of the 1.8×10^6 cells inoculated $<10\%$ were attached and viable on day 2. By day 4 in cultivation, the number of viable cells had declined to $<4\%$. Thereafter in the plates containing cells from the larger (older) rat groups, this decline continued to a point of "no growth" by days 6, 8, or 10. Growth in cells from the smaller (younger) rat groups rose sharply at this point reaching a plateau ($>20\%$ in 100–200 g groups) between days 12 and 16 in cultivation.

Results. Tissue from the liver of 20 groups of rats (10 with each size of resection) removed from 6 hr to 47 hr posthepatic resection yielded no surviving cells. Tissue removed at 48 hr, 72 hr, 4 days, and 5 days yielded cells with consistent growth in all inoculated plates (Table I). This growth declined with each increase in the interval from partial resection to the time of tissue removal, *i.e.*, from 125,250 ± 11,307 to 44,500 ± 8037/plate in the group with a 55% resection; and from 41,250 ± 6047/plate to 24,000 ± 2681/plate in the group with a 25% resection. Cells did not survive from tissue

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TABLE I. Cell Growth from Regenerating Liver Tissue.[a]

Interval post-resection, *i.e.*, liver removal (hr)	Hepatic resection (55%)		Hepatic resection (25%)	
	Rat wt (g)	Cell growth (cells/plate)	Rat wt (g)	Cell growth (cells/plate)
6	116.3 ± 5.5	NG	115.1 ± 3.8	NG
12	108.2 ± 3.7	NG	107.2 ± 3.4	NG
18	109.3 ± 3.7	NG	108.3 ± 4.7	NG
24	113.3 ± 4.1	NG	109.4 ± 3.6	NG
24	118.3 ± 1.1	NG	115.0 ± 6.5	NG
30	104.8 ± 5.9	NG	104.0 ± 3.8	NG
36	104.4 ± 4.8	NG	110.6 ± 3.9	NG
36	108.7 ± 4.2	NG	105.2 ± 3.5	NG
42	108.8 ± 2.6	NG	110.5 ± 1.8	NG
47	109.0 ± 4.7	NG	108.0 ± 2.1	NG
48	109.3 ± 3.2	125,250 ± 11,306.8	105.8 ± 3.1	41,250 ± 6046.7
72	113.8 ± 3.9	112,750 ± 10,370.8	111.1 ± 5.1	56,250 ± 8984.4
(days)				
4	105.8 ± 2.6	32,500 ± 2371.7	111.3 ± 3.4	24,000 ± 2681.4
5	110.3 ± 3.5	44,500 ± 8037.4	109.0 ± 4.7	NG
7	112.0 ± 2.3	NG	111.0 ± 2.2	NG

[a] Ninety male Long-Evans rats (wt, 100–120 g) were subjected to a 55% (approx) and 90 similar rats to a 25% (approx) hepatic extirpation. At intervals from 6 hr to 7 days thereafter, 6 rats from each group (55% resection and 25% resection) were sacrificed, and tissue was removed from the regenerating liver of a lobe (caudate) not contiguous with the previous resection sites. This tissue was combined (6 rats) and the cells were trypsin-dispersed and cultured for 16 days (see Method). Noted above are the mean cell counts with SD in 10 plates counted on day 16. Because of the marked influence of age on liver cell growth, the exact pre-extirpation weights of each group of rats was obtained and is noted above.

removed at 5 days and 7 days postresection (25% resection group) and at 7 days (55% resection group).

Discussion. Cells in the intact liver remnant following major hepatic resection may be primarily under the influence of extrahepatic factor(s) during this period (initial 48 hr) of rapid cell division. In the *in vitro* situation the same cells, following washing, trypsinization, etc., may entirely lose the influence of this factor(s). It is possible that after 48 hr in the intact (posthepatectomy) animal, this factor(s) has established permanent effects in remnant liver cells. Removal after this interval would not eliminate the stimulus to cell growth and cell division.

Alternatively, the cells removed from the remnant (posthepatectomy) liver may be unusually sensitive to trypsin, trauma, or other factors in the technique of cultivation. By 48 hr following resection, cells in the remnant liver may have assumed a more stable status which is resistant to these effects.

The effect of size of resection on growth of the remnant cells, *i.e.*, the larger the resection, the greater the initial growth potential of the remnant cells; is consistent with previous studies (6). The low level of the growth of these liver cells, removed from 48 hr to 5 days following partial hepatectomy, is in the range that has been noted in some specimens of normal liver from rats of this weight subjected to a somewhat larger (>60%) resection (7). This might be regarded as a return to near normal cultivation potential for these liver cells, following a period of inhibition of growth which persisted for 48 hr following liver extirpation.

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