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Formation of Colonies in Soft Agar Medium by Regenerating Liver Cells[1,2] (38591)

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Suspensions of cells from neoplasms, from established tumor cell lines, and from some tissue culture lines derived from normal cells have demonstrated the property of forming colonies when cultured in soft agar (1–3). The ability of such cultured cells to produce colonies in agar is regarded as an indication of malignancy (2) or transformation (3).

Bone marrow cells from various animal species and from man also form colonies in soft agar and the colony number is increased in the presence of a glycoprotein regulator termed colony stimulating activity (CSA) (4, 5). CSA is present in normal mouse serum (NMS) and is increased in serum obtained 4 hr after intravenous inoculation of endotoxin, producing endotoxin mouse serum (EMS) (6). The colonies formed from marrow are derived from colony forming cells (CFC) believed to represent hematopoietic stem cells, precursors of macrophages and granulocytes (4).

This report describes the cultivation of cells from trypsin-dispersed regenerating liver tissue (mouse and rat) cultured in soft agar medium. An attempt has been made to assess the effect of the serum factors (NMS and EMS), which have been shown to be stimulatory to marrow cells, on the colony forming properties of cells from regenerating liver tissue.

Methods. Regenerating liver tissue was obtained from seven groups of male Fischer rats (9–42 rats/gp) with weight ranges between 90 and 150 g (Tables I–II); and from 18 18–20 g C57 BL/6 male mice. An approximately 68% hepatic resection was performed in these animals under barbiturate anesthesia. Tissue was then removed from the hepatic remnant at intervals from 0 to 72 hr in the rat, and 0–168 hr in the mouse, by sacrifice. Regenerating liver remnants from three animals were combined to form the cell suspension for each culture. Fetal liver was obtained from six 16-day old Fischer rat fetuses; and from eighteen 19-day old C57 BL/6 mouse fetuses. Bone marrow samples were removed from the femurs of litter-mate C57 BL/6 mice. The liver tissue removed from the regenerating hepatic remnant (or fetal liver) was minced, trypsin-dispersed (15 min agitation in 0.2% trypsin) and the cells washed in Hanks' basal salt solution (BSS). Cell suspensions were centrifuged (950 rpm) and the cells in the pellet resuspended in Hanks' solution and counted. Marrow cells, spleen cells and peripheral blood leukocytes were prepared similarly.

EMS was obtained by bleeding mice 4 hr after intravenous administration of 5 μg of lypopolysaccharide, *E. coli* (Difco). Similar serum from rats, i.e., endotoxin rat serum (ERS) was prepared by the injection of 20 μg of endotoxin into 225 g rats. Designated cultures were supplemented with 40 μl of EMS, ERS, or NMS at the time of plating (Tables II and III).

The counted cell suspensions were placed in McCoy's 5a medium with 15% fetal calf serum (or human or rat serum, Table II) and 0.3% agar, introduced into 45 mm plastic petri plates (5×10^5 or 2.5×10^5 cells/plate), and incubated at 37° in 5% CO_2–95% air. Plates were scanned daily with a dissecting microscope. Colonies were visible on day 5 and were counted 7 days after plating.

The cellular composition of the initial cell suspensions (mouse) of bone marrow and

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TABLE I. COLONY FORMATION: CELL SUSPENSIONS OF LIVER TISSUE REMOVED AT DIFFERENT INTERVALS IN THE REGENERATIVE RESPONSE.

	Hours post resection (removal of liver)			
Inoculum/plate	0	24	48	72
(2.5×10^5 cells)				
100–110 g rats	3.0 (1.1)[a]	1.8 (1.4)	14.7 (2.1)	
120–130 g rats[b]	0.9 (.7)	1.1 (.7)	6.5 (1.6)	2.1 (1.4)
140–150 g rats	0.2	1.7 (.7)	5.5 (1.8)	
(5×10^5 cells)				
110–120 g rats	1.4 (.8)		11.6 (1.6)	5.7 (1.3)
140–150 g rats	0.8 (.8)	2.6 (1.5)	10.3 (.5)	
	42 hr	48 hr	54 hr	
90–100 g rats	10.5 (1.8)	40.7 (2.8)	28.7 (2.5)	

[a] Mean number of colonies/plate in 10 plates with (SD).

[b] Additional cultures in the 120–130 g rat series (only) included tissue removed at 12 hr (1.2 col/plate, SD 1.2), at 36 hr (2.3 col/plate, SD 1.4), and at 60 hr (3.9 col/plate, SD 1.6). Each cell suspension was formed from the liver remnant from three to four rats.

TABLE II. COLONY FORMATION: CELL SUSPENSIONS OF REGENERATING RAT LIVER WITH SERUM ADDITIVES.

	Cell types			
Additive to McCoy's media 5a	Normal liver	48 hr regen. liver	72 hr regen. liver	Fetal liver
15% Fet calf serum				
NRS (40 μl)	1.4 (1.2)[a]	11.2 (1.6)	5.7 (1.3)	0.2 (0.4)
EMS (40 μl)	1.2 (.79)	14.1 (2.9)	5.6 (1.3)	0.9 (0.7)
ERS (40 μl)	1.0 (.82)	15.9 (1.7)	7.4 (1.4)	1.4 (1.1)
15% Human serum		14.0 (2.1)		
15% Rat serum		19.5 (1.7)		

[a] Mean number of colonies/plate in 10 plates with (SD) The inoculum was 2.5×10^5 cells/plate of all cell types. NRS-normal rat serum, EMS-endotoxin mouse serum, ERS-endotoxin rat serum. Normal, 48 hr and 72 hr regenerating liver was from Fischer male rats (100–110 g).

TABLE III. MOUSE LIVER STUDIES.[a]

		EMS[b]	NMS[c]	No mouse serum
Regen. Liver: Hours Post Resection:	48	0	0	0
	72	18	6	6
	96	39	36	17
	120	26	15	1
	144	30	18	0
	168	86	46	6
Normal liver		1	0	0
Fetal liver		41	29	13
Marrow		350	148	0
Peripheral blood leukocytes		4	0	0
Spleen		3	2	1

[a] Colonies per 10^5 cells formed in soft agar at 7 days by incubating regenerating liver, normal liver, and other cell types with mouse serum additives.

[b] Endotoxin mouse serum.

[c] Normal mouse serum.

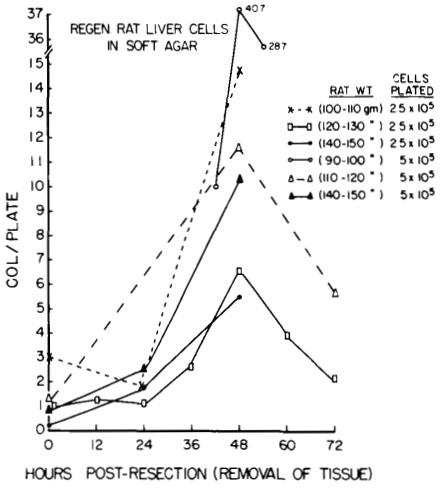

FIG. 1. Regenerating rat liver tissue was removed from the hepatic remnant at the intervals indicated in the abscissa, i.e., 0 to 72 hr. Colonies were counted at 7 days (see text). The ordinate indicates the number of colonies per plate, i.e., the mean of the total count/plate (10 plates). Standard deviations between plates are found in Table I.

fetal liver showed erythrocytic and granulocytic cells in all stages of maturation as well as cells with the characteristics of lymphocytes, monocytes and macrophages. The regenerating and normal liver suspensions were composed principally of macrophages, monocytes, segmented neutrophils and lymphocytes. A minor population (2–10%) of hepatocytes were seen in fetal, normal and regenerating liver cell suspensions. The spleen and peripheral blood preparations were composed largely of lymphocytes.

The cellular composition of mouse liver and marrow colonies was determined by aspirating individual colonies from the agar at 7 days and staining them (aceto-orcein) in addition to sectioning colonies fixed in the agar with Bouin's solution. Rat cell colonies were smaller and fragmented readily making differentiation of cell type difficult.

Results: In rat cell cultures (Table 1, Fig. 1) the greatest number of colonies consistently appeared when cells from tissue which was removed 48 hr postresection (liver) was employed, as opposed to tissue removed earlier or later in the regenerative response. This was seen in all (7) groups (Table I). Colonies appeared in the plates 5–6 days after inoculation and were composed of 50–500 cells/colony. In this tissue, i.e., 48 hr regenerating rat liver, the number of colonies was slightly increased by a larger cell inoculum (5×10^5 vs 2.5×10^5) and inversely related to animal weight. The effects of the addition to the medium of (a) EMS, or (b) ERS; or the substitution of (a) 15% human serum or (b) 15% rat serum, for the 15% fetal calf serum additive were negligible (Table II).

In regenerating mouse liver no colonies were formed from tissue removed 48 hr postresection, but all suspensions of cells from tissue removed 72–168 hr postresection formed colonies (Table III). These colonies were larger (500–2000 cells) than those formed from regeneraing rat liver and readily identifiable without magnification.

In the mouse, only regenerating liver and fetal liver formed a significant number of colonies in the absence of EMS or NMS. The peak of colony formation was seen in 96 hr regenerating liver tissue. Employing EMS and NMS the greatest number of colonies was formed by bone marrow, with fetal liver and regenerating liver yielding smaller and similar numbers of colonies (Table III). Few colonies were formed by cells from normal liver, spleen or by peripheral blood leukocytes. In all mouse tissues (except 48 hr regenerating liver), colony formation was stimulated by the addition of EMS and NMS to the medium, particularly by EMS.

The cellular composition of the colonies formed with cells from regenerating mouse liver, marrow and fetal liver was clearly distinguishable as either granulocytic, granulocytic–macrophage or macrophage. An additional colony type seen only in regenerating liver cultures (mouse) contained sheets of closely approximated epithelioid cells. The cell type of the rat liver colonies could not be clearly identified.

Comment. These studies indicate the presence in regenerating liver tissue of a small population of cells which form colonies in soft agar culture. The number of colonies formed in the regenerating rat cell cultures (and mouse cultures without EMS or NMS) appeared to be (1) directly related to the interval postresection at which the tissue

was obtained for culture and (2) possibly the animal age (rat). The addition of EMS and ERS to cultures of regenerating rat liver had no effect on colony formation. In the mouse, colony forming cells were found in regenerating liver in tissue removed 72–160 hr after partial hepatectomy and the number of colonies was increased by the addition of EMS and NMS. Thus, regenerating mouse liver contains cells with properties similar to those found in marrow cells, i.e., the ability to form granulocyte or macrophage colonies at 7 days in culture in the presence of NMS or EMS (6, 7). The finding of colonies in regenerating rat liver, not influenced by the serum additives and colonies in the mouse liver in the absence of these additives as well as the microscopic evidence of epithelioid colonies (mouse) suggests that there is a nonhematopoietic cell population in regenerating liver that can also form colonies.

Summary. The culture of actively regenerating liver tissue in soft agar reveals the presence of a cell population, not previously described, which includes (a) cells capable of forming hematopoietic colonies and (b) additional cells producing colonies with epithelioid characteristics.

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