V_t (ml/kg)	V_1 (ml/kg)	V_2 (ml/kg)	$1 - \frac{V_1 + V_2}{V_t}$ (%)	ΔG_{1-0} (kcal/mole)	ΔG_{2-1} (kcal/mole)
763 ± 27	163 ± 19	382 ± 47	29 ± 7	4.7 ± 0.7	2.1 ± 0.5

Conclusions and discussion. As mentioned before, the 2 aqueous compartments, whose volumes are V_1 and V_2, do not have any unambiguous anatomical correspondents, simply because they are defined only on the basis of the water accessibility for diffusion exchange. Nevertheless, in view of the rapidity with which the exchange between the external solution and the first tissular compartment (as expressed by the quasi-saturation time τ_1) occurs, one can infer that this compartment is extracellular, namely that it represents the intercellular space. This conclusion is further substantiated by the rather low free energy change ΔG_{1-0}, when D_2O passes from the external solution into this compartment. The second aqueous compartment of the nerve, into which the heavy water intake proceeds much more slowly, most probably represents the axoplasmic water. The fact that ΔG_{2-1} is even lower than ΔG_{1-0} can be tentatively accounted for by assuming that water passage through the conjunctive sheath surrounding the nerve is energetically more important than the passage through the axolemma. Anyhow, both ΔG_{1-0} and ΔG_{2-1} show that the intake of heavy water into the nerve is not accompanied by significant energy changes, so that one can conclude that at least 70% of the whole nerve water is almost free for rapid diffusion exchange. The remainder of up to 30% of the nerve water is not revealed by the deuterium oxide replacement, and thus it appears as either 'bound' or somehow 'obstructed' water. Maybe it is more correct to call this compartment 'invisible' from the viewpoint of the present technique. The slowness of the overall process of heavy water permeation into the tissue is to be attributed mainly to the spatial compartmentalization than to differences in the state of water. These conclusions are in very good agreement not only with similar investigations[6], but also with recent NMR measurements on the muscle[10,11].

A practical indication arising from this study is that in the investigations of the physiological effects of deuterium on the myelinated nerve, when deuteriation is obtained by simple immersion in heavy water, one should wait for several tens of minutes until heavy water replaces light water in the deeper tissular compartments.

It is to be observed that our measurements are practically incompatible with stirring, which means that the effect of the unstirred layer at the surface of the tissue is de facto included in the results. Anyhow, this effect leads to an apparent rate of exchange which is lower than the true one, a fact strengthening further the above conclusions.

10 P. S. Belton, R. R. Jackson and K. J. Packer, Biochim. Biophys. Acta *286*, 16 (1972).

11 M. M. Civan and M. Shporer, Biophys. J. *15*, 299 (1975).

Liver regeneration: cultural characteristics of remnant liver cells following a second partial hepatectomy

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Summary. When a 38% hepatic resection (rat) is followed in 24–72 h by a 30% hepatic resection (in the same animal), in vitro cell survival is observed in liver remnant tissue removed at any interval following the second resection.

Cells from the liver remnants of weanling or immature rats survive in culture when removed at any interval following a large hepatic resection[1,2]. In mature rats, however, employing the same primary culture techniques, only cells removed from the hepatic remnant at intervals greater than 46 h following hepatic resection survive in culture. This report describes the influence of a prior hepatic resection on the growth capacity of remnant liver cells (mature rat) removed during this early (< 46 h) interval.

Sixty 200 g male Wistar rats were subjected to a medial lobectomy (38% hepatic resection). At 3 subsequent intervals, i.e., 8 h, 24 h and 72 h, 20 of the same animals were subjected to a second (left) lebectomy. This would constitute a 30% hepatic resection in the intact rat, but more than that in this situation. 40 (control) rats had single 68% or 30% resections. At intervals of 12, 24, 36, and 48 h following the second hepatic resection, animals in the experimental groups were sacrificed and liver tissue removed for culture. At the same intervals following the single (control) resections, tissue was also obtained. Tissue was taken from the caudate lobe, an area not contiguous to prior resection(s).

Liver tissue from 5 rats in each of the 12 experimental and 8 control groups was combined, minced, the cells dispersed with trypsin solution (0.25%), suspended in Eagle's minimal essential medium (MEM) and counted. Each cell pool so formed was inoculated into 20 Petri dishes (1.8×10^6 cells/dish), containing MEM with 20% fetal calf serum (FCS) additive. Cultures were maintained at 37 °C in an incubator flushed with CO_2 and refed with the same media. After 2 days in culture, viable (attached) cell populations are reduced to approx. 5% of the inoculum. After 16 days in culture, cells attached to the plate were removed with trypsin solution, resuspended in MEM and counted with a hemacytometer.

As noted in previous studies[1,2] there was no cell survival in tissue removed 8, 24, and 36 h following a single hepatic resection, either 30% or 68% (table, a and b). A preliminary resection (38%) however, performed 24, 40 or 72 h prior to a 30% resection, resulted in survival

1 D. M. HAYS, Y. MATSUSHIMA, I. TEDO and A. TSUNODA, Proc. Soc. exp. Biol. Med. *138*, 658 (1971).

2 D. M. HAYS, Y. HIRAI, S. YOKOYAMA and K. NAKAJIMA, J. Surg. Res. *11*, 590 (1971).

Liver regeneration: effect of spaced resections

	Interval after single resection at which cells are removed for culture			
	8 h	24 h	36 h	48 h
a) Single 68% resection	NG	NG	NG	546[a] ± 24
b) Single 30% resection	NG	NG	NG	168 ± 32
	Interval after second resection at which cells are removed for culture			
	8 h	24 h	36 h	48 h
c) 38% resection followed in 24 h by 30+% resection	363[a] ± 37	243 ± 23	396 ± 30	413 ± 33
d) 38% resection followed in 40 h by 30+% resection	67 ± 14	153 ± 27	212 ± 32	214 ± 40
e) 38% resection followed in 72 h by 30+% resection	101 ± 5	140 ± 6	210 ± 6	755 ± 21

[a] Number of cells/plate × 10^3 (mean ± S.D.). NG = no growth. – Cell pools were formed from trypsin-dispersed regenerating liver tissue removed from 5 rats. The inoculum in each plate was 1.8 × 10^6 of these cells. The figure for each time interval (above) represents the mean ± S. D. in 20 plates with the same inoculum cultured for 16 days (see text.).

of cells removed at all 3 intervals, 8, 24, and 36 h following the second resection. As in previous studies[1,2], tissue removed 48 h following any size of resection (or resections) grew in vitro and maximum growth was always obtained in tissue removed at this interval post resection (table). Following the 40 h and 72 h (2nd) resections, cell survival was increased with each lengthening of interval post resection, i.e., 8 h post resection to 48 h post resection.

The relative failure of cells from liver to form a monolayer when they are removed during the initial interval (< 46 h) following 68% hepatic resection, employing this culture technique is of interest, as it is during this precise period that all of the principal indices of the in vivo replication of cells in the remnant liver during the regenerative response reach a peak and decline[3–7]. Cells from regenerating liver tissue cultured in soft agar also have minimal colony forming capacity when they are removed during this initial period following hepatic resection, and actively form colonies when they are removed later in the regenerative response[8,9]. This 'growth inhibition' interval appears to be longer in the mouse than in rats[8,9], as is the general regenerative response.

It might be assumed that this relative growth inhibition was related to the effect of the trauma of the liver excision per se on remnant cells, possibly producing an increased susceptibility to the action of trypsin, or in some other way directly inhibiting capacity for in vitro growth. From these studies however, it appears that this is not the case. The *initial* hepatic resection appeared to be the governing factor in establishing the growth potential of the regenerating liver cells in vitro. A second resection was not followed by a period of growth inhibition, a phenomenon always seen following a single resection.

[3] N. L. R. Bucher, J. F. DiTroia and M. N. Swaffield, Fed. Proc. *20*, 286 (1961).
[4] J. W. Grisham, Cancer Res. *22*, 842 (1962).
[5] K. Tsukada and I. J. Lieberman, Biol. Chem. *239*, 1564 (1964).
[6] K. Weinbren, Gastroenterology *37*, 657 (1959).
[7] G. A. Braun, J. B. Marsh and D. L. Drabkin, Metabolism *11*, 957 (1962).
[8] D. M. Hays, Y. Sera, Y. Koga and E. F. Hays, Proc. Soc. exp. Biol. Med. *148*, 596 (1975).
[9] E. F. Hays, F. C. Firkin, Y. Koga and D. M. Hays, J. Cell Physiol. *86*, 213 (1975).

Acceleration of muscle regeneration by bone marrow cells[1]

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Summary. Myogenesis was accelerated by addition of bone marrow cells to muscle minces cultured in diffusion chambers. This effect was inhibited by using gold suppressed bone Marrow cells.

It is generally agreed that muscle regeneration after injury is brought about by activated satellite cells and cleaved mononuclei (surrounded by a sarcoplasmic rim), which proliferate, fuse and synthesize myofilaments[2,3]. However, distally arising cells have not been excluded as myoblast precursors, and the present work was undertaken to test such a possibility.

The experiments were done on muscle minces cultured in diffusion chambers either alone or mixed with bone marrow cells. The method is nearer to in vivo conditions and is less technically demanding than conventional tissue culturing[4–7]. The chambers (capacity of 0.13 ml) were made of Millipore filters (0.22 μm and 0.45 μm pore size) cemented to both sides of a lucite ring (13 mm in diameter), with an access hole on the side. 2 chambers were implanted into each peritoneal cavity of young

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2 I. G. Burleigh, Biol. Rev. *49*, 267 (1974).
3 B. M. Carlson, Am. J. Anat. *137*, 119 (1973).
4 Z. Ben-Ishay, Scand. J. Haematol. *15*, 241 (1975).
5 K. O'Steen, Lab. Invest. *11*, 412 (1962).
6 D. J. P. Squires, Br. J. Haematol. *29*, 89 (1975).
7 B. Vernon-Roberts, J. D. Jessop and J. Doré, Ann. Rheum. Dis. *32*, 301 (1973).