observed on the unoperated side. No increased DNA synthesis can be seen in the interfascicular glial cells along the hypoglossal axons outside the regenerating nucleus. During this period with increased DNA synthesis mitotic figures can be found in the glial cells. In animals killed at 9 p.m. on the fourth postoperative day around one mitotic figure can be seen per section in the regenerating nucleus, and this corresponds to 2–3 mitotic figures per 1000 glial cells. The endothelial cells in the regenerating nucleus also show a DNA synthesis of nearly the same order as the glial cells.

In a second series material from a study of RNA synthesis in the regenerating *nucleus hypoglossus* was used. The experimental technique was exactly the same, but the tracers administered intracisternally were H^3-adenine and H^3-cytidine, which were given in daily injections at 9 p.m. during four days before sacrifice. The animals were sacrificed at 9 p.m. on the fifth day after the first injection. A total of 740 μc H^3-adenine and 300 μc H^3-cytidine was given in each experiment. The Carnoy fixed sections were incubated with ribonuclease in ammonium bicarbonate buffer according to EDSTRÖM[8] for 3×30 min at 37°C. Some sections were incubated after this extraction with deoxyribonuclease according to EDSTRÖM[7] for 3×60 min. The autoradiographic procedure was exactly the same as in the first series and the exposure time was kept constant for all sections, which made it possible to compare the DNA synthesis in four-day intervals from the second to the thirty-sixth postoperative day (Table II). All glial nuclei with ribonuclease resistant labelling extractable with deoxyribonuclease giving more than 10 grains in the autoradiographic emulsion over the nucleus were counted as labelled. This second series demonstrates that the DNA synthesis in glial cells rapidly decreases after the first postoperative week, and after the second postoperative week there is no significant difference between DNA synthesis on operated and unoperated sides. In all experiments the increase in DNA synthesis was restricted to the regenerating nucleus.

Table II. The number of labelled glial nuclei per ribonuclease treated section on the regenerating and control side of *nucleus hypoglossus*. Rabbits were injected with H^3-adenine and H^3-cytidine on four successive days and sacrificed 24 h after the last injection

Days after operation	6	10	14	20	28	36
Regenerating side	24.5	9.4	4.0	0.5	1.0	0.7
Control side	1.9	1.7	2.5	0.5	0.3	0.2

The morphological glial changes during and after the period with DNA synthesis in the regenerating nucleus were studied with Cajal's gold sublimate method for astrocytes, Tsujiyama's method for oligodendroglia, and Hortega's silver carbonate method for microglia. The astrocytes show a remarkable hypertrophy in the regenerating nucleus and this astrocytic reaction begins on the third day after nerve crushing and reaches its maximum during the ninth and fourteenth days. After the third week the astrocytosis rapidly decreases and 90 days after the nerve crush the astrocytosis in the regenerating nucleus is negligible. The microglial cells change less dramatically and the maximal microglial response is found between the third and fifth postoperative weeks. In the oligodendroglial cell population no significant changes can be seen during regeneration.

A detailed account of the experiments will be published later.

Zusammenfassung. Autoradiographie von Gliazellen, welche regenerierende motorische Nervenzellen umgeben, ergab eine stark erhöhte DNA-Synthese in den Gliazellen zwischen dem zweiten und sechsten Tag nach dem Nervenschaden. Morphologisch erscheinen typische Gliaveränderungen mit hochgradiger Hypertrophie der Astrocyten in der zweiten und dritten Woche nach der Operation.

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Protein Synthesis in the Early Stages of Liver Regeneration

A literature survey of radioisotopic investigations during regeneration after partial hepatectomy shows some lack of information on the behaviour of protein synthesis early after liver lobectomy, before significant tissue restoration occurs.

We refer in particular to the first post-operative day, when, compared to normal controls, generally only small changes have been reported in amino acid incorporation into regenerating liver proteins[1].

However, it is evident that active synthesis of proteins, most likely enzymes, necessary to trigger the later increase of the liver parenchyma, must take place at a molecular level long before the time the hepatic cells seem to be metabolically inert.

Such phenomena may in fact have been missed in previous researches either because of the tendency to take the regenerating liver for biochemical investigations long after the partial hepatectomy, when the growth rate has reached a maximum, or because the investigations are mainly carried out on the whole cell protein instead of on protein from subcellular fractions.

Some data supporting the above views may be derived from the research by several authors showing that, during the so-called pre-synthetic period, lasting from zero to 18 h after surgery, the apparently quiescent liver cell already synthetizes the enzymes necessary for nucleic acid replication. Generally, these investigations have been devoted more to the mechanism of the nucleic acid production and its inhibition by ionizing radiation than to

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the regeneration of the liver per se (for review, see CLERICI[2]).

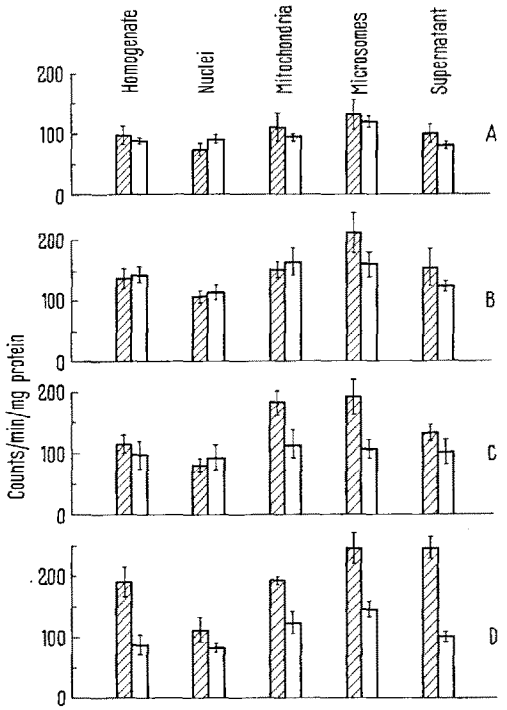
Therefore, it seemed worthwhile to undertake the present in vivo research in the hope of systematically defining both the behaviour of the protein synthesis in the early phases of the liver regeneration and the distribution among the subcellular structures.

After a 12 h fast, 20 male albino rats, Wistar strain, weighing about 100–150 g each, were hepatectomized according to HIGGINS and ANDERSON[3]. Four hours before sacrifice, each rat received an intraperitoneal injection of 0.5 ml of a solution containing 5 μC of DL-leucine-1-^{14}C (specific activity 3.8 mC/mM). Lots of five rats each were killed 4, 8, 16, and 40 h after surgery; their livers were perfused with ice-cold 0.25 M sucrose and homogenized with 9 Vol of the same medium.

After removal of a small aliquot, which was precipitated with trichloroacetic acid (TCA) (final concentration 1%), the homogenate was fractionated in a refrigerated centrifuge, following the technique of SCHNEIDER and HOGEBOOM[4], into nuclear, mitochondrial, microsomal and supernatant fractions, which were treated with TCA as above; the proteins of the four fractions and that of the homogenate were purified, plated and counted as previously described[5].

An identical procedure was carried out on 20 sham-operated rats which were utilized as controls.

Results obtained at all times were subjected to an analysis of variance according to SNEDECOR's[6] methods: the effect of the 'partial hepatectomy', the differences 'among preparations' (homogenate and subcellular fractions) and their interactions were taken into consideration.



DL-Leucine-1-^{14}C incorporation into liver protein of partially hepatectomized (shadowed bars) and sham-operated (empty bars) rats, 4 (A), 8 (B), 16 (C), and 40 (D) h after surgery, vertical lines representing standard errors.

The Figure, which summarizes the whole set of data (expressed as counts/min/mg of protein) shows that the experimental variability in the hepatectomized rats is greater than in the sham-operated controls and that in both conditions the highest specific activity is found in the microsomal fractions followed by the mitochondrial, supernatant and the nuclear ones. Furthermore, the specific activity of each fraction, except the nuclear ones, ranges above that of the whole homogenate.

The 'among preparations' effect is always statistically significant, though at different levels of significance (4 h = $P < 0.05$; 8 h = $P < 0.01$; 16 h = $0.01 < P < 0.05$; 40 h = $P < 0.01$). On the contrary, the 'partial hepatectomy' effect becomes relevant ($P < 0.01$) only in the group of animals sacrificed 16 h after surgery.

By observing the Figure it appears that such an effect would not have been evident by the mere observation of the DL-leucine-1-^{14}C incorporation rate into the protein of the whole homogenate, since the specific activity increase, as compared to controls, is definitely present only at the level of the microsomal and mitochondrial fractions.

Practically the same observations are valid for the results obtained in the 40 h group (partial hepatectomy = $P < 0.01$); however, in this case, all the subcellular fractions of the liver show an increased incorporating activity as compared to the controls. Such a property is also shared by the whole homogenate protein. The interaction between the two above effects bears no statistical significance either 16 or 40 h after surgery.

The present results are in good agreement with those of certain other investigations on the labeled amino acid incorporation into the total protein and the protein of some subcellular structures of the regenerating liver[7–9]; they also emphasize early and marked isotope uptake into the mitochondrial protein which may be tentatively interpreted as indicating an active synthesis of respiratory enzymes.

Riassunto. È stato dimostrato in vivo che i microsomi e i mitocondri di fegato rigenerante incorporano attivamente gli aminoacidi nelle loro proteine già 16 h dopo l'operazione, mentre tale effetto non è ancora rilevabile nell'omogenato totale.

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