PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 176, 371–377 (1984)

# Pulsed Electromagnetic Fields Increase the Rate of Rat Liver Regeneration after Partial Hepatectomy (41885)

V. OTTANI,* M. G. MONTI,† G. PICCININI,† L. PERNECCO,† P. ZANIOL,‡ A. RUGGERI,* AND B. BARBIROLI†

*Istituto di Anatomia Umana Normale, Via Irnerio 48, 40126 Bologna, †Istituto di Chimica Biologica, Via Campi 287, 41100 Modena, and ‡Istituto di Radiologia, Via del Pozzo, 41100 Modena, Italy*

*Abstract.* Pulsed extremely low-frequency electromagnetic fields interact with rat liver regeneration following partial hepatectomy when delivered to the rats immediately after the operation and every 12 hr thereafter. This interaction results first in an increased ornithine decarboxylase activity, an enzyme used as an early marker of cell growth. The rate of labeled thymidine incorporation into DNA is also increased by the treatments with magnetic fields during the early phases of liver regeneration. Glycogen depletion and lipid accumulation, two well-known early peculiar phenomena of liver regeneration following partial hepatectomy, are quantitatively decreased by the treatments with electromagnetic fields. The recovery to normal glycogen and lipid contents is completed within 5 days after surgery, instead of 7 days as found in control rats.

It is known that extremely low-frequency (ELF, 1–1000 Hz) pulsed electromagnetic fields interact with the living organisms and are able to modify several metabolic parameters of the cell (1–6). They are also being used to stimulate ligament (7) and bone healing (8) and to treat successfully several human bone diseases (9). However, very little is known not only of their mode of action but also of their precise effects on mammalian soft tissues.

A potentially fruitful approach to gain some insights into this problem would be the study of the effects of ELF electromagnetic fields on rat liver regeneration after partial hepatectomy. In fact this is a model for the study of controlled growth *in vivo* and a useful comparative system to study uncontrolled growth (10).

In this paper we report a remarkable increase of ornithine decarboxylase activity, an early marker of cell growth (11), induced in the regenerating rat liver by ELF pulsed electromagnetic fields. We show further that the same treatment induces an increase of the rate of DNA synthesis. Other parameters, such as glycogen and lipid contents, studied by stereological procedures performed on electron micrographs, show that treatment with pulsed electromagnetic fields determines a faster recovery of liver toward a normal metabolism.

**Materials and Methods.** Four-month-old male albino Wistar rats (350–370 g) were used in these experiments. Partial hepatectomy was performed under ether anesthesia with removal of the main lobes (68–70% of the liver) as described by Higgins and Anderson (12). All operations were performed at the same time of day (i.e., between 8:00 and 9:00 AM) after a fasting period of 12 hr. The portions of liver excised were employed to evaluate at zero time the parameters studied.

At the time intervals indicated in the single experiments, groups of rats were killed by decapitation, the livers were removed, rinsed in cold saline, blotted with absorbent tissue, and weighed.

*Treatment to the animals.* A pulsing electromagnetic field, homogeneous in a cylindrical volume of 30-cm diameter × 20-cm length, and generated by an apparatus made by one of us (P.Z.) was used. The shape of the pulse was a major sinusoidal halfwave (50 Hz, 6 mT) followed by few cycles of different frequency (400 Hz) and intensity (0.6 mT), as shown in Fig. 1. Thirty-minute treatments were delivered to the animals immediately after surgery and thereafter every 12 hr or as indicated in the single experiments. Control rats were left for the same length of time in the apparatus after having switched it off.

*Ornithine decarboxylase activity.* The livers were homogenized in 0.25 *M* sucrose with a Potter–Elvehjem homogenizer fitted with Teflon pestle. The homogenates (15% w/v) were centrifuged for 90 min at 105,000*g*. The resulting supernatant fraction was used for assay of ornithine decarboxylase activity.

Ornithine decarboxylase activity was mea-



0037-9727/84 $1.50

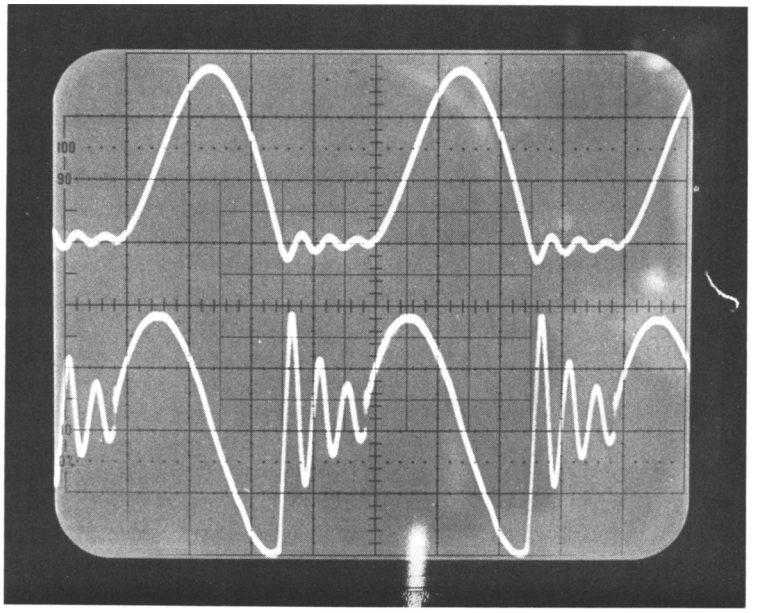

FIG. 1. Oscilloscopic traces of the pulsed electromagnetic field generated by the apparatus used in these experiments. Upper trace [$B(t)$] has been obtained by monitoring the pulsing electromagnetic field in air with a Hall probe connected to a Tektronic 466 oscilloscope (vert.: 20 G/div.; hor.: 5 msec/div.). Lower trace $\left[\frac{d\phi_B}{dt}\right]$ has been obtained by monitoring the pulsing electromagnetic field in air with a coil probe made of 50 turns of copper wire, i.d. 5 mm, length 15 mm, connected to a Tektronic 466 oscilloscope (vert.: 1 mV/div.; hor.: 5 msec/div.).

sured as previously described (13). The standard incubation mixture contained in a final volume of 0.5 ml: 25 $\mu$mole of Tris–HCl buffer, pH 7.1; 0.05 $\mu$mole of pyridoxal-5-phosphate; 1.25 $\mu$mole of dithiothreitol; 0.3 $\mu$mole of L-ornithine; 0.25 $\mu$Ci of DL-[1-$^{14}$C]ornithine (New England Nuclear (NEN) Chemicals, GmbH, Frankfurt, Main, Germany; sp act 47.2 mCi/mmole), and 0.2 ml of liver extract. The incubations were carried out in vials fitted with a disposable polypropylene center well attached to a rubber stopper for 60 min in a shaking water bath at 37°C. The released $^{14}CO_2$ was trapped into 0.2 ml of 1 *M* hyamine hydroxide in the center well. The reaction was stopped by injecting 1 ml of 10% (w/v) trichloroacetic acid into the reaction mixture, and the vials were left for an additional 15 min at 37°C to ensure the release of all $^{14}CO_2$ from the acidified medium. The center well and its contents were put directly into a scintillation vial containing 10 ml of toluene-base scintillation fluid and counted in a Packard Tri-Carb liquid scintillation spectrometer. Protein was assayed by the method of Lowry *et al.* (14).

*Incorporation of thymidine into DNA and measurement of haploid genomes.* To evaluate DNA synthesis, the rats of each group were injected intraperitoneally with 50 $\mu$Ci/100 g body wt of methyl[$^3$H]thymidine (NEN; sp act 16.2 Ci/mmole) in 1 ml of saline and killed by decapitation exactly 1 hr after the injection (15). The livers were quickly removed, dropped into cold saline, and blotted with filter paper, and then homogenized in 9 vol of distilled water. Perchloric acid was added to an aliquot of the homogenate to a final molarity of 0.5. After centrifugation the supernatant was saved and the residue was washed once with cold 0.2 *M* perchloric acid. The supernatants were combined and designated as the "acid-soluble fraction." The DNA in the pellet was separated from the RNA (16) and was assayed for radioactivity.

The DNA extracted as described was quan-

titatively assayed (17). The number of haploid genomes was calculated considering $3.25 \times 10^{-12}$ g of DNA the weight of one rat liver haploid genome.

*Electron microscopy and stereological procedure.* Perfusion *via* the hepatic portal vein (18), as well as immersion fixation were carried out for every single experiment using 2% glutaraldehyde in 0.1 *M* phosphate buffer, pH 7.4. In both fixations, segments from the tip of the lobus caudatus of unoperated animals and from regenerating lobes of hepatectomized rats, either treated or untreated, were removed. The segments were cut into 1-$mm^3$ blocks and immersed for 2 hr in 2% glutaraldehyde. After a rapid washing in phosphate buffer, the specimens were postfixed in 1% $OsO_4$ in 0.1 *M* phosphate buffer, pH 7.4, and then dehydrated in graded ethanols and embedded in Araldite. Thin sections were examined at the electron microscope and photographed at a primary magnification of 4000. Pictures to be used for stereological analysis were randomly selected from specimens either fixed in glutaraldehyde or in $OsO_4$. Areas to be analyzed by stereological analysis (19) were randomly selected choosing the upper left corner of the grid square and photographically enlarged to 1200×. Two square lattice systems were superimposed on the electron micrographs: the first, 11 × 11 cm, having a total number of 256 test points with a point spacing of 0.7 cm; the second, a double lattice 11 × 11 cm, having a total number of 1024 test points with a point spacing of 0.3 cm.

**Results and Discussion.** When partially hepatectomized rats are exposed, immediately after the operation, for 30 min to the electromagnetic field described, liver ornithine decarboxylase (ODC) activity shows a striking increase compared with the control rats (Fig. 2). In fact 3 and 6 hr after the operation the enzyme activity is, respectively, 234 and 250% of the corresponding values obtained from the untreated rats. This effect appears to be transient in nature as 9 and 12 hr after the operation no difference in the enzyme activity was observed between control and treated rats. However, if the same treatment is repeated 8.5 hr after the operation, a second increase of liver ODC activity is observed 3 hr later. This increase is of a smaller magnitude, i.e., 139% of the values observed either in untreated rats or rats treated only once immediately after the operation (Fig 2). Exposure to the same electromagnetic field did not have any effect on liver enzyme activity in intact or sham-operated rats (data not reported).

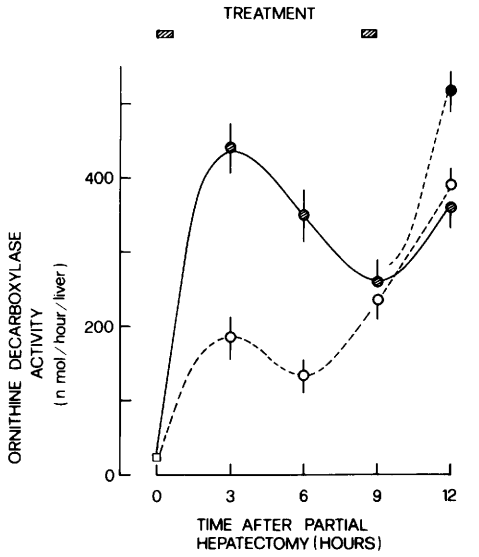


FIG. 2. Effect of ELF pulsing electromagnetic fields on ornithine decarboxylase activity of rat liver during early phases of regeneration following partial hepatectomy. Rats were treated with the electromagnetic field described in the text for 30 min, and treatment repeated 8.5 hr after surgery. Control rats (○); rats treated only once after surgery (▨); rats treated twice (●). Each point is the average of at least 9 rats and vertical bars represent the SEM.

ODC activity is induced by many hormones in their target tissues, the highest ODC levels paralleling the period of most rapid cellular growth and replication in a number of animal tissues. Direct evidence for the correlation of ODC activity and neoplastic growth has been obtained by comparing enzyme activity in normal and virus-transformed cells (for a recent review see Ref. (11)). Therefore it has been suggested that the mechanisms that regulate the ODC activity are an integral part of growth-control machinery. Furthermore, the extremely short half-life of ODC has suggested that this enzyme also plays a role in the regulation of growth. In this light our present results indicate that ELF pulsed electromagnetic fields are able either to amplify the regeneration of liver or to cause completion of regeneration to be reached in a shorter time.

To test these possibilities, we have studied

TABLE I. EFFECT OF PULSED ELF ELECTROMAGNETIC FIELDS ON THE INCORPORATION OF LABELED THYMIDINE INTO LIVER DNA DURING REGENERATION FOLLOWING PARTIAL HEPATECTOMY

| Time after partial hepatectomy (days) | Total uptake (dpm/100 mg liver × $10^{-3}$) | | Radioactivity in DNA (% of total uptake) | |
|---|---|---|---|---|
| | Control | Treated | Control | Treated |
| 0 | 40 ± 1.2 | — | ND | — |
| 1 | 132 ± 2.9 | 289 ± 7.8 | 8.1 ± 0.3 | 18.3 ± 0.8 |
| 3 | 143 ± 3.5 | 324 ± 11.1 | 20.5 ± 1.1 | 31.6 ± 1.3 |
| 5 | 186 ± 6.0 | 263 ± 7.3 | 18.7 ± 0.8 | 21.4 ± 0.9 |
| 7 | 80 ± 2.9 | 180 ± 5.1 | 5.6 ± 0.2 | ND |

*Note.* Rats were injected ip with [$^3$H]thymidine and pulsed for 60 min. Rats were treated with the electromagnetic field described in the text for 30 min every 12 hr, beginning immediately after surgery. Control rats were put, for the same length of time, into the apparatus which was switched off. Data are expressed as percentage of total uptake, defined as the sum of the radioactivities in DNA and in acid-soluble fraction at 1 hr after injection (15). Figures represent the average of at least six determinations performed on different occasions ± SEM (ND = not detectable).

the later phases of regeneration by measuring the rate of DNA synthesis and the number of haploid genomes from Day 1 to Day 7 after partial hepatectomy. Rats have been treated with the same pulsed electromagnetic field for 30 min every 12 hr, receiving the first treatment immediately after surgery.

The rate of DNA synthesis has been measured by the incorporation of labeled thymidine into DNA, and data are expressed as percentage of total uptake, defined as the sum of the radioactivities in DNA and in acid-soluble fraction at 1 hr after injection (15). This minimizes the changes in uptake of labeled thymidine due to permeability of cellular membrane which in turn would alter the specific activity of the TTP precursor pool for DNA.

Indeed, the pulsed electromagnetic fields used in these experiments have a positive effect on the rate of DNA synthesis. In fact, 1 day after the operation the labeled thymidine incorporated into DNA is 18.3% of total uptake in treated rats, while control rats had only an 8.1% label in the DNA (Table I). A smaller difference is found 3 days after the operation, and at the 5th day the rate of DNA synthesis is almost the same in both control and treated rats. Seven days after the operation the control animals are still synthesizing DNA, while the treated ones do not show any detectable label in DNA. These different patterns of the rate of DNA synthesis find direct confirmation from the accumulation patterns of total liver genomes (Fig. 3). In fact control rats show a constant increase of liver total genomes from the operation to Day 7, while in treated rats the accumulation is faster during the first postoperative days. The largest difference is

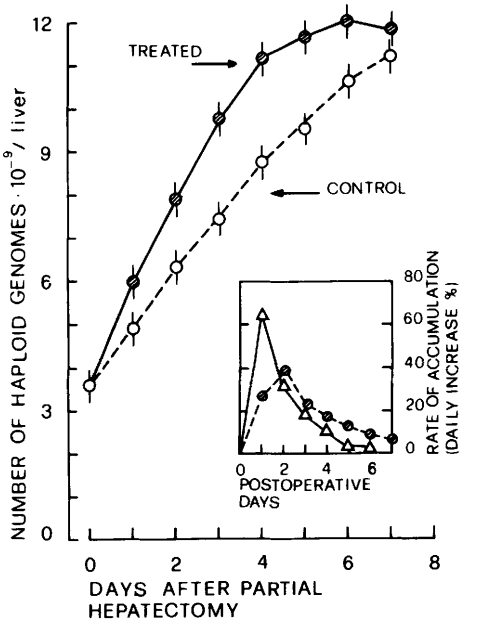


FIG. 3. Effect of pulsed ELF electromagnetic fields on the number of liver haploid genomes during regeneration following partial hepatectomy. For experimental details and treatments see legend to Table I and Materials and Methods. One liver haploid genome is considered 3.25 × $10^{-12}$ g of DNA. Each point represents the average of at least six determinations performed on different occasions. Vertical bars represent the SEM. The number of haploid genomes in the liver of intact rats was 13.1 ± 0.4 × $10^{-9}$ (average of eight determinations). The insert shows the rate of change during regeneration.

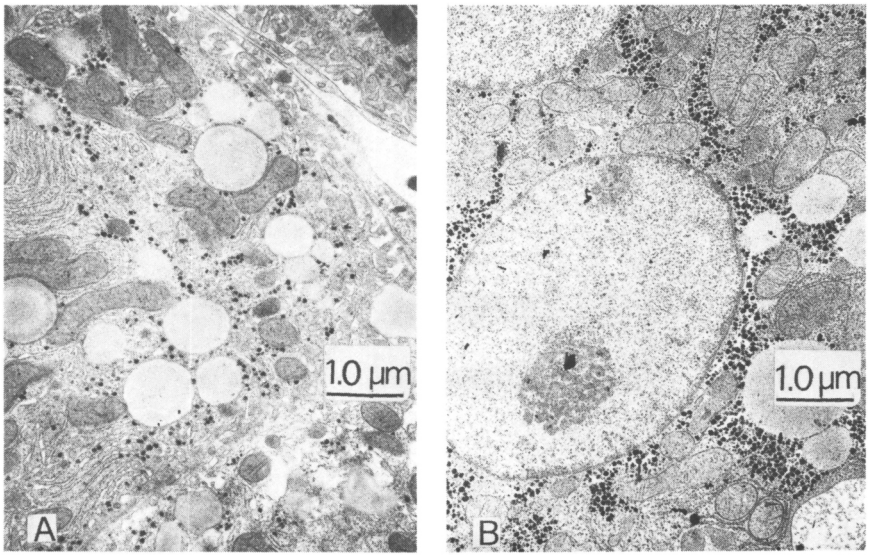


FIG. 4. Regenerating rat liver 1 day after partial hepatectomy. (A) Untreated controls; (B) rats treated with pulsed electromagnetic fields.

observed at the 4th day after surgery, and 3 days later the difference between the two groups is very small, if any. The total amount of labeled TdR (acid-soluble plus acid-insoluble counts) taken up by the cell during the pulse is remarkably increased by the treatments (Table I). This shows that magnetic fields used in these experiments have an influence on the accumulation of this molecule into the cell and hence possibly on its transport across cell membrane.

From a metabolic point of view the most striking and peculiar features of liver regeneration after partial hepatectomy are the gly-

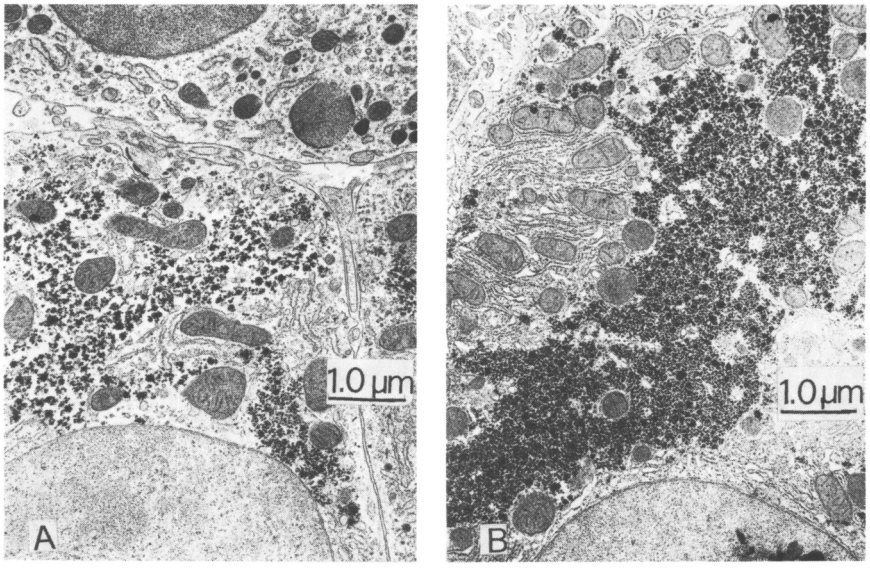


FIG. 5. Regenerating rat liver 5 days after partial hepatectomy. (A) Untreated controls; (B) rats treated with pulsed magnetic fields.

TABLE II. EFFECT OF PULSED ELF ELECTROMAGNETIC FIELDS ON GLYCOGEN AND LIPID CONTENTS OF RAT LIVER DURING REGENERATION FOLLOWING PARTIAL HEPATECTOMY

| Time after partial hepatectomy (days) | Glycogen volume density ($cm^3/cm^3$) | | Lipid volume density ($cm^3/cm^3$) | |
|---|---|---|---|---|
| | Control | Treated | Control | Treated |
| 0 | 0.201 ± 0.32 | — | 0.004 ± 0.05 | — |
| 1 | 0.044 ± 0.05 | 0.082 ± 0.07 | 0.084 ± 0.01 | 0.043 ± 0.05 |
| 5 | 0.149 ± 0.17 | 0.195 ± 0.10 | 0.021 ± 0.04 | 0.006 ± 0.02 |

*Note.* Rats were treated with electromagnetic fields as described in the legend to Table I and under Materials and Methods. Glycogen and lipid contents were determined by stereological analysis carried out on electron photomicrographs and are expressed as volume density. Figures represent the average of at least six determinations ± SEM.

cogen depletion during the first 24 hr after the operation and the almost parallel accumulation of lipid droplets within the same time (20–22). In order to ascertain whether the pulsed electromagnetic fields used in these experiments are able to influence also these metabolic parameters, we have studied liver glycogen and lipid contents by means of stereological analysis performed on electron micrographs. Examples of ultrastructural pictures used to perform these measurements are reproduced in Figs. 4 and 5 and data are reported in Table II.

One day after the operation both phenomena, i.e., glycogen depletion and lipid accumulation, are present in a more limited extent in the liver of rats treated with pulsed electromagnetic fields. Furthermore, the treated rats at the 5th day after surgery have already reached the zero time values, whereas control rat livers still have a lower glycogen and a higher lipid content.

Electromagnetic fields have been shown to have different and often contradictory effects on the living organisms including acceleration (23) of growth and its inhibition (24). However, it is important to stress that all results must be interpreted in strict relation to the particular intensity, frequency and wave form of the magnetic field and the treating protocol used.

In our experimental conditions the electromagnetic fields used have been shown to be able to increase the rate of liver regeneration following partial hepatectomy, apparently without interfering with the control mechanisms which regulate the end-point of regeneration.

1. Bawin SM, Adey WR. Sensitivity of calcium binding in cerebral tissue to weak environment electric fields oscillating at low frequency. Proc Natl Acad Sci USA **73**:1999–2003, 1976.
2. Tabrah FL, Guernsey DL, Chou SC, Batkin S. Effect of alternating magnetic fields (60–100 gauss, 60 Hz) on *Tetrahymena pyriformis*. Tower Int Technicomedical J Life Sci **8**:73–77, 1978.
3. Chiabrera A, Hinsenkamp M, Pilla AA, Ryaby J, Ponta A, Belmont A, Beltrame F, Grattarola M, Nicolini C. Cytofluorometry of electromagnetically controlled cell differentiation. J Histochem Cytochem **27**:375–381, 1979.
4. Grisset JD. Biological effects of electric and magnetic fields associated with ELF communications systems. Proc IEEE **68**:98–104, 1980.
5. Adey WR. Tissue interactions with nonionizing electromagnetic fields. Physiol Rev **61**:435–514, 1981.
6. Greenebaum B, Goodman ZM, Marrow MT. Magnetic field effects on mitotic cycle length in Physarum. Eur J Cell Biol **27**:156–160, 1982.
7. Frank C, Schachar N, Dittrich D, Shrive N, Dehaas W, Edwards G. Electromagnetic stimulation of ligament healing in rabbits. Clin Orthop **175**:263–272, 1983.
8. Barker AT, Lunt MJ. The effects of pulsed magnetic fields on the type used in stimulation of bone fracture healing. Clin Physiol P **4**:1–27, 1983.
9. Bassett CAL. Pulsing electromagnetic fields: A new method to modify cell behavior in calcified and noncalcified tissues. Calcif Tissue Int **34**:1–8, 1982.
10. Bresnick E. Regenerating liver: An experimental model for the study of growth. Methods Cancer Res **6**:347–397, 1971.
11. Bachrach U. The induction of ornithine decarboxylase in normal and neoplastic cells. In: Gaugas JM, ed. Polyamines in Biomedical Research. Chichester, Wiley, pp81–107, 1980.
12. Higgins GM, Anderson RM. Experimental pathology of liver. I. Restoration of liver of white rat following

partial surgical removal. Arch Pathol **12**:186–190, 1931.

13. Barbiroli B, Moruzzi MS, Tadolini B, Monti MG. Ornithine decarboxylase activity in regenerating liver from rats adapted to controlled feeding schedules. J Nutr **105**:408–412, 1975.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem **193**:265–275, 1951.
15. Barbiroli B, Potter VR. DNA synthesis and interaction between controlled feeding schedules and partial hepatectomy in rats. Science **172**:738–741, 1971.
16. Munro HN, Fleck A. The determination of nucleic acids. Methods Biochem Anal **14**:113–176, 1966.
17. Hopkins HA, Flora JB, Schmidt RR. Periodic DNA accumulation during the cell cycle of a termophilic strain of *Chlorella pyrenoidosa.* Arch Biochem Biophys **153**:845–849, 1972.
18. Wisse E. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. J Ultrastruct Res **31**,125–150, 1970.
19. Weibel ER. Stereological Methods. Vol. I. Practical Methods for Biological Morphometry. London, Academic Press, 1979.
20. Bucher NLR, Malt RA. In: Regeneration of Liver and Kidney. New England J Medicine, Medical Progress Series. Boston, Little, Brown, 1971.
21. Marks C, Markey C, Dyer R, Vaupel MR. An electron microscopic study of the effects of portacaval shunts on the ultrastructure of the rat liver after partial hepatectomy. Amer J Surg **129**:156–162, 1975.
22. Bartel H, Orkisz S, Kmiec B. Ultrastructure of hepatocyte in regenerating rat liver. Folia Morphol Warsz **4**:367–372, 1977.
23. Aarholt E, Flinn EA, Smith CW. Effects of low-frequency magnetic fields on bacterial growth rate. Phys Med Biol **26**:613–621, 1981.
24. Neurath PW. High gradient magnetic fields inhibits embryonic development of frogs. Nature (London) **219**:1358–1359, 1969.

---

Received September 6, 1983. P.S.E.B.M. 1984, Vol. 176.
Accepted April 18, 1984.