forward passivity phenomena, it seems reasonable to assert that models of motion, rhythm and electrical discharge based on physiochemical action, which have been often used in the verbally transmitted culture of physiology, deserve a wider audience and a more careful analysis than they have yet received.

Zusammenfassung. Durch Einführung einer Stahlnadel werden andauernde elektrische und mechanische Schwankungen erzeugt und zwar bei wechselnder Oberflächenoxydation und Reduktion eines Quecksilbertröpfchens, das in verdünnter Säure mit $K_2Cr_2O_7$ liegt. Die Schwankungen des Elektropotentials und des Widerstands wurden gemessen und zusammen mit den mechanischen Schwankungen photographiert.

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Phenobarbital Liver Microsomal Induction in MHV-3 Viral Hepatitis of the Mouse

Mouse hepatitis by MHV-3 Craig virus is characterized by parenchymal damage evolving rapidly towards a difused necrosis about 48 h from virus inoculation[1]. Previous works from this laboratory have shown that the earliest liver cell lesions involve lysosome membranes, while damage of mitochondria and of plasma membranes appears later[2–4]. Protein and RNA synthesis do not seem to be substantially altered even at an advanced stage of the infection[5,6]. The possibility of enhancing enzyme synthesis after phenobarbital treatment of mice in the presence of a progressed liver damage seemed to be of particular interest and has been actually the object of this research.

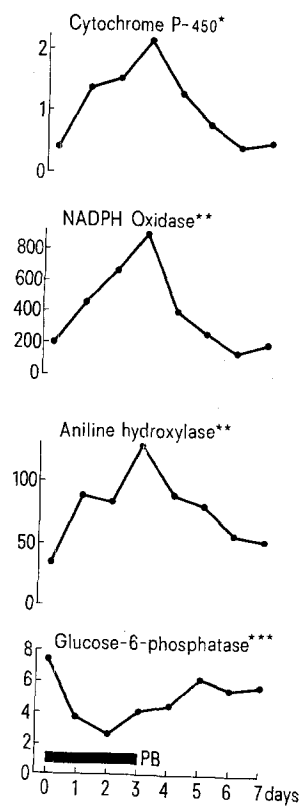

Fig. 1. Microsomal enzymes from mouse liver during and after phenobarbital treatment. Mean values of 2 concordant experiments. * mμmoles per mg of microsomal protein. ** activities expressed as mμmoles of substrate metabolized or product formed per mg of microsomal protein/h. *** μmoles of Pi liberated per mg of microsomal protein/h. ■, Phenobarbital (PB) 40 mg/kg i.p. twice a day.

Materials and methods. Male albino Swiss mice weighing 20–25 g were used. Treated animals were given 40 mg/kg i.p. twice a day of phenobarbital (PB) in 0.9% NaCl, while controls received an equivalent volume of 0.9% NaCl.

In the first series of experiments the course of PB induction in normal mice was examined. Groups of 6–7 mice fed ad libitum were killed by decapitation 1, 2 or 3 days after PB treatment and 1, 2, 3 or 4 days after cessation of treatment, as reported in Figure 1.

In a second series of experiments induction by PB of liver microsomal enzymes was studied in the course of hepatitis. Animals were given i.p. 0.1 ml suspension of infected mouse liver containing about 10,000 LD_{50} of the Craig strain of MHV-3 virus. Groups of 6–7 mice were sacrificed at 0, 24 and 48 h of PB treatment, and at 0, 24 and 48 h of infection with all the relative possible combinations. Blood was collected in heparinized tubes and plasma glutamic-oxalacetic transaminase (GOT) was determined according to Tonhazy et al.[7].

Pools of livers from the various groups of animals were weighed, chilled on ice and homogenized with 4 volumes of cold 1.15% KCl. The homogenate was centrifuged at 10,000 *g* for 20 min and then the supernatant fraction was again centrifuged at 105,000 *g* for 1 h in a Spinco Ultracentrifuge. The microsome pellet was finally suspended in 1.15% KCl so that 1.0 ml contained the microsomes from 1.0 g of wet liver.

Protein content was determined according to Lowry et al.[8]. The mean protein content of microsomes was found to be 26.5 mg $\pm$ 4.2 (S.D.) per gram of wet liver. No substantial difference as regards the protein microsomal

[1] F. De Ritis, M. Coltorti and G. Giusti, J. Infect. Dis. *101*, 219 (1957).
[2] A. Di Simone, R. Greco and M. Coltorti, Enzym. Biol. Clin. *9*, 157 (1967).
[3] M. Coltorti, G. Budillon, A. Di Simone and A. M. Barbieri, Enzym. Biol. Clin. *5*, 14 (1965).
[4] G. Budillon, C. Del Vecchio-Blanco and M. Coltorti, Enzym. Biol. Clin. *11*, 504 (1970).
[5] C. Del Vecchio-Blanco, G. Budillon and M. Carrella, Boll. Soc. it. Biol. sper. *43*, 1121 (1967).
[6] G. Budillon, C. Del Vecchio-Blanco, M. Carrella, V. Zappia and M. Coltorti, Proc. Soc. exp. Biol. Med. *126*, 409 (1967).
[7] N. E. Tonhazy, N. G. White and W. W. Umbreit, Arch. Biochem. *28*, 36 (1950).

content was observed between control and infected mice, and between the PB treated against the non-treated animals.

The following microsomal enzyme activities were assayed: cytochrome P-450 and NADPH oxidase were determined according to HART and FOUTS[9]; glucose-6-phosphatase was assayed substantially after DE DUVE et al.[10]; aniline hydroxylase was estimated with the method of KATO and GILLETTE[11] modified (the liberated para-aminophenol was directly determined in the incubation mixture, with the phenol reagent without ether extraction).

The histological picture of the liver at the various phases of infection was checked in a certain number of animals. A mild focal necrosis was observed at 24 h, and a massive liver necrosis at 48 h from the infection. The death of the mice occurred invariably between 48 and 72 h after virus inoculation. In accordance with the histological appearances, a massive increase of plasma GOT was found at 48 h of infection (Table).

Plasma glutamic-oxalacetic transaminase during hepatitis and phenobarbital treatment for 24 and 48 h (PB-24 and PB-48). μmoles of oxalacetate formed per ml/min at 37 °C. Mean values of 4 experiments ± S.D.

	—	PB-24	PB-48
Control	0.18 ± 0.02	0.22 ± 0.03	0.23 ± 0.02
24 h infected	0.26 ± 0.04	0.25 ± 0.05	0.25 ± 0.04
48 h infected	0.83 ± 0.10	0.90 ± 0.09	0.86 ± 0.12

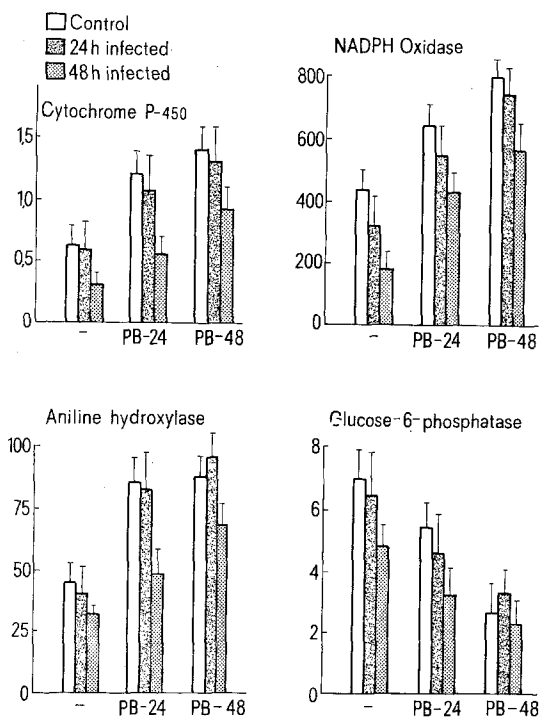

Fig. 2. Changes of liver microsomal enzymes in infected mice and in controls with and without phenobarbital treatment for 24 and 48 h (PB-24, PB-48). The enzyme activities are expressed as in Figure 1. Mean values of 4 experiments ± S.D. For the levels of the significance see the text.

Results. The daily treatment with phenobarbital in normal mice (Figure 1) caused a gradual increase of microsome cytochrome P-450, NADPH oxidase and aniline hydroxylase. After cessation of treatment these enzymes decreased so that the initial levels were reached after 3–4 days. A specular behaviour of glucose-6-phosphatase was observed, with enzyme levels decreasing following PB treatment and increasing after its suspension.

The following changes of microsomal enzymes were observed during MHV-3 infection (Figure 2): a significant reduction ($p < 0.05$) of cytochrome P-450, NADPH oxidase and aniline hydroxylase in the animals infected after 48 h in respect to uninfected mice, regardless of the PB treatment. These enzymes notably increased after PB treatment in infected as well as in healthy mice ($p < 0.05$ in mice PB treated for 48 h). Microsome glucose-6-phosphatase showed on the contrary a gradual decrease in the course of PB treatment both in normal and in infected mice. This decrease was signiticant ($p < 0.05$) in the animals PB treated for 48 h.

Discussion. The microsomal content of cytochrome P-450, NADPH oxidase and aniline hydroxylase appear to be markedly reduced in the mouse viral hepatitis at the stage of extensive liver cytonecrosis. The most significant finding of our research is indeed the phenobarbital stimulated elevation of these enzymes even at this stage of the disease. Phenobarbital is admitted to cause a RNA mediated de novo synthesis of enzyme protein, presumably reacting with a repressor compound[12]. Our results indicate the preservation of these mechanisms in spite of an extensive viral damage of the liver. This is well in keeping with the results of the research already mentioned on liver protein and RNA synthesis in mouse virus hepatitis[5,6]. The observed decrease of glucose-6-phosphatase after PB treatment in normal and infected mice might arise from a quantitative disproportion between rough endoplasmic reticulum where the enzyme is mainly located, which is not substantially influenced by PB treatment, and the smooth endoplasmic reticulum which is quantitatively augmented after phenobarbital enzyme induction.

Riassunto. Sono state studiate le variazioni di alcuni enzimi microsomiali del fegato di topi trattati con fenobarbital in varie fasi della epatite da MHV-3. È stato osservato che anche in fasi di avanzata citonecrosi epatica il trattamento con fenobarbital determina netti incrementi del citocromo P-450, della NADPH ossidasi e della anilina idrossilasi microsomiali. Nelle stesse condizioni invece la glucosio-6-fosfatasi mostra un decremento sia nei topi controllo che negli infetti.

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[8] O. H. LOWRY, N. J. ROSENBROUGH, A. L. FARR, R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[9] L. G. HART and G. R. FOUTS, Biochem. Pharmac. *14*, 263 (1965).
[10] C. DE DUVE, J. BERTHET, H. G. HERS, L. DUPRET, Bull. Soc. chim. biol. Paris *31*, 1242 (1949).
[11] R. KATO and J. R. GILLETTE, J. Pharmac. exp. Ther. *150*, 279 (1965).
[12] H. REMMER, Am. J. Med. *49*, 617 (1970).