Pentobarbital Detoxification by the Regenerated Liver of Partially Hepatectomized Rats

To demonstrate to medical students the role of the liver in the metabolism of certain drugs[1], the prolongation of pentobarbital-induced 'sleeping time' in partially hepatectomized rats has been scheduled yearly for some 20 years. After partial hepatectomy the liver regenerates rapidly from the 1st to the 3rd day[2]. During this initial 3 days no simultaneous reduction in sleeping time occurs. Therefore pentobarbital detoxification was determined before, during and after the initial phase of rapid liver regeneration following partial hepatectomy.

Methods. Pentobarbital sodium[3] was given i.p. to young adult male albino rats of a Wistar strain, in a dose of 35 mg/kg body weight, and the sleeping time (interval in minutes between disappearance and reappearance of the righting reflex) measured. Partial hepatectomies were performed by the method of HIGGINS and ANDERSON[4]. Control animals were laparotomized only. After the pentobarbital challenge, the rats were killed with chloroform and the liver removed, weighed and, in experiment 2, dried to constant weight in a forced-draft isotemp oven at 96 °C.

Experiment 1 comprised results obtained during several medical student laboratory periods on 184 rats, divided into 23 groups of 8 ± 2 rats per group. The distribution of pentobarbital challenges is shown in Figure 1. Body weight was 220 ± 33 g for 127 hepatectomized rats and 231 ± 33 g for 57 laparotomized controls. Experiment 2 consisted of 108 control animals, divided into 11 groups of 10 ± 1 rats per group, all laparotomized except 12 which served as day O-controls (Figure 1). Body weight of the controls was 284 ± 20 g. 161 rats, divided into 19 groups of 8 ± 2 rats per group, were hepatectomized. Their body weight was 268 ± 19 g. Experiment 2 was divided into 2 parts: in the 1st part 11 hepatectomized and 7 control groups were challenged with pentobarbital sodium, 35 mg/kg i.p., at times indicated in Figure 1. In the 2nd part, 4 groups of hepatectomized rats were given

[1] J. M. WALKER and C. B. WYNN PARRY, Brit. J. Pharmac. Chemother. *4*, 93 (1949).
[2] A. M. BRUES and B. B. MARBLE, J. exp. Med. *65*, 15 (1937).
[3] Obtained as Nembutal® Sodium Abbot, Pentobarbital sodium injection, USP.
[4] G. M. HIGGINS and R. M. ANDERSON, Archs Path. *12*, 186 (1931).

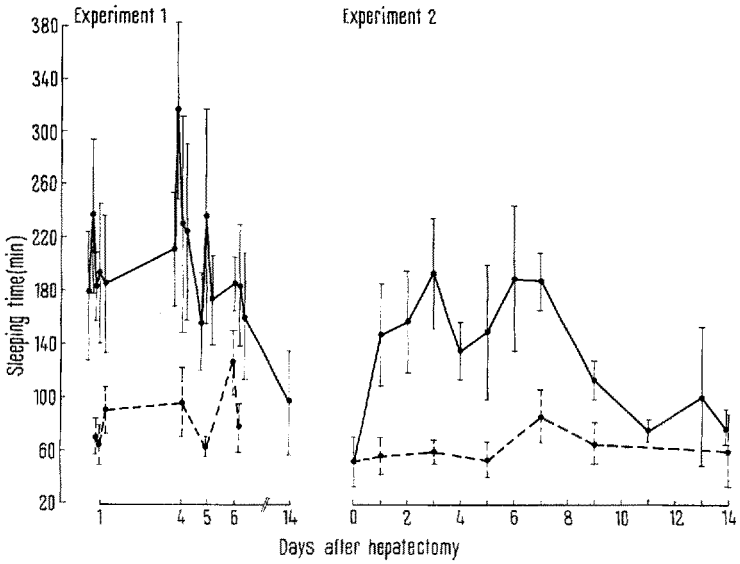

Fig. 1. Mean sleeping times (± standard deviations) induced by pentobarbital sodium in rats recovering from partial hepatectomy at increasing periods of time (solid bars and lines) and their laparotomized controls (interrupted bars and lines). For explanation of experiments 1 and 2 see text. Intact controls are indicated at day 0 for experiment 2 only.

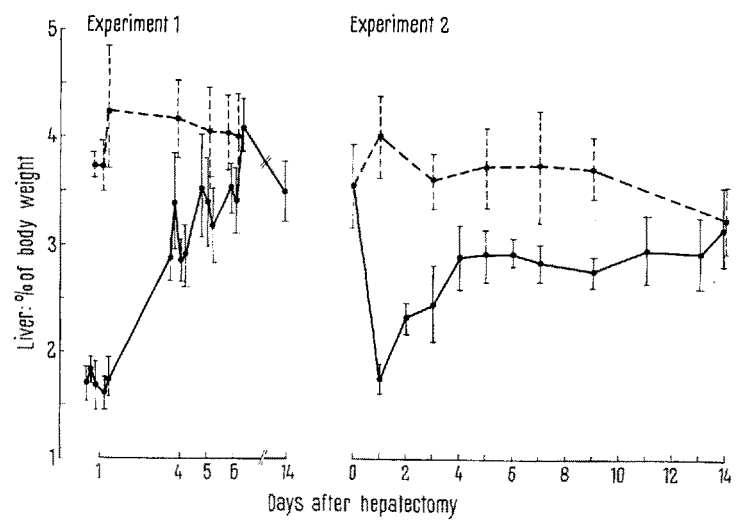

Fig. 2. Mean liver weights, as % of body weight (± standard deviations), in rats recovering from partial hepatectomy at increasing periods of time (solid bars and lines) and their laparotomized controls (interrupted bars and lines). For explanation of experiments 1 and 2 see text. Intact controls are indicated at day 0 for experiment 2 only.

27.5, 30.0, 32.5, and 40 mg/kg i.p. of pentobarbital sodium on each of days 1 and 4 post-hepatectomy, and 4 groups of controls were given 40, 45, 50, and 55 mg/kg 1 day after laparotomy.

Results and discussion. Experiment 1 showed that after hepatectomy the detoxification of pentobarbital as measured by sleeping time was decreased (Figure 1). In no instance was the sleeping time at 4, 5 or 6 days after hepatectomy significantly less than at 1 day. Nevertheless, weight regeneration of the liver was almost complete at 4–6 days (see Figure 2). The 1st part of experiment 2 confirmed these observations and indicated that, asynchronously with liver weight regeneration (Figure 2), sleeping time was reduced sharply between 7 and 9 days after hepatectomy (Figure 1). During the first 7 days, detoxification of pentobarbital apparently occurred at the same rate. Water content of the liver was increased from the 2nd to 5th day after hepatectomy (Figure 3).

Several doses of pentobarbital were tried on 2 different days to find if the dose-response curves for the 1st and 4th day after hepatectomy would overlap. The results are shown in Figure 4, and indicate almost complete overlap at these 2 days. Calculating the doses of pentobarbital as mg/g liver dry weight enabled us to see if any part of the experimental curves would overlap or form a continuation of the dose-response regression of the control animals. The results are shown in Figure 5, and demonstrate that (a) no overlap occurs in the hepatectomized rats at 1 versus 4 days and (b) that the regression at 1 day appears to be an extension of the regression in the controls. An extension of the dose-response study in the controls was not possible because a dose lower than 27.5 mg/kg did not produce hypnosis, while doses over 55 mg/kg were often fatal.

While the increased sleeping time 1 day after hepatectomy seemed to be the result solely of a loss of liver mass, this did not apply to the 4th day. The newly-formed liver tissue on day 4 seemed unable to metabolize the barbiturate. This would be readily explained if regeneration occurred by stem cell multiplication rather than a division of all hepatocytes. Although the division starts in certain zones of the liver acinus[5], no stem cell multiplication has been demonstrated[6].

The possibility remains that the existing metabolizing enzymes or enzyme-producing systems, which were left

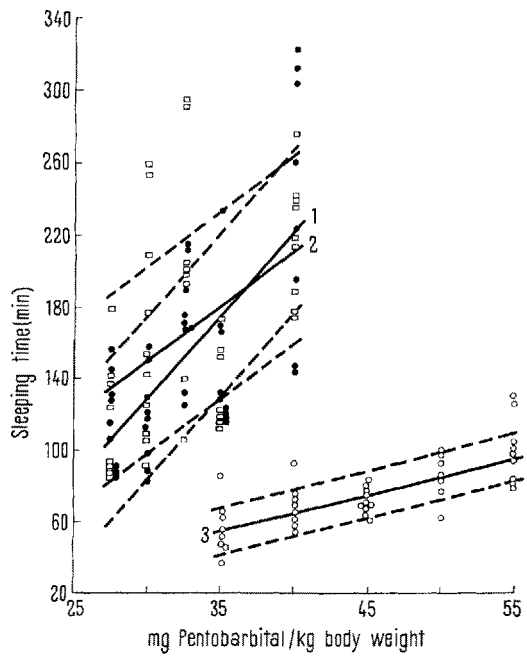

Fig. 4. Relation between dose of pentobarbital sodium, expressed as mg/kg body weight, and sleeping time in rats at 1 day (1, solid circles) and 4 days (2, open squares) post-partial hepatectomy and in controls at 1 day post-laparotomy (3, open circles). Indicated are the calculated linear regression lines (solid lines) ± their standard errors (interrupted lines).

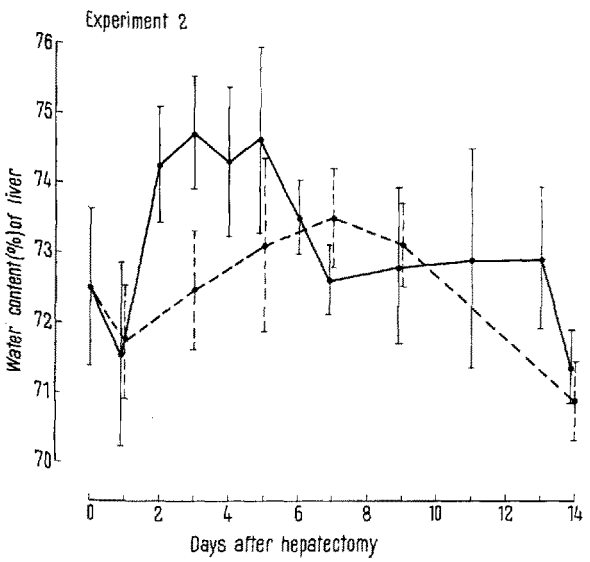

Fig. 3. Mean water content of liver, as % of liver wet weight (± standard deviations), in rats recovering from partial hepatectomy at increasing periods of time (solid bars and lines) and their laparotomized controls (interrupted bars and lines). Intact controls are indicated at day 0 for experiment 2 only.

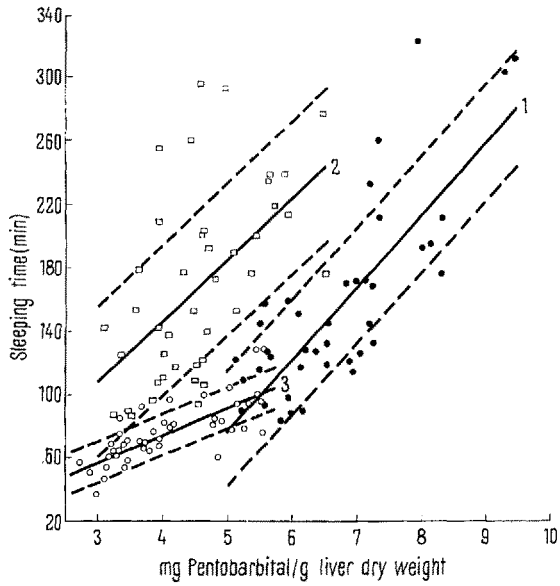

Fig. 5. Relation between dose of pentobarbital sodium, expressed as mg/g liver dry weight, and sleeping time in rats at 1 day (1, solid circles) and 4 days (2, open squares) post-partial hepatectomy and in controls at 1 day post-laparotomy (3, open circles). Indicated are the calculated linear regression lines (solid lines) ± their standard errors (interrupted lines).

on the 1st day in the remnant of the amputated liver, were transferred intact to, and divided over, the daughter cells of the multiplying hepatocytes. FOUTS et al.[7] reported equal activities of side chain oxidation for hexobarbital between the 2nd and 8th day after partial hepatectomy, but a comparison with the 1st day is not possible from their data and no liver weights were given. Their report of a sharp decrease in hexobarbital sleeping time between the 3rd and 5th day, in conflict with our results, remains therefore unexplained[8].

Résumé. La prolongation de l'anesthésie au pentobarbital fut constatée chez les rats pendant 1 semaine après l'hépatectomie partielle. Dans la suite, l'effet disparut. Le poids du foie était redevenu presque normal dès le 4ème jour. Les tissus hépatiques nouvellement formés ont donc été temporairement incapables de métaboliser le barbiturique.

J. M. PETERS, C. J. KRIJNEN, and H. D. MCEWEN

Department of Pharmacology, Queen's University, Kingston (Ontario, Canada), July 25, 1966.

[5] J. W. GRISHAM, Cancer Res. *22*, 842 (1962).
[6] N. R. KLINMAN and A. J. ERSLEV, Proc. Soc. exp. Biol. Med. *112*, 338 (1963).
[7] J. R. FOUTS, R. L. DIXON, and R. W. SHULTICE, Biochem. Pharmac. *7*, 265 (1961).
[8] The authors wish to thank Dr. E. M. BOYD, for professional advice. The project was assisted by a grant from the Medical Research Council of Canada. First author (J. M. PETERS) is a Fellow of the Medical Research Council of Canada.

Effect of Insulin on Carbon Dioxide Production in Adipose Tissue from Immature Rats

The purpose of this study was to determine the effect of immaturity on insulin-induced carbon dioxide production in rat epididymal fat pads. GLIEMANN[1] has recently reported that conversion of glucose-1-C^{14} to carbon dioxide in adipose tissue is greater in rats weighing 100–110 g than in those weighing 200–230 g. He also demonstrated relatively poor CO_2 production in older rats, an observation previously reported by HAGEN, BALL, and COOPER[2]. The direct relationship between CO_2 production and lipogenesis in adipose tissue has been shown by WINEGRAD and RENOLD[3].

It has also been known for some time that rats and rabbits are capable of synthesizing fatty acids in utero[4,5], but relatively little is known about fat metabolism in immature animals. Such data may prove to be important from the standpoint of human pharmacology, since it may eventually help to explain some of the differences between children and adults in response to drugs. The present report concerns the effect of insulin on CO_2 production in adipose tissue taken from young rats weighing 35–90 g. For purposes of comparison, a small number of experiments were performed using adult rats weighing 150–200 g.

Material and methods. The technique employed was essentially that described by BALL, MARTIN and COOPER[6], in which CO_2 production is measured manometrically in the Warburg apparatus. Male Sprague-Dawley rats were stunned by a blow on the head and decapitated by guillotine. Epididymal fat pads were removed, weighed, and transferred immediately to flasks containing 2.3 ml of calcium-free Krebs-Ringer bicarbonate solution at pH 7.4. Incubation, with shaking, was carried out for 30 min at 37.5 °C. Glucose, 0.1 ml, and glucogon-free insulin[7], 0.1 ml, were then added from the side-arm, resulting in a final concentration of 4 mg/ml and 0.1 U/ml respectively. Incubation was allowed to proceed for an additional 60 min, and the positive (or negative) pressure readings appearing in mm on the manometers were converted to μl of CO_2 evolved per 100 mg of wet tissue/h. Pressure readings prior to addition of the side-arm contents were not used in the calculations, but were obtained to assure that a net positive production of gas was not occurring in the absence of insulin.

Rats were grouped according to weight as follows:

Group		Weight
I	Immature	35 through 50 g
II	Immature	55 through 70 g
III	Immature	75 through 90 g
IV	Adult	150 through 200 g

An effort was made to allow each flask to contain roughly 100 mg of adipose tissue. 2 or more rats from group I were usually required to fill 1 flask, whereas the tissue from 1 adult rat was always sufficient to fill 2 or more flasks. For the adult group, the average value of the flasks representing the tissue from one rat was considered as 1 experiment. This was done in order to justify the assumption (for statistical analysis) that the values obtained were independent of each other.

All animals in groups II, III, and IV were fed water and Purina Laboratory Chow ad lib. Group I rats were fed as follows: Ia same as groups II, III, and IV; Ib same, except that the pellets were crushed to make the ration more readily obtainable; and, Ic milk and milk-soaked bread.

Results. The results are presented in the Table. The data from group Ia is not included in the Table. Since rats of this size are for the most part newly weaned, it was felt that their relatively poor performance (5.4 μl CO_2 $\pm$ 6.3 S.D., in 11 experiments) might be partially due to mechanical difficulty in obtaining adequate food from the

[1] J. GLIEMANN, Diabetes *14*, 643 (1965).
[2] J. M. HAGEN, E. G. BALL, and O. COOPER, J. biol. Chem. *234*, 781 (1959).
[3] A. I. WINEGRAD and A. E. RENOLD, J. biol. Chem. *233*, 267 (1958).
[4] W. H. GOLDWATER and D. STETTEN JR., J. biol. Chem. *169*, 723 (1947).
[5] G. POPJAK and M.-L. BEECKMANS, Biochem. J. *46*, 547 (1950).
[6] E. G. BALL, D. B. MARTIN, and O. COOPER, J. biol. Chem. *234*, 774 (1959).
[7] Kindly supplied by Eli Lilly & Company.