The Stimulation of Intestinal Cholesterogenesis in the Rat by Phenobarbital* (34125)

W. R. J. MIDDLETON[1] AND KURT J. ISSELBACHER

Department of Medicine, Harvard Medical School and the Medical Services (Gastrointestinal Unit), Massachusetts General Hospital, Boston, Massachusetts 02114

Phenobarbital has been shown in a number of animal species to cause marked hypertrophy of the smooth endoplasmic reticulum in the liver cell (1–3). The membrane proliferation is associated with increased concentration of microsomal protein (4) and with increased activity of microsomal drug-metabolizing enzymes (5–7). The activity of other microsomal enzymes, such as those involved in cholesterol synthesis from acetate (8), are also affected by phenobarbital and the rate of cholesterol synthesis in liver slices in the hamster has been shown to be increased fourfold after phenobarbital administration (3). The present study was designed to determine if phenobarbital also stimulates cholesterol synthesis in the small intestine of the rat, since the intestine as well as the liver is an important site of endogenous cholesterol production (9).

Methods. Female albino rats of the Charles River CD strain weighing between 180–220 g were maintained prior to study on standard rat chow (Ralston-Purina Co., St. Louis, Mo.).

The incorporation of acetate-2-^{14}C (New England Nuclear Corp., Boston, Mass.) into cholesterol was measured in slices prepared from liver and small intestine. Nine rats were given phenobarbital by intraperitoneal injection (80 mg/kg body weight/day) for 5 days and were fasted for the final 48 hr of this period. Nine matched controls were treated in an identical manner but received intraperitoneal saline instead of phenobarbital. The animals were killed by cervical dislocation. A Stadie-Riggs microtome was used to prepare slices from the right lobe of the liver. The slices were rinsed, blotted, and weighed (approximately 400 mg wet weight) and immediately transferred to an incubation flask. The terminal 10 cm of the small intestine was removed, washed with cold 0.9% NaCl, opened longitudinally, and gently blotted. It was then weighed, sliced into small pieces, and transferred to an incubation flask.

The effect of phenobarbital on the incorporation of acetate-2-^{14}C into CO_2, fatty acids, and cholesterol was studied at three levels of the small intestine in six rats given phenobarbital (as above) and in six control animals. Animals were not fasted. The entire small intestine was removed, suspended vertically, and an 8-cm segment was taken from each of three sites—immediately distal to the ligament of Treitz (upper), mid-small intestine (middle), and terminal ileum (lower). Slices were prepared from each segment as described above.

Tissue slices were added to 25-ml Erlenmeyer flasks containing 5 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) gassed with 95% O_2–5% CO_2. The incubation fluid contained glucose 28 μmoles, sodium acetate 10 μmoles, and acetate-2-^{14}C 1.25 μmoles (2.5 μCi) in 5 ml. The flasks were incubated at 37° for 2 hr in a Dubnoff shaker.

The incorporation of acetate-2-^{14}C into CO_2, fatty acids, and cholesterol was measured by a modification (10) of the method described by Siperstein and Guest (11). After incubation the contents of each flask were acidified and the evolved CO_2 was trapped in hyamine (Packard 10-X) placed in the

* This investigation was supported in part by a grant from the John A. Hartford Foundation, Inc., the RGK Foundation, and the National Institutes of Health (AM-01392 and 03014).

[1] Dr. Middleton was recipient of a Lilly International Fellowship in Medicine.

center well. After dissolution of the tissue with alkali, total lipids were extracted by the method of Folch *et al.* (12). The lower phase was saponified and ethanol added to make a 50% ethanolic solution. Nonsaponifiable lipid was extracted with hexane. The residue was acidified, extracted with hexane to remove fatty acids, and the hexane extract was backwashed with water. Sterols were precipitated from the first hexane extract as the digitonide (13). Radioactivity was counted in toluene POPOP [4 g 2,5-diphenyloxazole and 50 mg *p*-bis (2-5-phenyloxazolyl)-benzene per liter of toluene] in a Beckman LS 250 liquid scintillation counter. Results were expressed as mμmoles of acetate incorporated into CO_2, fatty acids, and digitonin-precipitable sterols (DPS)/g wet weight tissue/2 hr incubation.

Results. The rate of cholesterol synthesis in slices of liver and lower small intestine in fasting control and phenobarbital-treated rats is shown in Table I. Phenobarbital administration resulted in a highly significant increase in the rate of cholesterol synthesis in the lower small intestine. Cholesterogenesis was also increased in liver, the magnitude of increase in the fasting animal being comparable to that reported in the hamster (3).

The effect of phenobarbital on CO_2, fatty acid, and cholesterol production at three levels of the small intestine is shown in Table II. Cholesterol synthesis was 12–18 times more active in the terminal ileum than in the upper and middle small intestine, as previously reported (14). There was a uniform increase in cholesterol synthesis throughout the small intestine in the phenobarbital-treated animals, but the increase was significant only in middle and lower small intestine.

TABLE I. Effect of Phenobarbital on Acetate-2-^{14}C Incorporation into Cholesterol by Slices of Liver and Lower Small Intestine from Fasting Rats.

Animals	Acetate incorporation into cholesterol (mμmoles/g wet weight/2 hr: mean $\pm$ SD) Liver	Lower small intestine
Controls (9)	15.2 ± 12.7	134.8 ± 76.6
Phenobarbital-treated (9)	81.5 ± 35.9	332.9 ± 86.3
p[a]	<0.001	<0.001

[a] Determined by Student's t test.

Discussion. Consistent with previous observations in the hamster (3), the rate of cholesterol synthesis has been shown to be increased in liver slices from phenobarbital-treated rats. The administration of phenobarbital resulted also, however, in a highly significant increase in the rate of cholesterol synthesis in the small intestine. In view of the fact that phenobarbital did not affect the intestinal incorporation of acetate into fatty acids, a pathway which does not appear to involve the endoplasmic reticulum, it is suggested that the increased cholesterogenesis was associated in the intestine as in the liver (3) with increased activity of enzymes associated with the smooth endoplasmic reticulum. Electron microscopic studies are being conducted to determine if the enhanced cholesterol synthesis produced by phenobarbital in the intestine is associated with morphologic evidence of hypertrophy of the smooth endoplasmic reticulum.

The rate of cholesterol synthesis in liver slices from fasting phenobarbital-treated rats was five times greater than in control animals. It is of interest that this drug-enhanced hepatic synthesis of cholesterol in fasting animals did not attain the level of cholesterogenesis observed in liver slices of normally fed control rats (339.5 ± 141.8 mμmoles/g/2 hr; unpublished observations). Thus, factors which control the rate of hepatic cholesterol production continue to operate in the presence of phenobarbital. The profound depression of hepatic cholesterol synthesis in the fasting animal, in the present study to less than 5% of normal, was in distinct contrast to the effect of fasting on intestinal cholesterogenesis. Thus, in control animals, comparable rates of synthesis were observed in the lower small intestine in fasted (134.8 mμmoles/g/2 hr, Table I) and in unfasted animals (150.7 mμmoles/g/2 hr, Table II). The significance of the ***in vitro*** demonstration of enhanced cholesterogenesis produced by

TABLE II. Effect of Phenobarbital on Acetate-2-^{14}C Incorporation into CO_2, Fatty Acids, and Cholesterol by Slices of Small Intestine from Nonfasting Rats.

Product	Segment of small intestine	Acetate incorporation (mμmoles/g wet weight/2 hr: mean ± SD) Control (6)	Phenobarbital-treated (6)	p
CO_2	Upper	1143 ± 305	1615 ± 143	<.01
	Middle	1306 ± 435	3178 ± 571	<.001
	Lower	2588 ± 407	5146 ± 823	<.001
Fatty acids	Upper	92 ± 45	80 ± 32	>.5
	Middle	171 ± 67	260 ± 102	>.5
	Lower	180 ± 84	133 ± 34	>.5
Cholesterol	Upper	13.1 ± 3.4	24.6 ± 13.8	>.05
	Middle	5.4 ± 1.8	17.4 ± 8.6	<.01
	Lower	150.7 ± 57.8	309.3 ± 54.2	<.001

phenobarbital treatment has yet to be evaluated *in vivo*.

Summary. The administration of phenobarbital to rats (80 mg/kg/day for 5 days) was associated with an increased incorporation of acetate into cholesterol by liver and small intestine *in vitro*. The stimulating effect of phenobarbital on intestinal cholesterogenesis has not previously been reported. Its overall effect on systemic cholesterol metabolism remains to be determined.

1. Remmer, H. and Merker, H. J., Klin. Wochschr. **41**, 276 (1963).

2. Fawcett, D. W., J. Cell Biol. **23**, 30A (1964).

3. Jones, A. L. and Armstrong, D. T., Proc. Soc. Exptl. Biol. Med. **119**, 1136 (1965).

4. Remmer, H. and Merker, H. J., Science **142**, 1657 (1963).

5. Remmer, H., Arch. Exptl. Pathol. Pharmakol. **235**, 279 (1959).

6. Kato, R., Experientia **16**, 9 (1960).

7. Conney, A. H., Pharmacol. Rev. **19**, 317 (1967).

8. Bucher, N. L. R. and McGarrahan, K., J. Biol. Chem. **222**, 1 (1956).

9. Dietschy, J. M. and Wilson, J. D., J. Clin. Invest. **47**, 166 (1968).

10. McIntyre, N., Kirsch, K., and Isselbacher, K. J., J. Clin. Invest. In press.

11. Siperstein, M. D. and Guest, M. J., J. Clin. Invest. **39**, 642 (1960).

12. Folch, J., Lees, M. and Sloane-Stanley, G. M., J. Biol. Chem. **226**, 497 (1957).

13. Sperry, W. M. and Webb, M., J. Biol. Chem. **187**, 97 (1950).

14. Dietschy, J. M., J. Clin. Invest. **47**, 286 (1968).

Received April 21, 1969. P.S.E.B.M., 1969, Vol. 131.