Metabolism of Xenobiotics in Ruminants

Phenobarbital Induction of Liver Microsomal Nitrogen Demethylase

Robert M. Cook and K. A. Wilson

Phenobarbital decreases the storage of HEOD in the body fat of rats by a probable mechanism of induction of liver microsomal enzymes which metabolize HEOD. To determine the feasibility of treating livestock with phenobarbital to increase the rate of HEOD excretion, the cow, calf, sheep, goat, pig, and rat were treated with phenobarbital daily at the level of 25 or 30 mg per kg of body weight for 5 days. The increase in nitrogen demethylase activity in liver microsomes using aminopyrine as substrate was taken as a measure of enzyme induction. The percent increase in induced enzyme activity over that for control values was 550, 1000, 400, 12,500, and 400 for the cow, sheep, goat, calf, pig, and rat, respectively. Phenobarbital induces drug-metabolizing enzyme activity in livestock similar to that observed for the rat. This work indicates that it is feasible to treat livestock with phenobarbital to increase the rate of HEOD excretion.

We have demonstrated the efficacy of activated carbon as an antidote for dieldrin (HEOD) poisoning in ruminants (Wilson and Cook, 1970). Activated carbon acts by binding HEOD in the gut and increasing excretion in the feces. A second method would be to stimulate the excretion of HEOD or HEOD residues in the urine. Barbiturates and other chemicals decrease the storage of HEOD in body fat of rats (Braund *et al.*, 1968; Cueto and Hayes, 1965; Street *et al.*, 1966). The barbiturates probably exert their effect on HEOD storage by inducing the activity of enzymes in liver microsomes which metabolize HEOD to more polar compounds that can be excreted by the kidney.

The discovery that barbiturates decrease the concentration of HEOD in the body fat of rats suggests that barbiturates might be used to increase HEOD clearance from ruminants. To provide a basis for a trial to test this hypothesis, it was necessary to establish whether or not ruminants respond to phenobarbital by induction of drug-metabolizing enzymes similar to the response found in the rat. Experiments demonstrated that phenobarbital induces drug-metabolizing enzyme activity in the cow, calf, goat, sheep, and pig. Similar preliminary data have been reported (Zook and Cook, 1968).

EXPERIMENTAL PROCEDURE

Phenobarbital was administered orally once each day for 5 days at the level of 25 mg per kg of body weight to Holstein cows and 30 mg per kg of body weight to the other animals. Phenobarbital was mixed with 0.5 lb of grain and fed to Holstein cows. Rats were given an i.p. injection of an aqueous solution of phenobarbital. All other animals were drenched with an aqueous solution of phenobarbital. Liver samples were taken by biopsy from the cows. All other liver samples were taken at time of sacrifice.

The livers were homogenized in four volumes of ice-cold 1.15% KCl containing 0.2% nicotinamide, and the homogenate was centrifuged at 20,000 x g for 20 min. The 20,000 x g supernatant was centrifuged at 105,000 x g for 1 hr to isolate microsomes. The microsomal pellet was suspended by brief blending in 0.1M sodium phosphate buffer pH 7.4 containing 20% glycerol and stored under nitrogen at −20° C.

The change in nitrogen demethylase activity in liver microsomes was taken as a measure of the response to phenobarbital. Enzyme activity was determined by measuring the production of formaldehyde (Nash, 1953). Protein was determined by the Lowry method (1951). The enzyme incubation mixture contained 0.1M phosphate buffer pH 7.4, aminopyrine (10 mM), $MgCl_2$ (6 mM), isocitric acid (10 mM), NADP (0.5 mM), isocitric dehydrogenase (0.05 unit per ml), and microsomal protein (0.1 to 1.0 mg per ml). The reaction mixture was incubated 15 min at 37° C and stopped by adding 10% TCA. The specific enzyme activity is expressed as millimicromoles of formaldehyde formed per milligram of protein per minute.

RESULTS AND DISCUSSION

A preliminary experiment in which Holstein heifers weighing approximately 400 kg were fed varying amounts of phenobarbital formed the basis for our choice of a phenobarbital dose of 25 mg per kilogram of body weight daily for the Holsteins. The heifers were fed a single dose of phenobarbital at the level of 50, 40, 30 or 25 mg per kg of body weight. At the 50 mg per kg of body weight level, the heifer could be readily pushed off her feet with a firm push on the hipbone within 2 hr after the phenobarbital dose. When the 40 mg per kg of body weight dose was given, she could be readily pushed to her knees but always recovered. When heifers were fed a single dose of phenobarbital at the level of 30 mg per kg body weight, there was no clinical response. Heifers fed phenobarbital at the level of 25 mg per kg body weight daily for 5 days were weak in the hind legs after 3 days, but after 4 days of treatment appeared normal.

The normal *N*-demethylase activity relative to the rat is shown in Figure 1. The activity in the young calf and the pig was lower than in the rat or sheep, but the activity in a goat and, for comparison, an elderly man was much greater than

Dairy Department, Michigan State University, East Lansing, Mich. 48823.

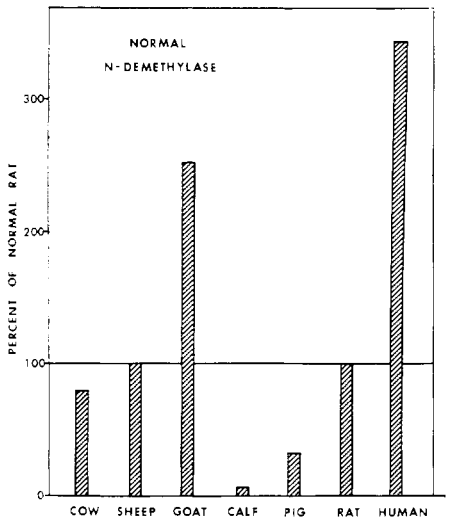

Figure 1. Normal nitrogen demethylase activity relative to the rat

the rat. *N*-Demethylase activity in the cow was slightly less than the rat. The response to phenobarbital is shown in Figure 2. *N*-Demethylase activity increased in all species, but the greatest response was observed with the sheep and pig (Figure 3). The pig had the highest induced *N*-demethylase activity, followed by the goat, sheep, and cow. Although the calf responds to phenobarbital treatment, the enzyme activity is less than half that found in the induced rat.

Phenobarbital treatment for 5 days markedly stimulates the activity of *N*-demethylase in all species studied. When phenobarbital was fed to a Holstein cow for 14 days, the enzyme activity was almost 10 times greater than pretreatment values (Figure 4). This shows that enzyme activity will continue to increase for 14 days and suggests that continued feeding of phenobarbital may result in even greater enzyme activity.

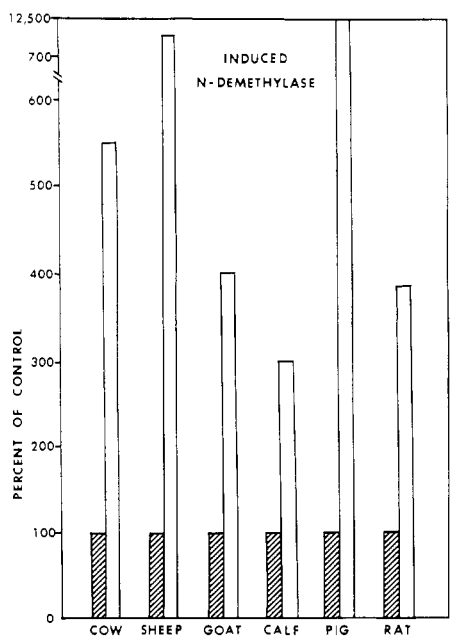

Figure 2. Phenobarbital-induced N-demethylase activity relative to normal activities

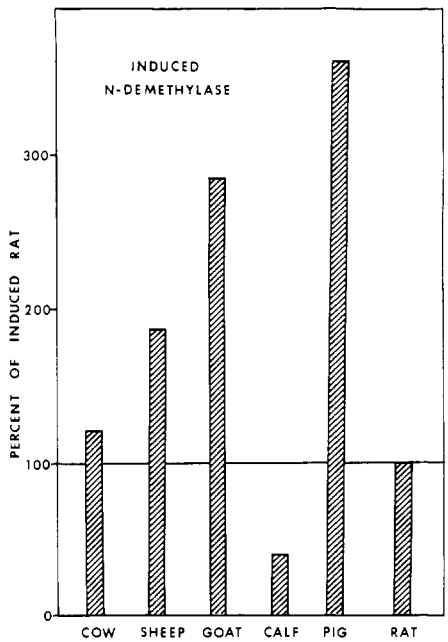

Figure 3. Phenobarbital-induced nitrogen demethylase activity relative to the rat

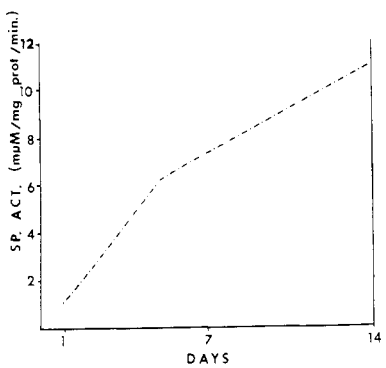

Figure 4. Effect of feeding phenobarbital 14 days on the activity of nitrogen demethylase

CONCLUSIONS

Phenobarbital induces liver drug-metabolizing enzyme activity in ruminants as well as in monogastric animals. This work provides a sound basis for studying the use of phenobarbital and other chemicals to increase the rate of excretion of HEOD from cattle.

ACKNOWLEDGMENT

The technical assistance of Barbara Purchas is gratefully appreciated.

LITERATURE CITED

Braund, D. G., Brown, L. D., Huber, J. T., *J. Dairy Sci.* **51** (982), p 152 (1968).
Cueto, C., Jr., Hayes, W. J., *Toxicol. Appl. Pharmacol.* **7**, 481 (1965).
Lowry, O. H., Rosbrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.* **193**, 265 (1951).
Nash, T., *Biochem. J.* **55**, 416 (1953).
Street, J. C., Wang, M., Blau, A. D., *Bull. Environ. Contam. Toxicol.* **1**, 6 (1966).
Wilson, K. A., Cook, R. M., J. AGR. FOOD CHEM. **18**, 437 (1970).
Zook, Barbara, Cook, R. M., *J. Dairy Sci.* **51** (981), p 151 (1968).

Received for review September 9, 1969. Accepted February 2, 1970. Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 4842.