12. Associated Electrical Industries.
13. G. Shaw, B. M. Smallwood, D. V. Wilson, *J. Chem. Soc.* **1966** (c), 921 (1966).
14. R. H. Hall, L. Csonka, H. David, B. McLennan, *Science* **156**, 69 (1967).
15. D. Klämbt, G. Thies, F. Skoog, *Proc. Nat. Acad. Sci. U.S.* **56**, 52 (1966); J. P. Helgeson and N. J. Leonard, *ibid.*, p. 60.
16. Z. Király, B. I. Pozsár, M. El Hammady, *Acta Phytopathol.* **1**, 29 (1966).
17. I thank J. R. Schramm for discussions leading to this work, E. Hacskaylo for a culture of the fungus, Gertrud Cowan for technical assistance, D. S. Letham and G. Shaw for samples of zeatin and zeatin riboside, respectively, and N. J. Leonard for samples of 6-dimethylallylaminopurine and its riboside. The project was supported by grant GE3612 and the mass spectra work by grant GP5234 from the National Science Foundation.

31 May 1967

Induction of Drug-Metabolizing Enzymes in Liver Microsomes of Mice and Rats by Softwood Bedding

Abstract. *Induction of three drug-metabolizing enzymes occurred in liver microsomes of mice and rats kept on softwood bedding of either red cedar, white pine, or ponderosa pine. This induction was reversed when animals were placed on hardwood bedding composed of a mixture of beech, birch, and maple. Differences in the capacity of various beddings to induce may partially explain divergent results of studies on drug-metabolizing enzymes. The presence of such inducing substances in the environment may influence the pharmacologic responsiveness of animals to a wide variety of drugs.*

Approximately 1 year ago during a long-term study of genetic and environmental factors affecting metabolism of hexobarbital (Evipal) in mice, an abrupt decrease in sleeping time was observed. This alteration took place when the animal room, cages, chow, and bedding were simultaneously changed. Because these experiments and those of others indicated that numerous environmental factors significantly affected metabolism of hexobarbital in mice, each of the new conditions was systematically investigated. It was discovered that red-cedar shavings caused decreased sleeping times and increased activity of the liver microsomal enzymes that metabolize hexobarbital. These changes were reversed when a mixture of beech, birch, and maple shavings replaced the softwood bedding (red cedar, white pine, or ponderosa pine). No alteration in the hexobarbital concentration in the brain at the time of restoration of the righting response occurred on any of the softwood beddings tested. Although the mechanism of the effect was not investigated, red-cedar bedding was reported to shorten sleeping time in mice treated with hexobarbital and pentobarbital (*1*).

Table 1. Sleeping times and activities of hepatic, microsomal hexobarbital oxidase, aniline hydroxylase, and ethyl morphine *N*-demethylase of NIH male mice kept 3 days on hardwood or red-cedar bedding (mean ± standard deviation). All activities are expressed in micromoles of substrates metabolized per gram of liver for 10 minutes, except for hexobarbital which is metabolized for 15 minutes. The number of animals is given in parentheses. All differences are significant ($P < 0.1$).

Bedding	Sleeping times (min)	Hexobarbital oxidase	Aniline hydroxylase	Ethyl morphine *N*-demethylase
Hardwood	35.3 ± 5.5(12)	0.46 ± 0.01(6)	0.35 ± 0.06(6)	3.8 ± 0.6(6)
Red cedar	16.0 ± 3.1(12)	0.95 ± 0.03(6)	1.00 ± 0.20(6)	7.0 ± 0.5(6)

Ten inbred and two outbred strains of adult male and female mice and male Sprague-Dawley rats (200 g) were used. Sleeping times were measured by stopwatch from the time of intraperitoneal administration of sodium hexobarbital (125 mg/kg for mice and 200 mg/kg for rats) to the time of restoration of the righting response. At the time that the righting response was restored, the animals were killed, and the brains and livers were removed.

The hexobarbital content of the brain was determined by extraction of the drug from brain homogenate into spectrally pure heptane containing 1.5 percent isoamyl alcohol according to a modification of the method of Cooper and Brodie (*2*). Next, the heptane was washed with critic acid buffer *p*H 5.5 to remove metabolites of hexobarbital. The hexobarbital in the heptane was then extracted into 0.8*M* phosphate buffer *p*H 11.0. Differences between readings at wavelngths 245 and 260 mμ in a Beckman DU spectrophotometer were related to readings at these wavelengths of a standard solution of hexobarbital. When the brain is homogenized in phosphate buffer *p*H 8 and the above procedure is followed, the blanks are close to zero. Recovery of a known quantity of hexobarbital added to the brain homogenate from a control mouse is approximately 90 percent.

The hexobarbital-metabolizing activity of the supernatant prepared from a 20 percent homogenate of liver centrifuged at 9000*g* was assayed as previously described (*2*). This supernatant (2 ml) was incubated for 15 minutes at 37°C with sodium hexobarbital (1 μmole), glucose-6-phosphate (25 μmole), nicotinamide-adenine dinucleotide phosphate (1.25 μmole), and $MgCl_2$ (50 μmole) in 3 ml of 0.2*M* phosphate buffer *p*H 7.5 to give a final volume of 5.0 ml. The amount of hexobarbital remaining after a 15-minute incubation was determined by the procedure described above. Similar studies were performed on the microsomal fraction prepared by centrifuging this supernatant for 60 minutes at 105,000*g*.

Twenty-four hours after NIH mice were placed on red-cedar bedding, their sleeping times diminished by one-third, and the activity of their hexobarbital-metabolizing enzymes in liver supernatant (centrifuged at 9000*g*) increased correspondingly. By 48 hours, the sleeping time had decreased by 66 percent of initial values, and the liver enzyme activity increased correspondingly (Fig. 1). Concentrations of sodium hexobarbital in the brain on these 3 days were similar: 36 ± 5, 39 ± 6, and 35 ± 5 μg per gram of brain, respectively. Alterations in sleeping time and enzyme activity were reversed when a combination of beech, birch, and maple bedding replaced the red-cedar shavings after 3 days (Fig. 1).

A further decline in sleeping time and increase in enzyme activity occurred after 1 day on the hardwood bedding (Fig. 1), but subsequently the values gradually returned to those existing prior to exposure of the mice to red-cedar bedding. Similar depression of sleeping time and elevation in the enzyme activity of the supernatant (centrifuged at 9000*g*) and microsomal fraction occurred in other strains of mice and in Sprague-Dawley rats when these animals were put on red-cedar, white-pine, or ponderosa-pine bedding. Two other drug-metabolizing enzymes, ethyl morphine *N*-demethylase (*3*) and aniline hydroxylase (*4*), were elevated, both in the supernatant (centrifuged at 9000*g*) and the microsomal fraction, in mice on red-cedar or white-pine bedding, as compared to their activities in mice on a mixture of beech, birch, and maple bedding (Table 1).

In an attempt to isolate the substance or substances responsible for

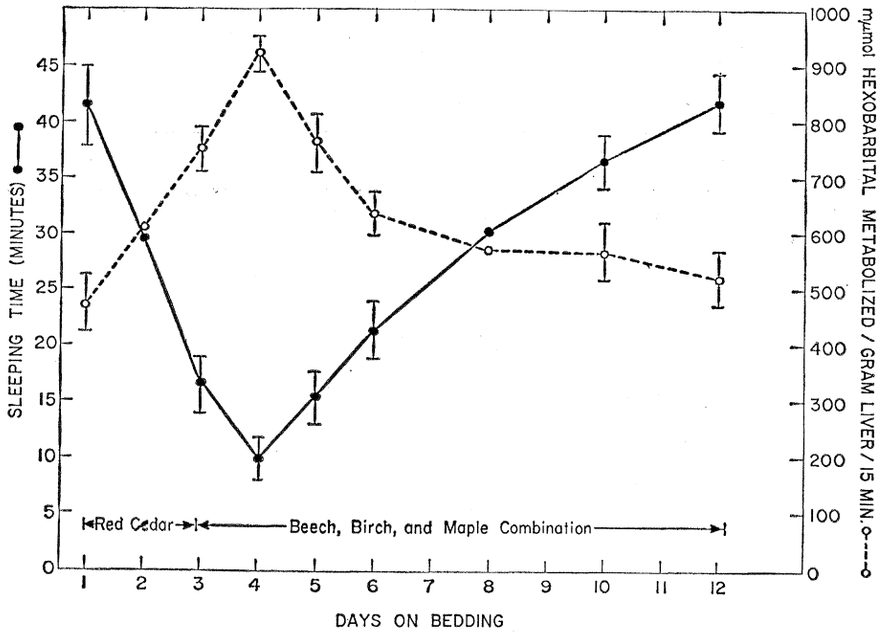

Fig. 1. Each point represents sleeping times (with standard deviations) of ten NIH male mice and hexobarbital-metabolizing activity of their 9000*g* liver supernatant. Standard deviations on days 2 and 8 are similar to those on other days. Mice were on red-cedar bedding for 3 days and then on a combination of beech, birch, and maple bedding for 9 subsequent days.

the induction of these drug-metabolizing enzymes, the red-cedar shavings were extracted in hexane for 2 hours at 25°C and for 30 minutes at 61°C. This treatment removed much of the inducing material, as indicated by the fact that mice kept on the dried, hexane-extracted, red-cedar bedding for 7 days exhibited only a 25 percent decrease in their sleeping times, as compared to an 80 percent decrease exhibited by mice kept on untreated red-cedar bedding. Both red-cedar heartwood and sapwood contain the inducing substance or substances. It is not clear whether induction in mice and rats follows ingestion or inhalation of these compounds.

These experiments offer an explanation for differences in the results of studies on drug-metabolizing enzymes in mice and rats. Animals kept on red-cedar or on white- or ponderosa-pine bedding exhibit higher activities of several microsomal drug-metabolizing enzymes than those kept on beech, birch, or maple bedding do. While the sleeping times are decreasing and the microsomal enzyme activity is increasing, the amounts of hexobarbital in the brain on awakening remain unaltered in mice put on softwood bedding; thus, the responsiveness of the receptor sites seems unaffected by softwood bedding.

It has been generally assumed that the activities of drug-metabolizing enzymes depend on the genotype of an animal, to which species and strain differences in these activities have been attributed (*5*). My experiments indicate that the concentrations of drug-metabolizing enzymes can be significantly affected by the environment of an animal, particularly by the presence of inducing substances in its natural habitat. These observations may be useful in investigating the evolution of drug-metabolizing enzymes in vertebrates and their induction in experimental animals kept on various types of wood bedding.

ELLIOT S. VESELL
Section on Pharmacogenetics,
Laboratory of Chemical Pharmacology,
National Institutes of Health,
Bethesda, Maryland 20014

References and Notes

1. H. C. Ferguson, *J. Pharm. Sci.* **55**, 1142 (1966).
2. J. R. Cooper and B. B. Brodie, *J. Pharmacol. Exp. Therap.* **114**, 409 (1955).
3. I. Nash, *Biochem. J.* **55**, 416 (1953).
4. R. Kato and J. R. Gillette, *J. Pharmacol.* **150**, 279 (1965).
5. G. E. Jay, Jr., *Proc. Soc. Exp. Biol. Med.* **90**, 378 (1955); A. McLaren and D. Michie, *J. Genet.* **54**, 440 (1956); G. P. Quinn, J. Axelrod, B. B. Brodie, *Biochem. Pharmacol.* **1**, 152 (1958).
6. I thank Wallace W. Holland for technical assistance, and B. B. Brodie, J. R. Gillette, and J. L. Holtzman for advice. Sodium hexobarbital was provided by Sterling-Winthrop, Rensselaer, N.Y.

14 July 1967

Bromophenols from Red Algae

Abstract. *3,5-Dibromo-*p*-hydroxybenzyl alcohol is reported as a natural constituent of* Odonthalia dentata *and* Rhodomela confervoides. *The amounts isolated, based on the fresh weight of the tissue, were 0.024 and 0.003 percent, respectively. A major phenolic compound in both algae was 2,3-dibromo-4,5-dihydroxybenzyl alcohol.*

Bromine was early recognized as an algal constituent (*1*), and by the 1920's many algae were known to concentrate it (*2*). This fact was exploited during World War II when bromine was extracted from *Rhodomela larix* (*3*).

The chemical nature of the accumulated bromine has been the subject of some dispute. Sauvageau (*4*) concluded that free bromine existed in specialized cells, "les bromuques," while Kylin (*2*) disagreed with the evidence presented. Later experiments on *Polysiphonia fastigiata* (known also as *P. lanosa*) provided unequivocal evidence for covalently bound bromine in algae (*5*). The occurrence and distribution of $FeCl_3$-reactive bromophenols in the Rhodomelaceae have been summarized (*6*).

The identification of these compounds remained a problem until Saito and Ando (*7*) demonstrated 5-bromo-3,4-dihydroxybenzaldehyde in *Polysiphonia morrowii*. The principal phenol of *P. lanosa* has been characterized as 2,3-dibromobenzyl alcohol-4,5-disulfate, dipotassium salt (*8*). Katsui *et al.* (*9*) have reported 2,3-dibromo-4,5-dihydroxybenzaldehyde (5,6-dibromoprotocatechualdehyde) and 2,3-dibromo-4,5-dihydroxybenzyl methyl ether in extracts of *Rhodomela larix*, while Matsumoto and Kagawa observed the same bromobenzylmethyl ether in *Odonthalia corymbifera* (*9*). Other brominated compounds are a sesquiterpenoid (laurenterol) from *Laurencia intermedia* (*10*), and an acetylenic, heterocyclic compound, laurencin, from *L. glandulifera* (*10*). We now describe the isolation and identification of a new natural product, 3,5-dibromo-*p*-hydroxybenzyl alcohol, from both *Odonthalia dentata* (L.) Lyngbye, and *Rhodomela confervoides* (Hudson) Silva (*11*).

After sorting and blotting the *Odonthalia*, 1 kg (fresh weight) was extracted twice with 8 liters of boiling 80-percent ethanol. The alcohol was evaporated (at reduced pressure), and the