ygen minimum of the eastern tropical Pacific have additional, perhaps heretofore undescribed, means of meeting their energy requirements in that comparatively vast area of vanishingly low concentrations of dissolved oxygen.

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2. Our observations are based on data gathered on Naval Undersea Center cruises *Minox* I (July 1970), *Minox* II (March 1972), and *Minox* III (October 1973). Some of the major findings from the *Minox* cruises and the general rationale for the *Minox* research program are described by W. A. Friedl, G. V. Pickwell, and R. J. Vent [in *Proceedings of a Symposium on the Prediction of Sound Scattering in the Oceans from Physical/Chemical/Biological Information*, N. R. Andersen, Ed. (Plenum, New York, in press)]. The low-oxygen area covered by our stations extends roughly from 13°N to 22°N, and from 100°W to 122°W. Seawater was collected in 8-liter polyvinyl chloride (Niskin) bottles. Samples for dissolved oxygen analysis were withdrawn anaerobically through rubber septa into 100-ml syringes. Fixing reagents were injected through additional rubber septa into the syringes, and we carried out an iodometric titration with a microburette, following the modified Winkler procedure of the Chesapeake Bay Institute [J. H. Carpenter, *Limnol. Oceanogr.* **10**, 141 (1965)]. Typically, dissolved oxygen concentrations at the depths of the oxygen minimum ranged from 0.08 to 0.02 ml/liter (equivalent to an oxygen tension of approximately 1.8 to 0.4 torr). Occasionally dissolved oxygen in our 100-ml samples was undetectable by this technique. In this region J. D. Cline and F. A. Richards [*Limnol. Oceanogr.* **17**, 885 (1972)] reported values for dissolved oxygen measured by means of a photometric technique consistently in the vicinity of 0.02 ml/liter. At DSL depths (400 m) temperatures ranged from 6.5° to 8°C and salinity averaged 34.5 per mil, whereas at the surface temperatures ranged from 18.5°C (Station Westfall, Table 1) to 28°C at the southernmost station, with corresponding surface salinities of 34.1 per mil and 32.6 to 33.5 per mil, respectively, depending on the season.
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4. Swim bladder oxygen values from a series of 15 oxygen-minimum fishes (five species) collected on *Minox* II during daytime (descended scattering layers) ranged from 57 to 88 percent. Values to 92 percent were obtained from additional specimens collected from ascended scattering layers at night [A. O. Valkirs, in *Scientific Results of the* Minox *Program*, G. V. Pickwell, Ed. (Technical Report, Naval Undersea Center, San Diego, in press)]. J. Kinzer [*Umsch. Wiss. Tech.* **22**, 733 (1967)] found myctophids and gonostomatids associated with the DSL in oxygen-depleted waters (0.08 to 0.04 ml/liter) of the eastern Arabian Sea and wondered what physiological mechanisms permitted such sojourns.
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7. Net depth was monitored throughout the haul, and the length of the towing cable was adjusted to maintain the net within the desired depth interval. Most diurnal hauls through the DSL sampled a stratum 45 to 95 m thick; nocturnal hauls in near-surface scattering layers seldom ranged over more than 50 m. Thirty-eight hauls made in the region of extremely low oxygen content were open an average of 57 ± 12 minutes, had an average velocity while open of 1.2 ± 0.1 m/sec, and filtered an average volume of 16,000 ± 3,800 m^3.
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13. For useful recent reviews, see A. Riggs, in *Fish Physiology*, W. S. Hoar and D. J. Randall, Eds. (Academic Press, New York, 1970), vol. 4, pp. 209–252; D. J. Randall, *ibid.*, pp. 253–292; K. Johansen, *ibid.*, pp. 361–411; ——— and C. Lenfant, in *Oxygen Affinity of Hemoglobin and the Red Cell Acid-Base Status*, M. Rorth and P. Astrup, Eds. (Munksgaard, Copenhagen, 1972), pp. 750–783; G. C. Grigg, in *Chemical Zoology: Deuterostomians, Cyclostomes, and Fishes*, M. Florkin and B. T. Scheer, Eds. (Academic Press, New York, 1974), vol. 8, pp. 331–368. Perhaps some of the specimens taken had some blood lactic acid from struggling in the net. Although the buffering capabilities of fish blood may be thought too limited to effectively maintain normal *p*H under this circumstance, there are as yet no more than about 20 fish species (marine and freshwater) in which the Root effect has been demonstrated. Hematocrit determinations made on two species gave low values (Table 1), and it thus seems unlikely that our low capacities or affinities were the result of lactic acid. By maintaining the equilibrating blood samples at 0°C and consuming only 30 seconds to transfer a blood sample to the analyzer syringe, we overcame any possible difficulties arising from red blood cell respiration.
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Epoxide to Olefin: A Novel Biotransformation in the Rumen

Abstract. *Studies with an insect juvenile hormone mimic and the insecticide dieldrin have shown that enzymatic processes in the rumen reduce the epoxide moiety in these compounds to an olefin. This reaction is apparently microbial in origin and does not involve an observable intermediate. Epoxide reductions in the digestive tract of ruminants and possibly other mammals may be important in the detoxication of biologically active epoxides, including pesticides, alkylating agents, and carcinogens.*

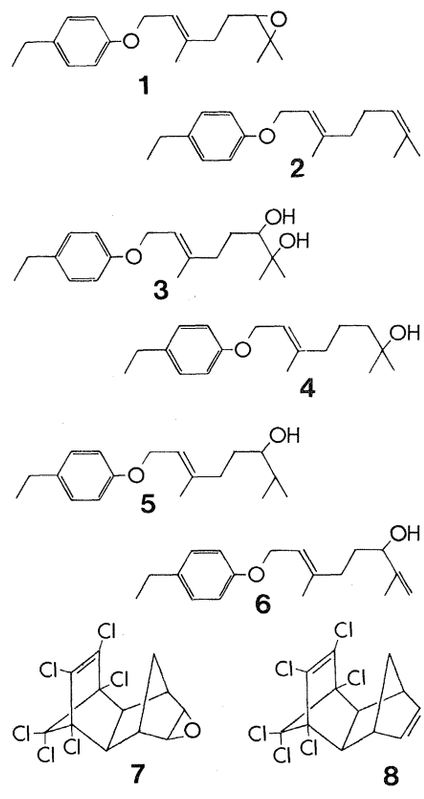
Enzymatic detoxication reactions protect mammals against the potentially toxic effects of foreign compounds to which they are almost continuously exposed. Oxidative, reductive, hydrolytic, and conjugative reactions transform the compounds to metabolites that usually have reduced biological activity or are more readily excreted from the body. Although most detoxications occur in the liver after absorption, it is advantageous to the organism if detoxication occurs before absorption, that is, in the case of oral exposure while the compounds are still in the alimentary canal. The epoxide moiety, whose presence in organic compounds often confers high biological activity (*1*), is reduced in the rumen in appreciable quantity to an olefin, a reaction which may represent a significant detoxication mechanism.

Studies on the metabolic behavior in steers of the insect juvenile hormone mimic 1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene (**1**) (Stauffer R-20458), labeled with ^{14}C in the phenyl ring, indicated that the compound was totally

metabolized and the label was excreted almost quantitatively in the urine and feces after oral treatment at 0.05 mg/kg (*2*). Analysis of the excreta revealed numerous metabolites, and in the feces one product behaved the same on thin-layer chromatography (TLC) as the diene analog **2**. The possibility that **2** was metabolically derived from **1** was intriguing because in vivo reductions of epoxide to olefin have not been reported in higher animals. However, **2** was a trace contaminant (less than 1.0 percent) in the ^{14}C-labeled preparation of **1** given the steer; thus the possibility that **2** had passed unabsorbed and unmetabolized through the digestive tract could not be totally discounted. This did not seem likely because **2** is highly lipophilic and should be readily absorbed through the gut wall and it undergoes rapid metabolism in other mammalian enzyme systems (*3*). Additional studies were therefore undertaken in an attempt to confirm these findings.

Both ^{14}C-labeled and unlabeled samples of **1** were purified by TLC to remove all traces of **2**. The resulting preparations contained < 0.1 percent of **2** as determined by gas-liquid chromatography (GLC) of the unlabeled preparation and TLC of the labeled compound, followed by radioautography and liquid scintillation counting (*4*). A 250-kg steer was given orally 20 g of the purified **1**, which was mixed with radioactive **1** as a tracer. Analysis of the excreta collected during the first 3 days after treatment revealed that, along with numerous other metabolites, 1.3 percent of the total radioactive carbon given the animal was recovered in the feces as the diene **2**. The isolated fecal metabolite gave essentially identical nuclear magnetic resonance (NMR) and GLC–mass spectral data as authentic **2** from synthesis (*5*); in addition, treatment of the product with one equivalent of *m*-chloroperoxybenzoic acid regenerated the epoxide and gave a product identical to **1** in TLC and GLC–mass spectral behavior. These findings were conclusive evidence that **1** was indeed metabolized to **2** in the steer, and it seemed likely that the transformation occurred within the digestive tract because no trace of **2** was detected in the urine of the treated animal.

Results from subsequent studies in vitro with ovine digestive tract fluids confirmed that **2** was generated within the alimentary canal. Fluids from the rumen, abomasum, and small intestine of freshly killed sheep were filtered through cheesecloth and were incubated under carbon dioxide with ^{14}C-labeled **1** at $1 \times 10^{-5}M$. After 3 hours of incubation at 39°C, the samples were extracted with ethyl acetate, and the extracts were analyzed by TLC. In the rumen samples, 36 percent of the starting epoxide **1** was converted to the diene **2**, but there was no similar reduction in abomasum or small intestine fluids. The conversion of **1** to **2** by the rumen fluid was clearly an enzymatic reaction because essentially no **2** was formed during the incubation of **1** with rumen fluid in which the enzyme activity had been destroyed by boiling. Incubation of **1** with rumen fluid at a higher concentration ($5 \times 10^{-4}M$) yielded sufficient **2** to permit isolation on a milligram scale and subsequent confirmation of structure by NMR, GLC–mass spectroscopy, and the formation of derivatives.



Studies with the chlorinated cyclodiene insecticide dieldrin (**7**) established that reductions of epoxide to olefin in the rumen are not limited to the epoxyterpenoid type of insect juvenile hormone mimics. Incubation of [^{14}C]dieldrin at $1 \times 10^{-5}M$ with ovine rumen fluid for 18 hours resulted in 5.2 percent conversion to the olefin aldrin (**8**). The metabolite was confirmed as aldrin by GLC–mass spectroscopy and by peracid oxidation back to dieldrin as confirmed by GLC–mass spectroscopy. As was expected, incubation of dieldrin with heat-deactivated rumen fluid yielded no detectable aldrin residues.

Because the rumen fluid incubations with **1** gave several metabolites in addition to the diene **2**, the possibility that one of these might be an intermediate in the conversion of epoxide to olefin was considered. The individual metabolites were isolated by TLC and again incubated with rumen fluid. None were converted to **2**, suggesting either that the epoxide reduction did not involve a stable intermediate or that the unidentified intermediate was rapidly converted to the olefin. Studies with the alcohols **3**, **4**, **5**, and **6** demonstrated that none of these compounds are intermediate in the reduction of **1** to **2** in the rumen. The alcohols were prepared in both radioactive and nonradioactive form by reported procedures (*6*), and the labeled alcohols were incubated with rumen fluid as described above for **1**. In addition, $1 \times 10^{-5}M$ concentrations of labeled **1** were incubated with rumen fluid in the presence of $1 \times 10^{-3}M$ concentrations of the appropriate unlabeled alcohol in an attempt to observe the potential hydroxy intermediate by the isotope trapping technique (*7*). Subsequent TLC analysis showed that none of the four labeled alcohols were converted to the diene **2** in significant amounts, and the isotope trapping studies likewise supported the conclusion that the epoxide reduction did not involve a hydroxy intermediate. The experiments with dieldrin support this conclusion also because no metabolites other than aldrin were detected in the sample extracts. The data thus suggest that the transformations of epoxide to olefin in the rumen do not proceed through a stable intermediate. No other information is available at this time concerning the mechanisms involved in this biotransformation.

Some epoxides are reduced to olefins by the microsomal fraction of mammalian liver (*8*), but clearly there is no hepatic involvement in the transformations reported here. Microbial reduction of some 3-keto-4,5-epoxy steroids to 3-keto-1,4-diene derivatives has been reported (*9*). This, and the fact that the rumen is rich in microbial activity, strongly suggests that the conversions of epoxide to olefin observed in my study are microbial in origin. However, initial attempts to culture rumen organisms capable of catalyzing this reaction have not been successful (*10*).

These studies establish only that conversions of epoxide to olefin occur in the rumen, a highly reductive environment (*11*), but such reactions may also occur in the digestive tract of other mammals. The diene **2** and related compounds have been observed by Hoffman *et al.* in the feces of rats treated intraperitoneally with very high doses of **1** (*12*). However, Hoffman *et al.* speculated that these products arose from **2** present as an impurity in the treatment preparation, and thus were not true metabolites of **1**. Because my studies show that conversions of epoxide to olefin do occur in the digestive tract of at least some

mammals, it seems possible that the diene analogs of **1** observed in the rat arose through biliary or intestinal (*13*) excretion of **1** or its metabolites (or both) from the intraperitoneal administration, followed by epoxide reduction in the intestine. Conversions of epoxide to olefin within the digestive tract may thus represent a significant metabolic pathway in mammals for potentially toxic epoxides, which include alkylating agents, carcinogens, and some pesticides. In the case of dieldrin, epoxide reduction is not a detoxication because the aldrin produced is itself toxic and its major metabolic transformation is epoxidation by liver oxidases back to dieldrin (*14*). However, reduction of the epoxide moiety in other compounds can be expected to diminish biological activity in cases where the olefin is not readily reepoxidized, or by allowing sufficient time for additional biodegradation to occur before reepoxidation. It also seems likely that reductions of epoxide to olefin in the digestive tract may function nutritionally in the reduction of oxidized foodstuffs such as fatty acid epoxides and cutin acid epoxides.

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Antagonism of Stimulation-Produced Analgesia by Naloxone, a Narcotic Antagonist

Abstract. *Analgesia produced by focal electrical stimulation of the brain is partially reversed by the narcotic antagonist naloxone. The absence of complete reversal does not appear to be caused by inadequate doses of naloxone since doses higher than 1 milligram per kilogram of body weight did not increase the antagonism. It is suggested that stimulation-produced analgesia may result, at least in part, from release of an endogenous, narcotic-like substance, such as that recently reported by other investigators.*

Focal electrical stimulation of the brain produces analgesia in the rat, the cat, and in man (*1–4*). This stimulation-produced analgesia exhibits several striking features in parallel with the analgesia produced by narcotic drugs. Both appear to exert their effects at sites surrounding the third ventricle, the cerebral aqueduct, and rostral portions of the fourth ventricle (*2, 5, 6*). Drugs that affect transmission in central monoamine pathways alter both morphine analgesia and stimulation-produced analgesia (*7, 8*). Morevoer, tolerance develops to the analgesic effect of brain stimulation and cross-tolerance between morphine and brain stimulation occurs (*9*). We report now that stimulation-produced analgesia can be partially blocked by the narcotic antagonist naloxone. This observation has important implications for the neural mechanisms of pain inhibition. A preliminary report of some of these findings has been made (*10*).

Forty-one male Sprague-Dawley rats were used. A single bipolar electrode constructed of twisted stainless steel wire (0.2 mm in diameter), Teflon-coated except at the cut cross section of the tips, was implanted in the periaqueductal gray matter, an area known to yield particularly potent and reliable analgesia (*1, 2, 11*). Analgesia was measured in a modified version of the D'Amour and Smith tail-flick test (*12*), in which one records the latency of the spinally mediated withdrawal reflex of the tail in response to the application of radiant heat. The apparatus and procedure have been described (*7*). The radiant heat source was adjusted to produce a baseline latency (BL) of 3.5 to 4.5 seconds. Following brain stimulation, if the animal did not respond within 7.0 seconds, the heat was automatically shut off in order to prevent tissue damage. The degree of analgesia (DA) due to brain stimulation was expressed as percentage and derived from the ratio of actual change in response time (T) from baseline to the maximum possible change according to a formula adapted from Benson *et al.* (*13*)

$$DA = 100\,(T - BL)/(7 - BL)$$

Following recovery from surgery, animals were screened to determine appropriate parameters of brain stimulation. Trains of 60-hertz sine-wave current 100 msec in duration were delivered at a rate of 3 per second for 20 seconds. For each animal, two current intensities were determined, one producing an intermediate degree of analgesia (30 to 60 percent), the other yielding total analgesia (100 percent). During screening, current intensity was raised in steps of 10 μa (peak-to-peak) until a 100 percent DA was observed. Current intensities above 200 μa were not employed. Once determined, current intensity was held constant throughout the experiment. Only animals exhibiting minimal motor and sensory side effects during stimulation were employed in these studies. In each test session, a BL was obtained by averaging three trials separated by 2-minute intervals. Analgesia was then assessed in three additional trials each immediately preceded by 20 seconds of brain stimulation. Since analgesia usually outlasted the period of brain stimulation, sufficient time was allotted between stimulation trials to permit pain responsiveness to return to prestimulation levels (normal BL).

For all experiments, animals were given three testing sessions, each separated by 2 days. These are referred to as predrug, drug, and postdrug sessions. In the predrug session, animals were injected with a matched volume of the naloxone vehicle (0.9 percent saline) and were tested 20 minutes later to obtain a BL score, then a DA score after brain stimulation. The drug session was identical except that animals received naloxone instead of vehicle. The postdrug session was identical to the predrug session.

In an initial experiment, nine animals were stimulated at an intensity yielding 100 percent DA. As shown in Fig. 1A, the predrug vehicle control did not affect analgesia. However, naloxone (1 mg per kilogram of body weight) reduced DA to a mean of 62 percent. Stimulation-produced analgesia was affected by naloxone in seven of the nine animals; and, in these, DA scores under the drug ranged between 7 and 89 percent. The DA was significantly higher in the postdrug session than in the drug session ($P < .05$, one-tailed t-test). In