results suggest that the species of fish represented by group A lived at or near the bottom of the water column, whereas those of group B lived above the bottom for at least part of their lives. From foraminiferal and molluscan paleoecological evidence, the depth of deposition of this sediment is estimated to be about 500 m.

Extension of the oxygen isotope technique to the study of fish ecology has several possibilities. Valuable information on fish migration may be forthcoming if oxygen isotope temperatures differ from the water temperature where the fish are collected. Small differences may reflect seasonal movement to waters of different latitude or depth.

Calcium carbonate laid down in equilibrium with fresh water has a markedly different oxygen isotope from that laid down in equilibrium with sea water. This difference is reflected in anomalously high isotopic temperatures. Anomalous results obtained from a fish's otolith may indicate that migration into rivers or estuaries is an important phase in the life-span of the fish.

Results of isotope measurements on otoliths may also be useful in determining the bottom temperature for studies in paleoecology. In deep-water sediments, benthonic fossil carbonate is often scarce. From results given in Table 2 it is reasonable to conclude that the lowest temperature obtained from a number of otoliths from a sediment sample is generally the bottom temperature. This has been demonstrated for sediments deposited at a depth of at least 500 m but may not necessarily be the case for sediments deposited at very great depths.

There is only a slight probability of a fish's remains being deposited in an area of bottom temperature warmer than that of its normal habitat. Of 40 otoliths from eight Tertiary sediments of different ages, not one recorded a temperature lower than the bottom temperature for the locality, as indicated by isotope measurements on other fossil forms.

IAN DEVEREUX
Geology Department, Victoria University, Wellington, New Zealand

References and Notes

1. H. C. Urey, H. A. Lowenstam, S. Epstein, C. R. McKinney, *Geol. Soc. Amer. Bull.* **62**, 399 (1951).
2. I. Devereux, in preparation.
3. The δO^{18} of the mass spectrometer CO_2 standard has been determined to ± 0.05 by comparison with international standards. Duplicate runs on the same sample are normally (90 percent) within 0.10 on δO^{18}. The total analytical error on each single result is therefore estimated conservatively at ± 0.2. Results given in the tables have been rounded off to the nearest 0.1.
4. S. Epstein, R. Buchsbaum, H. A. Lowenstam, H. C. Urey, *Geol. Soc. Amer. Bull.* **64**, 1315 (1953).
5. No major influx of fresh water occurs near the areas where the fish lived, and, although the oxygen isotope ratios of the waters have not been measured, salinity values indicated open ocean water; hence no corrections have been applied to the results.
6. I acknowledge the assistance given by J. Moreland, Dominion Museum, and L. Paul, Fisheries Research Laboratory, in selecting samples and supplying the ecological data necessary for this study.

14 November 1966

Flaxedil (Gallamine Triethiodide): Evidence for a Central Action

Abstract. *A central action of gallamine triethiodide has been demonstrated in cats with permanently implanted electrodes that permit direct repetitive stimulation and recording of afterdischarges from cerebral cortex. Systemically administered gallamine produced a consistent and reproducible augmentation of duration of afterdischarge at doses just sufficient to produce skeletal muscle paralysis. Simultaneous examination of expiratory carbon dioxide, blood pressure, body temperature, blood glucose, and direct cortical response to brief single stimuli failed to reveal any consistent peripheral change to which the centrally observed effect could be attributed.*

Gallamine triethiodide [1,2,3,-tri(2-diethylaminoethoxy) benzene triethiodide, Flaxedil] is a synthetic tubocurarine substitute first described by Bovet (*1*). It produces paralysis by blocking the action of acetylcholine at muscle end-plate receptors. Because of a belief that gallamine has no central action when administered in paralyzing doses, it is used, together with infiltration anesthesia, to immobilize animals for experiments involving the central nervous system. During such an experiment we noticed that afterdischarge obtained from the cerebral cortex of cats immobilized with gallamine was of much longer duration than that from comparable freely roving animals. We designed some experiments to determine the effect of gallamine on mechanisms of cortical afterdischarge.

Platinum electrodes held in a plastic plate were fixed over the pial surface of the suprasylvian gyrus of cats (2½ to 5 kg) of both sexes (*2*). Preparation of these animals for "acute" experimentation was done under ether anesthesia. Arterial, venous, and tracheal cannulae were fitted, and after primary closure all wounds were infiltrated with long-acting local anesthetic (Efocaine, Fougera). The animals were made com-

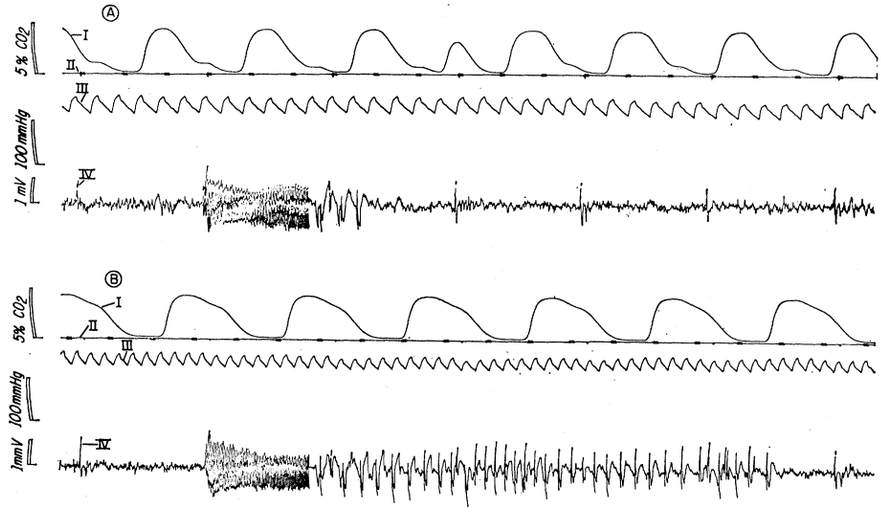

Fig. 1. (A) Afterdischarges at a threshold current of 1.5 ma are shown before the administration of any drug. *I*, Expiratory CO_2, calibration is 5 percent CO_2 (see spontaneous respirations); *II*, marks for one second; *III*, abdominal aortic blood pressure, calibration 0 to 100 mm-Hg; *IV*, electrocorticogram showing stimulus artifact (2½ seconds) and afterdischarge, calibration 1.0 mv. (B) The sequence and the stimuli are the same as in (A) except that (B) was obtained 15 minutes after intravenous administration of gallamine triethiodide (6.25 mg/kg). The animal was mechanically ventilated to near its level of carbon dioxide before administration of the drug, and the afterdischarge was of longer duration.

fortable in a Pavlov stand, and the experiments were started about 2 hours later, when fast activity induced by ether had disappeared from the electrocorticograms.

An experiment was terminated if the abdominal aortic blood pressure fell below 100 mm-Hg diastolic, or if deep muscle temperature could not be kept above 37.5°C by moderate heating. Carbon dioxide (CO_2) was continuously monitored in the physiological dead space. Ventilation of paralyzed animals was adjusted to match their expiratory CO_2 levels with those previously obtained under conditions of quiet spontaneous respiration. Arterial blood glucose was sampled every 3 hours by a glucose oxidase colorimetric test (Destrostix, Ames) and was consistently above 140 mg per 100 ml of blood.

All stimuli, except the 10-minute intervals between afterdischarges, were timed by a crystal-controlled digital clock that assured reproducibility of conditions to 0.01 percent in time. Every 3 seconds, a single stimulus was delivered to the cortex through the implanted electrodes. At 10-minute intervals, synchronous with the 3-second periods, a 30-hertz burst lasting 2½ seconds was delivered in the same manner. All stimuli were current-regulated pulses (0.5-msec) monitored with a Tektronix current probe. The direct cortical response, picked up by another pair of implanted electrodes, was amplified and displayed on an inkwriter and oscilloscope.

The intensity of the stimulating current was increased until an afterdischarge of 1 to 4 seconds was consistently evoked from cortex by each stimulation (2½ seconds). This intensity of current was held constant for the rest of the experiment. After a 1-hour control period when stable durations of afterdischarges were obtained, gallamine (6.25 mg/kg) was administered intravenously. The pinna reflex was tested 1 minute prior to each 10-minute epoch to assess the state of paralysis. At the end of the experiment the animal was given an overdose of pentobarbital. When examined, the cortex underlying the electrodes had a normal appearance in all cases.

A control afterdischarge is shown in Fig. 1A. An afterdischarge recorded after intravenous administration of gallamine (6.25 mg/kg) is shown in Fig. 1B. Durations of the afterdischarges were measured from the end of the stimulus artifact to the cessation of epileptiform activity.

The durations of 75 afterdischarges from three animals in relation to the time of administration of the drug are illustrated in Fig. 2. The increase in duration after administration of gallamine is significant to the 0.1 percent level [U test, Mann-Whitney one-tailed (*3*)].

Figure 3 shows the results from a single animal before and after administration of gallamine. Although paralysis occurred as soon as the compound was administered, changes in duration of afterdischarge were not observed until 10 to 20 minutes later. In this animal, afterdischarge persisted for 80 minutes after initial recovery from paralysis. Recovery from paralysis always preceded the return of the durations of afterdischarge to near the control values. The same dose of gallamine was again administered when the durations of afterdischarge had shortened to near the control values. A second and similar increase in durations of afterdischarge resulted.

Direct cortical responses (DCR) were evoked at 3-second intervals throughout the experiment and photographed. At the duration of afterdischarge (Fig. 1A and 1B), the DCR showed no remarkable changes. The normal appearance of DCR's throughout the experiment confirm the physiological state of the cortex. Longer duration of afterdischarge and generalized electrographic seizures, which occurred only in gallamine-immobilized animals, were accompanied by DCR changes.

In other animals, gallamine prolonged the duration of afterdischarge evoked at threshold from surgically isolated cortex. The average duration of 200 afterdischarges from freely roving animals was 4 ± 2 seconds. Similar preparations, when immobilized with gallamine, produced (in response to stimulation) afterdischarges of exceptionally long duration, 180 ± 30 seconds.

Central actions resulting from systemic administration of the drug have not been described. Furthermore, central actions of the drug have been considered unlikely because gallamine is a quaternary ammonium compound and the blood-brain barrier is known to be relatively impermeable to these compounds.

Central actions of gallamine have not been readily apparent because the situations in which the agent is used conceal central effects or disguise them as unexplained variables. When used as a skeletal muscle relaxant, gallamine is either administered on a regular time schedule or as required for immobilization. For example, gallamine is used as a skeletal muscle relaxant in clinical medicine but is always administered with a potent anesthetic agent. Profound depression (of the central nervous system) induced by the anesthetic agent conceals any central effect of gallamine. In animal experiments where gallamine is used to prevent spontaneous movement, data are not usually collected until some variable time after

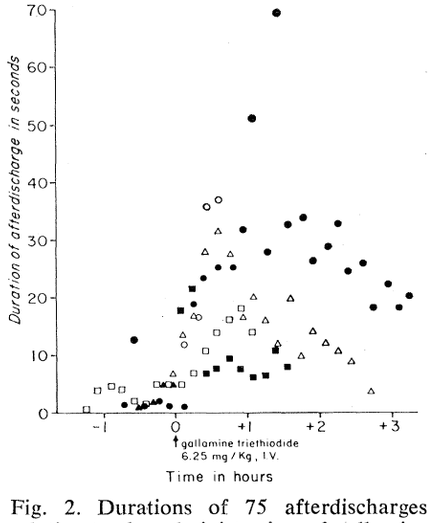

Fig. 2. Durations of 75 afterdischarges relative to the administration of gallamine triethiodide (Flaxedil) (6.25 mg/kg) intravenously at time zero. Each symbol designates a single experiment.

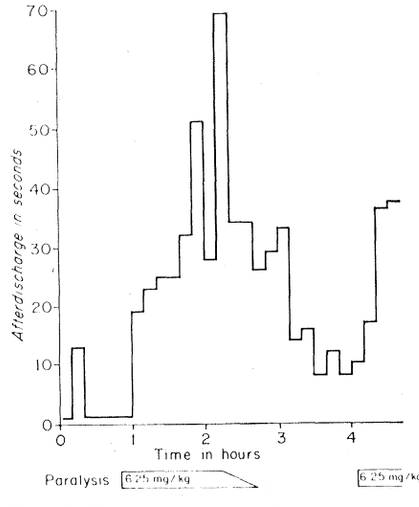

Fig. 3. Data from a single animal show the delay and persistence of the central effects of gallamine triethiodide (6.25 mg/kg) administered intravenously, with reference to the duration of the blockade of neuromuscular junction. Reproducibility of the effect is demonstrated by a second administration of the drug.

administration of the drug. In both cases, as shown by the time course of the central action of the drug, a significant and variable alteration of cortical function has probably taken place.

Unrecognized central actions of gallamine may be responsible for inconsistent or conflicting results from different laboratories. The significance of gallamine-induced alteration of central function is illustrated by a controversy concerning the reproducibility of duration of afterdischarge. Rosenbleuth and Cannon (*4*) and others (*5*) stated that if intensity, frequency, and duration of electrical stimuli to cortex were constant, constant durations of cortical afterdischarge were obtained. Other workers found variability even with constant stimulating parameters (*6*, *7*). To investigate the sources of this variability, Straw and Mitchell (*7*) considered species difference, region of cortex stimulated, intertrial interval, and presence of anesthesia. Many workers have not considered the use of gallamine as a possible variable in their experimental design. Using "chronically" implanted electrodes, we demonstrated the effect of this variable.

The action of gallamine in prolonging the duration of afterdischarge from both neurologically isolated and intact cortex is evidence for its direct central action. Peripheral action was probably not responsible for the central effect, in view of the fact that no change in any of the physiological parameters monitored could be related to the prolonged durations of afterdischarge observed with the use of gallamine. A slight to moderate tachycardia with no remarkable changes in aortic pressure was present (*8*) prior to the central effect but had disappeared while the central effect was still present. The acute cardiovascular changes with rapid administration of gallamine were avoided by slow administration of the compound over a 2-minute period. No central effects were noted during or immediately after administration of the drug. Changes in durations of afterdischarge did not become apparent until 10 to 15 minutes later. The effect increases to a maximum in ½ to 1 hour and may outlast recovery from paralysis by an hour.

Reproducibility of the effect in a single animal shows that the central action is reversible to a large degree and is related to the time of drug administration. Repeated heavy doses of gallamine (12.5 mg/kg) intravenously administered to another animal resulted in an afterdischarge which developed into a generalized electrographic seizure lasting 6 minutes. Thus, the central action of gallamine may be cumulative, as it is peripherally (*8*), producing instability of central mechanisms.

The fact that gallamine does have a direct central effect must now be taken into consideration in the interpretation of data from experiments with this agent. In addition, the use of this agent in humans predisposed to convulsive disorders should be reevaluated.

LAWRENCE M. HALPERN
RICHARD G. BLACK
Departments of Pharmacology and Neurological Surgery, University of Washington School of Medicine, Seattle 98105

References and Notes

1. D. Bovet, F. Depierre, S. Courvoisier, Y. de Lestange, *Arch. Int. Pharmacodyn.* **80**, 172 (1949).
2. S. K. Sharpless and L. M. Halpern, *Electroencephalogr. Clin. Neurophysiol.* **14**, 244 (1962).
3. S. Siegel, *Non-Parametric Statistics for Behavioral Sciences* (McGraw-Hill, New York, 1956), p. 312.
4. A. Rosenbleuth and W. Cannon, *Amer. J. Physiol.* **135**, 690 (1942).
5. W. Schalleck and A. Kuehn, *Proc. Soc. Exp. Biol.* **112**, 813 (1963); C. Bernhard and E. Bohm., *Brit. J. Pharmacol.* **10**, 288 (1955); E. Vastola and A. Rosen, *Electroencephalogr. Clin. Neurophysiol.* **12**, 327 (1960).
6. C. A. Berry, *Arch. Int. Pharmacodyn.* **145**, 197 (1965).
7. R. N. Straw and C. L. Mitchell, *Electroencephalogr. Clin. Neurophysiol.* **21**, 54 (1966).
8. B. E. Marbury, F. A. Artusio, Jr., W. C. Wescoe, W. F. Reiker, Jr., *J. Pharmacol. Exp. Therap.* **103**, 280 (1951).
9. We thank C. Bibbins and W. Johns for their expert technical assistance. This investigation was supported by research grant MH 12426-01 from NIMH and by research grant NB-4053 from NINDB.

28 November 1966; 31 January 1967

Ant Stridulations and Their Synchronization with Abdominal Movement

Abstract. *Two techniques for accurately correlating movements with vibrations produced by* Pogonomyrmex occidentalis *show three characteristics of the stridulations: alternation of mean chirp intensity, higher frequencies during the upstroke, and interruption of chirps into distinct pulses. These pulses are produced by briefly stopping the gaster during a chirp.*

Ants of the subfamily Myrmicinae stridulate by raising and lowering the gaster (posterior part of the abdomen) causing a series of ridges on the medial dorsal area of the gaster to rub against a scraper near the border of the preceding segment (postpetiole). Ants are much more sensitive to substrate vibrations than to aerial vibrations; and any transfer of vibrations from a stridulating ant to a receiving ant is almost certainly done by passing the "message" through a substrate (*1*). The only demonstrated function of the vibrations is that they attract other workers to the stridulating ant which, under experimental conditions, has always been trapped (*2*). Under natural conditions, ants must often be trapped by cave-ins and predators.

In that analysis of sound spectrograms showed that there are distinct differences in intensity and construction between the stridulation (or chirp) pro-

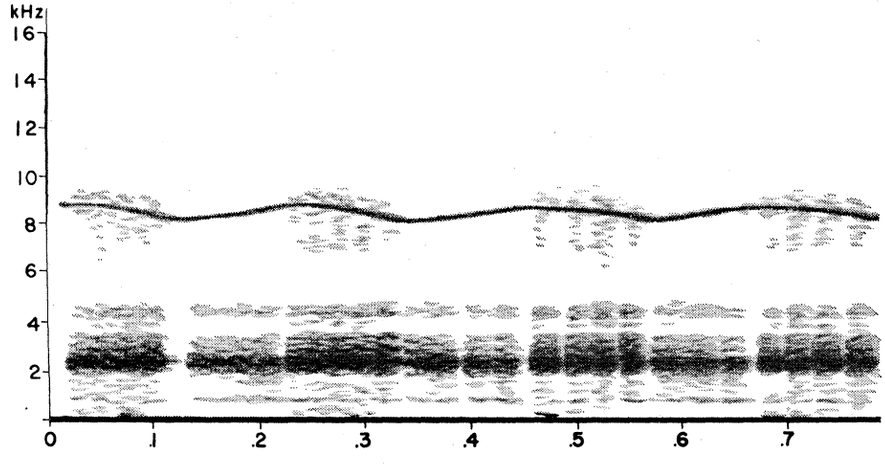

Fig. 1. Sound spectrogram of worker ant. Downward movement of upper line indicates upward movement of gaster. Abscissa, time in seconds; ordinate, frequency.