the transition of the inactive holotetramer to the active form is responsible for the slow spontaneous activation rate, but the binding of a maturation-inducing ligand to the inactive form overcomes this energy barrier and allows the transition to proceed; that is, in the classical sense the activation of threonine deaminase is catalyzed, or induced, by the binding of the ligand to the inactive form.

The concept of induced maturation is supported by the observation that a combination of isoleucine and valine or of isoleucine and threonine blocked the activation of the enzyme. Valine and threonine together, however, continued to promote normal maturation.

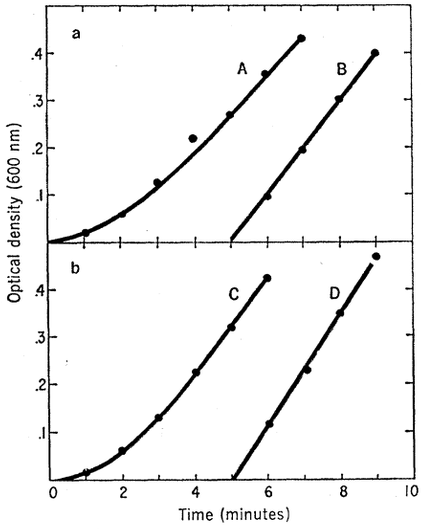

Fig. 2. (a) Activation of threonine deaminase by prior incubation with L-isoleucine. Enzyme (500 μg) was added to a reaction mixture (as described in Fig. 1) to which 1 μmole of L-isoleucine had been added. In curve A, 0.01 ml of this mixture was transferred immediately to another reaction mixture containing no L-isoleucine but containing 80 μmole of L-threonine (time 0). The dilution of the L-isoleucine was sufficient to eliminate most of its inhibiting effect at the concentration of L-threonine used. The activation period is observed. In curve B the enzyme was first incubated for 5 minutes in the buffer containing L-isoleucine before 0.01 ml of the mixture was diluted into the reaction mixture containing L-threonine. No activation period is observed. (b) Activation of threonine deaminase by prior incubation with L-valine. Curve C represents an assay performed as described for curve A, except that 1 μmole of L-valine was included in the first reaction mixture instead of L-isoleucine. Curve D represents an assay performed as described for curve B, except that 1 μmole of L-valine was substituted for the L-isoleucine in the first reaction mixture.

These results imply that at least two distinct stereospecific sites exist on the inactive holotetramer, one specific for either valine or threonine, and the other specific for isoleucine. The maturation process is effected when only one of these sites on the enzyme is filled, but when both sites are occupied this process is blocked. It is presumed that inhibition of the maturation process results from a steric hindrance which is imposed on the immature protein by ligands bound at both sites simultaneously and which prevents a conformational alteration required for the activation of the protein. An allosteric interpretation of this observation is also possible and cannot be easily ruled out at this time. Nevertheless, it is evident that the maturation-inducing ligands bind to the immature protein before the maturation process and that therefore the maturation of this enzyme represents a ligand-specific induction (*5*).

The functional protomer for threonine deaminase from *Salmonella typhimurium* is apparently a dimer composed of two identical polypeptide chains (*6*). A stable dimeric intermediate substructure of this enzyme can be obtained with the use of mild conditions (alkaline dialysis). These intermediate substructures are catalytically inactive even after they are reassembled into a form identical to the native enzyme in its gross molecular architecture. However, this immature, or inactive, protein can be irreversibly maturated, or activated, in the presence of those ligands for which there exist stereospecific binding sites on the enzyme. This maturation is apparently induced by the binding of these ligands before the maturation process itself. The existence in vivo of such a phenomenon would present an interesting correlation with the multiligand system (multivalent repression) involved in the regulation of the synthesis of this enzyme (*7*); it remains to be seen whether or not this process plays a significant regulatory role within the cell.

G. WESLEY HATFIELD
R. O. BURNS
Department of Microbiology and Immunology, Duke University School of Medicine, Durham, North Carolina 27706

References and Notes

1. M. H. Zarlengo, G. W. Robinson, R. O. Burns, *J. Biol. Chem.* **243**, 186 (1968).
2. R. O. Burns and M. H. Zarlengo, *ibid.*, p. 178.
3. P. Maeba and B. D. Sanwal, *Biochemistry* **5**, 525 (1966).
4. The inactive holotetramer exhibited an $S^0_{20,w}$ value of 8.4, which is the same as that observed for the native enzyme at the same protein concentration (4 mg/ml).
5. A. Worcel, D. S. Goldman, W. W. Cleland, *J. Biol. Chem.* **240**, 3399 (1965).
6. R. O. Burns, *Bacteriol. Proc.* **1968**, 119 (1968).
7. M. Freundlich, R. O. Burns, H. E. Umbarger, *Proc. Nat. Acad. Sci. U.S.* **48**, 1804 (1962).
8. Supported by NIH grant GM-12551. G.W.H. is a postdoctoral research fellow of the NIH. R.O.B. is recipient of a PHS research career development award.

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Receptor Potentials from Hair Cells of the Lateral Line

Abstract. *Intracellular recordings from hair cells in the tail lateral line of mudpuppy* Necturus maculosus *show receptor potentials less than 800 microvolts, peak to peak, from stimuli that are considered large compared to natural stimuli. The hair cells are in neuromasts that are sensitive at the time of recording and are identified by both in vivo and in vitro examination of intracellular staining.*

Microphonic potentials from the inner ear of many different species have been recorded and studied (*1*). Similar potentials have been observed in other labyrinth organs (*2*) and in lateral line organs (*3*). The source of these potentials recorded extracellularly was hypothesized to be the hair cells that are found in the inner ear and in all other sensory epithelia of the acoustico-lateral system (*4*). Receptor potentials have been sought in hair cells without success. We have recorded intracellular potentials from hair cells in the lateral line organ of the tail of the mudpuppy *Necturus maculosus*, and we have found a small component that responds to motion of the cupula (a 27-hz displacement was used); this response appears to have the characteristics of a receptor potential. The magnitude of cupular motion was about 2 μm at a distance of about 80 μm above the cupula hair-cell junction but of variable and unknown magnitude at the junction. The receptor potentials ranged downward from a peak of 800 μv (peak to peak). These potentials were recordable only from hair cells, and they were not an artifact of motion.

The neuromasts on the mudpuppy tail occur in groups called stitches with two to six neuromasts per stitch (Fig. 1). During an experiment a mudpuppy was immobilized with Flaxedil (1 mg

per 100 g of body weight), and the blood flow was monitored locally by examining capillaries near the tail neuromasts and in the gills. The preparation can be illuminated from below because the tip of the tail is translucent. Three glass pipettes were positioned above a stitch; one was the stimulating pipette, another was the recording pipette, the third was the monitoring pipette. The stimulating pipette (tip diameter, about 10 μm) can be positioned to move the cupula on only one of the neuromasts in a stitch. The recording pipette, usually filled with an 8 percent solution of Niagara Sky Blue dye, was positioned to enter a neuromast and to penetrate single hair cells. These micropipettes have a resistance of 300 to 500 megohm (measured in the tissue) and a tip diameter of 0.2 μm or less. They were filled immediately before use by injection (*5*). The outside of the pipette remains dry, we believe the tip remains very sharp. The monitoring pipette, usually filled with 3*M* KCl (~ 20 megohm) was positioned to enter a neighboring neuromast of the same stitch.

In the experimental procedure the monitoring pipette penetrated a neuromast to record extracellular nerve spikes (0.2 to 0.6 mv, peak to peak, in a noise background of about 0.1 mv). The nerve spikes were of one or two types and without external stimulation appeared to fire irregularly. The stimulating pipette was then positioned to displace the cupula of another neuromast in the same stitch. The neuromast was judged sensitive if the nerve spikes became phase locked to the 27-hz stimulus motion. The two types of nerve spikes locked to opposite phases of the stimulus. We interpret this response to mean that the monitoring pipette is monitoring the neural output from the whole stitch, that the two nerve spikes correspond to the two main myelinated nerve fibers (Fig. 1), and that their opposite sensitivity suggests that they innervate hair cells with oppositely oriented kinocilia (*6*). These results correspond quite closely to data reported from *Xenopus* (*7*). When a neuromast was determined to be sensitive to a physical stimulus, the recording pipette was advanced to penetrate a hair cell in the same neuromast. The stimulated neuromast was monitored to ensure that it was still sensitive to the stimulus even after penetration. Thus we could ascertain that the entire neuromast was functioning. The pipettes were positioned with a binocular microscope that permitted an underwater working distance of about 15 mm with a magnification of × 200. A whole neuromast was visible in the field and, with illumination from below, single cells were discernible.

Recording and monitoring pipettes could be connected to a high input impedance (10^{11} ohm) d-c amplifier (Bioelectric NF-1) and then to the amplifier of a Tektronix 502 scope. Both the d-c scope output and the a-c output at higher gain were recorded; the a-c output could be averaged with a Fabritek signal processor. The magnitude of the stimulus was also recorded. The recording pipette was usually filled with dye in order to mark the penetrated cells (*8*). While the electrode was within a cell and recording from it (~5 to 20 minutes), enough dye would leak out of the pipette for us to identify the cell as a hair cell in vivo (*9*). The intracellular potential was

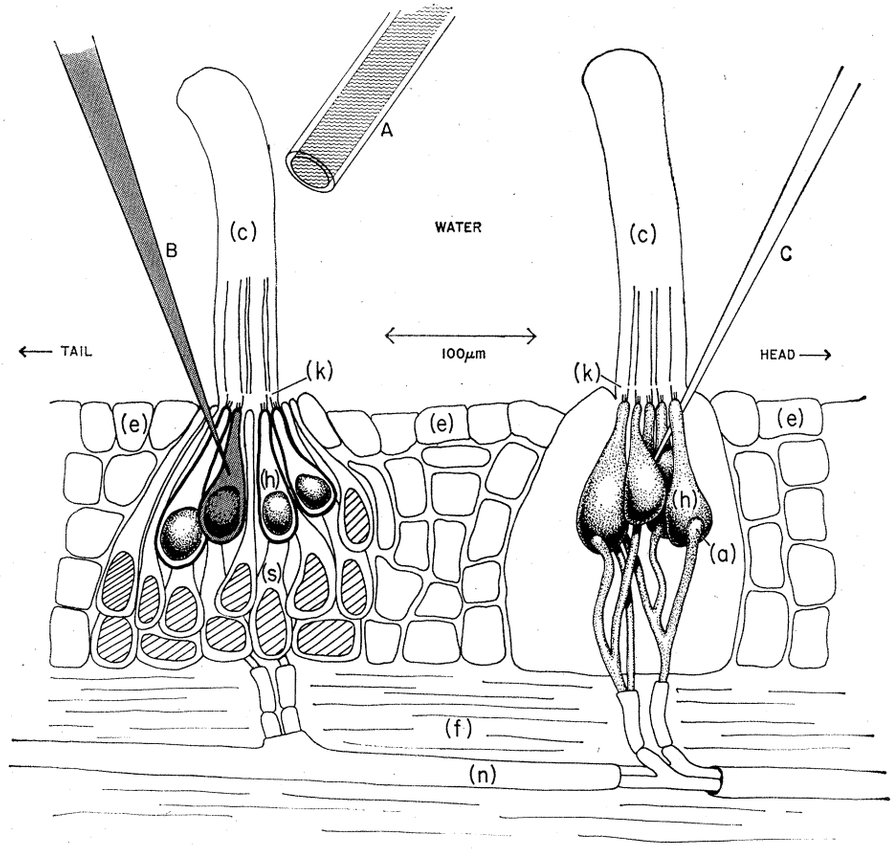

Fig. 1. Two neuromasts in the lateral line on the tail of *Necturus*. The section shown is through the skin along the rostrocaudal axis. Two to six neuromasts form a group called a stitch and are lined up approximately along the rostrocaudal axis, which is also the axis of motion sensitivity. The neuromast contains about eight hair cells (*h*) whose basal ends are roughly lined up in two rows along the rostrocaudal axis, and whose apical ends converge on a narrow region along the same axis. Each hair cell has a single kinocilium and a number of steriocillia protruding from its apical end (*k*). The assymetrically placed kinocilium may be oriented either toward the head or toward the tail with about half the hair cells of each neuromast oriented in each direction. The hair cells are surrounded and separated by a nest of supporting cells (*s*) that is embedded in the epithelial layer of the skin (*e*). A cupula (*c*) extends from the region of hair cells upward into the water. The hair cells are innervated by a nerve bundle running through a tough fibrous layer (*f*) just underneath the epithelial layer of cells. Two large myelinated afferent fibers innervate each stitch. Each fiber sends branches to each neuromast; in addition there are smaller fibers (not shown) whose branching and function are as yet uncertain. The two main fibers, lose their myelination just before entering the neuromast and form afferent synapses (*a*) on the basal ends of the hair cells. In a preparation the neuromasts are illuminated from below by transmitted light. Pipette A is used as a stimulator and as a source of externally applied chemicals. Pipette B is used for intracellular penetration of hair cells and recording and is often filled with an 8 percent solution of Niagara Sky Blue dye. Pipette C penetrates one neuromast of a stitch and is used to monitor nerve spikes from the two afferent nerves which can be excited from any neuromast in the same stitch.

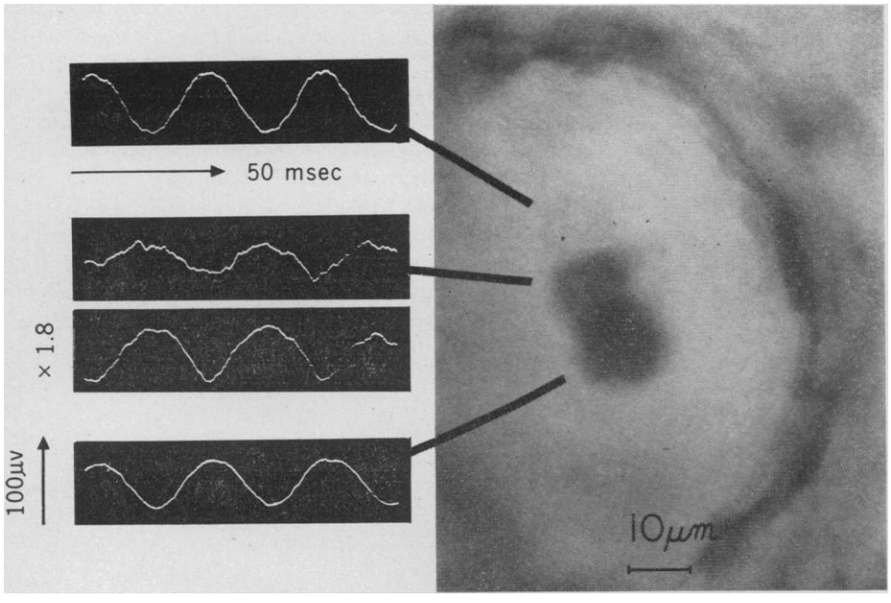

Fig. 2. Oppositely phased responses from different hair cells in the same neuromast are shown. (Right) Stained hair cells in a neuromast taken through one side of the binocular microscope. The air cells were stained by Niagara Sky Blue dye leaking out of the penetrating electrode during recording. (Left) Oscilloscope traces of the response from each cell averaged 128 times. The bottom cell and top cell, which hardly stained at all, showed the same phase response. The middle cell showed a response of opposite phase. The trace labeled × 1.8 indicates that the stimulus magnitude was increased by a factor of 1.8. The three penetrated cells were identified in vivo as hair cells. The two deeply stained cells retained stain during fixation and were confirmed to be hair cells by histological examination.

affected little if at all by this leakage, and a neuromast remained sensitive when Niagara Sky Blue dye was injected into the region of the hair cell. The pipette was not damaged by the staining process and could be used to penetrate other cells in the neuromast.

From 16 different animals 91 neuromasts were penetrated and judged sensitive to mechanical stimulation of the cupula. In each of these neuromasts, many cells were penetrated (indicated by negative shifts in the d-c potential) as the recording pipette was advanced. The a-c output from each of these cells was averaged over many stimulus cycles; 128 cells showed averaged a-c responses which were greater than 30 μv peak to peak. Although a few of these responses were as large as 800 μv peak to peak, most were in the lower range (73 less than 100 μv and 55 greater than 100 μv peak to peak). Of these cells, 28 were recorded from long enough to allow intracellular staining and visual identification of hair cells. Because of the stereoptic viewing the identification was good. In 12 of these the stained cells were also later identified histologically as hair cells. In no case did we obtain a response in a cell which was identified in vivo or in vitro as other than a hair cell. In one or two cases unresponding cells were stained and were identified as supporting cells. In control experiments the recording pipette was filled with potassium chloride or potassium citrate, and receptor potentials were recorded. Thus responses were not dependent on the electrolyte, although it did appear that intracellular responses were less stable when potassium chloride was used.

In the lateral line organs, approximately half of the hair cells have the kinocilium located toward the rostral side of the cell and the rest have their kinocilium toward the caudal side. This double orientation of hair cells, a characteristic of lateral line organs, may be the reason for the dominant double harmonic component of the lateral line–microphonic potential (*10*). This hypothesis implies that the intracellular receptor potential should have opposite phase in oppositely oriented hair cells. In testing this implication, it was necessary to ensure that the relation between the stimulator and the cupula did not change significantly when different hair cells of the same neuromast were penetrated. Any change in cupular position with respect to the stimulator is minimized during the same pipette penetration. There were five cases in which receptor potentials approximately 180° out of phase were recorded from different hair cells during the same penetrating pass of the recording pipette. Another way of minimizing stimulus was to move the stimulator above and away from the cupula. Multiple intracellular penetrations were possible under favorable stimulus conditions and oppositely phased responses were obtained (Fig. 2).

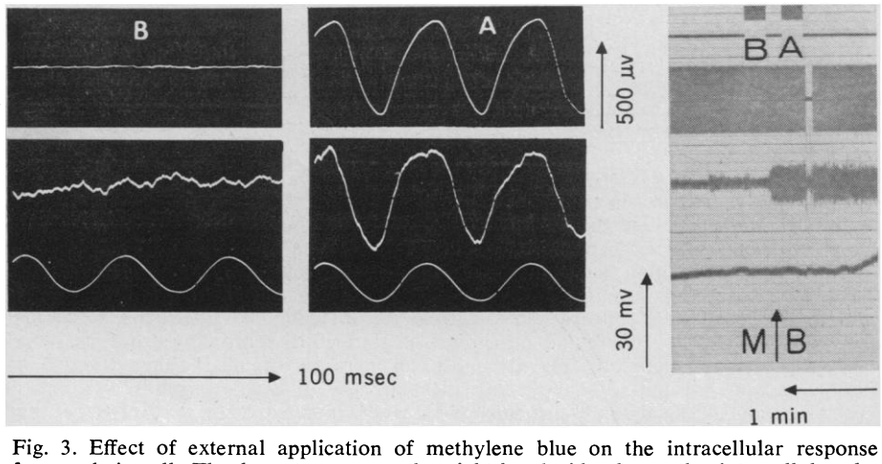

Fig. 3. Effect of external application of methylene blue on the intracellular response from a hair cell. The lowest trace on the right-hand side shows the intracellular d-c potential. This is the only trace to which the vertical scale applies. Above this are the a-c component of the intracellular potential with a gain of 12.5, a trace whose width corresponds to stimulus magnitude, and at the top is a trace showing the time intervals, *A* and *B*, during which average responses were obtained. The arrow at the bottom (*MB*) indicates the time at which methylene blue was applied. (Note that in these records time passes from right to left.) On the left-hand side are two series of oscilloscope traces showing, from bottom to top, the stimulus with arbitrary magnitude, a single response trace, and the average of 128 traces. Series A was taken immediately before application of the methylene blue and series B was taken immediately after. The stimulus is not changed from A to B, the d-c potential is seen to remain unchanged but the a-c response is rapidly abolished. This effect was reversible.

Because the stimulus for the lateral line organ is displacement of a cupula, it is important to establish that any intracellular response is not an artifact of motion. Such artifacts were produced when the recording pipette was just touching the skin surface. A noisy response was also seen when the pipette tip was up against a cell membrane. However, when the pipette tip was inside a cell, the response characteristics were more stable and less noisy. Methylene blue applied externally to the lateral line organs is readily absorbed by the cupula and rapidly proceeds down into the neuromast. Applied through the stimulating pipette, methylene blue rapidly abolished the receptor potential often without affecting the intracellularly recorded d-c potential (Fig. 3). It also destroyed the sensitivity of the whole neuromast. Both effects were reversible. Usually the receptor potential returned before the sensitivity of the neuromast as indicated by the monitored neural activity. Control experiments with water or Niagara Sky Blue dye in the stimulating pipette, instead of methylene blue, did not abolish the intracellular response and sensitivity of the neuromasts. We interpret the fact that the receptor potential was abolished by a known metabolic inhibitor as evidence that it is not a displacement artifact.

Békésy noted that small amounts of methylene blue did not abolish the microphonic potential when added to the cochlear perilymph of the guinea pig (*11*). There is danger in making inferences when discussing different sense organs in different species. Yet we would say that applying methylene blue to the cupula would correspond in the ear to applying methylene blue to the endolymph.

The receptor potential in the mudpuppy hair cell is small compared to that found in other sense organs (*12*). The stimulus was well above threshold compared to natural stimuli, and, in view of the stability of the receptor potential, it is unlikely that its size can be attributed entirely to cell damage. Whether such small potentials could directly activate the synapses between the hair cell and the afferent nerves is not known.

The size of the receptor potential may be one of the reasons that such potentials have not been seen in the cochlea. For, in the cochlea, the hair cells are oriented only in one direction, and consequently the current density above and below the cells is similar to the current density inside the cells. A jump in the microphonic potential will be recorded as a pipette penetrates a hair cell, but the jump could be smaller than the magnitude of the microphonic potential outside the cells and thus would not be easily detected. In the lateral line neuromast, with the hair cells oriented in opposite directions, the currents associated with these potentials will tend to cancel each other outside the cells. Thus, the a-c potential outside the cells is much smaller, and the potential change across the membrane is relatively much larger. This would make the transition between outside to inside a hair cell much easier to detect in the lateral line organ than in the cochlea.

GERARD G. HARRIS
Bell Telephone Laboratories, Murray Hill, New Jersey
LAWRENCE S. FRISHKOPF
Department of Electrical Engineering, Massachusetts Institute of Technology, Cambridge
ÅKE FLOCK
Gustaf V Research Institute, Stockholm 60, Sweden

References and Notes

1. I. Tasaki, H. Davis, D. H. Eldredge, *J. Acoust. Soc. Amer.* **26**, 765 (1954); E. G. Wever and M. Lawrence, *Physiological Acoustics* (Princeton Univ. Press, Princeton, N.J., 1954); Y. Katsuki, *Physiol. Rev.* **45**, 395 (1965).
2. H. de Vries and J. D. Bleeker, *Acta Oto-Laryngol.* **37**, 289 (1949); T. Furukawa and Y. Ishii, *Jap. J. Physiol.* **17**, 572 (1967).
3. R. Jielof, A. Spoor, H. de Vries, *J. Physiol.* **116**, 137 (1952); J. W. Kuiper, thesis, Groningen University, Netherlands (1956).
4. H. Davis, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 181 (1965).
5. H. van den Ende, *IEEE Trans. Biomed. Eng.* **14**, 266 (1967).
6. A hair cell—anywhere in the acousticolateral system—typically has, protruding from its apical end, two types of ciliary processes: a large number of stereocilia and a single asymmetrically located kinocilium.
7. G. G. Harris and A. Flock, in *Lateral Line Detectors*, P. H. Cahn, Ed. (Indiana Univ. Press, Bloomington, 1967), pp. 135–161; G. G. Harris and D. C. Milne, *J. Acoust. Soc. Amer.* **40**, 32 (1966).
8. A. Kaneko and H. Hashimoto, *Vision Res.* **7**, 847 (1967); F. S. Werblin, personal communication.
9. It might seem difficult that enough dye could leak out in this amount of time from a micropipette whose tip diameter is less than 0.2 μm, as checked by electron microscopy. Perhaps this negative ion becomes bound and can thus maintain a very sharp diffusion gradient.
10. A. Flock, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 133 (1965).
11. G. von Békésy, *Experiments in Hearing* (McGraw-Hill, New York, 1960), p. 488.
12. For example, bright flashes of light produce depolarizations of up to 30 mv in *Limulus* ommatidia [E. F. MacNichol, Jr., *Exp. Cell Res. Suppl.* **5**, 411 (1958)]; also H. Stieve, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 451 (1965); T. Tomita, *ibid.*, p. 559; C. Eyzaguirre and S. W. Kuffler, *J. Gen. Phys.* **39**, 87 (1955).

6 August 1969; revised 16 September 1969

Glyceryl Ether Metabolism: Regulation of Buoyancy in Dogfish Squalus acanthias

Abstract. *The ratios of concentrations of diacyl glyceryl ethers to triglycerides are increased significantly in the livers of weighted dogfish* (Squalus acanthias) *in comparison to an unweighted control group. The hydrostatic properties of the liver may be regulated through the metabolism of these two classes of lipids which have different specific gravities.*

Although glyceryl ethers are widely distributed among animals (*1*), no distinct use for these compounds in nature has been established since their discovery (*2*). Nevertheless, clues to a possible role for the glyceryl ethers in the life process may be found in studies of the class of fish in which they were originally discovered. For example, the fatty livers (Fig. 1) of the dogfish *Squalus acanthias* contain high percentages of diacyl glyceryl ethers (DAGE) that are metabolized very rapidly (*3*, *4*). The active turnover of these compounds suggests that their metabolism is intimately related to the role of the liver as a hydrostatic organ (*4*). We felt that the specific gravities of the two main hepatic lipids, DAGE and triglycerides (TG), might be significantly different so that buoyancy could be controlled by metabolic regulation of the ratio of DAGE to TG.

Table 1. The analysis of dogfish livers. The ratios of diacyl glyceryl ethers (DAGE) to triglycerides (TG) are less than 1 in the control group (unweighted fish) and greater than 1 in the experimental group in which 4-oz (114-g) weights were added to each fish ($t = 4.59$; d.f. $= 11$; $P < .001$) (*7*).

Fish	Weight		Lipid in liver (%)	Ratio (DAGE/TG)
	Fish (kg)	Liver (g)		
Experimental group				
A	2.7	189	57.2	1.25
B	3.4	337	66.8	1.37
C	3.4	266	64.7	1.58
D	2.7	177	59.3	1.10
E	2.7	196	62.2	1.59
F	2.7	246	62.6	1.03
G	3.0	460	72.2	1.08
Control group				
H	2.7	282	75.8	0.90
I	3.0	299	64.5	0.72
J	2.7	295	62.4	0.90
K	2.7	229	62.4	0.49
L	2.3	193	62.5	0.49
M	3.2	318	68.6	0.88