

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 mud-puppy 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.

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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 μ m, as checked by electron microscopy. Perhaps this negative ion becomes bound and can thus maintain a very sharp diffusion gradient.
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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)		
<i>Experimental group</i>				
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
<i>Control group</i>				
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

We isolated DAGE and TG from the liver by thin-layer chromatography (3) and established the purity of each fraction (3, 4). The following specific gravities (water at 25°C) were obtained: DAGE, 0.908 at 25°C and TG, 0.922 at 25°C. Though these differences are not great, 1 g of DAGE gives 14 percent more lift in seawater (specific gravity 1.026) than 1 g of TG.

The difference in the specific gravities of the two classes of lipids encouraged us to study the changes in the ratios of DAGE to TG that would result from an artificial increase in the body weight of experimental animals. Thirteen male dogfish *Squalus*

acanthias, caught in Puget Sound in July, were maintained for 2 days in circular tanks 2 m in diameter and 1.3 m deep. The water in the tanks was exchanged and aerated continuously. The fish were 80 to 90 cm long and weighed 2.7 to 3.4 kg. Lead weights (114 g) were suspended between the pectoral fins of seven fish selected at random. They were then returned to the tank with the unweighted fish. After 50 hours, all the fish were killed, and the livers were excised. Each liver was homogenized in a blender for 10 minutes. The resulting emulsion was centrifuged at 8°C for 30 minutes at 8000 rev/min. The DAGE were separated from TG by thin-layer chromatography. The layers were then charred with chromic-sulfuric acid, and the spots representing each glycerolipid were analyzed by photodensitometry (5). Peak areas were measured to establish the ratios of DAGE to TG (Table 1).

Triglycerides predominate in the livers of each unweighted fish, whereas the proportion of DAGE is significantly higher in each weighted fish. We postulate, therefore, that the ratios of DAGE to TG in the weighted group are increased to offset the increase in body weight. No correlation exists between

the ratios of DAGE to TG and the weights of the fish, the liver weights, and the percentage of oil. Individual anatomical features might contribute substantially to the variations observed (6).

We postulate that a regulatory mechanism, involving the selective metabolism of DAGE and TG, is used by *Squalus acanthias* in the maintenance of neutral buoyancy during vertical migrations. This regulatory mechanism may serve as a substitute for the commonly found gas-filled swim bladder.

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4 September 1969; revised 15 October 1969 ■

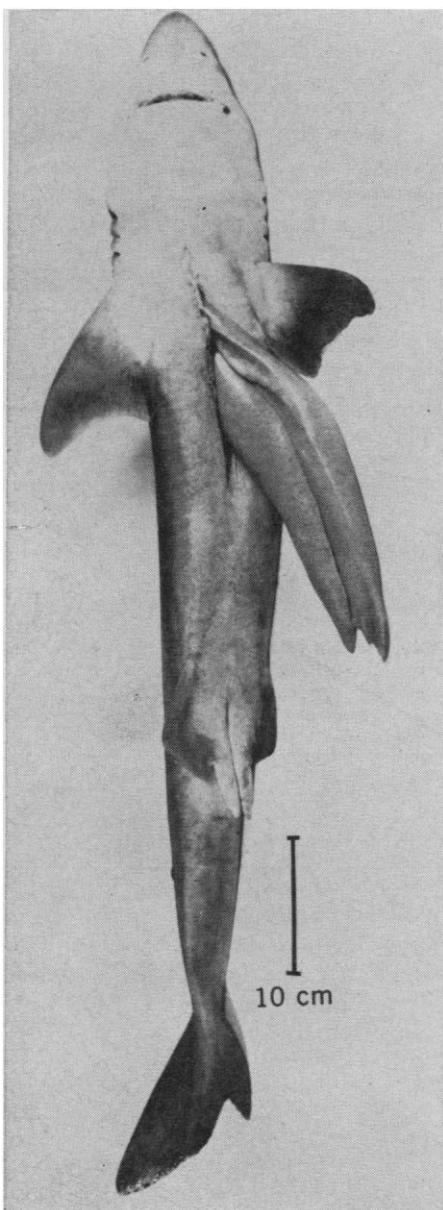


Fig. 1. Dissected dogfish *Squalus acanthias* illustrating the large liver in comparison to the body size.

Animal Cells: Noncorrelation of Length of G_1 Phase with Size after Mitosis

Abstract. The interval between mitosis and initiation of DNA synthesis (G_1) varied over a fourfold range for Chinese hamster ovary cells, an established line. This was not because of size differences. Synchronous cells of different sizes began DNA synthesis at similar times after mitosis. A novel technique of centrifugation for separating cells according to size is described.

Individual cells of synchronous mitotic mammalian cultures proceed through the mitotic cycle with different rates (1). Cells obtained by shaking monolayers growing in glass bottles (2) are initially more than 90 percent in metaphase, and yet they enter the DNA synthesis phase of the cycle (S) between 3 and 12 hours after division (Fig. 1). Thus, highly synchronous mitotic cells exhibit G_1 phases which vary over more than half of the cell cycle. The remainder of the mitotic cycle appears to vary less, the time of DNA synthesis through mitosis ($S + G_2 + M$) being relatively constant for many mammalian cell lines (1, 3). Thus the

variation in G_1 may account for much of the variation in intermitotic times.

The duration of G_1 might vary due to genetic, nutritional, or cell volume fluctuations within the population. We have examined the possibility (4, 5) that individual cells have G_1 durations dependent on initial cell mass. Velocity sedimentation of synchronous cells in early G_1 through Ficoll density gradients yields fractions of cells which differ in volume. These fractions are grown on glass cover slips and are exposed briefly to tritiated thymidine early and late during the period of DNA synthesis. Autoradiography of these cells indicates that there is no close relation