Table 1. Numbers of *G. ingens* captured in different ranges of oxygen concentration during day and night, and total numbers of 1-hour net tows taken in each range.

Oxygen concentration (ml/liter)	Individuals captured (No.)		Tows (total No.)
	Day	Night	
0.20–0.50	21	8	49
0.50–0.75	2	3	19
0.75–1.00	0	6	14
1.00–1.25	0	1	10
1.25–6.00	0	0	49

rates 100 to 200 percent at concentrations between 0.5 and 0.3 ml of oxygen per liter, producing a slight but not significant increase in the average curve; in the remaining 19 runs the slopes at this concentration were the same as those at higher oxygen concentrations. Thus, *G. ingens* from the minimum layer can maintain a nearly constant rate of oxygen consumption in seawater having down to the lowest oxygen concentration found where they live (0.20 ml/liter). All of the animals continued to consume oxygen until none was detectable. The animals continued swimming for less than 30 minutes after they exhausted the oxygen in the chamber, although they could be revived for up to 6 hours after this time.

The upper and lower curves of Fig. 1 show the extremes in respiratory rates observed. The upper curve represents one animal which was forced, by the action of a magnetic stirring bar, to swim at the maximum rate which it could sustain for the duration of the experiment (12 hours). The lower curve represents one animal which was not swimming at either the beginning or end of the experiment.

The animals used in these experiments lived in the laboratory for up to 3 months, during which time they showed no detectable change in respiratory rate and readily ate pieces of fish.

The results indicate that *G. ingens* lives aerobically in the oxygen minimum and requires, for long-term survival, at least 0.14 to 0.26 ml of oxygen per liter in its environment. Its ability to remain active under anaerobic conditions is strictly limited, however; this may explain why *G. ingens* is absent from the regions of the eastern tropical Pacific where oxygen concentrations of less than 0.1 ml/liter prevail at the depths which the species occupies elsewhere (*8*, *11*).

The ability of *G. ingens* to regulate its oxygen consumption at low oxygen concentrations is remarkable. Most crustaceans and fishes previously investigated cannot regulate oxygen consumption at concentrations below 1.2 ml of oxygen per liter (*12*), and the best regulators previously examined regulate at concentrations only down to 0.6 ml/liter (*13*). It is quite possible that other animals found in the minimum layer are also able to live aerobically in this nearly anaerobic environment. If this is the case, these populations may make a substantial contribution to the biological consumption of oxygen which is believed responsible for the formation and maintenance of these layers.

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13 March 1968

Transverse Tubular System in Glycerol-Treated Skeletal Muscle

Abstract. *Horseradish peroxidase used as an extracellular marker fills 98.5 percent of the central triadic elements (tubules) in normal muscle and 97.2 percent in muscle soaked in Ringer solution to which 400-millimolar glycerol is added. A glycerol-soaked muscle rapidly returned to normal Ringer solution has a disrupted transverse tubular system and has only 3.2 percent of triads filled with peroxidase.*

Frog skeletal muscle has an extensive network of transverse tubules that originate as invaginations of the plasma membrane, invade the muscle fiber at the Z-disk, and branch so as to surround the myofibrils. It seems likely that flow of current down these tubules is an essential link between excitation (that is, an action potential at the plasma membrane) and contraction deep in the muscle fiber (*1*). Frog skeletal muscle also has a set of electrical properties quite distinct from those of nerve; they have often been attributed to the transverse tubular system. In particular it has been suggested that the membrane of the transverse tubules increases the capacitance of muscle fibers (*2*), that the lumen of the tubules forms a restricted diffusion space in which flow of current can produce slow changes in ion concentration and thus slowly change membrane potential (*3*), and that the potassium conductance system, which rectifies in the direction opposite from that of squid nerve, may be located in the tubular membrane (*4*).

The most direct method of proving that these properties do indeed require an intact transverse tubular system would be removal of the system selectively. A recently described procedure (*5*) is said to disrupt the transverse tubular system. Moreover many of the electrical properties of the muscle fibers treated in this manner are those to be expected from an isolated sarcolemma lacking tubular infoldings (*6*). In particular the specific capacitance of these treated muscle fibers is strikingly low—close to the value previously predicted for the sarcolemma (*2*). So that one may interpret with confidence the properties of these treated fibers, the morphology of the fibers and the number of tubules still connected to the extracellular space (and thus presumably functional) must be determined.

The procedure for disrupting the transverse tubular system has two stages: (i) the muscle is soaked for 1 hour at room temperature (17° to 21°C) in Ringer solution made hypertonic by addition of 400 m*M* glycerol;

(ii) the muscle is then quickly returned to normal Ringer solution. In the glycerol-Ringer solution, the structure of the muscle has been reported to be normal (*5*); the electrical properties are those to be expected from a shrunken but otherwise normal muscle (*6*). Upon return to normal Ringer solution the structure of the transverse tubular system is disrupted, and the electrical properties change drastically. Examination of the morphology of treated muscle fibers [prepared for observation in the electron microscope in the usual manner (*7*)] showed that the sarcolemma was intact and had unit membrane substructure. The myofilaments were normal. The sarcoplasmic reticulum, however, was somewhat altered: the terminal cisternae were almost normal in appearance (Fig. 1, a and b) and the fenestrated collar and longitudinal tubules (that is, the reticulum in the middle of the sarcomere) were completely normal, but the intermediate cisternae (that is, the reticulum in the region of the A band–I band junction) were absent or severely broken, and the interfibrillar space in this region was often enlarged. Usually the central element of the triad (that is, the transverse tubule) was grossly disorganized and often completely absent (Fig. 1a). Occasionally, however, a relatively intact tubule could be observed (Fig 1b). Thus it seemed clear that some technique in addition to structural examination had to be used for determination of how many of the transverse tubules were still connected to the external medium and could thus be expected to contribute to the properties of treated muscle fibers.

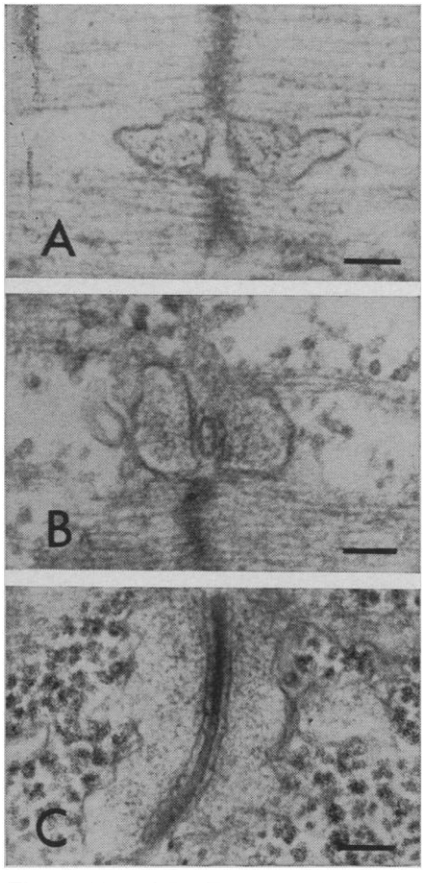

Fig. 1. (A and B) Frog sartorius muscle treated for 1 hour in Ringer solution plus 400-m*M* glycerol and then returned to normal Ringer solution: the central element of the triad (transverse tubule) is absent (A); selected triad in which the tubule remained (B). (C) Muscle, in glycerol-Ringer solution, showing normal triad with peroxidase filling tubule. Each scale is 0.1 μ.

Horseradish peroxidase proved to be a convenient and reliable marker of extracellular space in normal muscle (*8, 9*). The central element of the triad was filled with peroxidase in almost all the triads examined. Triads (1546 from nine fibers) were examined, and 98.5 ± 0.6 percent were filled with peroxidase (mean $\pm$ S.E.). However, not every sarcomere had a complete triad; in fact only 83.7 ± 1.7 percent of the sites where triads were to be expected (that is, sites between the myofibrils at the Z-disk) had transverse tubules. The remaining 16 percent of sites showed only lateral sacs (terminal cisternae) or glycogen granules. This finding is consistent with the described (*7*) structure of the transverse tubular system if the transverse tubules do not completely surround every myofibril. We used only surface muscle fibers of the sartorius; no observations were made on the few fibers lacking a dense ring of peroxidase around the external membrane.

Muscle fibers were examined by the peroxidase technique while they were in glycerol-Ringer solution for determination of whether glycerol itself was disrupting the tubular system. The muscle was transferred from the glycerol-Ringer solution to the 5-percent glutaraldehyde fixative (*7*) containing 400 m*M* glycerol. The necessity for use of this modified fixative is understandable if one considers the resemblance between the osmotic shock of transferral of a muscle from hypertonic glycerol-Ringer to normal Ringer solution, and that of transferral of a muscle from hypertonic glycerol-Ringer solution to a fixative designed for muscles in isotonic solutions. The structure of muscle fibers in glycerol-Ringer solution proved to be essentially normal (Fig. 1c), 97.2 ± 1.5 percent of the tubules being filled with peroxidase (1097 tubules from six fibers) and 85.8 ± 1.6 percent of the sarcomeres having triads. Thus one may reasonably expect that the properties of muscle fibers that require an intact transverse tubular system should be present in muscles still in glycerol-Ringer solution.

Muscle fibers having disrupted transverse tubular systems also were examined by the peroxidase technique. Only 2.6 ± 0.7 percent of sarcomeres had tubules filled with peroxidase (3779 sites from 15 fibers) versus 82.8 percent of the sarcomeres in normal muscle (combined data from normal muscles and from those in glycerol-Ringer solution). Thus only 3.2 percent ($2.6 \div 0.828$) of the transverse tubular system remained connected to the extracellular space in glycerol-treated muscle fibers.

Thus it seems safe to conclude that after such treatment with glycerol almost nothing of the transverse tubular system remains connected with the extracellular space. Furthermore, to the extent that glycerol has no direct pharmacological action on the sarcolemma, the electrical properties of glycerol-treated fibers should represent those of a simple cylinder of muscle membrane. Thus this preparation should allow separation of the properties of the tubular system from those of the sarcolemma.

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