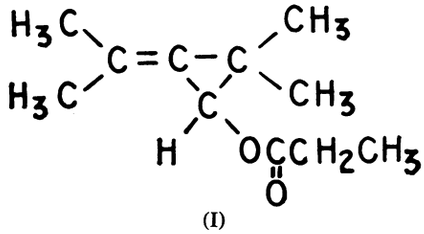
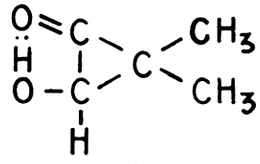
oxidation with periodic acid converted the neutral substance to a crystalline acid, mp 197° to 198°C, identified as dimethylmalonic acid by paper chromatography, infrared spectrum, and mixed melting point with a pure synthetic sample.

The only structure for the attractant consistent with the foregoing data is 2,2-dimethyl-3-isopropylidenecyclopropyl propionate (I).



(I)

The neutral substance formed on oxidation probably possesses the structure shown in (II).



(II)

That the attractant does indeed possess structure I was proved by synthesizing its hydrogenation product according to the following procedure. The reaction of 2,4-dimethyl-2-pentene with diazoacetic ester in the presence of copper powder (*10*) gave ethyl 2,2-dimethyl-3-isopropylcyclopropanecarboxylate (54 percent; bp 95° to 100°C at 20 mm-Hg; n_D^{25} 1.4315), which was saponified with ethanolic potassium hydroxide to the corresponding acid (66 percent), a colorless, viscous oil. The undistilled acid was decarboxylated with lead tetraacetate and iodine (*11*) to give 2,2-dimethyl-1-iodo-3-isopropylcyclopropane (66 percent; bp 52°C at 20 mm-Hg). Reaction of the iodide with silver propionate in dry benzene gave 2,2-dimethyl-3-isopropylcyclopropyl propionate (35 percent; bp 112°C at 20 mm-Hg; n_D^{25} 1.4352; analyzed exactly for $C_{11}H_{20}O_2$), whose infrared spectrum and elution time (5.9 minutes) by the previously cited gas chromatographic procedure were identical with those of the hydrogenated attractant.

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14 December 1962

Giant Muscle Fibers in a Barnacle, Balanus nubilus Darwin

Abstract. *Cross-striated muscle fibers of very large size have been found in the scutal-tergal adductor and depressor muscles of the large barnacle* B. nubilus. *Adductor muscle fibers are up to 2 mm thick. They are innervated by separate nerves, each supplying one end, but not the central region, with terminals; each fiber receives two or three excitor axons. Depressor muscle fibers are up to 1.4 mm thick and receive multiterminal innervation along their entire length; they are innervated by two excitor axons. Postsynaptic potentials are of small or large size and lead to small or large twitches; they do not show facilitation. The muscle fibers shorten to as little as one-sixth resting length.*

The study of the physiology of specialized cells has often been greatly aided by the use of examples of unusually large size. Giant muscle fibers might be expected to be of considerable value in muscle physiology. Fibers up to 600 μ in diameter, present in crab muscle, enabled Caldwell to study intracellular *p*H changes (*1*) but these fibers cannot be readily isolated for study and have not been widely used.

We have found fibers of at least twice this thickness in the scutal-tergal adductor and depressor muscles of the large barnacle *Balanus nubilus* Darwin. They are almost completely free from connective tissue and can very readily be isolated in good condition, with resting potentials from 78 to 86 mv. They can be partially isolated with the nerve supply intact.

The adductor contains about 25 giant fibers having maximum thickness ranging from 500 to 2000 μ. These are innervated by two separate nerves entering close to the two margins where the fibers are attached to the scutes. Each fiber receives several terminals from each nerve, and these are concentrated at the ends. In no case does the nerve cross the midline. Four axons in each nerve were stained clearly by methylene blue.

Intracellular recordings were made during graded electrical stimulation applied to the nerves. These gave similar results on each side. Most of the muscle fibers were doubly innervated, although some were triply innervated. The fibers were all excitatory. No evidence for inhibitory fibers has been found.

The smallest responses were postsynaptic potentials of 10 to 15 mv when recorded 3 mm from the attachment (peak zone); these were not present in all the muscle fibers. A second kind of postsynaptic potential of about 25 mv was observed in many muscle fibers. All gave a giant postsynaptic potential, 40 to 50 mv in magnitude, following maximal stim-

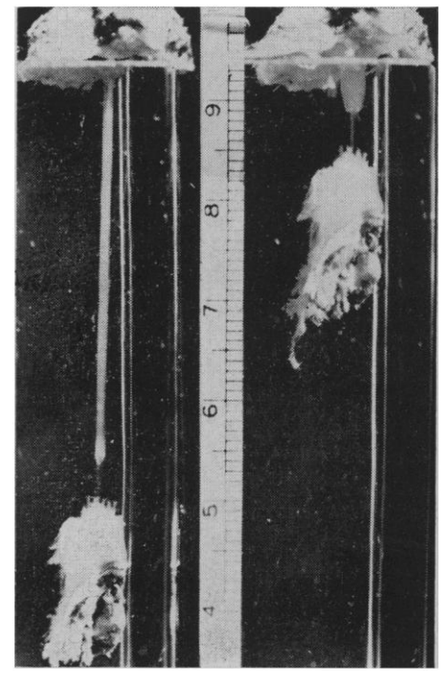

Fig. 1. Isolated single giant muscle fiber of m. depressor scutorum rostralis of *Balanus nubilus*. A portion of the shell is seen at the upper end, and a scute at the lower. The cut tendons of other giant fibers can be seen. The fiber was immersed first in barnacle Ringer solution (left) and then placed in barnacle Ringer (right) containing 200 mmole of potassium in place of sodium. Scale in centimeters.

ulation of the nerve. This in turn evoked small, graded spikes not larger than about 25 mv.

Recordings of tension changes were made from innervated single muscle fibers by cutting the attachment to the scute at one end and clamping it in a small holder attached directly to the peg of an RCA 5734 transducer tube. There was no or very slight mechanical response to a single, small post-synaptic potential, but there was marked growth during trains at increasing frequency. Large post-synaptic potentials gave rise to brisk twitches. The responses to stimulation of opposite ends were similar.

A brief tetanus of only one end leads to tight closing of the valves. Stimulating the two nerves together more than doubles the force developed. Fatigue occurs after about 8 minutes when stimulation is continuously applied to one end. However, the dual nerve supply enables the animal to maintain contraction of the adductors indefinitely, without neuromuscular fatigue, by alternating activation between the two ends.

The depressor muscles each contain 30 to 40 muscle fibers in the range of 500 to 1,400 μ maximum width. These in turn contain densely-packed, coarse fibrils, but do not show the internal partitions or folding of the surface, commonly found in large crustacean fibers and also present in the adductors of *B. nubilus*. There is very little connective tissue between fibers, so they can easily be separated. Each has a fine tendon, 3 to 4 mm long, at its scutal-tergal end (Fig. 1). This greatly facilitates preparation for physiological work.

A single fiber may be partially isolated with its nerve supply intact. Force was measured by clamping the tendon in a device attached directly to a transducer tube, at the same time as membrane potentials were recorded with an intracellular electrode. The nerve, which enters from the scutal end, was stimulated electrically on the scute. It supplies two excitor axons to each muscle fiber and gives off multiterminal endings along the whole length of the fiber. The electrical responses are of two sizes: one, 15 to 20 mv, gives rise to a weak twitch, (about 0.3 g), and the other, 25 to 40 mv, gives rise to a much larger twitch, up to 6 g (Fig. 2). Tetanus tensions up to 50 g per fiber (5 kg/cm^2) were obtained.

The capacity of the muscle for shortening is remarkable. Repeated, fully reversible contractions of single fibers to one sixth of the resting length were obtained (Fig. 1) by bathing the fibers alternately in high potassium Ringer and normal barnacle Ringer solutions (*2*).

These extraordinary muscle fibers could be extremely valuable in fundamental muscle research (*3*).

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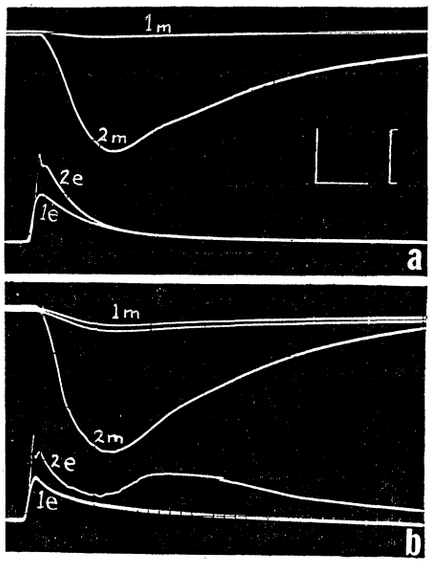

Fig. 2. Membrane potentials and force development in two (*a* and *b*) preparations of single fibers from m. depressor scutorum rostralis of *Balanus nubilus*. The muscle fibers were partially isolated, and their tendons were connected to a force transducer (force is registered on upper traces). An intracellular electrode was placed in the center of the fibers and used to record membrane potential changes (lower traces). (Note: Force [upper] traces start at level of zero membrane potential). Graded electrical stimulation, when applied to the nerve, produced two sizes of response, a pure end-plate potential (1*e*) giving rise to a small twitch (1*m*), and a larger end-plate potential (2*e*) evoking a small graded spike and giving rise to a much larger twitch (2*m*). Each muscle fiber was therefore dually innervated from "slow" and "fast" axons. In the fiber shown in *b* the small response was obtained twice in succession at a stimulus interval of 2 seconds. Note potentiation of mechanical response. Calibration: vertical bar, 20 mv; horizontal bar, 100 msec.; bracket, 1 g.

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2. Barnacle Ringer solution contained, in millimoles per liter, Na^+, 476; K^+, 8; Ca^+, 20; Mg^{++}, 12; Cl^-, 538; HCO_3^-, 10.
3. Supported by grant B-3819, U.S. Public Health Service. This work was done at the Friday Harbor Laboratory, University of Washington. A full account is in preparation.

28 September 1962

Feather Replacement in Birds

Abstract. *During natural molt in many birds, feathers of the old generation are passively pushed out of the follicles attached to the tips of the sheaths of incoming feathers.*

Recent observations on several species of birds reported here show that when feathers are replaced in natural molt, they remain temporarily attached to the sheaths of the new feathers and are passively pushed out of the follicles during growth of the incoming new feathers.

This is known to be the mechanism for replacement of natal downs during the first molt (*1*) and is also the normal mechanism of feather replacement during all molts in penguins and cassowaries (*2*).

Natural molt in all other birds, however, has been thought to be a two-part process entailing the passive loss of old feathers (ecdysis) and the subsequent growth of new feathers (endysis) (*3*). Because artificial plucking

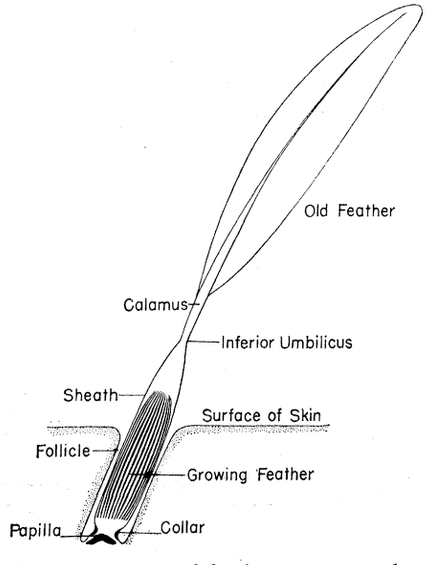

Fig. 1. Diagram of feather structures during molt; sheath and follicle below inferior umbilicus in longitudinal section.