drastic effects, such as the addition of substances causing crystallization of hemoglobin[14,15], as observed some days after a blood meal, should be taken into account and investigated, according to the requirements of the experiment.

Mixture of the blood with bug hemolymph while it is being extracted from the bug is conceivable. To estimate the maximum contamination with bug hemolymph, we squeezed out some 'empty' bugs that had not yet sucked and absorbed the hemolymph with filter paper: The maximum weight loss of the bug body weighing circa 20 mg amounted to 2 mg. A blood sample of 50 µl blood could therefore contain a maximum of 4% bug hemolymph; this estimate, however, is probably much too high, since strong squeezing during the blood extraction from the gaster of the bug can be avoided.

Applications. 1) Measuring the energy budget by means of doubly labeled water. For this method[16], blood samples have to be taken twice or several times from the experimental animals to allow measurement of the decrease of labeled hydrogen and oxygen in the blood. In experiments on flower-visiting bats[17] the blood extraction by means of Triatomidae yielded excellent results. Since it is only the ratio of the concentrations of labeled atoms which have to be evaluated for measuring the energy budget, neither a possible water loss during sucking time nor a slight but identical dilution of the blood through bug hemolymph or saliva would influence the results with this method.

2) Lymphocyte culture for karyological analyses: The blood obtained using bugs is suitable for lymphocyte cultures. We diluted a Heparin solution[18] up to a concentration of circa 1400 IU/ml with balanced salt solution. A small quantity of it (~ 50 µl) was taken in the syringe prepared for extracting the blood from the bug. After addition of culture media a dilution of about 50 IU/ml was reached. The syringe was rinsed with medium or fetal calf serum to obtain all the blood. So far, the lymphocytes of 13 bat species have been successfully cultivated by this method[19].

3) The method may well be suitable for other purposes, even though possible changes of blood composition caused by the bugs have to be tested in each case. This method may also be applicable for measuring hormone titers, for immunological and further biochemical analyses, e.g. allozyme-analyses and paternity tests, which have become increasingly important over the last few years[20–22].

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Shortening velocity of single muscle cells isolated from a molluscan smooth muscle

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Summary. The maximal unloaded shortening velocity (V_{max}) of smooth muscle cells isolated from the pedal retractor muscle of *Mytilus* was more than twice as large as that of the whole muscle, suggesting the presence of extracellular components which resist the contraction of the whole muscle. The V_{max} of the isolated cells was almost constant at cell lengths ranging between 0.5 and 0.83 l_0 (l_0, optimal length for tension generation) indicating that the intracellular resistance to contraction is negligible within this range of lengths.

Key words. Molluscan smooth muscle; single smooth muscle cell; unloaded contraction; shortening velocity; *Mytilus.*

The mechanical properties of smooth muscle do not necessarily reflect those of individual muscle cells because of the small size of the muscle cells, which are often arranged in a complex geometry, and the abundance of connective tissue in the whole muscle. Therefore, in deducing the mechanical characteristics of the contractile elements from those of the whole muscle, it is important to consider the mechanical arrangement of the muscle cells and the effect of connective tissue. Studies made on several smooth muscles to deduce the mechanical properties of individual cells from those of whole muscles have suggested practically linear relationships between the mechanical properties of the cells and those of the whole muscles[1–4]. In these studies, the lengths of the cells during contraction or other mechanical events were estimated by direct microscopic observation of the muscle or by measurements of the lengths of the cells dispersed from the muscle after chemical fixation. However, the best way to study the mechanical properties of individual cells is to measure directly these properties in isolated, live smooth muscle cells[5,6].

In the pedal retractor muscle (PRM) of a bivalve mollusc, *Mytilus edulis,* the length-tension relations have been examined in both whole muscles[7] and isolated cells[6]. The length-tension relation of the isolated cells have shown that the range of lengths over which active tension is developed was much wider in the isolated cells (0.17 to more than 2 l_0, where l_0 is the optimal length for tension generation) than in the whole muscle (0.35 to 1.8 l_0). Such a difference in the 'working range' would be accounted for by assuming that the cells are connected in series by long intercellular linkages. The effective length of such linkages would correspond to about 0.3 l_0. If such linkages exist, the

maximal shortening velocity of the whole PRM is expected to be smaller by about 30% than that of the isolated cells. In the present study, the shortening velocity of single smooth muscle cells isolated from the PRM was measured to see whether the isolated cells shorten faster than does the whole muscle.

Materials and methods. Single smooth muscle cells were isolated from the PRM of *Mytilus edulis* by the method described by Ishii and Takahashi[6]. One of the tapered ends of the cell was fixed by suction with a glass micropipette, and the other end was left free. The cells were electrically stimulated by using the micropipette as an anodal suction electrode. Rectangular pulses of 0.1 ms duration were used singly to induce twitch-like contractions (tentatively referred to as twitches) or repetitively at 50 Hz (duration, 2s) to induce completely fused tetanic contractions[6,7]. Free-end contractions of the cells were observed under a conventional light microscope and recorded on video tapes with a TV camera (Sony, AVC-1150D) and a video cassette recorder (Sony, VO-4900). The images of contracting cells recorded on video tapes were displayed field-by-field on a video motion analyzer (Sony, SVM-1110) and photographed so as to get a time resolution of 16.7 ms. The lengths of the cells were measured on photographic prints by using a digitizer (Graphtec, DT-1000) and a microcomputer (Oki, if800 model 30). After the cell lengths were plotted against time, shortening velocities of the cells during the initial phase of contraction were computed with the least-squares regression (fig. 2). Experiments were carried out at 20 ± 1 °C.

Results and discussion. Figure 1 shows video images of an isolated cell taken during a twitch (A) and a tetanus (B). By plotting the cell lengths against time, the shortening velocities were found to be almost constant at the early phases of both twitch and tetanus (fig. 2). The shortening velocity of twitch increased with the stimulus intensity above the threshold, but reached a plateau

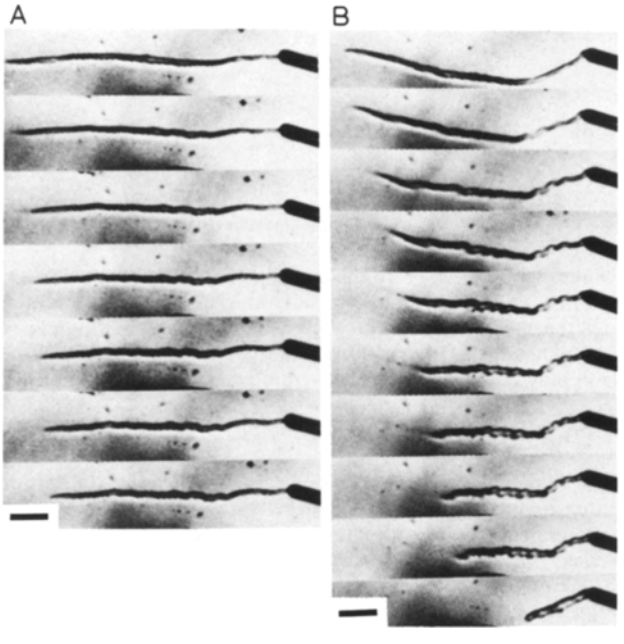

Figure 1. Video images of an isolated cell showing a twitch (A) and a tetanus caused by a repetitive stimulation at 50 Hz (B). In A, stimulus was given at the first (top) frame. In B, repetitive stimulation started at the first frame and continued throughout the sequence shown here. Intervals between the frames were 16.7 ms in A, and 33.3 ms in B, except the last frame in B which was taken 1267 ms after the onset of stimulation. A micropipette used to fix one end of the cell is seen on the right. Bars indicate 100 μm.

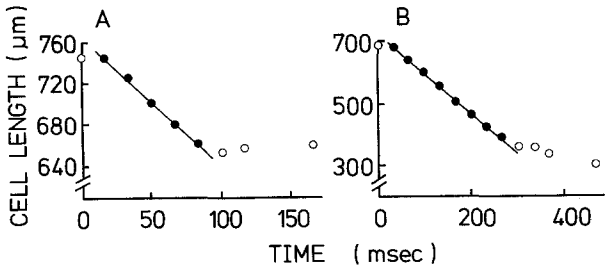

Figure 2. Cell length vs time after the onset of stimulation in a twitch (A) and a tetanus (B) elicited in the same cell. Plotted from the micrographs shown in figure 1. Data shown by filled circles were used for the least-squares regression (straight lines). Shortening velocities were 1090 μm/s (1.46 l_0/s) and 1020 μm/s (1.37 l_0/s) in A and B, respectively.

('maximal stimulation') (data not shown). Such a graded response to submaximal electric stimulations has also been reported in the smooth muscle cells isolated from the toad stomach[8], and is possibly due to small depolarizations caused by the stimulating pulses. The maximal shortening velocities of the isolated PRM cells in twitch and tetanus induced by supramaximal stimulations were 1.27 ± 0.30 and 1.27 ± 0.17 l_0 (mean ± SD, n = 15), respectively. l_0 refers to the optimal length for tension generation, and can be estimated for each cell from its natural length (l_s), since the length-tension relations of the isolated PRM cells have shown that l_0 corresponds to 1.2 times l_s[7]. The similarity of the shortening velocity in twitches to that in tetanuses suggests that, during the contraction, no significant load or resistance to shortening was imposed on the cells. In the isolated PRM cells, the isometric tension is much smaller (by about an order) in twitches than in tetanuses[6,7]. The shortening velocity of twitches must therefore be far more sensitive to any mechanical resistance than that of the tetanuses. Accordingly, the shortening velocity obtained in the present study may be regarded as the maximal shortening velocity (V_{max}) intrinsic to the contractile element.

The V_{max} of the whole PRM has been estimated at 0.4–0.6 l_0/s from the force-velocity relations[7,9]. The present results show that the V_{max} of the isolated cell is more than twice as large as that of the whole muscle. Such a large difference would not be accounted for solely by the cell-to-cell linkages with the effective length of 0.3 l_0 as predicted by the length-tension relations. Another likely factor responsible for the difference in the speed of shortening between the isolated cells and the whole muscle is the presence in the latter of resistive components that are mechanically parallel to the contracting muscle cells. These components include intercellular materials and nerve fibers as well as unstimulated muscle cells, and would have a significant effect on the shortening velocity of the whole muscle by resisting the contractile force, especially when the muscle contracts under a small load. Since the contribution of these components can hardly be assessed by studying the whole muscle, it seems generally important to use isolated cells if we are to determine the V_{max} of the contractile elements in any type of smooth muscle.

Another important characteristic of the contractions of the isolated PRM cells was that the shortening velocity was constant in tetanus even when the cells had shortened to about 50% of their initial length (fig. 2B). To examine the effect of cell length on the shortening velocity, twitches were induced successively in isolated cells at intervals of 10 s so that the cells did not resume their original lengths before the next stimulus was given and the initial lengths from which the contraction started decreased progressively (fig. 3A). Figure 3B shows the relation between the initial lengths and the shortening velocities in four cells. The shortening velocities seem almost constant over the lengths ranging between 0.5 and 0.83 l_0, although the velocities at lengths more than l_s (0.83 l_0) were not measured in the present

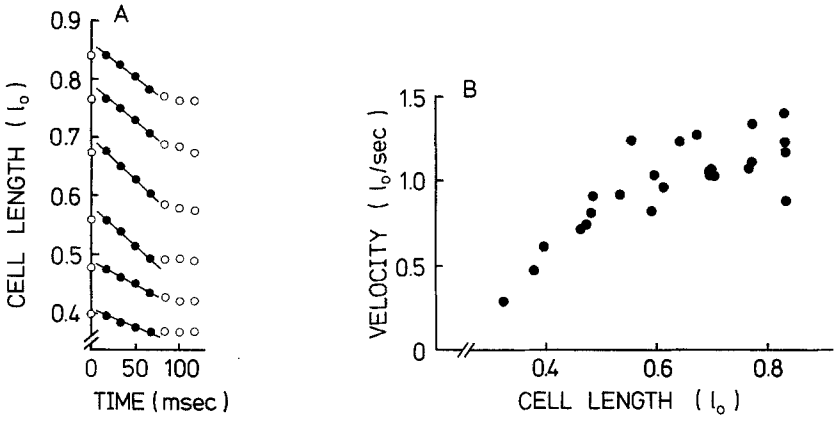

Figure 3. A. Twitches elicited in an isolated cell at various cell lengths. Single electric stimulation was repeatedly applied to the cell at intervals of 10 s so that the initial length from which the cell contracted decreased progressively (shown from top downwards) due to the residual contractions. Straight lines are the least-squares regressions of the data shown by filled circles. l_s, 840 μm. B. Relation between the shortening velocity and the initial cell length obtained from repeatedly induced twitches (as shown in A) in four isolated cells. The lengths and the shortening velocities were normalized to l_0 and l_0/s, respectively.

study because passive tension appeared transiently when the cells were stretched beyond this length[6]. This result shows a marked contrast with the relation between V_{max} and fiber length in striated muscle, in which V_{max} decreases sharply below 0.8 l_0[10]. It also suggests that the intracellular resistance to the contractile force, e.g. collisions between the thick filaments and the dense bodies[7] or increases in the intracellular pressure which might occur as the cells shorten, is negligible at lengths over 0.5 l_0, and the ascending limb of the length-tension relation of the isolated PRM cells depends largely on the amount of overlap between the thick and the thin filaments[6,7].

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Intercellular junctions of hyperplastic retinal pigment epithelium[1]

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Summary. In rats with retinopathies induced by excess fluorescent light or injections of urethane, the retinal pigment epithelium (RPE) undergoes focal hyperplasia. Neither intravascularly injected horseradish peroxidase or lanthanum nitrate penetrated the sensory retina at these hyperplastic sites. Electron microscopy revealed that this was due to the persistence of intact tight junctions among a single layer of hyperplastic cells facing the sensory retina. These junctions prevented intraocularly injected microperoxidase from passing as well. Cells within the hyperplastic foci were connected only by adherent junctions that presented no permeability barrier.
Key words. Retinal pigment epithelium; epithelium; permeability; hyperplasia; pathology.

Epithelia form barriers by means of their intercellular tight junctions. Given the physiologic importance of such barriers, we must consider changes in intercellular junctions and epithelial permeability when diseases affect them. In the eye, for example, a blood-retinal barrier exerted in part by the intercellular tight junctions of the retinal pigment epithelium (RPE) is disrupted in some retinal diseases[4]. These tight junctions and transport systems in the RPE plasma membrane control the passage of molecules and ions into and out of the sensory retina, and are probably important for maintaining photoreceptor function. Thus, we were interested when we noticed that in rats with experimentally induced hyperplasia of the RPE, a condition seen in human retinal disease[5], the RPE still prevented intravascularly injected tracers from entering the sensory retina. This report describes this phenomenon and the reason for its persistence in the face of extensive changes in the RPE sheet: a single layer of tight junctions remained where the hyperplastic focus faced the sensory retina.

Materials and methods. Five albino (Sprague-Dawley) and 9 pigmented (Long-Evans) rats were respectively exposed to fluorescent light or received s.c. injections of urethane by procedures that have been described[6,7]. Phototoxic rats were examined 9–12 months later, and urethane rats 8-12 weeks later. Observations in normal rats were drawn from those used in previous studies[8,9]. Tissue was obtained for electron microscopy by anesthetizing the rats (sodium pentobarbital, 40 mg/kg b.wt, i.p.) and removing the eyes into 2% formaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2--7.4; or by perfusing this fixative