using specific enzymes (EC 1.1.1.27 and EC 1.1.1.28, Boehringer, Mannheim). Blood samples were treated in the same way.

The results given in table 1 indicate that both L(+) and D(−)lactate were present in stomach contents at the remarkable mean concentrations of 6.18 and 4.56 g/l. The variability between individuals was higher for L than for D with coefficients of variation of 22.8% and 13.6%. The feeding ad libitum was assumed to be the main reason for this variability. The ratio of isomers L:D averaged close to 1:0.8 but varied from 1:0.5 to 1.0:1.1. It was thus apparent that D-lactate is normally produced in the stomach of rats, but for induction of metabolic (enzymatic) adaptation the absorption of the isomer as such would be necessary. Blood samples obtained at random from the hearts of 5 of the above animals contained 46±24 µmoles D-lactate/l, which suggested absorption.

Further evidence was provided by a different experiment in which rats were fed a test-meal containing DL-lactate. Groups of 6 rats were sacrificed after 30–180 min and D-lactate concentrations in blood samples from the vena portae and aorta were compared to similar samples from 10 untreated rats (0 min). The mean animal weight was 248±2 g and the mean intake of D-lactate 101±2 µmoles. The results summarized in table 2 show D-lactate already present in zerotime samples, which is in agreement with the observations given above. Within 30 min after lactate feeding portal D-lactate increased 6-fold, indicating rapid absorption. The portal peak level was reached at 90 min with about 10-fold concentration over normal, and after 180 min most of the stomach lactate appeared to have been eliminated. The comparison with the concentration of D-lactate in aortal blood indicates metabolism of this isomer. It is worth noting that within 30–120 min after lactate intake the D-lactate concentration in portal blood was about 2–3 times higher than in aortal blood. Thus the metabolic rate seemed to keep pace with absorption.

In conclusion, D-lactate appears to be a physiological isomer in the rat. This general result would explain the metabolic adaptation of rats to D-lactate reported previously[5], and may also indicate - at least for the rat - the physiological role of D(−)2-hydroxy acid dehydrogenase (E.C. 1.1.99.6) which was questioned up to now[7]. Furthermore, our data suggest that gastrointestinal production of D-lactate in monogastric mammals is more likely than intermediary generation of this isomer by glyoxalase activity[2]. Similar conditions to those shown for the rat may also exist in other monogastric mammals with a nonsecretory gastric (or pregastric) compartment.

Table 2. Concentrations of D(−) lactic acid in the blood after a test-meal

Time after test-meal (min)	Blood concentration of D(−) lactate (mmoles/l)* Vena portae	Aorta	Difference
0	0.31±0.13	0.27±0.14	0.04
30	1.86±1.03	0.64±0.17	1.22
60	1.57±0.60	0.53±0.27	1.04
90	2.94±1.62	1.19±0.33	1.75
120	1.40±0.60	0.76±0.20	0.64
180	0.77±0.47	0.71±0.51	0.06

* Results are means ± SD.

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Voltage clamp method on single cardiac cells from adult rat heart

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Summary. Voltage clamp experiments on isolated cardiac cells from adult rat hearts were carried out using an intracellular dialysis method. The fast inward current was recorded. Tetrodotoxin (TTX) at a concentration of 2.5×10^{-7} g/ml blocked this current to 30% of its initial value. Inward maximal fast current density was calculated to be 0.14–0.7 mA/cm^2.

The multicellular structure of myocardial preparations used in voltage clamp experiments is the main difficulty for qualitative analysis of membrane ionic currents[2-4]. Therefore, the purpose of the present paper was to record ionic currents on single cardiac cells using the intracellular dialysis technique[5].

Materials and methods. The experiments were performed on single myocytes from adult rat hearts isolated by a method described previously[6]. Disaggregation of the heart tissue was carried out by treatment with collagenase at a concentration of 0.8 mg/ml (type 1, Sigma) and Ca^{2+} free buffer at 37 °C by Langendorff perfusion. Cell suspensions were kept at room temperature in 5 ml buffer (pH 7.4) containing (in mM): 118 NaCl, 4.8 KCl, 0.9 $CaCl_2$, 1.2 $MgSO_4$, 25 $NaHCO_3$, 1 mg/ml bovine amniotic fluid (Serva), gently bubbled with 95% O_2–5% CO_2. This solution was designated in experiments as 'extracellular' solution. Cardiac cells were viable for 5–9 h. In the voltage clamp experiments we used the method of intracellular dialysis used for the neuroblastoma cells[5]. V-shaped plastic tubes with a conical pore on the tip were made for cardiac cell dialysis. The dimensions of the pore were: outer diameter 20–30 µm, inner diameter 10–15 µm. The pore was covered with an adhesive material consisting of 40% vaselin oil and 60% parafilm (USA) prepared by heating for about 1 h in a water bath. The tube was perfused with 'intracellular' solution (150 mM tris-PO_4, pH 7.3), reference and current passing electrodes (Ag-AgCl/3 M KCl) were placed in the output branch of the tube. The tip of the tube was immersed in the experimental chamber perfused with the

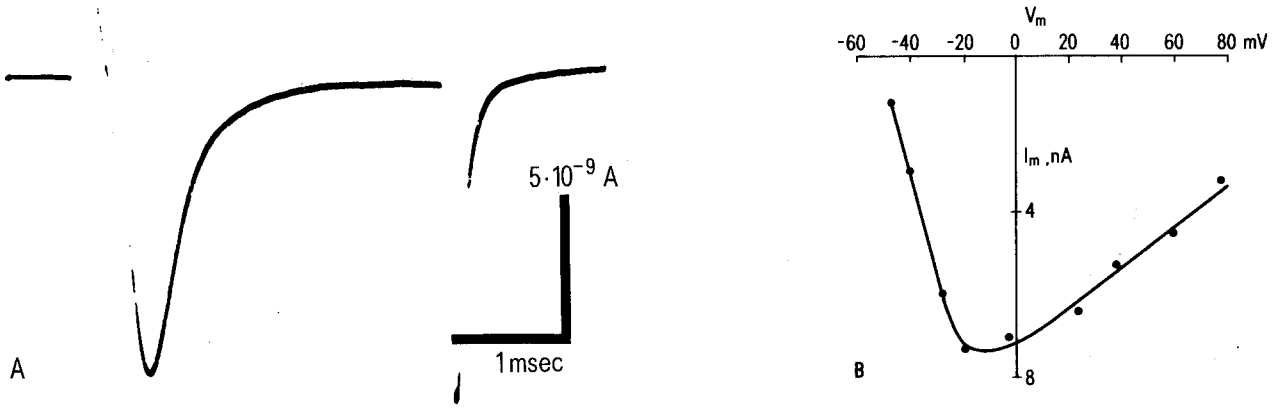

Fig. 1. The fast inward current in a single cardiac cell from adult rat heart. *A* Original recording of the fast inward current in response to membrane depolarization to −28 mV. Holding potential $V_h = -90$ mV. *B* Current-voltage relationship of the fast inward current $V_h = -90$ mV. Outside: Ca^{2+}-free buffer; inside: tris-phosphate.

extracellular solution. A few cells were then placed in the chamber and one of them was sucked on to the pore under visual control (inverted microscope, Diavert, Leitz). A suction effect was created with negative hydrostatic pressure inside the tube in comparison with the extracellular solution. After the cell was fixed in the pore part of the membrane was destroyed by a short more negative hydrostatic pressure jump. For voltage clamping and registration of ionic currents we used a special electronic circuit with leakage current and series resistance compensation. Ionic currents and membrane potential were recorded on a dual beam storage oscilloscope (5103N, D-13, Tektronix), equipped with a Polaroid camera (C-5A, Tektronix). For command pulse and holding potential supply 2-channel stimulator (302-T, WPI) was used. The cells were stimulated at 15/min.

Results and discussion. The dimensions of cells used in experiments were: 15–20 µm in width and 70–90 µm in length. About 90% of the living cells contracted spontaneously in the presence of 0.9 mM Ca^{2+}. To suppress cell contraction, Ca^{2+} from the extracellular solution was eliminated, since cell movements in the pore enhanced the leakage current. The experiments were performed at room temperature (20–22 °C).

The membrane potential was held at −90 mV. Figure 1,A shows a typical recording of the fast inward ionic current in response to a depolarization step. Duration of the capacity current was 300–400 µsec. Similar duration of the capacity current was obtained in the recent study carried out on the single myocardial cells of the rat[7]. The current-voltage relationship of the fast inward current is plotted in figure 1,B. In order to identify the fast inward current TTX (2.5×10^{-7} g/ml) was added to the extracellular solution. As shown in figure 2 TTX blocked the fast inward current to 30% of its initial value. Higher doses of the TTX (up to 5×10^{-6} g/ml) completely blocked this current within 1 min. The maximal value of the fast inward current varied between 2.4×10^{-9} A and 1.4×10^{-8} A (n=6). Average density of the maximum current was calculated to be 0.14 mA/cm²–0.7 mA/cm² (n=6), if we assume that the working surface of the membrane is cylindrical with a diameter of 15 µm and length of 30 µm. The membrane capacity (C_m) was calculated from capacitive transient in response to negative step of potential of 12 mV from holding potential and was found to be in the range between 3.32 µF/cm² and 12.8 µF/cm² (n=7). An average membrane capacity was calculated to be 9.5 ± 3.2 µF/cm² (mean ± SE, n=7). As discussed above, the membrane

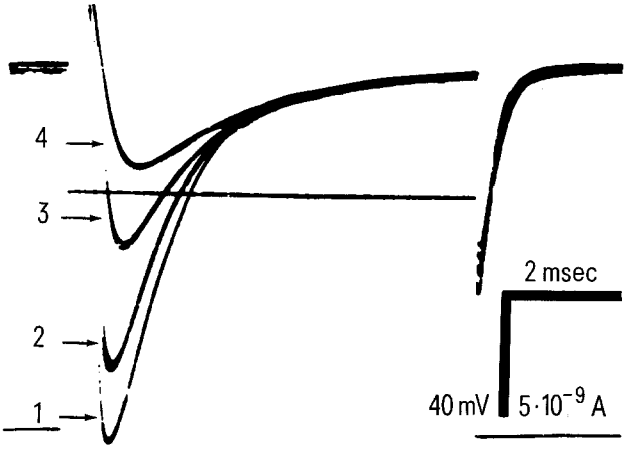

Fig. 2. Action of TTX (2.5×10^{-7} g/ml) on the fast inward current (upper traces). $V_h = -90$ mV, depolarization to −26 mV (lower trace). TTX was added to the extracellular solution. *1* Control; *2, 3, 4:* 0.5, 1, 1.5 min of TTX action. Outside: Ca^{2+}-free buffer, inside: tris-phosphate.

surface was assumed to be cylindrical in the C_m calculations. It should be pointed out that in all 7 cases 1 exponent with the time constant $\tau = 58.5\pm8.1$ µS (mean ± SE, n=7) was required to describe the capacitive transient.

The membrane current I_m can be expressed as $I_m = C_m$ dV/dt (Beeler et al.[3]), where dV/dt is the upstroke velocity of action potential. Combination of our magnitude of C_m with the dV/dt_{max} obtained in the rat myocardium[8] of 112 V/sec gives an expected inward current density in the range between 0.37 mA/cm² and 1.13 mA/cm².

The estimated membrane conductivity of the maximal current was $1.4\text{–}8.7\times10^{-3}$ S/cm² (n=6). Reactivation time constant τ_{re} of the fast current was found to be 10–50 msec (n=5).

In the experiment illustrated in figure 1,B we could not establish the reversal potential when the intracellular solution contained no Na^+. However, to test the efficiency of intracellular perfusion we varied the sodium intracellular concentration ($[Na]_{i_1} = 20$ mM; $[Na]_{i_2} = 50$ mM) and measured the value of the maximal inward sodium current I_{Na}. External sodium concentration $[Na]_o$ was 143 mM. I_{Na} is described as $I_{Na} = g_{Na}\,(V_m - E_{Na})$[9], where g_{Na} is the conductance for sodium ions, V_m is membrane potential, E_{Na} is reversal potential for the sodium ions.

If we assume, that g_{Na} is constant at various $[Na]_i$ then

$$I_{Na_1}/I_{Na_2}=(V_m-E_{Na_1})/(V_m-E_{Na_2}),$$

where I_{Na_1}, I_{Na_2} are maximal values of I_{Na} and E_{Na_1}, E_{Na_2} are the reversal potential at various $[Na]_i$. Reversal potential for the sodium ions was calculated from Nernst's equation:

$$E_{Na}=RT/F \cdot \ln [Na]_o/[Na]_i.$$

For these conditions the experimental value of I_{Na_1}/I_{Na_2} was found to be 1.21 and its calculated value obtained by using the Nernst's equations to determine $(V_m-E_{Na_1})/(V_m-E_{Na_2})$ was equal to 1.32. Therefore, we assume that this satisfactory agreement between the experimental data and the theoretical predictions points to an adequate intracellular perfusion.

Cardiac cells isolated enzymically from adult rat hearts are morphologically intact[6,10] and maintain electrical activity. The electrical response of the isolated cells appears to be similar to that observed in intact tissue; action potentials recorded from these cells exhibit fast upstrokes and low plateaus[11]. The fast upstroke is probably due to the fast inward TTX sensitive current identified in the present paper.

The results obtained in this study show the possibility of applying a voltage-clamp method to single cardiac cells. Combination of a voltage-clamp method with the intracellular dialysis technique may have advantages in studies of the dependence of the membrane ionic currents upon the intracellular medium.

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Cone myoid elongation and rod myoid contraction are inhibited by colchicine in the trout retina

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Summary. Retinal photoreceptors of lower vertebrates undergo photomechanical changes (elongation or shortening) in response to light or dark. Colchicine, a microtubule-disrupting drug, blocks cone, but not rod elongation. Instead, rod shortening is blocked by this drug, thus suggesting that different mechanisms mediating these responses are involved in rods and cones.

A remarkable adaptive feature of eyes in lower vertebrates is the translocation of photoreceptor (rod and cone) inner and outer segments and retinal epithelium pigment granules, seemingly to control the amount of light to which the photosensitive elements are exposed under various photic conditions. These movements are collectively called 'retinomotor responses' and have been extensively studied in the past 60 years [2-4]. The movements of rods and cones occur in opposite directions and appear to be mediated by contraction and elongation of the myoid, a specialized region of the photoreceptors located between the ellipsoid and the perikaryon.

The exact nature of the subcellular or supramolecular mechanisms underlying these movements is still poorly understood[4]. Couillard[5] and Burnside[6] proposed models based on studies of motility in unicellular organisms. In these proposals, a cooperative interplay of microfilament-mediated contraction and microtubule-mediated elongation accounts for these responses in photoreceptor myoids. Substantial evidence in favour of this model came from studies in which the presence of actin-like and myosin-like filaments, as well as microtubules, were reported in cone myoids and ellipsoids[6,7]. Moreover, it was shown that cytochalasin B prevents cone contraction during the dark-to-light transition, whereas colchicine disrupts microtubules and prevents cone elongation during the reverse transition in the retina of a fish capable of these responses[6,8,9].

The latter experiments allowed observations on cones only. Therefore, it remains crucial to know whether rod translocations are mediated by mechanisms similar to those postulated for cones. We report here that the retinomotor behaviour of rods in the trout retina is affected by colchicine in a manner opposite to that described in cones, thus suggesting that different mechanisms may be involved.

Young specimens of brook trout, *Salvelinus fontinalis* (14–15 cm in length), were kept for 1 week in a recirculating water tank at 8–10 °C prior to experiments, with a 12-h light period. The irradiance at the water surface was about 17.5 mW/cm^2. The right eye of each fish was injected intraocularly with 50 µl of 1 mM colchicine (Sigma) dis-

Rod and cone positions in the photoreceptor layer of light- and dark-adapted trout retina

	Dark → light		Light → dark	
	Cones	Rods	Cones	Rods
Control	0.30 ± 0.02	0.39 ± 0.06	0.56 ± 0.06	0.14 ± 0.04
Colchicine	0.29 ± 0.03	0.36 ± 0.05	0.27 ± 0.03	0.32 ± 0.03

Measurements of the distance between the external limiting membrane (ELM) and Bruch's membrane were made in 2 fish per experiment. The positions of double cone outer segment tips and rod ellipsoid tips within this space were expressed as ratios of the total distance. Owing to the dispersion of rods in 2 superimposed layers, only those within the vitread - most 30 µm from the ELM were measured. Each value represents the mean (± SD) of 10 measurements performed in transverse sections of ventral retina, at distances of every other 10 µm within a space of 200 µm.