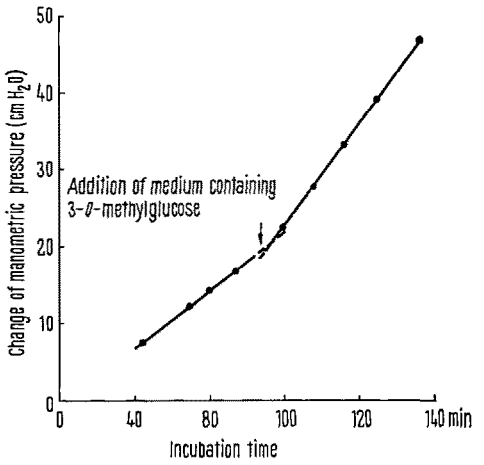
A 'two-compartment' diver technique, originally described by HAMBERGER[3], was used to study the respiratory rate of intestinal cells before and after addition of e.g. 3-*O*-methyl-glucose to the incubation medium. This was made possible by placing a capillary tube in the upper opening of the micro-diver. The capillary, sealed in its upper end by wax, contained the solution to be added to the original incubation medium. The 2 solutions were separated from each other by an air lock. Mixing of the 2 solutions was accomplished by lowering pressure in the floatation vessel, which expanded an air bubble in the upper end of the capillary tube and pushed the capillary solution into the incubation medium. The technique has been described in more detail elsewhere[3].

Incubation media: a) Na_2HPO_4-KH_2PO_4 buffer, pH 7.4, 37.5 m*M*; $AlCl_3$ 0.5 m*M*; $MgCl_2$ 0.5 m*M*; Na succinate 25 m*M*; cytochrome c 8.6×10^{-2} m*M*.

b) *Tris* HCl buffer, pH 7.4, 25 m*M*; NaCl 124 m*M*; KCl 5 m*M*; $CaCl_2$ 0.15 m*M*; $MgSO_4$ 0.5 m*M*; Na succinate 6 m*M*.

Results. The respiratory rates of cells from the tips and from the bases of villi were compared in 5 experiments. It was demonstrated that the oxygen consumption per unit area of the cell cluster was approximately twice as great for 'base cells' as for 'tip cells' ($(41 \pm 4.8) \times 10^{-4}$ and $(19 \pm 4.6) \times 10^{-4}$ µl/h × unit area, respectively; mean ± S.E.M., $n = 12$). In these experiments the incubation medium containing cytochrome c was used.

In 4 experiments the effect of adding 3-*O*-methylglucose (20 m*M* solution) to the incubation medium (*Tris* HCl buffer) was investigated. The final concentration of 3-*O*-methylglucose was approximately 5 m*M*. This produced an increased rate of oxygen consumption as illustrated in Figure 1.



The effect of 3-*O*-methylglucose on the respiratory rate of isolated intestinal epithelial cells. The experiment was performed with a 'two-compartment diver'[3]. The oxygen uptake is expressed as change in manometric pressure.

Discussion. During recent years several studies on isolated intestinal cells have been reported[4]. However, in none of these previous investigations have cells from different parts of the villi been isolated and studied separately. In this paper such methods are described and it proved possible to isolate and study oxygen consumption of intestinal cells from tips and bases of villi. Furthermore, these cells seem to be fairly intact since 3-*O*-methylglucose, a carbohydrate actively transported into cells but not metabolized[5], added to the incubation medium increased the rate of oxygen consumption of the cells. This may possibly reflect an augmented metabolic activity induced by the active transport of the sugar across the cell wall. With the present technique it thus seems to be possible to isolate and study the respiration of intestinal cells from different levels of the villi from the various consecutive sections of the intestinal tract before and after addition of different solutes[6].

Zusammenfassung. Der Sauerstoffverbrauch in isolierten Epithelzellen aus dem Dünndarm von Ratten wurde mit dem Kartesischen Taucher untersucht. Die Zellen aus den Villusbasen zeigten einen höheren Sauerstoffverbrauch als Zellen von den Villusspitzen.

L. HAMBERGER and O. LUNDGREN

Department of Physiology, University of Göteborg, S-40033 Göteborg 33 (Sweden), 1 July 1970.

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Effects of Drugs and Ions on a Primitive System of Spontaneous Contractions in a Sponge (*Euspongia officinalis*)

In Sponges, contractile responses of the oscular membrane following stimulation have been known for a long time[1–5]. Recent time-lapse cinematography of oscula indicates[6] that besides induced contractions Porifera show two discrete patterns of spontaneous activity: a) Short-term contractions arise here and there on the oscular membrane as rhythmic local pulsations (6 or 7 per h) which are occasionally propagated at 1 mm/min but are usually confined to restricted groups of cells (0.2 mm²). They last about 30 sec and take the form of slow, localized shrinkings. b) Long-term contractions (5 or 6 per 24 h) are synchronous and bring about tonic closure of the whole osculum for several minutes. Larger areas (5 mm²) are involved.

Only one type of contractile cells is revealed by electron microscopy[7–9], i.e. the mesenchymatous cells. They have microfilaments and form a network of connected elements[10]. The responses of these cells are reported to be unaffected by acetylcholine and adrenaline[4] and by the substitution of Na^+ by K^+ and of Ca^{++} by Mg^{++} in the external medium[5]. In order to see whether spontaneous and induced contractions behave in the same way, we

have now repeated these experiments while recording spontaneous contractions of the osculum.

The marine sponge *Euspongia officinalis*, Linné 1759, has numerous small oscular hillocks on its surface each of which is closed by a thin contractile sphincter membrane 2–3 mm in diameter. Excized oscular hillocks were set up under a light microscope (magnification × 35 or 100) in a small glass flow-chamber with constant light and temperature (20 °C). A slow stream of water ensured oxygenation and renewal of the medium without interrupting time-lapse cinematography (1 frame per 12 to 15 sec). 39 experiments were performed on 6 sponges collected by diving at Villefranche-sur-mer (France).

Acetylcholine and adrenaline. At concentrations of $10^{-5}M$ or $10^{-6}M$ these drugs act in the same way. They have no effect on the diameter of the osculum, which remains as at the beginning of the experiment (as reported by EMSON[4]). However, both acetylcholine and adrenaline increase the frequency and propagation of short-term contractions, which can increase from 6 or 7 local shrinkings to 15–17 propagated waves per h.

When acetylcholine and adrenaline act in succession (1 h each), the osculum shows a generalized tetanus-like closure as soon as the second drug reaches it. Short-term contractions disappear and are seen again only when normal sea water is replaced and the osculum is relaxing.

We conclude that acetylcholine and adrenaline may act as synergistic excitators of spontaneous rhythmic contractions. Such action on smooth muscle is not unusual either in invertebrates or vertebrates[11,12].

Parallel studies of material prepared for electron microscopy suggest that these 2 drugs may operate in the same way on the contractile cells. They induce depletion of all their large cytoplasmic vesicles (0.5–1.5 μ) which are normally filled with a homogenous and fairly electron-dense substance (Figure), and which have been proved to contain glycoproteins also bound to the cell coat and collagen filaments in extracellular spaces[13]. In normal sea water, extrusion of this material is observed only from some of the vesicles in some of the cells[10]. Here, whether due to the direct action of the drugs or to the increase of contraction frequency or to both, the depletion of vesicles would appear to confirm that there is some relation between short-term spontaneous contractions and the release and circulation of secretions (glycoproteins only?) through the intercellular spaces of the mesenchyme[6,10].

Sodium and potassium. In artificial sea water (according to PROSSER[5]), when all the Na^+ is omitted and an equiosmolar amount of sucrose or D-mannitol substituted for it, short- and long-term contractions fail to appear. The open osculum remains in a steady state and then all the contractile system appears to be disturbed for long enough to bring about persistent closure of the whole osculum as soon as normal sea water is replaced.

Similar disturbances of spontaneous contractions and recovery arose when we substituted K^+ for all the Na^+

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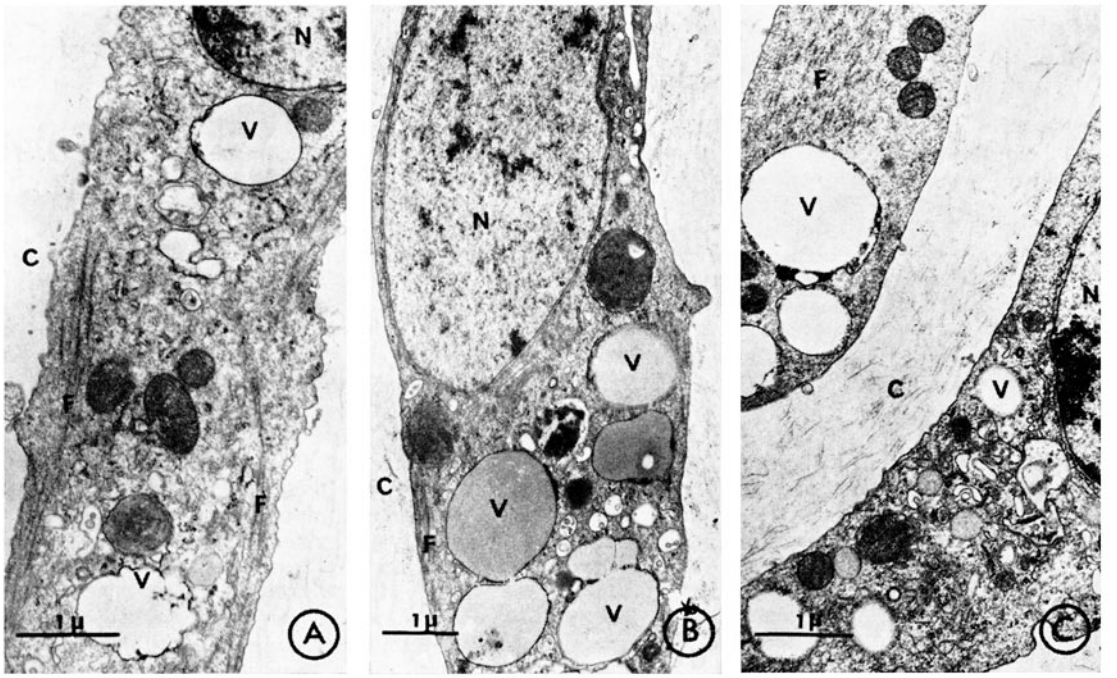

Mesenchymatous contractile cells. A) After 2 h in $10^{-5}M$ adrenaline (vesicles depleted). B) Control in normal seawater (glycoproteins containing vesicles filled). C) After 2 h in $10^{-5}M$ acetylcholine (vesicles depleted). C, collagen. F, microfilaments. N, nucleus. V, vesicles.

(with an excess of KCl in amount equiosmolar to the substituted NaCl). But this time, the substitution quickly induces the osculum to close with a contracture which later reverses in normal sea water only if it is not too prolonged (15 min).

Thus we cannot prove any equivalence of Na^+ and K^+ with regard to spontaneous activity and we can conclude only that pacemakers need Na^+, and that after the balance of monovalent ions has been disturbed the restoration of spontaneous activity is slow.

Calcium and magnesium. When calcium is omitted from artificial sea-water (and in view of its low concentration in normal sea water, not compensated for) short-term spontaneous contractions cease. An obvious feature is the constancy with which they are restored when normal sea water is replaced. However, during the action of Ca^{++}-free sea water, the steady state obtained may represent either the stable closure of a previously open osculum or the relaxation of a previously closed one.

Moreover, the total substitution of Mg^{++} for Ca^{++} (an excess of $MgCl_2$ for the $CaCl_2$ removed) causes the osculum to close.

Here again, we cannot prove any equivalence of Ca^{++} and Mg^{++} with regard to spontaneous activity. We can conclude only the Ca^{++} requirement for short-term contractions, and the easy recovery of pacemakers as soon as normal sea water is replaced after Ca^{++}-free medium.

Conclusions. The results of our experiments do not disagree with PROSSER's[5] report which points out 'the striking variant of ionic requirements for contractile responses' in sponges, and which leads the author to conclude that action potentials (never displayed) do not appear to be essential. But, from a comparative point of view, it is interesting to draw a parallel between that singularity of the contractile cells of sponges, and their classical features.

Indeed PROSSER's analysis and calculations show that in respect of organic solute concentrations 'sponge cells resemble the nerves and muscles of many marine invertebrates'. Similarly he reports 'ionic gradients which are in the same direction as in muscle' and gives a theoretical resting potential of 30–65 mV. For our part, our results emphasize the following points: the synergystic excitatory effects of acetylcholine and adrenaline on spontaneous short-term contractions related to the depletion of glycoproteins containing vesicles: the Na^+ and Ca^{++} requirements of the contractile pacemaker system[14].

Résumé. Chez *Euspongia officinalis*, l'acetylcholine et l'adrénaline agissent comme excitateurs des contractions spontanées, localisées et brèves, et favorisent en outre l'extrusion des glycoprotéines contenues dans les cellules contractiles. Na^+ et Ca^{++} sont nécessaires au fonctionnement des pacemakers.

M. PAVANS DE CECCATTY

Laboratoire de Biologie Tissulaire, Faculté des Sciences de Lyon, 16, Quai Claude Bernard, F-69 Lyon (France), 25 May 1970.

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The Lanthanides Ho^{3+} and Pr^{3+} as Inhibitors of Calcium Transport in Human Red Cells

MELA and CHANCE[1] have recently reported that the three-valent cations holmium (Ho^{3+}) and praseodymium (Pr^{3+}) inhibit the Ca uptake into mitochondria. The present work demonstrates that ATP-dependent outward Ca transport from human red cells is likewise blocked by these cations, although higher concentrations are required than in mitochondria.

$10^{-2}M$[2] stock solutions of the chlorides of Ho^{3+} and Pr^{3+} were prepared by boiling the oxides Ho_2O_3 and Pr_6O_{11} (Fluka, 99.9% purity) with twice the equivalent amount of $1N$ HCl and neutralizing with *tris* base to pH 7.

The ATP-dependent Ca transport was assayed in resealed ghosts. Human red cells contained in a small volume of citrated plasma were obtained one day after collection of the blood from the laboratory of the blood bank of the Swiss Red Cross. The cells were washed 4 times with saline at room temperature and the white cells were discarded. The red cells were hemolyzed at room temperature in a 5-fold volume of water containing 0.75 mM $CaCl_2$, 4 mM $MgCl_2$, 5 mM *tris*-Cl and 2 mM Na_2-ATP (Boehringer) neutralized with *tris*. After 135 sec the mixture was made isotonic by adding enough $3M$ KCl. The resealed cells were washed once without delay with ice-cold medium used in the transport experiment [(mM) 130 Na, 5 K, 2 Mg, 1 Ca, 20 *tris* as chlorides, pH 7.4]. Final suspensions had a hematocrit of about 0.3 and were incubated at 28 °C, samples being taken at intervals. Sampled cells were packed by centrifugation and processed without washing. An aliquot of packed cells was deproteinized with an equal volume of 10% trichloroacetic acid and an appropriate dilution of the filtrate was supplemented with 50 mM lanthanum-chloride for absorption flame photometry on an EEL instrument. The medium was treated in the same way.

Ho and Pr were present both in the hemolyzing fluid and in the washing and incubating medium. Concentrations are calculated from the added amount, possible complex formation with ATP being disregarded. Concentration-effect curves were obtained by measuring the Ca content of cells and medium at 0 and 15 min (see insert Figure 1). The change of the ratio [Ca]medium/[Ca]cells taking place during 15 min was taken as an estimate of the activity of the Ca-pump[3]. Full inhibition was reached with $10^{-3}M$ $HoCl_3$ or $PrCl_3$, the Ca movement being abolished completely at this concentration (insert Figure 1).

Red cell membranes were prepared by a method similar to that of GARRAHAN et al.[4]. 20 ml of washed cells were

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