(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.

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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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hemolyzed at room temperature in 1000 ml of 30 m*M* *tris*-Cl pH 7.0 with 1 m*M* *tris*-EDTA. The ghosts were washed 4 times with 15 m*M* *tris*-Cl pH 7.0 at 5 °C and finally frozen within a polyethylene tube in solid CO_2-ethanol and thawed slowly. They were stored for up to 1 week in 15 m*M* *tris*-Cl pH 7.0 at 2 °C. The suspensions contained about 3 mg protein per ml. Mg + Ca requiring ATPase activity of these membranes was assayed in the following medium: (m*M*) *tris* 60, choline 100, Mg 5, all as chlorides, Na_2-ATP 2, pH 7.4 or 6.9. Concentrations of Ca, Ho and Pr were varied. Ca-free, lanthanide-free controls contained 0.5 m*M* *tris*-EGTA. 0.2 ml of stroma suspension were incubated in 2.5 ml total volume at 37 °C for 1 h. ATPase activity was estimated by measuring the liberated inorganic phosphate (P_i) according to BERENBLUM and CHAIN[5]. Protein was determined according to the method of LOWRY et al.[6]. The K-free medium with low Na content (4 m*M*) was chosen in order to suppress the ATP-ase activity requiring Mg + Ca + Na or K[7] which is probably irrelevant for Ca transport. Mg + Na + K activated membrane ATPase was assayed in the same medium but replacing choline by 90 m*M* Na + 10 m*M* K and using 10^{-4} g/ml ouabain as inhibitor.

Figure 1 shows concentration effect curves for the inhibition of Ca transport by Ho^{3+} and Pr^{3+}. At Ho^{3+} concentrations above $2.5 \times 10^{-4} M$ the media turned

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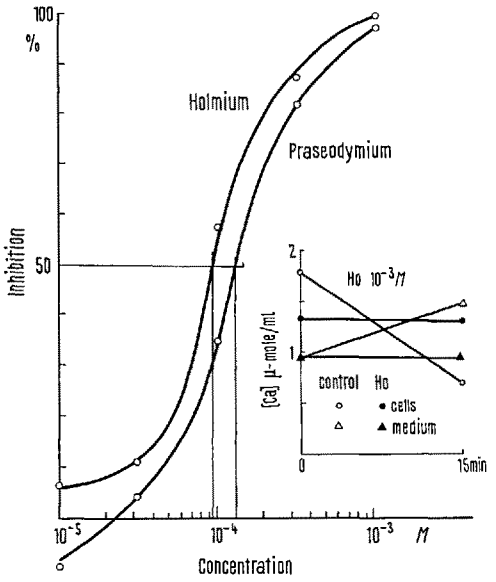

Fig. 1. Inhibition of active Ca transport in resealed human red cells by Ho^{3+} and Pr^{3+}. For method see text. Ho and Pr were present in hemolyzing fluid and medium. Abscissa: Ho and Pr concentration added to the samples. The insert shows the type of experiment used to construct the concentration effect curves. Open symbols = control; note uphill movement of Ca, leading to reversal of gradient between cells and medium. Closed symbols = sample with $10^{-3} M$ Ho. Temperature 28 °C, incubation time 15 min.

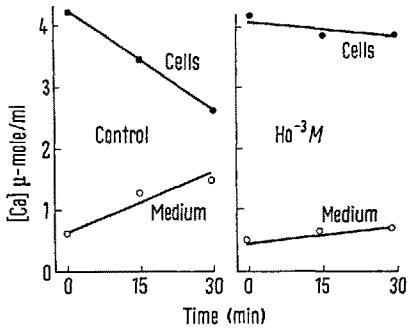

Fig. 2. Ca transport experiment as insert of Figure 1. Medium as described in text. The cells were loaded in a hemolyzing fluid containing 5 m*M* Ca, the Ca-concentration of the medium was reduced. The large gradient thus produced is nearly maintained in cells poisoned with Ho.

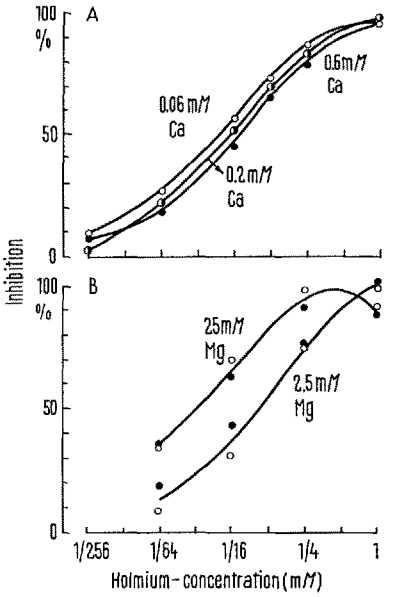

Fig. 3. A) Inhibition of Ca + Mg activated membrane ATPase by Ho. Medium (m*M*): Na 4, K 0, Mg 5, *tris* 60, choline 100, pH 6.9. Ca-concentration varied. 37 °C, incubation 1 h. 100% = difference between Ho-free sample at given Ca-concentration and sample with 0.5 m*M* EGTA. B) Same as A) with Ca-concentration 0.2 m*M*, pH 7.4. Two experiments with 2 different blood samples (dots and circles). Circles: Mg 25 m*M* replaces 25 m*M* choline. Dots: Mg 25 m*M* added without compensation.

Inhibition of membrane ATPases by $3.16 \times 10^{-4} M$ $HoCl_3$

Type of activation	µmole P_i/mg protein/h	
	Control	$3.16 \times 10^{-4} M$ $HoCl_3$
Mg	0.27	0.29
Mg + Ca	0.49	0.04
Mg + Ca + K	0.30	0.05
Mg + Na + K	0.21	0.0

Medium: (m*M*) *tris*-Cl 60, choline-Cl or KCl 100, or Na 90 + K 10, $MgCl_2$ 5, $CaCl_2$ 0.5, Na_2-ATP 2; pH 7.4, temperature 37 °C, incubation time 60 min, sample volume 2.5 ml, protein content of sample 0.25 mg/ml. Mg + Na + K-ATPase was tested by omitting Na and K and by adding 10^{-4} g/ml ouabain. 4 m*M* Na was always present due to Na_2-ATP. Ionic strength was maintained by choline in the absence of Na or K.

slightly opalescent. Nevertheless, complete inhibition was achieved only with $10^{-3}M$ suggesting that the concentration of the active form, which probably is the free cation, kept increasing beyond this point. The concentrations for 50% inhibition in these 2 experiments were $0.93\times10^{-4}M$ for Ho and $1.45\times10^{-4}M$ for Pr. Figure 2 demonstrates that failure to extrude Ca under the influence of the lanthanides reflects an interference with the transport mechanism and is not due to leakage of Ca from the cells. In this experiment an unusually large gradient of Ca from inside to outside was set up. It can be seen that this gradient remains nearly constant during 30 min in the cells poisoned with Ho, whereas in the control sample Ca leaves the cells in a downhill direction.

Figure 3A shows concentration-effect curves for the inhibition by Ho of the part of the Mg + Ca requiring ATPase not dependent on Na or K. The curves differ in position relative to the Ho concentration axis by less than a factor of two when the Ca concentration is varied by a factor of 10. At Ca 0.06 mM the apparent K_m for Ho was $0.67\times10^{-4}M$ and at Ca 0.6 mM it was $0.87\times10^{-4}M$ in this experiment. These values indicate that the affinity for Ho of the particular Ca + Mg activated ATPase system in the membrane and of the Ca transport system are quite similar. Since full inhibition is obtained with 1 mM Ho the possibility can be ruled out that complex formation with ATP, present in 2 mM concentration, is the cause. The parallel displacement to the right of the curves by Ca is compatible with, but does not conclusively prove competition between Ca and Ho for some site which, when occupied by Ca, is activated. The range of Ca concentrations accessible for such studies is narrow because the activation curve for Ca does not reach a plateau but declines at concentrations above 0.3–0.5 mM. Figure 3B shows that increasing the Mg-concentration, rather surprisingly, shifts the curve to the left.

In summary the 2 three-valent lanthanides tested inhibit both the active uphill Ca transport from resealed red cells and a membrane ATPase activated by Ca which, at least in part, might be involved in this transport. However, as can be learned from the experiment shown in the Table, other ATPases of the membrane, including the classical Na + K stimulated fraction, are inhibited as well. The enzymic activity requiring only Mg seems not to be affected even by high concentrations of Ho^{3+}. The insensitivity of the Mg-ATPase and the fact that Mg does not counteract but enhances the action of Ho^{3+} on the Ca + Mg activated ATPase (Figure 3B) suggest that the Mg-ATP accepting site in the membrane is not a likely point of attack of Ho^{3+}. It might be possible that the lanthanides interfere with the site specific for Ca. This assumption, however, leaves the question open as to why the Mg + Na + K activated ATPase is also affected.

The present finding affords, if nothing else, a convenient experimental tool to block the Ca transport mechanism without interferring with the passive permeability of the membrane for Ca.

Zusammenfassung. Die Lanthanide Holmium^{III} und Praseodymium^{III} hemmen den aktiven Ca-Transport der Erythrozytenmembran. Eine Konzentration von etwa $10^{-4}M$ ergibt 50% Hemmung. Eine Ca + Mg aktivierte Membran-ATPase des Erythrozyten zeigt gegenüber diesen beiden Kationen ungefähr die gleiche Empfindlichkeit wie der Transport.

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Changes in Intestinal Absorption of Glucose in Rats Treated with Ethanol

The majority of the clinical and experimental investigations which have been carried out so far on alcoholism are concerned with structural, functional and metabolic alterations elicited by ethanol in the liver[1-5], cardiocirculatory system[6,7] and central nervous system[8,9]. Conversely, little is known about possible variations in the intestinal absorption process which are brought about by ethanol. The purpose of the research project described in this paper was to ascertain whether subacute oral ethanol poisoning gives rise to alterations of glucose intestinal absorption in rats.

Materials and methods. The investigation was carried out on male albino rats of the Sprague-Dawley strain, weighing on average 180 g. The animals were divided into 4 groups. The rats in the first group were not treated with ethanol and were used as controls. The rats in the 2nd, 3rd and 4th groups were pre-treated with 40% ethanol for periods of 2, 7 and 20 days respectively. The alcohol was administered by gastric tube at a dose of 4 g/kg/day. All the rats were fed ad libitum. The experiment was carried out after 5 h fasting in the rats of the first group, and 5 h after the last ethanol administration in those of the 2nd, 3rd and 4th groups which had also fasted for 5 h.

The rats were anaesthetized with ethylurethane (1 g/kg i.p.) and then laparatomized. The small intestine was closed at the level of the duodenum and the ileum-cecal valve; 5 ml of a 20% solution of pure glucose were injected into the intestine. Each animal was re-opened 1 h after glucose administration. The small intestine, approximately 2 ml of aortic blood, and about 500 mg of liver tissue were taken from every animal.

Glycemia was determined in the blood by the Hugget and Nixon[10] enzymatic method.

Residual glucose in small intestine. The small intestine was washed and the washing water added to the contents

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