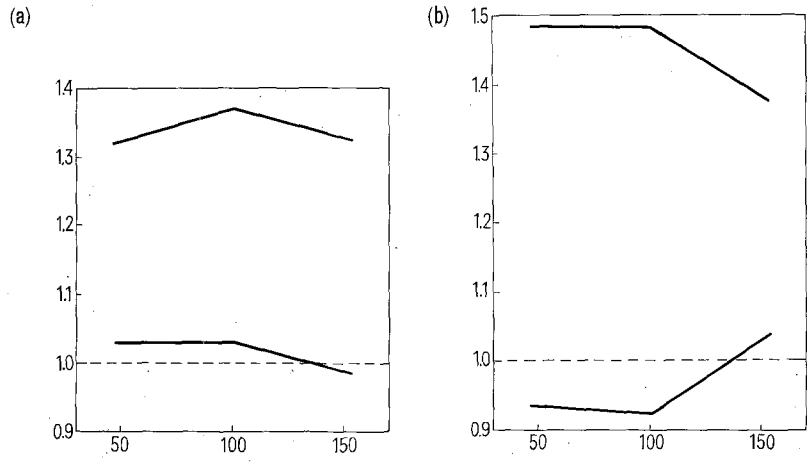

Fig. 2. 'Inoculum ratios' inactive/active (a) and inactive/non-infected (b) obtained from 12 experiments by comparing the ^{3}H-act.-D/^{14}C-TdR ratios for various times (min) after the beginning of the infection. The time symbols (abscissae) indicate the beginning of the pulse/chase; the lines the ranges of 2 standard deviations.

was no difference in the binding of act.-D by infected and non-infected cells, and the increase of binding after exposure to UV-inactivated viruses suggests a stimulation of DNA template activity in these cells. Act.-D specifically interferes with the production of RNA[12] by a mechanism involving intercalation of the chromophore of the antibiotic between G-C base pairs of the DNA double strand and chemical binding[2,3,13,14]. Assuming that act.-D plays the role of a non-specific model repressor[1-3], the quantitative analysis of its in vivo association with DNP may be considered a parameter for the earliest possible, albeit non-specific detection of changes of DNA template activity or, correspondingly, for the degree of complexing of DNA with chromosomal proteins[15], since transcription involves changes in the DNP complex[16,17].

The increase of binding of act.-D after exposure of cells to UV-inactivated viruses suggests that the viral coat protein was responsible for this stimulation, and that this process may be controlled by the intact viral genome.

Zusammenfassung. Nach Einwirkung UV-inaktivierter Polioviren wurde eine Zunahme der Bindung von Actinomycin-D, eines unspezifischen «Modellrepressors», durch HEp-2 Zellen um 15–21% beobachtet. Die Proteinhülle UV-inaktivierter Viren verursacht möglicherweise eine Stimulierung der Genaktivität der Wirtszelle, die normalerweise der Kontrolle des intakten Virusgenoms unterliegt.

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Lanthanum Inhibits Ca Inward Current but not Na-Ca Exchange in Cardiac Muscle

Two calcium transfer systems in the sarcolemma of mammalian cardiac muscle have been described: 1. a time- and voltage-dependent conductance system responsible for most of the inward charge transfer during the plateau phase of the cardiac action potential[1-4], and 2. a Na-Ca exchange system which is primarily responsible for extrusion of Ca from cardiac cells[5,6]. We report 2 series of experiments which provide additional evidence that these two Ca-transfer systems are separate, one being sensitive to external lanthanum ions while the other is not.

Methods. We carried out voltage clamp experiments in ventricular trabeculae (diameter 0.3–0.6 mm) isolated from pig and sheep hearts. The method, utilizing a sucrose gap for passing current through the preparation and intracellular microelectrodes for measuring and controlling the membrane potential, has been described previously[7]. The bathing solution had the following composition (mM/l): NaCl 137; KCl 5.4; $MgCl_2$ 1.05; $CaCl_2$ 1.8; glucose 5.0; *Tris*-HCl-buffer to pH 7.2 at 35 °C. $LaCl_3$ was added to give a final concentration of 0.4 mM/l.

For Ca efflux measurements guinea-pig auricles were loaded with ^{45}Ca in Tyrode's solution. The ^{45}Ca efflux from the resting auricles into nonradioactive solutions containing different Na- and Ca-concentrations was measured in the presence and absence of La (0.2-0.9 mM/l). The method has previously been described in detail[5].

Results and discussion. In the first series of experiments we studied the effect of La on the two components of inward current which flow during the cardiac action potential[4]. The first component is carried by Na ions (I_{Na}). In cardiac muscle, as in other excitable tissues, I_{Na} is rapidly

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activated and inactivated during depolarization[7–9] and is responsible for the fast upstoke of the action potential. The second current component which flows mainly during the plateau phase of the action potential is much slower than I_{Na} and is predominantly carried by Ca ions (I_{Ca})[1–4]. I_{Na} but not I_{Ca} is abolished by tetrodotoxin[1,7]. In the search for other substances which might affect the two components of inward current in cardiac muscle differently, we used the trivalent cation lanthanum because it is chemically quite similar to calcium and has a strong affinity to anionic Ca-binding sites in the membrane[10,11] La and Ca ions act similarly on the nerve membrane by shifting the Na conductance variables along the voltage axis in the depolarizing direction[12]. On the other hand, in presynaptic nerve terminals of squid giant synapses La blocks regenerative depolarizations which are presumably due to inward movement of Ca ions[13].

Figure 1 A illustrates the results of a double step voltage clamp experiment carried out with a single microelectrode impalement in a pig ventricular trabeculum. Panels a, b and c are the controls without La in the solution. In a and b the holding potential was set to −50 mV in order to inactivate I_{Na}[7,9]. In a first clamp step, the membrane potential was either displaced for 30 msec to 0 mV which produced inward current (panel a), or to +60 mV (panel b) which was above the reversal potential of this current and hence produced outward current. The second clamp step in a and b was to +3 mV and elicited exponentially decaying tails of inward current. The plateau of the action potential (panel c) is due to this slowly decaying inward current. Panels d, e and f were recorded after 20–25 min exposure to La-containing solution. The voltage clamp steps in d and e are the same as in a and b. The inward or outward currents at the first clamp steps were greatly reduced as were the tails of inward current at the second clamp steps. Correspondingly, the action potential had lost its plateau in the La-containing solution.

A plot of the current-voltage relations of the slow inward current measured in the absence and presence of La in the solution showed a marked inhibition of this current component by La (Figure 1B). Simultaneously measured contractions decreased concomitantly with the inhibition of the slow inward current by La. Similar results were obtained with 3 other preparations. In contrast to the inhibitory effect of La on the slow inward current, an increase in Ca in the bathing solution increases this current component[1–4]. In Na-free solution La inhibited the Ca-dependent regenerative depolarizations which can be elicited in these preparations by the application of constant current pulses[4]. In addition, ^{45}Ca uptake was measured in 8 paired sheep ventricular preparations stimulated at a constant rate of 0.5/sec. When one group of preparations was preincubated for 10 min in La-containing inactive solution and afterwards loaded for 15 min in La-containing radioactive solution ^{45}Ca uptake was reduced by 30 ± 4.6% (mean ± SE) as compared to the control group without La. All these results reinforce the earlier interpretation[1–4] that the slow inward current in mammalian cardiac preparations is predominantly carried by Ca ions.

The rapid initial I_{Na} in cardiac muscle seems to be similarly influenced by La as in other excitable tissues. The sigmoid curve relating membrane potential and the maximum upstroke velocity of the action potential (as a measure of I_{Na}[8]) was shifted along the voltage axis in the depolarizing direction by La, an effect similar to that of Ca[7,14]. In Figure 1A, f $(dv/dt)_{max}$ and the maximum height of the action potential were unaffected by La at a time when the plateau was abolished. These results

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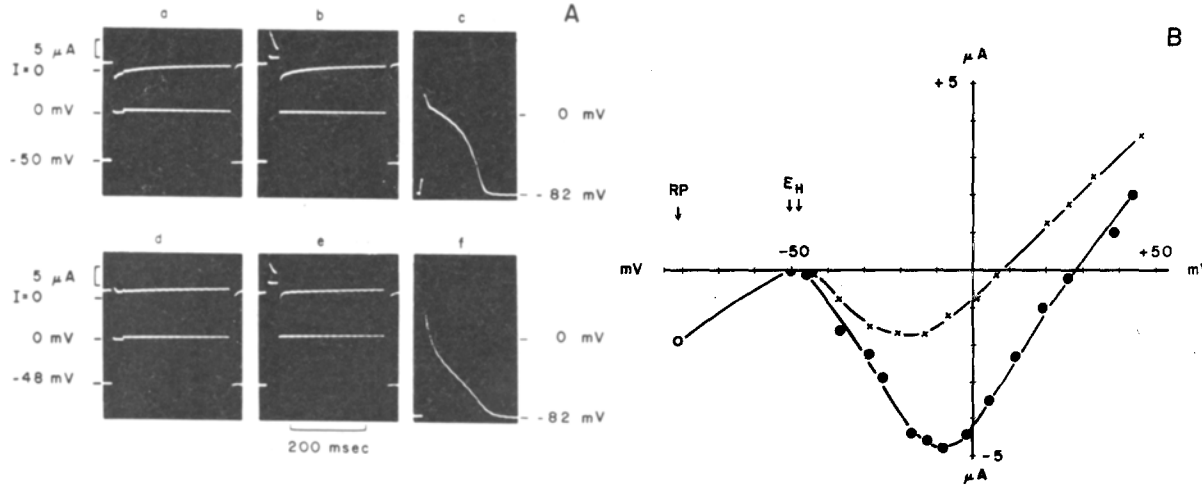

Fig. 1. A) Effect of La on the slow inward current and the plateau of the action potential measured in a pig ventricular trabecula. a–c, controls in Tyrode's solution; a, b, double step voltage clamps from a holding potential of −50 mV (I_{Na} inactivated); upper traces: membrane current, lower traces: clamp potential; first step (30 msec) to 0 mV produces inward current (a), first step to + 60 mV produces outward current (b); second clamp step always to +3 mV; note slowly decaying tails of inward current at the second clamp steps and the marked plateau of the action potential (c). d–f, Tyrode's solution containing 0.4 m*M*/l $LaCl_3$; same experimental procedure as in a–c; note marked decrease of the inward current tails at the second clamp step (d, e) and reduction of the plateau (f). B) Current-voltage relations of the slow inward current in the absence (filled circles) and presence (crosses) of 0.4 m*M*/l $LaCl_3$. The open circle indicates the current required to displace the membrane potential from the resting potential (RP) to constant holding potentials (E_H) at −50 mV in the absence and at −48 mV in the presence of La in order to inactivate I_{Na}. Plotted are maximum inward or minimum outward currents flowing during single step voltage clamps.

provide additional evidence that in mammalian cardiac muscle I_{Na} and I_{Ca} are separate current systems. Though they have similar effects on I_{Na}, La and Ca have opposite effects on I_{Ca}.

In the second series of experiments, we investigated the effect of La on the Na-Ca exchange system in left guinea-pig auricles. It has been shown that Ca efflux from cardiac muscle[5] or squid axon[15] is largely coupled to Na inward movement, presumably through a carrier-mediated transport system. Na and Ca ions compete for the carrier on both sides of the membrane. Therefore, the inwardly directed Na-concentration gradient across the membrane may provide the energy for uphill Ca transport from the interior of the cell into the extracellular space[5,15]. In squid axon La acts as an inhibitor of ^{45}Ca efflux[16]. We performed this series of experiments to assess whether La also inhibits the Na- and Ca-dependent ^{45}Ca efflux from cardiac muscle.

In the experiment shown in Figure 2, Ca efflux is expressed as fraction of ^{45}Ca lost per min from the preparation into the inactive rinsing solutions. During periods I, II and III, the rinsing solutions contained 0.2 m*M* La. As described earlier[5], Ca efflux decreased by approximately 80% when Na and Ca were removed from the solution (periods III and V) and increased promptly again after readmission of these ions (periods IV and VI). The results were the same in the absence and presence of La and irrespective of wheter La was applied during periods IV–VI instead of I–III. Therefore, we conclude that the Na-Ca-sensitive fraction of ^{45}Ca efflux from cardiac muscle is not affected by La. When La was added to the efflux media during one of the later efflux periods after period I, there was a transient increase in ^{45}Ca efflux and a decline to slightly lower steady state values. The same results were obtained with the lanthanide europium.

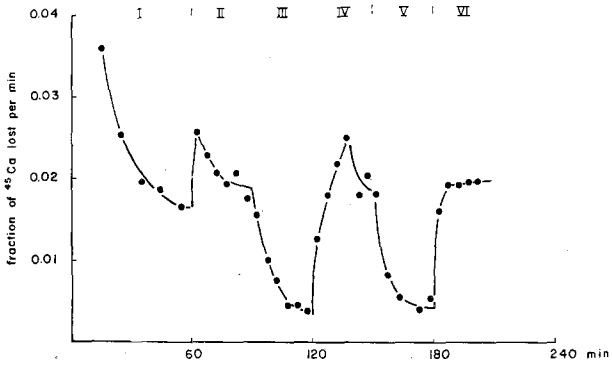

Fig. 2. Effects of changing $[Ca]_0$ and $[Na]_0$ on ^{45}Ca efflux from a guinea pig auricle in the presence and absence of 0.2 m*M*/l $LaCl_3$. Abscissa: time of tracer washout in min; ordinate: fraction of ^{45}Ca lost per min. The roman figures indicate experimental periods with different ion composition of the rinsing solutions. La was in the solutions during periods I–III. Period I: Na-containing, Ca-free solution; periods II, IV and VI: Na- and Ca- (1.8 m*M*/l) containing solution; periods III and V: Na- and Ca-free solution in which NaCl was isoosmotically replaced by choline Cl all solutions were buffered with *Tris*-HCl to pH 7.2 at 35 °C.

The lack of effect of lanthanides on the Na-Ca exchange system in mammalian cardiac muscle suggests that factors other than charge density or ionic radii determine the affinity of these carriers to ions. The electrostatic attraction should be much larger for the lanthanide ions than for Ca or Na ions which have ionic radii of similar size but much smaller charge densities[10]. The results suggest further that I_{Ca} and Na-Ca exchange in cardiac muscle are mediated by different mechanisms in the membrane since one can be inhibited by lanthanum while the other cannot[17].

Zusammenfassung. In Herzmuskelpräparaten sind zwei Systeme für den Ca-Durchtritt durch die Plasmamembranen zu unterscheiden: 1. Ein spannungs- und zeitabhängiger Ca-Einwärtsstrom, der während der Plateauphase des Aktionspotentials fliesst und durch La^{3+} gehemmt wird; 2. ein Na-Ca-Austauschsystem, das vor allem für den Auswärtstransport von Ca aus der Zelle verantwortlich ist, und durch La^{3+} nicht beeinflusst wird.

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Reversal of the Inhibiting Effect of 2- Chloroethyltrimethyl Ammonium Chloride on Chlorophyll Synthesis by Sulphur and Chlorine

The growth-retarding chemical, 2-chloroethyltrimethyl ammonium chloride (CCC) is known to preserve the loss of chlorophyll from detached leaves[1,2] and to inhibit chlorophyll synthesis[3,4]. The elucidation, however, of the action of CCC to inhibit turnover of proteins and to stimulate turnover of RNA, suggests that this compound may inhibit selectively synthesis of proteins catalyzing both the formation and degradation of chlorophyll[3].

The seeds of *Brassica campestris* were allowed to germinate and grow on petri dishes lined with filter paper moistened with 5 ml of distilled water or an equal volume of the test solution. The dishes were transferred to germination chamber maintained at about 28 ± 2 °C and illuminated from a light bank consisting of two 40 watts cool fluorescent lamps hanging at a distance of about 1 m. After 5 days of germination the length of the seedlings and chlorophyll content of cotyledons was determined. The chlorophyll from the cotyledons was extracted in 80% acetone and was determined by using the formula of Röbbelen[5].

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