

# SELECTIVE DEPOSITION OF LANTHANUM IN MAMMALIAN CARDIAC CELL MEMBRANES

## Ultrastructural and Electrophysiological Evidence

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### ABSTRACT

Perfusion of beating false tendons of the dog heart with ionic lanthanum produced drastic but reversible modifications of the excitability and the transmembrane action potential of Purkinje cells. Ultrastructural examination of these cells revealed the appearance of a fine extracellular precipitate detectable on unstained sections. In addition, specimens perfused with  $\text{La}^{+++}$  showed a striking increase in the contrast of the sarcolemma, particularly in gap junctions and in pinocytotic vesicles.  $\text{La}^{+++}$  deposits were restricted to the cytoplasmic leaflets of the sarcolemma; no precipitates were found at the plasma membrane of fibroblasts, endothelial and smooth muscle cells, or unmyelinated nerve fibers present in the same specimens. A selective deposition of  $\text{La}^{+++}$  was also observed in the sarcolemma of atrial and ventricular cells of dog, rabbit, and cat hearts, as well as in the membrane of the transverse tubular system of ventricular cells. Both the electrophysiological effects and the ultrastructural membrane deposits produced by  $\text{La}^{+++}$  disappeared when the specimens were subsequently perfused with phosphate-containing tyrode solution. These results tend to demonstrate that a distinctive feature of the sarcolemma of mammalian cardiac cells is the presence of regions with a high surface density of binding sites for polyvalent cations.

### INTRODUCTION

Among the various polyvalent cations known to bind at the cell surface, lanthanum has been found to form a deposit detectable with the electron microscope. The localization of lanthanum in the outer leaflets of plasma membranes of frog nerve fibers was first reported by Doggenweiler and Frenk (4). In addition, lanthanum used as colloidal suspensions (19) or in ionic form (7, 15, 16) acts as an electron-opaque tracer of the extracellular space. Revel and Karnovsky (19) used colloidal lanthanum to identify a previously undetected 20 Å intercellular space at the gap junctions of mouse heart and liver; we later obtained similar findings in a variety of epithelial and myocardial

tissues (13, 15, 16). Colloidal lanthanum has also been used to stain certain components of the surface of embryonic (9, 10, 14, 21) and differentiated cells (22).

This report presents ultrastructural and electrophysiological evidence indicating that the plasma membrane of living cardiac cells selectively binds ionic lanthanum ( $\text{La}^{+++}$ ), producing reversible modifications of the excitability and the action potential of mammalian cardiac cells.

### MATERIALS AND METHODS

Adult mongrel dogs, cats, and rabbits of either sex were used. Under nembutal anesthesia (33 mg/kg)

hearts were removed and immersed in a bath containing tyrode solution at 37°C. The composition of the normal tyrode (in mmol/liter) was: NaCl 137; KCl 2.7; CaCl<sub>2</sub> 1.8; MgCl<sub>2</sub> 0.5; tris-HCl buffer 5.0; glucose 8.3; pH 6.8–6.9. Papillary muscles with their attached false tendons from the right or the left ventricles, and fragments of atrial and ventricular muscle were placed in a small chamber filled with oxygenated flowing tyrode solution kept at 36–37°C. The electrophysiological studies were made only on the false tendon-papillary muscle preparation of the dog heart. The false tendon was either driven at a constant frequency (1–2/s, 1 ms pulses, just above threshold) or it was allowed to show its spontaneous activity. The transmembrane potentials of Purkinje fibers were recorded from the superficial cell layers through glass micropipettes (3 M KCl, 20–50 MΩ) connected to a P18 DC preamplifier (Grass Instrument Co., Quincy, Mass.) and displayed on a 502A oscilloscope (Tektronix Inc., Beaverton, Ore.). The rate of rise of the transmembrane potentials was measured through a modified P20 differentiator (Grass Instrument Co.). After the typical action potentials of Purkinje cells were recorded, the normal bathing solution was re-

placed by tyrode containing variable amounts of lanthanum chloride. The following concentrations were tested: 0.1; 0.25; 0.5; 0.75; 1.0, and 5.0 mmol/l. Periods of perfusion ranged from 8 min to 9 h. For the electron microscope studies a concentration of 5 mmol/liter throughout a 1-h period of perfusion was found to be the lowest for obtaining reproducible results.

At the end of the lanthanum perfusion time, some specimens were fixed in glutaraldehyde; others were subjected to an additional perfusion period of 30 min in which the La<sup>+++</sup> containing, tris-buffered tyrode was substituted by a La<sup>+++</sup>-free tyrode solution containing phosphate buffer, followed by fixation in glutaraldehyde. Controls were done by perfusing the false tendons for 1 h with normal tyrode without La<sup>+++</sup>. Specimens were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, for 1 h at room temperature. Subsequently, the fragments were placed in a cacodylate buffer and fixed in 1% osmium tetroxide in the same buffer for 1–3 h. Lanthanum was added only to the perfusion solution. Fixatives and dehydrating solutions were always free of lanthanum. After dehydration in alcohols, the frag-

TABLE I  
*Effects of LaCl<sub>3</sub> on Some Electrophysiological Properties of Cardiac Purkinje Cells*

Experiment number	LaCl <sub>3</sub> concentration	Changes in the action potential			Perfusion time to reach inexcitability	Decrease in spontaneous activity	Total time of perfusion with LaCl <sub>3</sub>
		Decrease in rate of rise	Decrease in amplitude	Increase in duration			
	<i>mM</i>	%	%	%	<i>min</i>		<i>min</i>
1	0.10		12.0	0	60	+	70
2	0.20		8.1	21.8	50		60
3	0.25				49	+	60
4	0.50	24.0	6.6	19.0	40	+	40
5	0.50	17.8	16.0	28.6	20	+	21
6	0.50				10		13
7	0.75					+	36
8	0.75	29.0	NO	27.8			60
9	1.00	22.5	5.4		14	+	15
10	1.00	13.5	2.9	30.4		+	10
11	1.00	9.0	6.0	25.0	14	+	17
12	1.00	35.5	16.0	27.8	43	+	60
13	1.00				27	+	60
14	1.00	30.5	5.3	0	59	+	65
15	1.00	22.0	9.7	23.3	34	+	36
16	1.00				30	+	30
17	1.00				37	+	38
18	1.00				6	+	10
19	5.00	30.0	2.0	16.7	6		60
20	5.00	6.5	NO	20.4	7	+	9
21	5.00	88.0	51.5	4.0	5	+	8

All figures correspond to the maximum effect produced by each La<sup>+++</sup> concentration independently of the total time of perfusion.

ments were flat-embedded in Epon. The blocks were oriented so as to obtain sections perpendicular or parallel to the long axis of myocardial cells. Tissue blocks were less than 2 mm in transverse diameter. Thin sections always included both peripheral and central cellular elements. Thin sections were observed with a Carl Zeiss EM 9S2 electron microscope without counterstaining; occasionally, brief staining with lead citrate was employed.

Observations on the ultrastructural localization of lanthanum in Purkinje cells of false tendons were based on a total of 16 experiments; those pertaining to ventricular and atrial cardiac cells of the dog, cat, and rabbit were based on three series of experiments. The electrophysiological results were collected from 21 experiments.

## RESULTS

### *Electrophysiological Findings*

In all the experiments performed, Purkinje cells were found to be susceptible to the different  $\text{La}^{+++}$  concentrations used. In order to evaluate the effects produced by this cation, various parameters of the electrical activity of the Purkinje cell membranes were measured. The changes produced are summarized in Table I. The resting membrane potentials are not included in the table since they

remained practically constant during the  $\text{La}^{+++}$  perfusion period (Fig. 1).

The first detectable change in the action potential was a decrease in the rate of rise, which developed gradually with time. Although the control values for the rate of depolarization ranged from 360 to 800 V/s, the maximal decrement produced by  $\text{La}^{+++}$  was independent of the original rate of rise. The initial changes in depolarization were not accompanied by any other modification of the action potential. Only when the decrement in the rate of rise became more pronounced did additional changes of the membrane properties start to appear (Fig. 1).

Other effects produced by  $\text{La}^{+++}$  on the transmembrane action potential were the modifications of both amplitude and duration (Fig. 1). In the majority of the experiments, the amplitude decreased, as shown in Table I. The percentage in the diminution of the amplitude was always smaller than that of the rate of rise, regardless of the  $\text{La}^{+++}$  concentrations used. In contrast, a gradual increase in the action potential duration was observed (Fig. 1). The magnitude of this increment was variable, but never exceeded 30% of the control values. The measurements of the

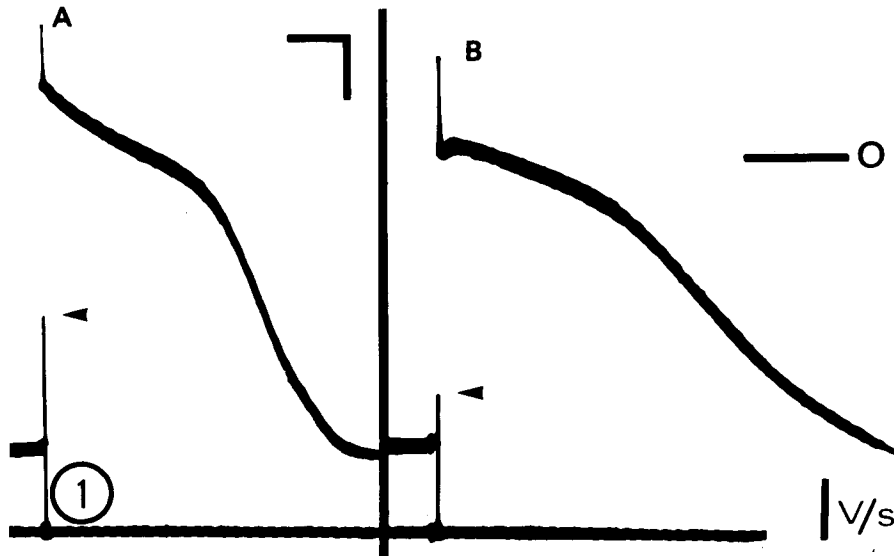


FIGURE 1 Changes produced by 1 mM  $\text{LaCl}_3$  on the action potential of a Purkinje cardiac cell. Upper traces, transmembrane potential; lower traces, differentiation to measure the rate of rise. A, under normal tyrode solution. B, after 17 min of perfusion with  $\text{La}^{+++}$ . Notice the decrease in the rate of rise (arrowheads) and amplitude, and the increase in duration. The change in resting membrane potential was negligible. Calibrations: upper, 100 ms and 20 mV; lower, 100 V/s.

duration were made from the upstroke to the end of the repolarization phase.

In regard to the excitability of Purkinje cells, it was repeatedly observed that perfusion with  $\text{La}^{+++}$  produced a gradual increase in the threshold, until the cell became inexcitable. Similarly, the spontaneous activity diminished its frequency and in some experiments was abolished. A partial or total recovery from the effects of lanthanum described above was observed when the preparation was perfused with phosphate-buffered tyrode solution. In general, no quantitative correlation between the magnitude of the electrophysiological changes of the cell membrane and either the  $\text{La}^{+++}$  concentration or the perfusion time could be established. However, it is possible to state that these changes kept a temporal relationship with the concentration of  $\text{La}^{+++}$ , since large concentrations (1.0–5.0 mM) showed their maximal effects within 5–10 min of lanthanum perfusion whereas small concentrations (0.10–0.75 mM) needed 13–60 min.

### *Ultrastructural Localization of Lanthanum Deposits*

Examination of myocardial tissues perfused with lanthanum revealed the appearance of an electron-opaque precipitate, both at the extracellular space and in certain regions of the plasma membrane of cardiac cells (Figs. 2, 3).

Of particular interest was the striking specificity of lanthanum localization in the sarcolemma of cardiac cells. In all specimens treated with 5 mmol of lanthanum chloride for 1 h, lanthanum deposits were present only in the surface membrane of myocardial cells and in the extracellular space; no precipitate was found at the plasma membranes of other cell types such as fibroblasts, endothelial cells, Schwann cells, and unmyelinated axons (Fig. 3), or arteriolar smooth muscle cells found in the same specimens. Lanthanum deposits were equally absent from the surface of collagen and elastic fibers. The specificity of lanthanum for

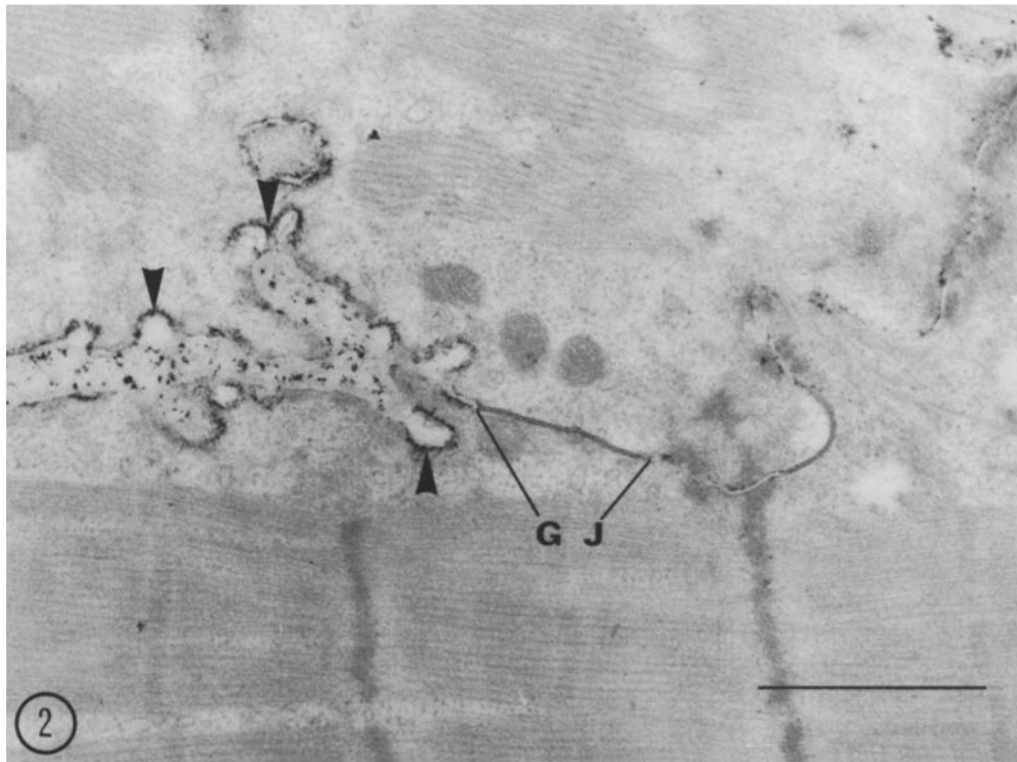


FIGURE 2 Purkinje cells from dog false tendon perfused for 1 h with 5 mM  $\text{LaCl}_3$  before fixation. Dense deposits are seen at the extracellular space, and in the membrane of pinocytotic vesicles (arrowheads) and gap junctions (*GJ*).  $\text{La}^{+++}$  deposits are mainly absent from other junctional regions and from nonspecialized segments of the sarcolemma. Specimen was fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Epon. Unstained section. Bar equals 1  $\mu\text{m}$ .  $\times 30,000$ .

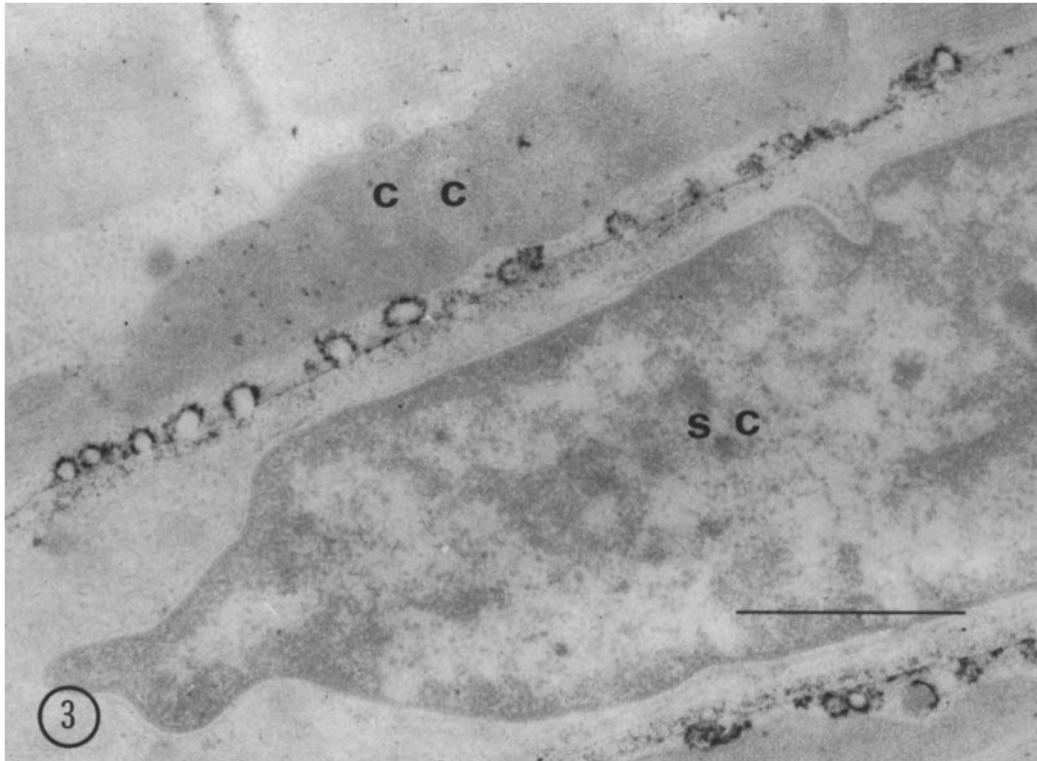


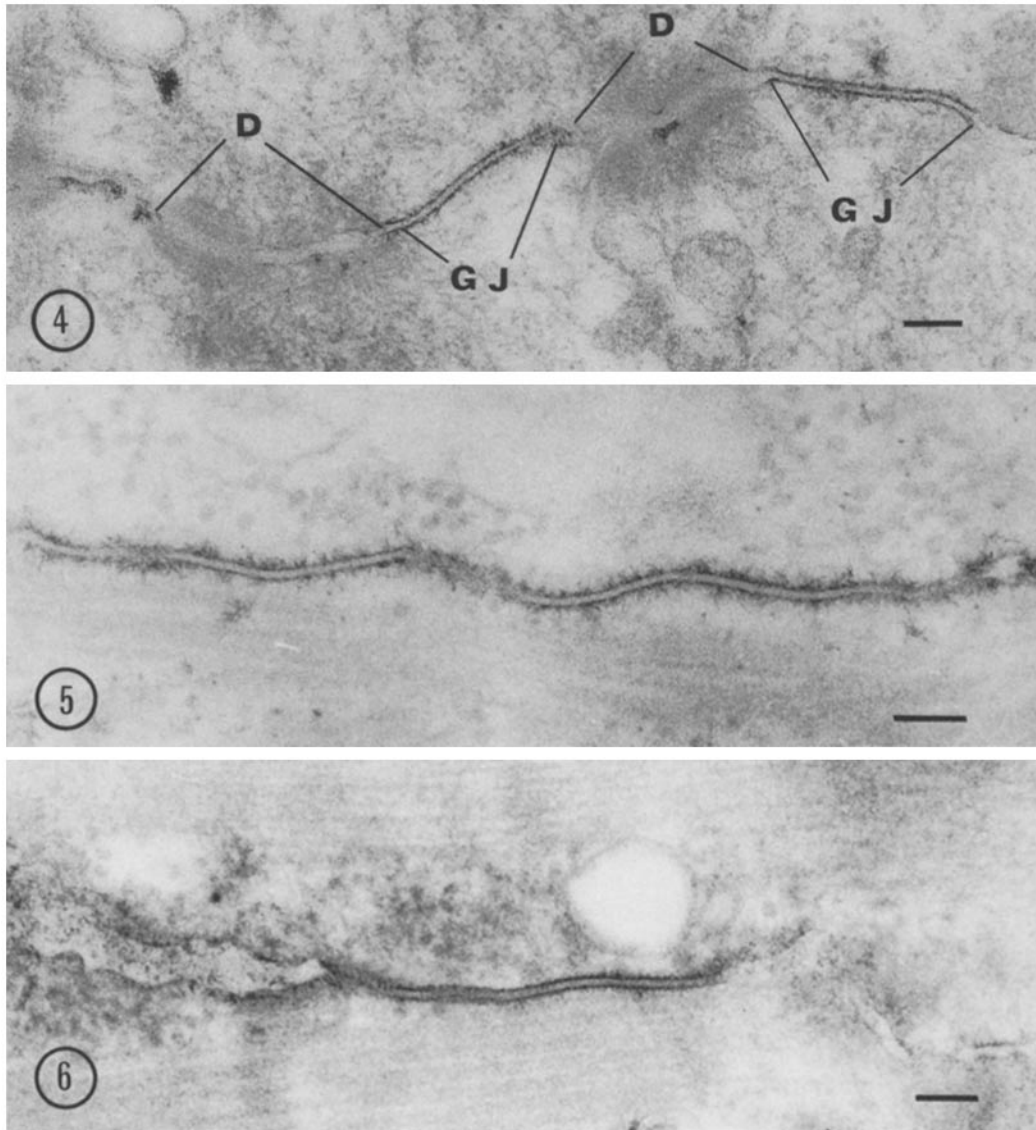
FIGURE 3 Dog false tendon. A large nucleus and a portion of the cytoplasm of a Schwann cell (SC) are seen at the center.  $\text{La}^{+++}$  precipitate is found at the extracellular space and at the numerous pinocytotic vesicles of two cardiac cells (CC). No deposits are seen at the plasma membrane of the Schwann cell. Other noncardiac cells from the false tendons perfused with  $\text{La}^{+++}$  also lack the electron-opaque precipitate in their outer membranes. Specimen preparation as for Fig. 2. Unstained section. Bar equals 1  $\mu\text{m}$ .  $\times 31,000$ .

cardiac cell membranes was maintained even when perfusion time was extended to 9 h.

A second finding was the preferential ability shown by certain regions of the sarcolemma of Purkinje cells to accumulate lanthanum. In Purkinje cells from false tendons, dense deposits outlined mainly the gap junctions and the numerous surface vesicles opened to the extracellular space (Figs. 2, 4-6). In general, other junctional elements of the intercalated disk in Purkinje cells, such as desmosomes and intermediate junctions, were free of lanthanum (Fig. 4). The selectivity of lanthanum deposition for gap junctions was repeatedly confirmed in 10 false tendons perfused for 1 h with tyrode solution containing 5 mmol  $\text{LaCl}_3$ . Under these conditions, lanthanum deposits in gap junctions were restricted to the cytoplasmic side of the junctional membranes (Figs. 2, 4-6). Gap junctions appeared therefore as two dense lines 25-30  $\text{\AA}$  thick, separated by a clear

space 100-120  $\text{\AA}$  wide. The latter consists of the middle and outer leaflets of the converging junctional membranes, as well as the 20  $\text{\AA}$  intercellular gap, as described by others (17, 19) in tissues treated with uranyl acetate or with colloidal lanthanum. In addition to gap junctions, both micro-pinocytotic vesicles and coated vesicles of cardiac cell membranes (6) showed lanthanum deposits (Figs. 2, 3). At these surface vesicles, the precipitate formed irregular patches over the membrane, in contrast with the nearly continuous and uniform dense lines outlined by lanthanum at the gap junctions.

The above-mentioned results were obtained in dog Purkinje cells and were confirmed in three experiments using Purkinje cells from false tendons of cat and rabbit hearts exposed for 1 h to tyrode solution containing 5 mmol of  $\text{LaCl}_3$ . Similar findings were observed in atrial and in ventricular cells of the dog, cat, and rabbit hearts perfused



**FIGURE 4** Junctional elements from a portion of an intercalated disk between two Purkinje cells.  $\text{La}^{+++}$  deposits are found at the cytoplasmic leaflets of two gap junctions (*GJ*); the membranes of two desmosomes (*D*) are essentially devoid of precipitate. Specimen preparation as for Fig. 2. Unstained section. Bar equals  $0.1 \mu\text{m}$ .  $\times 80,000$ .

**FIGURE 5** Gap junction between two ventricular cardiac cells from a cat papillary muscle. The cytoplasmic leaflets of the converging junctional membranes are outlined by a nearly uniform dense deposit. No precipitates are seen at the intercellular space. Specimen and section preparation as for Fig. 2. Bar equals  $0.1 \mu\text{m}$ .  $\times 100,000$ .

**FIGURE 6** Gap junction between two ventricular cardiac cells from a dog papillary muscle. A dense microcrystalline precipitate is found at the extracellular space and at the cytoplasmic leaflets of the gap junction. Specimen and section preparation as for Fig. 2. Bar equals  $0.1 \mu\text{m}$ .  $\times 100,000$ .

under the same conditions. However, in the latter specimens lanthanum deposits tended to have a uniform distribution throughout the entire sarcolemma (Fig. 7). Nevertheless, the precipitate was also restricted to the extracellular space and the plasma membrane of ventricular and atrial muscle cells. In ventricular cardiac cells the deposits outlined, in addition to the sarcolemma, the membrane of the transverse tubular system (T system). As illustrated in Fig. 8, the membrane of the T tubes showed prominent deposition of lanthanum. In all instances, the ability to bind lanthanum was restricted to the membrane of the T tubes; no deposits were found within the terminal cisternae or in the membranes of the sarcoplasmic reticulum (Fig. 8).

Intracellular lanthanum deposits were present only in blocks from specimens perfused for 9 h, which, in addition, showed morphological evidence of cell damage.

Precipitates were not seen in control specimens perfused for 1 h with normal tyrode or in cardiac cells from false tendons perfused first with 5 mmol  $\text{La}^{+++}$ , and washed subsequently with phosphate-containing buffer.

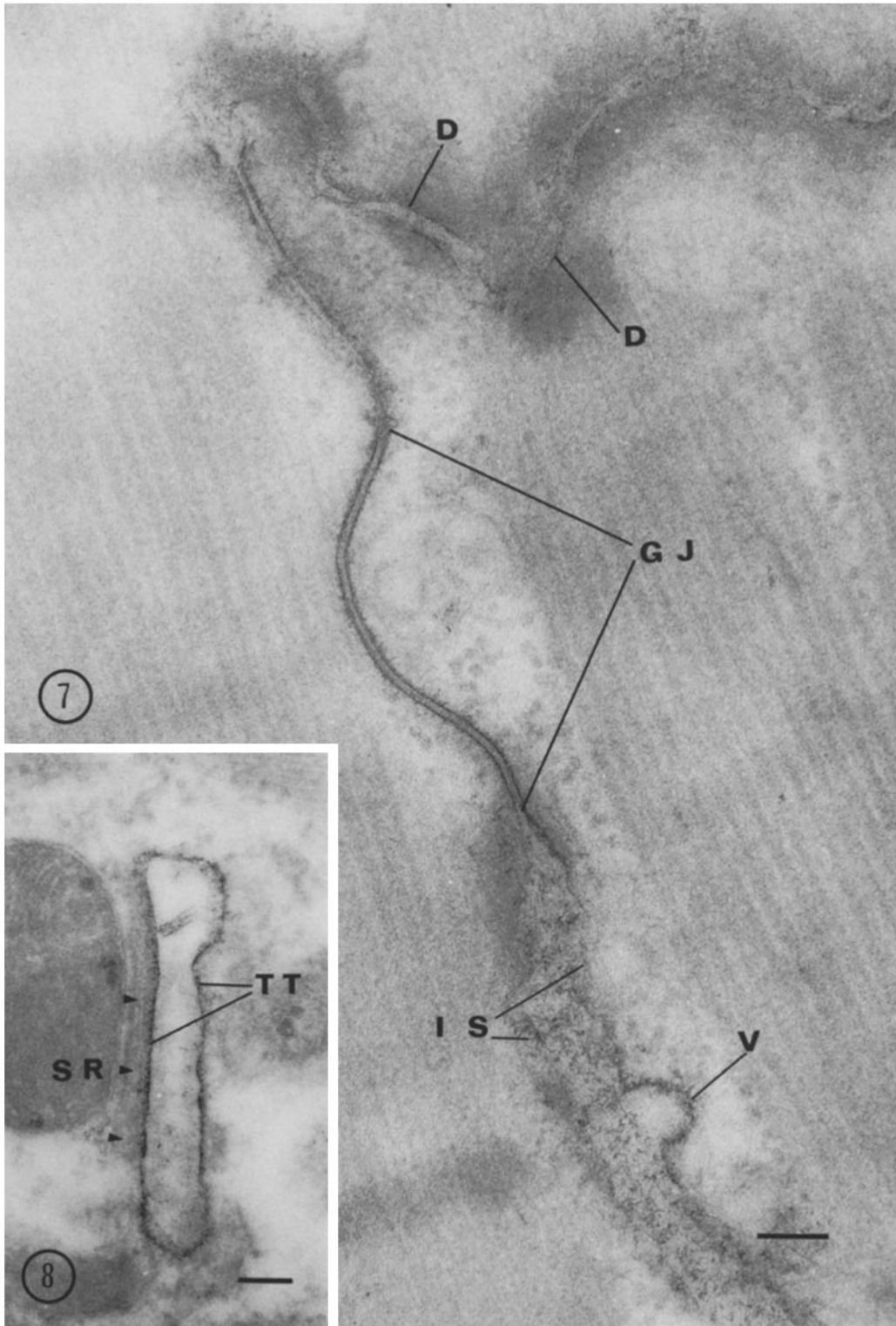
#### DISCUSSION

An interesting feature of the effects of ionic lanthanum on the electrophysiological properties of Purkinje cells is the decrement in the rate of rise of the action potential upstroke. This action suggests that  $\text{La}^{+++}$  might interfere with the mechanisms of the inward sodium current necessary for the development of the upstroke of the electrical response (1, 2, 3, 5, 8). This assumption is supported by the reports of Lettvin et al. (11) who first predicted that  $\text{La}^{+++}$  might function as a nerve-blocking agent by "locking" the sodium channels. Takata et al. (23) showed later, using the lobster axon membrane, that  $\text{La}^{+++}$  causes a progressive rise in threshold and a decrease in the height of the action potential as well as in its rates of rise and fall. They have shown, in addition, that the time courses of the ionic conductance changes for both sodium and potassium increase with lanthanum. The changes in amplitude and duration of the action potential, excitability, and spontaneous activity (see Table I and Fig. 1) reported here may be explained in terms of the above-mentioned interpretation, if the results obtained in nerve membrane are extrapolated to cardiac cell membranes. The increased duration of the Pur-

kinje cell action potential found by us confirms similar findings reported by Sanborn and Langer (20) in rabbit ventricular cardiac cells. These authors reported the displacement by lanthanum of calcium bound superficially, probably to the sarcolemma (20). In view of these results, the possibility exists that the regions where selective deposition of lanthanum was observed in the present study could represent the sites where contractile dependent calcium is normally bound.

The recovery of the electrical properties of Purkinje cells after washing out the  $\text{La}^{+++}$  with phosphate-buffered tyrode solution concomitant with the disappearance of the electron-opaque deposits can be explained in view of the chelating properties of phosphate groups. It is interesting to recall that the early report of Mines (18) on the action of  $\text{La}^{+++}$  on the electrophysiological properties of cardiac cells mentions the reversible nature of the binding of rare earths to myocardial cells.

Our ultrastructural observations indicate that lanthanum perfused into living heart tissues is bound not only at the extracellular space where it acts as an electron-opaque tracer, as reported previously for other tissues (16), but is also selectively bound at the sarcolemma of cardiac cells. The selectivity of lanthanum localization indicates that the sarcolemma possesses a high surface density of sites that bind polycations, particularly marked at the membrane of the gap junctions and the surface vesicles. The distribution of ionic lanthanum in gap junctions varies considerably from that obtained with the use of colloidal lanthanum in the fixative solutions (19). In the latter form, lanthanum permeates the narrow intercellular gap at the junctions, and also penetrates into part of the outer cytoplasmic leaflets of adjacent junctional membranes. In contrast, when we perfused the beating cardiac cells with lanthanum, most of which is in ionic form (12), deposits were found mainly at the cytoplasmic membrane leaflets of gap junctions. It was unusual in the present observations to see dense deposits at the 20 Å wide intercellular junctional space, either because lanthanum was washed out during the fixation and dehydration, or because the concentrations at the gap were too low to be detected with the electron microscope on thin sections. However, the presence of dense deposits at the cytoplasmic leaflets of the gap junction membranes indicates that  $\text{La}^{+++}$  entered freely into the narrow extracellular gap.





The deposition of lanthanum in cardiac cell membranes was observed in all specimens perfused with 5 mmol/liter  $\text{LaCl}_3$ , irrespective of the location of the cell with regard to the surface of the tissue fragment. The selectivity for cardiac membranes and the degree of lanthanum deposition was the same throughout the entire thickness of false tendons and blocks of atrial and ventricular tissues. We can rule out the artifactual washout of lanthanum from peripheral regions of the block as an explanation for the present results, since the selectivity for cardiac cell membranes was found at all levels of the blocks. Furthermore, we would like to emphasize that cell membranes of endothelial cells, fibroblasts, arteriolar smooth muscle cells, and nerve terminals were always free of precipitate, whereas the sarcolemma of all cardiac cells present in the same blocks showed prominent deposits of lanthanum.

The specificity observed could also be interpreted as the result of a selective washoff from non-cardiac cell membranes. Even if the latter possibility is valid, our results demonstrate clearly a particular high affinity of cardiac cell membranes for ionic lanthanum. There is no obvious explanation at present for the regional preference shown by gap junctions and surface vesicles to precipitate lanthanum. However, the above-mentioned observations seem to indicate that one of the distinctive features of the sarcolemma of mammalian cardiac cells is the presence of surface regions having a high density of polycationic binding sites.

#### ADDENDUM

After submission of the manuscript, Langer and Frank (1972, *J. Cell Biol.* 54:441) reported detailed observations on the displacement of calcium located at the surface of cultured rat heart cells, as a result of perfusion with lanthanum. However, under the conditions used by these authors, lanthanum formed non-specific electron-opaque precipitates both at the ex-

ternal lamina of cardiac cells and at the surface coat of fibroblasts.

Received for publication 26 September 1972, and in revised form 12 March 1973.

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FIGURE 7 Lateral and end-to-end junctions between two ventricular cardiac cells from a dog papillary muscle perfused with  $\text{LaCl}_3$ .  $\text{La}^{+++}$  deposits are found at the intercellular space (IS), and at the cytoplasmic sides of the membranes of a gap junction (GJ). Few precipitates are seen at the membranes of several desmosomes (D). (V) Pinocytic vesicle. Specimen preparation as for Fig. 2. Unstained section. Bar equals 0.1  $\mu\text{m}$ .  $\times 100,000$ .

FIGURE 8 A segment of a transverse tubule (TT) from a dog ventricular cardiac cell shows deposition of  $\text{La}^{+++}$  at the tubular membrane. No deposits are seen in the flattened cisternae of the sarcoplasmic reticulum (SR) closely apposed to the T tubule. Specimen preparation as for Fig. 2. Unstained section. Bar equals 0.1  $\mu\text{m}$ .  $\times 80,000$ .

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