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IONIC CURRENTS IN THE GUINEA-PIG TAENIA COLI

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SUMMARY

1. Short segments of portions of taenia coli of the guinea-pig averaging 54 μm × 219 μm × *ca.* 200 μm have been studied by a double sucrose-gap voltage-clamp technique.

2. The average total capacitance was 0·4 μF, corresponding to approximately 10^4 cells, if a specific membrane capacitance of 3 μF/cm^2 were assumed.

3. A significant resistance, averaging 11·4 kΩ, was in series with the membrane, and seriously limited the accuracy of the voltage control possible.

4. On depolarization, an early transient inward current was followed by a late maintained outward current.

5. The late current was carried mainly by K^+, because its direction could be reversed if the preparation were first depolarized in isotonic K_2SO_4 and held back to the original resting potential.

6. After appropriate corrections for residual capacitative and leakage currents, a reversal potential for the late current (E_b) was determined to be 15–20 mV more negative than the natural resting potential. It was not affected by the amplitude or the duration of the activating voltage step, but could be changed by prolonged applications of holding current.

7. At rest, the ratio of $P_{Na}:P_K$ was 0·16:1; for E_b it was 0·05:1.

8. The reversal potential for the transient early inward current (E_a) averaged 22 mV in Krebs–bicarbonate solution, but was shifted to about 35 mV when the late current was first suppressed with tetraethylammonium ion. The shift suggested that there was some overlap of the early and late currents.

9. Reduction of $[Na^+]_o$ to 50% of normal, or replacement of all Na^+ with dimethyldiethanol ammonium ion and choline ion, failed to cause any significant shifts in the reversal potential of the early current or reduce the magnitude of the early current.

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10. Reduction of $[Ca^{2+}]_o$ to 0·25 or 0·1 of the normal caused shifts of the E_a toward the negative and reductions in the early current. These changes can occur without changes in the maximum chord conductance of the early current, such as might happen in ordinary Krebs–bicarbonate solution, or in preparations which had been depolarized by prior treatment with isotonic K_2SO_4 and then held back to the original membrane voltage.

11. Increase of $[Ca^{2+}]_o$ to 5 times normal increased the early inward current, and the maximum chord conductances of the early and late currents, but did not shift the E_a.

12. In preparations pretreated with TEA, increasing $[Ca^{2+}]_o$ to 5 times normal shifted E_a toward 45 mV.

13. The various observations are interpreted to mean that the early current in the taenia coli is carried principally by influx of Ca^{2+}, and not by Na^+.

INTRODUCTION

The purpose of this paper is to present some results of voltage-clamp experiments on the guinea-pig taenia coli performed with a double sucrose-gap method. Because of inherent complexities of the preparation, there are serious limitations on the voltage control possible in this tissue (see Anderson, 1969; Kao & McCullough, 1975). However, some information that can be obtained relatively directly with a voltage-clamp method cannot be obtained by any other means. The information, although lacking in the quantitative accuracy that might be expected of a voltage-clamp study, is still useful in a qualitative manner for clarifying some unsettled problems in fundamental electrophysiological properties of the guinea-pig taenia coli. This paper contains a general description of some constant-current studies and some voltage-clamp studies. The main aim of the paper is an analysis of the ionic nature of the early and the late currents. Preliminary accounts of some of the work have been given at a Physiological Society meeting (Inomata & Kao, 1972), and at the Kiev Symposium on Physiology of Mammalian Smooth Muscles (Inomata & Kao, 1975).

METHODS

Male guinea-pigs, weighing 300–400 g, were anaesthetized with phenobarbitone (25 mg/kg, I.P.). Whole taenia coli were removed from the caecum upon laparotomy, and incubated in Krebs–bicarbonate solution at 37 °C. At 10–25 times magnification under a stereoscopic dissecting microscope, bundles of about 0·2 mm in diameter and 10–15 mm in length were dissected from the edges of the whole taenias to preserve as much of the natural surfaces as possible. These bundles, after further incubation for at least 30 min, were used individually in a double sucrose-gap apparatus for the study.

Details of the apparatus and the procedures have been described by Anderson (1969), and also in a previous paper dealing with the rat myometrium (Kao & McCullough, 1975). In essence, the preparation was isolated into three interconnected segments by two cuffs of high-resistance isotonic sucrose solutions. Because of the high extracellular resistance, current can be made to flow from one segment to another primarily by the intracellular path, and the preparation can be stimulated between the central segment (often called a 'node' because of its resemblance to an artificial node of Ranvier) and one end segment, and voltage can be recorded between the node and the other end.

The electrical resistance across each sucrose gap of 2·5–3 mm length was 75–100 MΩ. When a preparation was inserted, the gap resistance fell to less than 1 MΩ, initially, but tended to creep toward 3–4 MΩ after some 30–40-min washing in the sucrose solution. Although the short-circuit factor was on the order of 5%, the access resistance was relatively high, a factor which impaired the quality of the voltage control.

As shall be detailed in Results, the node in these experiments averaged 54 μm. There was a series resistance which averaged 11·4 kΩ. These were found to be acceptable compromises between a tolerable series resistance and a controllable segment of the preparation. With nodes longer than 100 μm, control was very poor, as evidenced by multiple spikes in a maintained depolarizing current step, and by notches in the current trace during a voltage-clamped step. With nodes shorter than 40 μm, the series resistance tended to increase to beyond 20 kΩ. Therefore, a node of *ca.* 50 μm with a series resistance not exceeding 15 kΩ was generally aimed for in each experiment.

Most of the solutions used are listed in Table 1. In experiments in which the relation of $[K^+]_o$ to resting potential was studied, $[K^+]_o$ was increased at the expense of $[Na^+]_o$, and all chloride was replaced by sulphate, all other ions being similar to those shown for solution J. The solution in the central channel perfusing the node was at 38° C, while those in all other channels were at room temperature of about 26° C.

In the results to be detailed, each node was tested under control and then under experimental conditions, every effort being made to keep the node the same in the two conditions (see Kao & McCullough, 1975, for procedures used). Because of this practice of using the same node, Student's *t* test of results was made on the basis of paired comparison.

RESULTS

The preparation

The taenia coli preparations were suspended in the centre of the muscle channel of the apparatus with just enough tension to remove slack, the procedures being similar to those used in earlier experiments on the uterine smooth muscle (Kao & McCullough, 1975). Whereas the myometrial preparations were ready for current or voltage clamping after 4–5 min of washing with sucrose, the taenia coli preparations always required at least 30 min of such preliminary washing (see also Kuriyama & Tomita, 1970). This delay suggests that in the taenia the extracellular diffusion either has a longer path or is impeded by some structure. Some properties of the taenia preparations (see p. 361) are undoubtedly related to this slow extracellular diffusion.

TABLE 1. Compositions of solutions used (m-mole/l.)

	A	B	C	D	E	F	G	H	I	J	K
	Standard		Na^+		Ca^{2+}			TEA		K^+ depolarized	
	HPO_4^-	Tris	Free	50 %	10 %	25%	5 ×	1 × Ca^{2-}	5 × Ca^{2-}	1 × Ca^{2+}	10 % Ca^{2+}
Na^+	143·4	133·4	0	71·7	143·4	143·4	122·0	66·7	55·3	0	0
K^+	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	5·9	149·3	149·3
Ca^{2+}	1·9	1·9	1·9	1·9	0·19	0·48	9·5	1·9	9·5	1·9	0·19
$Mg2^+$	1·2	1·2	1·2	1·2	1·2	1·2	1·2	1·2	1·2	1·2	1·2
Cl^-	126·9	153·1	126·9	126·9	126·9	126·9	156·9	153·1	156·9	0	0
HCO_3^-	25·0	—	25·0	25·0	25·0	25·0	—	—	—	25·0	25·0
HPO_4^-	1·2	—	1·2	1·2	1·2	1·2	—	—	—	1·2	1·2
SO_4^{2-}	1·2	1·2	1·2	1·2	1·2	1·2	1·2	1·2	1·2	64·6	64·6
DDA	—	—	118·4	71·7	3·4	2·84	—	—	—	—	—
Tris	—	10·0	—	—	—	—	10·0	10·0	10·0	—	—
TEA	—	—	—	—	—	—	—	66·7	66·7	—	—
Choline	—	—	25·0	—	—	—	—	—	—	—	—

All solutions containing HPO_4^- were buffered with bicarbonate and gassed with 5% CO_2–95% O_2. All Tris-buffered solutions were gassed with 100% O_2. All solutions contained 11 mM glucose/l. DDA is dimethyldiethanol ammonium.

The average node measured 54·2 ± 1·8 μm (node width ± S.E. of mean of 114 preparations) × 219·1 ± 0·94 μm (preparation width) × *ca.* 200 μm (preparation thickness). The individual taenia cells can be assumed to resemble two base-abutting right circular cones with 6 μm diameter bases and 50 μm heights. The volume of each individual cell is approximately 942 μm^3. Taking an average extracellular space of 35 %, an average node would contain about 1635 cells. An alternative estimate can be made on the basis of the observed total capacity, assuming that all capacity resides in the cell membrane. The average total capacity is 0·4 μF (see p. 352). Assuming a specific membrane capacity of 3 $\mu F/cm^2$ (Tomita, 1966), the average node would contain about 0·13 cm^2. With a volume/surface ratio of 1 μm, and the estimated cell volume of 942 μm^3, then the average node would contain upwards of 13,800 individual cells. The large discrepancy is not unexpected, since the estimates were made on rather crude assumptions. Were the cell membrane to be convoluted, the morphological estimate would have given too few cells. Were some capacitance to be in non-excitable membrane, the electrical estimate would have given too many cells. Therefore, the two values given might be considered as the limits, and a reasonable conclusion would be that some 10^4 cells were involved in an average node.

Constant-current conditions

1. *Resting and action potentials.* When one end of the taenia coli preparation was depolarized with an isotonic K_2SO_4 solution, a 'gap' potential developed between the node and the depolarized end, with the node being negative. The average 'gap' potential was −46·9 ± 0·3 mV (mean ± S.E. of mean in 230 preparations) and the highest −62 mV. This 'gap' potential is a composite of the short-circuited membrane potentials of the many individual taenia coli cells in the node, some liquid junction potentials, etc. However, because the short-circuit factor was of the order of 5 %, and because the observed 'gap' potential was very close to the resting potential observed on micro-electrode impalements (see also Holman, 1957; Bülbring & Kuriyama, 1963), we have accepted this 'gap' potential as representing the average resting potential of the cells in the node.

Action potentials were readily elicited by outward-current pulses injected into the node. The action potential almost always occurred singly, once the current was beyond threshold. Increases in the intensity and/or the duration of the current pulse did not usually cause multiple spike discharges. Only in rare instances in the early part of our experience, when relatively large preparations were used, was there a tendency for an occasional appearance of multiple spikes with increasing current strengths

or duration. The amplitude of the single spikes averaged $52{\cdot}9 \pm 0{\cdot}5$ mV ($n = 230$).

2. *Electrical constants of the preparation.* When inward currents were injected into the node, hyperpolarizing voltage responses were produced, which reached a steady state within 350–400 msec. Weak outward currents caused depolarizing responses similar to the corresponding hyperpolarizing responses. Outward currents beyond a threshold would elicit spikes. The time constant of the hyperpolarizing responses was $104{\cdot}2 \pm 2{\cdot}5$ msec ($n = 150$). An apparent input impedance, taken as the steady-state V/I (at the end of an inward current step) averaged $268{\cdot}9 \pm 4{\cdot}8$ kΩ ($n = 160$). The total capacity was $0{\cdot}40 \pm 0{\cdot}09$ μF ($n = 152$). Assuming the average area in the node to be 0·13 cm², a specific membrane resistance would be about 34 kΩ cm², and a specific membrane capacity would be about 3 μF/cm², values which are in good agreement with those found by Tomita (1970).

Constant-voltage conditions

1. *General descriptions and limitations of the method*

As has been shown for the pregnant-rat myometrium (Kao & McCullough, 1975), the isolated taenia coli preparation contains a significant resistance in series with the membrane. This series resistance has been estimated from the initial V/I relation on a 5 mV depolarizing step (Fig. 1), and averaged $11{\cdot}4 \pm 0{\cdot}25$ kΩ ($n = 160$). The membrane resistance estimated from dV/dI at 10 mV hyperpolarization (see Fig. 3) averaged $264{\cdot}0 \pm 0{\cdot}9$ kΩ ($n = 130$), a value which agreed well with the apparent input impedance estimated from constant-current records. Since the ratio of the series resistance : membrane resistance was 1 : 23, and since the nodal length of 54 μm was considerably shorter than the space constant of the taenia coli (1·8 mm, Tomita, 1970), it might be assumed that a reasonable degree of control was possible. In fact, however, uniform space clamp cannot be attained, because of the series resistance (which is largely the cleft resistance between cells; see Johnson & Lieberman, 1971). Loss of control was particularly evident when the ratio of series resistance : membrane resistance was low, such as occurred during the flow of inward current, or during the flow of larger outward current. An example of the latter instance is the 'dropping' of the outward current during a maintained voltage step (see below). The degree of voltage control cannot be properly estimated from the incomplete information available at present, and the only reliable way of assessing the accuracy of the method is to introduce a micro-electrode into the taenia cells within the node. Such checks, however, have not yet been successfully completed because of technical difficulties. Therefore, the information to be presented below

should be treated from a relative point of view, some properties of the node in a control condition being compared with similar properties of the same node in an experimental condition.

Fig. 1. 'Capacitative' current due to a 5 mV depolarizing step. The finite peak of the current is due to the presence of a significant resistance in series with the membrane. The slow rise is probably due to the presence of a small stray capacitance. The capacitative current decays exponentially, the average time constant being 4·9 msec. The steady-state current reflects the sum of the series resistance and the membrane resistance. See text for further details.

(*a*) *Currents associated with step-voltage functions.* Fig. 1 shows a typical record of the currents due to a 5 mV step depolarization. The initial 'capacitative' current was finite, and declined exponentially with a time constant of about 5 msec ($4{\cdot}86 \pm 0{\cdot}38$ msec in twelve preparations, as determined from semilogarithmic plots, Table 2). From this, the total capacity can be estimated to be $0{\cdot}43 \pm 0{\cdot}03$ μF. This value is comparable with that determined by a constant-current method in the same preparation.

Currents observed in the taenia coli (Fig. 2) were qualitatively similar to those in the myometrium (Anderson, 1969; Kao & McCullough, 1975), and those of many other excitable tissues. With pulses of about 100 msec, inward currents due to hyperpolarizing steps followed similar patterns, but currents due to depolarizing steps were complex, with an early transient phase of inward current followed by a late outward current. In many preparations of taenia coli, in which the total capacity was 0·4–0·5 μF, there was a tendency for the outward current to droop, even with a maintained voltage step. However, in small preparations, with total capacity of *ca.*

TABLE 2. Comparison of electrical constants of taenia coli preparation determined by constant voltage and by constant current method

		Constant voltage			Constant current,
Prep.	ΔV	τ_s (msec)	r_s (kΩ)	C_m (μF)	C_m (μF)
A	4	3·5	8·7	0·40	0·42
B	4	2·5	10·0	0·25	0·29
C	5	5·8	10·4	0·56	0·58
D	5	4·4	12·2	0·36	0·33
E	5	4·7	10·0	0·47	0·50
F	5	3·9	12·5	0·31	0·33
G	5	7·4	15·4	0·48	0·38
H	5	5·3	12·0	0·44	0·50
I	5	4·0	9·0	0·44	0·48
J	5	4·8	10·8	0·44	0·41
K	5	5·7	12·2	0·46	0·48
L	5	6·4	12·2	0·52	0·53
		4·86 ± 0·38	11·2 ± 0·53	0·43 ± 0·03	0·44 ± 0·03

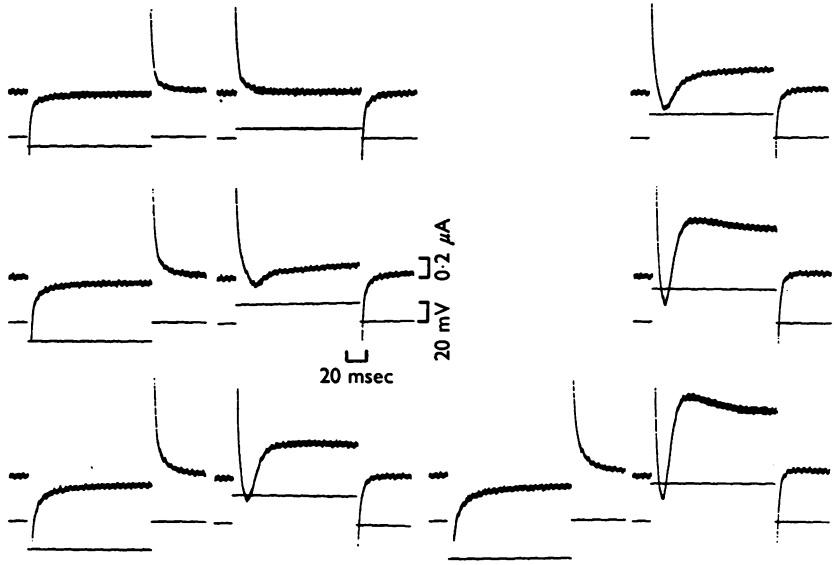

Fig. 2. Currents due to step voltage changes. The preparation was held at −42 mV, which was its natural resting potential. The steps were: −10 mV (hyperpolarizing) and +10 mV (depolarizing); −20 and +20 mV; −28 and +28 mV; +24 mV; +34 mV; and −38 and +38 mV. Note the net inward currents upon threshold depolarization. The outward current is flat except at large current levels. Calibrations hold for all frames.

0·2 μF, the outward current was maintained with no evidence of drooping, except when the outward currents were large. The fact that no droop was observed in small preparations, in which the technical aspects were probably superior, suggests that the droop itself cannot be a part of any significant inactivation process.

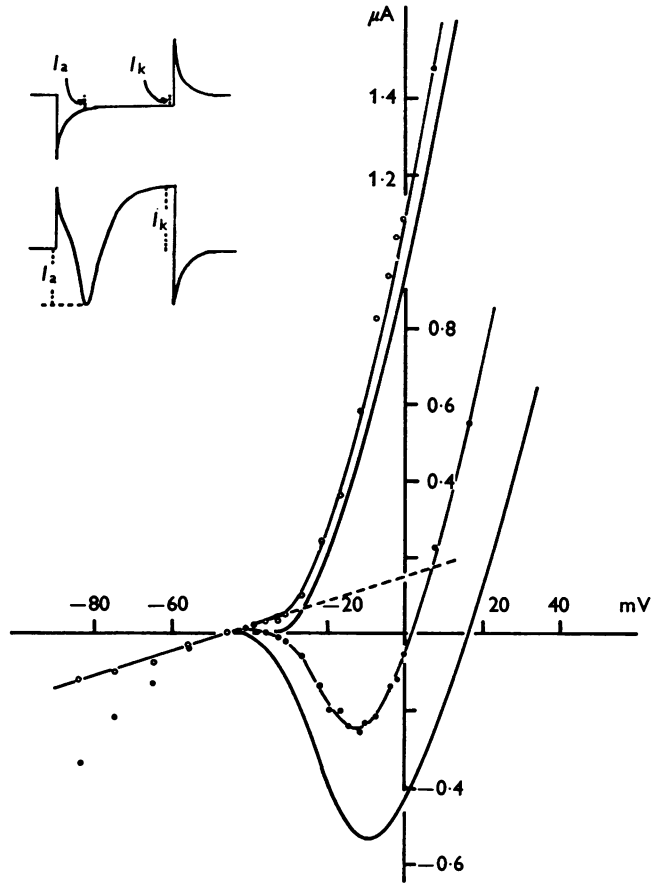

Fig. 3. Current–voltage relation of a typical experiment. Inset shows manner of obtaining the data. Filled circles are for the early current, and open circles for the late current. Thicker continuous curves are currents after correction for leakage and residue capacitative currents.

(*b*) *Current–voltage relation.* Fig. 3 shows the current–voltage relations of the early current and the late current in the voltage-clamped taenia coli preparation, before and after correcting for the leakage and capacitative currents. The late current was determined at about 100 msec after the beginning of the voltage step, when no capacitative current remained; and

the correction needed was that for leakage current. This correction was made by extrapolating the straight line from the hyperpolarizing side to the depolarizing side (see also Anderson, 1969; Kao & McCullough, 1975). For the early current, however, the peak was reached at 10–50 msec, at which time a small but appreciable capacitative current remained in addition to any leakage current. The correction for the early current was, therefore, made by alternating hyperpolarizing pulses with depolarizing pulses, and subtracting currents due to the former from those due to the latter, matching time and voltage for each.

Assuming a simple equivalent circuit, in which a resistance exists in series with the membrane, some estimates can also be made of the proportion of currents flowing through the resistive and capacitative elements at different times (see Kao & McCullough, 1975). Using average values of series resistance, $r_s = 11{\cdot}4$ kΩ, resistance of membrane, $r_m = 264$ kΩ; and capacity, $c_m = 0{\cdot}4$ μF, it is found that the current flowing through the resistive element is: at 20 msec, 0·99 of the total current; at 15 msec, 0·97; at 10 msec, 0·90; and at 5 msec, 0·68. Therefore, it can be concluded that for currents at later than 10 msec, most of it is attributable to the resistive elements, presumably ionic currents.

The leakage conductance estimated from the slope of the I/V of the late current at 10 mV hyperpolarization is 3·78 μmho.

The early current has a negative resistance region, and after corrections, a reversal potential at about 22 mV. As shall be made clear in a later section (p. 371), this voltage cannot be considered as representing any specific ionic equilibrium potential, because of an overlap of some late outward current with the early inward current.

2. *Late current*

Observations on the late current are being presented first, because some factors which affect the late current have an important influence in clarifying the ionic nature of the early current.

(*a*) *Ionic nature of the late current.* The experiments to identify the main charge carrier of the late current are modelled after those of Frankenhaeuser (1962) on the node of Ranvier, and are illustrated by one typical case in Fig. 4. A taenia coli preparation was first voltage-clamped in Krebs-bicarbonate solution, in which the late current was always outward (e.g. Figs. 2 and 3). The node was then bathed in isotonic K_2SO_4 (solution J), in which it became nearly completely depolarized, the 'resting' potential becoming −4 mV. This depolarized preparation was held by the feed-back system to the original resting potential in Krebs–bicarbonate (−47 mV). Step voltage were then applied as before. In this condition, an inward holding current was required, since the holding potential was substantially

negative to the voltage at which minimal holding current would be needed (see Kao & McCullough, 1975). In addition to this holding current, step depolarization elicited a late current which was inward, rather than outward as in normal conditions (Fig. 4). Only with large depolarizing steps did the late current become outward. Moreover, the tail current at the end

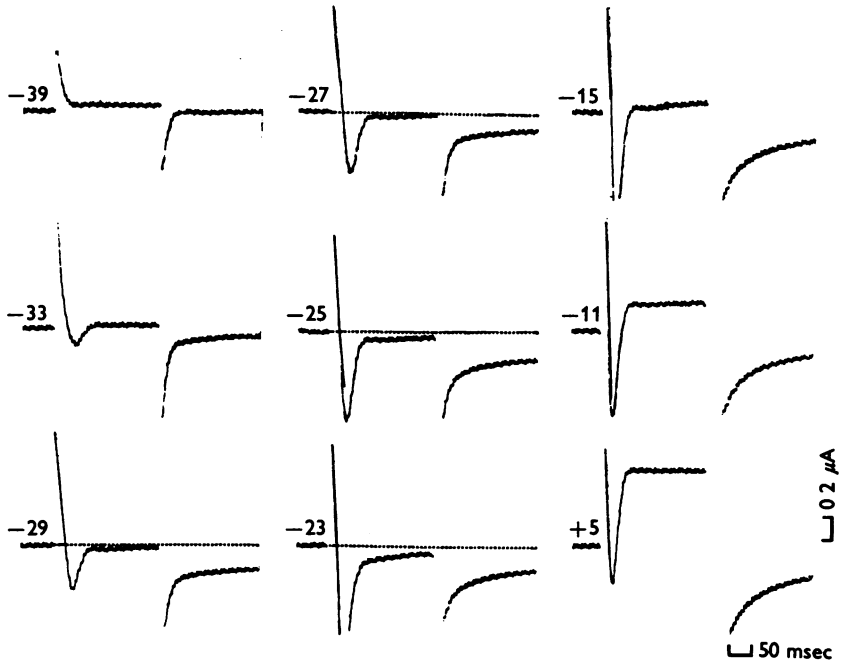

Fig. 4. Currents in a depolarized preparation. The preparation was depolarized in K_2SO_4, in which it attained a 'resting potential' of −4 mV. It was held back to −47 mV, requiring some inward holding current (not shown). Upon depolarization, the late current was inward for most voltage steps. The early current is due to Ca^{2+} influx (p. 366). Note also the long inward tail current upon repolarization.

of the voltage step was inward. The current–voltage relation (Fig. 5) shows that the late current is inward between the holding potential and the 'resting' potential of the depolarized state. As in the node of Ranvier, the explanation for the late inward current is that the electrochemical potential has been substantially altered by holding the depolarized preparation at a negative voltage, so that when the late-current conductance was increased by depolarization, K^+ moved inward, rather than outward as it would under normal conditions.

(*b*) *Reversal potential of the late current* (E_b). The 'instantaneous' tail current obtained by a two-pulse technique (e.g. Kao & McCullough, 1975) has been used to determine the reversal potential of the late current. Since the time constant of decay of the capacitative current in the taenia coli is

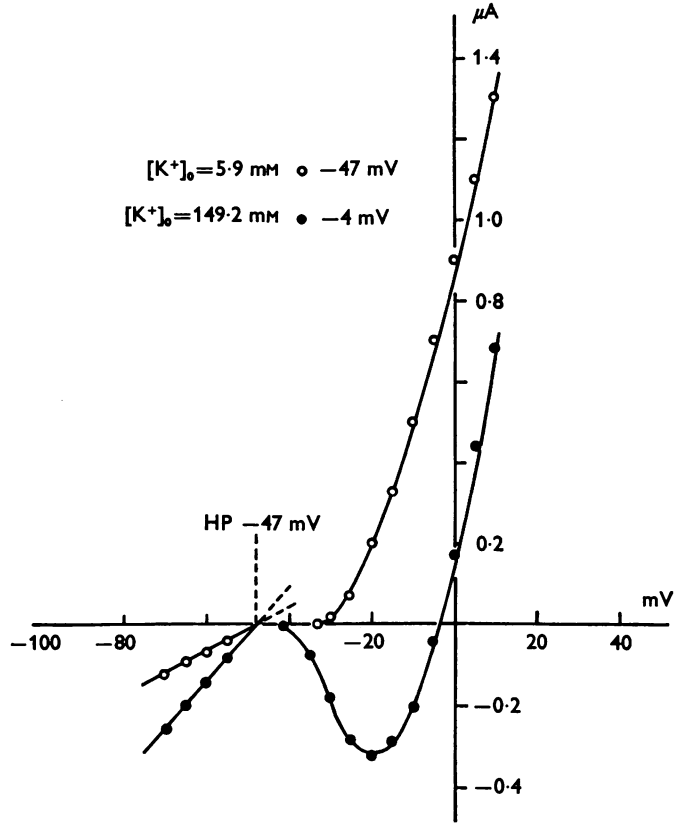

Fig. 5. Current–voltage relations of the late current in normal and in K^+-depolarized taenia coli. The normal current records for this preparation are not shown, but are similar to those in Fig. 2, and those for the depolarized state are shown in Fig. 4. Only the late current is shown; the reversal potential in the depolarized preparation is −4 mV.

about 5 msec (p. 353), a correction must be made for the capacitative artifact in the tail current (Fig. 6). It is clear from the net current–voltage curve that the relation is ohmic, and that the zero-current potential of the tail current represents the reversal potential of the late current. As shall become clear later (p. 362), although the late current is carried mainly by K^+, the cation selectivity of the membrane for K^+ is still relatively low

when compared with that of some other membranes. Therefore the term 'reversal potential' rather than the term 'equilibrium potential' is more appropriate. The reversal potential (E_b) is $-66{\cdot}5 \pm 0{\cdot}9$ mV (means ± S.E. of mean of thirty-five preparations), compared with the 'resting' potential of $-48{\cdot}6 \pm 0{\cdot}6$ mV.

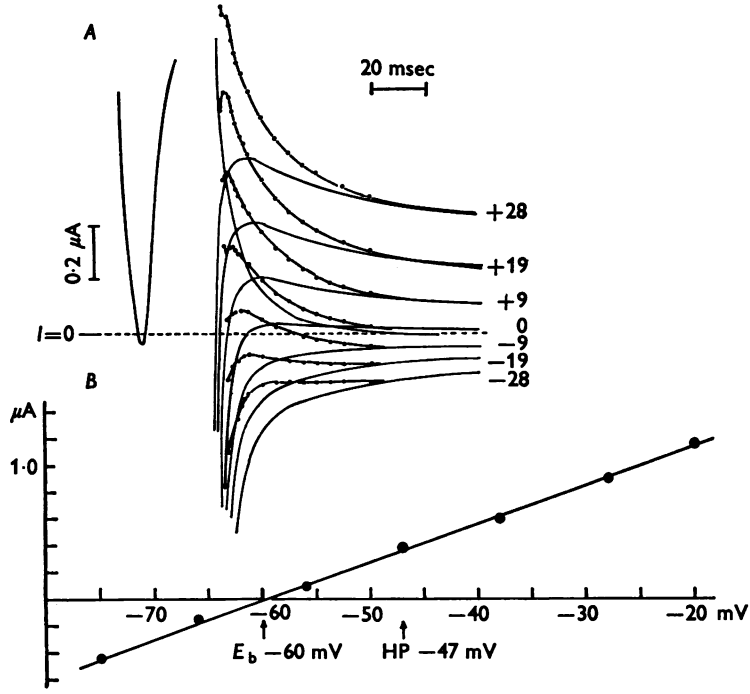

Fig. 6. Determination of reversal potential of late current (E_b) from tails of the late current. *A*, tracings of tail currents and correction. The thin continuous curves are tracings of actual currents. V_1 was +40 mV, and the same for all traces; it activated the early current and the late current. V_2 was variable (see number attached to each trace), and repolarized the membrane to different voltages, producing a family of tail currents. Tail current due to a hyperpolarizing step ($-V_1$) is also included. The curves with points are the corrected tail currents. Current values for *B* were taken at 3 msec from beginning of V_2. *B*, 'instantaneous' current–voltage relation. Corrected current values were plotted against membrane voltage repolarized by V_2. Note that the relation is ohmic, and that the zero-current potential is about 15 mV more negative than the natural resting potential, which was also the holding potential.

As shown in Fig. 7, the reversal potential is not affected by varying either the amplitude or the duration of V_1, the activating voltage step. Changing the amplitude of V_1 changed the amount of late current activated, and consequently, the slope conductance of the 'instantaneous'

tail current. The effect of the duration of V_1 on the tail current conductance is more complex. In some preparations, when the late current showed a droop, prolonging the duration of V_1 produced an effect of reducing the amount of the late current and its conductance. But in those preparations in which the late current was maintained, these features were not present. Since the droop in the late current is probably attributable to loss of voltage control, the tail current conductance probably is unaffected by the duration of V_1.

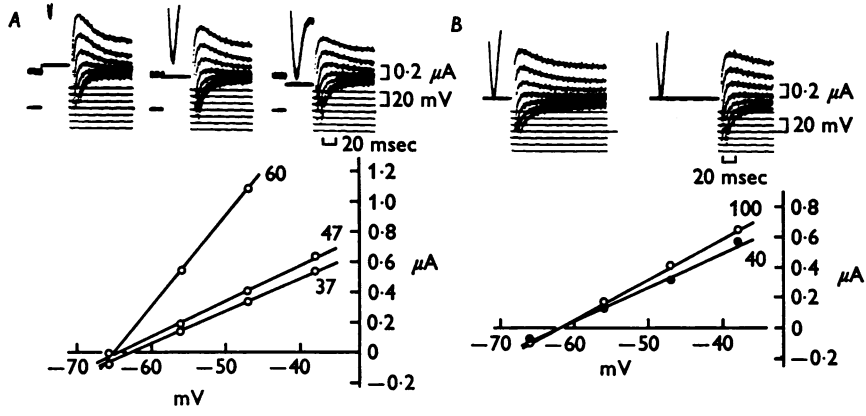

Fig. 7. *A*, relation between the amplitude of V_1 and E_b. Actual voltage and current records, obtained by multiple exposures, are shown in the top part. From left to right, the V_1 steps are +60, +47, and +37 mV. Bottom part shows the relation between membrane voltage and corrected tail currents. Although the slope conductance is different for the three different V_1, E_b is practically identical. *B*, relation between duration of V_1 and E_b. Top part shows the actual records, with V_1 of 40 msec (left) and of 100 msec (right). Bottom part shows the 'instantaneous' current–voltage relation. Note that E_b is the same.

(*c*) *Effect of holding potential on* E_b. As in the myometrium (Kao & McCullough, 1975), holding the taenia coli at its natural resting potential required negligible holding current, whereas holding the preparation at any potential other than the resting potential required some holding current. Because of the very small sizes of the individual cells in the taenia, any holding current applied for a sufficient time can be expected to produce some passive shifts in ionic distribution. Fig. 8 shows this effect in four preparations. In each, E_b was determined first when the preparation was held at its natural resting potential, then when the preparation was hyperpolarized, and again when it was depolarized. In each case, the preparation was held for 3–5 min before E_b was determined. When the preparation was hyperpolarized, the E_b became more negative, and the holding

potential and E_b were related by a line with a slope of unity. When the preparation was depolarized, E_b became less negative, but the line relating the two potentials had a slope greater than unity. The explanation of these results is that on hyperpolarization, some changes in $[K^+]_i$ were produced

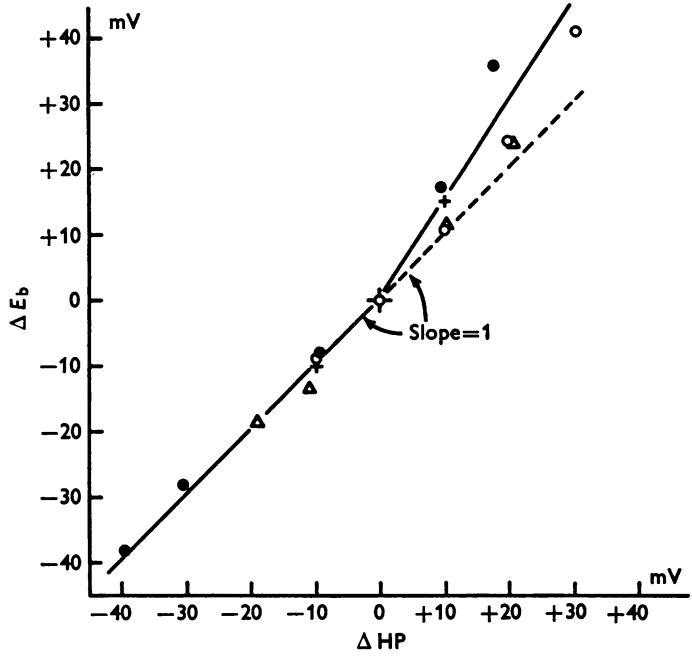

Fig. 8. Relation between changes in holding potential and changes in E_b. Four different preparations of taenia coli all held at their natural resting potential (at origin). When holding potential was made more negative, E_b became correspondingly more negative. When holding potential was made less negative, changes in E_b exceeded changes in holding potential. This is interpreted to mean that there was K^+ accumulation just external to the membrane.

by the imposed holding current; on depolarization, a similar change occurred, but, because of the presence of some diffusion lag, an accumulation of K^+ immediately outside the taenia cell membrane was possible, thereby producing a greater change in E_b than can be accounted for by a passive shift caused by the holding current (contrast with myometrium, Kao & McCullough, 1975).

(*d*) *Resting potential and* E_b *in different* $[K^+]_o$. The relation between $[K^+]_o$ and the resting potential and the E_b in the taenia coli has been determined by using solution containing different $[K^+]_o$, all chloride having been replaced by sulphate. The results are summarized in Fig. 9. These

findings are consistent with earlier observations (Kuriyama, 1963) in: (*a*) when $[K^+]_o \geqq 5{\cdot}9$ mM, the resting potential varied linearly with the log of $[K^+]_o$; and (*b*) when $[K^+]_o < 5{\cdot}9$ mM, the resting potential remained relatively fixed, with a slight positive shift when $[K^+]_o$ was made very low.

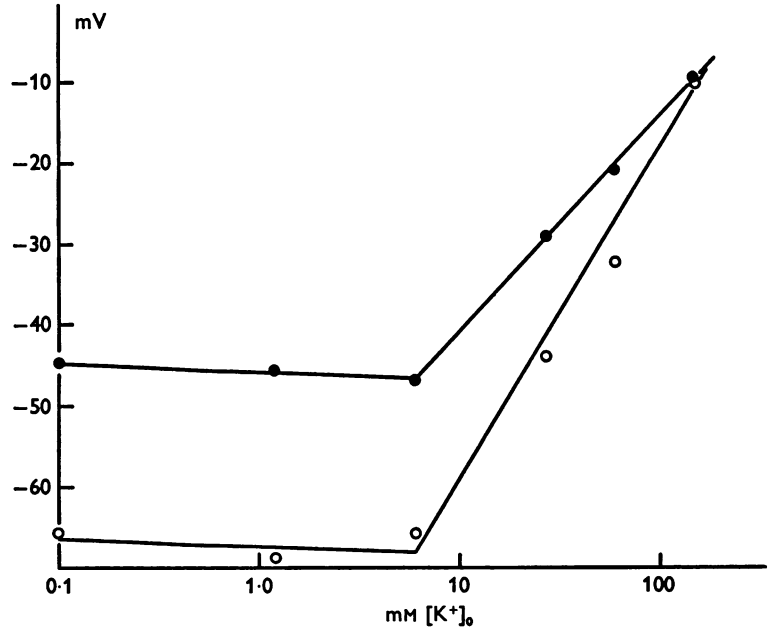

Fig. 9. Relations between $[K^+]_o$ (maintained by using K_2SO_4), and resting potential (filled circles) and E_b (open circles). $P_{Na}:P_K$ has been estimated to be 0·16 for resting potential, and 0·05 for E_b.

The slope of the curve relating resting potential and log $[K^+]_o$ is 25 mV per decade. E_b is about 20 mV more negative than the resting potential for $[K^+]_o < 5{\cdot}9$ mM. For $[K^+]_o > 5{\cdot}9$ mM, the difference between E_b and the resting potential became progressively smaller with increasing $[K^+]_o$. At $[K^+]_o = 149$ mV, E_b and the resting potential are the same. The slope of the curve relating E_b to the log of $[K^+]_o$ is 43 mV per decade. Assuming that the deviation of these curves from the ideal 61 mV (at 37° C) is due to some resting P_{Na}, the ratio P_{Na}/P_K (*b*, Hodgkin, 1958) can be estimated by

$$E = \frac{RT}{F} \ln \frac{[K^+]_o + b[Na^+]_o}{[K^+]_i + b[Na^+]_i},$$

using some concentration values from Casteels & Kuriyama (1966). For the natural resting potential, $b = 0{\cdot}16$, which is close to that (0·19) obtained by Casteels (1970) from flux measurements, and to one recalculated from Casteels & Kuriyama (0·13). For E_b, $b = 0{\cdot}05$.

(e) *Effect of tetraethylammonium on the late current.* As in nerve and skeletal muscle (e.g. Armstrong, 1969; Kao & Stanfield, 1970), TEA caused a prolongation of the spike in taenia coli cells, along with an increase in the amplitude of the spikes (Suzuki, Nishiyama & Inomata, 1963). TEA is effective on the taenia coli in a concentration as low as

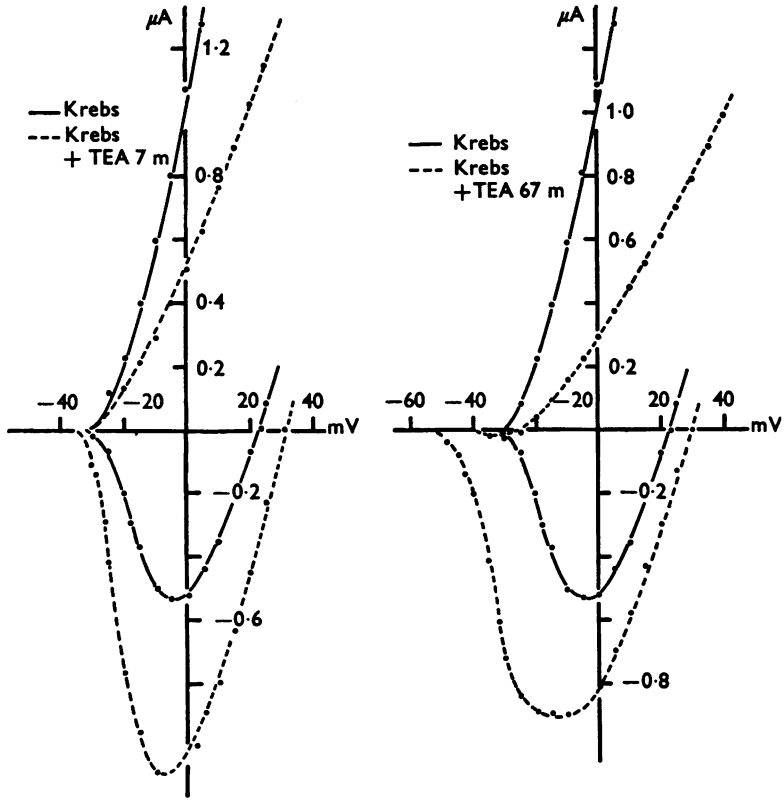

Fig. 10. Effect of tetraethylammonium (TEA) on the current–voltage relations of the early and late currents in the guinea-pig taenia coli. See text for details.

7 mM, and its effect on the late current is greater when the concentration is increased to 67 mM (Fig. 10). From Table 9 it can be seen that even in 67 mM TEA did not completely block the late conductance channel, but reduced $\bar{g}_b$ to about one half of that in the average untreated preparation. As shall be discussed in more detail later (p. 371), by reducing I_b, TEA caused a marked increase of the early current, I_a, as well as a shift of E_a toward the positive. These changes are believed to be due to technical imperfections of the voltage-clamp technique as applied to taenia coli.

(*f*) *Factors affecting the late current:*

(i) *Calcium.* Decreasing $[Ca^{2+}]_o$ to 0·25 or 0·1 of the normal 1·9 mM in Krebs solution had no significant effect on the late current, the maximum conductance, $\bar{g}_b$, remaining fairly constant (see Tables 5 and 6). A similar lack of effect was also observed in preparations previously depolarized in K_2SO_4 (Table 8, item 9). Increasing $[Ca^{2+}]_o$ to 9·5 mM, 5 times the normal concentration, significantly increased the late current, and the $\bar{g}_b$. The E_b was unaffected (Table 7). These effects are also seen in properties of the tail current, similar to those shown in Fig. 7.

(ii) *Manganese.* In 1, 2 and 5 mM, Mn^{2+} depressed the late current, and decreased the $\bar{g}_b$. The E_b was unaffected in 1 and 2 mM-Mn^+, but became less negative in 5 mM, in agreement with a depolarizing effect of the latter.

3. *Early current*

(*a*) $[Na^+]_o$ *and the early current.* There is considerable uncertainty as to the ion responsible for the depolarizing current of the spike in the taenia coli. There is some early evidence that the spike amplitude and the maximum rate of rise of the spike in spontaneously active preparations were both reduced when $[Na^+]_o$ was less than 5 mM (Holman, 1957; Bülbring & Kuriyama, 1963). More recently, from both intracellular recordings and extracellular recordings, it has been reported that removal or depletion of extracellular Na^+ did not reduce either the amplitude or the maximum rate of rise of spikes elicited by applied outward currents (Brading, Bülbring & Tomita, 1969). On the contrary, the spike amplitude was reported to be significantly increased. The change, opposite to one that might be expected of a Na^+-spike, was considered as a strong argument against the possibility of a Na^+-spike mechanism in the taenia coli (Brading *et al.* 1969). Since a hyperpolarization, as well as a significant reduction in the leakage conductance (Table 3), occurred in a low Na^+ medium (see below), the higher spike amplitude, by itself, is not sufficient argument against the possibility that a reduced Na^+ influx could still be responsible for the spike. We have attempted to re-examine the problem of the ionic nature of the early current by using the voltage-clamp technique, which should provide relatively direct information on the reversal potential for the early current (E_a), the magnitude of the early current (I_a), as well as the conductance associated with the early current ($\bar{g}_a$).

Two types of experiments were performed, each with slightly different procedure. In the first type, a solution containing no Na^+ (solution C, Table 1) was used, and the node, after a control run in Krebs–bicarbonate solution, was bathed in it for 20–30 min. At the end of this period, both current-clamp and voltage-clamp runs were conducted. In the second type of experiment, a solution containing 50% of the usual $[Na^+]_o$ was used

(solution D), and the node, after a control series, was bathed in it for 5 min before the current-clamp and voltage-clamp runs were conducted. In the first type, using a Na^+-free medium, the purpose of the long wash was to attempt to reduce the concentration of Na^+ in the extracellular space, without great concern for the $[Na^+]_i$. In the second type, the objective, based on an assumption that the extracellular $[Na^+]_o$ can be brought to an equilibrium with that in the test solution within the test period, was to maintain the $[Na^+]_i$ the same as in Krebs–bicarbonate solution, and to see whether a change in the reversal potential of the early current would match some alterations predicted on the basis of a Na^+-selective membrane (see Hodgkin & Huxley, 1952).

TABLE 3. Effect of Na^+-free solution on voltage-clamped taenia coli

	$[Na^+]_o$		
	149 mM	0 mM	*P*
1. Resting potential (mV)	$-45{\cdot}1 \pm 1{\cdot}4$ (8)	$-54{\cdot}5 \pm 1{\cdot}4$ (8)	0·01
2. Spike amplitude (mV)	$50{\cdot}8 \pm 1{\cdot}7$ (8)	$58{\cdot}6 \pm 1{\cdot}7$ (8)	0·001
3. E_a (mv)	$19{\cdot}5 \pm 3{\cdot}0$ (8)	$21{\cdot}3 \pm 3{\cdot}2$ (8)	0·3
4. I_a (μA)	$-0{\cdot}38 \pm 0{\cdot}04$ (8)	$-0{\cdot}55 \pm 0{\cdot}07$ (8)	0·04
5. G_1 (μmho)	$5{\cdot}9 \pm 0{\cdot}8$ (8)	$4{\cdot}9 \pm 0{\cdot}6$ (8)	< 0·001
6. $\bar{g}_a$ (μmho)	$32{\cdot}2 \pm 2{\cdot}2$ (6)	$33{\cdot}2 \pm 2{\cdot}4$ (6)	> 0·3

All values are mean ± S.E. of mean with number of preparations in parentheses. *P* values were obtained on basis of paired comparison. Item 3 is the reversal potential of early current. Item 4 is the maximum early current, with the negative sign indicating it is inward. Item 5 is the slope conductance of V/I at the end of a voltage step, taken at 10 mV hyperpolarization from a curve of current–voltage relation. Item 6 is the maximum chord conductance, defined as $g_a = I_a/(E-E_a)$.

Na^+-free solution. The effects of replacing all Na^+ with dimethyldiethanol ammonium and choline ions on the taenia coli are summarized in Table 3 and Fig. 11. There is a significant hyperpolarization, and a significant increase in the spike amplitude. In the voltage-clamp mode, the leakage conductance (G_1) is significantly reduced and the early inward current (I_a) is significantly increased, changes which are consistent with the hyperpolarization and the increase in spike amplitude under constant-current conditions. There is, however, no appreciable shift in the reversal potential of the early current (E_a), nor is there any alteration in the conductance associated with the early current ($\bar{g}_a$).

50% $[Na^+]_o$. Provided that the concentration of Na^+ outside the membrane can be altered appropriately, and that $[Na^+]_i$ remained constant, a 50% reduction of the $[Na^+]_o$ should produce a shift of 18·4 mV of the E_a toward the negative, if the early current were carried by Na^+. The

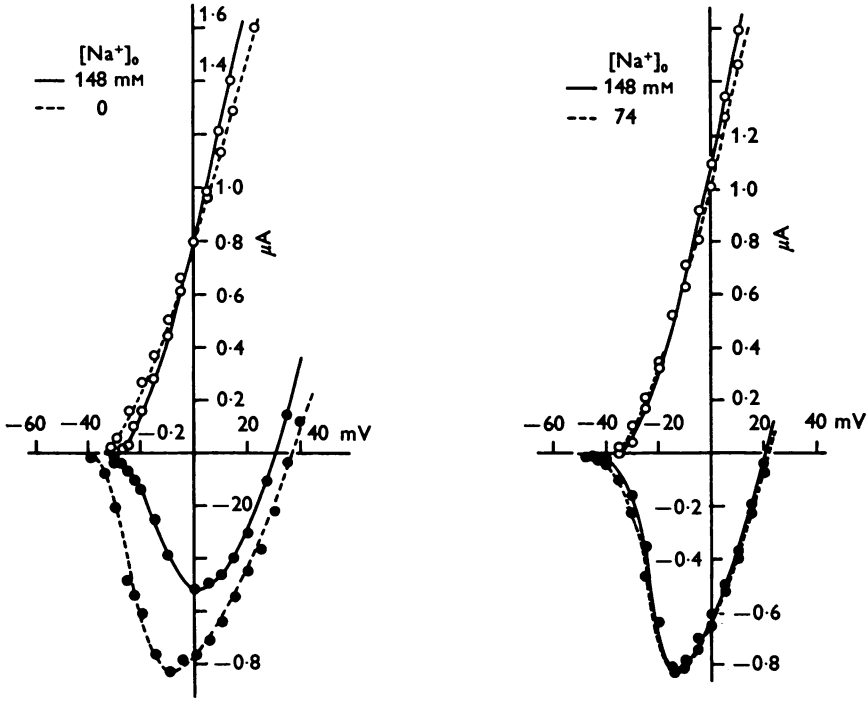

Fig. 11. Current–voltage relations in Na^+-free solution and in 50% $[Na^+]_o$ solution. These curves are based on currents after appropriate corrections for leakage and capacitative currents. See text for details.

results of this series of experiments are summarized in Table 4, and a typical example of the current–voltage relation is shown in Fig. 11. It is clear that within the test period employed, a 50% reduction of $[Na^+]_o$ had no effect on any of the electrical properties of the taenia coli.

(*b*) $[Ca^{2+}]_o$ *and the early current.* The objectives of these experiments are similar to those for the 50% reduction in $[Na^+]_o$. We have avoided experiments with a Ca^{2+}-free medium, because limited experience with the taenia coli confirmed earlier experience on the pregnant-rat myometrium which became rapidly and irreversibly depolarized in media buffered to contain 10^{-6} M $[Ca^{2+}]_o$ (Kao & McCullough, 1975). However, since the $[Ca^{2+}]_o$ is normally 1·9 mM, it has been possible to reduce as well as to increase the $[Ca^{2+}]_o$ without causing appreciable osmotic effects.

TABLE 4. Effect of 50% $[Na^+]_o$ on voltage-clamped taenia coli

	$[Na^+]_o$ 149 mM	$[Na^+]_o$ 71 mM	P
1. Resting potential (mV)	$-46{\cdot}2 \pm 2{\cdot}6$ (5)	$-52{\cdot}2 \pm 1{\cdot}9$ (5)	0·07
2. Spike amplitude (mV)	$54{\cdot}6 \pm 2{\cdot}5$ (5)	$54{\cdot}0 \pm 2{\cdot}3$ (5)	0·1
3. E_a (mV)	$18{\cdot}8 \pm 1{\cdot}5$ (5)	$17{\cdot}4 \pm 1{\cdot}7$ (5)	0·2
4. I_a (μA)	$-0{\cdot}56 \pm 0{\cdot}07$ (5)	$-0{\cdot}56 \pm 0{\cdot}08$ (5)	0·3
5. G_1 (μmho)	$4{\cdot}3 \pm 0{\cdot}4$ (5)	$3{\cdot}8 \pm 0{\cdot}3$ (5)	0·3
6. $\bar{g}_a$ (μmho)	$34{\cdot}7 \pm 2{\cdot}3$ (4)	$38{\cdot}0 \pm 1{\cdot}2$ (4)	
7. $\bar{g}_b$ (μmho)	$23{\cdot}8 \pm 1{\cdot}6$ (4)	$21{\cdot}8 \pm 1{\cdot}9$ (4)	
8. E_b (mV)	$-64{\cdot}5 \pm 4{\cdot}2$ (4)	$-64{\cdot}5 \pm 4{\cdot}2$ (4)	

Conventions are similar to those in Table 3. Item 7 is the maximum chord constance of the late current, which has been identified as mainly a K^+ current. Item 8 is the reversal potential of the late current.

0·48 *and* 0·19 *mM*-$[Ca^{2+}]_o$. These concentrations represent 0·25 (solution F) and 0·1 (solution E) of the normal $[Ca^{2+}]_o$. Although the spike amplitude was significantly reduced in both solutions, a significant depolarization occurred only in 0·19 mM-$[Ca^{2+}]_o$ (Tables 5, 6). Were Ca^{2+} to be the only charge carrier for the early current, a shift of $-18{\cdot}4$ mV in the reversal potential could be expected in the 0·48 mM-$[Ca^{2+}]_o$ solution, and a shift of -30 mV in the 0·19 mM solution. The effects shown in Tables 5 and 6 were obtained within a similar test period as that used for testing the influence of $[Na^+]_o$. It can be seen that in 0·48 mM-$[Ca^{2+}]_o$, E_a is shifted toward the negative, I_a is reduced (Fig. 12), but $\bar{g}_a$ is not affected. In 0·19 mM-$[Ca^{2+}]_o$, the changes in E_a and I_a are greater, but, importantly, $\bar{g}_a$ is significantly lowered. As in the myometrium (Kao & McCullough, 1975), in the reduced $[Ca^{2+}]_o$ solutions the peak of the early current is reached more slowly.

9·5 *mM*-$[Ca^{2+}]_o$. This concentration represents a fivefold increase in the $[Ca^{2+}]_o$ (solution G). For a Ca^{2+}-selective membrane, a shift of 21 mV (toward the positive) of the reversal potential could be expected. An example of the I–V relation in this high $[Ca^{2+}]_o$ solution is shown in Fig. 12. Table 7 summarizes the effects of such a solution. The resting potential

TABLE 5. Effect of 10% $[Ca^{2+}]_o$ on voltage-clamped taenia coli

	$[Ca^{2+}]_o$		
	1·9 mM	0·19 mM	*P*
1. Resting potential (mV)	−47·7 ± 0·9 (7)	−44·1 ± 1·5 (7)	0·05
2. Spike amplitude (mV)	58·0 ± 1·9 (7)	49·0 ± 1·87 (7)	0·007
3. E_a (mV)	23·9 ± 1·7 (7)	5·7 ± 2·0 (7)	< 0·001
4. I_a (μA)	−0·46 ± 0·07 (7)	−0·24 ± 0·04 (7)	< 0·001
5. G_1 (μmho)	4·6 ± 3·3 (7)	3·3 ± 0·6 (7)	< 0·001
6. $\bar{g}_a$ (μmho)	31·9 ± 3·9 (7)	25·1 ± 2·9 (7)	0·02
7. $\bar{g}_b$ (μmho)	23·3 ± 2·1 (7)	19·9 ± 2·5 (7)	0·07
8. E_b (mV)	−63·3 ± 2·3 (7)	−62·1 ± 1·9 (7)	0·1

Conventions similar to those in Tables 3 and 4.

TABLE 6. Effect of 25% $[Ca^{2+}]_o$ on voltage-clamped taenia coli

	$[Ca^{2+}]_o$		
	1·9 mM	0·48 mM	*P*
1. Resting potential (mV)	−47·9 ± 0·9 (9)	−46·5 ± 0·9 (9)	0·06
2. Spike amplitude (mV)	53·0 ± 1·8 (9)	49·4 ± 1·8 (9)	0·001
3. E_a (mV)	21·7 ± 1·6 (9)	10·2 ± 1·5 (9)	< 0·001
4. I_a (μA)	−0·48 ± 0·06 (9)	−0·38 ± 0·05 (9)	0·02
5. G_1 (μmho)	4·4 ± 0·3 (9)	3·8 ± 0·3 (9)	< 0·001
6. $\bar{g}_a$ (μmho)	34·0 ± 2·0 (10)	30·1 ± 1·8 (10)	0·1
7. $\bar{g}_b$ (μmho)	22·4 ± 1·2 (10)	21·9 ± 1·1 (10)	0·3
8. E_b (mV)	−65·1 ± 0·5 (9)	−65·6 ± 0·5 (9)	0·3

Conventions similar to those in Tables 3 and 4.

became more negative, and the spike amplitude was significantly increased. The magnitude of the early current (I_a) was increased. These changes in the spike amplitude and I_a are probably attributable to a fairly significant increase in $\bar{g}_a$. In spite of these changes, the E_a remained essentially unaffected. Unlike published observations made in constant-current studies (Brading *et al.* 1969), in which the membrane resistance estimated from inward currents was reduced in a medium containing 12·5 mM-$[Ca^{2+}]_o$, our observations with 9·5 mM-$[Ca^{2+}]_o$ indicate that the membrane resistance (estimated from G_1) was not affected.

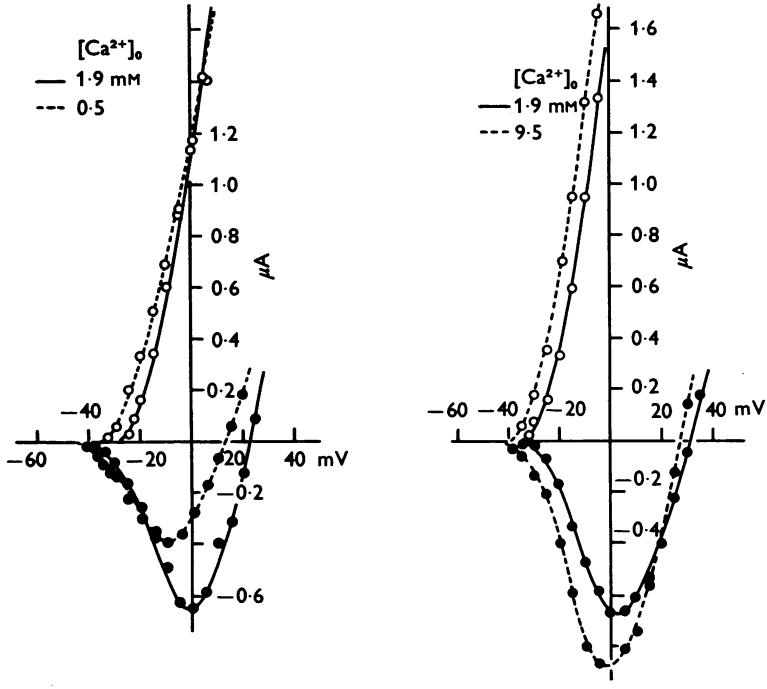

Fig. 12. Current–voltage relation in low and high $[Ca^{2+}]_o$ solutions. These currents have been corrected for leakage and residue capacitative currents. Note that reversal potential for early current was shifted by reducing $[Ca^{2+}]_o$, but not by increasing $[Ca^{2+}]_o$. See text for details.

(*c*) *Effect of* 0·19 *mM-*$[Ca^{2+}]_o$ *on taenia coli depolarized with* K_2SO_4. The results presented above of reducing $[Ca^{2+}]_o$, although suggestive of a role of Ca^{2+} as a charge carrier for the early current, are complicated by an effect on the early-current conductance (Table 5). To strengthen the view that Ca^{2+} has a charge-carrying function, it would be necessary to show that E_a and I_a can be affected by changes in $[Ca^{2+}]_o$ without concomitant changes in any conductances. The experiments were performed on taenia

coli preparations which had been depolarized in a high-K^+ solution (solution J), and held back to the original resting potential in Krebs–bicarbonate solution, from which step-voltages were applied.

TABLE 7. Effect of $5 \times [Ca^{2+}]_o$ on voltage-clamped taenia coli

	$[Ca^{2+}]_o$ 1·9 mM	$[Ca^{2+}]_o$ 9·5 mM	P
1. Resting potential (mV)	$-49{\cdot}7 \pm 1{\cdot}2$ (8)	$-54{\cdot}3 \pm 1{\cdot}0$ (8)	< 0·001
2. Spike amplitude (mV)	$54{\cdot}3 \pm 1{\cdot}7$ (10)	$58{\cdot}8 \pm 1{\cdot}9$ (10)	< 0·001
3. E_a (mV)	$27{\cdot}3 \pm 3{\cdot}6$ (8)	$29{\cdot}4 \pm 3{\cdot}6$ (8)	0·15
4. I_a (μA)	$-0{\cdot}53 \pm 0{\cdot}08$ (8)	$-0{\cdot}76 \pm 0{\cdot}11$ (8)	0·01
5. G_1 (μmho)	$3{\cdot}4 \pm 0{\cdot}2$ (8)	$3{\cdot}2 \pm 0{\cdot}3$ (8)	< 0·3
6. $\bar{g}_a$ (μmho)	$15{\cdot}7 \pm 2{\cdot}8$ (7)	$33{\cdot}4 \pm 4{\cdot}5$ (7)	0·01
7. $\bar{g}_b$ (μmho)	$18{\cdot}9 \pm 1{\cdot}8$ (7)	$23{\cdot}9 \pm 2{\cdot}5$ (7)	0·003
8. E_b (mV)	$-67{\cdot}4 \pm 2{\cdot}1$ (7)	$-66{\cdot}6 \pm 1{\cdot}9$ (7)	0·27

Conventions similar to those in Tables 3 and 4.

In comparison with taenia coli preparations in Krebs-bicarbonate solution, the depolarized preparations have a leakage conductance some 2–2·5 times higher, and a $\bar{g}_a$ two thirds as high (Table 8). $\bar{g}_b$ was about the same. E_a in the depolarized preparations averaged 22·7 mV, which is in good agreement with that found in a Na^+-free solution (21·3 mV, Table 3).

When $[Ca^{2+}]_o$ was lowered from 1·9 to 0·19 mM (solution K), I_a was reduced to about one half, and E_a was shifted by -11 mV. None of the conductances were affected (Table 8), showing that a charge-carrying role of Ca^{2+} can be separated from any conductance-influencing role.

(*d*) *Effect of changing* $[Ca^{2+}]_o$ *ont aenia coli treated with tetraethylammonia ion.* One obstacle to a clear conclusion that Ca^{2+} carries charges during the early-transient current is the absence of any shift in the E_a when $[Ca^{2+}]_o$ was increased by fivefold. A possible reason for failing to observe such a shift might lie in some overlap of an outward current with the early-inward current. Thus, if the outward current had not been affected by changes in $[Ca^{2+}]_o$ (Tables 5–7), then the apparent reversal potential of the early current might remain fairly well fixed, as much determined by the

threshold for activating the late current as by the driving forces on the early current. To obviate this possible difficulty, some experiments were conducted in which the taenia coli preparation was first treated with tetraethylammonium ion (TEA) (see p. 363).

TABLE 8. Effect of 10% $[Ca^{2+}]_o$ on K_2SO_4-depolarized and voltage-clamped taenia coli

	$[Ca^{2+}]_o$		
	1·9 mM	0·19 mM	*P*
1. Holding potential (mV)	−46·3 ± 1·9 (6)	−46·3 ± 1·9 (6)	—
2. I_H (μA)	−0·4 ± 0·7 (6)	−0·3 ± 0·3 (6)	0·3
3. E_a (mV)	22·7 ± 2·8 (6)	11·2 ± 1·6 (6)	0·01
4. I_a (μA)	−0·46 ± 0·01 (6)	−0·24 ± 0·04 (6)	0·06
5. G_1 (μmho)	9·9 ± 1·5 (6)	9·2 ± 1·4 (6)	0·06
6. $\bar{g}_a$ (μmho)	20·6 ± 2·9 (6)	20·6 ± 2·1 (6)	0·3
7. $\bar{g}_b$ (μmho)	26·6 ± 2·2 (6)	24·3 ± 2·5 (6)	0·3
8. E_b (mV)	−11·8 ± 3·0 (6)	−12·0 ± 2·3 (6)	0·3
9. Max I_b	−0·1 ± 0·9 (6)	−0·1 ± 0·9 (6)	0·3

Item 1 is the holding potential, which is the same as the resting potential in Krebs solution before depolarization. Item 2 is the holding current needed to hold depolarized preparation at holding potential. Items 3–8 have same meaning as in Tables 3 and 4. Item 9 is the peak inward late current.

As is clear from Table 9, in comparison with preparations in Krebs solution containing 1·9 mM-$[Ca^{2+}]_o$ the TEA-affected preparations show the following differences: the spike amplitude is higher, E_a is more positive, I_a is larger, $\bar{g}_a$ is considerably larger, and $\bar{g}_b$ is smaller (see also Fig. 10). The last change is the TEA action, and all the other changes can be explained by some overlap of an outward current with the early inward current under normal conditions, because of technical imperfections introduced by the series resistance: when the outward current was reduced by TEA, the inward current (I_a) increased, the spike amplitude was higher, and E_a moved toward more positive levels. Without attempting to resolve the difficult question of whether the observed effects are due to an imperfect space clamp entirely, or whether there exists some new early

outward current, the relative effects of changing $[Ca^{2+}]_o$ can still be seen (Table 9). It is clear that, in contrast to the effect of increasing $[Ca^{2+}]_o$ on preparations bathed in normal Krebs solution where no shift of E_a was observed (Table 7), the same procedure on TEA-affected preparations shifted the E_a by 12·5 mV toward more positive levels (Fig. 13). Although the shift is less than the 21 mV shift which can be expected of a Ca^{2+}-selective membrane, it is a highly significant shift in the correct direction. Even though the $\bar{g}_a$ is already considerably larger in the TEA-affected preparations, increasing $[Ca^{2+}]_o$ significantly increased it further. Increasing $[Ca^{2+}]_o$ also increased $\bar{g}_b$, which had been depressed by TEA.

TABLE 9. Effect of 5 × $[Ca^{2+}]_o$ on TEA-affected taenia coli

	$[Ca^{2+}]_o$ 1·9 mM	$[Ca^{2+}]_o$ 9·5 mM	P
1. Resting potential (mV)	−47·3 ± 2·8 (7)	−50·0 ± 3·3 (7)	0·09
2. Spike amplitude (mV)	70·4 ± 2·7 (8)	79·9 ± 3·4 (8)	< 0·001
3. E_a (mV)	33·8 ± 4·2 (6)	46·3 ± 4·5 (6)	< 0·001
4. I_a (μA)	−1·4 ± 0·3 (6)	−2·0 ± 0·4 (6)	0·02
5. G_1 (μmho)	5·1 ± 0·5 (6)	4·7 ± 0·7 (6)	0·1
6. $\bar{g}_a$ (μmho)	58·0 ± 8·8 (6)	63·5 ± 10·3 (6)	0·02
7. $\bar{g}_b$ (μmho)	13·5 ± 1·2 (6)	15·4 ± 1·6 (6)	0·02

All experiments were conducted in solution containing 67 mM-TEA. Compared with data in Krebs solution at $[Ca^{2+}]$ = 1·9 mM (Table 7), spike amplitude is larger, E_a is more positive, I_a is larger, G_1 is larger, $\bar{g}_a$ is larger, and $\bar{g}_b$ is smaller.

(*e*) *Other experiments:*

(i) *Mn^{2+}.* In some tissues which have been shown by other means to have a Ca^{2+}-spike, Mn^{2+} exerts a blocking action on the spike mechanism (e.g. Hagiwara & Nakajima, 1966). We have conducted some experiments to see whether Mn^{2+} has a specific effect on the early current in the taenia coli, and the results can be summarized as follows: in 1 or 2 mM concentrations, Mn^{2+} produced observable effects only after 5 min or longer; in 5 mM, the effects were more rapid. In general, the spike amplitude was reduced and the spike duration prolonged; E_a was shifted toward the negative; I_a was reduced; the leakage conductance (G_1), the conductances of both the early ($\bar{g}_a$) and late ($\bar{g}_b$) currents, as well as the slope conductance of the late-tail current ('instantaneous'), were all

markedly reduced by Mn^{2+} in all concentrations. E_b was not affected by 1 or 2 mM-Mn^{2+}, but was reduced by 5 mM, consistent with a depolarizing effect of the latter. Fig. 14 shows the effect of 2 mM-Mn^{2+} on the current–voltage relation of one preparation.

From these observations, it is clear that although Mn^{2+} exerted some blocking actions on the early current in the taenia coli, its effects are so widespread on other electrical properties that it cannot be used as a specific tool for confirming a Ca^{2+} nature of the spike in the taenia coli.

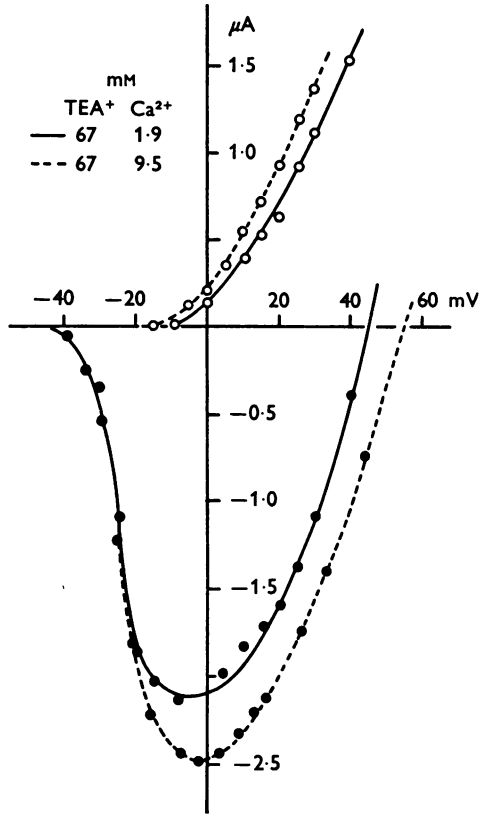

Fig. 13. Current–voltage relation of TEA-pretreated taenia coli in normal (1·9 mM) and high (9·5 mM) $[Ca^{2+}]_o$. These currents have been corrected for leakage and residue capacitative currents. Note that the reversal potential of the early current is shifted toward more positive levels (contrast with Fig. 12).

(ii) *D-600*. This compound, α-isopropyl-α-[(*N*-methyl-*N*-homoveratryl)-γ-aminopropyl]-3,4,5-trimethoxyphenylacetonitril, has been suggested as having a selective action on the calcium channels in some tissues (Fleckenstein, Grün, Tritthart & Byron, 1971). The effect of D-600 on the current–

voltage relation of the taenia coli is illustrated in Fig. 14. It is clear that at 10^{-5} M, I_a was lowered, and E_a was shifted toward the negative. The late current was unaffected. The reduction of the inward current is consistent with a reduction in spike amplitude, and can be explained by a reduction of some conductance. On the surface, the shift in E_a is not consistent with such a view. However, as has been shown in the TEA-affected preparations,

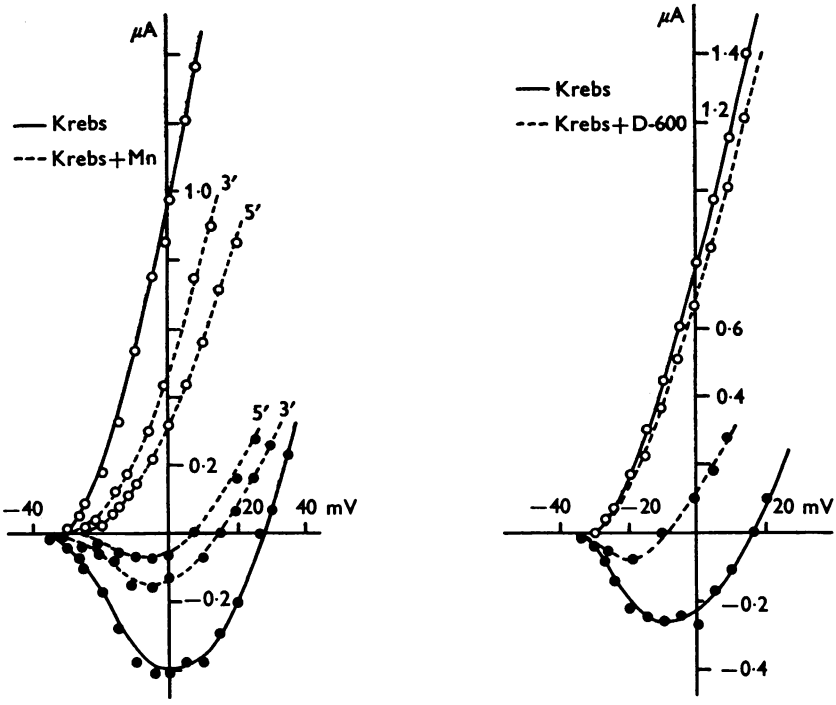

Fig. 14. Current–voltage relations of taenia coli treated with Mn^{2+} (2 mM) and with D-600 (10^{-5} M). The currents have been corrected for leakage and residue capacitative currents. In Mn^{2+}, effects at two different times are shown.

because of the limitations imposed by the series resistance, there is some overlap of the outward current with the early inward current. Since D-600 did not affect the outward current, it is understandable that as a result of a reduced I_a, a shift of the E_a toward the negative can occur. On the available information, it is not possible to be more specific about the action of D-600 on the ionic conductances of the taenia coli, but it is clear that, unlike those of Mn^{2+}, the actions of D-600 are confined mainly to the early current. One discouraging observation is that such actions are not reversible within the 15–20 min of attempted recovery.

DISCUSSION

Overlap of currents. The observations that in TEA-affected taenia coli preparations there are marked changes in the early current, as well as a suppression of the late current, raise some questions about the nature of the currents for which the bulk of the results represent. In another smooth-muscle preparation, the ostrogen-treated non-pregnant myometrium of the guinea-pig, Vassort (1974) concluded, on the basis of similar TEA effects, that there was an early outward current overlapping with the inward current, in addition to the classical late outward current. Such a conclusion would be applicable to the present experiments if the TEA effects were observed on single taenia coli cells. However, in view of the almost certain knowledge that a uniform space clamp cannot be obtained because of the cleft resistance in the present preparations, the observations on the TEA effects can just as well be ascribed to some inhomogeneity of the membrane potentials of the individual cells. As a result of the TEA action, the membrane conductance during the late current would be lower, and the space clamp must improve. Moreover, with a marked reduction of the outward current, which overlapped the inward current, I_a would increase, and E_a would shift toward more positive levels. Conversely, when I_a is reduced and the late current unaffected, then E_a would be shifted toward the negative, without there being any changes in the driving forces (such as occurred in preparations treated with D-600).

Because of insuperable technical limitations in the taenia coli preparation, the question of whether at the cellular level there are new currents in addition to the classical early and late currents (Hodgkin & Huxley, 1952), or whether there is a real overlap of these currents, must remain in abeyance. Such a question can be resolved only when a preparation is developed for investigation that has either single taenia coli cells, or small groups of cells with negligible cleft resistance between them.

K^+ as the charge-carrier of the late current. The observation that the late current can be made to reverse its direction when the preparation was immersed in an isotonic K_2SO_4 solution is clear indication that the main charge-carrier for the late current is K^+. The reversal potential is some 15–20 mV more negative than the natural resting potential, where it is considerably less than the K^+ equilibrium potential estimated on the basis of chemical determinations (Casteels & Kuriyama, 1966). A possible reason for this discrepancy is that even when the late current channel is activated, the ratio of P_{Na}/P_K is still 0·05, suggesting that some shunting by Na^+ is present. For this reason, the designation of E_b is used to indicate that the reversal potential is not strictly the K^+ equilibrium potential.

In the myometrium, it was difficult to be certain that the late current

was distinctly separate from the early current, because the latter could not be selectively blocked by tetrodotoxin (see Kao & McCullough, 1975). In the taenia coli, although the same problem exists, a separation of the two currents is made more probable by the observations that the two currents seemed to have different thresholds for activation (Fig. 4). Other evidence in support of separate mechanisms for the two currents is the effects of reducing $[Ca^{2+}]_o$ which can often produce marked effects on the early current without affecting the late current.

Ca^{2+} as charge-carrier of the early current. In a previous paper on the myometrium of the pregnant-rat uterus, it was concluded that the early current was carried chiefly by Na^+, and that Ca^{2+} had an important role in influencing the conductance changes (Kao & McCullough, 1975). The conclusion was based, in part, on the observations that (*a*) reduction of $[Na^+]_o$ by 50 % caused a shift in E_a by an amount close to that expected of a Na^+-membrane, and (*b*) removal of all external Na^+ caused the early current to change from inward to outward. Similar experiments have now been performed on the taenia coli preparations, and the observations are entirely different from those made on the myometrium. Although a longer extracellular diffusion lag is present in the taenia coli, it is unlikely that such a lag is the entire explanation for the observations in the taenia coli. In the experiments on 50 % $[Na^+]_o$, it is quite possible that within the test period no significant alteration of $[Na^+]_o$ had taken place (see also the flux data of Brading, 1971). Hence, the absence of any changes in the voltage-clamped preparation (Table 4) is understandable. However, when a Na^+-free medium was used, and the test period was prolonged, some changes in $[Na^+]_o$ must have occurred (even if the extracellular environment did not become Na^+-free) for the leakage conductance to be significantly reduced and the spike amplitude to be significantly increased (Table 3). Yet, there was neither a shift of the E_a toward the negative, nor a reduction of the inward current, both changes being expected if Na^+ were to be an important charge-carrier for the early current.

The experiments with changing $[Ca^{2+}]_o$ further support the idea that Ca^{2+} rather than Na^+ may be a principal charge-carrier for the early current. Thus, the negative shifts of E_a and the reductions of I_a on lowering $[Ca^{2+}]_o$ are consistent with the conclusion. Moreover, any conductance-influencing role of external Ca^{2+}, which might complicate the situation, has been largely eliminated in the depolarized preparations which showed a similar shift in E_a and a similar reduction in I_a. The observation that increasing $[Ca^{2+}]_o$ to 9·5 mM in TEA-affected preparations shifted E_a toward the positive further confirms the view that influx of Ca^{2+} contributes to the early current.

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