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Ionic basis of intestinal electrical activity

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JOB, DONALD D. *Ionic basis of intestinal electrical activity.* Am. J. Physiol. 217(5): 1534–1541. 1969.—A rapid collection technique is described which allows for the fractionation of perfusate which is either labeled itself or passes over an isotopically labeled segment of intestinal muscle. Efflux of sodium is maximal early in the repolarizing phase of a slow wave. Influx of sodium is maximal during the depolarization phase. The repolarization phase of intestinal slow waves is attributed to an electrogenic sodium pump. Effects of temperature and inhibitors indicate that slow-wave amplitude and pump activity are metabolically driven. The depolarization phase is attributed to passive sodium influx. Spikes are associated with peaks of calcium influx.

intestinal slow waves; ion fluxes; electrogenic pump; smooth-muscle electrophysiology; Ca^{++} spikes

THE SMALL INTESTINE exhibits small electrical oscillations (slow waves) of the membrane potential. Action potentials or spikes usually occur near the peak of slow waves.

The slow waves of the cat small intestine originate in the longitudinal muscle layer (1, 9). They are reduced or blocked by metabolic inhibitors (1, 5), by anoxia (6), by low extracellular sodium, and by ouabain (5, 10, 17). The effectiveness of these agents has suggested to previous investigators the possible role of active transport of sodium in slow-wave electrogenesis (5).

The present study was undertaken to determine the ionic basis of the intestinal slow waves and action potentials and to explore possible mechanisms for the ion movements. The ion fluxes during the electrical events were determined directly by employing a rapid-collection technique which allowed the collection of many fractions of radioactively labeled perfusate during one slow-wave cycle. The collection technique was similar to that of Spyroupolus et al. (15). Several investigators have reported correlations between observed fluxes and the action potential of the heart. Pak et al. (13) concluded, however, that the efflux of potassium ions during the repolarization phase of the ventricular action potential reported previously (11) was the result of mechanical squeezing of the extracellular space associated with the contraction initiated by the electrical activity.

Dynamic tracer studies of the intestinal slow wave has two advantages over those in the heart muscle. First, the duration of the slow wave at body temperature is 4–5 sec and this can be extended to 8–10 sec by lowering the temperature. Second, slow waves can be recorded independently of spikes and, therefore, of muscle contraction.

MATERIALS AND METHODS

A segment of intestine was removed from the cat under α-chloralose (65 mg/kg) anesthesia and placed in oxygenated Tyrode solution. The mucosa and submucosa were removed and the muscle was slipped over a Plexiglas rod and secured. The mounted muscle was either placed in an isotope-loading medium at room temperature for equilibration for at least 2 hr (efflux experiments) or was placed directly into the muscle chamber shown in Fig. 1 for perfusion with isotopic Tyrode solution (influx experiments). The last 30 min of loading for efflux experiments was done at the temperature to be used for the flux studies. For influx experiments the muscle was equilibrated in nonradioactive Tyrode solution.

The perfusion chamber consisted of a split channel so that the longitudinal muscle layer was bathed around the entire circumference of the segment. The integrity of the underlying circular layer was preserved to provide circumferential synchrony of the slow waves in the longitudinal fibers (9). Recording of electrical activity was by means of a pressure electrode. This electrode consisted of 2 mm outer diameter glass tubing drawn down to 50- to 75-μ tip diameter. This was filled with 2% agar-saline and a silver chlorided silver wire (1). The chamber was situated between the perfusion reservoirs and the collecting carousel as shown in Fig. 2. Several 1-min samples of tissue washout were obtained before and after each fraction collection. The loaded muscle was washed for at least 8 min with nonradioactive perfusate prior to collecting fractions so that the contribution to the flux pattern from the extracellular space was minimal as determined from washout curves for each ion studied.

The collecting carousel rested on a turntable the speed of which was adjusted with a silicon-diode rectifier motor-control unit. Each revolution of the turntable closed a switch which generated a signal on one channel of a Grass polygraph. The pressure electrode recording was displayed on the second channel. The speed of the turntable was adjusted so that each revolution as indicated by the timing mark corresponded to the same point on each successive slow wave. Usually 10–20 slow waves were averaged and 15 fractions were obtained per slow wave.

The isotopes ^{24}Na and ^{42}K were obtained from the University of Illinois Triga Mark II reactor in the carbonate form (14 and 13 mc/g, respectively). They were converted to the chloride salt. Experiments were performed using singly labeled solutions or a combination of one short-lived isotope and one long-lived isotope (^{45}Ca, Tracerlab, or ^{14}C-labeled inulin, Volk Radiochemical). Within 24 hr the

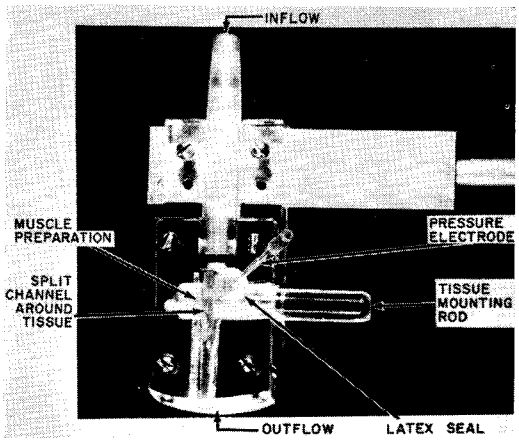

FIG. 1. Muscle chamber. Chamber consists of two blocks of Plexiglas which have been symmetrically machined to allow for fluid flow over muscle. Two blocks are bolted together over cylinder of muscle and over inflow tube to form a water-tight seal. Pressure electrode is supported on muscle by a small clamp which is flexibly attached to supporting metal bar.

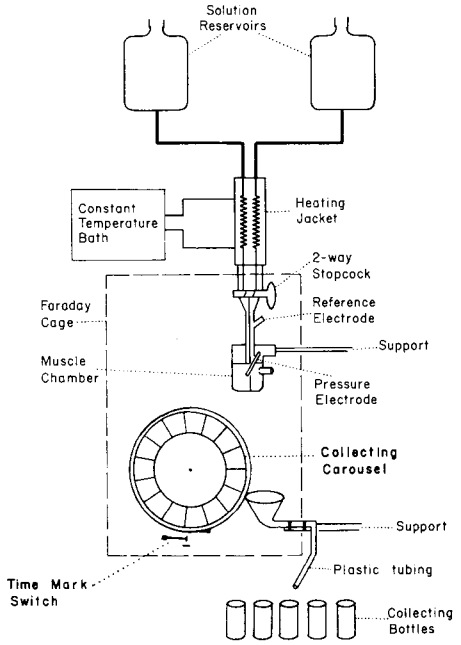

FIG. 2. Fraction collection system. Tyrode solution perfused tissue by gravity flow from solution reservoirs. Solution was warmed to a preset temperature. Washout curve was obtained between carousel collections by using collecting bottles shown at bottom of figure.

aqueous samples of ^{24}Na or ^{42}K were counted on a Tri-Carb 314E liquid scintillation counter utilizing Čerenkov radiation. A portion of this sample was resuspended in the scintillation medium of Bruno and Christian (2). These were counted more than 20 half-lives later to obtain the activity of the long-lived isotope.

The electrical activity and the timing mark from the turntable were fed into two channels of an FM tape recorder (TMC) during the collection period. These signals were later fed from the magnetic tape into a computer of average transients (CAT, Technical Measurements Corporation) for averaging. The sweep of the CAT was triggered by the timing mark generated by each revolution of the turntable. The computed average of the electrically recorded slow waves taken during the collection period was plotted by an $x - y$ plotter. The scale of this plot was obtained by estimating the average delay between the end of one sweep of the CAT and the triggering of the next and comparing this to the total sweep time (4, 8, or 16 sec). This fraction gave the percent of the total slow wave averaged and was designated as a fraction number according to the number of fractions of perfusate collected per cycle (usually 15). The intermediate values were adjusted accordingly.

The count data from the fractions were corrected for background, decay, counter dead time, and sample volume by an IBM 7094 digital computer. The same program computed the standard deviation for each point and plotted the results. This plot which represents the average unidirectional flux per slow wave was then compared directly to the plot of the average configuration of the electrical activity for the same slow waves.

RESULTS

Sodium and potassium effluxes. Fractions of the perfusate collected from a muscle loaded in ^{24}Na-Tyrode solution showed a pattern such as is illustrated in Figs. 3 and 4. A peak in sodium efflux occurred during the repolarization phase of the slow wave. Although the precise shape and position of the peak varied from one experiment to the next the tendency was the same, that is, maximum efflux occurred during the repolarization phase. This efflux peak was reduced by ouabain to the same extent by which the slow wave was reduced (two experiments). Its position was not significantly altered by changing the temperature, rate of perfusion, or by small contractions. It is noted that the efflux is maximal at the beginning of the repolarization phase and declines before repolarization is completed. This was found to be the case in all 18 experiments.

To take account of the variability, all of the experiments were pooled in the following way. The peak of the averaged electrical slow waves for each experiment was determined from the CAT output to the nearest fraction number. The corresponding number on the activity versus fraction number graph was designated as fraction number 3 and all other fraction numbers were renumbered accordingly. Thus, fraction number 3 in each experiment corresponds to the peak depolarization. The entire collection cycle was arbitrarily divided into five parts of three fractions each. The three fractions with the highest activity from each experiment were then assigned to one of the five sections of the cycle. The distribution found is tabulated in Table 1. Since the repolarization phase (R) generally constitutes at least two-thirds of the entire slow-wave cycle, the sections II, III, and IV comprise the repolarization phase, R, and sections I, and V, the depolarization phase, D, respectively. R and D are the sum of scores from the sections of which they are composed. Assuming a 3:2 distribution of scores in repolarization and depolarization phase a value for chi-square of 5.70 was obtained. This value for chi-square indicates that the probability (P) of obtaining this distribution purely by chance is very small ($P < .02$). It is concluded that

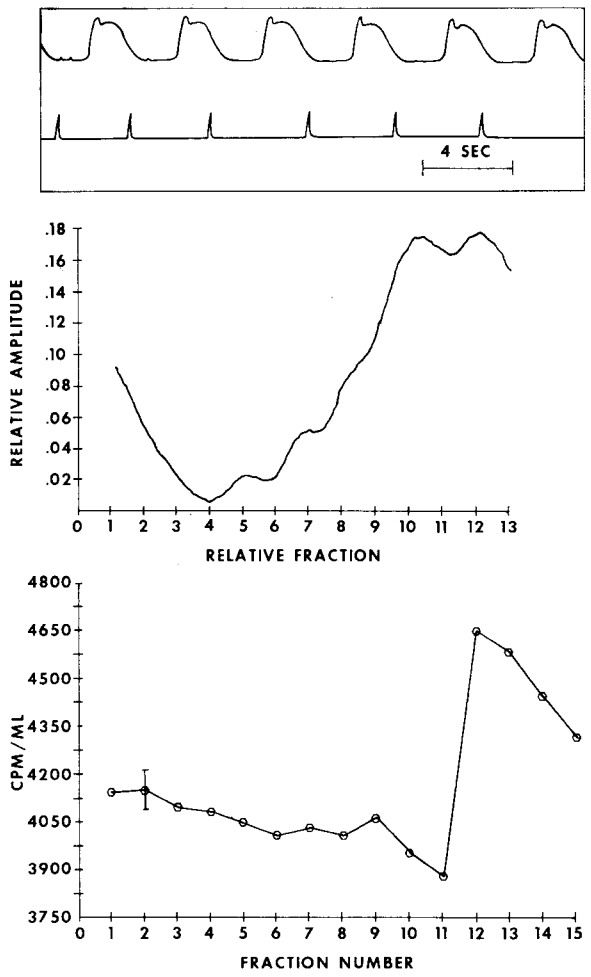

FIG. 3. Sodium efflux during repolarization. Electrical and flux records are average of 26 cycles. Enclosed record is of original polygraph record taken during collection period. Spikes in lower portion are generated on each revolution of turntable and hence are not of equal intervals. Upper record is average electrical activity. Bottom record shows perfusate activity. Computed standard deviation (± 1 SD) which is nearly identical for all fractions is shown in fraction 2. Relative amplitude in this and subsequent figures reflects any changes in gain in plotting system from that usually used, highest gain giving a peak amplitude of 1.0. Relative fraction number is determined by dividing average period of slow waves by total number of fractions collected in each cycle.

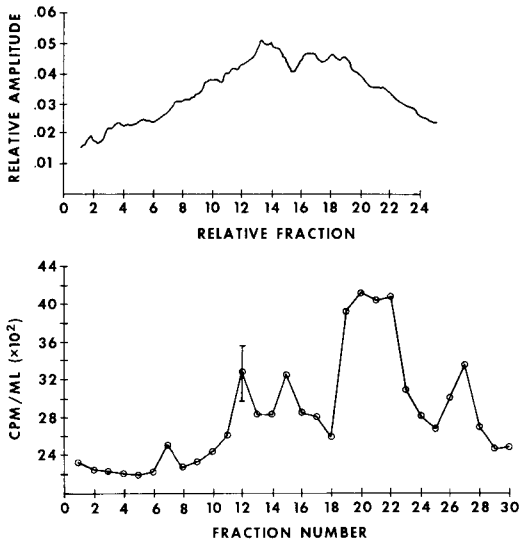

FIG. 4. Sodium efflux during repolarization with 30 fractions. Electrical and flux records are average of 13 cycles. Upper record is averaged electrical activity and lower, perfusate activity. Representative computed standard deviation (± 1 SD) is indicated in fraction 12.

there is a significant peak in Na^+ efflux during the repolarization phase.

The efflux of potassium ions is illustrated in Fig. 5. In this experiment the temperature was lowered to 31.5 C with a consequent appearance of two components to the slow wave. This phenomenon of component separation by low temperature in the intestine was noted on several occasions. The efflux of potassium was maximal during the late component of the slow wave, i.e., after the initial rise. At higher temperatures the potassium efflux pattern was more variable than the sodium pattern, but an analysis of all of the experiments in the same way as was done for sodium revealed a significant ($P < .02$) efflux during the repolarization phase (Table 1). This result suggests that potassium efflux may also play a role in slow-wave electrogenesis.

Sodium and potassium influx. The tissue content of isotope could not be sampled for dynamic influx changes because the synchrony is limited at a given time to a very short ring of muscle. Instead, the nonloaded muscle was perfused with isotope and the activity in the perfusate was counted. This activity represented the difference between that originally in the perfusate and that taken up by the tissue. In spite of inherent difficulties in this technique, reproducible influx patterns were obtained. The influx of ^{24}Na ions is shown in Fig. 6. This figure shows maximal influx during the depolarization phase. Maximal influx is downward in the figures since the radioactive ions entering the cells are depleting the perfusate in contrast to the efflux experiments. The distribution for the pooled results of all of the experiments is included in Table 1. The results suggest that passive influx of sodium ions may contribute to the depolarization phase of the slow wave. The influx of potassium ions during the slow wave did not show a significant deviation from a random distribution.

Calcium fluxes and spikes. In the absence of spikes the calcium ion fluxes could not be correlated significantly with any particular phase of the slow wave although a transient increase in efflux was often, but not invariably, observed in the latter portion of the repolarization phase. These results are summarized in Table 1. In muscle preparations exhibiting spikes, the calcium fluxes were more definitive. The maximum influx and efflux of calcium ions were temporally associated with the spikes. Increased calcium exchange observed by many investigators during contraction could contribute to these fluxes. Figure 7 illustrates calcium influx peaks corresponding to the spikes in the average electrical record. The original record shows several large spikes. The potassium influx which was obtained together with the calcium influx exhibited an erratic change in influx during the spike. This could have been due to a passive response to the transient changes in membrane potential.

The role of potassium ions in spikes was related to the calcium flux. When an efflux peak of ^{42}K occurred, it was

TABLE 1. *Fluxes in absence of spikes*

	Repolarization			Depolarization		R	D	χ^2 3:2	No. Exp	P
	II	III	IV	V	I					
Na^+ efflux	17	10	14	5	8	41	13	5.70	18	<.02
K^+ efflux	13	9	7	3	4	29	7	6.35	9	<.02
Na^+ influx	5	5	5	11	14	15	25	8.43	9	<.01
K^+ influx	5	3	7	4	5	15	9	.06	6	>.80
Ca^+ efflux	5	3	7	6	3	15	9	.06	8	>.80
Ca^+ influx	3	5	7	6	5	16	11	.006	9	>.95
Inulin-^{14}C efflux	7	6	3	8	3	16	11	.006	9	>.95

Fractionation of approximately 15 waves per experiment into three periods of repolarization (R) and two of depolarization (D). R and D give sums of average fluxes in these two phases. χ^2 and P compare flux differences for R and D.

usually (six of seven experiments) simultaneous with the calcium efflux peak and may have been only a passive response.

Inulin-^{14}C. The validity of the preceding results rests on the assumption that the tissue is not artificially producing changes in the flux patterns by contracting and periodically squeezing isotope from the extracellular space. A control for mechanical artifact was to load the tissue extracellular space with ^{14}C-labeled inulin and follow its efflux simultaneously with ^{24}Na or ^{42}K fraction collections. An experiment in which small spikes were observed on the early depolarization phase of the individual slow waves is shown in Fig. 8. It is seen that, whereas the inulin efflux is variable, there is no peak corresponding to the sodium efflux peak during the repolarization phase. The decreased efflux of inulin in fractions 12 to 14 probably reflects the relaxation phase of a small contraction, and would explain the earlier than usual decline in the sodium peak if the extracellular space were increased following the squeezing due to contraction. The results of all nine inulin efflux experiments are summarized in Table 1.

No correlation was found between Na^+ fluxes and Ca^{++} fluxes during a spike. This lack of correlation further supports the contention that the spikes are due to Ca^{++} and that the Ca^{++} fluxes observed are not the result of movement artifact; if they were, K^+, Ca^{++}, Na^+, and inulin should all appear in the same place. Analytical procedures used for slow waves were not applicable for spikes.

Temperature effects. The relation between temperature, rates of rise and fall, and frequency of the slow waves was studied on a segment of circular and longitudinal muscle mounted on a Plexiglas rod in a tissue bath. Rates of rise and of fall of slow waves were affected similarly by temperature, each having an average Q_{10} of 2.5 ± .1 (N = 3) over the temperature range of 26–36 C. The frequency change plotted against the reciprocal of temperature is shown in Fig. 9. The frequency between the temperatures 25 and 35 C increased by a factor of 2.73; by comparison, the change in rate of repolarization over the same temperature range was 2.67. The values for the Q_{10}s are similar to those reported previously (1) for frequency change. The high value of Q_{10} and the fact that the rate of repolarization exhibits an optimum is suggestive of the involvement of an enzymatic reaction in the repolarization phase. The nonlinear nature of the curve suggests a multicomponent repolarization phase. This is in contrast to the linear response

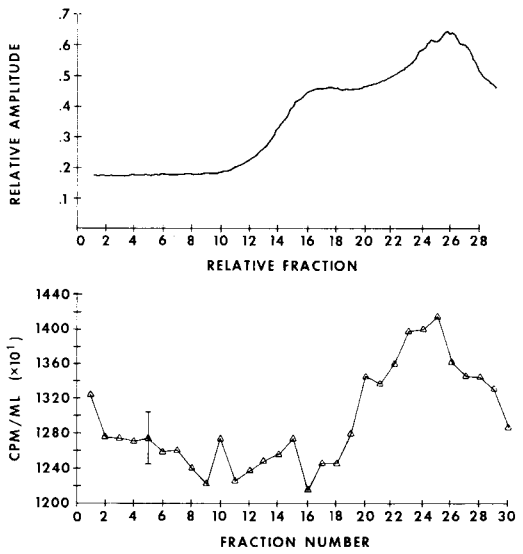

FIG. 5. Potassium efflux during repolarization phase. Electrical and flux records are average of 16 cycles. Upper record is of average electrical activity, and lower record is of perfusate activity. Computed standard deviation (± 1 SD) representative of all fractions is shown in fraction 5. Temperature was lowered to 31.5 C, which led to appearance of two components in electrical record.

of the frequency which suggests a single rate-limiting step. After the tissue was stored in the cold for several hours, the optimal temperature decreased in proportion to the time spent in the cold. For this reason the optimum was lower than body temperature (38 C) as in Fig. 9.

Inhibitors of active fluxes. The foregoing results suggest that the electrogenesis of the slow waves may involve an enzymatic reaction and that this reaction may be part of an electrogenic sodium pump. It has already been established that ouabain blocks the slow waves (5) as does dinitrophenol (DNP) (1). Daniel (5) has shown in perfused dog intestine that anoxia also blocks slow waves. In the present system, anoxia produced by bubbling nitrogen gas through the bathing medium or application of 10^{-4} M sodium cyanide blocked the slow waves within a few minutes. Iodoacetic acid, however, did not block the slow waves in over 30 min at a concentration of 10^{-4} or 10^{-3} M. This would suggest that the energy requirement of the pump is derived from oxidative phosphorylation. The inhibition of slow waves by 10^{-4} M

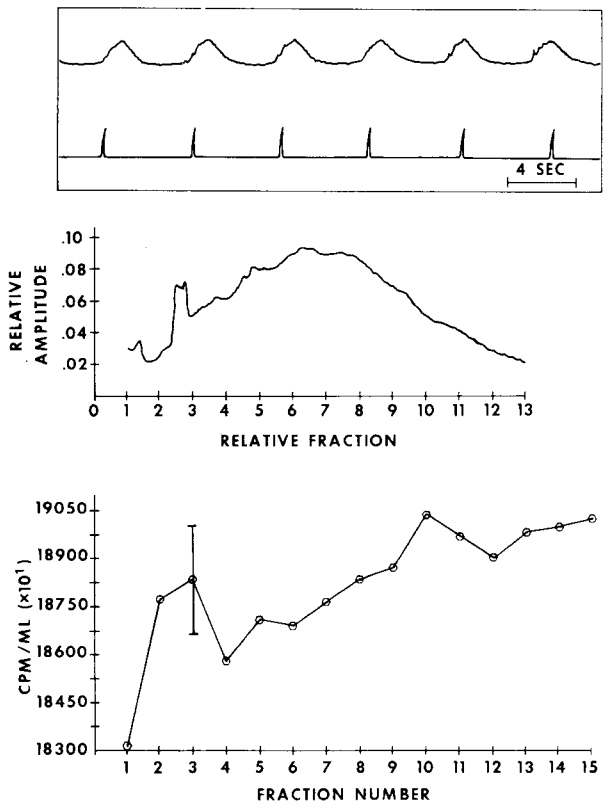

FIG. 6. Sodium influx during depolarization phase. Enclosed record is a portion of polygraph trace taken during experiment. Upper record is sum of 23 electrical cycles. Small depolarization-repolarization peak on depolarization phase of average slow wave is seen to correspond to similar electrically recorded peaks on individual slow waves. Lower record is of perfusate activity. Values with least number of counts represent maximum influx since perfusate collected is being depleted of isotope. Approximate standard deviation (± 1 SD) representative of all fractions is indicated in fraction 3.

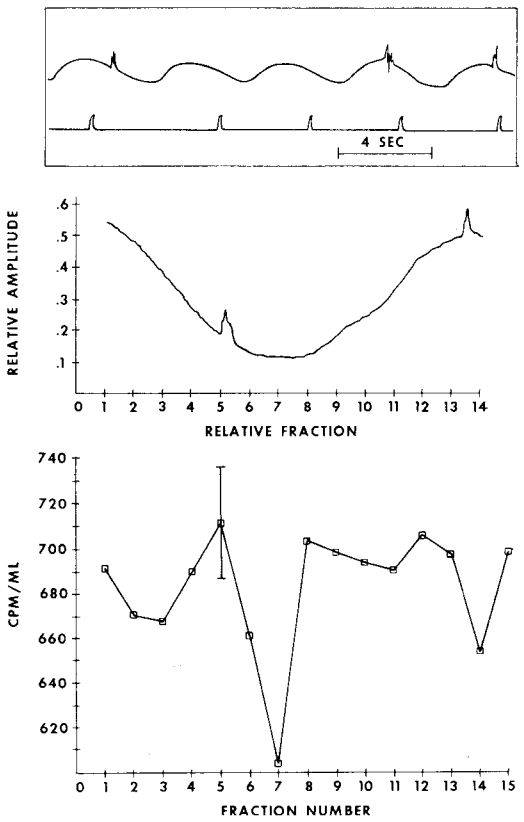

FIG. 7. Calcium influx during spikes. Enclosed record is a portion of polygraph trace obtained during experiment. Relatively large spikes were present in three individual slow waves out of a total of 14 and these appeared both on repolarization phase and near peak. Upper record is average electrical activity and lower record, perfusate activity. Computed standard deviation (± 1 SD) representative of all fractions is shown in fraction 5.

dinitrophenol and by 10^{-4} M pentachlorophenol (PCP) also supports this contention.

Inhibitors of passive fluxes. Several agents which have been reported to interfere selectively with processes involving conductance changes were studied. Tetraethylammonium chloride (TEA) is an inhibitor of potential-dependent K^+ conductance changes (7) in several tissues. At low concentrations (<1 mM) TEA had no effect on intestinal electrical activity. When high concentrations of TEA (20 mM) were used in conjunction with 1 mM atropine, the rates of both rise and fall of the slow waves were reduced by 70–80% in 15 min. During the same period the rates of both rise and fall of the spikes were reduced by 30–40%. The dramatic prolongation of repolarization phase reported for taenia coli (16) was not observed. Either the repolarization phase of slow waves and of spikes is much less sensitive to TEA, or potassium conductance changes in this tissue play a less important role in electrogenesis than in other tissues.

DISCUSSION

System homogeneity. The conclusions drawn from the flux studies rest on two assumptions. The first is that the tissue is functionally homogeneous. The presence of nerve fibers and ganglion cells is not considered to seriously contribute to inhomogeneity. The arguments for this assumption are set forth in the following section. Multiple electrode recording has shown (9) that when the circular muscle is intact, the slow waves of the longitudinal muscle are in near synchrony around the circumference of the preparation. Another factor in electrical homogeneity is the synchrony of cells in the longitudinal axis. An indication of this synchrony is obtained from comparing the time for the slow-wave front to traverse the length of tissue sampled to the total time for one slow-wave cycle (6 sec). The former is the channel width (0.4 cm) divided by the conduction velocity (1.0 cm/sec (9)), or 0.4 sec. The error in assuming longitudinal synchrony is therefore only 6–7%. The channel width was chosen to permit collection from a ring of nearly synchronous fibers in the longitudinal direction but still allow sufficient area for a reasonable flow rate over the tissue. Since the circular muscle has been shown to be unable to generate slow waves (9) it is further assumed that it follows passively the activity in the longitudinal fibers. For reasons outlined in the subsequent section, the validity of this assumption would not appear to be important.

Diffusion limitation. The second and perhaps the more critical assumption is that the ions which leave (or enter) the membrane will be collected in a short time compared to the duration of the electrical event. This requirement imposes a definite limit on the thickness of the tissue. Because of the

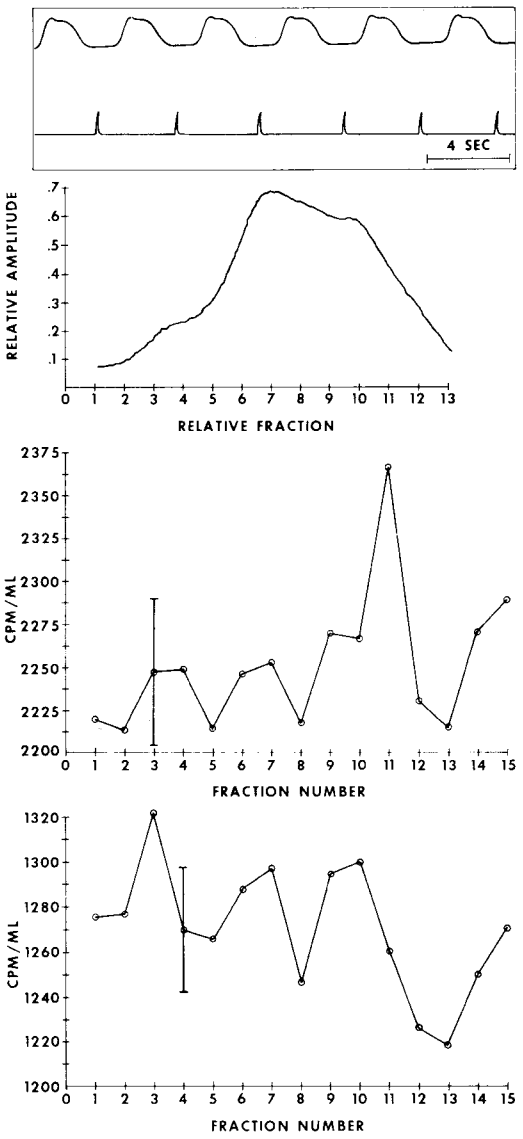

FIG. 8. Inulin-^{14}C efflux with sodium efflux. Enclosed record is a portion of polygraph record. Very small spikes were observed in 8 of 40 slow waves averaged. These occurred most frequently just prior to depolarization phase of individual slow waves. Upper record is average electrical activity and middle and lower records are perfusate activity of ^{24}Na and inulin-^{14}C, respectively. Approximate standard deviation (± 1 SD) representative of all fractions is indicated for both ^{24}Na and inulin-^{14}C, in fractions 3 and 4, respectively.

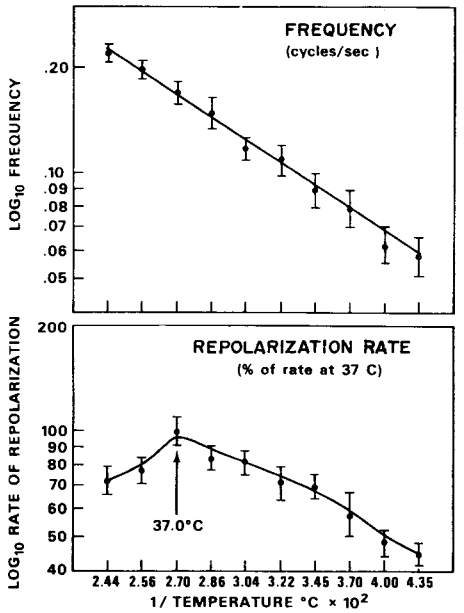

FIG. 9. Temperature effect on frequency and rate of repolarization. Slopes of repolarization rate were obtained graphically from a polygraph record. Value given at each reciprocal temperature is average of 4–5 slow waves. Frequency is average of same 4–5 slow waves at each temperature. Bar for each point indicates ± 1 SE for separate experiments (N = 4).

first requirement for synchrony, however, pure longitudinal muscle could not be used since synchrony requires the circular and longitudinal muscle layers to be intact. The presence of the circular muscle increased the thickness to more than 0.6 mm. The diffusion of tracer through a tissue of this thickness requires considerably more than a few seconds.

The analysis of the diffusion limitations can be formulated in the following way. The periodic flux changes which occur at the surface of the tissue appear in the perfusate with a short phase lag due to an unstirred layer and to the serosal membrane. Assuming electrical homogeneity, the flux changes in deeper layers of the muscle occur simultaneously but due to diffusion time, appear in the perfusate with a phase lag and amplitude decrement proportional to the distance from the surface. The extent of this phase lag and amplitude decrement has been solved for the case of heat flow in a slab by Carslaw and Jaeger (3). The change in flux at a depth of 60 μ will appear in the perfusate with a phase lag of 90° and a decremented amplitude of approximately 50%. A 90° phase lag would shift the peak by nearly four fraction numbers, but the largest amount of flux change arising from within the muscle will be from those cells closest to the surface (<60 μ deep) and will therefore shift the peak considerably less. The time between the passage of the perfusate over the muscle and its collection in the carousel below was determined from a typical flow rate of 80 ml/min to be less than 0.5 sec (or a shift of less than two fraction numbers). With a combination of a phase lag and a collection lag it is likely that the collection of isotope would appear in one and possibly two fractions beyond the actual event recorded electrically. The method of data analysis which groups the 15 fractions into five groups of three allows for variability of up to two fraction numbers within each group. When the data of all experiments were shifted by one fraction number so as to align all the slow-wave peaks to fraction 4 rather than fraction 3 (simulating a lag of one fraction number or 0.4 sec for a 6-sec slow wave), equally significant ^{24}Na and ^{42}K efflux peaks occurred during repolarization and a ^{24}Na influx peak during depolarization.

Interpretation of flux patterns. The first step in interpretation of the influx and efflux curves is to make a visual comparison of the flux curves and the average electrical slow wave for a particular experiment. In the experiments presented it is apparent that the flux patterns and electrical patterns are often irregular in shape.

One factor in causing irregularities is that each compartment of the collecting carousel does not sample exactly the

same part of the slow wave on each revolution. This difficulty prompted the use of the computer to average the electrically recorded slow waves. Irregular peaks in the averaged electrical record often resulted when two or three slow waves were out of register with the others, as confirmed by examination of the original polygraph records. When this was the case, the experiments were discarded.

The presence of spikes raises an additional problem of interpretation because the spikes may initiate contraction. The experiment with sodium and inulin, Fig. 8, illustrates small irregularities presumably due to the presence of spikes in the preparation and, in addition, a general trend toward decreasing efflux of both inulin and sodium, presumably associated with the time course of the small contraction, was exhibited. This perturbation did not however affect the appearance of the sodium efflux peak during the repolarization phase.

It is concluded that the flux patterns are subject to many variabilities. The presence of the underlying circular layer may contribute to their irregularity. Also, there may be additional, more subtle components involved in the slow-wave electrogenesis which complicate the flux pattern. Potassium ion efflux, for example, appears to contribute to the electrical event. Confirmation of the validity of single experiments was obtained by repeating experiments with the same isotopes but with different tissue, temperatures, flow rates, and minor variations in handling the fractions, and other variables which could change from experiment to experiment. Also, doubling the number of fractions obtained per experiment confirmed the presence of peaks which appeared in only 1 or 2 fractions out of 15.

Estimate of flux magnitudes. It is desirable to know whether the magnitude of the flux observed is sufficient to account for the observed electrical activity. The slow-wave amplitude from a single muscle cell was assumed to be 10 mv (a typical value obtained using potassium chloride-filled glass microelectrodes) and the capacitance of the cell membrane to be 5.0 μf/cm^2 (10). The charge, Δq, in coulombs (coul) transfer is calculated to be

$$\Delta q = 5.17 \times 10^{-13} \text{ moles/cm}^2 \text{ of cell surface}$$

The number of moles observed to move in a typical sodium efflux experiment is calculated as follows. The area under the flux peak was obtained using a compensating polar planimeter. The base line was roughly estimated from the character of the overall flux curve. The counts under the peak, in Fig. 4 given by the product of the ordinate, the abscissa, and the area, equal 8.55 counts/ml. Correcting for counting efficiency of ^{24}Na (30%) and the specific activity of the loaded muscle, the actual efflux of all sodium ions labeled and unlabeled can be computed. After 2 hr of loading in ^{24}Na-Tyrode solution the tissue had reached approximately 30% of the specific activity of the loading solution, as determined by the uptake curve for sodium 24. The specific activity of the sodium 24 loading solution was 3.11×10^9 dps/mole. At the time the collection was midway (13 min) the tissue activity had dropped by approximately 5% so the tissue activity was only 25% of that of the loading medium. Converting counts to disintegration and correcting for reduced specific activity of the tissue, one obtains the corrected efflux, CE, of radioactive and nonradioactive sodium, as follows:

$$\begin{aligned} \text{CE} &= (8.55 \text{ counts/ml})/(3.11 \times 10^9 \text{ dps/mole} \times .25 \times .30) \\ &= 3.67 \times 10^{-8} \text{ moles-sec/ml} \end{aligned}$$

This value must be multiplied by the volume of solution which was collected during the peak activity and divided by the number of slow waves which were taken to obtain this volume. In the experiment being considered, the peak was measured over five fractions, each of which contained 12.3 ml of perfusate and sampled the perfusate for approximately 0.333 sec. This gives a total volume taken during the 26 cycles of 61.5 ml. Each fraction sampled the perfusate for a total of 26×0.333 sec during the experiment so that the total time the efflux peak was sampled was 5×8.66 sec. The efflux M is therefore:

$$\begin{aligned} M &= 3.67 \times 10^{-8} \text{ moles-sec/ml} \times 61.5 \text{ ml}/43.3 \text{ sec} \\ &= 5.20 \times 10^{-8} \text{ moles} \end{aligned}$$

The total surface area of all of the cells which are in the open perfusion channel was estimated using cell dimensions reported by other investigators (10), and assuming loose packing (each fiber surrounded by four others rather than six). The mean of three determinations of the longitudinal layer thickness was 0.15 mm. On this basis the total surface area was calculated to be 270 cm^2. In order to express the flux in terms of square centimeters of cell surface for comparison to the electrical event, the flux M must be divided by the surface area. Hence,

$$\begin{aligned} M &= 5.20 \times 10^{-8} \text{ moles}/270 \text{ cm}^2 \\ &= 1.93 \times 10^{-10} \text{ moles/cm}^2 \text{ of cell surface} \end{aligned}$$

Another sodium efflux experiment gave a lower value of 1.69×10^{-11} moles/cm^2. These values are 100–1,000 times greater than the fluxes of 5×10^{-13} moles/cm^2 calculated from the electrical activity. Similar calculations for sodium influx in Fig. 6 and calcium influx in Fig. 7 gave values of 3.80×10^{-8} moles/cm^2 and 6.50×10^{-7} moles/cm^2, respectively. These values would be reduced to the extent that the surface volume calculation is underestimated by not assuming a hexagonal array and by not introducing a factor for surface invaginations. Consideration of surface irregularities would, however, also influence the calculated capacitance measurements (10).

Assuming that the system can be approximated by two homogeneous compartments (intra- and extracellular space) it can be concluded that the magnitudes of sodium influx and efflux and calcium influx are more than sufficient to account for the electrical events attributed to the fluxes of these ions. The fact that the flux magnitudes are in excess of the requirements suggests that these ions can move in both directions through the membrane. Deviation from the two compartment system should not influence the calculations by more than a factor of 4 or 5 judging from the effect of arbitrarily resolving the washout curves into more than two components.

Proposed model for slow waves. The efflux of sodium ions during the repolarization phase is not only against the electrochemical gradient for sodium but also is opposite in

direction to the change in electrical driving force. It is reasonable therefore to state that the increased efflux of sodium ions during the repolarization phase is due to an active process. That an active process is involved in slow waves is also supported by the studies of the effect on slow waves of inhibitors of oxidative phosphorylation (cyanide, anoxia, DNP, PCP); by the effects of specific inhibitors of active ion transport (ouabain); by the effect of sodium substitution in the medium (17, 12); and by iontophoresis of sodium ions into the longitudinal muscle cells (12).

The character of the efflux pattern of sodium is not, however, what one would predict for a simple transport of ions which charges the membrane to a greater negative potential. In all the experiments for sodium efflux the peaks were significantly narrower than the duration of the repolarization phase of the slow wave. This is in contrast to the influx curve for sodium and the influx curves of potassium and calcium; that is, the influx peaks are at least as broad as the event with which they are presumably associated. No single model can be supported at present which would account for the short efflux peak for sodium ions.

The data of this and the accompanying paper (12) suggest that the depolarization phase of the slow wave is due to the passive inward diffusion of sodium ions. The repolarization phase appears to be due, at least in part, to the active extrusion of sodium ions by a ouabain-sensitive ATP-requiring pump. The maintained hyperpolarized state is proposed as being due to either a balance between Na^+ extrusion and Na^+ leak or one-to-one coupling of Na^+ extrusion and K^+ uptake. In the former case the control of the oscillation may lie in a cyclical production of ATP by mitochondria (4). In the latter case the ATP/ADP ratio could influence the degree of coupling between sodium extrusion and potassium uptake so that as the ratio decreased, the coupling would become more nearly one-to-one and finally reach one-to-one at the height of the repolarization phase. This type of affinity change for potassium with ATP concentration is deduced from Robertson's data (14) on brain Na- K-ATPase. Alternate mechanisms would involve specific conductance increases to sodium during the depolarization phase and to potassium during the late repolarization phase.

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