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THE PHARMACOLOGY OF THE ETHANESULPHONATE ANION

BY

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A method of preparing analytically pure sodium ethanesulphonate, $C_2H_5SO_3Na.H_2O$, in quantity is described. Physiological saline solutions were prepared in which a proportion of the sodium chloride normally present was replaced by an equimolar concentration of sodium ethanesulphonate. The effects of partial or total replacement of sodium chloride were examined upon a series of isolated organs, and the guinea-pig ileum preparation was found to be particularly sensitive to the change. Evidence is presented that these low-chloride solutions depolarized the cell membrane of smooth muscle, and that this effect might have been due to an alteration of the chloride ion potential.

The present work arose from a study of ventricular fibrillation in the Langendorff preparation of the rabbit heart. Alterations of the calcium or potassium ion concentrations in the solution perfusing this preparation could readily be made, without affecting any other properties of the perfusate. However, chloride ion forms such a high proportion of the total solute that any significant alteration of its concentration might change the tonicity, the *p*H, or the ionic strength of the solution.

Kärki (1958) replaced sodium chloride with a mixture of sodium sulphate and sucrose when he studied the effects of lowering the chloride ion concentration; however, this procedure might have altered the ionic strength of the solution, since the monovalent chloride ion was replaced by the divalent sulphate ion. We decided to examine sodium ethanesulphonate in order to find out whether it was pharmacologically inert: if it were, it might be used instead of sodium chloride to prepare low-chloride solutions. The ethanesulphonate was preferred to the methanesulphonate because the first member of a homologous series often has anomalous properties.

METHODS

Langendorff Rabbit Heart.—Rabbit hearts were perfused at 37° with the solution used by McEwen (1956) in a conventional Langendorff apparatus, and similar perfusion solutions were prepared in which a quarter, a half, or all the sodium chloride was replaced by an equimolar concentration of sodium ethanesulphonate. It was observed that calcium was precipitated from such a solution in which all the sodium chloride had been replaced when the solution was left standing for many hours. The solutions containing ethanesulphonate were therefore prepared from stock immediately before they were required; one stock solution contained all the solutes except calcium, and the second contained 20 g. of anhydrous calcium chloride dissolved in water to give 100 ml. of solution.

Rabbit Atria.—Atria were dissected carefully from freshly excised rabbit hearts, and set up at 29° in an isolated organ bath of conventional design (vol. 40 ml.). The original solution was that used by McEwen (1956), and changes were made in which a quarter, half, or all the sodium chloride was replaced by sodium ethanesulphonate.

Rat Ventricle Strip.—A strip of muscle 8 mm. long and 3 mm. broad was cut from the right ventricle of a freshly excised rat heart and suspended in Locke solution in an isolated organ bath (vol. 40 ml.). The muscle strip was cut from the pulmonary artery towards the apex of the ventricle parallel to the junction with the left ventricle; it was held flat in the bath by two spring clips, so that it presented the greatest area for oxygenation. The preparation was mounted to work a straw lever. Since it was not spontaneously active at 29°, it was stimulated through two electrodes placed parallel to the strip, one on each side of it; when a momentary current was passed the muscle gave an all-or-none response.

Rabbit Aorta.—A piece of rabbit aorta was cut in a spiral and unwound to form a long narrow strip of muscle which was set up in an isolated organ bath (vol. 40 ml.). The preparation had a rather slow response to drugs, and was gently stretched for two hours in normal Locke solution to develop tone. Submaximal doses of noradrenaline at 30 min. intervals produced slow contractions; this stimulation was repeated when all the sodium chloride in the solution was replaced by sodium ethanesulphonate.

Frog Rectus.—The conventional preparation was set up in a mixture of 5 vol. Locke solution with 2 vol. of water, and submaximal responses to acetylcholine were obtained. The sodium chloride in the Locke solution was then entirely replaced by sodium ethanesulphonate, and the same dose of acetylcholine was reapplied.

Guinea-pig Ileum.—The preparation was set up in conventional manner at 34° in Locke solution, and two test solutions were examined. The first of these was prepared with ethanesulphonate instead of chloride as in the other experiments which have been described; the second had normal sodium chloride, but ten times the normal concentration of potassium chloride.

Guinea-pig Taenia Coli.—Our colleague, Dr. G. Burnstock, was making an examination of the taenia coli muscle at this time, using the sucrose gap technique. He was kind enough to replace the sodium chloride in his normal perfusion solution with sodium ethanesulphonate whilst he recorded the membrane potential, the action potentials and the tension of the muscle.

Surface Tension and Densities of Solutions.—It was important to know whether the surface tension of Locke solution was altered when it was prepared from sodium ethanesulphonate instead of sodium chloride. A glass capillary tube was freed from grease in hot sodium dichromate solution, and washed through ten times with distilled water and four times with double-distilled water before drying at 110°. This tube, when cool, was dipped into the solution to be tested, which rose up to form a meniscus. The liquid level was then adjusted so that the meniscus came to a fixed point on the glass (in case the bore of the tube was not constant) and the capillary rise was recorded.

The densities of the solutions were determined by weighing in a density bottle.

Preparation of Sodium Ethanesulphonate

Sodium ethanesulphonate was required in substantial amounts, because of its high molecular weight relative to sodium chloride. The following procedure gave excellent yields of the pure salt and could be adapted to large scale preparation of the compound. A hot solution of the commercial barium salt (106 g. $(C_2H_5SO_3)_2Ba.H_2O$ in 1 l. H_2O) was treated with a slight excess of a hot solution of sodium sulphate (42 g. Na_2SO_4 in 400 ml. H_2O) and the precipitated barium sulphate was removed by filtration. The filtrate, which should give no precipitate with sodium sulphate solution, was concentrated in an open dish on a steam-bath until a thick crust of crystals formed on the surface of the hot solution. The cooled product formed an almost solid but damp mass of crystals, which was extracted with boiling methanol (500 ml.). The hot extract was filtered, cooled to 0° and the crystals collected. The insoluble material from the first extraction was re-extracted with the methanolic mother liquor from the first crop of crystals, the extract filtered and concentrated on the steam-bath until crystals appeared in the hot solution. On cooling to 0°, this gave a second crop of crystals. The total crude product so obtained (about 80 g.) was dissolved in boiling methanol (600 ml. +2 ml. H_2O) and filtered; it was concentrated until crystals appeared in the hot solution, and then cooled to 0°. This product was crystallized again in exactly the same manner; about 60 to 65 g. (70 to 76% yield) of sodium ethanesulphonate, free from barium and sulphate, were obtained. The salt was dried at atmospheric pressure over calcium chloride in a desiccator. (Found: C, 16.2; H, 4.4; H_2O, 10.0. Calculated for $C_2H_5SO_3Na.H_2O$: C, 16.0; H, 4.7; H_2O, 12.0%.) The method of recrystallization described was used because the salt crystallized so readily from its saturated solution in hot methanol that it was difficult to filter the solution. The salt could also be crystallized from a more dilute solution in methanol by the addition of acetone, but this method gave products with a variable content of water of crystallization. The anhydrous salt was hygroscopic and, as the analysis shows, it was difficult to dehydrate the hydrated salt completely at 100° *in vacuo*.

Results

A Locke solution, in which half the sodium chloride was replaced by sodium ethanesulphonate, will be referred to as "Locke (½E)"; similarly if all the sodium chloride in McEwen solution was replaced the solution will be referred to as "McEwen (E)."

Langendorff Rabbit Heart.—McEwen (½E) solution produced no marked change in the performance of this preparation, but when

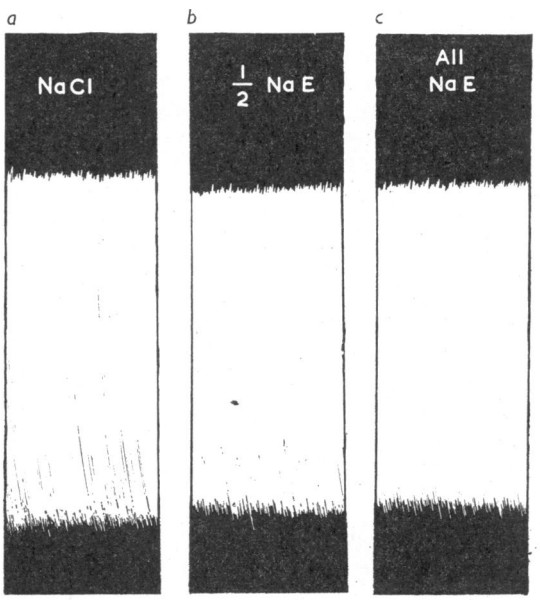

FIG. 1.—Isolated rabbit atria beating spontaneously at 29° in: (*a*) normal McEwen solution (NaCl); (*b*) McEwen solution in which half the sodium chloride was replaced by sodium ethanesulphonate (½NaE); (*c*) McEwen solution in which all the sodium chloride was replaced by sodium ethanesulphonate (All NaE).

McEwen (½E) solution was perfused there was always a fall in the coronary flow. The amplitude of the contractions sometimes diminished at this stage of an experiment, and the heart failed quickly in McEwen (E) solution; none of these effects could be readily reversed by changing back to normal McEwen solution.

We decided that the experiments which we had originally planned would not be possible, because the heart died in McEwen (E) solution. At the same time we thought it worth while to study the effect of replacing chloride by ethanesulphonate upon a number of other isolated organs.

Rabbit Atria.—Very little alteration was observed in the amplitude of contraction of rabbit atria when the solution was changed from normal McEwen to McEwen (½E) (Fig. 1), although in McEwen (E) solution there was an increased spontaneous rate of beating. Atria left overnight in the latter were still contracting the following morning at a quarter of their original amplitude.

Rat Ventricle Strip.—This preparation was driven at 40/min. for 0.5 min. periods, and then allowed 1.5 min. to recover (Fig. 2*a*). In normal Locke solution there was no spontaneous activity during the undriven periods (Fig. 2*a*), but activity was observed as soon as the bath was washed out with Locke (E) solution (Fig. 2*b*). This spontaneous rhythm continued even if all electrical stimuli were withdrawn (Fig. 2*c*), but could be abolished by the addition of acetylcholine to the bath.

Rabbit Aorta.—Isolated rabbit atria and rat ventricle behaved normally when all the sodium chloride in the solution was replaced by sodium ethanesulphonate. We therefore considered that the Langendorff heart preparation might have died because the coronary flow failed and not because ethanesulphonate had any direct toxic action upon the muscle.

Fig. 3 shows the contractions produced by submaximal doses of noradrenaline upon the rabbit aorta strip. When a change was made to Locke (E) solution the contraction due to noradrenaline was potentiated; however, after this contraction the muscle did not relax again to its original length. The aorta remained still more contracted after a second dose of noradrenaline in the modified solution, but relaxed again as soon as the bath was washed out with normal Locke.

Frog Rectus.—Submaximal acetylcholine contractures of the frog rectus were potentiated when

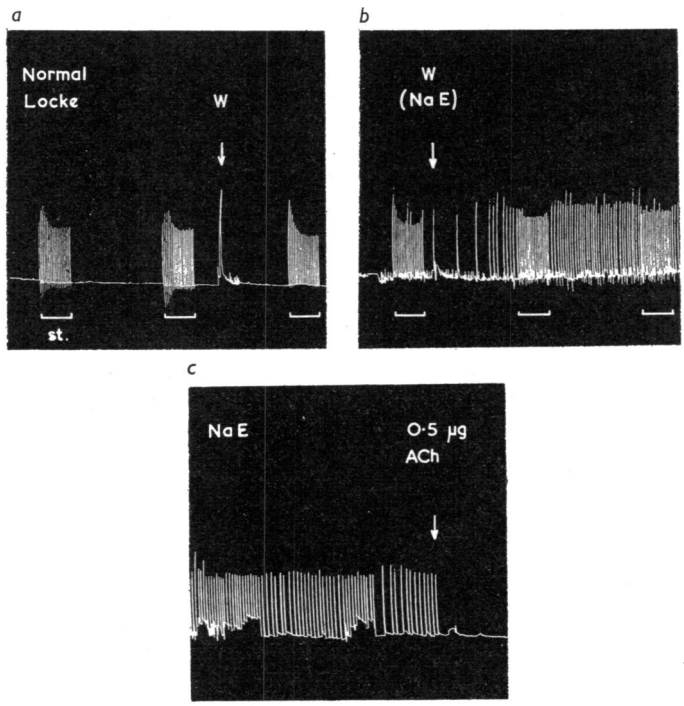

FIG. 2.—Isolated rat ventricle strip at 29°. (*a*) Driven for 30 sec. (for duration of horizontal bar) in Locke solution with 90 sec. rest periods: at W the bath was washed out with fresh Locke solution. (*b*) Driven as in (*a*). At W(NaE) the bath was washed out, and the normal Locke changed to a solution in which all the sodium chloride had been replaced by sodium ethanesulphonate. (*c*) No stimulation: the spontaneous activity in ethanesulphonate Locke was arrested by acetylcholine (ACh).

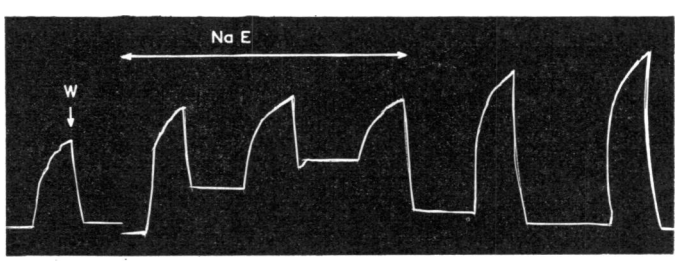

FIG. 3.—Rabbit aorta strip. Contractions in Locke solution after submaximal doses (0.3 µg.) of noradrenaline. When sodium ethanesulphonate Locke solution (NaE) was used the contractions were potentiated but the muscles did not relax fully.

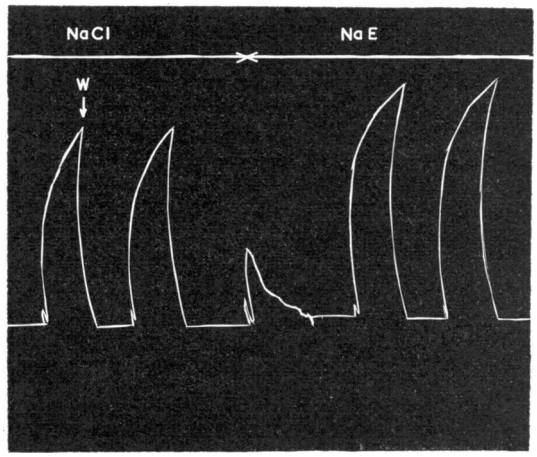

FIG. 4.—Frog rectus. Contractures after submaximal doses of acetylcholine (0.15 μg.). When the sodium chloride (NaCl) in the solution was replaced by sodium ethanesulphonate (NaE) there was a small contraction, and the response to acetylcholine was then potentiated. Bath volume, 5 ml.

the sodium chloride in the Ringer was replaced by sodium ethanesulphonate. The muscle contracted a little at the moment when the solutions were changed (Fig. 4), although this contracture was not maintained.

Guinea-pig Ileum.—A similar, but more powerful, contraction was observed when the Locke solution surrounding a piece of guinea-pig ileum was changed to Locke (E) solution. Again the contraction was not maintained, although it lasted much longer in this preparation than in the frog rectus.

Fig. 5*a* shows submaximal contractions of guinea-pig ileum produced by acetylcholine. When the muscle was fully contracted the kymograph was switched off and the bath was washed out with normal Locke solution (Fig. 5*a*, W). However, after the third contraction the bath was washed out with Locke (E), and the muscle did not relax but contracted still more. The kymograph was turned on at once, and the muscle was observed to relax slowly. When acetylcholine was applied in exactly the same way as before it had no effect.

This action of Locke (E) solution was entirely reversible, and Fig. 5*b* shows three acetylcholine contractions produced in normal Locke solution 1 hr. after the tracing in Fig. 5*a*. After the third contraction the bath was washed out with normal Locke solution ; when the muscle had relaxed the bath fluid was changed to one containing 10 times the normal potassium concentration. The muscle contracted, and then relaxed in a very similar fashion to the tracing in Fig. 5*a* ; once again acetylcholine was without effect.

Guinea-pig Taenia Coli. — The taenia coli preparation was composed of smooth muscle cells, and in this respect it was similar to the guinea-pig ileum. Depolarization of the muscle was observed when the modified Ringer solution was introduced during a sucrose-gap experiment ; action potentials were then discharged, tension was developed and was maintained for several minutes if the muscle was left in the ethanesulphonate Ringer. During this time added acetylcholine caused no additional depolarization, no increase in rate of firing of action potentials nor any increase in tension.

Surface Tension of the Solutions.—The surface tensions of Locke solution and Locke (E) solution were observed to be the same. However, when the two solutions were successively used in the same experiment, the gas bubbles from the oxygen tube were larger in Locke (E) than in normal Locke solution. This might have been due to a difference of surface tension which was too small to be measurable by the method described.

DISCUSSION

The first preparation studied was the rabbit heart, and although this died as soon as it was perfused with an ethanesulphonate solution isolated atrial or ventricular muscle appeared to function normally in the same solution. We concluded that the modified saline did not have any direct toxic action upon cardiac muscle and that the perfused hearts might have died from some other cause ; possibly this was the fall in coronary flow which had been observed when as little as half the sodium chloride had been replaced. We therefore decided to study the behaviour of arterial muscle, and observed that a strip of rabbit aorta would not relax fully when it was placed in a solution prepared with ethanesulphonate. This would explain the diminished coronary flow observed in perfused hearts, if the coronary vessels had behaved in a similar fashion.

The frog rectus preparation responded almost normally in ethanesulphonate solution, but the guinea-pig ileum was very sensitive to the change. Further, the effect on the ileum was very similar to that of an increased potassium concentration in the bath fluid. Since a high potassium concentration almost certainly acted by depolarizing the cell membrane, we thought that the sodium ethanesulphonate solution might act in a similar fashion, and we are very grateful to Dr. G. Burnstock, who tested this idea.

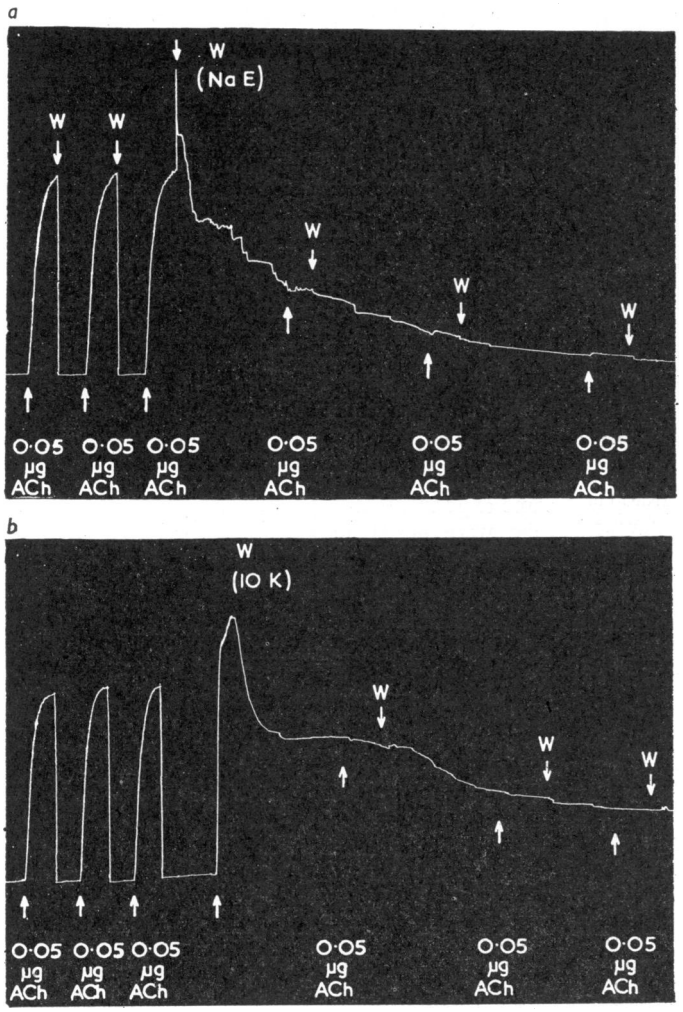

FIG. 5.—Guinea-pig ileum. Contractions after submaximal doses of acetylcholine: (*a*) after the third dose of acetylcholine the bath was washed out with Locke solution (E) prepared with sodium ethanesulphonate; (*b*) 1 hr. after Locke solution (E) had been replaced by normal Locke solution, acetylcholine produced its usual effect: after the third dose of acetylcholine the bath was washed out with normal Locke solution, and when the muscle had relaxed the fluid was changed to one containing ten times the normal potassium concentration (10K). Bath volume, 25 ml.

Dr. Burnstock observed a depolarization as soon as the sodium chloride in the solution superfusing the taenia coli was replaced by sodium ethanesulphonate; this led to the discharge of action potentials, and the muscle tension increased. Acetylcholine added at this stage did not cause further depolarization and did not increase the rate of firing of action potentials; it had no effect on the tension of the muscle, which in all respects behaved like the guinea-pig ileum preparation.

We therefore know that ethanesulphonate solution depolarized the cell membrane of smooth muscle; a similar action would explain all our other observations. Thus acetylcholine produced a contracture of the frog rectus preparation by depolarization, and this effect would be potentiated in ethanesulphonate solution if it partially depolarized the muscle. A short weak contracture of the rectus, when the solutions were changed, would also follow a partial depolarization; the noradrenaline contractions of the rabbit aorta would also be potentiated in the manner observed if noradrenaline acted by depolarization. Finally, a small depolarization of atrial muscle would reduce the membrane potential towards the threshold, and this action on the pacemaker would increase the spontaneous rate; a similar effect on the ventricle strip preparation would cause spontaneous activity. Both effects were observed.

However, in cardiac muscle the observations suggested that depolarization was only partial: that is to say, the membrane potential approached the threshold but did not pass it. Similarly in the frog rectus depolarization could only be partial or a much more powerful contracture would have been observed when the change was made to ethanesulphonate solution. On the other hand there was a much more complete depolarization in smooth muscle, so that many action potentials were fired and a powerful contraction took place. Why should there be this difference between smooth and other types of muscle?

In 1943 Goldman discussed the movement of ions in an ideal cell membrane, and Hodgkin and Katz (1949) were able to derive a very useful equation founded on his treatment. They showed that the electrical potential E across the membrane would be equal to

$$E=\frac{RT}{F}\ln\left[\frac{P_{K}(K)_{i}+P_{Na}(Na)_{i}+P_{Cl}(Cl)_{o}}{P_{K}(K)_{o}+P_{Na}(Na)_{o}+P_{Cl}(Cl)_{i}}\right] \quad \ldots\ldots \text{(I)}$$

where R = gas constant;
T = absolute temperature;
F = Faraday constant;
$(K)_i$, $(Na)_i$, and $(Cl)_i$ are ionic activities inside the cell;
$(K)_o$, $(Na)_o$, and $(Cl)_o$ are ionic activities outside the cell;
P_K, P_{Na}, and P_{Cl} are permeability constants for the ions in the cell membrane.

This equation involved a number of simplifying assumptions: E was the potential difference when there was no net ionic current through the membrane and when the ionic concentrations on each side of the membrane were uniform; that is to say, when there was no concentration gradient in the immediate neighbourhood of the membrane itself. Furthermore the ions in this ideal membrane were assumed to move under the influence of electrical and diffusion forces only.

However, all the results of the present work may be interpreted on the basis of this treatment simply as the result of changes in $(Cl)_o$. When a change was made from normal to ethanesulphonate solution, the chloride ion concentration outside the cell was reduced from about 140 mM. to 10 mM.; the activity of the chloride $(Cl)_o$ was similarly affected. Reduction of $(Cl)_o$ would reduce E according to equation (I), by reducing the value of the expression

$$P_K(K)_i + P_{Na}(Na)_i + P_{Cl}(Cl)_o \quad \ldots\ldots\ldots \text{(II)}$$

However, the significance of a change in $(Cl)_o$ would be determined by the relative values of the three permeability constants P_K, P_{Na}, and P_{Cl}.

In a resting membrane P_{Na} was much the smallest of these constants, and expression (II) approximated to

$$P_K(K)_i + P_{Cl}(Cl)_o \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{(III)}$$

In smooth muscle P_{Cl} and P_K are thought to be comparable (Burnstock and Straub, 1958; Holman, 1958) so that changes in $(Cl)_o$ would alter E significantly; this was the situation which was observed in the taenia coli preparation, where the ethanesulphonate solution produced a profound depolarization. Moreover, should P_{Cl} be smaller than P_K in cardiac and skeletal muscle a smaller change in E would be expected from the same change of $(Cl)_o$; this is a likely interpretation of the observations on these tissues.

It is a pity that more evidence is not available concerning the ratio, $P_K : P_{Cl}$, in different cell membranes, as this information could confirm or confound the present suggestion. It is also possible that the action of ethanesulphonate was not primarily to alter $(Cl)_o$, but that the values of P_K, P_{Na}, and P_{Cl} were changed in the unusual environment. Should the ethanesulphonate solution diminish P_K and P_{Cl} selectively (so that P_{Na} assumed greater significance) E would become smaller, and this was the effect observed. A greater or lesser effect upon P_K and P_{Cl} in different tissues could again explain the observed differences between skeletal, cardiac, and smooth muscle.

This possibility could be tested by examining a short series of homologous sodium alkylsulphonates. In the series $C_nH_{2n+1}SO_3Na$ the physical properties should change in a regular manner as the value of n is increased; for example, solubility should decrease and surface active properties increase progressively. However, the latter should not become prominent for members with n less than 8, and micelle formation would not be expected to become apparent until n exceeded 12. Consequently, if a short series (say, $n=2$ to 6) were tested on isolated preparations as we have tested the ethane member, and no progressive change in the physiological effects was observed, it could be concluded that short-chain alkylsulphonate ions were pharmacologically inert and that the effects which we have observed were due simply to a change in the value of $(Cl)_o$. Alternatively, if the physiological effects changed progressively as the series was ascended, it would be clear that they depended not only upon the change in $(Cl)_o$ but also upon some specific action of the alkylsulphonate anion, such as an effect on the permeability properties of the cell membrane of the muscles, or an entry into the cell.

Long-chain alkylsulphonates would be unsuitable for replacing chloride because of their well-known surface active properties, but if short-chain alkylsulphonates could be shown to have no other effect than that due to a reduction in $(Cl)_o$ they might be used to replace chloride in the physiological solutions for some isolated preparations, though not for all as our results with the Langendorff rabbit heart demonstrated.

References

Burnstock, G., and Straub, R. W. (1958). *J. Physiol. (Lond.)*, **140**, 156.
Goldman, D. E. (1943). *J. gen. Physiol.*, **27**, 37.
Hodgkin, A. L., and Katz, B. (1949). *J. Physiol. (Lond.)*, **108**, 37.
Holman, M. E. (1958). Ibid., **141**, 464.
Kärki, N. T. (1958). Ibid., **141**, 366.
McEwen, L. M. (1956). Ibid., **131**, 678.