Effect of Different Bathing Media on the Short-Circuit Current Across the Intestine of the Rat and Guinea-Pig

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Summary. The changes in short-circuit current occurring when one or both solutions bathing the intestine of rat or guinea-pig mounted in flux chambers were recorded. The results with the guinea-pig can be explained in terms of diffusion potentials arising from the ionic replacements, and an electrogenic sodium pump, sensitive to ouabain, in the contraluminal membrane of the cell. In the rat, the situation is more complicated, and the enterocyte probably possesses an electrogenic sodium pump in the brush-border membrane.

The short-circuit current technique has been very helpful in the understanding of sodium-pumping mechanisms in the intestine[2]. In principle, further information on the nature of sodium pumps could be obtained by replacing the ions bathing one of the faces of the tissue and registering the subsequent changes in short-circuit current. Obviously, if a permeable ion is used as a replacement, a diffusion potential will arise from the movement of this ion across the tissue and will be opposed by a diffusion potential of sodium. But judicious control of the different conditions should permit interpretations of such data in terms of active and passive fluxes.

Methods. Segments of rat or guinea-pig ileum were opened longitudinally, rinsed and mounted between 2 plexiglass hemi-chambers, fitted with electrodes to record the potential and the short-circuit current across the tissue[2]. The chambers were attached to thermostatically controlled reservoirs, and fluid was circulated by means of a gaslift system.

After mounting the chambers, both faces of the tissue were first bathed with a glucose-containing Krebs bicarbonate buffer (119 m*M* NaCl; 4.7 m*M* KCl; 2.5 m*M* $CaCl_2$; 1.2 m*M* KH_2PO_4; 1.2 m*M* $MgSO_4$; 25 m*M* $NaHCO_3$; 11.1 m*M* glucose), gassed with 95%/5% O_2/CO_2. When the potential difference and short-circuit current had stabilized, readings were taken, the solution at one or both faces of the intestine was changed, and the new potential difference and the short-circuit current were recorded.

Results and discussion. The results are presented in the Figure for convenience in the form of percentage changes occurring after changing the bathing media. Since the principal interest of this study is of a qualitative and comparative nature, the contribution of junction potentials[3] to the results have been ignored; this is justified by the absence, in most instances, of equal and opposite effects when the same change is made on opposing sides of the tissue. Both rat and guinea-pig tissues generate a potential of 6–9 mV (serosa positive) under the control conditions, requiring currents of 100–140 μAmps for nullification. The tissue resistance (R) varied with the different conditions, but no obvious pattern emerged; the values when sodium was removed from both sides of the tissue are clearly not reliable.

Passive fluxes of cations occur when different solutions bathe each face of the membrane, giving rise to diffusion potentials. In addition, sodium pumping mechanisms, if electrogenic in nature, would generate potentials. The results with the guinea-pig can be explained in terms of 2 diffusion potentials together with a serosally-orientated electrogenic sodium pump, specific for this cation and sensitive to ouabain. If a mucosally-orientated pump exists, as proposed by other workers[4], it must be electroneutral and probably unaffected by ouabain. It must also be assumed that the tissue is relatively impermeable to choline and that it is more permeable to potassium than to sodium, a fact that has considerable experimental basis[5].

Thus, in these terms, replacement of the serosal medium with a potassium solution leads to a reduction in short-circuit current, since the diffusion of potassium towards the mucosa is greater than that of sodium towards the serosa. When ouabain is also added to the serosa, the polarity is reversed since the electrogenic sodium pump is now abolished and the potassium flux exceeds that of sodium (the flux ratio being *a.* 1.5:1). When choline bathes the mucosa, a reduced but finite potential remains, presumably indicating that the majority of the sodium that enters the enterocyte is pumped preferentially back to the serosal solution, rather than diffusing towards the mucosa. This explanation is confirmed when ouabain is added to the serosal medium: the sodium can then no longer be pumped back, so it passes towards the mucosa, causing an inversion of the polarity. Since no other electrogenic mechanism is active under these conditions, the sodium diffusion potential is thus seen to be of approximately the same magnitude as the resting potential.

Two other points emerge from these results. First, ouabain inhibits from both sides, though it is more effective in the serosal solution, as it acts at the outer face of the contraluminal membrane of the enterocyte, and only passes slowly across the cell[2]. In some other species, it has apparently no action when added to the mucosal medium[6]. Secondly, replacement of chloride by sulphate only influences the current when the serosal solution is concerned. This presumably indicates that the anions do not generate notable diffusion potentials and that chloride movements only actively contribute to serosal-mucosal fluxes, in accordance with the concept of an electroneutral pump concerning $Na^+ + Cl^-$ in the luminal membrane[4]. Thus, in confirmation with earlier observations[7], the mucosal membrane of the guinea-pig intestine appears to be relatively permeable to sulphate. Nevertheless, not too much emphasis should be laid on the interpretation of experiments with the sulphate medium, since the concomitant reduction in calcium ion activities may provoke considerable changes in membrane permeability[8].

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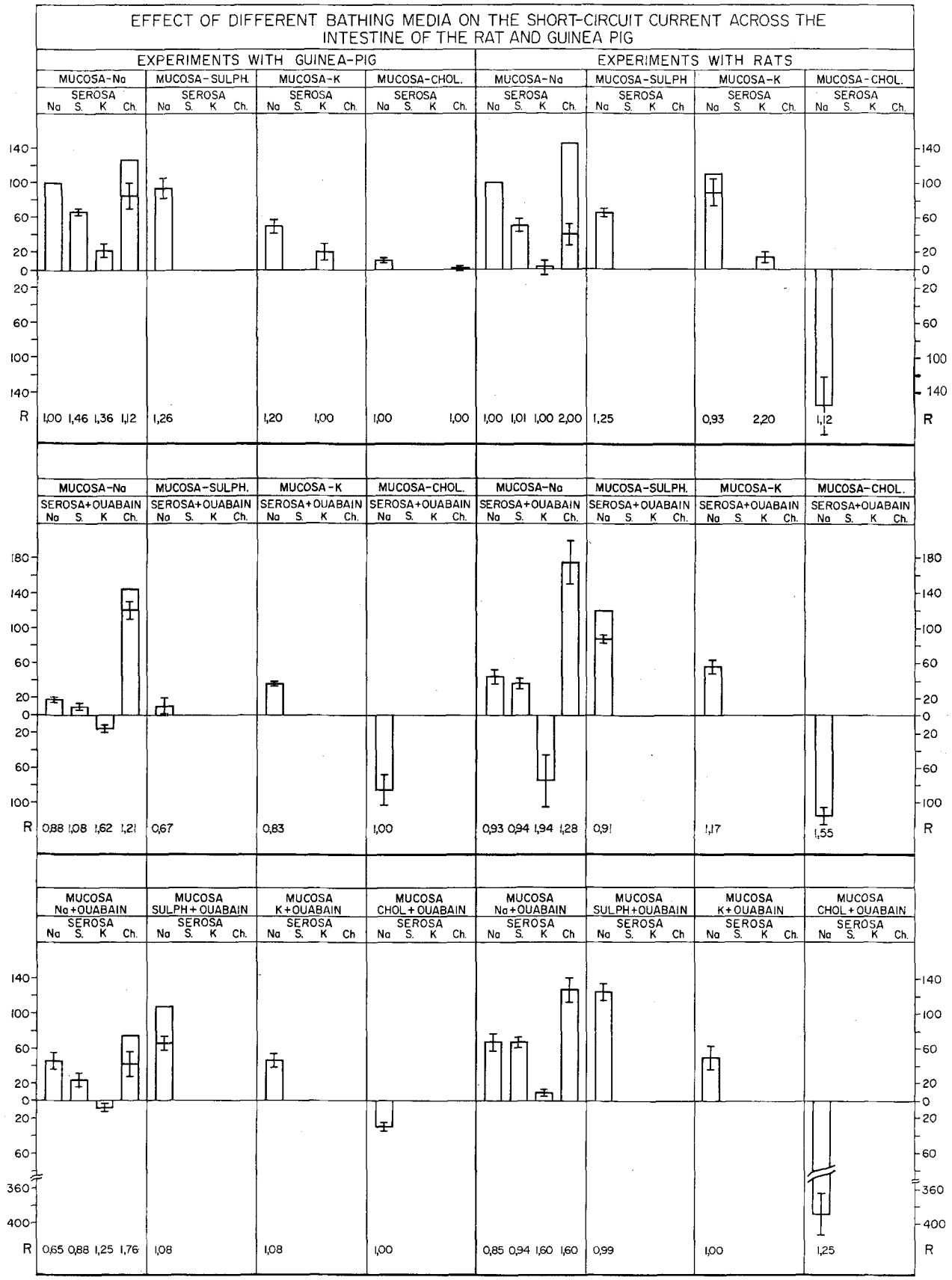

Changes in short-circuit current across guinea-pig or rat intestine clamped in flux chambers when either serosal or mucosal (or both) solutions were replaced. Replacement solutions were chloride-free, sulphate-substituted buffer (S or Sulph), sodium-free, potassium-substituted buffer (K) or sodium-free, choline-substituted buffer (Ch or Chol). All media contained 0.2% glucose. In the lower 2 panels, 1 m*M* ouabain was added either to the serosal or to the mucosal medium. Results are presented as percentage of control value following replacement of the solution, and following stabilization. Where an obvious spike potential occurred, followed by later stabilization at a different value, this is presented in the form of a double column. The values are the means ± SEM of at least 5 animals in each case. The values of R refer to the final tissue electrical resistance, calculated simply as the ratio of the p.d. to the short-circuit current, the control being considered as unity[16].

Some important differences were encountered between the behaviour of rat and guinea-pig ileum, and the results cannot easily be explained in terms of the model applied to the guinea-pig. Replacement of the serosal solution with potassium abolished the short-circuit current, indicating that the potassium diffusion potential is equal to the sum of the sodium diffusion potential and the potential of the electrogenic sodium pump. This could imply that the difference in the permeabilities of the tissue for the two ions is greater than in the guinea-pig, or that the pump is less active in the rat. Neither of these explanations could account for the maintenance of an almost normal current when potassium bathes the mucosal face. This latter result suggests the existence of a mucosally-directed electrogenic sodium pump, as confirmed by the large inversion of polarity when the mucosal face is bathed with choline. Thus the serosally-orientated electrogenic pump appears to be less active in the rat and there probably exists an electrogenic mucosally-orientated sodium pump. Finally, the reduction when sulphate is placed in the serosal chamber suggests the participation of a chloride diffusion potential, in accordance with the low permeability of the rat intestine to sulphate[7], or possibly the existence of an electrogenic chloride secretion mechanism in the luminal membrane, analogous to that of the jejunum[9], which would be inhibited when sulphate bathes the serosa. However, the other observations suggest that a cation rather than an anion is extruded electrogenically across the brush border.

Ouabain partially inhibits the serosally-orientated electrogenic pump in the rat, a species known to be rather insensitive to cardiac glycosides[10]. Indeed, the fact that ouabain affects the short-circuit current without influencing Na^+-dependent amino-acid transport[11] constitutes evidence in favour of the existence of a second sodium pump, independent of a ouabain-sensitive ATPase, as argued elsewhere[11].

When potassium + ouabain bathe the serosal face of the tissue, there is an important inversion of polarity, probably as a consequence of the high permeability to potassium, coupled with a partial inhibition of the Na^+-K^+-ATPase pump. Unexpectedly, there is no increase in the inversed polarity, as occurred in the guinea-pig, when ouabain is added to the choline medium in the serosal chamber.

Finally, there is an enormous inversion of polarity when the mucosa is bathed with choline and ouabain, and the sodium is retained at the serosa. This intriguing result, which was confirmed repeatedly, often using paired tissues, signifies that under certain conditions, ouabain interacts directly with the luminal face of the mucosa, though not by inhibiting a Na^+-K^+-ATPase-dependent ion flux (since in this case, the inverted potential would be decreased, not increased). One possibility is that an electrogenic recapture of sodium ions is inhibited. It is known that the coupled entry of sodium ions and amino-acids or monosaccharides is an electrogenic process[12,13], and there is some evidence, in certain species[14], that energy resulting from the action of Na^+-K^+-ATPase might be involved in the process. The fact that there is no analogous occurrence when potassium + ouabain bathe the mucosal face is consistent with this explanation, since the events surmised would probably be inhibited by potassium[15]. On the other hand, the effect of the mucosal ouabain is so huge that a purely physical explanation, such as a change in the permeability characteristics of the junction complexes, must be considered. Evidently, the influence of ouabain on the luminal membrane of the rat intestinal mucosa would repay further study.

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Capillary Lengths and Areas, and Intercapillary Distances in Tissue Near the Human Knee[1]

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Summary. Quantitative stereological electron microscopy has been used to investigate the capillary lengths, surface areas and intercapillary distances in the tissues around the human knee, the synovial membrane, synovial capsule, fat and tendon. The vascularity of these regions was much less than in other areas of the body, especially muscle.

The principles of stereology[2-4] have not yet been extensively applied to describing the quantitative morphology of blood capillaries. Such applications are essential if the findings of physiology are to be integrated with those of morphology in order to comprehend, at the fine structural level, the detailed functioning of the various capillary-types and the tissues they serve.

A start has been made in this direction. It has been shown[5] that the lengths, widths and depths of the close junctions in dog skeletal muscle allow one to calculate the capillary filtration and diffusion coefficients with very good agreement with those found by experiment; the vesicular numbers, etc., give good agreement with experimental results, using a Brownian-motion model. With the fenestrated capillaries of the cat jejunum[6], it was found that the filtration and diffusion coefficients so calculated were many orders of magnitude greater than those found by experiment. This indicated that these capillaries correspond to the tunnel-capillaries of INTAGLIETTA and DE PLOMB[7]; the continuous capillaries of the muscle are tube-capillaries, where the permeability is controlled by the endothelium. In tunnel-capillaries the endothelium has no influence on permeability, except for cells and the largest macromolecules: it is the interstitial connective