# ACTIVE SODIUM TRANSPORT THROUGH THE RAT INTESTINAL MUCOSA STUDIED BY THE AUTOMATIC CONTINUOUS VOLTAGE CLAMP METHOD

S. T. Metel'skii

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Electrophysiological methods have begun to be used in the study of active transport processes in epithelial tissues and, in particular, the short-circuit current method [14], which enables total flows of actively transported ions to be measured. For this method to be put into practice, the ion concentrations in the two solutions must be equal and the potential difference on the preparation must be zero. To compensate the potential difference on a preparation of epithelial tissues to zero, a current is passed through the object in the corresponding direction (short circuit), the strength of which can be varied manually [8], by means of an electromechanical clamp [1], continuous clamps — simple [7] or with manual [4] or automatic [5] compensation of the resistance of the solution, and also be means of a discrete voltage clamp [3].

The rat small intestine has particular features which create difficulties when the voltage clamp method is used on it. It differs from the relatively compact epithelium of frog's skin type in the lower spontaneous potential difference and resistance, and from loosely textured epithelia of the rabbit's small intestine type by its smaller size and more "delicate" tissue, so that edge damage effects must be more marked. The presence of fairly thick, unmixable layers near the surface of the mucosa has a significant effect on the results [12], and for that reason its influence must be minimized, although no serious attention has been paid to this problem in any electrophysiological investigation so far undertaken.

There are no publications in which the use of the short-circuit method to study active sodium transport through the wall of the rat small intestine and allowing for the features described above has been described. We describe below a sufficiently simple apparatus, which we have worked with for several years and which satisfies modern demands.

The electrical part of the apparatus consists of a two-cascade differential amplifier (Fig. 1). To control the voltage on the preparation an additional differential amplifier is provided, the signal from which is controlled by means of a digital voltmeter. The electric current passing through the preparation is recorded as a voltage drop on a small resistance, the signal from which is led to the input of the differential amplifier. The circuit provides for the use of this signal to compensate the resistance of the solutions and submucosa, although usually we do not use compensation of series resistance on the rat intestine. The signal from the output of the current amplifier is monitored by means of an oscilloscope and recorded on a self-writing potentiometer. The preparation of rat small intestine, divided along the contramesenteric line [2], is placed in the form of a partition between two similar half-chambers (Fig. 2), with capacities of 70 and 200 µl, to one of which is glued a latex ring. The two half-chambers have water jackets connected to a thermostat. The temperature of the preparation is monitored in its immediate vicinity by means of a graduated MT-54 microthermistor, the resistance of which was measured by means of a slide-wire bridge. Both half-chambers were perfused independently in two channels from a peristaltic pump. The solutions are oxygenated with $O_2$ on entering the pump. The potential was recorded by means of EVL-1M electrodes. The current was passed through chlorided silver wires, twisted into a coil. All electrodes were separated from the solutions by gaps of 1.5% agar in 1M KCl (Fig. 2, 3).

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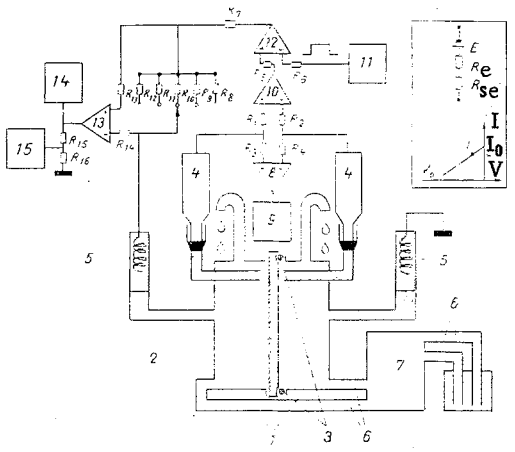


Fig. 1. Block diagram of apparatus: 1) half-chambers; 2) preparation; 3) latex tightening ring; 4) potential electrodes; 5) current electrodes; 6) tubes for perfusion; 7) peristaltic pump; 8) voltage amplifier (amplification factor 20) 1K284 UDIA; 9) digital voltmeter; 10) first cascade of clamp (amplification factor 15) K1284 UDIA; 11) stimulator; 12) second cascade of clamp (amplification factor 140) K153 UD2; 13) current amplifier 1K284 UDIA; 14) oscilloscope; 15) automatic writer; $R_1$-$R_4$) each 1 mΩ; $R_5$) 3 kΩ; $R_6$) 1.8 mΩ; $R_7$) 33 kΩ; $R_8$) 32.5 Ω; $R_9$) 65 Ω; $R_{10}$) 130 Ω; $R_{11}$) 260 Ω; $R_{12}$) 520 Ω; $R_{13}$ and $R_{14}$) 3 kΩ; $R_{15}$) 20 kΩ; $R_{16}$) 40 Ω; Shading: with dashes — Ringer's solution, with dots — agar, horizontal — 1 M KCl. In box, top right corner: equivalent electrical circuit of preparation on dc (E — emf, $R_e$ — resistance of epithelial layer, $R_{se}$ — resistance of subepithelial tissues) and current—voltage characteristic curve of preparation ($V_o$ — potential difference on preparation with current equal to 0; $I_o$ — true short-circuit current). In a real experiment, we are located at the point 1 of the current—voltage characteristic curve.

With a decrease in size of the preparation the contribution of edge damage increases in proportion to the radius of the preparation, so that edge effects on the rat intestine ought to be about three times greater than on the rabbit intestine. To reduce the contribution of the edge effect and to increase the repeatability of the results, first, a latex tightening ring was used (Fig. 1-3), by means of which the tightening force is distributed more evenly aroung the perimeter of the preparation. Second, the half-chambers are pressed together by means of two long springs (Fig. 3), mounted on two guides, along which the half-chambers also moved. The springs create a tightening force of about 10g force. In addition since the length of the springs is much greater than the thickness of the preparation, the tightening force is independent of the thickness of the wall of the segment of intestine studied.

Perfusion of the empty chamber continues until the asymmetry potential (potential difference between the electrodes in the absence of the preparation) ceases to fluctuate with an accuracy of 0.1 mV in the course of 1-2 h. After the end of the experiment the asymmetry is measured once again. In good experiments the champed potential and the asymmetry potential change in the course of the experiment by not more than ±0.1 mV.

Results obtained on the mucosa depend strongly on the intensity of mixing of the solutions [12], and for that reason a rate of perfusion was chosen in these experiments such that the results were almost independent of it. For a volume of the mucosal half-chamber of 70 µl, and with a temperature of 26°C, the optimal rate of perfusion was 3.1 ml/min. This rate corresponds to the highest achieved in [13]. During the study of active sodium transport ordinary Ringer's solution containing 80 mM $Na_2SO_4$ instead of 141 mM NaCl is used, for as a result of this replacement the rate of reabsorption of sodium is unchanged [6] and the short-circuit current becomes equal to the flow of actively transported sodium [9].

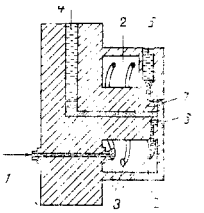

Fig. 2

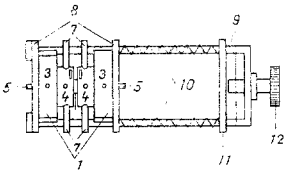

Fig. 3

Fig. 2. Left half-chamber in section (side view): 1) inlet pipe; 2) water jacket; 3) silicone tube; 4) channel for current electrode; 5) channel of potential electrode; 6) experimental chamber; 7) outlet for solution. Oblique shading — transparent plastic, with dashes — Ringer's solution, with dots — agar, horizontal — 1 M KCl.

Fig. 3. Half-chamber with tightening device (viewed from above): 1) half-chambers; 2) tightening ring; 3) channels for current electrodes; 4) channels for potential electrodes; 5) inlet pipe; 6) outlet pipes; 7) pipe for water jacket; 8) tightening plates; 9) guides; 10) springs; 11) spring support; 12) screw control.

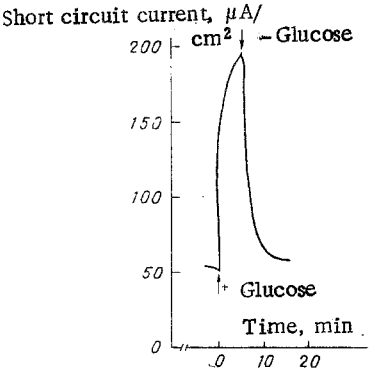


Fig. 4. Dynamics of change in short-circuit current through wall of rat small intestine at 37°C in response to addition of 10 mM glucose to mucosal solution.

The viability of the preparation, assessed from its ability to respond by an increase of short-circuit current to addition of glucose to the mucosal solution (Fig. 4), is preserved in individual experiments for 10-12 h (at a temperature of 26°C).

Distortion of the results on account of resistance of the subepithelial tissues present in the preparation, and connected in series with the resistance of the epithelial layer (Fig. 1, in box) was specially investigated. According to the equivalent electrical circuit of the intestinal wall preparations, its current—voltage characteristic curve is described by the equation

$$I = I_0 + V/(R_e + R_{se}).$$

As a result of the presence of resistance of the subepithelial tissues, even if the potential difference on the preparation is kept at zero, the potential difference on the epithelium is compensated short of zero by an amount $\Delta V = I \cdot R_{se}$, and we measure the apparent short circuit current $I_1 = I_0 - \Delta V/(R_e + R_{se})$. Hence, $I_0 = I_1[1 + R_{se}/(R_e + R_{se})]$. Hence, to calculate the true short circuit current $I_0$ we need to multiply the apparent current $I_1$ measured experimentally by $1 + R_{se}/(R_s + R_{se})$. To estimate this value, the epithelium was removed from the intestine [10]. Removal of the greater part of the epithelium was confirmed morphologically and also with reference to a functional characteristic (absence of responses of short-circuit current to glucose — Fig. 4). It was shown that the resistance of the subepithelial tissue is 10.1 $\Omega \cdot cm^2$, and accordingly $R_{se}/(R_e + R_{se})$ is 0.31, in good agreement with the analogous estimate of 33% obtained recently [11]. Allowing for series resistance by multiplying the

values of the short circuit current obtained by 1.31 is thus perfectly adequate when the use of manual compensators of serial resistance changing in the course of the experiments is ineffective, and a clamp with automatic compensation of resistance is complex and only a single one of its kind may be in existence.

The method can be used both in experiments to study mechanisms of active transport through various epithelial tissues (intestine, urinary and gall bladders, amphibian skin, retina) and also under clinical conditions to assess the functional state of resected segments of intestine.

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# COMPARISON OF THE IMMUNODEPRESSANT ACTION OF MICROBIAL DEAMINASE FROM DIFFERENT SOURCES

E. A. Kozlov and T. A. Tsvetkova

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Asparaginase is an enzyme used in the treatment of several human diseases connected with malignant transformation of lymphocytes [8]. It was initially held that asparaginase acts only on malignant cells, growth of which is dependent on asparagine. Clinical studies have shown that asparaginase also inhibits proliferation of normal tissue cells and has an immunodepressant action, inhibiting rejection of grafted organs and tissues [7, 10]. The enzyme also inhibits blast transformation of lymphocytes stimulated by phytohemagglutinin (PHA) [4]. However, the mechanism of the immunodepressant action of asparaginase has not yet been completely explained. The most widely held explanation of this phenomenon is the ability of the enzyme to catalyze hydrolysis of asparagine, thus making it in short supply for lymphocytes [11]. This view is supported by data showing that asparagine, added to the medium, prevents the inhibitory action of asparaginase on blast transformation of lymphocytes [11]. However, glutamine [6] also has a similar action. It has also been shown that asparaginase from *Erwinia carotorova*, which has higher glutaminase activity than the the enzyme from *Escherichia coli*, is a more powerful immunodepressant [5]. To study the role of glutaminase activity in

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