METHOD OF STUDYING HYDROCHLORIC ACID SECRETION BY THE ISOLATED GASTRIC MUCOSA USING A TWO-CHANNEL pH-METER

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A method of recording the secretion of hydrochloric acid by means of a two-channel system in symmetrical halves of the same area of isolated gastric mucosa is suggested. The system has important advantages over the one-channel method. A special feature of this method is that the effect of various substances on the rate of secretion of H^+ can be more clearly differentiated and the wastage of animals is substantially reduced. The block diagram of the apparatus for two-channel recording of H^+ secretion and the design of the four-chamber cuvette are described.

KEY WORDS: isolated gastric mucosa; H^+ secretion; two-channel pH-meter.

The method of recording the kinetics of H^+ secretion by means of a two-channel system in two symmetrical halves of the same piece of isolated gastric mucosa has important advantages over the one-channel system extensively used by several workers [1-3].

By means of a two-channel system it is possible to differentiate more clearly between the effects of different stimulators and inhibitors on the rate of H^+ secretion, i.e., it is possible to monitor tests on symmetrical halves of the same piece of gastric mucosa, thereby greatly shortening the duration of the experiment and substantially reducing the wastage of animals.

EXPERIMENTAL METHOD

A four-chamber polar cuvette was designed and the pH-meter-22 made by the firm of Radiometer (Denmark) was modernized. By means of a cuvette, 60-80% of the area of one piece of isolated gastric mucosa can be divided hermetically into two symmetrical halves (Fig. 1) and it consists of two parts: "serous" and "mucous". Each part is divided by a partition into two symmetrical chambers, for the test and nutrient solutions, respectively. The top and bottom parts of the cuvette also have windows with a partition for fixing the isolated peice of gastric mucosa. After fixation of the tissue and assembly of the polar cuvette, the gastric mucosa is hermetically divided into symmetrical halves. As a result, four independent chambers are formed: two "mucous" and two "nutrient". The "mucous" chambers are closed, the "nutrient" are continuously flowing. The gastric mucosa was bathed correspondingly with "mucous" and nutrient solutions. The nutrient solution contained (in mM) NaCl 84.6, KCl 3.2, $CaCl_2$ 1.8, KH_2PO_4 0.8, and $NaHCO_3$ 17.8. The solution was continuously saturated with a mixture of 95% O_2 and 5% CO_2. The "mucous" solution contained (in mM): NaCl 102.4, KCl 3.2, $CaCl_2$ 1.8, and $MgSO_4$ 0.8. The solutions were constantly stirred.

In the "mucous" chambers the quantity of H^+ produced was measured by means of two insulated glass electrodes. The single-channel radiometer-22 pH-meter was suitably modernized for two-channel working and for the use of two independent glass electrodes and one calomel comparison electrode (Fig. 1)†. The individual potential of each glass electrode was recorded in turn by the amplifier of the pH-meter through the operation

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† To prevent diffusion of the "mucous" solution through the bend of the calomel electrode the bend was filled with 3% agar–agar in saturated KCl solution.

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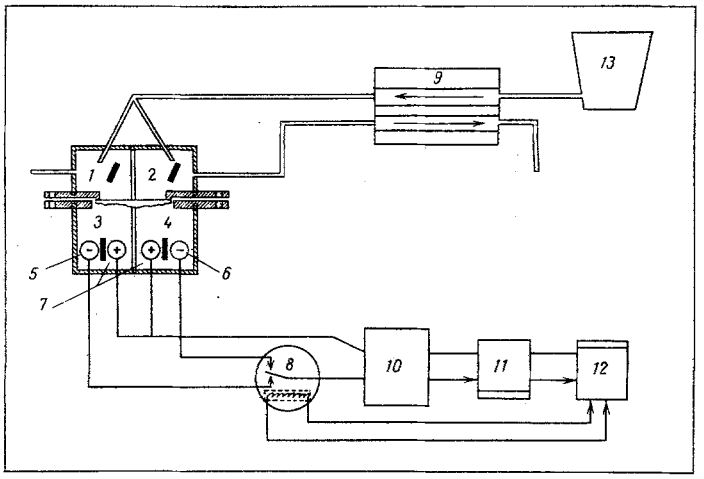

Fig. 1. Scheme of apparatus for simultaneous two-channel recording of H^+ secretion in two parts of the same piece of gastric mucosa: 1) "serous" chamber of first channel, 2) "serous" chamber of second channel, 3) "mucous" chamber of first channel, 4) "mucous" chamber of second channel, 5, 6) glass electrodes, 7) calomel electrodes, 8) polarization relay, 9) peristaltic pump, 10, 11) radiometer 22 pH-meter, 12) KSP-4 recording potentiometer, 13) oxygenator.

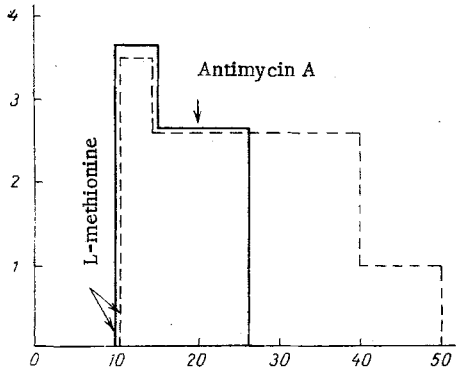

Fig. 2. Effect of methionine on H^+ secretion in isolated gastric mucosa and inhibitory action of antimycin A on methionine stimulation determined by a two-channel H^+ recording system. Abscissa, time (in min); ordinate, quantity of H^+ (in μeq/cm^2/h). Continuous line represents experiments with addition of antimycin (50 μM) after preliminary addition of methionine (100μM); broken line shows control with methionine only. Arrows indicate addition of substances.

of a polarization relay, synchronized with the KSP-4 three-point recording potentiometer (modification 41.340, 50.042, time for indicator to travel along entire scale 2.5 sec). The precision vibrating-reed converter of the radiometer-22 pH-meter with a working voltage of 20 V used as the polarization relay. The source of direct current was a stabilized rectifier with a working voltage of 20 V, fixed to the chassis of the recording potentiometer. The pick-up distributor of the three-point potentiometer consisted of an alternating polarity distributor of the potential of the stabilized source of direct current with an interval of 12 sec. Circulation of the "serous" and "mucous" solutions and the regulation of their rate of flow were carried out by means of the LKB (type 4912A) peristaltic pump.

EXPERIMENTAL RESULTS

Results illustrating how this method can be used for the differential study of H^+ secretion in the isolated gastric mucosa are given in Fig. 2. In the experiment represented, L-methionine was added to the gastric

mucosa of a frog (in the hibernation phase) in both "nutrient" chambers after complete exhaustion of spontaneous secretion. Clearly methionine caused almost identical stimulation of H^+ secretion in both halves of the mucosa. Against this background, antimycin A, an inhibitor of mitochondrial respiration, was added to one of the nutrient chambers. Under these circumstances, the stimulation of H^+ secretion by methionine in the half of the mucosa whose serous side was in contact with the added inhibitor, was inhibited completely after 5-6 min, whereas the other half, without addition of antimycin A, continued to secrete acid for 30 min. It will be clear from this example that the stimulation of acid secretion in both halves of the mucous membrane coincided with sufficiently high accuracy, and the stimulation of secretion itself was evidently connected with the function of the mitochondrial respiratory chain.

The use of a two-channel system for recording H^+ secretion in symmetrical halves of the same piece of gastric mucosa thus has definite advantages over the single-channel scheme. To begin with these advantages are expressed as minimal scatter of the data (the very substantial individual variations in the rate of H^+ secretion are leveled out) and data on differences in the physiological state of the two parts of the same piece of gastric mucosa can be obtained quickly and simultaneously. Another advantage of this method is that it can be used for the spectrophotometric measurement of the redox level of the cytochromes in the gastric mucosa by the use of a dual-beam system with simultaneous recording of the H^+ secretion.

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RAPID METHOD OF STANDARDIZING CHOLAGOGUES IN MICE

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A rapid method of selection and quantitative estimation of the specific activity of cholagogues in mice is described: The gall bladder is removed from the animals, weighed initially together with bile and then without it, so that the difference is the quantity of bile collected. The accuracy of the method was tested in experiments in which insulin, dehydrocholic acid, and 2-mercaptobenzthiazole (Mebetizole) were administered. The method as described can be used for pharmacological screening.

KEY WORDS: cholagogues.

Cholagogue activity is usually determined either in chronic experiments on animals with fistulas [7, 8] or in acute experiments on anesthetized rats [1, 5, 6]. However, the high cost of the animals, the complexity of the operations, the long duration of the postoperative period, and also the low sensitivity of anesthetized rats to the substances tested (which distort the results of the corresponding determinations) all combine to restrict the usefulness of these methods and to make essential the development of more simple methods in which cheaper biological material can be used. The writer suggests a rapid method of standardization of cholagogues on mice which he has developed.

After enteral or parenteral administration of the cholagogues to mice, at the height of their action (30, 60, or 90 min after administration) the unanesthetized animals animals are killed by bleeding from the carotid arteries. Laparotomy is performed, the liver exposed, and a No. 75 silk ligature is tied around the cystic duct,

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