Effects of Barium on the *in Vitro* Frog Gastric Mucosa* (32661)

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The H^+ secretory rate of the gastric mucosa is inversely related to its electrical resistance and at high H^+ rates the resistance is about 100 ohm cm^2 (1). The low resistance is predicted on the basis of a theory postulating separate electrogenic transport mechanisms for the H^+ and Cl^- and is not compatible, without *ad hoc* postulates, with nonelectrogenic theories (2). Agents or procedures used in the past that markedly increase the resistance result in a concurrent marked reduction in secretory rate (3,4). In a previous analysis (5) it was shown that if a marked increase in resistance occurred with little change in the H^+ rate, doubt would be cast on the electrogenic theories of H^+ and Cl^- transport and/or the assumption that Cl^- and HCO_3^- move across the nutrient membrane as free ions because improbably high emf's would be demanded. It was found during the course of studies on the role of external Ca^{++} on the gastric mucosa (6) that Ba^{++} in the presence of normal Ca^{++} results in a marked increase in resistance without a marked decrease in H^+ secretory rate. The purpose of this paper is to present these studies. A preliminary report appeared elsewhere (7).

Methods. The experiments were performed on Rana Pipiens with an *in vitro* method described elsewhere (5). Two pairs of electrodes were used, one pair for sending current across the mucosa and the other for measuring the potential difference (PD). Resistance was determined as the change in PD per unit of applied current. The nutrient bathing solution contained (in m*M*): Na^+ 102; K^+ 4; Ca^{++} 1; Mg^{++} 0.8; Cl^- 82.6; HCO_3^- 25; phosphate 1.0; glucose 10, and histamine 10^{-4} *M*, and the secretory solution: Na^+ 102; K^+ 4; Cl^- 106. Both sides were gased with 95% O_2 - 5% CO_2. The ratio of the volume of the nutrient bathing solution to the area of the gastric mucosa was 10.0 ml/1.3 cm^2. With this method the PD, resistance, and H^+ rate are maintained at relatively constant levels for periods of over 2 hours. The Ba^{++} was added as 0.1 *M* $BaCl_2$ except for concentrations of 5 m*M* or higher in which case $BaCl_2$ replaced NaCl.

Results. Figure 1 shows the effects of adding successive increments of Ba^{++} to the nutrient solution (final concentrations are noted). The initial Ba^{++} concentration of 0.005 m*M* produced a small increase in resistance and after 0.5 m*M* the resistance increased to about 80% of its maximal value and after 5.0 m*M* it started decreasing. The H^+ secretory rate was maintained at a high and relatively uniform level until the Ba^{++} concentration was increased to 5 m*M* after which it decreased. The PD showed relatively little change. In 11 experiments performed similarly to that shown in Fig. 1, the threshold for an increase in resistance was found to be between 0.005 and 0.01 m*M*. The concentration necessary to produce a maximal increase in resistance was usually 2.0 m*M* or less and 1.0 m*M* resulted in an increase to 80% or more of the maximal value. Following an increase of the resistance to a maximum the concentration necessary to produce a sub-

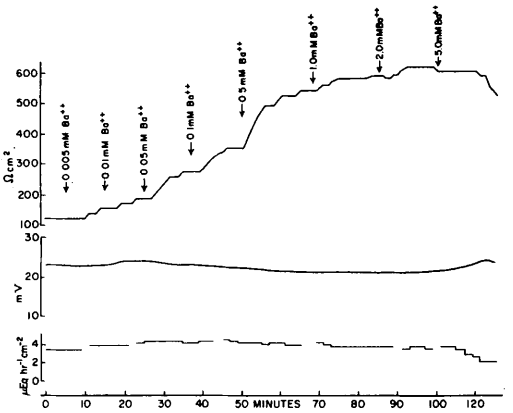

FIG. 1. Effect of additions of Ba^{++} to the nutrient solution (values are final concentration) on the resistance, PD, and H^+ secretory rate of gastric mucosa.

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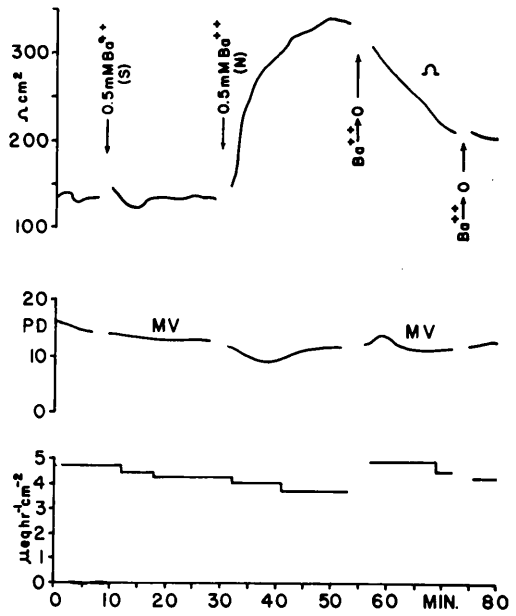

FIG. 2. Effect of addition of 0.5 m*M* Ba^{++} to secretory fluid and subsequent addition to nutrient fluid. The nutrient side was washed with Ba^{++}-free nutrient at times indicated by arrows (the Ba^{++} remained in the secretory fluid for duration of experiment).

sequent decrease in resistance (see 5 m*M* Ba^{++} in Fig. 1) was greater than 3.0 m*M* and usually a decrease did not occur until the concentration was 5 m*M* or greater.

Removal of Ba^{++} by washing with Ba^{++}-free nutrient solution as seen in Fig. 2 results in a return of the parameters toward their control levels. In some experiments there was complete reversal of the Ba^{++} effects while in others the reversal was only partial.

Addition of Ba^{++} in one step to a final concentration of 1 m*M* resulted in a rapid rise in the resistance, the steady state level being reached within 10–20 min. Two groups of experiments were performed in which measurements were made for periods of 100 min (6 mucosae) and 20 min (7 mucosae) after the addition of 1 m*M* Ba^{++}. These results are presented in Table I. In both groups there was relatively little change in the PD. In the first group the H^+ rate decreased but even after 100 min was about 70% of the control level. In the second group the H^+ rate was 87% of the control level. In both groups the increase in resistance was significant (method of paired variates, $p<.001$).

The question naturally arises as to whether the addition of Ba^{++} to the secretory fluid would have comparable effects. Figure 2 shows an experiment in which 0.5 m*M* Ba^{++} was added to the secretory solution without producing a significant effect on the parameters. In a total of 10 experiments it was found that the addition of Ba^{++} in concentrations up to 20 m*M* or greater had no effect on the parameters.

Discussion. Since the addition of Ba^{++} on the secretory side had no effect it might be assumed that the effect upon addition of the Ba^{++} to the nutrient side resulted from changes in the nutrient membrane of the mucosal cell layer. It is usually assumed that the sites for the active Cl^- and H^+ transport are located on the lumen-facing membrane (8) and since there was relatively little effect on the H^+ rate with Ba^{++} concentrations giving maximal increases in resistance it seems unlikely that the increase in resistance is due to a change in the secretory membrane. However, these arguments do not rigorously exclude the possibility that the increase in resistance occurs in the secretory membrane. If the increase in resistance occurs in the secretory membrane doubt is cast on the electrogenic theories of Cl^- and H^+ transport

TABLE I. Effect of Addition of Ba^{++} (1 m*M*) to the Fluid Bathing the Nutrient Side of Gastric Mucosa on PD, Resistance, and H^+ Secretory Rate (Standard Deviation Given in Parentheses).

No. of mucosa		PD (mV)	R (ohm cm²)	H^+ rate (μeq/hour cm²)
6	Control 0, no Ba^{++}	22.5 (±5.7)	190 (±42)	3.1 (±1.1)
	30 min after Ba^{++}	19.5 (±5.8)	363 (±84)	2.5 (±1.1)
	100 min after Ba^{++}	21.3 (±4.7)	350 (±75)	2.2 (±1.4)
7	Control 0, no Ba^{++}	15.9 (±4.2)	84.1 (±32.8)	4.59 (±0.75)
	20 min after Ba^{++}	13.9 (±5.6)	274 (±131)	4.0 (±0.53)

while if the increase occurs in the nutrient membrane doubt is cast on the assumption that Cl^- and HCO_3^- moves across the nutrient membrane as free ions because improbably high emf's would be demanded (5). Further work needs to be done before an analysis of the implications of this finding for the mechanisms of ion transport would be profitable.

Conclusion. The addition of Ba^{++} to the fluid bathing the nutrient side of the frog gastric mucosa resulted in a marked increase in resistance and relatively little change in the H^+ rate and the PD. Addition of Ba^{++} in comparable and higher concentrations to the secretory fluid had no effect.

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Serologic Screening of Rhesus and Grivet Monkeys for SV_{40} and the Foamy Viruses (32662)

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The spontaneous appearance of virus cytopathic effect (CPE) in many cultures has been a critical problem in the use of monkey kidney cell cultures for virus vaccine production or research purposes. Although a number of viruses have been isolated and identified, their detection in monkey kidney cell cultures from this laboratory has been limited mainly to two virus CPE groups (1). Group one includes the syncytial CPE, induced primarily by measles virus; SV_5 (parainfluenza 5 as it is now classified); and the foamy viruses. The vacuolating CPE comprises the second group of which SV_{40} is the causative agent. In order of frequency of occurrence, the foamy viruses and SV_{40} comprise the bulk of viruses isolated from monkey kidney cell cultures.

The serological studies of Ruckle (2) and the fluorescent antibody studies of Carski (3) indicate a direct correlation between the absence of antibody to foamy virus in monkeys and the absence of this virus in their kidney cell cultures. With these criteria, by use of serological techniques it should be possible to select monkeys free of foamy viruses for tissue culture production.

This paper describes in detail the development and routine application, over an extended period, of a complement fixation-antibody screening test, which has been used for the rapid and effective selection of monkeys for production of tissue cultures free of the foamy and SV_{40} viruses.

Materials and Methods. Monkeys. Both rhesus, *Maccaca mulatta,* and grivet, *Cercopithecus aethiops,* monkeys, which are used for poliovirus vaccine production, were tested in this study. These monkeys were trapped and housed by a procedure described by Vickers (4), and shipped to this laboratory. Upon arrival, the monkeys were isolated in single-cage units and test-bled, and sera were taken for testing. At the end of a 6-week quarantine period, selected monkeys were bled again and killed, and their kidneys were removed aseptically for tissue culture preparation.

Tissue cultures. Kidney cell cultures derived from both rhesus (RMK) and grivet monkeys (GMK), and from rabbits (RbK), were prepared by the method of Youngner (5). The growth media consisted of lactalbumin hy-