novirus type 27 by conjunctival inoculation, followed in 3 months by a rechallenge with type 27, and a month later by a heterotypic cross-challenge with type 26. Sequential serum specimens were fractionated by density gradient ultracentrifugation or by gel filtration; pools containing a preponderance of IgM, IgA or IgG were prepared from each specimen; and homotypic and heterotypic HI and neutralization antibody titers were measured in each serum specimen and immunoglobulin pool.

No 19S IgM homotypic or heterotypic antibody activity was detected.

Titers of IgG and IgA antibody rose sharply 2 weeks after the initial inoculation, and the pattern of their appearance was much the same as the pattern of appearance of antibody in unfractionated serum. No significant boosts of antibody titers were demonstrable after rechallenge or cross-challenge. Antibody remained present in serum and in "IgG" and "IgA" pools for the 18-week duration of the study.

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Secretion by Guinea Pig Gastric Mucosa *in vitro*.* (31614)

R. L. SHOEMAKER,† G. SACHS, AND B. I. HIRSCHOWITZ

Division of Gastroenterology, Department of Medicine, and Department of Physiology, University of Alabama Medical Center, Birmingham

The nature and activity of the pumps in the mammalian stomach have not been measured *in vitro*. Reports describing the properties of the secreting guinea pig mucosa(1), and of the *in vitro* cat and rat mucosa(2,3) seem to indicate that there are certain marked distinctions between the *in vitro* mammalian preparations.

This paper will therefore outline some of the characteristics of the guinea pig preparations, with particular emphasis on ionic requirements for H^+ and Cl^- transport.

Methods. Guinea pigs (*Cavia porcellus*) of approximately 800 g weight were killed by CO_2 narcosis and the stomach removed. The serosa and external muscle layers were dissected off and the mucosa mounted in a lucite chamber as previously described for *Rana pipiens*(4). The nutrient bathing solutions were of the following composition in mM: Na^+ 132, K^+ 6, Ca^{++} 1.0, Mg^{++} 1, Cl^- 115, HCO_3^- 25, HPO_4^{--} 1.0, glucose 20 and the secretory in mM: Na^+ 152, K^+ 6, Cl^- 158. The solutions were gassed by bubbling 95% O_2 and 5% CO_2. For ion substitution experiments, sulfate was substituted for chloride and choline was substituted for sodium. Measurements of hydrogen rate were by the pH stat method, transmembrane potential difference (P.D.) by calomel electrodes with renewable KCl junctions, and resistance by sending 10 microamps of current in either direction and the short-circuit current (I_{sc}) as the current required to reduce the P.D. to zero after 30 seconds.

Fluxes were determined using Cl^{36} or Na^{22}

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TABLE I. Average Parameters Measured in Guinea Pig Gastric Mucosa.

No. of experiments	H^+ rate, $\mu E/cm^{-2}/hr^{-1}$	P.D., mv	Resistance, Ω cm^2	Short circuit current, $\mu a/cm^{-2}$
34	3.08 ± .81	7.48 ± .78	104 ± 5	57.2 ± 5.1

and counting in a Nuclear Chicago scintillation counter.

Results. The gastric mucosa as obtained *in vitro* produced in general a spontaneous secretion maintained for at least 120 minutes. However, if no spontaneous secretion was obtained, the mucosa was unresponsive to stimulation and furthermore the established rate in a secreting mucosa was not altered by histamine (10^{-4} M), gastrin pentapeptide (10^{-7} M), or mecholyl (10^{-6} M).

Table I summarizes the parameters found in 34 experiments. The P.D. observed *in vitro* was low compared to the P.D. obtained *in vivo* (40 mv) using a KCl bridge. Various modifications were inefective. The orientation of the P.D. (lumen negative) may be accounted for by nutrient (N) → secretory (S) chloride transport or S → N cation transport. Measurement of Cl^- flux showed a net N → S flux of 2.21 μEq/cm^2 per hour. Sodium flux S → N was less than one μEq per hour per cm^2. With I_{sc} of 57 microamps per cm^2, these data suggest that most of the current is carried by chloride as in the frog.

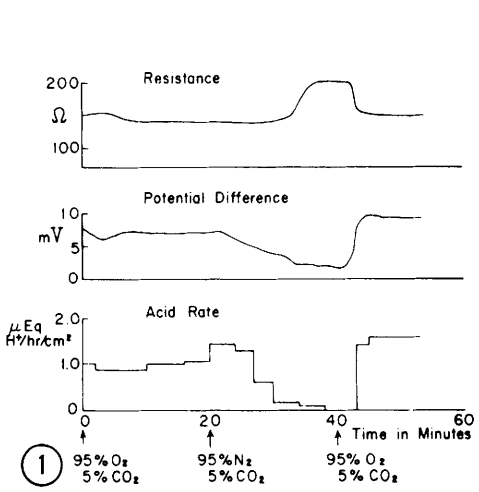
Fig. 1 illustrates the effect of anoxia on guinea pig gastric secretion *in vitro*. With the onset of anoxia, there was within 20 minutes a fall of H^+ rate to zero, a fall of P.D. and rise of resistance. Readmission of O_2 resulted in reestablishment of the H^+ rate and P.D. within 5 minutes with fall of resistance. SCN^- at 10 mM also inhibited acid secretion.

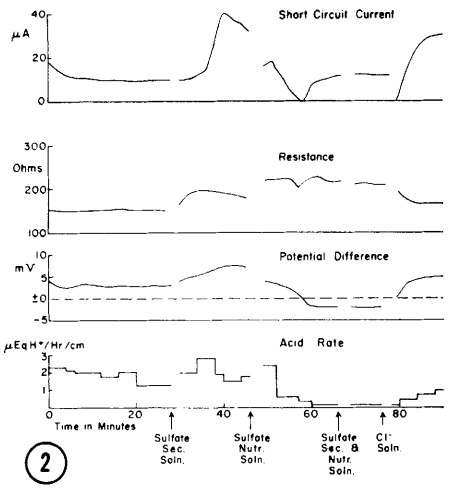
Fig. 2 shows the effect of SO_4'' substitution on the mucosa. Removal of Cl^- from the secretory side alone resulted in a rise in P.D., I_{sc} and resistance. This is presumably due to an increased N → S Cl^- gradient. Readmission of Cl^- to the secretory side, with removal of Cl^- from the nutrient side resulted in fall of H^+ rate to low values, with inversion of P.D. and a rise in resistance. Substitution by SO_4'' on both sides had no further effect, and Cl^- readmission resulted in rapid return of P.D. to normal orientation, with an increase in acid rate.

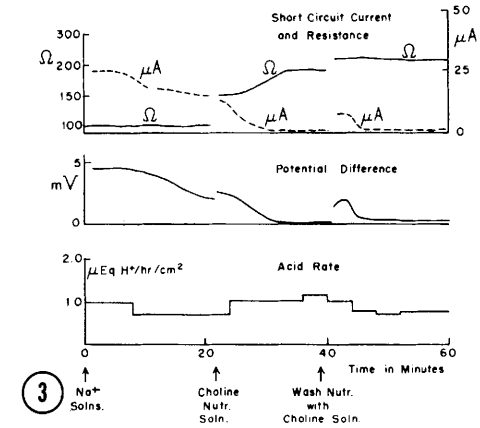
Cation removal had significant effects on the secretory parameters of guinea pig. Removal of K^+ from both sides of the mucosa resulted in acid inhibition, reversed by readmission of K^+ on the nutrient side. The results of Na^+ removal from the nutrient side alone are shown in Fig. 3. Little change was observed in the secretory rate, but there was a fall of the P.D. to zero with a rise of resistance. In this respect, the results are similar to removal of Na^+ from both sides in *Rana pipiens* gastric mucosa. Apart from a transient rise in P.D., little effect was noted on further washing.

Removal of Na^+ from the secretory side alone is shown in Fig. 4. Apart from a slight rise in the resistance there are no maintained effects. Further washing with choline did not significantly change the parameters. However, subsequent removal of Na^+ from the nutrient side, resulted in a rapid reversal of the P.D., and rise in resistance with a maintained acid rate. Subsequently as the H^+ rate fell, the P.D. rose towards zero. Readmission of Na^+ reestablished the acid rate and the normal orientation of the P.D. In the same Figure is shown the effect of 10^{-4} M ouabain. This concentration resulted in inhibition of acid rate after 40 minutes. Ouabain in other experiments also had a delayed effect on acid, with but little effect on the P.D.

Discussion. With the appearance of reports on other *in vitro* mammalian gastric mucosal preparations, it is pertinent to review in detail some of the properties of the *in vitro* guinea pig mucosa. It is unfortunate that when obtained in the resting state, this system was not sensitive to stimulation. Another problem has been the low P.D. obtained *in vitro*. Although the highest P.D. recorded was 21 mv, this was not reproducible, and the P.D. in general was less than 10 mv. This leads to the conclusion that there has been some damage to the transport mechanisms perhaps due to relative anoxia. In spite of several







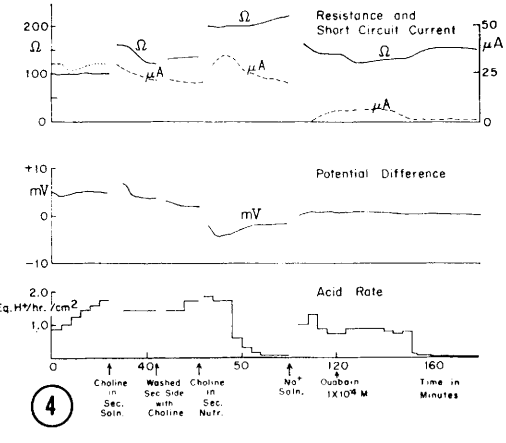


FIG. 1. Effect of anoxia on secreting guinea pig mucosa.

FIG. 2. Effect of SO_4'' substitution for Cl^- on secretory, nutrient or both sides of guinea pig gastric mucosa.

FIG. 3. Effect of choline substitution for Na^+ on nutrient side of gastric mucosa.

FIG. 4. Effect of Na^+ removal from the secretory and subsequently both sides of the guinea pig gastric mucosa, and effect of 10^{-4} M ouabain on secretion in Na^+ solutions.

modifications of technique and medium, oxygenation and temperature, no consistent effect on the P.D. was observed. However, experimentally, the induced changes in the P.D. corresponded to changes observed in other species.

The parameters obtained with secreting guinea pig mucosa showed that essentially all of the I_{sc} could be accounted for by net Cl^- flux and that the Na^+ pump contribution was minor. This is in sharp contrast to the results obtained in the resting cat mucosa where there appears to be an equivalence of the Cl^- and Na^+ mechanisms(2).

To explain these differences it seems reasonable to assume that in the resting mucosa *in vivo* there are two major transport systems, Cl^- directed N → S, and Na^+ S → N, which together determine the orientation and magnitude of the P.D. The actual P.D. observed in a particular *in vitro* system will depend on the relative contribution of these pumps. Thus, if *in vivo* the magnitude of the Na^+ and Cl^- systems are equal, with a P.D. of 60 mv, and *in vitro*, the Na^+ pump is 100% inhibited, the observed P.D. will be of the

order of 30 mv. If further inhibition of the Cl^- mechanism occurs, the P.D. will be even less, and with a maintained H^+ rate, and virtual absence of Na^+ and Cl^- transport, the P.D. will be inverted. Thus in the cat, both Na^+ and Cl^- systems are equally suppressed, whereas in guinea pig, the Na^+ mechanism is abolished *in vitro*, according to the above hypothesis.

In SO_4'' conditions, guinea pig mucosa behaves as if the H^+ mechanism is predominant, and as if there is very little net Na^+ or SO_4'' transport, since the P.D. is inverted with only a low H^+ rate.

The effect of Na^+ removal was also of interest. Removal of Na^+ from nutrient side alone resulted in fall of P.D. to zero, with maintained H^+ rate. Under these conditions guinea pig gastric mucosa behaves somewhat similarly to frog mucosa with Na^+ removed from both sides(5). As previously discussed, this is interpreted most simply as a unitary H^+ mechanism under these conditions, whereas in SO_4'', the H^+ mechanism acts as if it is electrogenic. With Na^+ absent from both sides of the mucosa the guinea pig showed an inverted P.D. that was dependent on a maintained H^+ rate. Na^+ readmission reversed those effects. It seems therefore, that in the absence of Na^+ guinea pig gastric mucosa acts as if the H^+ mechanism is predominant and electrogenic, *i.e.*, similar to SO_4'' conditions. Stated alternatively, Na^+ appears essential for Cl^- transport in this system.

Summary. The secreting *in vitro* guinea pig gastric mucosa showed an H^+ rate of 3 μE cm^{-2} hr^{-1}, and an I_{sc} of 57 μa cm^{-2} hr^{-1}. The latter was accounted for by net N→S Cl^- transport and H^+ was sensitive to SCN^- and N_2. Substitution of SO_4'' for Cl^-, and choline for Na^+ inverted the P.D. if H^+ was maintained. An explanation for *in vitro* species differences is proposed.

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Susceptibility of HeLa Cells in S-Phase to Inhibition of DNA Synthesis by Poliovirus Infection.* (31615)

W. W. ACKERMANN, C. D. POWERS,† AND D. WAHL

Department of Epidemiology and Virus Laboratory, School of Public Health, University of Michigan, Ann Arbor

Since the original observation that poliovirus infection inhibited DNA synthesis in the host cell(1-3), several investigations have concerned themselves with the competence and availability in the infected cells of the reaction components which yield DNA. These studies were prompted by a view that the virion or some product of its vegetative phase acted directly in the DNA primed polymerase reaction to prevent DNA synthesis(4-6). Observations of this type, of a negative nature to date, fail to explain the mechanism of the viral inhibitory effect.

It has also been suggested that the viral action might be directed to some cellular control mechanism that normally governs the rate and sequence of macromolecular synthesis in the uninfected cell(7-9). DNA synthesis in HeLa cells is restricted to approximately 5 hours of the 18-hour reproductive cycle of the cell(10). Conditions which would act to prevent cells from entering the S-phase (DNA productive phase) would inhibit DNA synthesis without acting directly upon the

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