Inhibition of the Rabbit Ileal Motility *In Vitro* by Human Saliva[1] (34933)

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Many investigators over the last 30 years have observed inhibition of gastric acid secretion in animals given iv injections of gastric and salivary secretions. Brunschwig, (1) Code, (2) and Menguy (3) have demonstrated the existence of an antisecretory principle in gastric or salivary secretions. Menguy (3) presented evidence indicating that the salivary glands are the source of the inhibitor and suggested the name "sialogastrone" for the inhibitory agent. The action of saliva on gastrointestinal motility has not been tested, however. The present paper presents the results of a study of the effect of a salivary extract on the motility of the rabbit ileum *in vitro*.

Materials and Methods. Saliva from young human adults of either sex was collected in glass breakers after at least 3-hr fasting. The saliva was immediately placed in Union Carbide dialysis tubing (pore diameter = 24 Å) and dialyzed against multiple changes of distilled water at 4° for 24 hr. After dialysis the samples were lyophilized and stored at −18° until used. Three to four milligrams of extract were obtained from each milliliter of saliva collected.

To determine whether bacteria proliferating in the saliva during collection was responsible for the effect of the extract on the ileum, one collection of saliva was made into beakers containing penicillin. Subjects spat alternately into beakers containing no penicillin and beakers containing 200,000 units (= 120 mg) of penicillin G (Sigma). These samples were dialyzed and lyophilized in the same manner as the other samples.

Adult albino rabbits of either sex were killed by a sharp blow to the head. The most distal 10–15 cm of the ileum were immediately removed and washed in Krebs–Ringer bicarbonate solution (KRb). The segment was divided to give two pieces weighing 2–4 g and measuring 5–7 cm. Both strips were suspended in muscle baths containing 50 ml KRb at 38°. The solution contained 100 mg/100 ml dextrose and was aerated by 95% O_2–5% CO_2. Osmolarity was 300 mOsm ± 5 mOsm, pH 7.3 ± .1. One end of a strip was fixed to the bottom of the bath, while the other was connected by a stainless-steel hook and cotton thread to a Grass Instruments force-displacement transducer, model FT103. Activity of the strip was monitored by a Beckman type RB dynograph. Sensitivity of the dynograph was set at 200 μV and a 3.5 mm/g deflection.

Ten milligrams (unless otherwise indicated) of the salivary extract dissolved in 1 ml KRb were added to the bath after the activity of the strip had become regular. The response of the strip was recorded for several minutes after which the bath was drained and flushed three times before being refilled with fresh KRb. The procedure was then

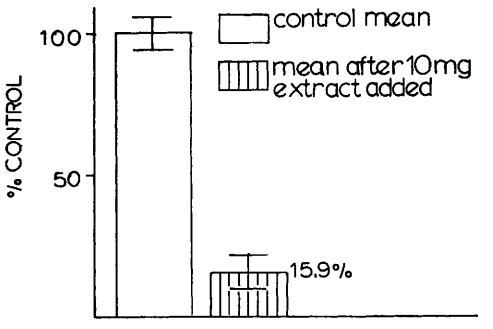

FIG. 1. Comparison of mean amplitudes of contractions in 35 trials before and after addition of extract to the media. Figures are expressed as percentage of control mean. Confidence limits of 99% are indicated.

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repeated from three to six times on each strip. Each repetition is referred to as a trial.

Results. In the first series of experiments 14 strips were prepared from seven rabbits. Four strips showed insufficient or irregular activity and were not included in the study. Thirty-five trials were performed on the remaining 10 strips. In all trials the addition of the dissolved salivary extract was followed by a period during which the magnitude of the contractions was greatly decreased while the rhythm of contraction remained essentially unchanged. The mean amplitudes of the preparations before and after the addition of the extracts are shown in Fig. 1. The data represent the mean amplitude during the 3 min immediately before the addition and the mean for the 1-min period after the addition

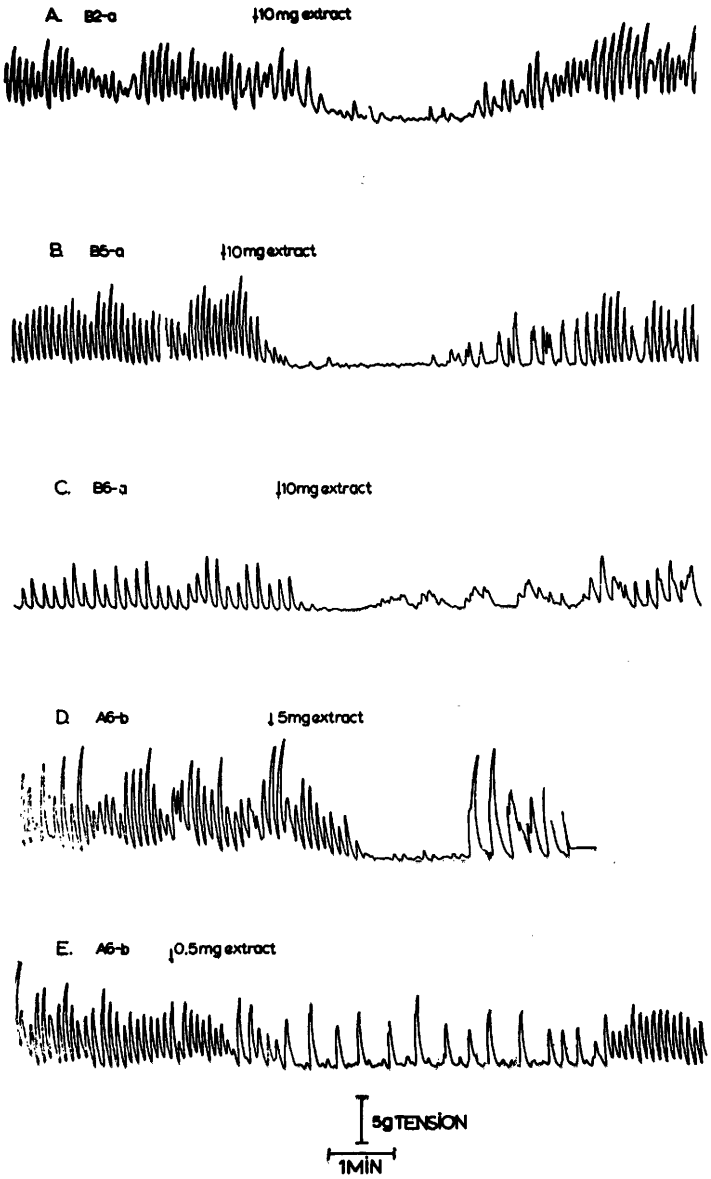

FIG. 2. Recordings of typical responses of ileal strips upon the addition of extract. The responses of three different strips to addition of 10 mg extract are shown in A, B, and C. D and E show the responses of a strip (A6-b) to 5 mg extract and 0.5 mg extract.

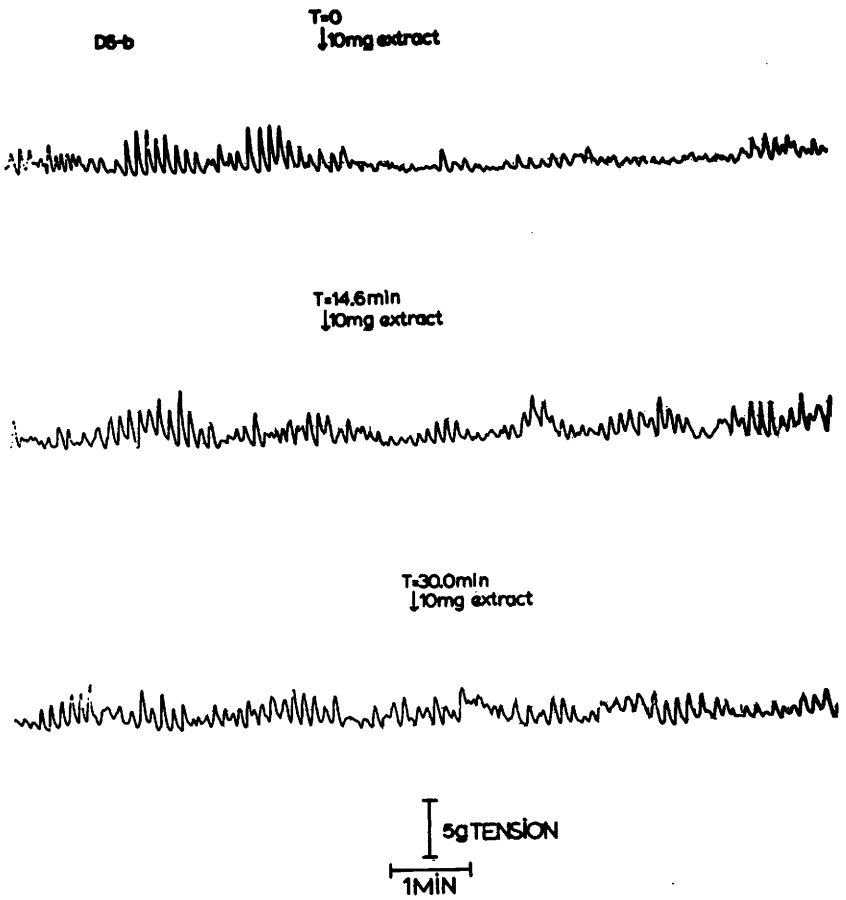

FIG. 3. Response of a strip (D6-b) to three 10-mg additions of extract demonstrating tachyphylaxis. Bath medium was not changed between additions. The second and third additions occurred 14.6 min and 30.0 min after the first addition.

of the extract during which the most marked inhibition occurred. The figure represents the pooled data from all 35 trials. Figure 2 shows three typical records of responses of strips to addition of 10 mg extract.

First signs of inhibition were seen from 20 to 60 sec after addition of the extract. Duration of the inhibition varied widely, lasting between 1 and 5 min after which the strips generally regained near normal activity while still in the presence of the extract; however, some strips showed continued irregular activity. All strips recovered normal activity after the baths were flushed and refilled with fresh KRb. An apparent tachyphylaxis was often observed; after several trials on the same strip, the latent period of response was lengthened, the maximum change in amplitude of contraction was lessened, and recovery was more rapid. After the strips had recovered while extract was still in the bath, a second addition of extract had no effect on the strips (Fig. 3).

In a previous series of experiments using the same technique, preliminary indications of a dose-response relationship were observed with significant inhibition occurring after addition of 0.5 mg extract (Fig. 2-E). Preliminary experiments have also been performed to determine whether or not the inhibitor is heat stable. Heating the extract in KRb to 100° for 10 min decreased its inhibitory effect, although significant inhibition was still evident. Redialyzing the heated extract did not alter its effect on the strips.

Evidence has been presented by Baume *et*

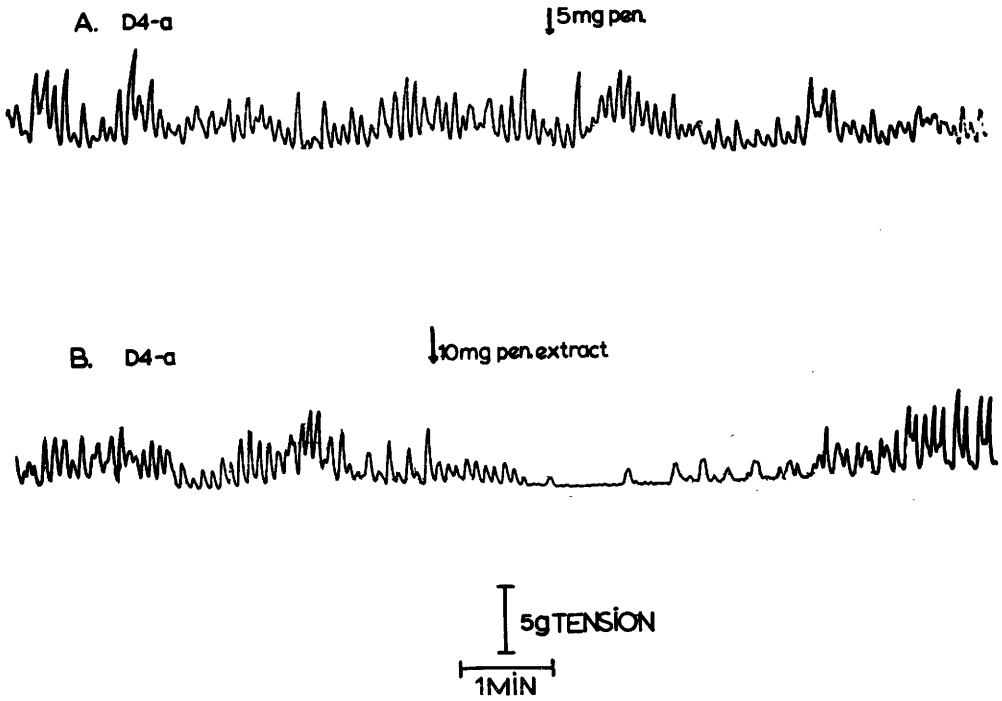

FIG. 4. Response of a strip (D4-a) to addition of 5 mg penicillin (A) and 10 mg extract collected in the presence of penicillin (B).

al. (4) indicating that the inhibitory effect of saliva on gastric acid secretion may be due to the presence of bacteria in the extract. When growth of bacteria in the saliva during collection was prevented by the presence of penicillin, the extract had no capacity to inhibit rat gastric acid secretion. To determine if bacteria were responsible for production of the inhibitor of motility, a series of experiments was performed using saliva collected in beakers containing 200,000 units penicillin G. The effect of the extract collected with penicillin was of the same order as that produced by extract from saliva collected in beakers without penicillin at the same time from the same subjects. Assuming all penicillin added to the collecting beakers was present in the extract after dialysis, which is unlikely, the maximum amount of penicillin in 10 mg of extract would be 4.3 mg. Five milligrams penicillin G in 1 ml KRb added to the baths had no effect on the motility of the strips. Figure 4 shows records of the response to addition of penicillin and the addition of extract collected with penicillin.

Discussion. The results indicate that human saliva contains an inhibitor of rabbit ileal smooth muscle. Addition of 10 mg of the lyophilized aqueous extract to baths containing 50 ml of KRb in which ileal strips were suspended reduced the force of the contractions to the limit of the sensitivity of the recording device. The fundamental rhythm of the contractions remained unaltered suggesting the contractile mechanism rather than the electrical activity of the muscle was affected by the extract. It is doubtful that the inhibitor is of bacterial origin although the role of bacteria cannot be rigorously excluded. The results of the series of experiments using extract collected in the presence of penicillin are not conclusive since the saliva was collected by expectoration and, therefore, was contaminated during passage through the mouth.

The effect of the inhibitor on the musculature of other areas of the gastrointestinal tract and its effect when administered intraluminally remains to be tested. However, Malhotra's (5) observation that addition of

whole saliva to a test meal slowed the rate of emptying upon being introduced by a cannula into the stomachs of human subjects suggests that saliva possibly affects gastric motility, directly or indirectly, when given intraluminally.

Summary. Dialyzed, lyophilized human saliva has been found to inhibit the motility of rabbit ileum *in vitro*. Significant decrease in the force of contraction of ileal strips is observed at salivary extract concentrations as low as 1 mg/100 ml. The inhibition is reversible and is produced by a substance found in saliva in which bacterial growth has been prevented by penicillin.

1. Brunschwig, A., Van Prohaska, J., Clark, T. H., and Kandell, E. V., J. Clin. Invest. **18**, 4 (1939).

2. Blackburn, C. M., Code, C. F., Chance, D. P., and Gambill, E. E., Proc. Soc. Exp. Biol. Med. **74**, 233 (1950).

3. Menguy, R., and Berlinski, M., Amer. J. Dig. Dis. **12**, 1 (1967).

4. Baume, P. E., Baxter, C. H., and Nicholls, A., Aust. Ann. Med. **17**, 42 (1968).

5. Malhotra, S. L., Amer. J. Physiol. **213**, 169 (1967).

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