INFLUENCE OF pH ON TONE AND MOTILITY OF ISOLATED STRIPS OF RAT FUNDUS AND PYLORUS COMPARED WITH OTHER PARTS OF ALIMENTARY TRACT

By

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In the course of estimations of 5-hydroxytryptamine (5HT) on the isolated rat fundus preparation according to Vane (3), we noticed that, at times, addition of solutions not containing any 5HT but strongly acidic produced stimulant effects. Stomach strips are known to be inhibited by acids (2). Evans and Underhill (1) reported relaxation of intestine with acids. Hence we investigated in detail the response of rat fundus strip to changes in the pH of the bathing fluid and compared it with pylorus, duodenum, ileum and colon.

METHODS

All tissues were taken from white rats, stunned with a blow on the head and decapitated. Ninety rats were used in these experiments. Strips of fundus were prepared according to Vane (3) preserving the longitudinal muscle layer. Pyloric strips were cut either in the same way as fundus, or spirally to preserve the circular muscle. Other tissues like duodenum, ileum and colon were used in 5 cm long bits. In experiments comparing two different tissues, they were taken from the same rat.

The tissues were kept bathed in well-oxygenated Mg-free Tyrode solution at 37°C. Simple levers with frontal writing points were used to record the contractions magnified seven times. In experiments where two tissues were studied simultaneously, the inflow and outflow tubes of the two organ baths were connected together so that the baths could be filled or emptied simultaneously. To expose the tissues to a solution of different pH, the baths were emptied completely and the solution of set pH, previously warmed to 37°C was poured into one of them, which immediately reaches the other through the communication.

Solutions : The pH of Tyrode solution was between 7.5 and 8. Solutions of pH 10, 9, 8, 7, 6, 5, 4, 3 5, 3, 2.5, 2, 1.5 and 1 were prepared by adding to Tyrode solution, sodium hydroxide to raise the pH, or hydrochloric acid to lower it. In a few experiments sulphuric acid was used to acidify instead of hydrochloric acid. The pH of the fluids was set with the help of Direct reading Cambridge pH indicator.

Atropine sulphate (T. H. Smith), mepyramine maleate (May and Baker) and lysergic acid (LSD-25, Sandoz) were used in some experiments as antagonists. In some, reserpine (SERPASIL, Ciba) was given intraperitoneally to rats at 0.3 mg/kg for 2 days.

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RESULTS

Effects of application of solutions of different pH for 3 minute periods on strips of fundus and pylorus

In 12 experiments, fundal and pyloric strips taken from the same stomach were exposed to solutions of different pH ranging from 10 to 1 for three minute periods. Fig. 1 shows the record from one such experiment.

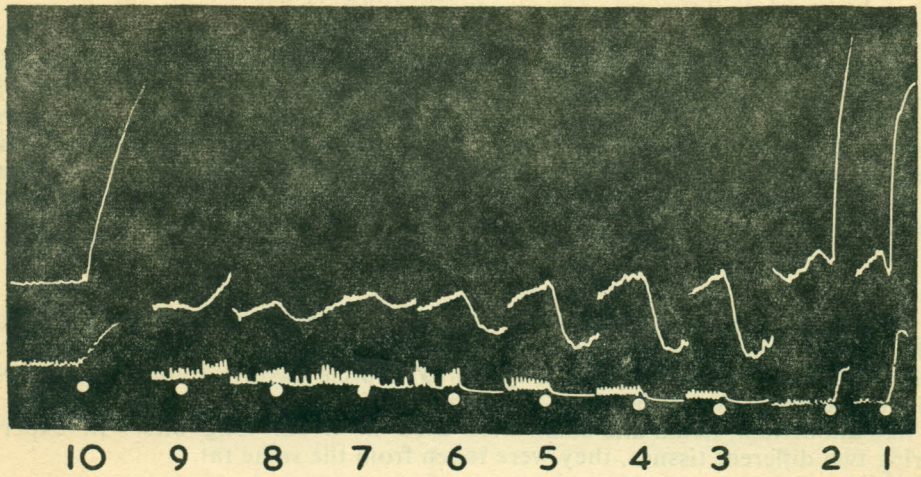

Fig. 1. Effects of application of solutions of pH 10 to 1 for 3 minute periods on isolated strips of rat fundus (top) and pylorus (bottom). At the white dots solutions of pH noted on the abscissa are applied. The tissue is bathed in the intervals with Tyrode solution of ph 7.8.

Effects on fundus : Alkaline solutions stimulated the fundus greater at pH 10 than 9. Acid solutions caused significant changes only from pH 6 downwards. With pH 6 to 4, fundus immediately relaxed for a minute or two, but tended to regain tone during the last minute. It relaxed more at pH 4 than at 6. At pH of less than 4, fundus contracted instead of relaxing. At pH 3 out of 12 experiments, there was stimulation in 7, slight relaxation followed by stimulation in 3, and only relaxation in 2 experiments. The experiment of fig. 1 showed only relaxation at pH 3. Solutions of pH 2.5, 2 and 1.5 always caused a marked contraction. The contraction at pH 2.5 or 2 was more than that obtained at pH 1.5 or 1.

Effects on pylorus : Pyloric strips also showed an increase in tone in alkaline pH (fig. 1). Normal movements of pylorus ceased on application of acid solutions of pH 6 to 3. Marked relaxation shown by fundus at pH 6 to 4 was absent with pylorus, although slight loss of tone occurred as the experiment proceeded. In some experiments, at pH 3 when the fundal strip contracted, the pyloric strip remained quiescent. Pylorus also was stimulated at pH 2, 1.5 or 1. While the pylorus appeared to contract more at pH 1 than at 2, the fundus seemed to contract more at pH 2 than at 1.

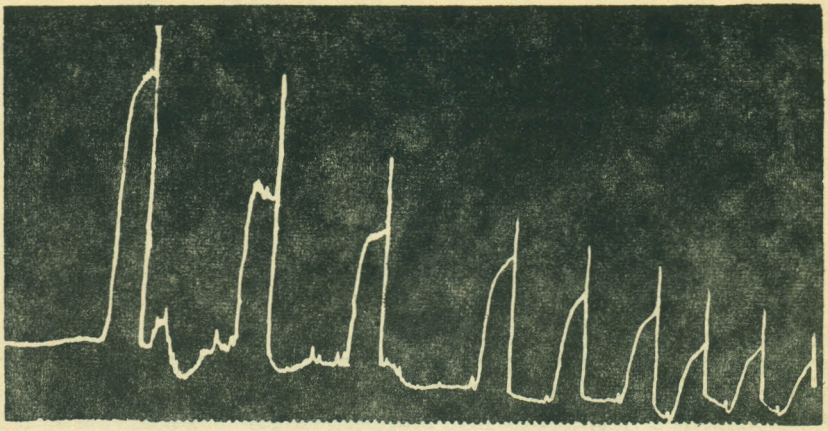

Fig. 2. Effects of repeated application of solution of pH 2.5 on isolated rat fundal strip for 2 minute periods. Tissue bathed in intervals in Tyrode solution of pH 7.8.

Effects of repeated application of solution of pH 2.5 or 9 on fundus : In four experiments the stimulant action produced by acids on fundal strip was further studied by repeatedly exposing it to pH 2.5. Fig. 2 shows the record from one such experiment. Fundus markedly contracted during the 2 minute period of exposure to pH 2.5 at the first application and returned to the base line in about 20 minutes. Subsequent applications of this pH resulted each time in a progressively reduced height of contraction and a quicker return to base line. Though the tissue was bathed in Tyrode solution during the intervals it gradually tended to lose tone as shown by the decline in base line. In three experiments the stimulant action of alkaline solution of pH 9 was similarly studied. Even here the height of contraction and the time taken to relax diminished with each subsequent application.

Effect on isolated tissues from rat alimentary tract, of exposure for 10 minute periods to solutions of progressively lower pH

Since fundus appeared to regain tone towards the end of the three minute period of exposure to acids in the previous group of experiments, and to obviate the effect of sudden change from pH of Tyrode to the pH of the test solution, the following procedure was adopted in the rest of the experiments described here. After taking records with the tissues in Tyrode solution, the bath was emptied and refilled with a solution of pH 9. Thereafter, the solution in the bath was replaced at the end of each 10 minute period by a solution of next lower pH i. e. 8, 7, 6, 5, 4, 3.5, 3, 2.5, 2 and 1.5 in that order. Lastly the solution of pH 1.5 was replaced by Tyrode solution once more. In each of these experiments, strips of fundus were compared simultaneously with either pylorus or one other part of the intestines In a few ileum was compared with other parts of intestines. Table I summarises the effects of gradual change of pH from 9 to 1.5 produced on different tissues. The results in detail are as follows.

TABLE 1

Effect of change of pH every 10 minutes on the tone of isolated tissues from alimentary tract

pH	9	8	7	6	5	4	3.5	3	2.5	2	1.5
Fundus	++	+	0	∓	∓	—	++	+	+	+	+
Pylorus	+	0	0	0	—	—	—	+	+	+	+
Duodenum	+	0	0	∓	∓	∓	—	—	—	—	+
Ileum	+	0	0	∓	—	—	—	—	—	—	—
Colon	++	+	0	∓	—	—	—	+	+	+	+

\+ contraction
— relaxation
∓ relaxation followed by regain of tone
0 no change

Effect on fundus : Mere replacement of the Tyrode solution of the bath by washing and refilling, produced immediately a slight loss of tone which was gradually regained as shown at *W* at the beginning of the experiment of fig. 6. Vane (3) observed that rat fundal strip gains tone in the bath fluid probably due to accumulation of metabolites, and that this tone is lost on washing. The changes in tone to be described hereafter on application of solutions of different pH, are different from these slight changes obtained on mere washing.

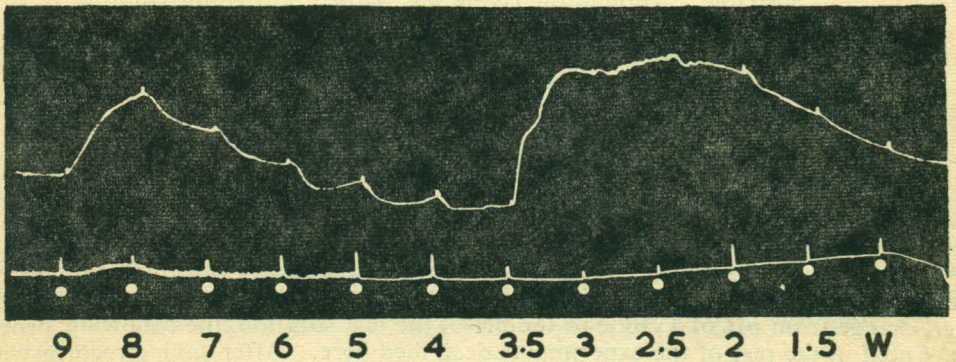

Fig. 3. Records from isolated strips of rat fundus (top) and pylorus (bottom). At the first white dot, the Tyrode solution of bath is replaced with solution of pH 9. Subsequently every 10 minutes, at the white dots, the solution in the bath is replaced with solution of next lower pH noted on the abscissa, and at *W* with Tyrode. Tprode pH 7.8.

In all 26 experiments, the tone of the fundal strip increased when the pH of the bathing solution was changed to 9. This is shown in fig. 3. As the bathing solution was changed step by step to one of lower pH every 10 minutes, the tissue gradually relaxed

back to the starting level with pH 8 or 7. Subsequent change to pH 6, 5 and 4 produced immediately on change, a rapid relaxation. But small spontaneous movements of the fundus reappeared soon and the tissue tended to regain tone Out of 23 experiments where pH 3.5 was used, fundus showed, in 16 slight relaxation which was immediately followed by rapid increase in tone, in 4 only contraction, and in another 3 only relaxation. When the bathing fluid was changed from one of pH 3.5 to 3, fundus always contracted over and above that already produced at pH 3.5. At pH 2.5 further increase in tone was produced with pH 2 this tone was either maintained or slightly lowered and all spontaneous movements ceased. With pH 1.5, fundus lost tone slowly and showed no spontaneous movements. Replacement of this highly acid fluid with Tyrode solution produced further loss of tone, but the tissue appeared to be dead as it did not recover spontaneous activity even after half to one hour.

Effects on pylorus : Seven spiral and nine longitudinal pyloric strips were studied. No significant difference was noticed whether the strips were taken spirally or longitudinally. Pylorus exhibited small contractions in Tyrode solution. As the pH of the bathing fluid was changed step by step into acid side, these contractions disappeared immediately on each such change, but reappeared after 5 or 6 minutes (fig. 3). Between pH 5 and 3.5 they were completely inhibited. The tone of pylorus is slightly increased in alkaline pH 9. Gradual relaxation followed as the pH is lowered from 9 to 3. The tone slightly increased with pH 2.5 and 2. At pH 1.5, the tone built up at pH 2.5 and 2 was lost in 11 experiments, but showed further augmentation in 5 experiments. Neither the relaxation nor the contraction of the pylorus was as great as that of the fundal strip. Further, in experiments where fundal and pyloric strips were studied simultaneously, pylorus always contracted at a pH lower than that at which the fundal strip started contracting.

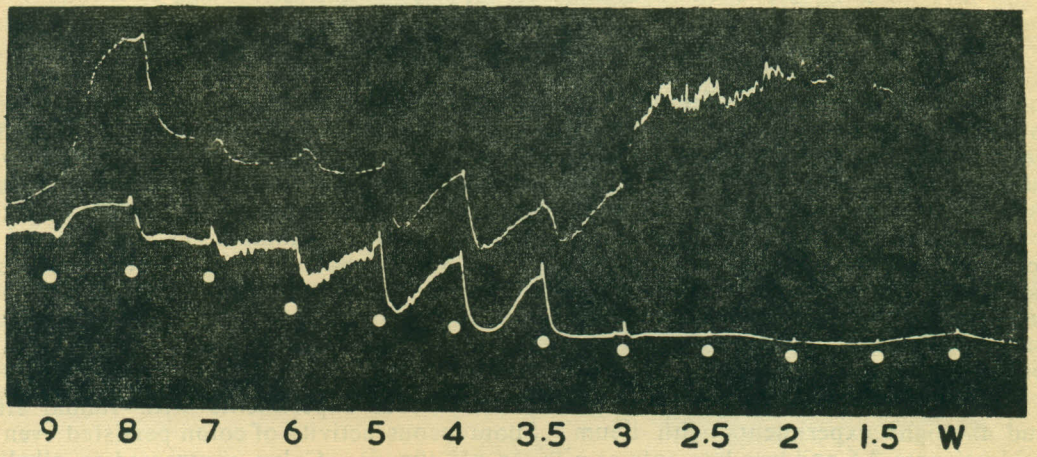

Fig. 4. Records from isolated rat fundal strip (top) and duodenum (bottom). At the first white dot Tyrode solution of the bath is replaced with solution of pH 9. Subsequently every 10 minutes, at the white dots, the solution in the bath is replaced with a solution of next lower pH noted on the abscissa and at *W* with Tyrode. Tyrode pH 7.5.

Effects on duodenum : Duodenum was compared with either the fundal strip or a bit of ileum in 14 experiments. Fig. 4 shows the effects of gradual change in pH on duodenum in the lower trace. Its spontaneous activity disappeared as the pH was lowered below 5. Duodenum also showed an increase in tone with pH 9 and a gradual relaxation at each lowering of pH. The tissue however regained tone partially upto pH 4. From pH 3.5 downwards it showed a complete loss of tone followed again by a small increase at pH 1.5. Duodenum differed from pylorus and fundus in that it did not show marked increase in tone in pH range below 4. It differed from pylorus in that its changes in tone were of much greater magnitude at each pH.

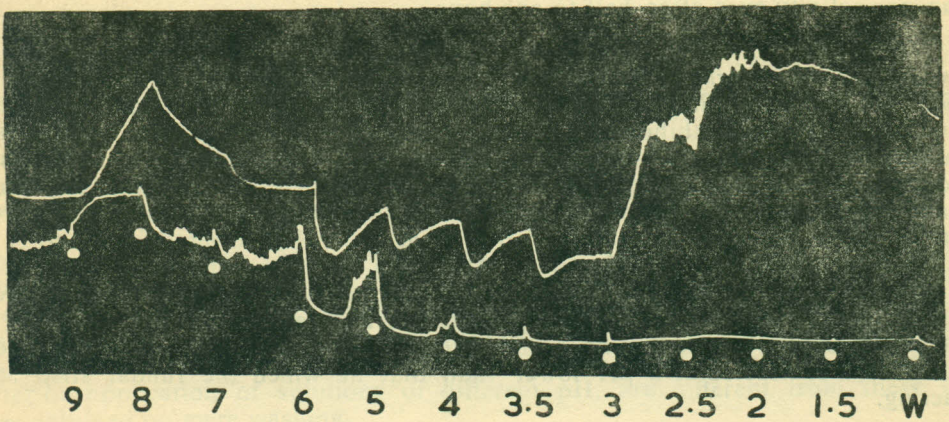

Fig. 5. Records from isolated rat fundal strip (top) and ileum (bottom). At the first white dot the Tyrode solution of the bath is replaced with solution of pH 9. Subsequently every 10 minutes, at the white dots, the solution in the bath is replaced with a solution of next lower pH noted on the abscissa and at *W* with Tyrode. Tyrode pH 7.6.

Effects on ileum : Ileum was compared, in three experiments with fundal strip, in ten experiments with duodenum and in eight with colon. Ileum showed peristalsis till the pH was lowered to 5, and thereafter it lost its spontaneous activity. It showed an increase in tone in alkaline pH 9 and gradually relaxed as the pH was lowered (fig. 5). It relaxed steeply on exposure to pH 6, but soon regained its tone. At pH 5, in ten experiments it lost tone and failed to regain it, while in another 11 experiments it regained the lost tone partially. At pH 4 or 3.5 in most experiments it relaxed and failed to regain tone. Below pH 3.5 it remained relaxed and even the slight gain of tone observed at pH 1.5 with duodenum was generally absent. It tends to relax completely at a pH slightly higher than that at which duodenum relaxes completely.

Effects on colon : Colon was compared in four experiments with fundal strip and in eight experiments with ileum. Spontaneous activity of colon persisted even in acid pH upto 3.5 and was lost only at a lower pH (fig. 6). Colon contracted in alkaline pH 9 and gradually relaxed as the pH was lowered. It relaxed and partly regained tone upto pH 5 and failed to do so at pH 4. At pH below 3.5, colon showed an increase in tone unlike ileum which showed only relaxation at this pH.

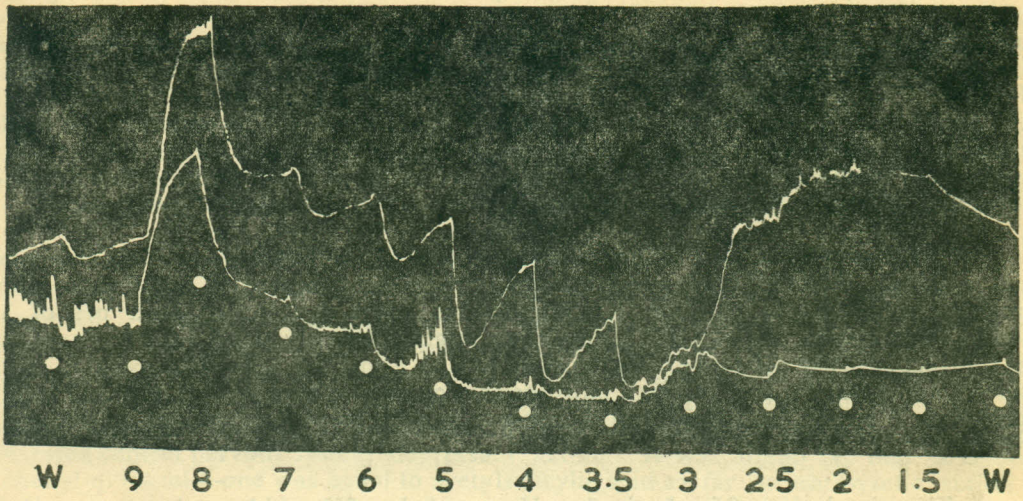

Fig. 6. Records from isolated rat fundal strip (top) and colon (bottom). At *W* the bath is emptied and refilled with Tyrode solution. At the second white dot the Tyrode solution in the bath is replaced with solution of pH 9. Subsequently every 10 minutes the solution in the bath is replaced with a solution of next lower pH noted on the abscissa. Tyrode pH 7.7.

Effects of acidification with sulphuric acid. In four experiments sulphuric acid was used for acidification and the responses of fundus and pylorus to changes in pH did not differ from those obtained when hydrochloric acid was used.

Effects on fundus and pylorus taken from reserpine treated rats. In 12 experiments fundus or pylorus taken from rats previously treated with reserpine for two days to deplete the stores of 5HT did not show any significant difference in their response to changes in pH from 9 to 1.5, compared with tissues of untreated animals.

Effects of atropine, mepyramine and LSD-25. In three experiments atropine and mepyramine were added to the bathing fluid throughout the experiment. The presence of atropine in concentration of 0.1 μg/ml or mepyramine of 0.1 μg/ml, or the two together in concentrations of 1μg/ml did not affect either the spontaneous activity of the fundal and pyloric strips or their responses to gradual change of pH from 9 to 1.5.

The effect of addition of LSD-25 to the bathing fluid was studied similarly in five experiments. The presence of LSD-25 in concentrations of 0.01 μg/ml or even 2μg/ml in the bathing fluid added 90 minutes before, did not affect the response of the fundus to change in pH. LSD-25 in concentration of 2 μg/ml raised the basal tone of the fundus slightly.

DISCUSSION

Gorman *et al* (2) record that stomach strips are inhibited on addition of acids to the bath. However no correlation has been established between the pH attained in the

bath and the response of the tissue. Generally buffer solutions are used for bathing isolated tissues. Addition of strong acids directly to the bath would give rise to variable response from the tissue, as it would take a little time before the pH becomes uniform throughout the bath. Besides, while large quantities of acids are necessary to lower the pH from 7.5 to 5, or from 3 to 1.5, very small amounts of acid are enough to change the pH from 5 to 3, which appears to be the critical pH where the responses of the fundus change from maximum relaxation to increase in tonus. Our experiments show that the responses of fundus depend more on the actual pH obtained in the bathing fluid, than on the amount of acid added to bath.

There appears to be a sequence in the response of different parts of rat alimentary tract to acids. While all of them relax, some of the tissues showed in addition a stimulant effect in highly acid solutions. While fundus starts contracting at a pH of 3.5, pylorus starts contracting at pH 2.5, duodenum contracts feebly at pH 1.5 and ileum does not contract at lower pH range. Colon contracts at a pH of 3.5. Normally it is the stomach and proximal part of small intestine that come into contact with acid pH and the significance if any of this differential response to acid in the intact animal is yet to be investigated.

The stimulation of fundus in the pH range below 3.5 could not be due to release of an active chemical substance like 5HT, acetylcholine or histamine from the tissue, since the presence of their antagonists in the bath did not make any change in the response of the fundus to changes in pH. The presence of 5 HT, or its depletion by previous treatment with reserpine also did not influence the response of fundus and pylorus to acids. The changes are probably due only to the H ion concentration because identical results were obtained with sulphuric acid or hydrochloric acid.

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