Effect of Intraluminal Pressure on Enterochromaffin Cells in the Rat Duodenum.* (26532)

JACK W. COLE, JOSEPH SCHNEIDER AND ANNE MCKALEN
Department of Surgery, Western Reserve University School of Medicine and the University Hospitals, Cleveland, Ohio

It is generally agreed that, in mammals, the mucosa of the gastrointestinal tract is a major site of 5-hydroxytryptamine (5-HT) production(1,2,3). Furthermore, there is considerable experimental evidence supporting the hypothesis that the enterochromaffin cells (E.C. cells) within the mucosa are the principal, if not the sole source, of this amine (4,5,6). The pharmacological activity of 5HT has been well described in a wide variety of organs and tissues, in both man and animals, but as yet its physiological role has not been elucidated.

Bülbring and Crema(7) have shown that 5HT may be important in the peristaltic activity of the intestinal tract. Their studies in guinea pigs indicate that 5HT is released from the intestinal mucosa in response to increased intraluminal pressures. They suggest that the enterochromaffin cells might be neurosecretory cells in which "deformation leads to the release of 5HT."

The present study was undertaken to determine if histochemical alterations occur in the enterochromaffin cells of the duodenal mucosa of rats in response to increased intraluminal pressures.

Methods. White Carworth rats (200-400 g) were anesthetized with intraperitoneal 2% sodium pentobarbital (0.2 cc/100 g). The abdominal cavity was opened and a segment of duodenum, 3-4 cm in length was isolated by transection. The mesentery of the isolated segment remained intact. The proximal and distal ends of the segment were carefully cannulated with small glass cannulae. The distal cannula was connected to a water manometer to record the intraluminal pressure in the isolated bowel. The proximal cannula was connected to a sphygmomanometer bulb to permit introduction of air into the bowel and to maintain the desired luminal pressure. The isolated duodenal segment was then subjected to a predetermined pressure (20-80 mm H_2O) by insufflation of air and the pressure maintained for varying intervals of time (10-36 minutes). Portions of the experimental segment of duodenum were then quickly excised and placed immediately in 10% formalin. Control tissue was obtained from duodenum adjacent to the isolated segment. Proximal and distal sections of the duodenum were used alternately as experimental and control tissues, to compensate for possible differences in the enterochromaffin cell population at different levels of the duodenum. A total of 10 rats was used.

Following fixation, the experimental and control tissues were dehydrated with an ascending series of alcohols, cleared in benzene, infiltered and embedded in paraffin. Sections of experimental and control tissues were cut at 4 μ and mounted on the same slide. These sections were deparaffinized with xylene, and hydrated with a descending series of alcohols to water. The Gomori-Burtner methenamine silver method(8) was used for identification of the argentaffin cells.

Five millimeter lengths of the stained tissue were marked off with India ink. The enterochromaffin cells within the 5 mm lengths of tissue were then counted by independent observers and recorded. All slides were coded until the E. C. counting was completed.

Results. The results shown in Table I indicate a significant decrease in number of recognizable enterochromaffin cells in the mucosa subjected to increased intraluminal pressure as compared to controls ($P<0.0001$). When pressures of 80 mm of H_2O were maintained for longer than 20 minutes the resultant edema and ecchymosis rendered the tissue unsuitable for study. Tissue sections analyzed in this report demonstrated satisfactory preservation of histological detail.

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TABLE I. Enterochromaffin Cell Counts in 5 mm Duodenal Mucosa at Varying Pressures and Times.

No. of exp.	Pressure, mm of H_2O	Time, min.	Enterochromaffin cell counts/5 mm mucosa		
6 Control	—	20	13	11	12
Exp.	20	20	7	6	5
12 Control	—	20	13	9	10
Exp.	20	20	7	4	5
9 Control	—	25	10	11	10
Exp.	20	25	5	5	4
13 Control	—	25	9	6	7
Exp.	20	25	9	6	6
4 Control	—	30	11	9	10
Exp.	40	30	6	5	5
5 Control	—	30	11	9	10
Exp.	40	30	7	5	6
10 Control	—	30	11	11	13
Exp.	40	30	5	5	5
11 Control	—	30	15	11	11
Exp.	40	30	7	5	6
7 Control	—	10	11	9	8
Exp.	80	10	9	6	5
8 Control	—	20	7	5	4
Exp.	80	20	4	4	3

There was no apparent correlation between amount of pressure and/or length of time the pressure was maintained and extent to which the E.C. cell counts were reduced.

Discussion. It is concluded from these observations that there is a diminution in number of recognizable E.C. cells in the duodenal mucosa of rats following increased intraluminal pressure. These findings provide histochemical support for the hypothesis that pressure is one mechanism for mediating the release of 5HT from intestinal mucosa.

A reduction in actual number of E.C. cells per unit tissue is not inferred from these findings. They simply indicate that pressure upon the mucosa has resulted in a release of the argentaffin positive material (presumably 5HT) from E.C. cells, making recognition impossible by the histochemical technic employed.

It may be significant that within the range of pressures used, the mucosa was never found completely devoid of E.C. cells, suggesting that after an initial period of accelerated release, rate of formation of 5HT and its release were in equilibrium. It is noteworthy, that in guinea pigs, intraluminal pressures of 20-40 mm of H_2O maintained for 3 hours, did not deplete 5HT content of the mucosa(9).

Additional histological evidence supporting the concept that pressure and peristalsis may be related to release of 5HT by the E.C. cells may be found in Pearse's(10) observations in intestinal tuberculosis and Crohns disease. In cases where the bowel had become rigid he observed an increase in number of argentaffin cells with an increase in size and number of granules they contained. In our study, for lack of suitable methods of quantitation no attempt was made to document the degrees of granulation in the various cells counted. Our impression was, however, that E.C. cells in the experimental groups averaged fewer cytoplasmic granules than E.C. cells studied in the control sections.

Summary. The number of recognizable enterochromaffin cells in the mucosa of the rat duodenum is significantly reduced in response to increased intraluminal pressure. It is suggested that these findings offer histochemical corroboration for the concept that pressure is one mechanism mediating release of 5-hydroxytryptamine from intestinal mucosa.

1. Erspamer, V., *Pharmacol. Rev.*, 1954, v6, 425.
2. Bertaccini, G., *J. Physiol.*, 1960, v153, 239.
3. Feldberg, W., Toh, C. C., *ibid.*, 1953, v119, 352.
4. Benditt, E. P., Wong, R., *Proc. Inst. Med. Chicago*, 1957, v21, 279.
5. Barter, R., Pearse, A. G. E., *J. Path. Bact.*, 1955, v69, 25.
6. Shepherd, D. M., West, G. B., Erspamer, V., *Nature*, 1953, v172, 357.
7. Bülbring, E., Crema, A., *J. Physiol.*, 1959, v146, 18.
8. Burtner, H. J., Lillie, R. D., *Stain Techn.*, 1949, v24, 225.
9. Bülbring, E., Lin, R. C. Y., *J. Physiol.*, 1958, v140, 381.
10. Pearse, A. G. E., 5-hydroxytryptamine, Edited Lewis, Pergamon Press, London, 1958, p158.

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