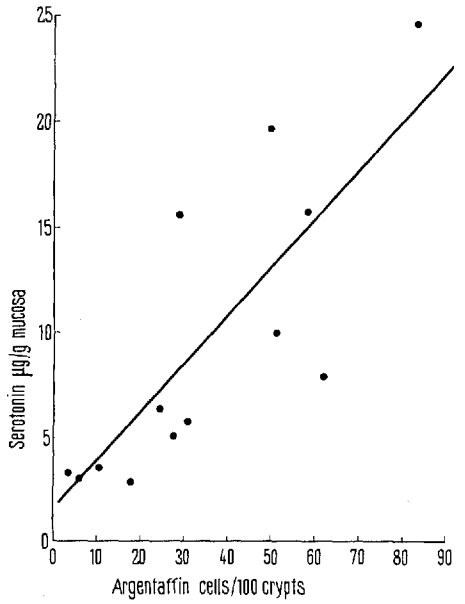

Fig. 2. Regression line demonstrating the relationship between the mean serotonin concentration (µg/g mucosa), and the number of argentaffin cells throughout the gastrointestinal tract of the normal rat. t, 4.446; $P < 0.001$; r, + 0.80.

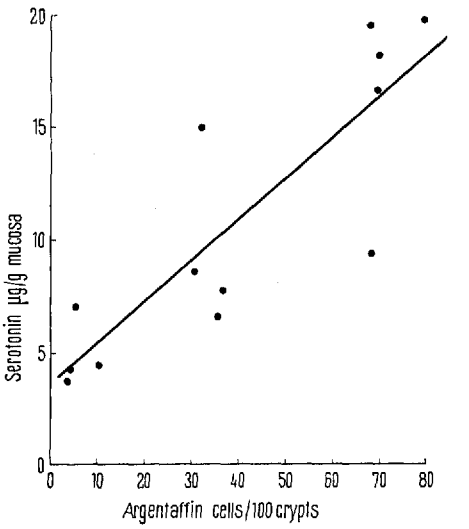

Fig. 3. Regression line demonstrating the relationship between the mean serotonin concentration (µg/g mucosa), and the number of argentaffin cells throughout the gastrointestinal tract of the sulfamerazine pretreated rat. t, 5.335; $P < 0.001$; r, + 0.85.

groups are indicated in the Table. Mean values and the range are presented because of the small number of rats in each group.

The relationships between the mean AG cell counts, and the fluorometrically determined serotonin values for each of the tissues are indicated in Figures 2 and 3 for the untreated, and the sulfamerazine pretreated rats respectively. For the untreated control rats the plot has a correlation coefficient $r = +0.80$, which is statistically significant ($t = 4.466$, d.f. II, $P < 0.001$), and in the sulfamerazine treated rats the curve has a correlation coefficient $r = +0.85$, which is statistically significant ($t = 5.335$, d.f. II, $P < 0.001$). These data are in keeping with the suggestion that serotonin is the material stored within the AG cell granules. In comparing the slopes of the 2 lines, there was no significant difference between them.

From this report there is some suggestive evidence that the increased serotonin concentrations reported in some intestinal areas following sulfamerazine[9] could be due to a higher level of serotonin/cell, and a larger population of visable AG cells[14].

Zusammenfassung. Eine direkte Korrelation zwischen Serotoninspiegel und der Zahl der argentaffinen Zellen im gastro-intestinalen Trakt normaler Ratten und solcher, die nach Sulfamerazinbehandlung einen höheren Serotoninspiegel aufweisen, wird festgestellt.

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[14] This research was supported by research grants from the National Science Foundation, (No. GB 6105), and from the American Medical Association Education and Research Foundation awarded to J. H. THOMPSON. Dr. M. A. VERITY kindly prepared the slides.

The Effect of Reserpine upon Gastrointestinal Serotonin in the Sprague-Dawley Rat

It is well known that reserpine causes a release of serotonin from the gastrointestinal tract[1-4], however, there are certain discrepancies in the literature concerning its degree. In the present study, the effects of reserpine were observed on 14 areas of the rat gastrointestinal tract to determine the degree of response to reserpine from one anatomical area to another.

Male and female Sprague-Dawley rats from the Charles River Laboratories (breeding shed 1), ranging in weight from 200–300 g were maintained on normal Purina rat chow, with a tryptophan content of 0.22%. The rats were housed in colony cages and exposed to a regular 24 h light/dark cycle (light: 05.00–19.00). In order to avoid any possible circadian influences on bowel serotonin, the rats were always killed between 08.00 and 09.00 on the day of assay by decapitation. The tissues sampled are indicated in Figure 3. In the experiments on the dose and time response to reserpine, only upper jejunal samples were taken. Upper jejunum was selected because of the ease of removal, the availability of duplicate samples,

[1] A. PLETSCHER, P. A. SHORE and B. B. BRODIE, Science *122*, 374 (1955).
[2] V. ERSPAMER, Experientia *12*, 63 (1956).
[3] R. K. SANYAL and G. B. WEST, J. Physiol. Lond. *144*, 525 (1958).
[4] K. S. KIM and P. A. SHORE, J. Pharmac. exp. Ther. *141*, 321 (1963).

and the narrow range in variability between one segment to the next. Serotonin was assayed by the method of BOGDANSKI, PLETSCHER, BRODIE and UDENFRIEND[5], and the results expressed as μg/g mucosa, wet weight. Exact details of our experimental methods and their efficacy, have been published previously[6].

Injections: the rats were randomly divided into 2 groups, and injected i.p. with either reserpine (Serpasil®, Ciba Pharmaceuticals Ltd.) or normal saline. Reserpine was injected in doses ranging from 0.5–20.0 mg/kg (see individual experiments) while all doses of saline were 2.0 ml/kg. Fasting has been shown to elevate bowel mucosal serotonin levels[7], and as rats treated with reserpine fail to eat or drink due to sedation, the saline injected control animals were isolated from both food and water following injection.

Jejunal mucosal serotonin levels following reserpine as a function of time. The dose of reserpine most commonly used to produce 'Maximal' serotonin depletion in rabbit brain[8,9], rabbit bowel[1], guinea-pig duodenum[10], and rat bowel[2,3], has been 5.0 mg/kg. Using this dosage, the concentration of jejunal mucosal serotonin as a function of time is indicated in Figure 1. Maximal depletion is seen to develop at 4 h in both sexes reaching values of 59% and 58% depletion respectively in male and female animals. PLETSCHER et al.[1] originally demonstrated in the rabbit small bowel, that maximal serotonin depletion (75% approximately), developed 16 h following reserpine, 5.0 mg/kg. Thus, not only the degree, but also the time of onset of maximal serotonin depletion differs between rabbits and rats. However, most of the experiments in rats concerning the effects of reserpine upon serotonin bowel stores[2,3,11] and/or function[4], have been performed at 16–24 h after drug injections: a time period (presumably based on the original work of PLETSCHER et al.[1] in rabbits) when amine levels are 67% (male), and 70% (female), of control levels (Figure 1). Thus, SANYAL and WEST[3] asserted that reserpine depleted rat bowel serotonin, but these authors do not give any actual tissue levels, nor do they indicate the time lapse involved. ERSPAMER[2] noted a 30% amine depletion at 16 h, which is similar to that reported here, while MORAN and WESTERHOLM[11] found reserpine to be without effect at 24 h.

The small but definite increase in serotonin levels seen in both sexes 30 min after reserpine is not significantly different from values at zero time and is probably an artifact associated with differing degrees of reserpine absorption. Certainly low concentrations of reserpine strongly inhibit the spontaneous release of catecholamines from isolated chromaffin cell granules[12], and increase rabbit stomach fundal serotonin[13].

Jejunal mucosal serotonin concentration as a function of reserpine dose. The jejunal mucosal serotonin concentrations 4 h following graded doses of reserpine are indicated in Figure 2. As there was no difference between the male and female values the results were pooled. Maximal serotonin depletion developed with 2.5–5.0 mg/kg, a dose similar to that reported for rabbit bowel[1].

The topographical distribution of serotonin throughout the gastrointestinal tract following reserpine. The topographical distribution of serotonin throughout the gastrointestinal tract 4 and 16 h following reserpine 5.0 mg/kg is indicated in Figures 3 and 4. In each case the drug treated animals are compared to saline injected, fasted control rats. At both time periods, significant serotonin depletion is apparent in all tissues assayed with the exception of the stomach fundus and body at 4 h. The values obtained for the 16 h fasted animals are similar to those already published[7], and indicate the importance of fasting the saline injected control rats. If the data from Figures 3 and 4 are regrouped as % depletion of the control values (Table), the bowel tissues can be divided anatomically into 3 regions. Depletion is greater in the stomach and colon (with the exception of the transverse colon), at 16 h, while in the small bowel and appendix maximal depletion develops at 4 h. Presumably this dissociated effect between the stomach and colon on the one hand, and the small bowel on the other, depends upon differing rates of serotonin synthesis after reserpine induced depletion. Certainly, the half lives of serotonin in the stomach (17 h), and intestines (11 h), of rabbits are different[14].

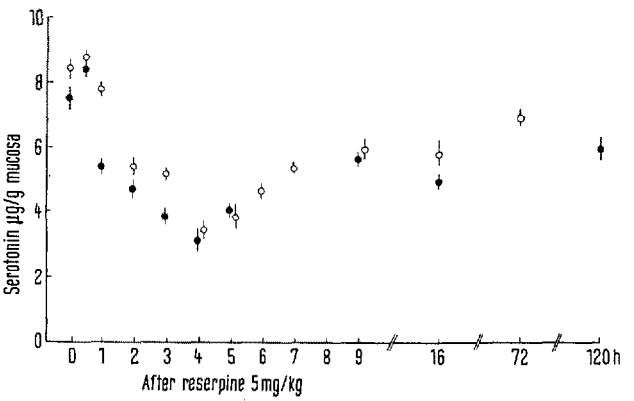

Fig. 1. Upper jejunal serotonin concentration in μg/g mucosa in male (●), and female (o) rats following reserpine 5 mg/kg i.p. Each point represents the mean ± 1 S.E. for 10–15 animals.

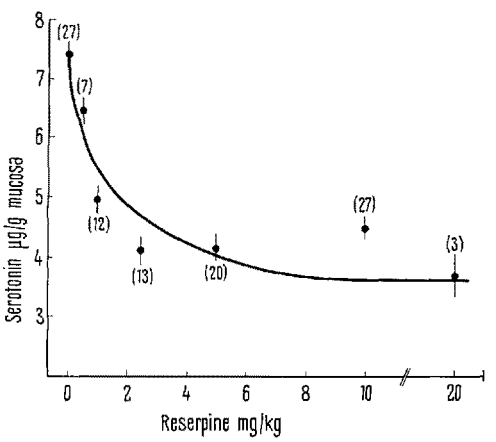

Fig. 2. Upper jejunal serotonin concentration in μg/g mucosa in male and female rats at 4 h following graded reserpine dosage. Values are represented as mean ± 1 S.E. The number of rats in each group is indicated in brackets.

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[6] J. H. THOMPSON, Ir. J. med. Sci. *490*, 411 (1966).
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[13] A. PLETSCHER, in *5-Hydroxytryptamine* (Ed. G. P. LEWIS; Pergamon Press Inc. 1958), p. 84.
[14] S. UDENFRIEND and H. WEISSBACH, Proc. Soc. exp. Biol. Med. *97*, 748 (1958).

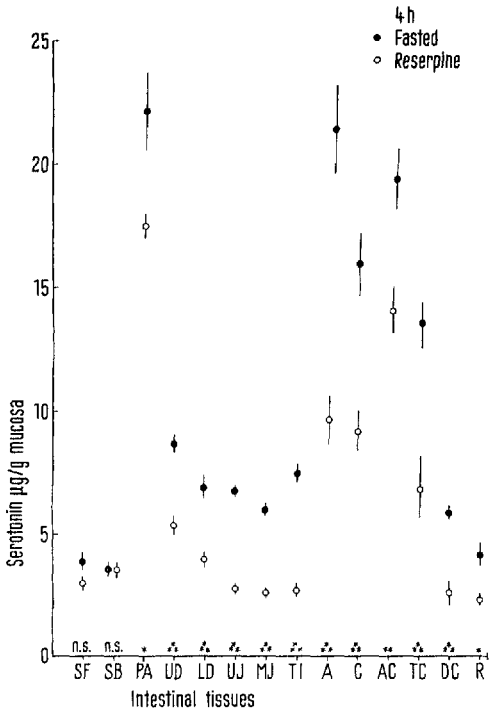

Fig. 3. Mean values ± 1 S.E. for stomach fundus (SF), stomach body (SB), pyloric antrum (PA), upper and lower duodenum (UD, LD), upper and mid jejunum (UJ, MJ), terminal ileum (TI), appendix (A), cecum (C), ascending, transverse and descending colon (AC, TC, DC), and proximal rectum (R), serotonin 4 h following reserpine, or saline injections in male and female Sprague-Dawley rats. Each point is the mean of 15–20 animals. *P* values are indicated: NS, non-significant; $\overset{x}{xx}$, < 0.001; xx, < 0.01; x, < 0.05.

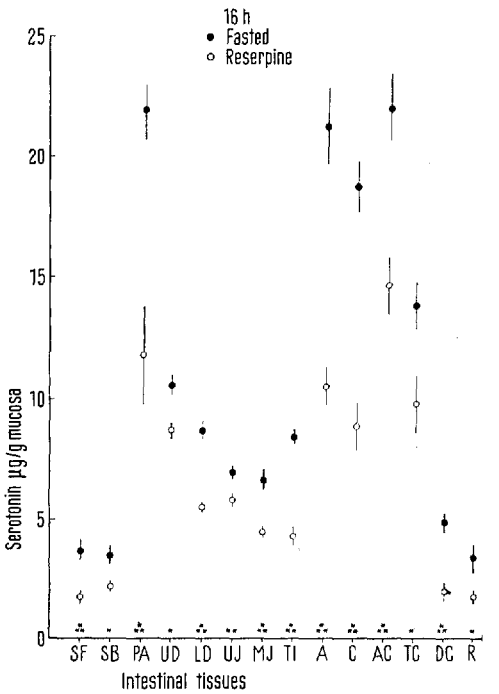

Fig. 4. Mean values ± 1 S.E. for stomach fundus (SF), stomach body (SB), pyloric antrum (PA), upper and lower duodenum (UD, LD), upper and mid jejunum (UJ, MJ), terminal ileum (TI), appendix (A), cecum (C), ascending, transverse and descending colon (AC, TC, DC), and proximal rectum (R), serotonin 16 h following reserpine, or saline injections in male and female Sprague-Dawley rats. Each point is the mean of 15–20 animals. *P* values are indicated: $\overset{x}{xx}$, < 0.001; xx, < 0.005; x, < 0.01.

The results presented in this paper demonstrate that reserpine is a potent depletor of bowel serotonin in the rat, which is in agreement with ERSPAMER[2], and SANYAL and WEST[3]. The failure of some investigators to demonstrate a depletory effect of reserpine on bowel amine levels is almost certainly due to the failure to assay the serotonin at the time of maximal depletion. There are several interesting differences between the degree and rate of serotonin depletion and recovery in the bowel and brain. After reserpine, about 90% of the brain serotonin disappears[8], while only 60% disappears from the bowel (Figure 1). Moreover, brain serotonin declines rapidly (about 75% depleting within 30 min), and after maximal depletion is reached at 4 h, recovery is slow[9], while in the bowel a sharp peak of depletion is evident (Figure 1), and recovery is more rapid. Presumably, the failure to deplete bowel serotonin more than 60% is due to the fact that a greater percentage of the amine is bound. No data is available at present on this point for the rat. However, GAL, DREWES and BARRACLOUGH[15] could only produce a 60% depletion of bowel serotonin in rats by combining a tryptophan deficient diet and reserpine[16].

Percentage depletion of mucosal serotonin following 5 mg/kg reserpine compared to saline injected control rats at 4 and 16 h post injection. Data has been regrouped from Figures 3 and 4.

Tissue	4 h	16 h	Depletion time (h)
Stomach fundus	22	54	16
Stomach body	0	37	16
Pyloric antrum	25	46	16
Upper duodenum	38	18	4
Lower duodenum	42	37	4
Upper jejunum	59	17	4
Mid jejunum	57	32	4
Terminal ileum	65	49	4
Appendix	55	51	4
Cecum	43	53	16
Ascending colon	27	36	16
Transverse colon	49	32	4
Descending colon	54	60	16
Rectum	44	58	16

Zusammenfassung. Der Serotoningehalt der Schleimhaut von 14 gastrointestinalen Geweben von Sprague-Dawley Rattenmännchen und -weibchen wurde nach i.p. Injektion von Reserpin spektrophotofluorimetrisch gemessen. Die Verteilung von Serotonin im Magendarmkanal 4 und 16 h nach der Reserpininjektion zeigte, dass Magengewebe und Kolongewebe nach 16 h mehr entleert waren als nach 4 h, während Dünndarm und Appendix nach 4 h mehr entleert waren als nach 16 h.

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[16] This research was supported by U.S.P.H.S. grant No. AM 07909, to J. H. THOMPSON.