

### The subcellular distribution of serotonin in the gastrointestinal tract of the immunosympathectomized mouse

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WE HAVE previously reported increased serotonin levels in some areas of the gastrointestinal tract of the immunosympathectomized mouse.<sup>1</sup> These elevated serotonin levels appear to be the result of an increased concentration of serotonin per argentaffin cell rather than of a larger population of cells.<sup>2</sup> The present study was undertaken to determine whether the increased serotonin levels after immunosympathectomy were due to an increase in the bound amine, in the free amine, or possibly in both.

White Swiss-Webster mice, obtained from the randomly bred, closed colony at UCLA, were divided into four groups of mixed sexes: anti-nerve growth factor injected group,  $\gamma$ -globulin injected controls, handled controls, and unhandled controls. The first group was injected s.c. in the first 6 days of life with 0.02 ml anti-nerve growth factor (anti-NGF)\* serum/g body wt.; the second group received 0.02 ml  $\gamma$ -globulin† per g body weight instead of the anti-NGF serum; the third group was handled and weighed but not injected, and the fourth was left unhandled, apart from cage cleaning, until the time of assay. All assays were performed on mice between 330 and 370 days of age, the mice being maintained singly in mouse pans and fed commercial Purina rat chow with a tryptophan content of 0.22 per cent.

The mice were decapitated between 9.00 and 10.00 a.m. on the day of assay, and after decapitation all procedures were performed at 1° in a refrigerated laboratory. The first 6 in. of small bowel, and the large bowel from 1 in. distal to the ileocecal valve to the proximal rectum, were rapidly excised and immediately immersed in cold 0.25 M sucrose,  $2 \times 10^{-3}$  M EDTA, and  $2 \times 10^{-3}$  M pargyline hydrochloride (Eutonyl; Abbott Laboratories, Chicago). The bowel sections were opened longitudinally, cleaned, and blotted dry, and the mucosa separated from the underlying tissues by scraping.<sup>3</sup> The mucosal samples were weighed, then homogenized on ice in one of two ground-glass homogenizers (one for small bowel mucosa and the second for large bowel samples). Care was taken to standardize the procedure and eliminate possible sources of error.<sup>4,5</sup> The homogenate was divided into two equal aliquots; one was assayed for its total serotonin content and the other was ultracentrifuged at 100,000 g in a Spinco refrigerated ultracentrifuge for 25 min to separate the bound and free amine. All samples, plus standard serotonin solutions and blanks, were extracted by the method of Bogdanski *et al.*<sup>6</sup> and the serotonin concentration was determined spectrophotofluorometrically as previously described.<sup>3</sup>

Results are indicated in Table 1. Since there was no statistically significant difference between the values obtained for the three control groups, their data were pooled. An "ultracentrifuged recovery" was determined for each mouse by comparing the serotonin concentrations of the total homogenate with the sum of the particulate and supernatant amine levels. In all instances this ranged between 95 and 98 per cent. The concentration of serotonin in both the small and large bowel samples was significantly elevated in the immunosympathectomized mice compared to the control animals, the difference being greater for the small bowel ( $P < 0.001$ ) than for the colon ( $P < 0.05$ ). There was a significant increase in the particulate-bound amine in both the small intestine ( $P < 0.005$ ) and the large intestine ( $P < 0.05$ ) of the immunosympathectomized mice compared to the controls; the supernatant fractions remained unchanged.

It is not known why there is about 10 per cent less particulate serotonin in the large bowel compared to the small intestine. However, this may reflect a basic tissue difference, a differential effect of the anti-NGF serum,<sup>7,8</sup> or an inequality of homogenization since the colonic mucosa is less easily homogenized.

The data presented suggest that the increased serotonin levels observed in the gastrointestinal mucosa of the immunosympathectomized mice are due to an increase in the particulate-bound amine.

\* Abbott Laboratories, Chicago, Ill. This antiserum has a potency of 22,000 antiunits/ml, based on an assay preparation of sensory ganglia from 8-day-old chick embryos.

† Purified bovine  $\gamma$ -globulin containing 17.6% protein diluted in normal saline.

TABLE 1. TOTAL CONCENTRATION AND SUBCELLULAR DISTRIBUTION OF SEROTONIN IN THE MUCOSA OF THE INTESTINE OF THE MOUSE\*

	N	Total	Particulate	Supernatant	Per cent particulate
Small intestine					
IMS	6	16.82 $\pm$ 0.87	14.82 $\pm$ 0.80	1.07 $\pm$ 0.14	93
Controls	11	13.36 $\pm$ 0.41	11.55 $\pm$ 0.55	1.14 $\pm$ 0.24	91
P value		< 0.001	< 0.005	N.S.†	
Large intestine					
IMS	7	70.16 $\pm$ 1.45	56.83 $\pm$ 1.65	12.07 $\pm$ 1.41	82
Controls	13	57.30 $\pm$ 3.92	45.30 $\pm$ 3.39	10.76 $\pm$ 1.08	81
P value		< 0.05	< 0.05	N.S.†	

\* Data are presented as mean values  $\pm$  1 S.E.

† Not significant.

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