Bowel 5-hydroxytryptamine levels in the immunosympathectomised mouse

SIR,—Mammals treated at birth with the antiserum to Levi-Montalcini's nerve growth factor (NGF) show an almost complete absence of peripheral sympathetic nerves in organs supplied by the thoracic ganglia (Cohen, 1960; Levi-Montalcini & Booker, 1960; Levi-Montalcini & Angeletti, 1962; Levi-Montalcini, 1964; Vogt, 1964; Zaimis, Berk & Callingham, 1965). Levi-Montalcini & Angeletti (1962) demonstrated that in such immunosympathectomised animals, the noradrenaline content was markedly decreased in various tissues. Subsequently, Hamberger, Levi-Montalcini, Norberg & Sjöqvist (1965), using fluorescence microscopy, showed that in the peripheral effector organs of the superior cervical and stellate ganglia of the rat, a complete loss of fluorescent axons and terminals occurred in immunosympathectomised animals. Levi-Montalcini & Angeletti (1962) have reported lower levels of monoamine-oxidase activity in the gastrointestinal tract of immunosympathectomised mice, whilst cells exhibiting a yellow fluorescence typical of 5-hydroxytryptamine (5-HT) were found in increased numbers in the intestines of immunosympathectomised rats compared to controls (Hamberger & others, 1965). Iversen, Glowinski & Axelrod (1966) have recently reported increased 5-HT levels in mouse bowel associated with no depression of monoamine oxidase activity.

Presented here are mucosal 5-HT levels for 14 bowel areas in the gastrointestinal tract of the immunosympathectomised mouse.

Five litters of mixed sexes from the same randomly bred, closed colony of Swiss-Webster white mice maintained at UCLA were obtained within a three week period. The first was injected subcutaneously on the first six days of life with 0·02 ml anti NGF serum per gram of body weight;* the second received 0·02 ml/g 0·9% saline in place of the anti NGF serum and the third was weighed and handled but not injected. The fourth and fifth litters were left unhandled apart from cage cleaning until the time of assay. All assays were made on mice 160–180 days old. The diet was Purina rat chow with a tryptophan content of 0·22%. Mice were killed between 8 a.m. and 9 a.m. on the day of assay by exsanguination after ether induction, and the following tissues were rapidly excised: oesophagus, stomach fundus and body, pyloric antrum, upper and lower duodenum (first and fourth centimetre from pyloric sphincter), mid jejunum, mid and terminal (last centimetre) ileum, appendix (caecal tip), ascending, transverse, and descending colon, and proximal rectum. After removal the segments were opened longitudinally, cleaned, blotted dry, and the mucosa separated from the muscle, and assayed spectrophotofluorometrically for 5-HT content (Thompson, 1966). Where tissues were available duplicate samples were run. P values between the immunosympathectomised group, and the three control groups (Table 2) were compared with the pooled figures from the normal, the saline injected, and the handled mice. This procedure was justified since the analysis of variance with unequal groups (Emmens, 1948) demonstrated no significant difference between the normal, the saline injected, and the handled mice. At the time of assay, specimens of the posterior thoracic wall were removed for sympathetic ganglia cell counts in the immunosympathectomised, and the saline injected mice. The tissues were fixed in bichromateformaldehyde and serial histological sections cut at 10 μ and stained with cresyl violet.

Table 1 gives the weight and sex of the immunosympathectomised and saline injected mice, as well as the mean ganglion cell counts on serial sections of four

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

ganglia of the thoracic sympathetic chain. The reduction in nerve cell density is 85%. The results presented in Table 2 demonstrate an increased level of mucosal 5-HT in the proximal gastrointestinal tract of the immunosympathectomised mice, compared to the normal, the saline injected, and the handled animals. Such differences range from a $P < 0.02$ (pyloric antrum, and lower duodenum) to $P < 0.001$ (stomach body, mid jejunum, and ileum), with the upper duodenal samples exhibiting no significant difference. The data presented for the large bowel uniformly shows no significant difference in all tissues sampled. Undetectable values for the oesophageal mucosa are not unexpected due to the paucity of argentaffin cells present (Ham, 1965).

Hamberger & others (1965) also demonstrated increased fluorescence typical

TABLE 1. MEAN GANGLION CELL COUNTS OF FOUR THORACIC SYMPATHETIC GANGLIA. THE MEAN IMMUNOSYMPATHECTOMISED (IMS) CELL COUNT AS A PERCENTAGE OF THE MEAN SALINE INJECTED GROUP CELL COUNT IS INDICATED

IMS group			Saline group			
Sex	Body weight	Ganglion cell count	Sex	Body weight	Ganglion cell count	IMS cell count as % of control
M	25·5	56	F	26·0	367	
M	20·5	69	F	26·0	549	
F	26·0	90	M	32·5	455	
M	25·0	74	F	26·5	463	
F	24·0	82	M	31·5	462	
M	30·0	82	F	24·0	538	
F	22·0	78	M	31·0	754	
M	31·0	85			—	
Mean ganglion cell count		77			513	15%

TABLE 2. GASTROINTESTINAL 5-H LEVELS IN μG/G MUCOSA FOR THE FOUR GROUPS OF MICE. [Values are expressed as means ± standard error. The number of samples is shown in brackets. In calculating the P values the IMS group was compared to the pooled data from the normal, the saline injected, and the handled mice (see text for explanation).]

Tissue	Mouse groups		P
	IMS	Controls	
Oesophagus	0 (8)	0 (29)	—
Stomach fundus	22·72 ± 0·97 (5)	15·96 ± 0·95 (22)	<0·01
Stomach body	20·12 ± 2·62 (6)	11·79 ± 0·79 (22)	<0·001
Pyloric antrum	84·93 ± 8·45 (8)	66·60 ± 2·92 (28)	<0·02
Upper duodenum	27·11 ± 2·17 (7)	23·66 ± 1·37 (29)	NS*
Lower duodenum	20·11 ± 2·93 (7)	15·69 ± 0·63 (27)	<0·02
Mid jejunum	18·89 ± 1·96 (8)	14·94 ± 0·92 (29)	<0·001
Mid ileum	13·95 ± 1·40 (6)	9·35 ± 0·52 (23)	<0·001
Terminal ileum	16·87 ± 3·36 (6)	10·16 ± 0·73 (22)	<0·001
Appendix	12·57 ± 2·30 (6)	15·81 ± 1·11 (26)	NS*
Ascending colon	54·74 ± 1·55 (7)	47·50 ± 3·01 (24)	NS*
Transverse colon	51·93 ± 3·07 (6)	45·09 ± 1·56 (28)	NS*
Descending colon	47·95 ± 3·41 (6)	42·92 ± 2·39 (23)	NS*
Proximal rectum	44·78 ± 1·81 (6)	41·88 ± 3·47 (17)	NS*

* Not significant.

of 5-HT in the small intestine (exact location unspecified) of immunosympathectomised rats compared to controls. Iversen & others (1966) who reported increased bowel 5-HT levels in immunosympathectomised mice compared with controls did not indicate which portion of the small bowel was involved.

There appear to be two possible mechanisms for this increase in small bowel mucosal 5-HT in the immunosympathectomised animals. Firstly, incomplete degradation of 5-HT by monoamine oxidase could be postulated, in view of the lower levels of this enzyme reported by Levi-Montalcini & Angeletti (1962) in the small bowel after immunosympathectomy. However, Iversen & others (1966) assert that after this treatment no alteration of monoamine oxidase occurred in the bowel. Secondly, although it is generally considered that argentaffin cells do not migrate up the villus, little is known about their ageing processes. The demonstration by Dupont, Biggers & Sprinz (1965) of a decreased transit time of epithelial cells in the jejunum of immunosympathectomised rats suggests that some similar effects may be operating for the argentaffin cell.

The differential changes in 5-HT levels seen between the large and small bowel samples may be related to a selectivity in the effects of the anti NGF serum on the sympathetic nervous system. This has already been demonstrated both by Vogt (1964) and Zaimis & others (1965) who have shown in rodents that the thoracic, coeliac and mesenteric ganglia are not equally affected by the anti NGF serum.

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