On the other hand, SHIBA et al.[5] found that mitomycin-C selectively inhibited the synthesis of DNA. It is also known that actinomycin-D inhibits the formation of mRNA from DNA. Thus both these compounds have a direct effect on the cells, hence the change in the GAT/GNase ratio is seen earlier than that with Endoxan-Asta.

In conclusion it may be said that the change in the plasma GAT/GNase ratio might possibly be useful for the testing of compounds which may have antitumour activity.

Summary. Three known antitumour drugs have been tested for their effect on the GAT/GNase ratio of Ehrlich Ascites cells and host plasma. It was observed that all these drugs had changed the ratio of the 2 types of glutaminases from below 1.0 to the normal value of 1.0, this was accompanied with an increase in the survival time of the tumour-bearing animals. There was, however, no effect on the plasma GAT/GNase ratio of normal animals in the presence of the 3 antitumour compounds tested.

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Are there Somatostatin-Containing Nerves in the Rat Gut? Immunohistochemical Evidence for a New Type of Peripheral Nerves

It has been generally accepted that the gut receives a dual innervation by cholinergic and adrenergic nerves, exerting, respectively, an excitatory and inhibitory effect in the control of gut motility[1-4]. More recently BURNSTOCK and collaborators have advanced the concept of a purinergic innervation to represent the main inhibitory system antagonistic to the excitatory cholinergic nerves (see[5]). Finally, immunohistochemical studies by NILSSON et al.[5] have demonstrated that, in the mouse, Substance P, or a Substance P-like substance, is present in nerves forming a dense network mainly around the ganglion cells of the myenteric plexus. Thus, at least part of the Substance P present in the gut[6,7] and originally isolated from this tissue by VON EULER and GADDUM[6] is of neuronal origin.

In the present paper we report the existence of probable nerves containing somatostatin or somatostatin-like immunoreactivity in the rat gut. This peptide was originally isolated from the hypothalamus and has an inhibitory action on the growth hormone release[8]. However, somatostatin is present not only in the hypothalamus[9-11], but has recently also been found in various peripheral tissues with immunohistochemistry[12] and radioimmunoassay[13].

Material and methods. Antibodies to somatostatin were prepared by coupling synthetic somatostatin to human α-globulin as described previously[14].

Male albino rats (6, wt. 150–200 g) were perfused with ice-cold 4% formalin, prepared according to PEASE[15] as described previously[16]. After rinsing, cryostat sections were cut from the stomach, duodenum, jejunum, ileum, colon and rectum. The sections were incubated with somatostatin antiserum pretreated with human α-globulin, diluted 1:20, for 30 min, rinsed in phosphate buffered saline (PBS), incubated with fluoresceinisothiocyante conjugated sheep antirabbit immunoglobulin (Statens Bakteriologiska Laboratorium, Stockholm, Sweden), diluted 1:4 for 30 min, rinsed in PBS, mounted and examined in a Zeiss Junior fluorescence microscope. Consecutive sections were incubated with somatostatin antiserum adsorbed with somatostatin (control serum) or with Substance P. All sera contained 0.3% Triton X-100[17].

Results and discussion. After incubation with somatostatin antiserum pretreated with human α-globulin, principally two types of structures exhibited a positive immunofluorescence. Firstly, cells mainly localized in the lamina propria of the gut mucosa but partly also among the gland cells were green fluorescent. However, except in certain cases described below, cells with a similar localization were immunopositive not only after control serum followed by FITC conjugated antibodies but also after incubation with FITC conjugated antibodies alone. Thus, many, but probably not all, fluorescent cells seen after incubation with somatostatin antiserum cannot be considered as containing somatostatin. On the other hand, in the stomach morphologically characteristic cells, mostly sending out short, thick processes, were observed only after incubation with somatostatin antiserum, but not in the controls. These cells may thus represent true somatostatin-containing cells confirming the radioimmunological results of ARIMURA et al.[13]. The localization of somatostatin in cell bodies in the intestinal wall will be discussed in detail in a following paper[18].

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Secondly, a positive immunofluorescence was seen in dot and fibre-like structures often with a varicose appearance, in all probability representing nerve terminals. They were present in all parts of the intestine but not in the stomach. In no instances were these fibres observed after incubation with control serum. Furthermore, fluorescent fibres with an identical distribution were observed after incubation with somatostatin antiserum pretreated with Substance P. The fibres were mostly seen in the basal part of the lamina propria of the mucosa in all parts of the small and large intestine with the highest numbers in the jejunum and ileum (Figure 1). Only rarely did the fibres approach the apical parts of the propria in the villi. Positive fibres were also observed around the ganglion cells of the myenteric (Auerbach's) plexus with rather high numbers in the ileum and colon (Figure 2) and less in the other parts. Somatostatin positive nerves occasionally penetrated into the muscular layer of the mucosa, especially in the colon (Figure 3) and rectum but very rarely in the rest of the intestine. Submucosal plexuses were observed, e.g. in the duodenum, ileum and colon (Figure 4).

In unpublished[19] parallel experiments on consecutive sections with antibodies to Substance P[20], a markedly different distribution of strongly immunofluorescent fibres was observed (see also[5]). The Substance P-positive fibres were much more numerous and formed a very dense plexus around the ganglion cell bodies of the myenteric plexus, especially in the stomach, but were also seen in the muscular layer, especially the circular one, in the entire lamina propria of the mucosa, i.e. also in the most apical parts of the villi, and finally around cell bodies of the submucosal ganglion layer. These findings are in agreement with earlier immunohistochemical work by NILSSON et al.[5,20].

The origin of the somatostatin containing nerves is at the moment unclear. No somatostatin positive nerve cell bodies could be identified in the myenteric plexus, but it cannot be excluded that some positive cell bodies in the lamina propria may be of neuronal origin. Furthermore,

[19] The immunohistochemical analysis of the distribution of Substance P in the gastrointestinal tract is carried out by the authors cited under ref.[5]. A full account of this work on different parts of this organ system in different species will be published elsewhere by these authors.

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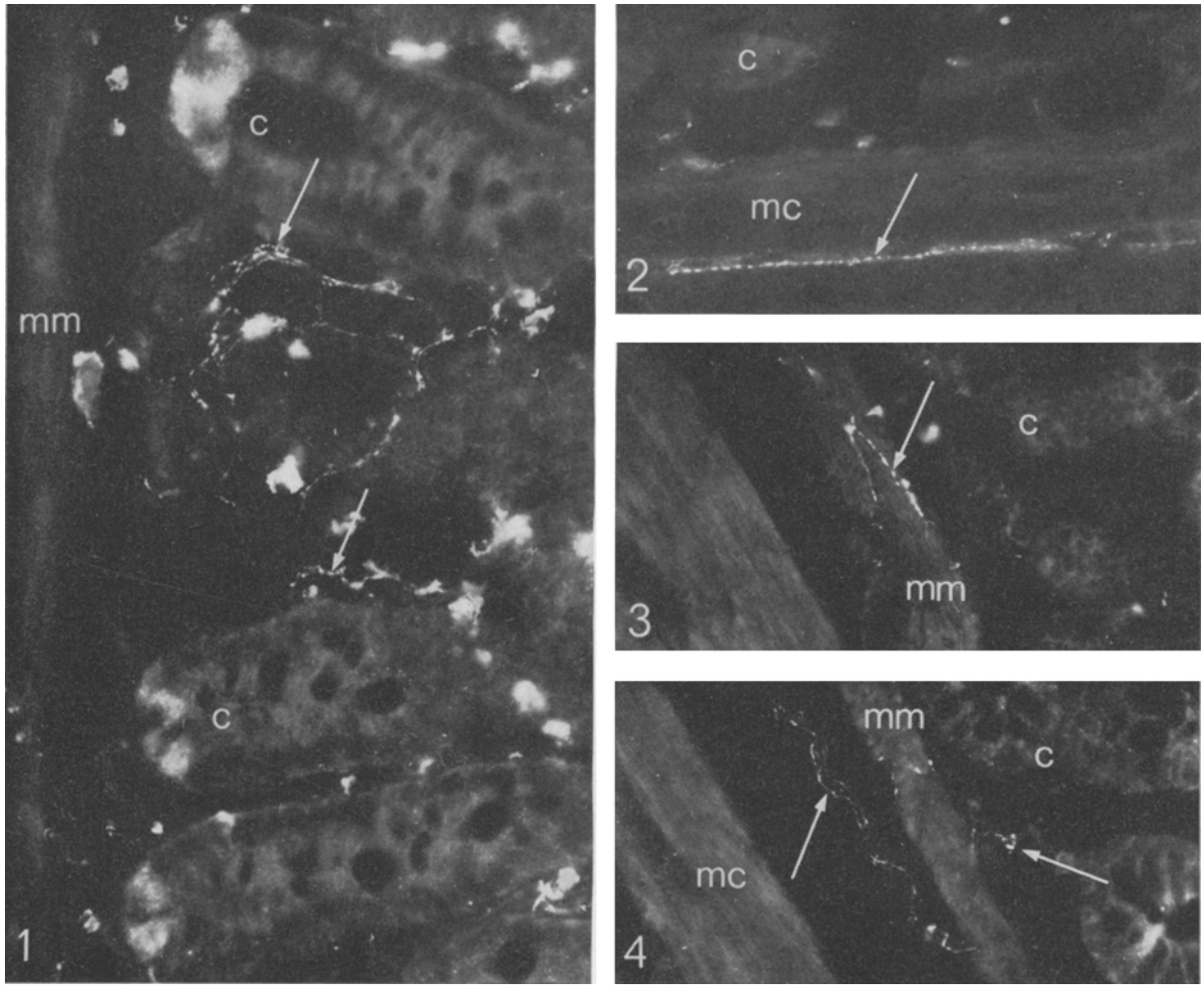

Figs. 1–4. Immunofluorescence micrographs of the rat intestine after incubation with somatostatin antiserum. Plexuses of positive nerve fibres (arrows) are seen in the lamina propria of the mucosa of the jejunum (Figure 1), in the myenteric plexus of the colon (Figure 2), in the lamina muscularis mucosae of the colon (Figure 3) and in the submucosa of the colon (Figure 4). C, bottom of crypts; mm, lamina muscularis mucosae; mc, circular layer of the muscularis externa. The fluorescent cellular structures in Figure 1, e.g. at the bottom of the crypts, exhibit an unspecific fluorescence. Magnifications 400 × (Figure 1) and 330 × (Figures 2–4).

the lack of an immunopositive reaction in cell bodies may only indicate that the sensitivity of the technique is insufficient. Further studies are clearly necessary to establish the localization of the entire somatostatin neurons. Such studies should also include other tissues such as spinal ganglia, thus persuing the possibility that the somatostatin containing nerves are of sensory nature. Preliminary studies, e.g. on the pancreas, thyroid gland, salivary gland, liver, kidney, adrenals, spleen and thymus, have not demonstrated somatostatin positive nerves in these tissues. Thus, so far, somatostatin-containing nerves seem to be present only in the hypothalamus[9-11], the posterior pituitary[18] and the intestine.

The presence of somatostatin or a somatostatin-like peptide in probable nerves with a clearly different distribution from those of the Substance P-containing nerves (and of cholinergic and adrenergic nerves) indicates the presence of a further type of nerve in the gut. The functional rôle of these nerves is at present unknown. Quantitatively, these new nerves, however, seem to be of less importance than, for instance, the Substance P-containing systems. Some somatostatin-containing nerves seem related to ganglion cells, others are found between smooth muscle cells, whereas those in the lamina propria could influence blood vessels. It may be pointed out that somatostatin in the hypothalamus inhibits growth hormone secretion[8] and that in the pancreas this peptide inhibits insulin and glucagon secretion[21-28]. Finally, it should be emphasized that, although the immunoreaction is specific in the sense that it is abolished by pretreatment of the somatostatin antiserum with somatostatin, the possibility of a cross reaction with a similar peptide cannot be excluded[29].

Summary. Antibodies to somatostatin, a recently isolated hypothalamic peptide inhibiting growth hormone release, were used in immunohistochemical studies on the gastrointestinal tract. Somatostatin containing cells in the stomach, and somatostatin-containing nerves in the small and large intestine, could be demonstrated. These findings give evidence of a new type of nerve in the gut.

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Effect of *Mycobacterium bovis* and *Mycobacterium tuberculosis* on the Take and Survival of Chickens with Transplanted MC-29 Hepatoma

The therapeutic effect of *Mycobacterium bovis* (BCG) and of mycobacterial cell walls has been demonstrated in several experimental tumor systems[1-11]. The use of BCG in the therapy of human tumor patients has also given promising results in a number of cases[12]. No data, however, are available about the effect of BCG in experimental transplantable tumor systems of a certain virus origin and having an epithelial character.

The present investigation was designed to test the effect of BCG (Paris strain) and of *Mycobacterium tuberculosis* (W-115 strain[13,14]) on a transplantable chicken tumor system of a proved virus origin.

Newly hatched Hunnia hybrid chickens kept on a standard diet were used. They were selected for the different groups at random and were controlled daily. A post mortem examination of every chicken was performed.

The transplantable MC-29 hepatoma established from a primary hepatoma induced by the oncogenic MC-29 RNA virus isolated in Bulgaria[15] was used as tumor system. We transplant this tumor every 14th day i.m. and s.c. and

Table I. Comparative tumor take and tumor growth of chickens after transplantation of MC-29 hepatoma with and without BCG

Series of experiment	Treatment	No. of animals	Tumor at the site of transplantation	Mean $\frac{\text{tumorweight}}{\text{bodyweight}} \times 100$
1	Only tumor	11	10/11[a]	9.3 ± 8.3[b]
	Living BCG + tumor at the same site	12	3/12	2.1 ± 1.2
2	Only tumor	10	10/10	10.0 ± 2.2
	Heat killed BCG + tumor at the same site	15	12/15	16.7 ± 4.7

[a] Number of animals with tumor/number of animals tested. [b] Standard error of the mean.