Immunohistological localization of melatonin in the rat digestive system

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Summary. The highest amount of melatonin (M), localized immunohistologically, was found in rectum, decreasing in the order: colon, duodenum, caecum, esophagus, stomach, ileum and jejunum. No M was found in the liver, spleen or pancreas. The distribution of M corresponds with the localization of serotonin-producing argentaffin cells.

Melatonin (N-acetyl-5-methoxytryptamine) was first time isolated from the pineal gland by Lerner et al.[3]. For many years melatonin was regarded as being synthetized exclusively in the pineal gland[4]. However, in recent years, the melatonin synthetizing enzyme, hydroxyindole-O-methyltransferase has also been found in the retina and the Harderian gland[5]. In vitro synthesis of melatonin from serotonin has been demonstrated in the retinal tissue[6]; melatonin was identified using bioassay in many areas of the brain[7], and by radioimmunoassay melatonin has been found in the Harderian gland and the retina[8]. Immunohistologically, melatonin has been localized in the outer nuclear layer of the retina, in the optic nerve, chiasma and tract, in the suprachiasmatic nucleus[9], and in the Harderian gland[10]. We have also evidence supporting the concept that melatonin may be synthetized in other areas than the pineal gland. N-acetylated indolealkylamine (later identified as melatonin) has been found in the retina 3 weeks after bilateral sectioning of the optic nerves and 6 weeks after pinealectomy[11]. Recently a report has been published which suggests that melatonin may also be synthetized in enterochromaffin cells of the gastro-intestinal tract. Using thin layer chromatography, Kvetnoy et al. have demonstrated the presence of melatonin in an extract of the mucosa of human appendices[12]. In a biological test system, this extract produced a lightening of the frog skin melanophores which was comparable to the effect of melatonin[13].

In order to investigate the cellular localization of melatonin in the digestive system, we have investigated immunohistologically esophagus, stomach, intestines, liver, spleen and pancreas in adult male rats. Using double

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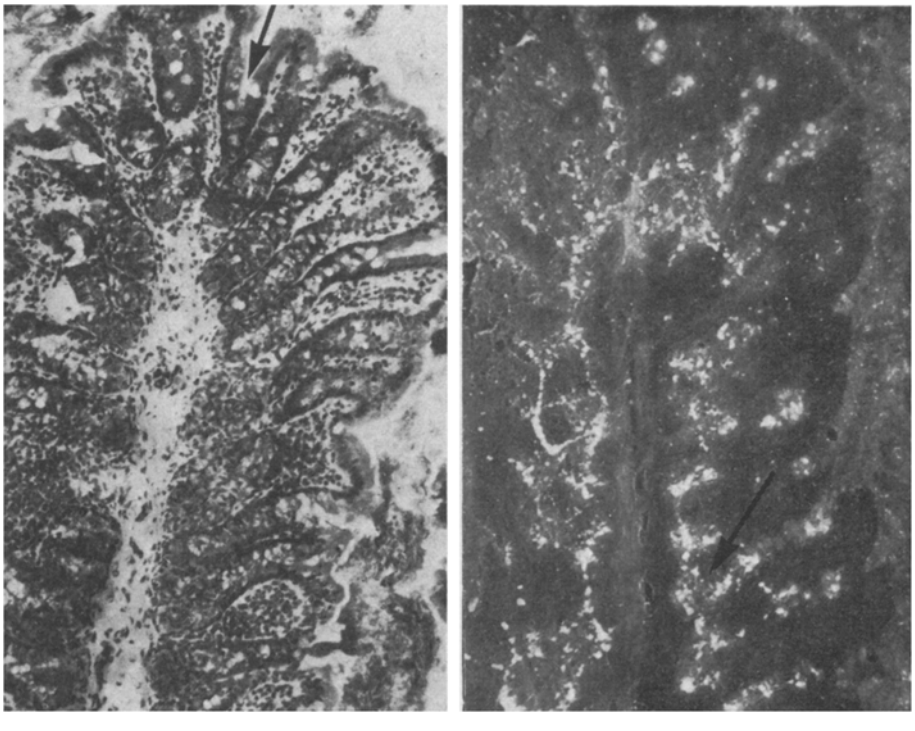

A Cresyl violet staining of the rat mucosa in the colon. ↓ Lieberkühn's crypts. × 200.

B Fluorescein labelled antibody technique. ↓ Specific fluorescence indicating melatonin in the middle and basal portions of the Lieberkühn's crypts.

Crossreactivity of antisera (calculated on a weight basis as equivalence at 50% displacement of ^{3}H-melatonin)

Antigen	Melatonin-M-BSA
Melatonin	100.0
N-Acetylserotonin	1.3
6-Hydroxymelatonin	< 0.1
5-Methoxytryptamine	< 0.1
N-Acetyl-1-tryptophan	< 0.1
Bufotenine monooxalate hydrate	< 0.1
N-Acetyl-1-tryptophanamide	< 0.1
Serotonin Creatinine sulphate	< 0.1
5-Methoxyindoleacetic acid	< 0.1
5-Hydroxy-N-methyltryptamine oxalate	< 0.1
N-Methyltryptamine	< 0.1
5-Methoxytryptophol	< 0.1
Tryptamine hydrochloride	< 0.1
5-Methyltryptamine hydrochloride	< 0.1
N-Methylserotonin hydrogen oxalate	< 0.1

antibody technique, the tissues were prepared according to the method described in a previous paper[11]. The first antibody, a highly specific anti-melatonin antibody (table), has been prepared according to Grota and Brown[14]; the second antibody, a fluorescein labelled antibody, was used as described by Coons et al.[15]. Comparable serial sections were stained by cresyl violet for identification of cellular details. The specificity of the staining was determined in 3 separate tests: a) the antimelatonin serum was saturated with melatonin; b) the different specific antiserum (antitestosterone) were used for comparison; and c) the reaction was performed with second antibody, without using the specific antimelatonin serum[11].

No specific fluorescence was found in the liver, spleen or pancreas. However, fluorescence indicating melatonin was observed throughout the whole digestive system. In the esophagus, melatonin is mostly present in the basal epithelium but some also is found in the circular muscles. In the stomach, the fluorescence was registered in the glandular portion of the wall. One of the highest amounts of melatonin was found in the duodenum, mostly in the Lieberkühn's crypts and the Brunner's glands but a considerable amount was also localized in the villi. On the other hand, the jejunum was found to be almost melatonin free, with only a few fluorescent particles scattered in the glandular portion. More melatonin than in the jejunum, but far less than in the duodenum, was registered in the ileum. Fluorescence was distributed mostly in the Lieberkühn's crypts and villi. In the caecum, colon and the rectum, the distribution of fluorescence was almost identical but there was a rising quantity of melatonin toward rectum, where it reached the highest concentration. Most fluorescence was observed in the higher and apical portions of the Lieberkühn's crypts (figure, A, B). The exact localization of melatonin in respect to the type of cell containing the N-acetylated indolealkylamines will require a further study.

The distribution of melatonin (higher in the stomach and duodenum, low in the jejunum and ileum and rising again toward rectum) corresponds to the distribution of serotonin-producing argentaffin cells[16,17]. Moreover, the localization of fluorescence in the Lieberkühn's crypts (higher and apical portion) corresponds closely to the localization of argentaffin cells[16]. Serotonin is known to facilitate the peristalsis[16]. It is therefore possible that melatonin, a derivative of serotonin, also participates in some aspect of intestinal physiology. Physiological studies investigating the role of melatonin in the digestive processes are in progress.

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Cytological effects of some medicinal plants used in the control of fertility[1]

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Summary. The effects induced upon the cell cycle of Allium cepa meristems by 2 medicinal plants used in the control of fertility were studied. Infusions of Aristolochia triangularis induces typical c-mitotic figures. On the other hand, Stevia rebaudiana have no specific toxicological effects upon the cell cycle.

Rural and indigenous peoples of Paraguay employ several medicinal plants in the control of fertility[4]. The effectiveness of these treatments has not been confirmed yet, and many international institutions are highly interested not only in the effects of these plants upon fertility, but in research concerning their general biological effects[5]. Chaudhury[6] found that several plants used by primitive peoples of India in order to prevent pregnancy significantly decrease the fertility of adult female albino rats. Many drugs with specific toxicological effects upon mitosis (i.e. colchicine, vinblastine and podophyllotoxine) are isolated from plants which have been employed by primitive people for generations[7]. Finally, Wiesner and Yudkin[8] and Robson[7] have proposed that the so-called

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