Varieties of Dopamine-Containing Cell in the Duodenal Mucous Membrane of the Sheep

The presence of dopamine in mast cells of ruminants has been inferred from their chromaffinity and from their presence in tissues whose content of dopamine is high[1]. Cells characterized as mast cells by histochemical and ultrastructural studies have been reported in each layer of the duodenal wall[2], as well as in a variety of other tissues in ruminants[3,4]. During an investigation of the fluorescence properties of monoamine-containing cells in intestinal epithelium it became apparent that in the duodenum of the sheep two morphologically distinct cell types were present, both of which appeared to contain dopamine. This view was confirmed by the application of several histochemical staining procedures.

Studies were carried out on tissues obtained from healthy adult sheep within minutes after death. Specimens were taken from areas of duodenum containing submucosal glands and from skin, lung and liver capsule and were either freeze-dried or fixed in cold 10% neutral formalin. Sections of freeze-dried tissues were examined for the presence of amine fluorophores using the formaldehyde-induced fluorescence technique[5] and were subsequently stained with acid fuchsin and toluidine blue. Spectral characteristics of intracellular fluorescence were studied using a Leitz-Schoeffel microspectrofluorometer. These were compared with the fluorescence properties of a variety of biogenic amines, each at a known concentration within a cell model comprising Sephadex beads of small diameter embedded in Araldite, sectioned to an appropriate thickness, and treated with formaldehyde vapour[6]. In addition, sections of tissue either freeze-dried or fixed in aqueous formaldehyde were stained by each of the following: haematoxylin and eosin; toluidine blue, either alone or following acid fuchsin; PAS, with and without prior digestion with diastase; Alcian blue (pH 1.0 + 2.5); Giemsa; and Masson's Trichrome.

In freeze-dried specimens of duodenum fixed in formaldehyde vapour, cells with green fluorescent granules were found to be present in all tissue layers but were most numerous in the mucous membrane. Fluorescent cells in the muscularis externa and in the connective tissues investing submucosal glands were small and either elongated or irregular in shape; they showed a diffuse cytoplasmic fluorescence more commonly than individual fluorescent granules. Similar cells in the lamina propria showed a finely granular appearance throughout the cytoplasm (Figure 1). All such cells were prominent after staining with toluidine blue and their granules were seen to be strongly basophilic and metachromatic. Cells which were indistinguishable from the above fluorescent duodenal cells, in both fluorescence properties and staining reactions, were found in connective tissues of the skin, lung and liver capsule. On present criteria the cells were therefore identified as duodenal mast cells.

A second type also containing green fluorescent granules was seen in the duodenum, but in the mucous membrane only. The cells were distinctive for they contained large granules (Figure 1) which were shown subsequently to be strongly acidophilic. On the basis of their appearance in stained preparations the cells are referred to here as acidophilic cells. They could be readily distinguished from mast cells for they were large and oval in profile, with rounded and eccentric nuclei (Figure 2). In tissues fixed in aqueous formalin the cells were less well stained and their granules less obvious. Histochemical staining reactions failed to demonstrate the presence of proteoglycans in acidophilic granules following either method of tissue preparation, although the surrounding cytoplasm was moderately PAS-reactive in freeze-dried specimens.

A difference was seen in the distribution of the two cell types in the duodenal mucosa although here they were approximately equal in number. Mast cells were evenly distributed in the lamina propria and only occasionally were located also within the epithelium of a mucosal gland. In contrast, the acidophilic cells were predominantly intraepithelial (Figure 2), particularly at the intestinal sur-

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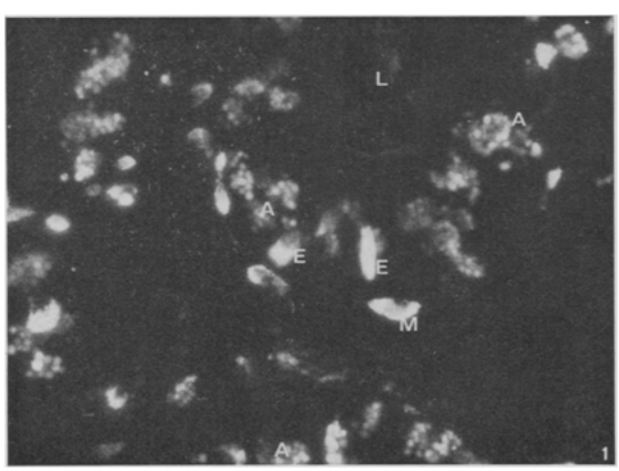

Fig. 1. Formaldehyde-induced green fluorescence of large granules of acidophilic cells (A) in epithelium lining the intestinal lumen (L) above, and a mucosal gland, below. The fine granules of mast cells (M) in the lamina propria also emit a green fluorescence, while enterochromaffin cells (E) emit a yellow fluorescence. × 530.

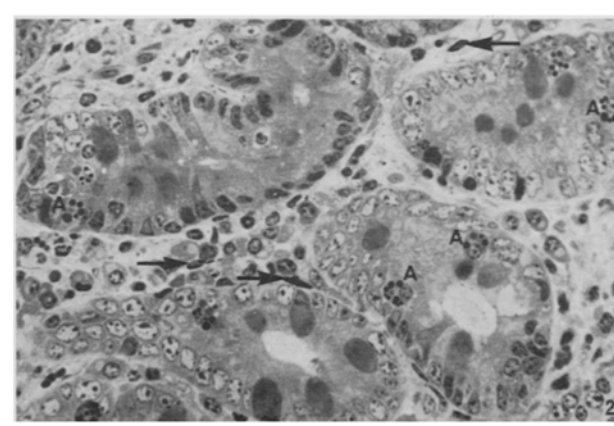

Fig. 2. Acidophilic cells (A) are more common in the epithelium than in the lamina propria, whereas mast cells (→), identified by their basophilic and metachromatic granules, are almost exclusively located in the lamina propria. Toluidine blue. × 240.

face and in the ducts of submucosal glands which in the sheep open directly into the lumen of the duodenum[7]; in the lamina propria they were mainly subjacent to the surface epithelium.

Green fluorescence emitted by granules of the mast cells and acidophilic cells was studied and compared, first, with the yellow fluorescence of enterochromaffin cells seen in the same preparations and, secondly, with the formaldehyde-induced fluorescence of each of adrenaline, noradrenaline, dopamine and 5-hydroxytryptamine incorporated in the cellular model system. Microspectrofluorometric analysis showed that the yellow fluorescence of enterochromaffin cell granules was indistinguishable from that emitted by the 5HT-containing model. The green fluorescence of mast cell granules and of acidophilic cell granules was indistinguishable in both absorption and emission characteristics, and approximated most closely to the fluorescence spectra of either dopamine or noradrenaline in the cellular model used. Reliable methods for distinguishing between the formaldehyde-induced fluorescence of dopamine and noradrenaline are not yet available[8]. However, in view of the high concentration of dopamine, and the insignificant content of other catecholamines in extracts of the mucous membrane of the sheep duodenum[9], it is concluded that the most likely fluorophore in both mast cells and acidophilic cells of sheep duodenal mucosa is dopamine-derived.

Ruminants appear to be unique in that their mast cells contain dopamine[10]. It is clear that the presence of dopamine in cells other than mast cells in the duodenum of the sheep must also be considered. Possibly some of the mast cells here undergo a transformation in appearance accompanied by a propensity for epithelial migration when exposed to an intestinal environment. It is more probable, however, that acidophilic cells belong to a distinct cell type with the capacity to elaborate, store, or transport dopamine, whatever its involvement in intestinal activities.

Zusammenfassung. Es wurden zwei Arten Dopamine enthaltende Zellen in der Mucosa des Schafduodenums aufgefunden. Eine Art ist morphologisch und histochemisch vom Mastzellen-Charakter und die andere mit acidophilen Granulierungen.

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Alteration in Catecholamines in Local Cerebral Cortex Lesions

Local freezing of the cerebral cortex produces[1,2] an epileptogenic focus in animals and thus provides a model to study experimentally induced epilepsy[3]. Usually abnormal discharges are observed as early as 3 h after a lesion is made and may last for several weeks[2,4]. Histologically the damaged area shows dense gliosis, dropping out of neurons, and pallor of the superficial layers of the cerebral cortex[2]. Surrounding the lesion basophilic cells are seen which stain for RNA as well as for gamma globulins[5,6]. There is also local destruction of the blood brain barrier[7].

Although decreased levels of glutamic acid, glutamine and glutathione have been reported[8], little else is known about the neurochemical changes associated with epileptogenic foci[9,10]. Since dopamine and norepinephrine are intimately involved in modulating electrical activity in brain[11,12], the possibility was entertained that these biogenic amines might be altered after freeze lesions. In a previous study, FALCK et al.[13] using the histofluorescent technique[14,15] to study normal rabbit cortex, described normal cortex as having a fine network of adrenergic nerve terminals as well as adrenergic innervation of pial vessels and radial arteries penetrating the cortex. In beginning this investigation of freeze lesions, it appeared feasible to use the same technique to examine in situ the distribution of biogenic amines under neuropathological conditions.

Procedure. 12 New Zealand adult male rabbits weighing 2 to 3 kg were operated under nembutal anesthesia. A 3 × 3 mm burr hole was made on the right side over the motor cortex. The dura was left intact and a piece of dry ice was applied to the region for 1 min, covered with Gelfoam and closed. The animal recovered uneventfully. In 4 additional rabbits, used as controls, surgery was performed without making a lesion.

Animals were maintained postoperatively for 1 or 2 weeks under normal conditions before the brains were removed for histofluorescent study. In 2 animals with freeze lesions, reserpine was injected (4 mg/kg) i.v. 18 h prior to killing them. Blocks encompassing the area of the lesions were removed and processed according to the method of FALCK[15]. Comparable blocks of motor cortex from controls were taken. In 2 animals with freeze lesions, the tissues were not exposed to paraformaldehyde vapors in order to assess the amount of autofluorescence. Eight μm sections were cut through the extent of the blocks and sections were examined under the Leitz UV microscope.

Results. The lesions show an infiltration of macrophages which autofluoresce a brownish yellow color in the UV microscope (Figure, A). In the deeper layers of the cortex bordering the lesions there are motor cortex cells which stand out from the background and fluoresce a bright green (Figure, A). Some of these cells are pyknotic and in a state of disintegration. The green fluorescence in these cells is not dependent on exposure to paraformaldehyde but is slightly reduced by reserpine.

In the area of damage there are many swollen and distorted green fluorescent varicosities which are seen intermingled with normal appearing fluorescent nerve endings. The excessive accummulation of catecholamines is demonstrated in Figure, C and D, in cross sections of the same nerve terminals of adjacent sections. Distal to the lesions as well as in sections taken from control animals, the varicosities have a normal appearance (Figure, B). The abnormal amount of fluorescence seen in the swollen terminals as well as the normal appearing fluorescence in the fine varicosities are abolished by reserpine and are absent when the paraformaldehyde vapors are omitted.

Discussion. This preliminary study demonstrated in freeze lesions of the cerebral cortex the abnormal presence