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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[7] G. C. SCHOFIELD and A. M. ATKINS, J. Anat. *108*, 210 (1971).
[8] K.-A. NORBERG, M. RITZEN and U. UNGERSTEDT, Acta physiol. scand. *67*, 260 (1966).
[9] A. BERTLER, B. FALK, N.-A. HILLARP, E. ROSENGREN and A. TORP, Acta physiol. scand. *47*, 251 (1959).
[10] D. E. SMITH, Int. Rev. Cytol. *14*, 327 (1963).

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.

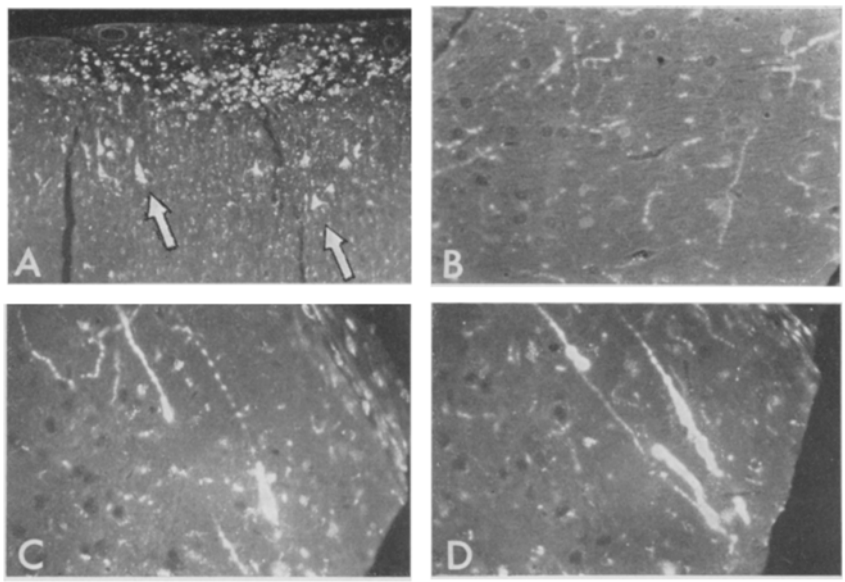
Procedure. 12 New Zealand adult male rabbits weighing 2 to 3 kg were operated under nembutal anesthesia. A 3 × 3 mm burr hole was made on the right side over the motor cortex. The dura was left intact and a piece of dry ice was applied to the region for 1 min, covered with Gelfoam and closed. The animal 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



A) Motor cortex region in rabbit. There is an infiltration of macrophages in the area of the freeze lesion. Around the border of the lesion autofluorescent motor cortex cells stand out the background (arrows). ×100. Prints made from Kodak High Speed Film.

B) Normal appearing nerve endings distal to the lesion. ×250.

C and D) Border of freeze lesion. Cross sections of the same nerve terminals in adjacent sections with excessive accumulation of noradrenaline producing the distored swollen appearance. In the same field there are also a few normal appearing nerve endings. ×250.

of motor cortex cells that autofluoresce and the excessive accumulation of norepinephrine in the varicosities of nerve terminals surrounding the lesion.

The autofluorescent cells around the lesions are not present immediately after damage but appear approximately 1 week after injury[16]. They are possibly dying cells and are probably the same basophilic, pyknotic cells described in freeze lesions with routine histological stains[2,5] and with immunofluorescence[6].

Although the autofluorescent cells have the same green appearance seen in catecholamine containing cells, their emission spectra by microspectrofluorometry are not exactly the same as that of catecholamines (R. KATZMAN, personal communication). They are also seen after stab wound lesions of the cortex[16]. Previous histofluorescent studies have not described these autofluorescent cells in other regions of the brain.

The appearance of the swollen nerve terminals in the cortex due to accumulation of norepinephrine is similar to what has been described after injury to peripheral nerve[17–19] and after interruption of catecholamine containing fiber tracts[20,21]. The cell bodies of the catecholamine containing terminals in the cerebral cortex are reported to be located in the loecus coerulus[21], although all the origins are not known. It is of interest to note that just injury to the cerebral cortex does not produce the extremely swollen terminals since they are not present after stab wound lesions of the cortex[16]. It is possible that the oedema which is produced by a freeze lesion[7] stimulates the flow of catecholamines.

When electrophysiological indices are used, noradrenaline has been reported to be primarily inhibitory in function[22–24]. The manner in which the alteration of noradrenaline in a freeze lesion may contribute to the development of an epileptogenic focus is currently under further investigation.

Zusammenfassung. Nachweis, dass corticale Läsionen eine Akkumulation von Noradrenalin an der Läsionsstelle hervorrufen.

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[1] L. F. NIMS, C. MARSHALL and A. NIELSEN, Yale J. biol. Med. *13*, 477 (1941).
[2] F. MORRELL, Am. med. Ass. Arch. Neurol. Psychiat. *1*, 141 (1959).
[3] F. MORRELL, in *Basic Mechanisms of the Epilepsies* (Eds. H. H. JASPER, A. A. WARD JR. and A. POPE; Little Brown and Co., Boston 1969), p. 357.
[4] F. MORRELL, Epilepsia *1*, 538 (1960).
[5] F. MORRELL, in *CIOMS Symposium on Brain Mechanisms and Learning* (London, Blackwell 1961), p. 375.
[6] F. P. BOWEN and A. ROTH, Abstract. Am. Ass. Neuropath., New Haven (1970).
[7] I. KLATZO, H. WISNIEWSKI and D. E. SMITH., J. Neuropath. exp. Neurol. *23*, 18 (1964).
[8] S. BERL, D. P. PURPURA, M. GIRADO and H. WELSCH, J. Neurochem. *4*, 311 (1959).
[9] L. S. WOLFE and K. A. C. ELLIOTT, in *Neurochemistry* (Eds. L. PAGE and J. H. QUASTEL; Chas. C. Thomas, Springfield, Ill. 1963), p. 694.
[10] R. A. LOWELL, in *Handbook of Neurochemistry* (Ed. A. LAJTHA; Plenum Press, New York 1971), vol. *6*, p. 63.
[11] G. B. KOELLE, in *Basic Mechanisms of the Epilepsies* (Eds. H. H. JASPER, A. A. WARD JR. and A. POPE; Little, Brown and Co., Boston 1971), p. 195.
[12] A. DAHLSTRÖM, in *Basic Mechanisms of the Epilepsies* (Eds. H. H. JASPER, A. A. WARD JR. and A. POPE; Little, Brown and Co., Boston 1971), p. 212.
[13] B. FALCK, G. I. MCHEDLISHVILI and Ch. OWMAN, Acta pharmac. toxic. *23*, 133 (1965).
[14] B. FALCK, N.-A. HILLARP, G. THIEME and A. Torp, J. Histochem. Cytochem. *10*, 348 (1962).
[15] B. FALCK and C. OWMAN, Acta Univ. lund. *117*, 1 (1965).
[16] F. P. BOWEN and C. MYTILINEOU, Neurology *22*, in press (1972).
[17] A. DAHLSTRÖM, J. Anat. *99*, 677 (1965).
[18] A. DAHLSTRÖM and K. FUXE, Z. Zellforsch. *62*, 602 (1964).
[19] A. DAHLSTRÖM and J. HAGGENDAL, Acta physiol. scand. *67*, 278 (1966).
[20] A. DAHLSTRÖM and K. FUXE, Acta physiol. scand., Suppl. *62*, 232, 1 (1964).
[21] U. UNGERSTEDT, Acta physiol. scand. Suppl. *367*, 1 (1971).
[22] G. W. ARBUTHNOTT, J. Physiol., Lond. *186* (1966).
[23] J. L. MALCOLM, P. SARAIVA and P. J. SPEAR, Int. J. Neuropharm. *6*, 509 (1967).
[24] G. W. ARBUTHNOTT, J. Neurochem. *16*, 599 (1969).
[25] I wish to express my thanks to Dr. CATHERINE MYTILINEOU, Dr. LUCIEN CÔTÉ and Mrs. IRENE TAR for their assistance in this study.