patients, certain immunologically competent cells may "forget" their early exposure by mutation to a pattern corresponding to a tissue antigen with a concomitant loss of homeostatic controls on number of cells(13). Precisely how such cells arise and proof of their existence remain to be determined. On the other hand, the abnormal immunological reactions in SLE may result simply from autologous antigens altered by contact with microbial agents or their toxins. Such alterations may change certain cellular structures so that they are no longer recognizable by antibody producing cells, and these altered autologous antigens may provide the direct incitant for autoimmune disease. The antibodies produced may be cross reactive with normal structures and thus the auto-immunization process initiated. Certain disease processes elicit elevated levels of tissue autoantibodies(14), but whether these autoantibodies are similar to those seen in SLE patients remains to be determined. The normal agglutinin and antistreptolysin O levels found in SLE patients in this study represent antibodies to foreign antigens and cannot contribute to the question whether the etiology of autoimmune disease is to be attributed to altered normal cellular constituents. The results indicate, however, that SLE patients are not greatly abnormal in their production of so-called normal antibodies to foreign antigens.

*Summary.* SLE patients do not possess higher levels of blood group isoagglutinins, *Proteus* OX-2 agglutinins or antistreptolysin O than other patients or normal individuals. The immunological hyperreactivity of these patients may be restricted, therefore, to circulating autoantibodies and possibly to the delayed or cellular hypersensitivity reaction.

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## Localization of Acetylcholinesterase in the Neurohypophysis and its Functional Implications.* (26423)

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The neurosecretory theory of functioning of the hypothalamico-neurohypophyseal system is now generally accepted. According to this concept, vasopressin and oxytocin, or their precursors, are elaborated within cell bodies of neurons of the supraoptic and paraventricular nuclei, and transported *via* the

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corresponding axons through the infundibular stalk to axonal swellings (the Herring bodies) and terminations in the neurohypophysis, where hormones are stored until secreted(1). Secretion of individual hormones in response to physiological and artificial stimulation is mediated by neural pathways which converge in the hypothalamus. On the basis of pharmacological evidence, it is likely that cholinergic neurons are involved in bringing about secretion of both vasopressin(2,3) and oxytocin(4). Since known cholinergic neurons stain selectively for acetylcholinesterase (AChE) by the acetylthiocholine (AThCh) method(5,6), Abrahams *et al.* (7) examined serial sections of several regions of the dog hypothalamus stained by this technic in order to identify cholinergic pathways in question. Unexpectedly, perikarya of supraoptic and paraventricular neurons themselves were found to be stained distinctly for AChE, but fibers terminating upon them showed no significant staining; among other possible interpretations, it was suggested that the same neurons might liberate at the neurohypophyseal level both acetylcholine (ACh) and endocrine products, with the former providing the stimulus for release of the latter. Recently the same proposal was offered independently by Gerschenfeld *et al.*(8) to explain their electron microscopic observations that individual neurosecretory axonal terminals in the toad neurohypophysis contain 2 distinct populations of granules: (a) large, electron-opaque granules, which could be traced back through the infundibular stalk to the hypothalamus, and presumably represented endocrine secretions, and (b) small, less dense vesicles, confined to terminals, which resembled synaptic vesicles noted previously at various presynaptic terminals where they may represent packets of neurohumoral transmitters(9), including ACh(10).

Since the neurohypophysis itself had not been examined in the histochemical study of AChE of the hypothalamic nuclei(7), it was of considerable interest to determine whether tracts and terminals of the hypothalamico-neurohypophyseal fibers contain significant concentrations of the enzyme.

*Materials and methods.* The neurohypophysis and infundibular stalk from 7 cats were stained selectively for AChE and nonspecific cholinesterase (ChE) or butyrocholinesterase (BuChE) activities; in most cases, intermediate lobe and adenohypophysis and part of the adjacent hypothalamus were left attached.† The hypophysis was usually sectioned immediately after sacrifice of the cat and excision; in a few instances, it was frozen in isotonic saline solution and used the following day. Fresh frozen sections were cut in the sagittal plane generally at 15 $\mu$ (occasionally at 20 $\mu$) thickness, placed on slides, and carried through the standard staining procedure(5,6). In one instance, all 10 combinations of substrates and inhibitors were employed to determine the possible presence in tissues under study of non-cholinesterase enzymes which can attack thiocholine esters used as substrates(6); however, none was detected. Except for the first exploratory specimen, where no inhibitors were used, other specimens were stained with various inhibitor-substrate combinations which always included pre-incubation with di*iso*propylfluorophosphate (DFP) and incubation with AThCh for the selective localization of AChE. Incubation times were varied from 30 to 240 minutes. Following development with $(NH_4)_2S$, which converts the white precipitate of copper thiocholine sulfate at sites of enzymatic activity to copper sulfide, and gold-toning, sections were dehydrated and mounted in Permount. Occasional sections were counterstained with hematoxylin-eosin.

Neurohypophyses from 3 cats were fixed in Bouin's fixative, frozen-sectioned, and stained for neurosecretory substance (NSS) by Bargmann's modification of Gomori's chrome-hematoxylin method, as given by Pearse(11).

*Results.* Findings were quite consistent in all 7 hypophyses studied. The distribution of AChE in the neurohypophysis was

† We wish to thank Drs. S. D. Erulkar and E. Koenig for help in obtaining the specimens. Photomicrographs were taken by Mr. E. F. Glifort.

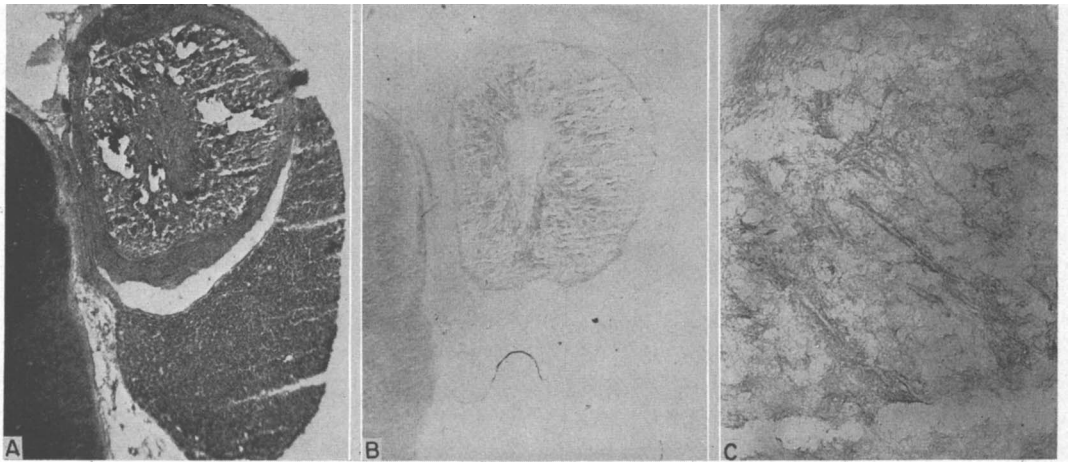


FIG. 1. Section (15 $\mu$) of cat neurohypophysis (above), adenohypophysis (below), and adjacent hypothalamus (left) stained for AChE activity by 120 min. incubation in AThCh medium preceded by 30 min. incubation with $10^{-8}$M DFP for selective inhibition of non-specific ChE. A. Magnification × 13, counterstained with H & E. B. Magnification × 13, no counterstain. C. Portion of neurohypophysis, magnification × 80, no counterstain. All staining in B and C represents AChE activity.

observed most precisely in 15 $\mu$ sections stained by incubation with AThCh for 120 minutes following pre-incubation with $10^{-8}$ M DFP for 30 minutes. Such a preparation, counterstained with hematoxylin-eosin, is shown in Fig. 1A, and without counterstaining in Fig. 1B; in the latter, and in the corresponding photomicrograph at higher magnification (Fig. 1C), all staining represents AChE activity.

Fiber tracts of the infundibular stalk showed very faint staining. Throughout the infundibular process of the neurohypophysis, there was light but definite staining (Fig. 1B), which appeared to represent fiber tracts (Fig. 1C) along which were seen numerous vesicular swellings reminiscent of Herring bodies (12, plate VI) seen with specific staining for NSS. For comparison, sections of the superior cervical ganglion and nodose ganglion were stained simultaneously under identical conditions. Neurohypophyseal fibers were stained with an intensity similar to that of the afferent vagal fibers of the nodose ganglion; in both these types of fibers, intensity of staining for AChE was considerably lighter than that of the cholinergic preganglionic fibers, but much greater than that of the virtually unstained postganglionic adrenergic fibers of the superior cervical ganglion; photomicrographs of these structures stained identically, have been published previously(6).

Varying degrees of staining for AChE were noted in adjacent hypothalamic tracts and nuclei, and intense staining was present in scattered nerve trunks in the immediate vicinity of the hypophysis. However, intermediate and anterior lobes of the pituitary appeared to be virtually devoid of AChE activity (*cf.* Figs. 1A and 1B). The only distinct staining for non-specific ChE (BuChE) noted in the area of the neurohypophysis was in rather poorly defined structures at its borders; these have been identified tentatively as interstitial cells. Similarly stained structures were noted occasionally in and at the periphery of the adenohypophysis.

The pattern of deep purple staining obtained with the chrome-hematoxylin method for NSS was similar to that for AChE, and, while less distinct, resembled that seen in published photomicrographs (12, plate VII).

*Discussion.* The present results, along with those of an earlier study in which AChE was demonstrated histochemically in neuronal cell bodies of paraventricular and supraoptic nuclei of the dog(7), indicate that enzyme is present in moderate concentrations throughout the lengths of the neurons com-

prising the hypothalamico-neurohypophyseal tract. In intensity of staining, they resemble the afferent vagal neurons, and contrast with both the known cholinergic neurons of the cat, which contain uniformly high concentrations of AChE, and with the majority of adrenergic neurons of the same species, which contain practically none. Although it is generally considered that the neurohumoral transmitter of primary afferent fibers is not ACh, recent work has suggested that in response to stimulation an ACh-like compound was liberated by the central terminals of vagal afferent fibers in the superior cervical ganglion of cats in which functional cross-anastomosis had been accomplished with the peripheral preganglionic cervical sympathetic trunk.‡ In connection with the latter findings it was suggested that under normal conditions, ACh might represent not the primary neurohumoral transmitter of vagal afferent fibers, but that its release and action immediately at presynaptic terminals might promote release of an unidentified synaptic transmitter. The present findings are consistent with previous proposals(7,8) of operation of a similar mechanism at terminals of the hypothalamico-neurohypophyseal tracts, *i.e.*, that in response to impulses conducted along the axons from hypothalamic nuclei. ACh is liberated at terminals within the neurohypophysis where it brings about the release of oxytocin or vasopressin, and that this local action of ACh is terminated by its hydrolysis by AChE of the axonal terminals. In this situation there is, of course, no postsynaptic site at which ACh could act. The function of perikaryonal AChE in the neurons under discussion, and in acknowledged cholinergic neurons, remains conjectural. It was proposed earlier(13) that it might represent recently synthesized enzyme in transit to its functional site at axonal terminals, *i.e.*, a sequence similar to that of neurosecretory processes as described above. However, recent study(14) has failed to confirm this suggestion.

*Summary.* Neurohypophyses of 7 cats were stained for AChE activity by AThCh method. In the infundibular stalk, fibers showed light staining, whereas fibers, their vesicular swellings, and terminations showed moderate staining in the infundibular process of the posterior lobe. No significant AChE activity was noted in the anterior or intermediate lobe. Thus, in conjunction with findings of an earlier study(7), neurons of the hypothalamico-neurohypophyseal tract appear to contain light to moderate concentrations of AChE throughout their full lengths. These findings are consistent with previous proposals(7,8) that liberation of endocrine secretions of the neurohypophysis is controlled by liberation of ACh from the same terminals.

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