Acetylcholinesterase in Human Thymus Cells

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Acetylcholinesterase (AChE) was long thought to be an enzyme found specifically at the sites of nerve synapses and neuromuscular junctions. It has also been found to occur, however, in cells that are not involved with neurotransmission. This study presents the ultrastructural localization of AChE activity in human thymus cells, using the indirect thiocholine method. Cytochemical demonstration of the enzyme was based on the coupling of acetylthiocholine iodide to the precipitation of heavy metal salts. AChE activity was selectively revealed in the perinuclear cisternae, within the endoplasmic reticulum, and in the Golgi complex of thymic lymphocytes and epithelial cells. Evidence of the presence of reaction product in the latter cells was also found in vesicles that opened into the extracellular space. This is the first demonstration of AChE in human thymus cells. Its possible physiologic role in the thymus gland is discussed.

ACETYLCHOLINESTERASE (AChE; EC 3.1.1.7) is the enzyme that hydrolyzes acetylcholine (ACh), thereby terminating the action of this neurotransmitter in synapses and motor end plates (neuromuscular junctions). AChE is found not only in nervous tissue, however, but also in all mammalian erythrocytes and in rodent megakaryocytes and platelets, as well as in certain other cells that are not involved with neurotransmission.

The function of this enzyme in nonnervous tissues is as yet unknown. Using cytochemical methods, we investigated the appearance and distribution of AChE in human thymus cells at the ultrastructural level.

MATERIALS AND METHODS
Normal thymus tissue was obtained from two patients, aged 1 and 29 years, undergoing surgery for congenital heart disease (patients were advised of procedures and attendant risks). The fresh tissue samples were prefixed in 1.44% glutaraldehyde in 0.1 mol/L cacodylate buffer at pH 7.2, washed in the buffer, and incubated in a staining medium containing 0.003 mol/L acetylthiocholine iodide (Sigma Chemical Co, St Louis) as a substrate for AChE. Controls consisted of tissue pieces incubated without substrate or with butyrylthiocholine iodide (Sigma). Other samples were incubated in the presence of 10⁻⁵ mol/L BW284c51, a specific inhibitor of AChE, or of 10⁻³ mol/L eserine, an inhibitor of both AChE and nonspecific cholinesterase. After incubation, the material was washed in 0.1 mol/L acetate buffer at pH 5.2 adjusted to 300 mosm with NaCl, treated with 0.25% ammonium sulfide, postfixed in 1% OsO₄, dehydrated, and embedded in Epon. Sections were cut with a diamond knife and stained with lead citrate or were examined in the electron microscope without counter staining.

RESULTS
AChE activity was cytochemically demonstrable both in the lymphocytes and in the epithelial cells of a human thymus gland. In small lymphocytes, the enzyme was found only in the perinuclear cisternae (Fig 1) and in a few channels of the endoplasmic reticulum (ER). In large lymphocytes, it was found in the ER (Fig 2) and in the cisternae and in the vesicles of the Golgi complex (Fig 2, insert). The reaction varied greatly in intensity, and some lymphocytes were nonreactive (Fig 1). The epithelial cells showed the fine granular AChE reaction product in the perinuclear space, within the ER, in the Golgi complex, and in the vesicles on the periphery of the cytoplasm (Figs 3 and 4). Numerous nonreactive vesicles were present throughout the perinuclear cytoplasm (Fig 4). In addition to this intracytoplasmic localization, the most consistent concentration of AChE

DISCUSSION
AChE plays an essential role in cholinergic mechanisms of neurotransmission. It has recently been revealed that the enzyme is much more widely distributed than its known substrate ACh. There is no ready explanation, however, for why AChE activity should be present in human erythroid cells, rodent megakaryocytes and platelets, or other cells that are not involved with neurotransmission. The observations that we describe reveal that AChE is synthesized in the lymphocytes and in the epithelial cells of the human thymus.

As far as we know, no evidence of AChE synthesis in the human thymus gland has yet been reported in the literature. Recently, Szélényi et al demonstrated the association of AChE activity with the outer membrane of T lymphocytes derived from peripheral blood, both of normal donors and of patients suffering from chronic lymphoid leukemia, by means of colorimetric and radiometric techniques.

Our cytochemical results may be complementary with these observations. As we have revealed in glutaraldehyde-fixed thymic lymphocytes, AChE activity is localized within...
Fig 1. In two small thymic lymphocytes (L^*), reaction product for AChE is limited to the perinuclear cisternae (pns). Note the nonreactive lymphocytes (L^o). Section weakly counter-stained with lead citrate. (Original magnification x15,000; current magnification x9,750. Bar = 1 μm.)

Fig 2. Portions of two thymic lymphocytes. Fine granular AChE reaction product is localized within the perinuclear cisternae (pns) and the endoplasmic reticulum (ER). Insert, Golgi region (G) of a large thymic lymphocyte also displaying fine AChE's granular reaction product. Sections counterstained with lead citrate. (Fig 2: original magnification x44,000; current magnification x28,600. Bar = 1 μm. Insert: original magnification x33,000; current magnification x21,480. Bar = 1 μm.)
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Fig 3. Thymic epithelial cell (Ep) surrounded by lymphocytes (L). AChE reaction product is localized in the perinuclear cisterna, endoplasmic reticulum, and vesicles on the periphery of the cytoplasm. Heavy deposits of AChE reaction product are concentrated in the outer extracytoplasmic space (arrows). Lead citrate counterstaining (original magnification x12,000; current magnification x7,800. Bar = 1 μm).

Fig 4. Portion of the thymic epithelial cell (Ep). Fine granular AChE reaction product is localized within the perinuclear cisterna (pns), endoplasmic reticulum channels (ER), and vesicles (v*) on the periphery of the cytoplasm. Numerous nonreactive vesicles (v') are present throughout the perinuclear cytoplasm. Dark deposits of AChE reaction product are concentrated in the outer space (arrow heads) between an Ep and a lymphocyte (L). Section counterstained with lead citrate. (Original magnification x30,000; current magnification x19,500. Bar = 1 μm.)
the perinuclear cisternae, in the channels of the ER, and in the Golgi complex. This seems to be an indication that the enzyme is endogeneous, since these organelles are responsible for protein synthesis and its intracellular transport. We have not proven, however, that AChE synthesized in the thymic lymphocytes proceeds from the intracytoplasmic membrane system to the cell surface, since we have as yet seen no evidence of membrane-bound AChE. Even so, it is worth noting that the apparent lack of membrane-bound AChE in our experimental system may be due to glutaraldehyde inactivation. It is also worth citing as a parallel the fact that AChE can be demonstrated by the cytochemical method in the cytoplasm of human erythroblasts but not in human erythrocytes, of whose outer membrane this enzyme is an integral part. These results, taken together with our own, lead us to believe that the AChE that we have revealed in the thymic lymphocytes might be a precursor of the membrane-bound form revealed by Szelenyi and co-workers in peripheral T lymphocytes.

In thymic epithelial cells, AChE activity was revealed within the perinuclear cisternae, in the ER, in the Golgi complex, and in the vesicles that discharge the enzyme into the extracellular space. Similarly, cytochemical studies on glutaraldehyde-fixed adrenal gland tissue, by Somogyi et al., have localized AChE activity within the perinuclear cisternae and the ER of chromaffin cells. The authors occasionally observed an apparent joining of the ER channels with the cell membrane, giving the impression that there might be connections between the extracellular space and the space within the ER. They did not, however, present evidence of AChE activity in vesicular structures. Carmichael has recently demonstrated, however, that AChE activity is in fact associated with vesicular structures of chromaffin cells. These observations correlate with the biochemical data of Gratzl et al., who found two forms of AChE, a membrane-bound form as well as a soluble form, within the secretory vesicles isolated from the adrenal medulla. The physiological study by Mizobe and Livett confirms the structural basis for the synthesis and extracellular release of AChE in chromaffin cells.

Although our experimental material is quite different, the distribution of AChE activity in the thymic epithelial cells that we have revealed appears to be comparable to AChE localization in the adrenal medulla.

The function of AChE synthesized by the thymic lymphocytes and epithelial cells is quite unclear. It is possible that AChE synthesized in the human thymus is used for the conduction of nerve impulses in cholinergic synapses of the organ. However, it is not clear from our observations whether the enzyme is transported to the regions of the gland innervation.

As mentioned earlier, it is generally accepted that AChE is not exclusively concentrated in the cholinergic neurons or their effector cells; it is found in both noncholinergic neurons and in nonnervous tissues.

The mechanisms bearing a resemblance to the conduction of the stimuli by excitable membranes of nerve and muscle cells were recently found in human T lymphocytes. It is possible that by controlling ACh concentration in the thymus tissue, AChE modulates cell-to-cell communication by regulating the passage of ions or small molecules or both across membrane surfaces by means of mechanisms similar to that used in neurotransmission.

On the other hand, it has been proposed that the distribution of AChE may parallel that of certain biologically active peptides, and AChE has been shown to be capable of hydrolyzing these compounds. The possibility that AChE may act in the thymus gland by the orderly degradation of some biologically active peptides cannot be excluded.

Finally, although the function of AChE in human thymus gland is unclear, the ability to demonstrate its presence should stimulate further investigation.

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REFERENCES

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