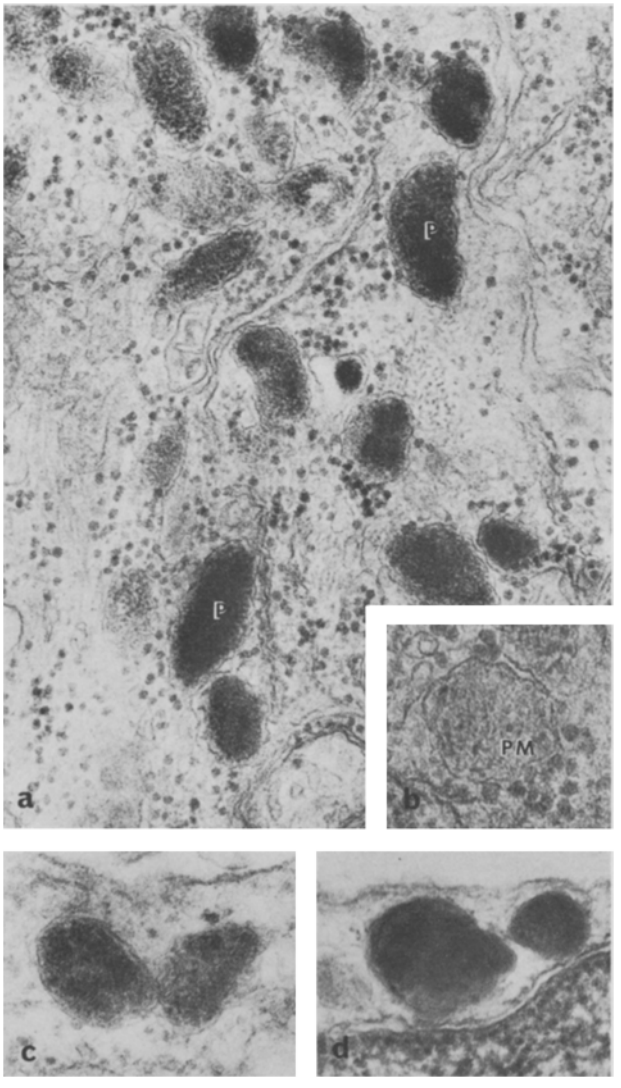

a Pigment granules in choroidal melanophore of the albino goldfish (P). × 50,000. *b* Premelanosome (PM). × 90,000. *c* Pigment granules after incubation with Dopa. × 47,000. *d* Pigment granules after incubation with Dopa and iodoacetoamide. × 47,000.

to Hishida et al.[14] with the slight modification, namely, that the incubation time was shortened from 24 h in their method to 3 h, to avoid the autoxidation of Dopa. This treatment produced full melanization in most of the pigment granules (figure, d). These results indicated that tyrosinase is present in an inhibited state in these pigment granules[14,15]. In several animal species, the albinism has been attributed in part to the inhibition of melanin formation caused by the presence of tyrosinase inhibitor[12].

As described briefly in the previous paper, the particulate or granular type of premelanosome inner structure has recently been reported in the melanocytes of certain skin-color mutants of the mouse and of mammalian melanomas[8]. Additionally, this type of premelanosome was reported in the melanophores of the melanomatous xiphophorin fish[16] and in the melanocytes in café-au-lait spots of neurofibromatosis[17] and in lentigines of Leopard syndrome[18] in man. The albino goldfish seems to be a suitable material for studying the formation of these unusual premelanosomes.

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2 Present address: Biological Institute, Faculty of Science, Shinshu University, Matsumoto 390, Japan.
3 M.S.C. Birbeck, Ann. N.Y. Acad. Sci. *100*, 540 (1963).
4 A.S. Breathnach and S.V. Poyntz, J. Anat. *100*, 549 (1966).
5 J.J. Eppig, Jr, Z. Zellforsch. *103*, 238 (1970).
6 M.S. Forbes, R.A. Zaccaria and J.N. Dent, Am. J. Anat. *138*, 37 (1973).
7 H. Imaki and W. Chavin, Cell Tiss. Res. *158*, 363 (1975).
8 I.K. Takeuchi, Naturwissenschaften *62*, 488 (1975).
9 T. Kajishima and I.K. Takeuchi, J. exp. Zool. *200*, 349 (1977).
10 W.A. Turner, Jr, J.D. Taylor and T.T. Tchen, J. Ultrastruct. Res. *51*, 16 (1975).
11 J.E. Dowling and R. Gibbons, J. Cell Biol. *14*, 450 (1962).
12 I.K. Takeuchi and T. Kajishima, Cell Tiss. Res. *155*, 383 (1974).
13 C. Ide, Z. Zellforsch. *131*, 171 (1972).
14 T. Hishida, H. Tomita and T. Yamamoto, Embryologia *5*, 335 (1961).
15 T. Hama, C. r. Soc. Biol. (Paris) *163*, 236 (1969).
16 U. Vielkind, J. exp. Zool. *196*, 197 (1976).
17 K. Jimbow, G. Szabo and T.B. Fitzpatrick, J. invest. Derm. *61*, 300 (1973).
18 J. Bhawan, D.T. Purtilo, J.A. Riordan, V.K. Saxena and L. Edelstein, J. cutan. Path. *3*, 207 (1976).

Histochemical localization of cholinesterases in the neural tissue of the pineal in the rhesus monkey[1]

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Summary. The neural tissue of the monkey pineal contains both acetyl and butyryl cholinesterases. Acetylcholinesterase was localized in the cisternae of the nuclear membrane, rough endoplasmic reticulum, on the plasma membrane of the neurones, and on the axolemma of both non-myelinated and myelinated fibres. The enzyme was not found in the axo-somatic or axo-dendritic synapses. It is therefore suggested that the pineal neurones have a cholinergic function rather than a cholinoceptive one.

The pineal of the rhesus monkey is distinguished by the presence of a distinctive and substantial mass of neural tissue comprising neurones, axons, dendrites and glial cells[2,3]. Ultrastructural and experimental studies have shown that the pineal-neurones and their dendritic processes are innervated by nerve fibres originating from, or coursing through the habenula[3]. The present studies were carried out to determine the distribution of cholinesterases in the neural tissue of the pineal with a view to understanding its functional role.

14 healthy, adult female rhesus monkeys, each weighing 5–6 kg, were used in the present studies. The pineals of 6 monkeys were processed[4] for studying the distribution of specific (acetyl) and nonspecific (butyryl) cholinesterases by optical microscopy. The pineals from the remaining 8 monkeys were processed[5] for studying the ultrastructural

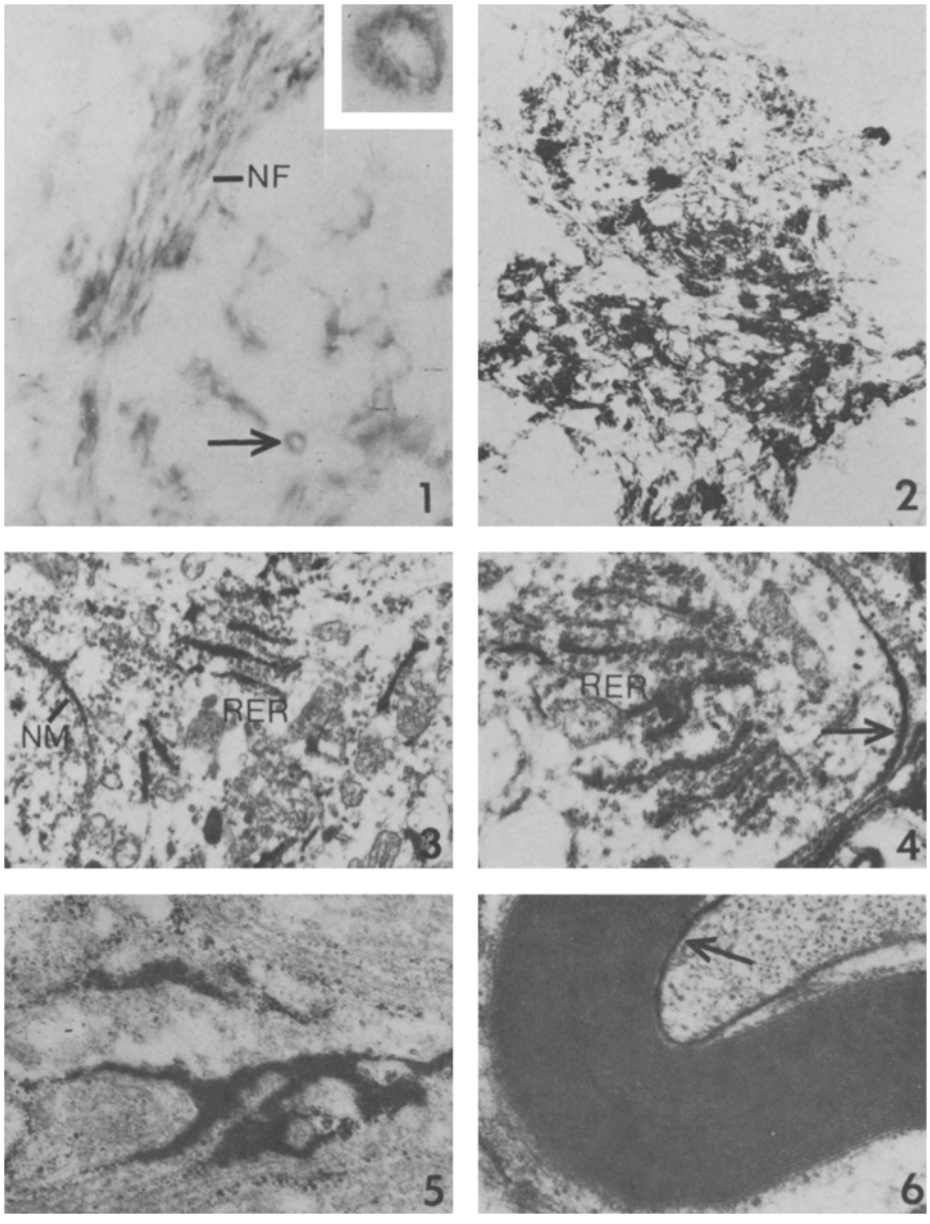

Fig. 1. Photomicrograph showing acetylcholinesterase activity in neurones (arrow) and nerve fibres (NF) of the pineal ×65. Inset. A neurone × 1600.

Fig. 2. Butyrylcholinesterase activity in the neural tissue of the pineal. ×65.

Fig. 3. Electronmicrograph of a neurone showing acetylcholinesterase activity in the cisternae of nuclear membrane (NM) and rough endoplasmic reticulum (RER). × 11,500.

Fig. 4. Acetylcholinesterase activity in the rough endoplasmic reticulum (RER) and plasma membrane (arrow) of a neurone. × 18,000.

Fig. 5. Acetylcholinesterase activity in the inter-space and axolemma of a non-myelinated nerve bundle. × 46,000.

Fig. 6. The axolemma (arrow) of a myelinated nerve fibre showing acetylcholinesterase activity. × 28,000.

distribution and localization of acetylcholinesterase. Toluidine blue-stained sections (1 µm) of pineal tissue embedded in araldite were examined by optical microscopy. Ultrathin sections were stained with uranyl acetate and examined under a Philips EM 300 electron microscope.

Optical microscopic studies revealed that acetylcholinesterase was localized in neurones and in nerve fibres, while butyrylcholinesterase was diffusely distributed throughout the neuropil of the pineal (figures 1 and 2).

Acetylcholinesterase was localized ultrastructurally in the cisternae of the nuclear membrane, rough endoplasmic reticulum (figure 3), on the plasma membrane (figure 4) of the neurones, in the interspaces between the axolemma of non-myelinated axon bundles (figure 5) and on the axolemma of myelinated nerve fibres (figure 6). The enzyme could not be localized in the axo-somatic or axo-dendritic synapses.

The distribution of acetylcholinesterase in the pineal neurones resembles that described in cholinergic nerve cells of the central nervous system[6,7] in that the enzyme is restricted to the cisternae of the nuclear membrane, rough endoplasmic reticulum and the plasma membrane of the neuronal perikaryon and of the axolemma. The absence of acetylcholinesterase in the axo-somatic or axo-dendritic synapses indicates that the pineal neurones are cholinergic rather than cholinoceptive.

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2 W.E. Le Gros Clark, J. Anat. *74*, 471 (1940).
3 G.F.X. David, B. Umberkoman, K. Kumar and T.C. Anand Kumar, in: Brain-Endocrine Interaction II, p. 365. Ed. K.M. Knigge, D.E. Scott, H. Kobayashi and S. Ishii. S. Karger, Basel 1975.
4 V. Navarathnam, P.R. Lewis and C.C.D. Shute, J. Anat. *103*, 255. (1968).
5 G.F.X. David, J. Herbert and G.D.S. Wright, J. Anat. *115*, 79 (1973).
6 P.R. Lewis and C.C.D. Shute, J. Microsc. *89*, 181 (1969).
7 V. Navarathnam and P.R. Lewis, Brain Res. *18*, 411 (1970).