was used. Fluorescence was excited by the green line (546 nm) of a 200 W Hg lamp and the barrier filter passed wavelengths longer than 580 nm. Exposure time for the negative (100 ASA film) was 120 sec.
Chromocenters in interphase nuclei glowed brilliantly. In the metaphase M-chromosomes, the D-bands showed with marked contrast (figure) and were similar to the respective Q-bands[6–10]. The most intense band was on the long arm, close to the centromere, and 2 major and one minor bands were on the opposite side of the centromere, on the arm having the secondary constriction. This observation, combined with that on human chromosomes, lends support to the notion that D-bands are similar to Q-bands in all cases.

Enzymatic patterns in reptilian brain. Histochemical characterization of the optic tectum

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Summary. The enzymatic patterns present in the optic tectum of 4 species belonging to different reptilian orders seem related to the degree of structural and functional organization reached by the nervous centre, as in other vertebrates. In particular the AChE localization in reptiles is representative of a evolutionary sequence in the distribution of this enzyme in the optic tectum along the tetrapode series.

The optic tectum of non-mammalian vertebrates is usually a well-developed brain region which in several classes reaches high levels of structural and functional organization[1–3]. Following up a series of research on histoenzymological characterization of nervous centres in lower vertebrates[4–8], we have studied the histochemical localization of 5 enzymatic activities in the optic tectum of 4 species representative of the main reptilian orders. To our knowledge the histochemical studies on reptilian optic tectum have up till now only used turtle species[9,10].
Materials and methods. We have studied in the optic tectum of lizards (Lacerta muralis), snakes (Natrix natrix), turtles (Pseudemys scripta) and alligators (Caiman sclerops) the following enzymatic activities: acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), monoamine oxidase (MAO), lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH).
For AChE and BuChE demonstration, the brains were fixed for 3–4 h in 10% formol saline at 4 °C, rinsed for 20 min in 0.1 M acetate buffer (pH 6), frozen and cut in the cryostat in transverse or sagittal planes. The sections, 30 µm in thickness, were incubated for 90 min at 22–24 °C in the media of Gerebtzoff[11] or Karnovsky and Roots[12] containing alternatively acetylthiocholine or butyrylthiocholine iodide as substrate. In order to test the actual nature of the enzymatic activity revealed, the following inhibitors were routinely used in a pre-incubation bath (45 min in acetate buffer containing the inhibitor) and in the final medium: the selective AChE inhibitor BW 284C51 5×10^{-5} M, the selective pseudocholinesterase inhibitor iso-OMPA 3×10^{-5} M and the inhibitor of all cholinesterases eserine 3×10^{-5} M[13–16]. For MAO, LDH and SDH demonstration, the unifxed brains were immediately frozen and cut in the cryostat at 20 µm thickness. The sections, adherent to coverslips, were briefly dried in the air and incubated for 45 min at 35 °C in the media of Glenner et al.[17] for MAO or the standard media for LDH and SDH[17]. As control of MAO reaction, some sections were treated in the pre-incubation bath (20 min at 35 °C) and in the final medium with the MAO inhibitor nialamide at 5×10^{-5} or 1×10^{-4} M concentration.
Results. AChE activity is usually weak in deeper tectal layers, and weak to moderate in the stratum griseum centrale while it is always present in the stratum fibrosum and griseum superficiale of Huber and Crosby[18], showing noticeable differences in distribution patterns among the different reptilian species examined. In Natrix (figure 1) the histochemical reaction is weak and uniformly spread all over the layer. In Lacerta (figure 2) and Pseudemys a similar distribution pattern exists: the middle band of the stratum fibrosum and griseum superficiale, largely corresponding to the stratum fusiforme retinum of Leghissa[2], shows a very strong reaction while the 2 adjacent plexiform layers exhibit much lower AChE activity. In Caiman (figure 3), the stratum fibrosum and griseum superficiale shows a clear laminar pattern with 2 bands of strong AChE activity severed by a wider band with moderate reaction. In the stratum opticum, the histochemical reaction is moderate or negative; in Lacerta and Pseudemys the stratum opticum is overlaid by a thin stratum zonale[1] showing moderate reaction. BuChE activity is not appreciable in the optic tectum of Pseudemys and Caiman; a very weak reaction for BuChE is present in Natrix and a more intense one in Lacerta (figure 4), both localized at level of fibrous tectal layers. The histochemical controls have confirmed the respective localization of AChE and BuChE: the reaction recorded with media containing acetylthiocholine is not sensitive to iso-OMPA while is inhibited by BW 284C51; the contrary

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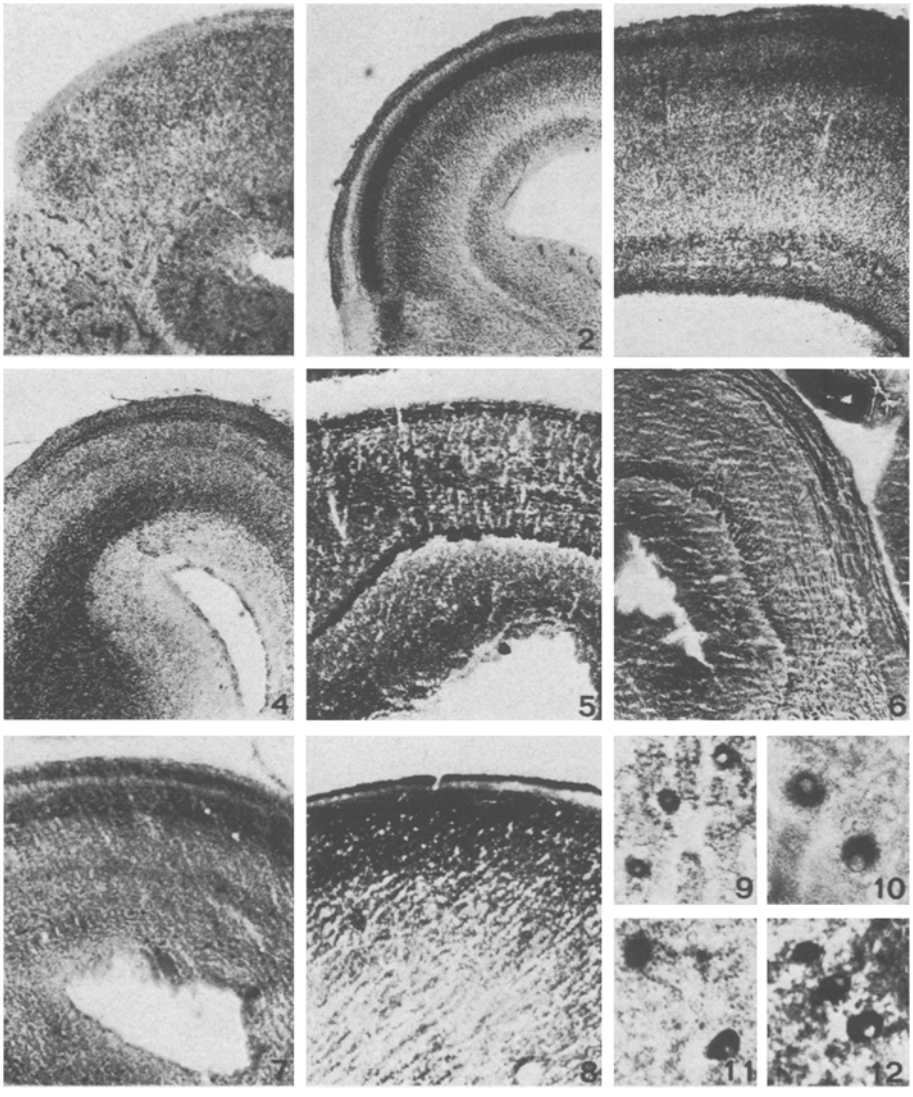
is true for media containing butyrylthiocholine; both the 2 types of reaction are inhibited by eserine. Finally the 2 methods of Gerebtzoff, and Karnovsky and Roots, have given largely equivalent results.

MAO activity in the optic tectum exhibits comparable features in the 4 reptilian species examined. The enzymatic activity is mainly localized in fibrous layers of the tectum: the histochemical reaction is moderate to strong in the stratum fibrosum periventriculare, the stratum album centrale and the stratum opticum (figures 5 and 6), with some differences in the reaction intensity among the different species. In the sections treated with nialamide, no distinct reaction is observable.

The features of LDH and SDH localization are similar in the optic tectum of the different reptilian species (figures 7 and 8). These enzymes appear more concentrated in cellular than in plexiform and fibrous layers and usually 1 of the 2 enzymes shows a distribution pattern similar to that of AChE. In Pseudemys and Lacerta the histochemical reaction for LDH and SDH shows almost equivalent intensity, while in Caiman LDH activity seems to be higher, and the contrary occurs in Natrix. In enzyme-rich layers, the histochemical reaction for LDH and SDH, like the reaction for AChE, is equally intense in neuronal cell bodies and in the surrounding neuropil with the exception of the neurons of the mesencephalic trigeminal root. These large, globe-shaped nerve cells exhibit strong reaction for SDH and particularly for LDH (figures 9–12) and the neuronal cell bodies are clearly outlined on the background of the surrounding neuropil.

Discussion. The present observations add to the available data on enzymatic patterns present in reptilian optic tectum, and confirm and extend the observations previously carried out on turtles[9,10]. Our results reveal some variability in AChE distribution among the different reptilian orders, even if the enzymatic activity is always prominent in the stratum griseum and fibrosum super-



Figs. 1–3. Histochemical localization of AChE in the optic tectum of Natrix (figure 1, Gerebtzoff method), Lacerta (figure 2, Gerebtzoff method) and Caiman (figure 3, Karnovsky and Roots method). ×30. Fig. 4. Histochemical localization of BuChE in the optic tectum of Lacerta (Gerebtzoff method). ×30. Figs. 5 and 6. MAO activity in the optic tectum of Pseudemys (figure 5) and Lacerta (figure 6). ×28. Fig. 7. LDH activity in the optic tectum of Lacerta. ×30. Fig. 8. SDH activity in the optic tectum of Natrix. ×45. Figs. 9–12. Dehydrogenase localization in the neurons of the mesencephalic trigeminal root. Fig. 9. SDH in Lacerta. Fig. 10. SDH in Caiman. Fig. 11. LDH in Lacerta. Fig. 12. LDH in Pseudemys. ×90.

ficiale. If we compare AChE localization in the optic tectum of reptiles with that of amphibians and birds[7,10,19–21], a clear evolutive sequence is observable. In all cases, the enzymatic activity is prevailingly localized in the stratum fibrosum and griseum superficiale, but its distribution pattern shows progressive lamination in connection with the concurrent development and differentiation of the same stratum, which reaches a maximum in the birds[22,23]. This process of lamination is emphasized by the AChE distribution pattern in the series frogs-lizards and turtles-alligator-birds, while the snakes seem to possess a distribution pattern not clearly related to the structural organization of the stratum fibrosum and griseum superficiale. In this connection, however, one must remember that the optic tectum of snakes, and in particular the stratum fibrosum and griseum superficiale, shows reduction in differentiation and structural organization in comparison with the other reptiles[2,18,24]. Since the stratum fibrosum and griseum superficiale constitutes the main receptive layer for sensitive discharge, mainly the retinal one, the features of AChE distribution strongly suggest a precise correlation between the enzymatic activity and the mechanisms of reception and modulation of sensitive input to the tectum. The uneven BuChE distribution in reptilian optic tectum confirms the extreme species-dependence of this enzymatic activity, as previously pointed out in birds and mammals[25].

The preferential MAO localization at level of fibrous layers of the tectum seems to be a common feature in vertebrate optic tectum; a similar kind of MAO distribution exists in teleosts[26], amphibians[7] and birds[10,21]. In addition, the distribution pattern observed in reptilian optic tectum is in good agreement with the localization of serotonine and catecholamine-containing nerve terminals, as revealed by the method of formaldehyde-induced fluorescence[27–29].

The dorsal tectal areas and in particular the stratum fibrosum and griseum superficiale are provided with high activity of oxidative enzymes as previously pointed out for SDH in other reptilian species[9,30]. In reptilian optic tectum, a connection seems to exist between these enzymes and AChE distribution; in fact the areas provided with high AChE activity show substantial activity of LDH and/or SDH. This kind of relationship is usually present in many nervous regions of other vertebrates[25]. In conclusion, the optic tectum in reptiles, as in other vertebrates[6,7,9,10,19–21], exhibits a close relationship between the level of structural and functional organization, and the distribution patterns of some enzymes connected with energy metabolism and other enzymes connected with the specific neural function.

The results of the present work, together with other studies on enzyme distribution in the vertebrate nervous system, provide further support for the research-line of the chemical mapping of the brain, as stated by Friede[25].

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Nuclear changes in cultured human dystrophic muscle

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Summary. Nuclear size in myotubes in cultured human dystrophic muscle has been found to be significantly greater than in normal muscle. These findings are discussed in relation to the pathogenesis of muscular dystrophy.

Significant enlargement of the muscle nucleus has been observed in biopsies from children with Duchenne muscular dystrophy (DMD)[3] and in some male fetuses at risk for DMD[4]. This finding was interpreted in terms of modified nucleo-cytoplasmic relationships. The present study was undertaken to determine if similar changes in the muscle fibre nucleus occur during the early stages of dystrophic muscle development in vitro.

Materials and methods. Myogenic cell lines were prepared from fresh muscle biopsies of 6 normal and 2 dystrophic children. The samples were processed as soon as possible after biopsy and were first washed in phosphate buffered saline solution containing 100 units/ml Penicillin, 200 mg/ml Streptomycin and 2.5 µg/ml Amphotericin B (Mg and Ca ion free). The explants were clotted using a 1:1 mixture of filtered chick embryo extract and cock plasma for a few minutes. Once clotting had taken place, the explants were overlaid with Hams F-10 containing 10% fetal calf serum (FCS) and the same concentration of antibiotics. Explants with well developed myotubes were used for secondary cultures. These were set up following trypsinisation (0.25% trypsin in Dulbecco – pH 7.4). The differentiation of myoblasts was examined after 15 and 25 days culture in 10% FCS on coverslips subsequently stained with haematoxylin and eosin.

1 The authors wish to thank Mrs L. McGregor and Miss L. Andruzczenko for technical assistance. This work was supported by the Muscular Dystrophy Group of Great Britain. D. V. is in receipt of a scholarship from the State Scholarship Foundation of Greece.
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