tion is evident in the case of soluble fraction, an inhibition is clearly shown by the particulate and the nuclear ones. Treatment of the enzyme with Mg^{++} leads to a small activation in all the cases.

These results are in good agreement with those of Wang[11] and Strittmatter[12,13] and strongly suggest that different enzyme forms are present in different cell compartments of the chick liver. Since 3 electrophoretically different acid phosphatases are present in the whole homogenate[14], it is particularly interesting to point out which of the enzyme bands are present in each subcellular fraction. The electrophoretic analysis reported in the figure confirms that 3 enzyme activity bands, here labelled P1, P2 and P3, are detected in the whole homogenate (H). The particulate fraction shows the presence of the 2 slower bands P1 and P2, whereas the soluble one contains only the fast migrating band P3. All the enzymatic bands are present in the nuclei. The migration differences between the electrophoretic bands of samples N and P and those of H are due to the preliminary purification procedures. It has been demonstrated by Allen and Gockerman[2] that the physical and physicochemical treatments leading to the purification of the subcellular fractions of rat liver cells strongly influence the electrophoretic mobilities of the acid phosphatases. Also in the case of chick the electrophoretic behavior depends on the preliminary treatment of the samples. So, the total homogenate, which is not subjected to purification procedures like the nuclear and the particulate fractions shows an electrophoretic migration of bands lower than that of samples N and P. When the particulate and the soluble fractions are mixed together, the distribution of bands typical of the total homogenate is restored, though the migration on the gel is increased according to that of N and P samples.

These electrophoretic results confirm what was suggested by the inhibition experiments reported here and furthermore demonstrate that acid phosphatase activity in the particulate fraction is due to 2 almost electrophoretically distinct molecule types, whereas that of the soluble fraction is due to only one.

Previous work on the heterogeneity of acid phosphatase demonstrated the existence of 2 molecular forms of this enzyme in the mitochondrial-lysosomal fraction of rat liver homogenate and of only one molecular form in the soluble fraction of the liver of some mammalian species[8-10]. Our results demonstrate that a similar heterogeneity is also found in bird liver and the subcellular distribution of acid phosphatase molecular forms is similar.

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The localization of acetylcholinesterase in the optic lobe in the developing chick embryo

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Summary. The localization of acetylcholinesterase (AChE) in the optic lobe in the developing chick embryo was studied histochemically and biochemically. The histochemical reaction of AChE increased remarkably between stage 42 and 44 especially in the neuropile. The increase of the biochemical activity of the AChE in the synaptic membrane fraction occurred at a later stage than that in the microsomal fraction. These findings can be interpreted as the result of axonal transport of the enzyme from the synthetic to the synaptic site.

It is known that a high concentration of AChE is present in the optic lobe in non-mammalian vertebrates[2]. Especially, it has been suggested that the enzyme plays an important role in relation to intrinsic neuronal activity in the optic tectum[3,4]. Boell et al.[5] and Filogamo[6]studied the change in the localization of tectal AChE in the developing tadpole and the chick embryo respectively, so as to follow the relationship between the distribution of the enzyme and synapse formation. On the other hand, Ciani et al.[4] studied AChE in the quail optic tectum by electron microscopy and suggested that the enzyme was released from the site of synthesis to the neuropile. In this report, in order to relate the localization of AChE in the developing chick embryo optic lobe with the cell structure, the changes in the subcellular distribution of the enzyme activity were compared with the changes in its histochemical localization.

Materials and methods. Fertilized eggs from the White Leghorn strain of chicken were incubated at 37.5 °C (±1 °C) and approximately 80% relative humidity until used. The developmental stages were determined at the time of sacrifice according to the description of Hamburger and Hamilton[7]. Experiments were performed from stages 38 to 45+. For AChE histochemistry, the brains were fixed in buffered fixative (pH 7.4), containing 4% paraformaldehyde, at 4 °C for 16 h, and washed with Holt's Gum-Sucrose solution at 4 °C for 24 h. The fixed brains were fast-frozen and cut into 10-μm sections with a cryostat. Sections were processed according to the method of Karnovsky and Roots[8]. For the quantitative determination of AChE in subcellular fractions, both optic lobes were homogenized in ice-cold 0.32 M sucrose and fractionated according to the method of Inoue et al.[9] with some modifications.

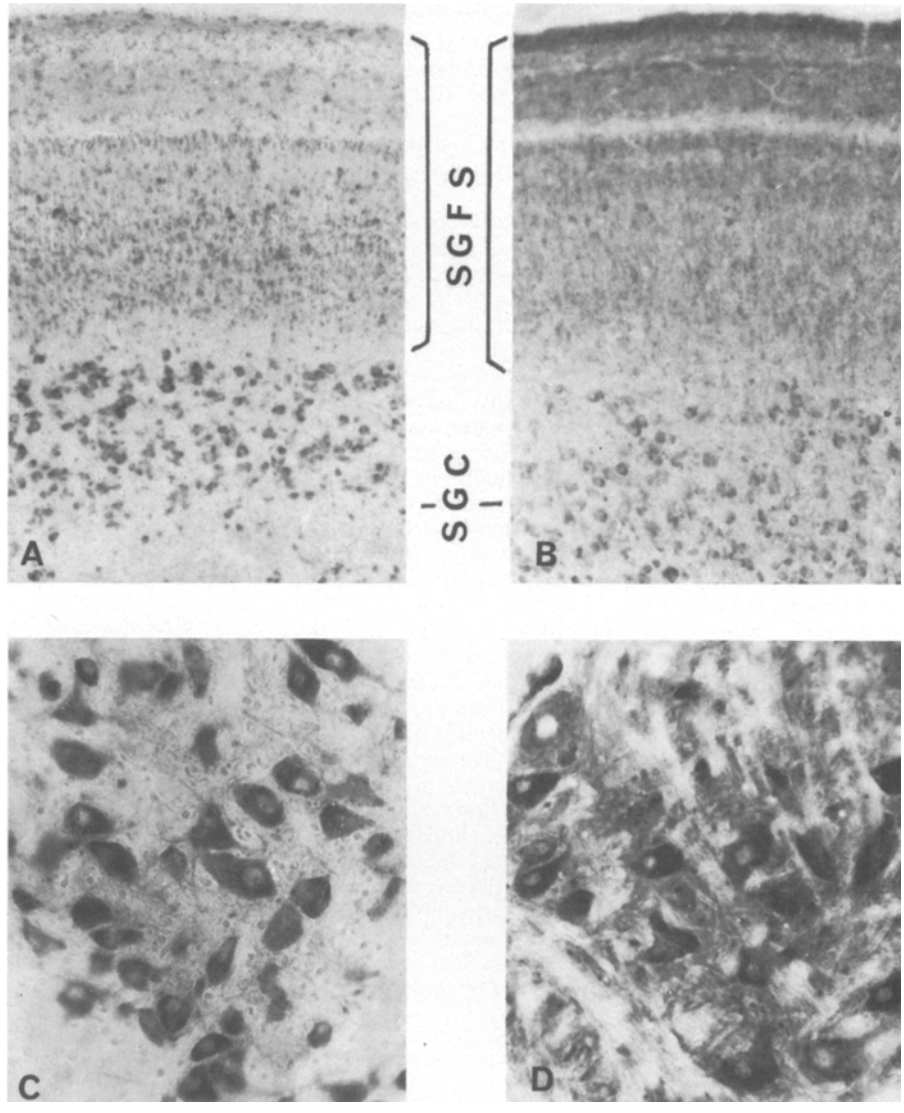
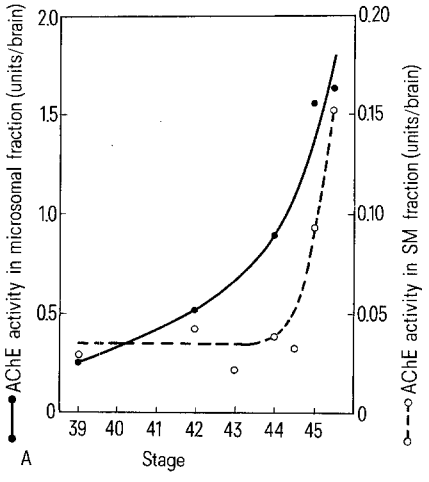


Fig. 1. *a, b* Histochemical localization of AChE in the chick embryo optic tectum at stage 42 (a) and at stage 44 (b). × 100. *c, d* Histochemical localization of AChE in the nucleus isthmi pars magnocellularis at stage 42 (c) and at stage 45 (d). × 250. Notice the more intense reaction of the enzyme in the neuropile at later stages in both areas. SGFS, stratum griseum et fibrosum superficiale; SGC, stratum griseum centrale.



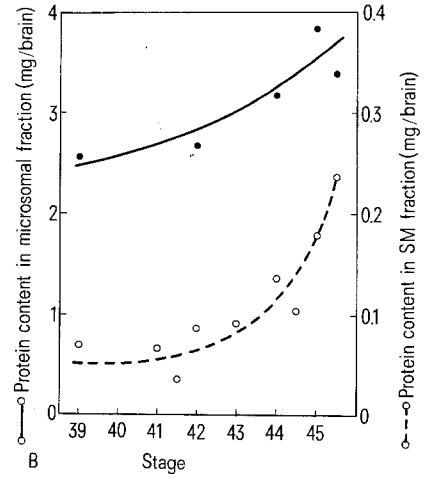


Fig. 2. Changes in the AChE total activity (A) and changes in protein content (B) in subcellular fractions of the chick embryo optic lobe. The increase of the AChE activity in the SM fraction (○---○) is found at later stages than that in the microsomal fraction (●——●).

The crude mitochondrial fraction, which was subjected to osmotic shock, was resuspended in 0.32 M sucrose and subfractionated on a discontinuous sucrose density gradient (50,000 × g, 1 h). The layer which formed at the interface between 0.8 M and 1.0 M sucrose was used as the synaptic membrane fraction. The enzyme activity and protein content were measured by the methods of Ellman et al.[10] and Lowry et al.[11] respectively.

Results and discussion. The histochemical reaction of AChE in chick embryo optic lobe increased rapidly between stages 42 and 44, and this increase was especially remarkable in the neuropile of the tectum and the mesencephalic nuclei. In the tectum, the reaction of the neuronal cell bodies can be recognized in all the layers at stage 42; however, it is obscured by the more intense reaction in the neuropile of the various layers, with the exception of the stratum griseum centrale, at stage 44 (figure 1, a and b). In the nucleus isthmi pars magnocellularis, also, the more intense reaction of the enzyme in the neuropile at stage 45 compared with that at stage 42 is noticeable (figure 1, c and d). These results, specially in the tectum, are the same as those which Filogamo[6] observed. The point to notice in particular is that the reaction in the neuropile increases as development proceeds.

On the other hand, the biochemical activity of AChE in the optic lobe rose gradually from about stage 40 to hatching, in the microsomal fraction, while that in the synaptic membrane fraction (SM) rose rapidly from about stage 44 to hatching (figure 2, a). Namely, the increase of the AChE activity in the SM fraction was found at later stages than that in the microsomal fraction. The increase in the protein content in the optic lobe SM fraction also occurred at a later stage than that in the microsomal fraction (figure 2, b). The biochemical activity in the microsomal fraction and the SM fraction can be considered as reflecting the enzyme levels within the cell body and synaptic site. So the changes of AChE activity in the subcellular fractions of the chick embryo optic lobe are consistent with the concept that AChE is synthesized primarily in the cytoplasm of the neuronal cell body, and is then transported to the synaptic site by axonal flow. This concept has been suggested by Austin et al.[12] on the basis of the rates of regeneration of AChE in rat subcellular fractions following DFP inhibition. The change in protein content in the subcellular fractions reveals that other proteins are also transported by axonal flow. It is also possible to consider that the histochemical reaction of AChE in the neuropile is due to the enzyme activity of a neuronal network which contains synaptic contacts. Accordingly, the increase in the histochemical reaction of AChE in the neuropile of the developing chick optic lobe can be interpreted as the result of the transport of the enzyme from the cytoplasm of the neuronal cell body to the synaptic site.

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La croissance cellulaire de quelques tissus au cours des trois derniers stades larvaires de *Pieris brassicae* L. (Lepidoptera: Pieridae)

The cellular growth of certain tissues in last three instar larvae of *Pieris brassicae* L. (Lepidoptera: Pieridae)

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Summary. The cellular growth of certain tissues in last 3 instar larvae of *Pieris brassicae* L. was studied in relation to moult, body growth and also with variations in the size of prothoracic gland.

Trager[2,3] a étudié d'une façon détaillée la croissance cellulaire chez *Lucilia sericata, Bombyx mori* et *Aedes aegypti* en relation avec la croissance de l'animal. Il montre que toute la croissance larvaire chez *Lucilia sericata* est due à une augmentation de la taille des cellules et non à une multiplication de celles-ci. Par contre, chez *B. mori* et *Ae. aegypti*, certains tissus subissent une croissance cellulaire, d'autres une multiplication cellulaire. La croissance se fait de 2 façons: elle peut être proportionnelle à l'augmentation de la taille du corps ou bien être plus rapide que celle-ci. La taille des cellules qui se multiplient augmente proportionnellement moins rapidement que la taille du corps. Les résultats obtenus conduisent Trager à l'hypothèse suivante: les tissus larvaires qui s'histolysent entièrement durant la nymphose tendent à croître par une augmentation de taille de leurs cellules, tandis que ceux qui persistent jusqu'à l'imago croissent par multiplication cellulaire. Plusieurs auteurs ont également confirmé cette hypothèse[4–8]. Cependant ce type de croissance n'a été étudié que par quelques auteurs[9,10]. Nous traitons ce problème au cours des 3 derniers stades larvaires de *Pieris brassicae*, analysant la consommation de la nourriture, la croissance pondérale et linéaire, la surface interne de l'intestin moyen et la croissance des cellules épitheliales de l'intestin moyen, des cellules du corps gras, des cellules épidermiques et des oenocytes.

Matériel et méthodes: Les chenilles ont été élevées selon les techniques habituelles[11,12]. Pour les observations histologiques, le premier segment abdominal des chenilles à divers stades est fixé au Bouin et inclu à la paraffine, les coupes de