found that brain PEase differs from PEase of other organs in that a considerable fraction is insoluble. This fraction can be rendered soluble by treatment with Triton X-100. One possible explanation of our findings is that PEase-inhibition has an effect on this insoluble enzyme or interacts with the proteinlike inhibitor reported by CHEUNG[21].

A more accurate insight into the causal relationships discussed in this paper will emerge from further work on isolated isoenzymes and total PEase preparations from various organs.

Zusammenfassung. Die Hemmung der cAMP-Phosphodiesterase in verschiedenen Organen der Katze durch Hydergin und DHE wurde in vitro bestimmt. In Konzentrationen von $10^{-4}M$ bis $10^{-6}M$ wurde ein Hemmeffekt nur im Gehirn beobachtet. Diese Wirkung wird besonders deutlich, wenn man das infolge der Enzymhemmung nicht umgesetzte cAMP berechnet.

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Ontogenetic Differences between Acetyl and Butyrylcholinesterase Isozymes in the Chick Embryo Cerebellum

Multiple forms of acetylcholinesterase (AchE: E.C.3.1.1.7) and butyrylcholinesterase (BuchE: E.C.3.1.1.8) have been detected in neural tissue and the serum of many vertebrate species[1–5]. Total AchE synthesis increases during periods of accelerated brain development[6], with the appearance of synaptic vesicles, and with bioelectric activity in the spinal cord[7]. Rapid increases of total AchE and BuchE content are known to occur in the chick cerebellum between the 10th and 14th day of incubation[8]. This interval corresponds to the first occurrence of extensive body movements by the chick embryo[9]. In *Ambystoma punctatum* significant increases in AchE also first occur with motile behavior[10,11]. Thus, AchE and BuchE isozymes have been considered as indices of vertebrate neural and neuralmuscular development. This report describes changes in AchE and BuchE isozymes during this period of rapid neural development in the chick embryo cerebellum by the techniques of isoelectric focusing.

Embryos were obtained from a genetically homogenous strain of White Leghorn chickens and incubated under standard conditions. Tissues were collected from 10- and 14-day-old embryos and prepared for enzyme analysis[12,13]. A 110 ml isoelectric focusing column was prepared according to manufacturer's instructions[14]. Twice the recommended amount of Ampholytes (pH 3–10) were used to increase protein solubility with 0.1 ml of the supernatant being added after the column was partially filled. Conditions of 4 °C, 600 V, and 10 mA were maintained with the cathode at the top of the column. When the current stabilized at 1–2 mA the column was emptied in 40 drop fractions. The pH of each fraction was recorded and then adjusted to pH 8.0 to eliminate any pH effect on the enzyme reactions. Separate experiments demonstrated that Ampholytes had no effect on the basic enzyme reaction and that different homogenizing media[12] did not significantly alter the pH gradient. 6 replicates were analyzed for each age. Comparisons were made between the isoelectric point of each isozyme within an isozyme complex (either AchE and BuchE) by F-ratió analysis and DUNCAN's multiple range test and between isozyme complexes (AchE and BuchE) with the student *t*-test.

Three isozymes were resolved for both AchE and BuchE in the 10-day-old cerebellum (Figure 1). The activities between each isozyme complex were significantly different ($P \leq 0.05$). AchE isozymes had isoelectric points similar to those of the BuchE isozymes. Analysis within isozyme complexes demonstrated that the isoelectric points of the different isozymes of each enzyme were significantly different ($P \leq 0.001$).

Five isozymes of AchE and BuchE were detected in the 14-day-old cerebellum (Figure 2). Enzymes activities were significantly different ($P \leq 0.05$) between AchE and BuchE. The isoelectric points between AchE and BuchE isozymes I, II, and III were significantly different ($P \leq 0.01$); AchE and BuchE isozymes IV and V had

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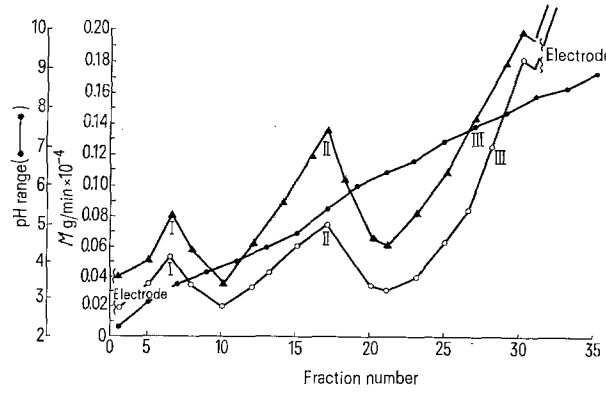

Fig. 1. 10-day-old cerebellum isoelectric focusing of AchE and BuchE isozymes. Key: ●, pH; ▲, AchE; and ○, BuchE.

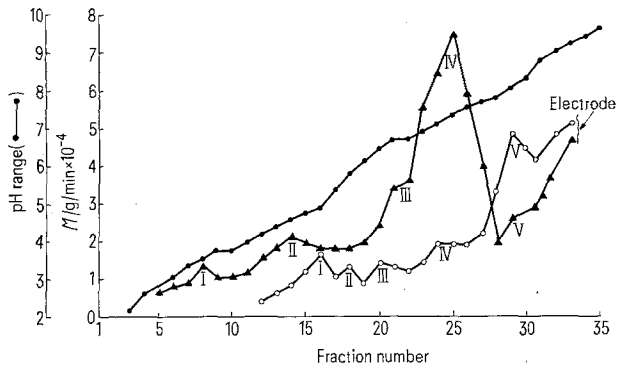

Fig. 2. 14-day-old cerebellum isoelectric focusing of AchE and BuchE isozymes. Key: ●, pH; ▲, AchE; and ○, BuchE.

similar isoelectric points. Analysis within each isozyme complex indicated that the isoelectric points of different isozymes of each enzyme were significantly different ($P \leqq 0.01$). The isoelectric point occurred in the same fraction ± 1 in each of the 6 replicates. Because the curves were superimposable, it was possible to further verify that isozymes I, III and V of BuchE and II of AchE were always present and were not artefacts.

Studies using acrylamide gel electrophoresis had previously indicated the presence of only 3 AchE isozymes in the chick embryo between 3 and 18 days of incubation[2]. The detection of 2 additional AchE isozymes and 5 BuchE isozymes in the present study could be due to several factors: reduction in the activity of AchE isozymes due to cross linking in the acrylamide gels[15], and the greater sensitivity of the isoelectric focusing technique.

In the 10-day-old chick embryo cerebellum the isoelectric points of the 3 AchE isozymes were similar to those of 3 BuchE isozymes. It is not possible from the present data to determine whether a single enzyme exists with several substrate specificities or if different proteins exist with identical isoelectric points. These results suggest a dimeric structure for both AchE and BuchE under the control of 2 genes. However, a tetramer structure is still possible.

It has been reported that AchE is a tetramer which can be dissociated into dimers and monomers[3]. In the 14-day-old chick embryo cerebellum 5 isozymes of both AchE and BuchE were found which is consistent with AchE[16] and BuchE[17]. Isozymes had isoelectric points different from the 3 BuchE isozymes suggesting that different proteins are present. The results described here for 14 days are consistent with the LDH model[18] in which 2 different polypeptides may combine into as many as 5 different tetramer complexes. Since 2 enzymes are present, both tetramers would require 2 structural genes each. The switch from the 10 day isozyme complex to the 14-day-old isozyme complex of AchE and BuchE may be interpreted as a differentiating step requiring gene control. These biochemical changes in isozymes of AchE and BuchE neuroenzymes occur during a period of intense cerebellar differentiation that is associated with the ontogeny of movement.

Résumé. Les isozymes d'acétylcholinestérase et de butyrylcholinestérase du cervelet des embryons de poulet de 10 à 14 jours furent séparés par la méthode de mise au point isoélectrique. 3 isozymes de l'un et de l'autre enzyme étaient présents dans les embryons de 10 jours. Dans les embryons de 14 jours il y avait 5 isozymes différents le uns des autres et de ceux qui étaient présents dans les embryons de 10 jours. On estime donc que les changements apparus dans la quantité, dans les points isoélectriques, et dans les niveaux d'activité maxima des isozymes neurotransmetteurs sont liés à la maturation du cervelet et à l'acquisition du mouvement pendant la morphogénèse de l'embryon. Les résultats sont exprimés en accord avec le modèle LDH pour la différentiation de l'isozyme.

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Sterols and Sterol Biosynthesis in the Slug *Aplysia depilans*

Opisthobranchia have been little successful in attracting the attention of biochemists. As a result hardly anything is known about the origin and the composition of the sterols present in this group. In fact our knowledge of this subject in the subclass Pleurocoela is confined to the single datum that the sterols of *Aplysia kurodai* seem to consist mainly of cholesterol.[1] For this reason we decided to study the sterol composition and sterol biosynthesis of *Aplysia depilans*.

Three specimens of *Aplysia depilans*, with a fresh weight of 400 g, were collected in the neighbourhood of Stazione Zoologica at Naples, Italy. The animals were each injected with an aqueous solution of sodium acetate-1-^{14}C (NEN Chemicals, specific activity 1.0 mCi/41.0 mg). The total dosage administered amounted to 400 μCi. The animals were maintained in well-aerated seawater for 82 h and then fixed in ethanol.

Lipids were extracted from the animals[2], purified[3] and separated into phospholipids and neutral lipids[4,5]. The neutral lipids were separated into various lipid classes by means of column chromatography on Florisil[6].

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