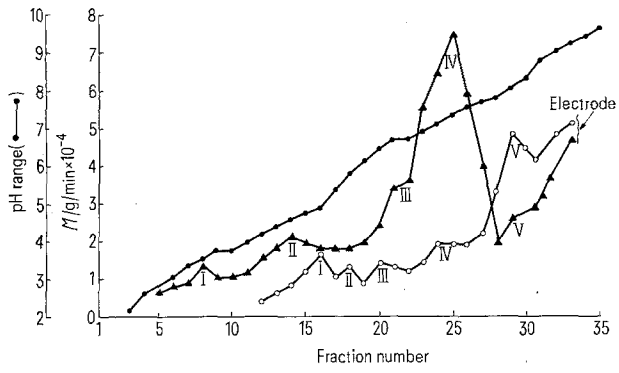

Fig. 2. 14-day-old cerebellum isoelectric focusing of AchE and BuchE isozymes. Key: ●, pH; ▲, AchE; and ○, BuchE.

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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5 February 1973.

15 Y. DEGANI and T. MIRON, Biochim. biophys. Acta *212*, 362 (1970).
16 E. M. SCOTT and R. F. POWERS, Nature, New Biol. *236*, 83 (1972).
17 R. SCHIFF, J. P. LEWIS and C. E. CROSS, J. Histochem. Cytochem. *20*, 472 (1972).
18 C. L. MARKET, *Cytodifferentiation and Macromolecular Synthesis* (Edited M. LOCKE; Academic Press, New York 9163) p. 65.
19 This work was supported by U. Wyoming, Division of Basic Research and Research Coordination Council Grants.

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].

1 T. TANAKA and Y. TOYAMA, Mem. Fac. Eng., Nagoya Univ. *11*, 182 (1959).
2 D. J. VAN DER HORST, A. H. VAN GENNIP and P. A. VOOGT, Lipids *4*, 300 (1969).
3 CH. TERNER, E. I. SZABO and N. A. SMITH, J. Chromat. *47*, 15 (1970).
4 S. R. LIPSKY, A. HAAVIK, C. L. HOPPER and R. W. MCDIVITT, J. clin. Invest. *36*, 233 (1957).
5 D. J. HANAHAN, J. C. DITTMER and E. WARASHINA, J. biol. Chem. *228*, 685 (1957).
6 K. K. CARROLL, J. Lipid Res. *2*, 135 (1961).

Table I. Quantities and radioactivities of the isolated lipid fractions of *Aplysia depilans*

Lipid fraction	Quantities		Radioactivity	
	mg	Total lipids (%)	Specific radioactivity (dpm/mg)	Radioactivity incorporated into total lipids (%)
Total lipids	1041.8	100	47,374	100
(% of fresh weight)	0.26			
(% of total dosage of radioactivity administered)			5.56	
Phospholipids	158.3	15.2	43,079	13.8
Neutral lipids	716.5	68.8	50,233	72.9
Hydrocarbon fraction	36.9	3.5	475	0.4
Fraction containing sterolesters and methylesters of fatty acids	224.2	21.5	45,323	20.5
Triglycerides	77.8	7.5	38,320	6.0
Sterol fraction	94.7	9.1	27,720	5.3
Remaining fraction	258.9	24.9	61,183	32.1

The fraction containing sterolesters was saponified with 1.5 N KOH in 80% methanol under the usual conditions, after which the sterols were isolated. Free sterols and sterols from the esters were purified further by means of TLC using benzene-diethylether-ethanol-acetic acid (50 + 40 + 2 + 0.2) and isopropylether-acetic acid (96 + 4) as the moving phases.

Radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrometer, Model 2420. The scintillation medium consisted of toluene with 0.4% Omnifluor (NEN Chemicals).

Gaschromatography of the 3β-sterols was carried out on a Becker instrument, Model 420, with flame ionisation detection. The glass columns (200 × 0.38 cm I.D.) were either filled with Chromosorb G (100–120 mesh) deactivated with PVP and coated with NPGS[7] or with Chromosorb W (100–120 mesh) deactivated with DMCS and coated with SE-30[7]. Column temperature was 205 °C. Hydrogenation, acetylation and preparation of trimethylsilylethers of the sterols was performed as described elsewhere[7]. Sterols were identified by means of their steroid numbers[8].

Lipids were separated into various fractions, and the specific radioactivity of each fraction was determined. The results are given in Table I.

This Table shows that lipid content of *Aplysia* is low. This holds even in comparison with the corresponding value of other molluscs, as contents of about 1% are commonly met here. It is striking that triglycerides are only present in relative small amounts, and further that the fraction containing sterolesters and methylesters of fatty acids together with the remaining fraction, containing among other fatty acids, make up nearly half of the total lipids.

The Table also shows that 5.56% of total dosage of radioactivity administered was incorporated into lipids. Incorporation of radioactivity into the various lipid fractions shows a picture similar to that of the distribution of weights, mentioned above.

The fraction containing sterolesters and methylesters of fatty acids was saponified and yielded 7.9 mg of sterols, which possessed specific radioactivities of 11,205 and 11,328 dpm/mg after one and two purifications respectively. The crude sterol fraction was purified and contained 45.1 mg of sterols. The specific radioactivity of these sterols was determined and amounted to 22,410 and 21,701 dpm/mg after one and two purifications respectively. From the foregoing it can be concluded that *Aplysia* is able to synthesize from acetate a variety of lipids including sterols.

Chromatograms of the hydrogenated sterols showed only 2 peaks corresponding with sterols containing 26 and 27 carbon atoms and making up 0.5% and 99.5% of the total sterols respectively. In chromatograms of the TMS ethers 6 peaks were present; the 1st one representing a C_{26} sterol, the next 3 ones C_{27} sterols, the 5th one a C_{28} sterol, while the 6th one, with steroid numbers 32.18 and 32.81 on SE-30 and NPGS, respectively, could not be identified. The proportional composition is given in Table II.

In conformity with the single datum in the literature[1], cholesterol is the main sterol in *Aplysia*, but as could be expected some other sterols are present in addition.

Zusammenfassung. *Aplysia depilans* ist fähig, Lipide, einschliesslich der Sterinen, aus Azetat zu synthetisieren. Cholesterin ist das wichtigste Sterin (87%), aber auch andere Sterine, bisher bei Opisthobranchia unbekannt, wurden gefunden.

Table II. Proportional composition of the 3β-sterols of *Aplysia depilans*

Sterol	%
C_{26} sterol	0.5
22-*cis*-cholesta-5,22-dien-3β-ol	2.6
cholest-5-en-3β-ol	87.4
cholesta-5,24-dien-3β-ol	8.3
24-methylenecholest-5-en-3β-ol	0.4
unidentified (C_{29}?)	1.1

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[7] P. A. VOOGT, Archs. int. Physiol. Biochim. *79*, 391 (1971).
[8] P. A. VOOGT, Neth. J. Zool. *22*, 489 (1972).