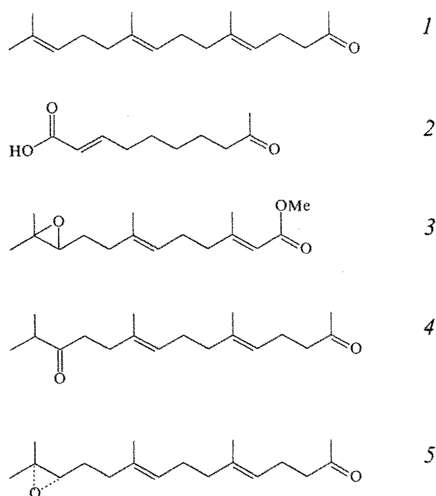

vitro. It was known that high concentrations of *3* in the honeybee [10] had an inhibitory effect on vitellogenesis.

In the present communication, we wish to report the biological activity of two natural derivatives of farnesylacetone *1*, namely the diketone *4* and the epoxide *5*, previously isolated with *1* [11] from the brown alga *Cystophora moniliformis*. The material and methods used in this work have already been described elsewhere [3]. All experiments have been repeated twice and in the two sets performed in triplicate, the results obtained being similar in each parallel determination. The methylasic system used was prepared from crab testis and had an activity of 90 dpm/mg protein.

With diketone *4*, a maximum inhibition of 45% is noticed on the transmethylations of calf thymus histones at a concentration of 270 ng/ml and 14% with *E. coli* B tRNA, concentration 300 ng/ml. Using epoxifarnesylacetone *5*, the observed maxima of inhibition are 49% for histones (with 270 ng/ml) and 14% for tRNA (450 ng/ml). The choice of crab testis as a source of methylases for these preliminary experiments was obviously determined by the fact that farnesylacetone *1* is a natural inhibitor in this animal [3] (see also [2] for details on the androgenic glands of crustaceans).

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1. Nau, F.: Biochemie *58*, 629 (1976)
2. Ferezou, J.P., et al.: Experientia *33*, 290 (1977); Ferezou, J.P., Barbier, M.: Helv. Chim. Acta *61*, 669 (1978)
3. Tekitek, A., et al.: C.R. Acad. Sci. Paris *285* (D), 825 (1977); FEBS Lett. *80*, 348 (1977)
4. Rojas, M., et al.: C.R. Acad. Sci. Paris *288* (D), 1335 (1979)
5. Callow, R.K., Johnston, R.C.: Bee World *41*, 152 (1960); Barbier, M., Lederer, E.: C.R. Acad. Sci. Paris *250*, 4467 (1960)
6. Pain, J.: Bee World *58*, 41 (1977)
7. Torossian, C.: C.R. Soc. Biol. Fr. *159*, 2518 (1965)
8. Nayar, J.K.: Nature *197*, 923 (1963); Sannasi, A.: Life Sci. *8*, 785 (1969)
9. Rojas, M., Barbier, M.: Experientia (in press)
10. Rutz, W., et al.: J. Insect Physiol. *22*, 1585 (1976)
11. Kazlauskas, R., Murphy, P.T., Wells, R.J.: Experientia *34*, 156 (1978)
12. Charniaux-Cotton, H., in: Organogenesis (Haan, R., Ursprung, H., eds.). New York 1965

The Induction of Ion Channels Through Excitable Membranes by Acetylcholinesterase

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It has been established that acetylcholine (ACh) is rapidly hydrolysed by acetylcholinesterase (AChase, EC 3.1.1.7) at cholinergic membranes during the process of synaptic transmission [1]. Therefore, the investigation of the effect of ACh on the conductivity of bilayer lipid membranes in the presence of AChase may be useful to understand the involvement of this enzyme in the conduction process [2, 3].

We have recently found [4] that various molecular forms of AChase [5], purified from electric-organ tissue of *Electrophorus electricus* by affinity chromatography [6, 7], interact with liposomes prepared from phospholipids such as dipalmitoyl phosphatidylcholine (DPPC), sphingomyelin, and phosphatidylserine, which have been demonstrated to be present in electroplax membranes [8]. Association has been demonstrated both by photon energy transfer [9] and by sucrose gradient centrifugation according to [10]. Thus, DPPC fully binds tailed forms, 14 S and 18 S AChase, up to ionic strengths corresponding to 1 *M* NaCl, while partial binding is observed of 11 S AChase, the catalytic subunit tetramer which is devoid of the tail [4].

Black-film lipid membranes were made according to [11, 12] using L-α-phosphatidylcholine type II-S from soybean (SIGMA), a lipid mixture. The formation of bilayers at 20–22 °C in KCl or NaCl solutions buffered at pH 6.8–7.5 (data presented are for 1 *M* KCl and pH 7.4) was controlled by measurement of conductivity and capacitance. It is known [12] and was confirmed by us, that ACh, applied to such membranes under our conditions, has no effect on ion conduction.

Tailed 14 S+18 S AChase was added in amounts of 10^{-6} g or below, corresponding to ca. 10^{-11} moles of active sites [7], to one of the two electrolyte compartments. Incorporation of AChase into the corresponding monolayer was controlled by the resulting increase in surface pressure.

No conductance changes were observed in the absence of ACh. However, after addition of (unstirred) 5×10^{-6} *M* acetylcholine chloride (SIGMA), channels of amplitude $(2.2 \pm 0.2) \times 10^{-10} \Omega^{-1}$ appeared reproducibly. Slow channels of lifetime 50 ms, taken from the lifetime distribution, were usually composed of faster channels following each other "in queue", i.e., with silent interruptions at (and possibly below) the experimental time resolution of 0.5 ms. In addition, and most often, we saw fast events below 1 ms, sometimes as "bursts" of high frequency. Examples are collected in Fig. 1. Membrane potentials attracting

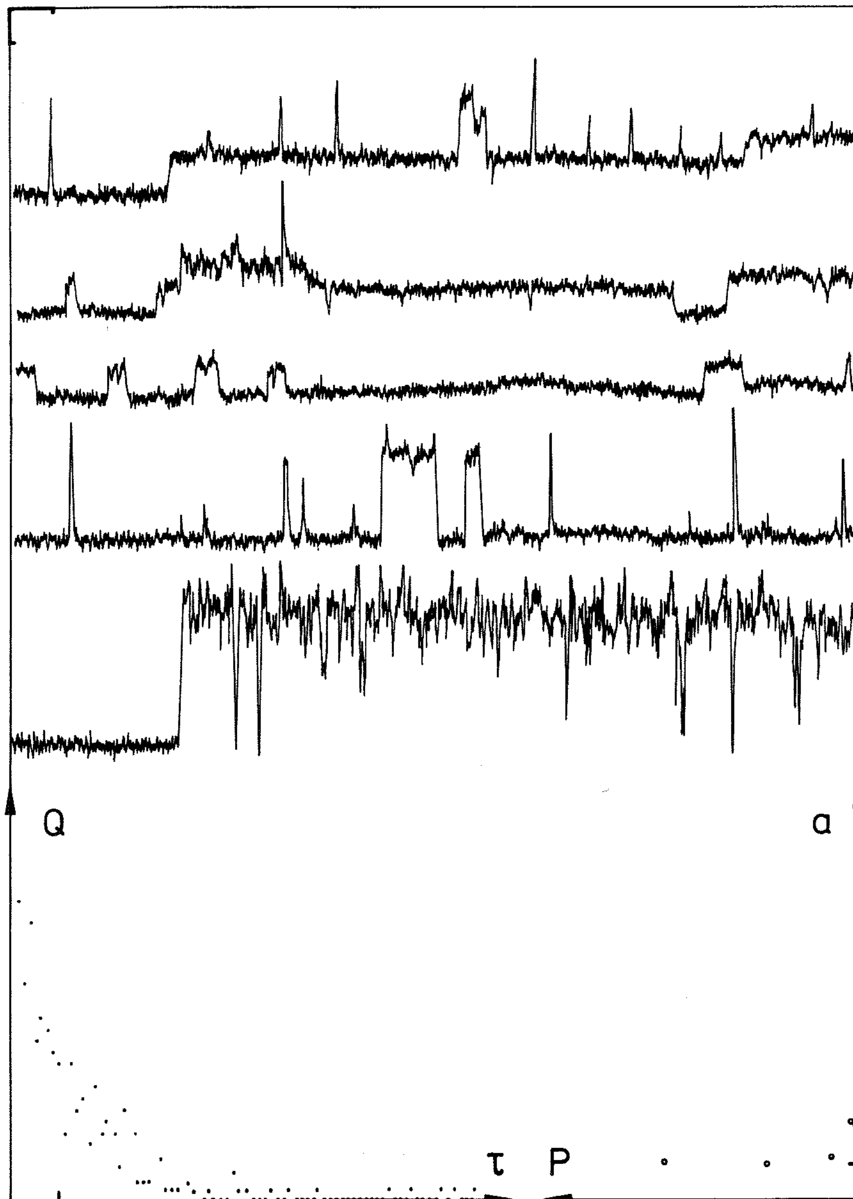

Fig. 1. Acetylcholine-induced channels through the elementary membrane [3] lipid bilayer & acetylcholinesterase. The channel conductivity $2 \times 10^{-10}\,\Omega^{-1}$ in 1 *M* KCl is indicated top left ([) and as ordinate in the amplitude histogram *P*(*a*) of a queued-up single-channel state; the abscissa of its apparent lifetime histogram *Q*(τ) indicates the time scale of 50 ms (⌴). From top to bottom: superimposed channels of long lifetime up to many seconds, below 1 ms fast-kinetic channels show up, too; fast-kinetic channels tend to queue-up; queued-up single-channel state; fast-kinetic and queued-up two-channel states; fast-kinetic [26] channels of fourfold conductivity

ACh^+ to the surface increased the response. Little or no activity was seen when ACh was added to the electrolyte compartment on the other side of the bilayer. Corresponding results were obtained for K^+, Na^+; for soybean, dioleyl, and other lecithins; for 18 S, 14 S, 11 S AChase; as well as for varied quantities.

Channels for monovalent cation conductance, predicted [13] and verified [14] at cholinergic membranes, exhibit an elementary event in excitation not only at synaptic but also at axonal membranes [15] with a probability related to the deterministic "macroscopic" ion permeability [16]. The lifetimes and amplitudes for the ion channels induced by ACh acting on AChase in vitro are very similar to those observed in vivo.

A possible mechanism for channel opening may involve ACh hydrolysis by AChase. This may rapidly produce a local pH decrease [16a] which may, in turn, shift the phase transition temperature [17] of the lipids by protonation of the head group favouring electrostatic transition to a state of different symmetry [18]. In fact, similar conductance increases have already been reported at the lipid phase transition [19]. It is also of interest that Podleski and Changeux [20] earlier reported an ACh-induced depolarization of the innervated membrane of the electroplax which they ascribed to a local pH decrease by enzymic hydrolysis of the agonist by AChase.

Whatever the relationship of AChase to the bungarotoxin-binding receptor [21] may be, our data clearly demonstrate that AChase has the capacity to induce ion channels which are very similar phenomenologically to those seen in vivo. AChase [22, 23] may thus play a direct role [24] in producing ACh-induced conductance changes at some of the numerous loci [22] where it occurs in vivo.

Hermann Träuble was on the way [25], but left us just four years before we arrived. The third of July is his day.

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1. Nachmansohn, D.: Chemical and Molecular Basis of Nerve Activity. New York: Academic Press 1959; Katz, B.: Nerve, Muscle, and Synapse. London: McGraw-Hill 1966; Changeux, J.-P.: Mol. Pharmacol. *2*, 369 (1966)
2. Del Castillo, J., et al.: Science *153*, 185 (1966); Leuzinger, W., Schneider, M.: Experientia *28*, 256 (1972); Jain, M.K., Mehl, L.E., Cordes, E.H.: Biochem. Biophys. Res. Commun. *51*, 192 (1973)
3. Kaufmann, K.: Int. J. Quant. Chem. *12*, Suppl. 2, 169 (1977)
4. Kaufmann, K., Silman, I.: Neurochem. Int. *2*, 205 (1980)
5. Rieger, F., et al.: Eur. J. Biochem. *34*, 539 (1973); Dudai, Y., et al.: Proc. Nat. Acad. Sci. USA *69*, 2400 (1972)
6. Dudai, Y., Herzberg, M., Silman, I.: ibid. *70*, 2473 (1973)
7. Dudai, Y., et al.: Biochim. Biophys. Acta *268*, 138 (1972)

8. Rosenberg, P., et al.: J. Neurochem. *29*, 561 (1977)
9. Vaz, W., Kaufmann, K., Nicksch, A.: Analyt. Biochem. *83*, 385 (1977)
10. Watkins, M.S., Hitt, A.S., Bulger, J.E.: Biochem. Biophys. Res. Commun. *79*, 640 (1977)
11. Montal, M., Müller, P.: Proc. Nat. Acad. Sci. USA *69*, 3561 (1972); Boheim, G., Kolb, H.A.: J. Membr. Biol. *38*, 99 (1978)
12. Schindler, H., Quast, U.: Proc. Nat. Acad. Sci. USA *77*, 3052 (1980)
13. Katz, B., Miledi, R.: J. Physiol. (London) *224*, 665 (1972)
14. Neher, E., Sakmann, B.: Nature *260*, 799 (1976); Sakmann, B., Patlak, J., Neher, E.: ibid. *286*, 71 (1980)
15. Conti, F., Neher, E.: ibid. *285*, 140 (1980)
16. Hodgkin, A.L., Huxley, A.F.: J. Physiol. (London) *117*, 500 (1952)
16a. Silman, I., Karlin, A.: Proc. Nat. Acad. Sci. USA *58*, 1664 (1967); Katchalski, E., Silman, I., Goldman, R.: Adv. Enzymol. *34*, 445 (1971)
17. Träuble, H., in: Structure of Biological Membranes, p. 509 (eds. Abrahamsson, S., Pascher, I.). New York: Plenum Press 1977
18. Janiak, M.J., Small, D.M., Shipley, G.G.: J. Biol. Chem. *254*, 6068 (1979)
19. Antonov, V.F., et al.: Nature *283*, 585 (1980); Boheim, G., Hanke, W., Eibl, H.: Proc. Nat. Acad. Sci. USA *77*, 3403 (1980)
20. Podleski, T.R., Changeux, J.-P.: Science *157*, 1579 (1967)
21. Changeux, J.-P., Kasai, M., Lee, C.-Y.: Proc. Nat. Acad. Sci. USA *67*, 1241 (1970)
22. Silver, A.: The Biology of Cholinesterases. Amsterdam: North-Holland 1974
23. Rosenberry, T.L.: Adv. Enzymol. *43*, 103 (1975); Silman, I.: TIBS *1*, 225 (1976); Massoulié, J.: ibid. *5*, 160 (1980)
24. Kaufmann, K.: Habilitationsschrift. Univ. Göttingen 1980; Neurochem. Int. *2*, 111 (1980)
25. Träuble, H.: Naturwissenschaften *58*, 277 (1971)
26. Eigen, M., in: Nobel Lectures. Chemistry 1963–1970, p. 170. Amsterdam: Elsevier 1972

Pteridine Excretion from Cells as Indicator of Cell Proliferation

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Pteridines are ubiquitous in natur. They seem to play a central role in cell metabolism. However, due to their occurrence in trace amounts and to their chemical instability they were only marginally studied. So far, only the functions of a few pteridines are known [1]. Here we report that pteridines are excreted from proliferating cells independent of their sources. The excretion of these pteridines is apparently coupled to cell growth and, thus, an indicator of cell proliferation.

Bacteria like *E. coli* excrete monapterine (Fig. 1) during their logarithmic growth phase. Interestingly, at the switch from logarithmic growth phase to the stationary phase the excretion of pteridines experiences a burst, whereas in the stationary phase *E. coli* hardly releases any pteridines. Correspondingly (Table 1) *Physarum polycephalum* (slime mould), *Calanchoe tubiflora, Solanum tuberosum* (germinating potatoes), *Acetabularia mediterranea*, Ehrlich ascites-bearing mice [2] or humans with invasively growing tumors (Fig. 2, Table 1) excreted pteridines during proliferation [3].

The association of cell proliferation and pteridine excretion is supported by several other findings: Regeneration of the tail of *Triturus* species is accompanied by elevated concentrations of tetrahydrobiopterin in the regeneration bud [4]. Patients with malignant tumors excrete up to a 20-fold enhanced amount of neopterine (Fig. 2) [3]. Viral infections, which generally stimulate nucleic acid metabolism lead to en-

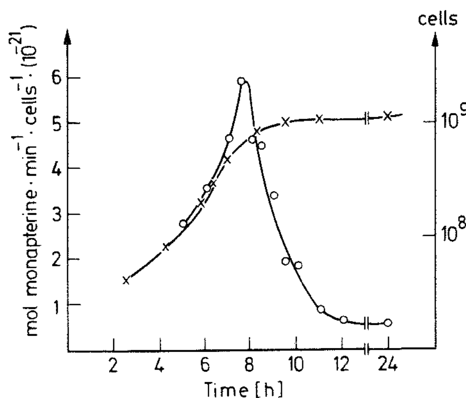


Fig. 1. Monapterine secretion by *E. coli*. *E. coli* was grown in mineral medium (M9) with glucose [8]. At indicated times 1 ml samples were withdrawn, the bacteria removed by centrifugation and the fluorescent pteridines were measured [3] in the medium. x—x growth, o—o excretion rate of monapterine. The excretion rate was determined from the amount of monapterine per volume plotted against the time in minutes. The slopes were taken as rates at the given times. The rates were then related to the number of cells

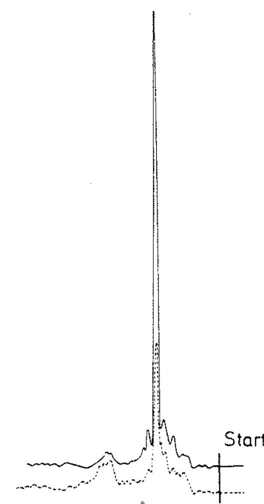
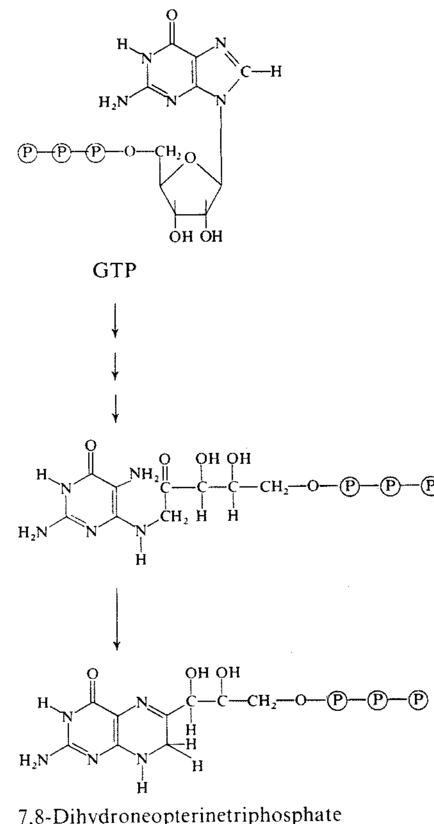


Fig. 2. Neopterine excretion in human urine. HPLC chromatogram. Prepurification, HPLC analysis and fluorescence detection (excitation 380 nm, emission 450 nm) as previously described [3]. ---- healthy proband, — patient with bronchus carcinoma



7.8-Dihydroneopterinetriphosphate