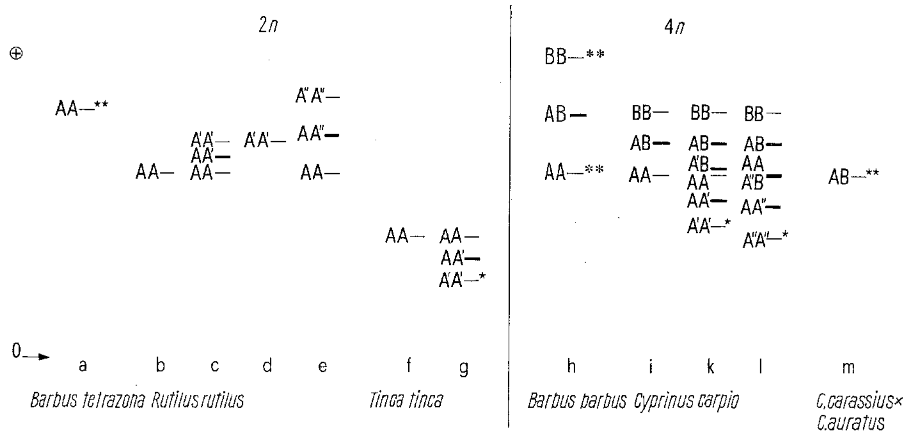

Fig. 2. Schematic presentation of the various S-AAT isoenzyme phenotypes observed and tentative designation of the subunit coposition. Species in which no variantr were observed are marked by 2 asterisks, those in which variants occurred only in the heterozygous state are marked by 1 asterisk.

in the diploids and of 2 loci in the tetraploids seems to be established.

The existence of a diploid-tetraploid relationship has also been established in the order *Isospondyli* (clupeoid and salmonoid fish)[26], and a number of genes coding for different isoenzyme systems (e.g. LDH[26], M-NADP-IDH[27], and SDH[28]) have been shown to be duplicated in the tetraploids. It may be mentioned that this is also valid for S-AAT. The findings of ODENSE[11] in the herring as a diploid species can be interpreted under the assumption that one gene locus with different alleles for S-AAT exists in this species. These data are confirmed by our own results: of 69 herrings investigated, 65 showed a single electrophoretic band (homozygous wild type), and 4 individuals the assumed heterozygous pattern, consisting of 3 bands. *Salmo trutta* and *Coregonus lavaretus*, as representatives of the tetraploid group, are apparently endowed with 2 gene loci for S-AAT: in 60 specimens of *Salmo trutta* and 41 specimens of *Coregonus lavaretus*, 3 electrophoretic bands were consistently found. One individual of *Coregonus lavaretus* showed an assumed heterozygous pattern with 5 bands.

Thus, the S-form of AAT proves to be another useful genetic marker for the demonstration of gene duplication as the consequence of polyploidization. In contrast to some other genes, which have apparently disappeared during evolution of the tetraploids (genes coding for 6-PGD in *Isospondyli* and for SDH in *Ostariophysi*[24]), duplicated S-AAT genes were apparently conserved or modified in such a way that their products fulfill a useful function. From this, it may be concluded that some selective pressure may be responsible for the maintenance of certain duplicated genes in the tetrapolids[29].

Zusammenfassung. Die S-AAT wurde bei Fischen der Familie *Cyprinidae* elektrophoretisch dargestellt. Die Isoenzymmuster lassen bei den diploiden Vertretern dieser Fischfamilie auf einen Genlocus, bei den tetraploiden auf 2 Loci schliessen. Analoge Verhältnisse bestehen offensichtlich auch bei Fischen der Ordnung *Isospondyli*.

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Antennal Proteins Involved in the Neural Mechanism of Quinone Inhibition of Insect Feeding

Findings about several aspects of the energy-transduction mechanism involved when certain quinones inhibit feeding in 2 species of insects were previously reported[1–10]. These chemical messengers react with sulfhydryl groups of macromolecules in nerve membranes in chemosensitive sensilla especially on the antennae of the insects. Such reactions, as monitored by ultraviolet difference spectroscopy[3,5], change the conformation of macromolecules in isolated nerve-membrane fragments. This change in vivo presumably allows altered ion flow through the receptor membrane[9], and this may bring about the generation of an action potential in the neuron[11]. Because our previous studies indicated that proteins (i.e. especially sulfhydryl-containing moieties) were selectively involved in the receptor macromolecules, the binding affinity of the feeding inhibitor, 2-methyl-1, 4-naphthoquinone, for antennal proteins was further investigated. We now present evidence of the resolution, by disc gel electrophoresis, of protein-containing bands from homogenized antennae of *Periplaneta americana* which appear to possess the properties of receptor chemical(s) for this feeding-inhibitory naphthoquinone. Results from in situ exposure of protein-containing materials in the antennae to 2-(^{14}C) methyl-1, 4-naphthoquinone indicated that 2 closely associated bands in the electrophoretically resolved Triton X-100-soluble proteins contained relatively high amounts of label (Figure A) in contrast to a low degree of count associated with saline-soluble proteins (Figure B).

Materials and methods. Antennae of live cockroaches were held in a $2.4 \times 10^{-6} M$ aqueous solution of the radio-labelled naphthoquinone (9.7 mC/mM) for 0.5 h. The antennae then were rinsed extensively with tap water which degrades naphthoquinone on the surface of the antennae. The antennal proteins were extracted according

to the methods of DAVIES[12]. Treated antennae (100 mg wet wt/ml) were homogenized in 0.9% NaCl. The homogenate was centrifuged at 100,000 g for 60 min, and the pellet obtained was washed twice in the saline and centrifuged after each wash. The resultant supernatants were combined. The pellet was further extracted with 0.6% Triton X-100 in 0.9% NaCl by mechanical homogenization and ultrasonification. This latter extract was then centrifuged at 100,000 *g* for 60 min, and the supernatant was saved. To obtain solutions with a concentration of proteins suitable for analysis by disc gel electrophoresis, the saline and detergent extracts were concentrated by use of coarse Sephadex G-25. The protein content of the concentrated solutions was determined according to LOWRY et al.[13]. The extracts were fractionated by disc gel electrophoresis as described by DAVIES[12]. At pH 8.3, the proteins moved toward the anode. The gels were stained, sliced and prepared for liquid scintillation counting according to MUTO[14]. Each sample was counted at 95% efficiency for 20 min.

Results and discussion. Binding of the radiolabelled naphthoquinone to the saline-soluble proteins did not seem to indicate selective binding. In contrast, counts bound to certain detergent-soluble proteins did support selective complexing. The observed greater binding affinity of the quinone for Triton X-100-soluble than for saline-soluble proteins agrees with our previous findings[5]. Of the total counts applied in the solutions per replicate gel column (10,200 cpm per 200 µg saline-soluble protein and 80 µg TX-100-soluble protein) the percentage bound to the saline- and Triton-solubles was 1.6 and 4.5, respectively. The high binding capacity of certain detergent-extracted proteins becomes even more apparent when the amounts of bound label are compared on the basis of quantity of protein applied per gel column. Though about 2.5 times more saline-soluble protein was run per column, the detergent-soluble proteins per column contained about 3 times as much bound radioactivity.

In this in situ study, physically accessible proteinaceous macromolecules which function naturally in chemoreception would be expected to especially interact with the quinone. From these results, we conclude that the 2 Triton X-100-soluble proteinaceous bands which selectively bound the quinone (Figure A) contain natural receptor sites for the feeding inhibitor.

Our various previous studies[1–10] of this energy transduction indicated that important initial in vivo energy transfer involves a basically reversible phenomenon. This characteristic seems especially reasonable in such primary interaction of relatively low concentrations of chemical messenger with sensory nerves. Under such conditions of reversibility, the degree of complexing of some concentration of messenger with receptor sites would be expected to reach equilibrium at some relatively low level per µg protein compared to levels with increasing degrees of irreversibility of complexing. In this regard, several types of meaningful reactions between natural messenger and groups of receptor sulfhydryls of differing reactivities in neurons are apparently supported by our previous studies[10]. Some of the secondary interactions, associated especially with the more potent messengers, obviously are particularly characterized by less ready reversibility. The summation of complexing interactions of such varying reversibilities, upon extensive exposure of sensory neurons to messenger, probably is represented in our level of bound label in the double band of protein in Figure A[15].

Zusammenfassung. Es wird gezeigt, dass der radioaktiv markierte Frasshemmstoff Methylnaphthochinon in den Antennen von *Periplaneta* an 2 spezifische, mit Triton X-100 extrahierbare, elektrophoretisch trennbare Proteine gekoppelt wird.

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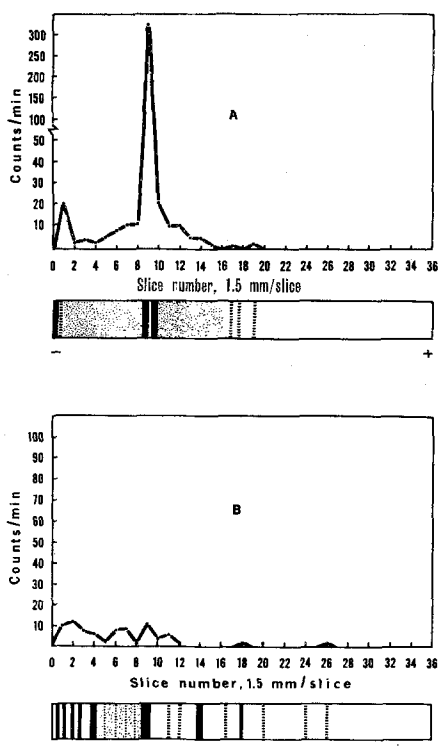

Polyacrylamide gel electrophoresis- and bound radioactivity-patterns of Triton X-100-extracted (A) and saline-extracted (B) proteins from antennae (of living insects) held in an aqueous solution of 2-(^{14}C) methyl-1,4-naphthoquinone ($2.4 \times 10^{-6} M$) for 0.5 h.

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