anilides (data not shown). We have also observed foci of intense direct immunofluorescence in some vascular and perivascular round cell infiltrates in the central nervous system and striated muscle from anilide-treated animals when challenged directly with the FITC goat anti rabbit serum. This unpublished observation suggests the occurrence of immune complexes in certain tissue structures; this feature was never seen in slices from control animals.

Discussion. The specific response to anilides in skin tests and the characterization of anilide-specific antibodies in the sera from anilide-treated rabbits by immunoadsorption and solid phase radioimmunoassay strongly support the immunogenic character of fatty acid anilides when administered to rabbits either p.o. or i.p. Furthermore, the identification of 'anilide-dependent antigens' in different tissue structures, as shown by the immunofluorescence studies, introduces the possibility of several immunopathogenic mechanisms of cell injury, which may be relevant for the pathogenesis of the toxic oil syndrome. For instance, the activation of the complement pathway by immunocomplexes, could have caused acute respiratory distress[16] in toxic oil consumers when challenged with volatile aniline derivatives in the kitchen. The neurological disorders in rabbits[11], which bear a close resemblance to the experimental neurotoxicity of eosinophilic extracts[17], might represent an antibody-directed eosinophilic neurotoxicity, which could have been operative in TOS patients. In addition, the production of antibodies able to recognize components of otherwise normal structures could have been involved in the induction of scleroderma-like autoimmune processes[18] which are characteristic features of the chronic stage in TOS[10].

It remains however, a matter of debate to establish how direct the relationship may be between the experimental pathology and immunogenicity of anilides in rabbits and the clinical manifestations in TOS patients. Although there is no evidence that anilides act as liposomes in the clinical situation, there is epidemiological data showing a high comsumption of emulsified oil (mayonnaise) among affected people. In a preliminary study it was found that 70% of the sera from acute phase TOS patients had specific IgE antibodies for the same synthetic anilides used in our experimental work[19]. Moreover, all these acute phase sera had IgG antibodies specific for the fatty acid anilides (C. Lahoz, personal communication). It is, however, unfortunate that these preliminary findings were not followed by a more comprehensive study of the acute phase sera that might still be available. The only other report related to this issue described the absence of specific IgE antibodies for aniline derivatives in subacute and chronic phase sera[20]. Thus, in the absence of additional confirmatory data from human studies, no clear cut conclusion can be drawn as to the relevance in humans of our experimental findings in rabbits.

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Influence of sulfhydryl reagents on the structural and molecular organization of crayfish photoreceptor microvilli

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Summary. SDS-polyacrylamide gel electrophoresis of isolated photoreceptor membrane from crayfish compound eyes in the absence and presence of the sulfhydryl-reducing agent 2-mercaptoethanol revealed major qualitative and quantitative differences in electrophoretic behaviour of polypeptides. Two peptides of 37 and 69 KD show abnormal migration patterns under the experimental conditions, indicating a possible significance of disulfide bridges for the structural integrity of invertebrate photoreceptor membrane.

Key words. Sulfhydryl; photoreceptor; microvilli; compound eye; crayfish.

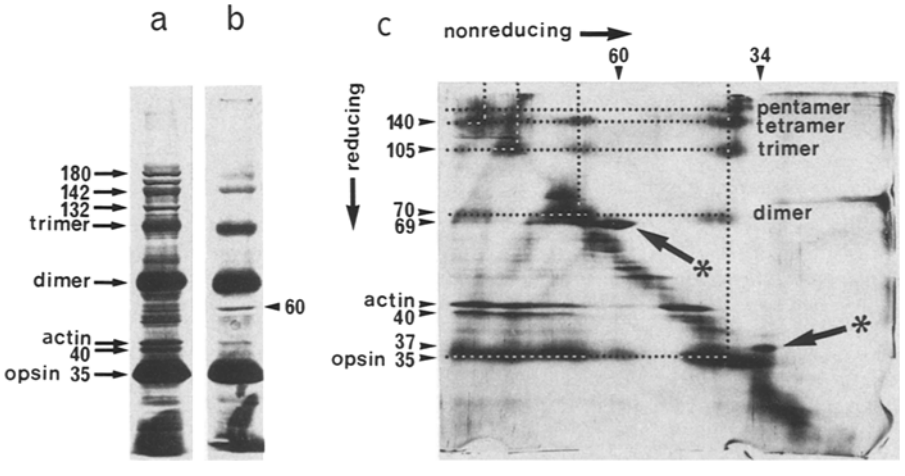
The photoreceptors of the compound eyes of arthropods exhibit a highly organized system of microvilli containing the light-harvesting photopigment rhodopsin. These microvilli are supported by a single axial filament crosslinked to the photoreceptor membrane via numerous 3 nm bridges[1,2]. Photoreceptor membrane also undergoes a circadian turnover, most dramatic among crustaceans, possibly involving the breakdown and reassembly of the membrane cytoskeleton[2,3].

Among the agents serving to stabilize the cytoskeleton ultrastructurally are Ca^{2+}-chelating agents[1,4], the calmodulin-antagonist trifluoperazine, and specific inhibitiors of thiol proteases. Inversely the sulfhydryl reagents 2-mercaptoethanol and dithiothreitol lead to a rapid disorganisation of the microvilli. It has therfore been argued, that the central axial cytoskeleton of photoreceptor microvilli may be composed of a neurofilament which is degraded by a Ca^{2+}-activated thiol-protease[1,2]. So far, however, actin has remained the only identified cytoskeletal protein in rhabdomeral photoreceptor microvilli in both arthropods[4] and cephalopod eyes[5]. Actin appears to be associated with a small set of integral membrane glycoproteins, from which it fails to separate upon detergent treatment[4]. Also, attempts to demonstrate the presence of a protease in isolated preparations of rhabdomeral photoreceptor membrane have consistently failed[4]. It was the aim of the present study to find a reason for the ultrastructural observation that microvillar membrane rapidly disintegrates after treatment with sulfhydryl reagents in situ. The method of crossed electrophoresis in the presence and absence of reducing agents[6] was used to assess the role of inter- and intramolecular disulfide bridges for the organization of photoreceptor membrane.

Materials and methods. Rhabdoms were isolated from crayfish retinae *(Cherax destructor)* by gentle homogenization and subsequent centrifugation on a sucrose gradient from 5–45% (w/v) as described previously[4] and washed once in homogenization medium. All steps were carried out under dim red light and at 4 °C. Prior to use, all solutions were deoxygenated by gasing them with nitrogen. Samples were prepared for electrophoresis under non-reducing conditions by dissolving the rhabdoms from about 10 animals in 50 to 100 µl electrophoresis buffer [50 mM TRIS-HCl pH 6.8, 2.3% SDS, 8 M urea (ultrapure), 10% (v/v) glycerol, 5 mM ascorbic acid] and incubating them overnight at 4 °C under nitrogen to prevent oxidation. Immediately before electrophoresis, the sample was divided into 2 equal aliquots and diluted 1:1 with either the same buffer or electrophoresis buffer containing additional 5% (v/v) 2-mercaptoethanol. Electrophoresis was carried out on 7.5–15% polyacrylamide gradient slabgels according to Laemmli[7]. Gels were left for 2 days at room temperature before use to ensure the complete polymerization of their constituents.

The electrode buffer (0.025 M TRIS, 0.192 M glycine, 0.1% SDS) was extensively degassed before use and gels were run overnight at room temperature. For 2-dimensional electrophoresis, the first dimension was carried out on 10% cylindrical gels under non-reducing conditions as described above. Immediately after electrophoresis, the gels were removed from the tubes and equilibrated in sample buffer containing reducing agent [50 mM TRIS-HCl pH 6.8, 2.3% SDS, 10% glycerol, 5% (v/v) 2-mercaptoethanol] for 10min. Subsequently the gels were placed on top of a 10% slabgel with a 4.75% stacking portion and secured in place by 1% agar in sample buffer. Electrophoresis was carried out at 8 mA initially until the dyefront had reached the separation gel and continued at 25 mA. Gels were stained with Coomassie Blue or an ultrasensitive silver stain to visualize minor traces of proteins[8]. Molecular weights were calculated from the electrophoretic mobilities of opsin and opsin polymers, assuming a monomeric molecular weight of 35,000.

Results and discussion. Figure 1 demonstrates the qualitative and quantitative differences that were consistently found between samples run in the presence and absence of reducing agents. The total number of protein bands is drastically reduced as a consequence of non-reducing electrophoresis, as revealed after ultrasensitive silver staining, whereas much dye-binding material remains in the stacking portion of the slabgel (fig., b). Most apparent is the appearance of a major 60 KD component after electrophoresis under non-reducing conditions only detectable in trace amounts after electrophoresis in the presence of reducing agents. A 132 KD and a 180 KD component are completely absent after electrophoresis without 2-mercaptoethanol. The 60 KD component is also stained by Coomassie Blue and therefore represents a major polypeptide of the preparation. The amounts of other membrane constituents such as actin and a 40 KD component associated with the detergent-insoluble membrane-matrix[4] are also drastically reduced. The 132 KD peptide is one of the 2 major complex glycoproteins of the rhabdomeral photoreceptor membrane,



SDS-polyacrylamide gel electrophoresis of total crayfish photoreceptor membrane proteins under reducing (a) and nonreducing (b) conditions as described[6] on 7.5–15% gradient gels, silver stained. Samples were run on the same gel, about 5 cm apart to prevent interference of 2-mercaptoethanol. Lanes a and b correspond to identical amounts of protein.

The positions of major peptides absent after electrophoresis under nonreducing conditions (b) are indicated as well those of actin, opsin and opsin oligomers. The arrow in (b) indicates the position of a peptide that is not apparent after electrophoresis under standard conditions. Molecular weights are given in KD.

(c) crossed electrophoresis on 10% gels. The nonreducing dimension is from upper left to right, separation under reducing conditions was subsequently performed from top to bottom. The dotted lines define the coordinates of opsin and its polymers, from which molecular weights were calculated. Molecular weights indicated on top of the figure correspond to apparent electrophoretic mobilities. Asterisks indicate the positions of 2 peptides with abnormal migration behavior. Proteins with an increased migration behavior under nonreducing conditions appear on the right hand side of the diagonal. The position of high molecular weight peptides is obscured by opsin oligomers.

which binds a number of lectins and is associated with the membrane matrix (in preparation). Crossed electrophoresis in the absence and presence of reducing agents reveals 2 major polypeptides with abnormal migration behavior and apparent molecular weights of 37 and 69 KD, both of which exhibit a considerably reduced electrophoretic mobility in the absence of 2-mercaptoethanol. Under reducing conditions these peptides migrate close to the monomers and dimers of opsin (mol.wt 35 KD and 70 KD), but are clearly distinct as becomes apparent after 2-dimensional electrophoresis (fig., c). Opsin is characterized by its tendency to form oligomers by hydrophobic interactions under any electrophoretic conditions[9] and by its property to form ill-defined banding patterns. This electrophoretic behavior was used to assess the coordinates of opsin and its oligomers in 2-dimensional gels and to estimate approximate molecular weights of other components in relation to opsin (fig., c). The figure c also demonstrates that opsin will undergo renewed polymerization and depolymerization upon re-electrophoresis in a second dimension. In non-reducing electrophoresis both 37 and 69 KD peptides show increased mobility (apparent mol.wts: 34 KD and 60 KD) and migrate well ahead of opsin and its dimer, thereby indicating an asymmetric molecular shape. The larger of these peptides is possibly identical with the 60 KD band seen in 2-dimensional electrophoresis without reducing agent (fig., b), whereas the smaller one is not well enough resolved from the monomeric opsin band in 1-dimensional techniques. Both peptides do not appear to be composed of smaller subunits but they may be related polypeptides and are probably intramolecularly bonded via disulfide bridges. Under nonreducing electrophoretic techniques a molecular asymmetry caused by a disulfide bridge would result in a higher electrophoretic mobility (right hand side of fig., c) which is sensitively recognized using gradient gels[6]. The 37 KD peptide is probably identical with a component of similar molecular weight which is resolved in 2-dimensional isoelectric focusing and exhibits an isoelectric point slightly more acidic than opsin[4] which, however, is irrelevant for electrophoretic behavior under denaturing conditions. Both the quantitative loss of polypeptides on polyacrylamide gels without prior reduction and the existence of 2 peptides with apparent intrasubunit disulfide linkages in the photoreceptor membrane sufficiently explain the ultrastructural observation of rapid disorganization after treatment with sulfhydryl-reducing agents. The selective loss of a complex 132 KD glycoprotein from the detergent-lysed mixture of photoreceptor polypeptides in the absence of reducing agents during electrophoresis also indicates that this protein may participate in a disulfide-based supramolecular structure. It has been reported however, that such high molecular weight complexes can form randomly between sulfhydryl groups of unrelated proteins if they are solublized in the absence of an antioxidant but it was also shown that this can be prevented effectively by either blocking native sulfhydryl-groups chemically or by carefully excluding oxygen from all handling procedures. Although great care was taken to observe this rule it cannot be excluded completely that quantitative changes observed are partly due to oxidation during handling or electrophoresis. Therefore ascorbic acid was included in some experiments in the electrophoresis buffer as an antioxidant, however, no changes were observed.

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Retroviruses released from a human tumor xenograft in nude mice induce colony-stimulating factor (CSF) activity in human fibroblastic cells

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Summary. A human colony-stimulating factor (CSF)-producing tumor transplanted into athymic nude mice released retroviruses in vitro. The viruses induced CSF activity in human fibroblastic cell lines.
Key words. Fibroblastic cells, human; mice, nude; retrovirus; colony-stimulating factor; CSF-producing tumor; tumor xenograft.

Xenotropic infection with murine type C retroviruses is not uncommon in human tumor cells when the tumors have been transplanted into athymic nude mice[1-3]. The tumors inoculated into nude mice and then established as cell cultures in vitro have been shown to release xenotropic murine leukemia virus[1]. It is not clear at present what role, if any, the retrovirus plays in the development of tumors in nude mice by primary human tumor cells. The effects of xenotropic infection of the virus on functional properties of the human tumor cells have not been well investigated.

In 1977, we described a human lung cancer, when transplanted into athymic nude mice, which produced granulopoietic factor(s) (colony-stimulating factor[CSF])[4]. While the patient with this cancer showed only a slight increase in peripheral blood granulocytes, the nude mice into which the tumor was transplanted developed a remarkable granulocytosis. In the plasma of the tumor-bearing mice, and in extracts of the xenografts, high CSF activities were demonstrated. The tumor cells isolated fromt he xenografts also released CSF activity in vitro in culture[5]. While it is not known whether the primary human