able to degrade aflatoxin. Previous studies found that various *Aspergillus* species (including a toxigenic *A. flavus* isolate) and *Penicillium* species were capable of degrading aflatoxin[12]. However, no studies of microbial degradation of SAD have been reported. Therefore, it is difficult to estimate whether mutual degradation could explain the decreased mycotoxin levels found in mixed cultures. We found that incubation of the culture supernatant fluid of *P. oxalicum* with aflatoxins did not degrade the aflatoxins. Whatever the mechanism no simple relationship between growth potential of the competing molds and secondary metabolite formation is likely to offer a complete explanation since in the above studies both competing molds can be reisolated as a higher percentage of the culture than would be expected based on the relative percentages of secondary metabolites formed.

Limitations in nutrient supply or space obviously play a role in the selection of mold species which can successfully invade a host but, apparently, there is no direct correlation between competition in substrate invasion and toxin production.

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Pharyngeal cavity and the gills are the target organ for the repellent action of pardaxin in shark

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Summary. Pardaxin, an active principle of the repellent secretion of the Red Sea flatfish, *Pardachirus marmoratus*, elicited severe struggling, mouth paralysis, and transient increase in urea leakage from the gills only when administered to the medium bathing the shark's pharyngeal cavity and gills. An apparatus was constructed which prevents a mixing of the outflow from shark's gills with water bathing its surface skin. It is concluded that in sharks the gills and/or the pharyngeal cavity are the target organ for the repellent action of pardaxin.

Key words. Gill function; elasmobranchs; repellents; fish toxin.

In the 19th century ichthyologists noted that the flatfish *Pardachirus marmoratus* (Soleidae), which inhabits the shallow waters of the Red Sea and the Indian Ocean, could exude a milky fluid from a series of glands located along their dorsal and anal fins[1,2]. It has been demonstrated only recently that predatory sharks exposed to this fluid open their mouths abnormally wide (mouth paralysis) and are generally repelled. It is considered a naturally occurring weapon of self-defense against predation[3,4].

The toxicity of the flatfish secretion has been attributed to a protein of a mol.wt of Mr = 13,100. This toxin, named pardaxin (PX), was found to be a monomeric chain, with a helical structure and four disulfide bridges[5]. However, recently active compounds composed of steroids and sugar complexes were also identified[6,7].

In fractionation of the flatfish secretion the collection was based upon the number of drops per tube. Surprisingly, it was found that the tubes with the active fraction contained only about one half of the expected volume[8]. This surface tension-reducing property was the first indication of the detergent-like action of the flatfish toxin. The detergent character of PX was finally demonstrated in a lipid bilayer membrane where it produced ion-conducting channels[9] and by an analysis of PX's secondary structure. All of its 12 amino acids of N-terminus are of a hydrophobic quality[10]. These findings indicated that PX's biological activities as well as its detergent quality are within the same molecule. In fish, detergents are known to produce an impairment of chemoreceptors and cytotoxicity in gills and pharyngeal skin[11,12], and sodium lauryl sulfate was shown to have repellent effect in sharks[13]. The pharyngeal skin and the gills are known to play an important role in ion osmoregulation[14,15].

Pardaxin administered to the dogfish shark *(Squalus acanthias)* caused the shark to struggle and to open its mouth as if gasping, and diminished the spiracular rate. Since these effects were associated with an increase in the leakage of urea it was suggested that PX acts upon the gills[16].

However, in sharks, the head possesses not only the gills, ordinarily considered part of the cardiovascular system[17], but also the lateral-line sensory system. Chemical sensors are also within the receptors of the olfactory bulb[17,18]. Their input is supplied by several thousands nerve fibers running from the lateral-line organs of the head, which includes the ampullae of Lorenzini[20]. Therefore, experiments were designed to determine whether the response to PX is mediated through the lateral-line system or via the pharyngeal cavity and the gills.

Materials and methods. Pardachirus marmoratus fish (Pisces; Soleidae) were collected from the Red Sea (Eilat, Israel). *P. marmoratus* secretion (PMC) was obtained by the method described by Clark and Chao[21]. Pardaxin (PX), the ichthyotoxic component isolated from PMC, was prepared according to Primor et al.[22] and stored in a lyophylized form.

Female dogfish sharks, *Squalus acanthias*, weighing 4–5 kg were taken from Frenchman's Bay, Maine, for study at the Mount Desert Island Laboratory. They were maintained in

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Marine livecars in a fasted state and used within 3–4 days of capture.

In vivo gill perfusion systems. In the present study, the target of PX's action and its effect on gill permeability to urea was determined utilizing two different perfusion systems: the dry- and the wet-head apparatuses.

The dry-head apparatus. A gill perfusion apparatus, similar to that described by Boylan[23] was constructed. In this apparatus the surface skin on the shark's head is kept dry while seawater passing over the pharynx and the gills is recirculated through a pair of tubes connected to a constant temperature system at 15 ± 1 °C. This closed system consisted of 2 l of perfusate. PX was administered to the perfusate at 25 μg ml^{-1}. Five fish were used to observe the effect of PX including two fish previously injected with ^{14}C-urea. Since PX activity involves disruptions of gill membrane function[16,24] leakage of urea from the gills was measured. The dorsal aortae of the dogfish were catheterized via the caudal artery (using polyethylene tubing PE 90, Clay Adams Co., Parsippany, N.J.) for the purpose of injecting ^{14}C-urea and blood sampling. Following injection of 200 μCi of ^{14}C-urea the dogfish shark was kept in a tank with running seawater for 3 h (for the purpose of distribution of the injected isotope). Every 2 min prior to and after adding of PX to the perfusate, 0.5 ml blood samples and 0.2 ml perfusate were taken. Three samplings were made before PX was given and five thereafter. Since in fish, it is hard to estimate the gill area from where urea is transported it is inappropriate to express its transport in terms of flux (usually given per area). Instead, a leakage of urea from the fish to the seawater was determined. The following calculation was applied:

$$\frac{\text{change in the cpm in the perfusate}}{\text{specific activity in blood}} \times \text{sampling time (given in h)}$$

There is no significant (less than 2%) difference in quench as ^{14}C-urea was added to 0.2 ml of seawater or blood. Therefore, quench corrections for seawater were not made in these experiments. ^{14}C-urea was determined by liquid scintillation spectrometry and expressed in equivalents per h, per kg b.wt and corrected for background (table).

The wet-head apparatus. This apparatus was constructed to test the possibility that PX may act on the shark's head area other than the gills and the pharyngeal cavity. In this system, the surface skin of the shark's head is immersed in a chamber containing 2 l of seawater. Its pharyngeal cavity and the gills are perfused with seawater from a container located 1 m above the fish through a pair of tubes attached into each spiracle. The outflow is removed by a pair of chambers tightly attached to each of the gill's clefts and through a tube inserted in the shark's mouth and pumped through the constant temperature bath (15 ± 1 °C) back to the container (fig.). The total perfusate volume consists of 2.5 l. Pardaxin (25 μg ml^{-1}) was added either to the perfusate (into the container located above the fish), or into the medium where the shark's head was immersed (five experiments of each). The respiratory movements were counted in fish positioned in dry- and wet-head apparatuses by removing the tubes attached to each spiracle and counting the spiracle movements. Since the shark responds to PX by violent struggles, it often damages the chambers of the gill outflow of the wet-head apparatus. Therefore, the dry-head apparatus was used in studies with the isotope injected fish.

Results. Under the conditions of the dry- and wet-head systems, the respiratory movements continued for about 6 h within its normal rate of 50–55 min^{-1}.

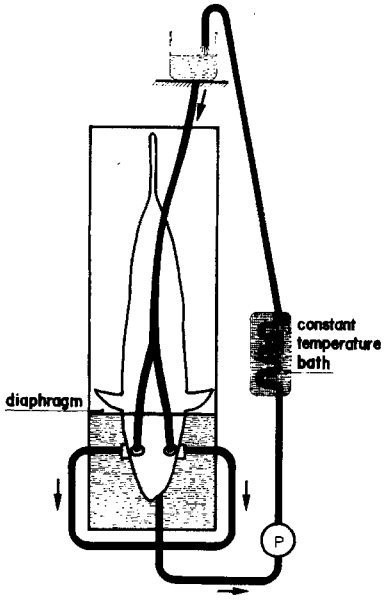
PX, administered to the perfusate at 25 μg ml^{-1}, caused the fish to struggle and to open its mouth widely within 2–3 sec. The dogfish gills are known to have low permeability to urea[23,25]. The addition of PX produced a marked increase in urea leakage from the shark to the perfusate which rose about 50-fold within 2 min and then declined to over 4-fold within 10 min, as compared to the control periods (table). The decline in leakage to urea cannot be explained by a rapid inactivation of the toxin. PX's potency is maintained for a period of 1–2 h, as within this time the perfusate given to other dogfish shark produced a similar effect (struggle and mouth opening). It suggests a transient action in spite of the continuous presence of PX. However, PX left for 5–6 h in seawater at 15 ± 1 °C failed to produce these effects. At the administered dose, PX was not lethal and the tested dogfish recovered. The transient mode of PX action may correlate with the proposed action to repel predators rather than cause death.

These results indicate that the pharyngeal cavity and the gills may be the targets of PX action. However, the possibility remains that the lateral-line sensory system located on the surface skin of the shark's head is also involved in PX action. To test the latter possibility, the wet-head apparatus was con-

The effect of pardaxin (PX) on leakage of urea in an in vivo perfused gills in *Squalus acanthias*

			PX added				
Control periods Periods before PX added			Experimental periods Periods after PX added (min)				
6	4	2	2	4	6	8	10
180.0	150.0	160.0	8400.0	5600.0	2400.0	1000.0	710.0

The surface skin of the shark's head was kept dry while the seawater was passed through the pharynx and the gills through tubes connected to each spiracle and circulated through constant temperature system (15 ± °C). The shark was injected with 200 μCi of ^{14}C-urea through its caudal artery. Samples of perfusate were taken every 2 min for a period of 6 min before pardaxin (25 μg ml^{-1}) was added, and every 2 min after it was administered to the perfusate for a period of 10 min. The result was given in units of equivalent urea h^{-1} kg^{-1} b.wt and are the means of data from two fish.



A scheme of an apparatus used to locate the action of padaxin in *Squalus acanthias*. The surface skin of the shark's head is immersed in a chamber containing 2 l of seawater. Its pharyngeal cavity and the gills are perfused with seawater from a container located 1 m above the fish through a pair of tubes attached into each spiracle. The outflow is removed by a pair of chambers tightly attached to each of the gill's clefts and through a tube inserted in the shark's mouth and pumped through constant temperature bath (15 ± 1 °C) back to the container. The total perfusate volume consists of 2.5 l. Experiments (five each): a) PX administered into the water bathing shark's head; and b) PX administered into the perfusate (shark's pharynx and gills) at a concentration of 25 μg ml^{-1}.

structed (fig.). PX (25 μg ml^{-1}) administered to the medium bathing the shark's head skin surface did not elicit observeable behavioral responses (within 10 min). However, when added to the medium bathing the shark's pharynx and the gills, PX immediately caused the shark to struggle severely.

Discussion. Free-swimming sharks previously were noted to respond to *Pardachirus* fish by avoidance, struggling and mouth paralysis while attempting to bite them[3] and PX also was shown to elicit a similar effect when applied to a shark positioned in a tank[16]. Therefore, it is likely that the struggling and mouth paralysis elicited by administration of PX into the medium of the pharyngeal cavity and the gills is related to its repellent action in sharks. At present, it is difficult to draw a connection between a transient increase in gill permeability to urea and the induced behavioral responses. Merely by increasing the seawater temperature from 15 °C to 30 °C evokes a 15-fold increase in the leakage of urea in sharks[23]. However, these sharks did not struggle or show mouth paralysis similar to those produced by PX. Therefore, an increase in gill permeability to urea alone does not appear to be the direct stimulus for the above PX-induced behavioral effects but rather resulted from an impairment of chemoreceptors situated in the gill and/or pharyngeal cavity. In the whole fish it is difficult to separate the water body which bathes the gills from these which flow through the pharyngeal cavity. Therefore, presently we cannot conclude as to PX acts on both of these organs or on one of them to produce its noxious effect. However, it was rather unexpected to find that the shark's main sensory system located at the head surface does not mediate PX's repellent action. In *S. acanthias* the gills are known to have respiratory and nonrespiratory function[17]. This study suggests that they could also mediate sensory responses to repellent substances. The in vivo results presented in this study are with agreement with the in vitro results from the isolated killifish gill-like epithelia.

In those studies PX elicited a net sodium flux from seawater side toward the body and an about equal leak on both sides to inulin[24]. The possible biological significance of the detergent property of the Moses sole secretion, could be that it promotes its spreading and absorption from the water into which it is released by the flatfish and facilitates its noxious action upon the gill and/or pharyngeal cavity membranes of predators.

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Sahara stopover in migratory flycatchers: fat and food affect the time program

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Summary. Migrating spotted flycatchers, resting and feeding in an oasis, have longer stopover periods when fat reserves on arrival are low. In the laboratory migratory activity could likewise be suppressed by a combination of two factors: low fat reserves and the possibility of feeding.

Key words. Bird migration; stopover; fat reserves; time program.

The temporal course of fall migration in long-distance migrants is partly based on an endogenous[2], genetically determined program[3,4], which defines the onset and end of migration in inexperienced young birds. So far all efforts to manipulate this program experimentally by altering environmental factors[5] or the physiology[6-9] of the birds have failed. Food deprivation experiments at the beginning of or during the migration period did not affect migratory activity unless fat reserves