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Invasion of the vertebrate skin by cercariae of *Trichobilharzia ocellata*: penetration processes and stimulating host signals

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Abstract The penetration of *Trichobilharzia ocellata* cercariae into the skin of their duck hosts was described using electron microscopy and histology. The behavior patterns of the cercariae on their exposure to human skin differed only little from those known for *Schistosoma mansoni* cercariae. After their attachment to living human skin the cercariae crept to wrinkles within a mean of 8 s, and full penetration was achieved within a mean of 4.0 min (83 s to 13.3 min). Tail shedding occurred as early as within a mean of 6.5 s of the first penetration attempts. It was supported by a muscular sphincter at the cercarial hindbody. The skin-surface stimuli for cercarial penetration were contained in the lipid fraction of the duck and human skin surface; hydrophilic components were effective only in some *T. ocellata* isolates. The penetration-stimulating components of duck-skin lipids were exclusively free fatty acids with the same chemical characteristics known to stimulate penetration of *Schistosoma* species. Skin-surface lipids of the abnormal human host, with their higher fatty acid contents, stimulated higher cercarial penetration rates than did skin lipids of the natural duck host. Fatty acids as penetration stimuli may offer advantages for *T. ocellata* cercariae by increasing the specificity for an invasion of terrestrial vertebrates, which is additionally determined by cholesterol and ceramides as signals for attachment and enduring contact behavior.

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Introduction

Cercariae of the duck blood fluke *Trichobilharzia ocellata* actively penetrate their duck host's skin. They also invade human skin and cause cercarial dermatitis. How the cercariae find and recognize their hosts has been studied in some detail (Neuhaus 1952; Wright 1974; Feiler and Haas 1988a, b), and the results show that the host-finding strategy of *T. ocellata* cercariae differs considerably from those of the four mammal-invading *Schistosoma* species studied thus far (reviewed by Haas 1992, 1994; Haas and Haberl 1997). After leaving the snail intermediate hosts, *T. ocellata* cercariae enter a phase of dispersal and microhabitat selection employing a complex intermittent swimming behavior (Feiler and Haas 1988a). Then they tend to cling to the water surface or to solid materials in an energy-saving resting posture, whereby they do not respond to water currents or to touch. However, they react sensitively to minor shadow stimuli with long, negative, phototactically oriented forward swimming bursts, which may lead the cercariae down from the water surface toward the feet of ducks. The shadow simultaneously induces an increased readiness of the parasites to attach to warm substrates (the threshold for a full response is a temperature difference of 1 °C) and to duck skin (Feiler and Haas 1988a).

The chemical signals that stimulate the attachment to duck skin have been identified as the lipids cholesterol and ceramides (Feiler and Haas 1988b). The use of these compounds as signals for attachment has been interpreted as adaptation to invade ducks as hosts (Haas 1992, 1994). The cercariae may have specialized on lipids as host signals as the glandless skin of duck feet, with its lipid-laden stratum corneum, may release fewer diffusable components into the surrounding water than does the skin of land-living mammals. In addition, ceramides and cholesterol are lacking in bird uropygial gland contents, which are distributed on bird feathers. Therefore, the cercarial response to these compounds may

avoid useless attachment to bird feathers. However, as both components also occur on the mammalian skin surface, *T. ocellata* cercariae also readily attach to mammalian skin.

After their attachment to the skin, *T. ocellata* cercariae remain on this substrate by responding as well to warmth and to cholesterol and ceramides (Feiler 1985). Then they penetrate the skin. Skin invasion and the host responses have been studied using histology methods and recovery in tissue squashes (Brackett 1940; Macfarlane 1949; Olivier 1953; Olivier and Weinstein 1953; Yang et al. 1965; Bourns et al. 1973; Shimuzu et al. 1981; Appleton and Brock 1986). Further migration via the lungs and liver to the intestine has been analyzed using organ preparation and histology (Bourns et al. 1973; Ellis et al. 1975) and detection of radioisotope-labeled cercariae in compressed host tissues (Haas and Pietsch 1991). However, only sparse information is available on cercarial behavior patterns during penetration, and the chemical stimuli for the penetration response are unknown. In this study we examined the behavior of *T. ocellata* cercariae penetrating the skin of their duck hosts and that of the abnormal human host and we identified the host cues that stimulate skin penetration.

Materials and methods

Our stock of *Trichobilharzia* is synonymous with *T. szidati* (Neuhaus 1952) and can be considered as *T. ocellata* La Valette 1855 (Odening 1996). The stock was isolated in its snail host *Lymnaea stagnalis* from fish ponds near Höchstadt/Aisch (Germany) and was maintained using domestic ducks (*Anas platyrhynchos*) as final hosts and laboratory-reared *L. stagnalis* as intermediate hosts. Some experiments on the penetration-stimulating effect of hydrophilic skin extracts were also performed with cercariae from field-collected snails. Cercariae were used in the experiments in dechlorinated tap water (DTW) within the first 3 h of their departure from the snail hosts.

Penetration into skin and agar substrates

Penetration of the cercariae into living human skin was observed by placement of about ten cercariae in 50 µl DTW on the skin of the forearm and observation of the cercarial behavior under a dissecting microscope at room temperature (25–27 °C). Skin surface temperatures varied between 29.4° and 32.5 °C. Many itching papules appeared on the exposed skin areas after the experiments, indicating successful cercarial penetration. Penetration into duck skin was studied by fixing of the foot webs of anesthetized ducks in a spread position, placement of nickel-plated brass rings (13-mm inner diameter according to Smithers and Terry 1965) on the web skin, and introduction of about 1000 cercariae in 500 µl DTW onto the skin areas. The ducks were killed with an overdose of the anesthetic and the marked web skin areas were processed for histology at 8, 16, 24, and 32 min after exposure to the cercariae.

The effect of chemical agents on cercarial penetration was studied using modifications of the methods described by Haas and Schmitt (1982a) and Haas et al. (1987). In brief, the skin fractions or chemicals were integrated into 0.4% agar (agar agar SERVA for electrophoresis; Serva, Heidelberg, Germany), adjusted to pH 7.0 with 5 m*M* phosphate buffer, and poured into flat-bottomed wells (7-mm diameter) of microtiter plates at 50 µl/well. After solidification the agar substrates were layered with 20 µl DTW containing 20–60 cercariae. After incubation for 1 h at 35 °C the percentage of cercariae that had penetrated was determined.

Fractionation of skin

The uppermost layers of duck-foot skin or human skin were scraped off with a scalpel as described by Haas et al. (1987). The scrapings were homogenized and separated into lipophilic and hydrophilic extracts using the extraction methods of Folch et al. (1957) as modified by Haas et al. (1987). Duck foot-skin lipids were fractionated by thin-layer chromatography (TLC) as described by Haas et al. (1987). In brief, 20 × 20-cm precoated TLC plates (Silicagel 60, layer thickness 0.25 mm; Merck, Darmstadt, Germany) were developed successively in hexane (to 19 cm), in toluene (to 19 cm), and, finally, (twice) in hexane/diethylether/acetic acid at 70:30:1 (to 10 cm). Three lipid mixtures were removed from the plates and offered to the cercariae: free fatty acids at a flow rate (R_f) of 0.31–0.38, more polar lipids at R_f 0–0.3 (including free sterols, diacylglycerols, phospholipids, ceramides, and glucosylceramides), and nonpolar lipids at R_f 0.4–1.0 (including triacylglycerols, wax esters, sterol esters, and hydrocarbons). Amino acid analyses of hydrophilic skin extracts were performed by high-performance liquid chromatography as described by Haas et al. (1995).

Histology and electron microscopy

Cercaria-exposed duck-foot webs were fixed in Bouin's solution and embedded in Araldite, and 12-µm sections were subjected to Azan staining according to Heidenhain. For scanning electron microscopy, cercariae or duck skin samples were fixed in 2.5% glutaraldehyde, postfixed in 1% osmic acid, dehydrated, and dried in a critical-point drier. After being coated with a 10-nm gold layer the samples were examined with a Hitachi S-500 SEM operating at 25 kV. For transmission electron microscopy the cercariae were fixed in 2.5% glutaraldehyde in 100 m*M* phosphate buffer (pH 7.4) for 45 min, washed in phosphate buffer, postfixed with 4% OsO_4 in phosphate buffer, then dehydrated in acetone and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate and then examined in a Zeiss 9-S electron microscope operating at 60 kV.

Results

Penetration processes

After their attachment to living duck web or human skin, most cercariae performed a short period of leech-like creeping behavior. On human skin, only 5 of 50 cercariae penetrated immediately at the attachment site without creeping. During creeping, which lasted 8 s in mean (0–80 s, Fig. 1A), the cercariae crawled to wrinkles and eventually followed them over various distances before they started penetration movements. All penetration sites were located in wrinkles or at the skin surrounding the orifices of hair follicles (8 of 50 observed entries). The creeping cercariae did not seem to be attracted to hair follicles, as many cercariae crept over the follicles and later penetrated into wrinkles and as not a single penetration of several hundred observed penetrations occurred within follicular canals. However, cercariae that showed penetration movements seemed to exert a strong stimulatory effect on creeping individuals. It was often observed that a creeping cercaria started penetration immediately (one to three body widths) next to an individual in the process of penetrating. This did not seem to occur by chance. When about 10 cercariae were placed on a skin surface area measuring 4–5 mm in

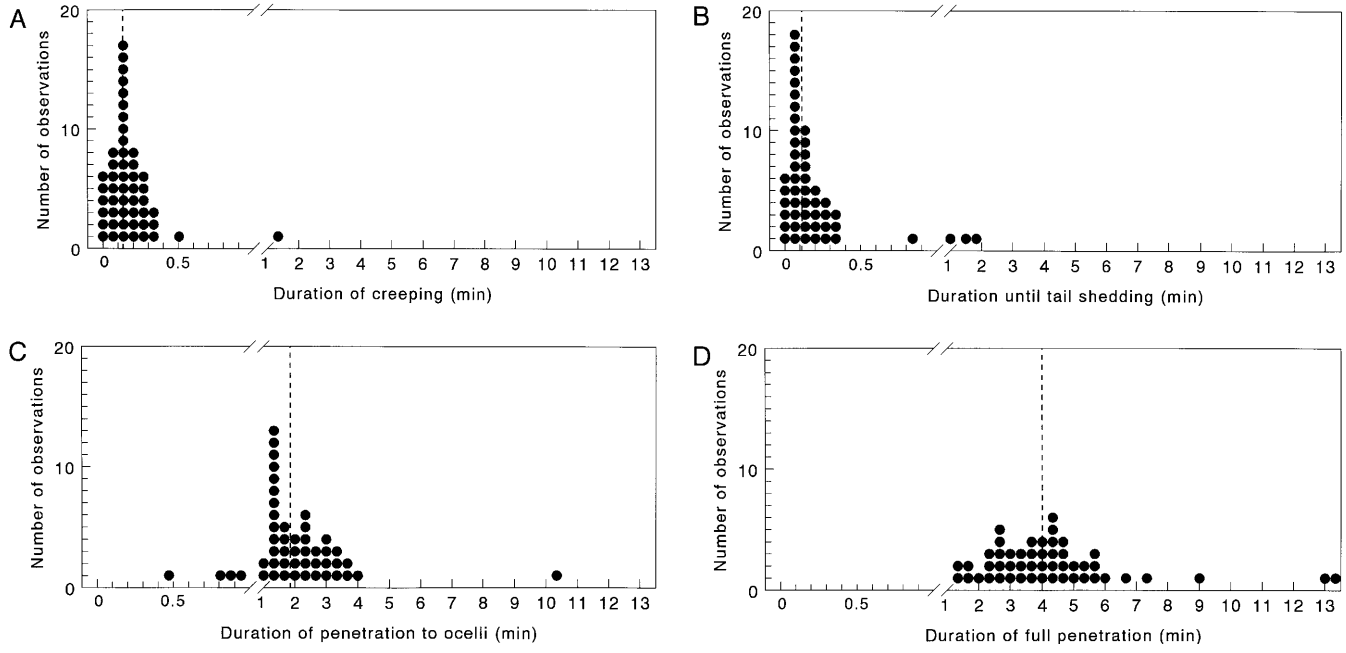


Fig. 1A–D Entry of *Trichobilharzia ocellata* cercariae into living human skin. **A** Duration of creeping between attachment and first penetration attempts. **B** Duration between first penetration attempts and shedding of the tail. **C** Duration of penetration movements until ocelli enter the penetration pore. **D** Time needed for full penetration, i.e., until the hindbody presses against the penetration pore during pushing movements. *Vertical lines* represent median values

diameter, 3 of 50 replicates showed the penetration of 5 cercariae into the same penetration site, and in 6 replicates, 4 individuals penetrated together. It could not be determined whether penetrating individuals attracted the creeping individuals or whether they just stimulated penetration behavior.

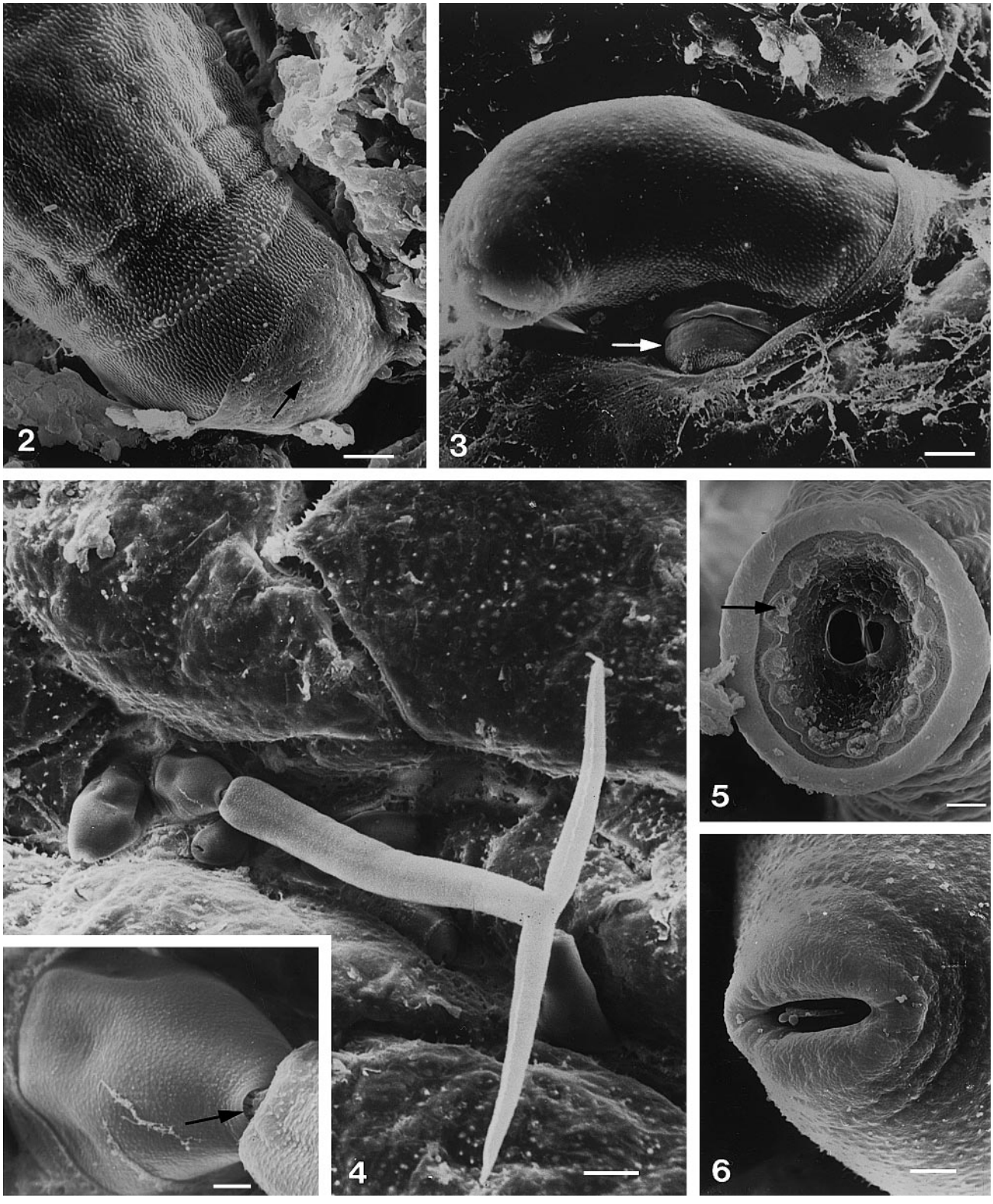
Penetration started with a close attachment of the oral sucker to the skin surface. Then, intense contractions and elongations of the sucker created an entry site, whereby the spines gave mechanical support and the acetabular gland contents were secreted (Figs. 2, 8–10). Histology showed a distinct lysis of the stratum corneum (Figs. 9, 10). After the onset of penetration movements the tail was normally shed on the human skin after a mean of 6.5 s (0–105 s, Fig. 1B). However, tail shedding during creeping (2 of 50 observed penetrations) or immediately together with the first penetration movements also occurred (1 of 50 observations). The sudden tail shedding seems to be supported by the anatomy of the tail-body junction. A tegument-like layer separates most of the hindbody from the tail, and connecting muscle fibers enter to a distance of only 4 μm into the tail stem (Figs. 4, 5, 7). The hindbody ends with a collar that contains circular muscle fibers (Figs. 5, 7), which obviously plays a role in the closure of the cercarial hindbody after tail shedding (Fig. 6). A contraction of this collar might even participate in the tail-shedding process by disrupting the connecting muscle fibers. Perhaps Fig. 4 shows this process.

The penetration occurred normally in a nearly surface-parallel direction. The thrusts of the oral sucker were accompanied by contractions and elongations of the body. The spined ventral sucker supported the squeezing of the body into the opening (Fig. 3), and the hindbody seemed to contribute a great deal toward pushing the cercaria into the opening via pressure against the skin surface and elongation. The cercariae had entered the living human skin within a mean of 4.0 min; the fastest full penetration occurred within 83 s (Fig. 1D). After penetration, most cercariae travelled just below the stratum corneum in a surface-parallel direction before they entered deeper layers. Histology showed that the pre- and postacetabular glands were nearly empty, whereas the head gland retained its contents during this phase of migration (Fig. 11).

Host stimuli for penetration

Warmth could be excluded as a stimulus for cercarial penetration. The cercariae never showed penetration attempts when applied in 25 °C water on various surfaces (agar, glass, silver sheet, petroleum jelly) warmed up to temperatures ranging between 26° and 45 °C.

The chemical signals of skin that stimulate cercarial penetration behavior were determined by a study of cercarial penetration into agar substrates containing human and duck skin fractions. In most experiments the cercariae responded only to skin lipids, whereas hydrophilic skin extracts had no effect on penetration (Table 1A). The lipids of the human skin surface resulted in higher penetration rates than did those of the duck-skin surface. Only cercariae of two isolates also responded to hydrophilic duck-skin components (data not shown). However, the responses were not consistent enough to



Figs. 2–6 Scanning electron micrographs of *T. ocellata* cercariae penetrating into the foot skin of a duck. **Fig. 2** Anterior end in the penetration site. Note the spines and acetabular gland secretions (*arrow*). *Bar* 10 μm. **Fig. 3** The spined ventral sucker (*arrow*) supports the penetration. *Bar* 10 μm. **Fig. 4** Shedding of the tail. One of six penetrating cercariae has not yet shed its tail. *Bar* 50 μm. The *insert* shows that the hindbody is connected to the tail by muscle fibers (*arrow*). *Bar* 10 μm. **Fig. 5** Hindbody of a cercaria after removal of the tail. A collar encloses an area with 14 muscle-fiber ends (arrow) and the openings of the 2 protonephridial canals. *Bar* 5 μm. **Fig. 6** Hindbody of a cercaria after contraction of the collar. *Bar* 5 μm

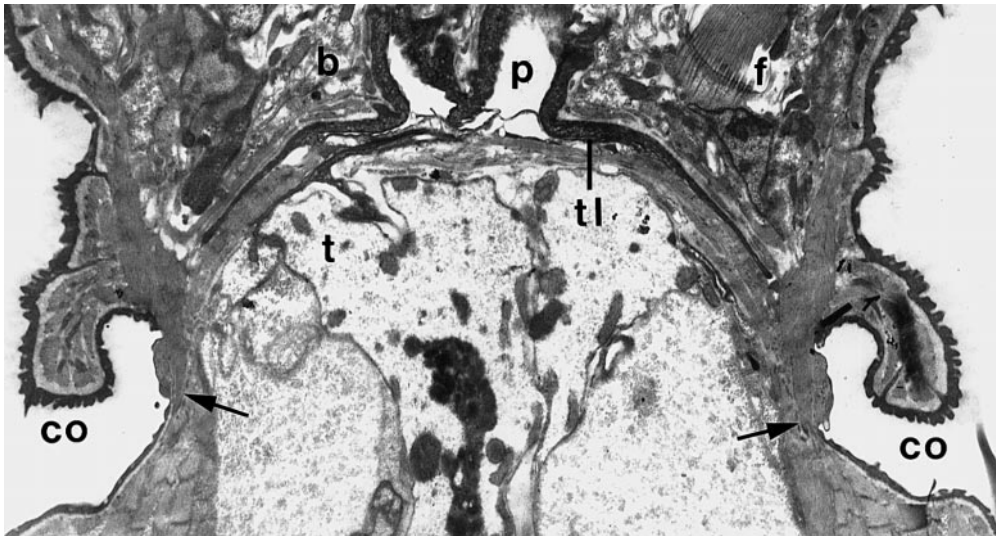
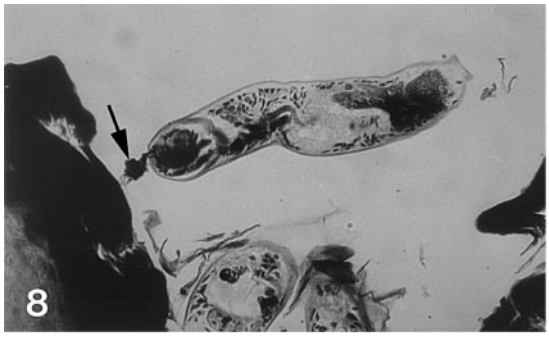
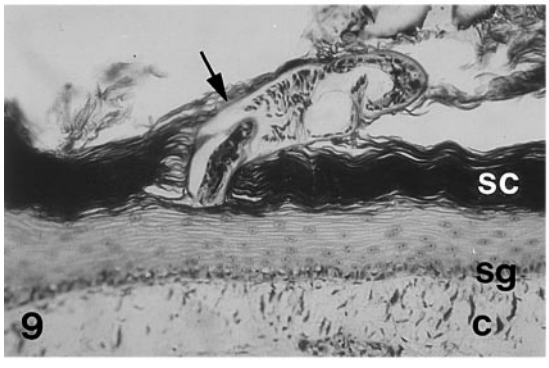
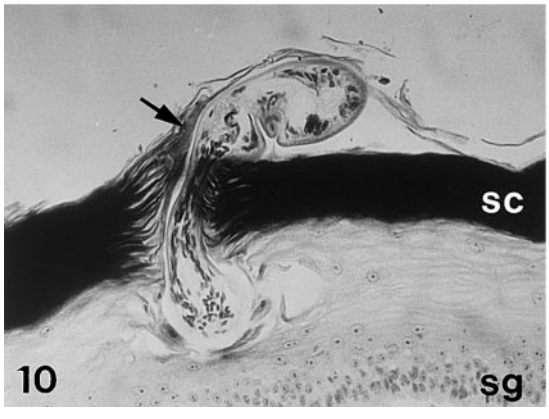
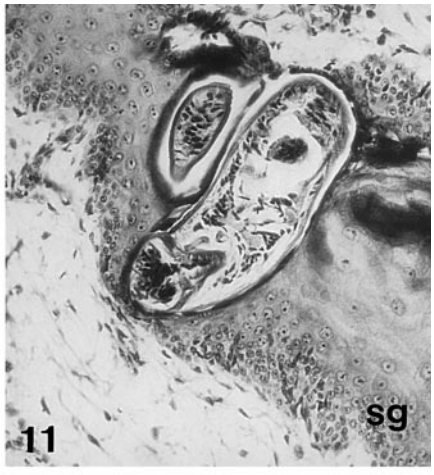


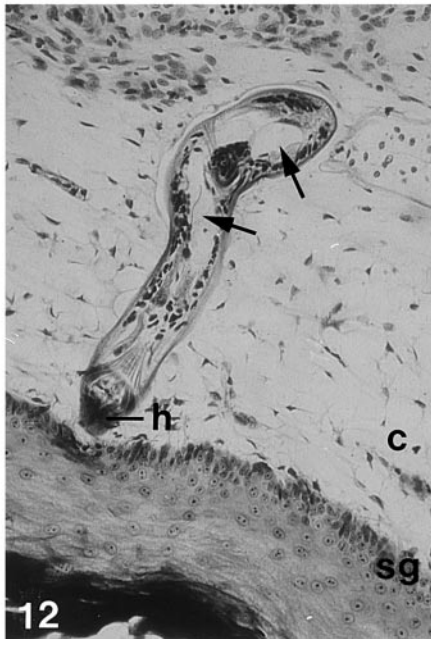
Fig. 7 Transmission electron micrograph of the tail-body junction of a *T. ocellata* cercaria (×4300). The cercarial body (*b*) is separated from the tail (*t*) to a large extent by a tegument-like layer (*tl*), but peripheral longitudinal muscle fibers of the body end within the tail (*arrows*). The collar of the hindbody (*co*) contains circular muscles, which may contribute to sealing of the hindbody after shedding of the tail (*f* Flame cell, *p* protonephridial canal)











Figs. 8–12 Histology sections of *T. ocellata* cercariae penetrating into the foot skin of a duck. **Fig. 8** Cercaria adhering to the skin surface via secretions of the pre- and postacetabular glands (*arrow*). **Figs. 9, 10** Cercaria penetrating the stratum corneum. The tissue is lysed and secretions of the postacetabular glands are distributed around the cercaria (*arrows*). **Fig. 11** Cercaria after penetration of the stratum corneum in a position parallel to the stratum germinativum. **Fig. 12** Cercaria in the corium after penetration of the stratum germinativum. The pre- and postacetabular glands are nearly empty (*arrows*), whereas the head gland retains most of its contents (*c* Corium, *sc* stratum corneum, *sg* stratum germinativum, *h* head gland)

Table 1 Influence of duck-foot and human skin surface fractions and their components on the penetration of *Trichobilharzia ocellata* cercariae into agar[a]

	Concentration	Penetration, %	Confidence limits, 95%
A. Hydrophilic and lipophilic skin extracts:			
Hydrophilic extract, duck	0.8 mg/ml	0.6	0–2.7
Lipophilic extract, duck (low conc.)	0.8 mg/ml	5.7*	3.2–9.3
Lipophilic extract, duck (high conc.)	1.6 mg/ml	40.6*	33.9–46.4
Mixture of both extracts, duck	0.8 mg/ml each	6.5*	3.8–10.3
Hydrophilic extract, human	0.8 mg/ml	0	0
Lipophilic extract, human	0.8 mg/ml	50.0*	43.6–56.3
Mixture of both extracts, human	0.8 mg/ml each	52.0*	40.9–55.1
Control		0	0
B. TLC fractions of duck-foot skin-surface lipids:			
Crude lipids	0.5 mg/ml	17.5*	15.2–22.2
Nonpolar lipids	0.5 mg/ml	0	0
Polar lipids	0.5 mg/ml	0	0
Fatty acids	0.05 mg/ml	82.8*	78.4–86.1
Control		0	0
C. Cholesterol and ceramides (pure chemicals):			
Cholesterol	0.5 mg/ml	0	0
Ceramides	0.5 mg/ml	0	0
Lipids, duck-foot skin	1.0 mg/ml	34.6*	27.8–41.5
Control		0	0
D. Aliphatic hydrocarbon chains with 0, 1, and 2 hydrophilic end groups (pure chemicals):			
Dodecane	0.05 m*M*	1.1	0.3–2.5
Dodecanoic acid	0.05 m*M*	43.8*	38.6–48.5
1,12-Dodecanedioic acid	0.05 m*M*	0	0
1-Dodecanol	0.05 m*M*	54.3*	49.0–59.0
2-Dodecanol	0.05 m*M*	0	0
1,12-Dodecanediol	0.05 m*M*	0	0
Control		0	0
E. Saturated monocarboxylic acids, effect of chain length (pure chemicals):			
Ethanoic acid	1.0 m*M*	0	0
Butanoic acid	1.0 m*M*	0	0
Hexanoic acid	1.0 m*M*	0.3	0.0–1.3
Octanoic acid	1.0 m*M*	0.4	0.1–1.1
Nonanoic acid	0.5 m*M*	3.2*	2.2–4.5
Decanoic acid	0.5 m*M*	23.4*	20.8–26.1
Undecanoic acid	0.5 m*M*	23.3*	20.7–26.0
Dodecanoic acid	0.5 m*M*	37.0*	34.0–40.1
Tridecanoic acid	0.5 m*M*	12.9*	10.9–15.2
Tetradecanoic acid	0.5 m*M*	4.4*	3.2–5.9
Hexadecanoic acid	1.0 m*M*	0	0
Octadecanoic acid	1.0 m*M*	0	0
Eicosanoic acid	1.0 m*M*	0	0
Control		0	0
F. Unsaturated monocarboxylic acids, effect of number of double bonds (pure chemicals):			
Octadecanoic acid	0.1 m*M*	0	0
cis-9-Octadecenoic acid	0.1 m*M*	2.4	0.9–5.2
cis-9,*cis*-12-Octadecadienoic acid	0.1 m*M*	18.4*	13.8–23.8
cis-9,*cis*-12,*cis*-15-Octadecatrienoic acid	0.1 m*M*	24.0*	18.9–29.8
Control		0	0
G. Unsaturated monocarboxylic acids, effect of *cis*-/*trans*-isomery (pure chemicals):			
cis-9-Hexadecenoic acid	1.0 m*M*	28.9*	23.5–35.0
trans-9-Hexadecenoic acid	1.0 m*M*	2.7	1.0–5.6
cis-9,*cis*-12-Octadecadienoic acid	1.0 m*M*	35.8*	30.1–42.3
trans-9,*trans*-12-Octadecadienoic acid	1.0 m*M*	1.3	0.3–3.5
Control		0	0

* $P < 0.001$ vs control (χ^2 test)
[a] Percentage of penetration of 270–910 cercariae (5–20 replicates)

allow an analysis of the chemical nature of the hydrophilic stimuli. Amino acids could be excluded as stimuli, as they had no effect when offered to the cercariae at the same concentration determined within the hydrophilic skin extracts (data not shown).

A separation of duck-foot skin-surface lipids by TLC revealed that the penetration-stimulating activity was exclusively contained in the fatty acid fraction (Table 1B). The fractions containing more polar lipids (including free sterols, diacylglycerols, phospholipids,

ceramides, and glycosylceramides) and more nonpolar lipids (including triacylglycerols, wax esters, sterol esters, and hydrocarbons) were without any effect on the penetration response. Cholesterol and ceramides, which intensively stimulated attachment (data not shown), had no effect on penetration when offered as pure chemicals (Table 1C).

The penetration-stimulating structure of fatty acids was determined by the offering of pure chemicals to the cercariae. Aliphatic hydrocarbon chains stimulated penetration only when they contained both a hydrophilic and a lipophilic end group (Table 1D). Hydrocarbons with two hydrophilic or two lipophilic end groups were without effect. As the hydrophilic end group, a hydroxylic group was as effective as a carboxylic group. Saturated fatty acids stimulated penetration only at chain lengths of between 9 and 14 carbons; a maximal response was obtained with dodecanoic acid (Table 1E). The penetration-stimulating effect of unsaturated fatty acids increased with the number of double bonds (Table 1F), whereby *cis*-isomers had a considerably stronger stimulatory effect than did *trans*-isomers (Table 1G). All penetration-stimulating components, including human and duck skin surface lipids, had an impairing effect on the cercariae as reflected by reduced mobility, a swollen appearance, and, eventually, death. This was not the case for the other skin components.

Discussion

The behavior of schistosomatid cercariae during penetration of vertebrate skin has been intensively studied in *Schistosoma mansoni* (Stirewalt and Hackey 1956; Stirewalt 1959). *Trichobilharzia ocellata* cercariae showed similar behavior patterns. However, they seem to differ from *S. mansoni* in their early tail shedding just as they start to penetrate human skin. *S. mansoni* cercariae often retain their tails until they have achieved full penetration. Vigorous tail movements seem to support their entry, whereas *T. ocellata* cercariae use their tailless hindbodies to push the body into the opening of the skin. The mechanism underlying the tail shedding of *T. ocellata* cercariae seems to be similar to that suggested for *S. mansoni* (Howells et al. 1975); in both species, shedding is supported by a sphincter muscle at the cercarial hindbody.

The time required by *T. ocellata* cercariae for full penetration into human skin also corresponds to that reported for *S. mansoni* during penetration into various mammalian skin types (Stirewalt and Hackey 1956). Only newborn mice and hairless mice were penetrated more quickly by *S. mansoni* cercariae (average 2.7–3.1 min, fastest entries within 0.7–0.8 min). Our finding that *T. ocellata* cercariae can enter the human skin as rapidly as within 83 s indicates that a very short contact of humans with contaminated water may result in cercarial dermatitis. However, most cercariae needed more time for penetration (average 4 min). Furthermore, we could press fully penetrated cercariae out of their penetration canals by rubbing the skin just after the experiments, and penetrating cercariae died when the skin was dried. This means that some protection from cercarial dermatitis can be achieved when the skin is rubbed to full dryness immediately after water contact.

On human skin, *T. ocellata* cercariae often penetrated in groups at the same penetration sites, and there is no doubt that this was the result of an interaction among the individuals. However, we could not decide whether cercariae in the process of penetrating attracted nearby creeping individuals or whether they only stimulated penetration. Stirewalt (1971) described group penetrations in *S. mansoni* cercariae and suggested "that some diffusible substance related to the penetration process is a penetration stimulus to nearby cercariae." A cooperation of the cercariae during penetration would seem to offer advantages, e.g., in immune evasion processes and by saving energy and acetabular gland contents. However, it is difficult to imagine how this cercarial interaction on the skin might have evolved. The cercariae are widely distributed in their aquatic habitats, and it may be a very rare event that the few cercariae attaching to the relatively large-dimensioned vertebrate skin actually meet each other. Indeed, in vitro experiments in our laboratory showed that creeping *S. mansoni* cercariae were strongly attracted by human skin surface extracts but not by cercarial acetabular gland secretions (Stoll et al. 1997; Stoll et al., unpublished results). This suggests that an attraction to penetration sites might be brought about by skin components, which may be liberated by the penetrating organisms.

The penetration of *T. ocellata* cercariae is mainly stimulated by fatty acids, whereas warmth as a single stimulus has no effect. Our findings that some *T. ocellata* isolates responded in addition to hydrophilic skin components seem to point to different physiological strains and need further investigation. Host cues that stimulate schistosomatid cercarial penetration into birds have been analyzed only for two gull-invading marine species. *Austrobilharzia terrigalensis* penetrated in response to free sterols, whereas fatty acids had only a weak effect (Clegg 1969), and *A. variglandis* responded maximally to fractions of chicken skin containing cholesterol, fatty acids, and triacylglycerols, but other lipid fractions also had a significant stimulatory activity (Zibulewsky et al. 1982). Therefore, bird-invading schistosomatids seem to have evolved a similarly high diversity of host-recognition strategies as those found in other trematode species (Haas 1994; Haas and Haberl 1997). The possibility also remains that the identification of terrestrial vertebrates generally differs between marine and freshwater species. The freshwater-living *T. ocellata* cercariae actually penetrate in response to free fatty acids with the same chemical characteristics as do the four mammal-invading *Schistosoma* species whose host-finding behavior has been analyzed thus far (reviewed by Haas 1992, 1994; Haas and Haberl 1997).

At first sight it is not intelligible why *T. ocellata* cercariae rely on fatty acids as penetration stimuli. Our various analyses of duck-foot skin lipids revealed only low amounts of free fatty acids. Indeed, the fatty-acid-rich human skin-surface lipids stimulated *T. ocellata* cercarial penetration with a higher level of intensity than did duck-foot skin lipids. However, the response to fatty acids also seems to bear some advantages. First, it may act as an additional "compatibility filter" in the host selection process (Combes 1991) that may contribute to restriction of the invasion to terrestrial vertebrate hosts with a higher probability than a response to ceramides and cholesterol alone. Second, fatty acids might contribute to transformation and immune evasion processes of *T. ocellata* schistosomula in a fashion similar to that suggested for *S. mansoni* cercariae (Salafsky and Fusco 1987). Indeed, *T. ocellata* cercariae synthesized the same types of prostaglandins, leucotrienes, and hydroxyeicosatetranoic acids in similar quantities as did *S. mansoni* cercariae when incubated in linoleic acid, and the obtained eicosanoid fractions of both species inhibited superoxide production of human neutrophils with the same intensity (Nevhutalu et al. 1993).

The response of *T. ocellata* cercariae to fatty acids might provide an approach for a specific control method similar to that suggested for *Schistosoma* cercariae (Haas 1984). Our data on the chemical characteristics of the stimulating fatty acids, such as the effect of the chain length, double bonds, and *cis*-/*trans*-isomery, suggest that the molecules fit into a specific fatty acid receptor similar to that described for *S. mansoni* cercariae (Haas and Schmitt 1982a, b). In addition, *T. ocellata* cercariae were impaired by all penetration-stimulating components under hypoosmotic conditions, as were *S. mansoni* cercariae. Therefore, fatty acids seem to stimulate not only penetration behavior of *T. ocellata* cercariae but also a transformation of the tegument for immune evasion. Fatty acid analogues that intensely stimulate penetration behavior may thus affect the host-invasion capability of *T. ocellata* cercariae with a level of intensity similar to that with which they affect *S. mansoni* cercariae (Haas 1984). They may therefore be considered as potential candidates for slow release into *Trichobilharzia*-infested waters for the control of cercarial dermatitis in humans.

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