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# Specific Binding of Human Low-density Lipoprotein to the Surface of Schistosomula of *Schistosoma mansoni* and Ingestion by the Parasite

**Maria W. Bennett*‡ and John P. Caulfield*†‡‖**

*From the Program in Cell and Developmental Biology,* and the Departments of Pathology,† Harvard Medical School, Tropical Public Health,‡ Harvard School of Public Health, and Rheumatology/Immunology,‖ Brigham and Women's Hospital, Boston, Massachusetts*

***Low-density lipoproteins (LDL)[1] may be important in human schistosomiasis because LDL bound to the surface of the parasite inhibits the binding of anti-schistosomal antibodies. Low-density lipoproteins also may serve as a source of lipids for the parasite membrane synthesis. Here LDL fluorescently labeled with carbocyanine dye (DiI-LDL) was used to measure the specificity of binding of LDL to the surface of schistosomula of* Schistosoma mansoni *and to examine the distribution of the LDL particles over time. DiI-LDL binding was saturable and specific, with strong inhibition by unlabeled LDL and apoB but not by apoA1, bovine serum albumin, or IgG, and only weak inhibition by high-density lipoproteins. Half of the bound DiI-LDL was displaced by unlabeled LDL. DiI-LDL remained bound on the surface of schistosomula for up to 36 hours in culture. However parasites also ingested both DiI-LDL and a second fluorescent LDL, Bodipy-LDL. Over time, both fluorophores appeared throughout the worm tissues, suggesting the LDL particles were breaking down and that the fluorophores and lipids originally contained within the LDL particle were partitioning throughout the worm. Thus human LDL appears to bind to the surface of schistosomula specifically. Ingested LDL appears to be broken down and may serve as a source of host lipids for the parasite. (Am J Pathol 1991, 138:1173–1182)***

*Schistosoma mansoni* is one of the species of schistosomes that cause schistosomiasis, a disease affecting more than 200 million people worldwide. Cercariae enter through the skin from fresh water and transform into larval schistosomula, which migrate through the lungs to the liver. Fully developed adults live in the portal blood vessels for years. However the schistosome surface can be recognized and attacked by the host immune system in cell-mediated, antibody-dependent reactions. The parasite also can defend itself against both recognition and attack. The surface is covered by two lipid bilayers that overlie a syncytium called the tegument. The tegumental membranes are synthesized in 'cell' bodies or cytons lying beneath the tegument and two subjacent muscle layers.[1–3] The cytons then package the tegumental membranes into multilaminate bodies that are transported to the surface where the bodies fuse with the tegumental membranes. The lipids for all of the schistosome's membranes, including the tegumental membranes, must be derived from the host because the parasite cannot synthesize cholesterol or fatty acids *de novo*.[4]

Schistosomula of *Schistosoma mansoni* bind human low-density lipoprotein (LDL) *in vitro*.[5–9] Low-density lipoprotein is present in human plasma in a concentration of approximately 4 mg/ml and consists of particles that are 78% lipid. Each particle is made up of a core of cholesterol ester surrounded by a phospholipid and apoprotein B-100 (apoB) shell.[10–12] Low-density lipoprotein bound to schistosomes may assist parasite survival in humans in two ways. First the bound LDL may supply the worm with cholesterol and fatty acids for the biogenesis of its membranes. Second, *in vitro*, the bound LDL partially blocks the binding to the parasite surface of anti-schistosomal antibodies from the serum of infected patients.[5,6] Thus LDL binding to the worm may mask par-

[1]Abbreviations used in this paper: Apoprotein A1 (apoA1); Apoprotein B (apoB); Apoprotein E (apoE); 1,1′-dioctadecyl-3,3,3′, 3′-tetramethylindocarbocyanine complex (DiI); low density lipoprotein (LDL); high density lipoprotein (HDL); relative fluorescence (RF).

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Address reprint requests to John P. Caulfield, Harvard Medical School, Seeley G. Mudd Building, Room 517, 250 Longwood Ave., Boston, MA 02115.

asite antigens and serve as a mechanism for immune evasion.

Previous studies from this laboratory used antibodies to apoB to measure LDL binding to the worm *in vitro*.[5,6] These studies showed that LDL bound in a time-dependent and saturable manner and with partial charge dependence. The specificity and long-term distribution could not be assessed, however, because the labeling technique did not permit this. Here we used LDL labeled with a carbocyanine dye partitioned into the lipid component of the particle (DiI-LDL)[13,14] to visualize LDL binding to the worm under various conditions. The specificity of LDL binding to the surface of schistosomula was determined by measuring inhibition of DiI-LDL binding with unlabeled LDL and other lipoproteins as well as IgG and bovine serum albumin (BSA). Displacement of bound DiI-LDL was tested with unlabeled LDL. Furthermore DiI-LDL and another fluorescently labeled LDL (Bodipy-LDL) were used to follow the fate of LDL bound to the parasite over time. In particular, we examined the parasite's ability to internalize both forms of the labeled LDL.

## Materials and Methods

### Parasites and Culture Conditions

Schistosomula of a Puerto Rican strain of *Schistosoma mansoni* maintained in *Biomphalaria glabrata* snails were obtained by vortexing cercariae.[15] For each experiment, 10,000 to 50,000 freshly transformed schistosomula were washed six times in Roswell Park Memorial Institute (RPMI) 1640 medium containing 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mmol/l (millimolar) L-glutamine, and 20 mmol/l HEPES buffer (RPMI-PS; Gibco, Grand Island, NY) under sterile conditions and then cultured overnight in 1.5-ml polypropylene centrifuge tubes at 37°C in 5% $CO_2$ in culture medium consisting of RPMI-PS containing 10% defined fetal calf serum (Hyclone Laboratories, Inc., Logan, UT). Parasites lacking gross movement or ciliary beating in flame cells or exhibiting surface blebs were considered nonviable. After overnight culture, 90% to 95% of the parasites were viable. Parasites were approximately 20 hours after transformation at the beginning of incubation with labeled LDL. Roswell Park Memorial Institute-PS medium was used for all binding assays and for washing. Unless indicated otherwise, approximately 500 to 1000 worms were used for each culture condition and each experiment was repeated three times.

### Reagents

Purified human LDL, high-density lipoprotein (HDL), apoproteins A1 and B100 (apoA1 and apoB), goat-anti-human apoA1, and goat-anti-human apoB were purchased from Chemicon International, Inc. (Temecula, CA); rhodamine-conjugated rabbit-anti-goat IgG from Cappel Organon Teknika Corp. (West Chester PA); human IgG (hIgG) and normal goat serum from Jackson Immunoresearch Labs, Inc. (West Grove, PA); fluorescently labeled human LDL-1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbocyanine complex (DiI-LDL), provided at a concentration of 200 μg/ml with 28 to 49 DiI molecules/600,000 $M_r$ LDL particle from Molecular Probes (Eugene, OR). Low-density lipoprotein-didecylnoic acid boron dipyrromethene difluoride contained 23 fluorophores/600,000 $M_r$. Low-density lipoprotein particle (Bodipy-LDL) was a gift from Dr. Rosaria Hoagland of Molecular Probes.

### Binding Assays

Parasites were cultured at 37°C in 1 to 20 μg (6.7 to 133 μg/ml) of DiI-LDL, with or without 100 μg of unlabeled purified human LDL in a total volume of 150 μl RPMI-PS. After 3 hours, schistosomula were washed three times with RPMI-PS and the relative fluorescence (RF) of the bound fluorophore was measured. To examine the extent of binding over time, parasites were cultured at 37°C in 67 μg/ml DiI-LDL with or without 100 μg of LDL for 0 to 48 hours, washed, and the RF was measured.

To examine the rate of loss of bound DiI-LDL, schistosomula were incubated for 3 hours with 67 μg/ml DiI-LDL in a total volume of 300 μl at 5°C or 37°C, washed, cultured at 37°C, aliquots removed at the indicated times, and the RF measured. The ability of LDL to displace bound DiI-LDL from the worm surface was tested by incubating schistosomula in DiI-LDL (67 μg/ml) for 3 hours at 37°C, washing, and incubating in culture medium containing 200 μg unlabeled LDL. At various times up to 3 hours, aliquots were removed and the RF was measured.

To measure the ability of various proteins to inhibit DiI-LDL binding to the surface of the worm, schistosomula were incubated with 20 μg (67 μg/ml) DiI-LDL alone or together with the test protein in a total volume of 300 μl for 3 hours at 37°C. The test proteins were LDL (1 mg), HDL (1 mg), apoA1 (200 μg), apoB (100 μg), hIgG (200 μg), or BSA (1 mg). After washing, the RF was measured.

To measure the amount of HDL binding, the presence of apoB antigen in the HDL preparation, as well as apoA antigen in the LDL preparation, schistosomula were cultured in 60 μg of LDL or HDL in 300 μl RPMI-PS for 3 hours, washed, and incubated with antibodies to apoA1 or apoB, for 20 minutes at room temperature. After washing, worms were incubated in rhodamine-conjugated

rabbit-anti-goat IgG (1:40 dilution) for 20 minutes at room temperature, washed, and the RF measured.

To examine the distribution of Bodipy-LDL, schistosomula were incubated in 133 μg/ml Bodipy-LDL for 18 hours at 37°C, washed three times with RPMI-PS, and examined by fluorescence microscopy.

## *Microscopy*

Fluorescently labeled parasites were examined with a Leitz Orthoplan (Rockleigh, NJ) microscope equipped with a Ploem illumination system with an H2 cube for fluorescein and an N2 cube for DiI and rhodamine. The RF was measured using a Leitz MPV compact photometer from an approximately 200 $\mu m^2$ area of the surface that excluded the area over the cecum to eliminate signal from the ingested fluorophore. For each condition, the fluorescence from 15 schistosomula was measured, corrected for background, and averaged. Parasites were photographed with Kodak Tri-X film (Eastman-Kodak, Rochester, NY) exposed at ASA 1600 and developed with Diafine (Acufine, Chicago, IL) developer.

# *Results*

## *Binding Characteristics of DiI-LDL*

### *Saturation*

Schistosomula cultured in medium containing DiI-LDL bound the DiI-LDL on their surfaces (Figures 1A, B; see below). The relative intensity of the surface fluorescence as measured with a photometer attached to the microscope increased for both total LDL binding and nonspecific binding up to 100 μg/ml. Specific binding was determined by subtraction of the nonspecific binding from the total. Saturation of specific binding of DiI-LDL to the worms was reached by 100 μg/ml (Figure 2).

DiI-LDL binding was time dependent (Figure 3), increasing sharply for the first 3 to 6 hours and then increasing more slowly. Thus, during a 48-hour period, the slope decreased approximately eightfold, from four RF units per hour in the first 6 hours to 0.5 RF units per hour between 24 and 48 hours. The binding was specific because the binding of DiI-LDL to the worm was blocked by unlabeled LDL. The rapid increase in the first 3 hours represented binding to binding sites present at the beginning of the incubation. The longer term increase was probably due to the expression of new binding sites because the surface area of the membrane increases by 300% during the first 4 days after transformation.[16]

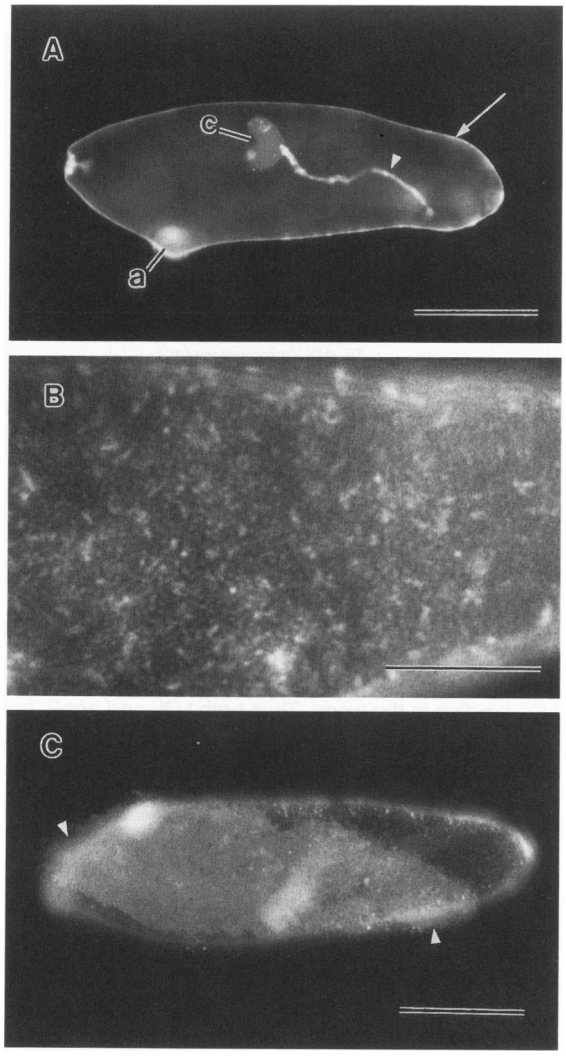


**Figure 1. A–C:** *DiI-LDL binding to the surface and gut of schistosomula. Worms were incubated with 67 μg/ml DiI-LDL in 300 μl of RPMI-PS at 37°C for 3 hours.* **A:** *Fluorophore is present in the esophagus (arrowhead), cecum (c), and on the surface of the tegument (arrow). The fluorescence over the acetabulum (a) is more intense due to the increased membrane folding.* **B:** *High magnification of surface of tegument, showing fluorophore concentrated on spines and in aggregates. The worm in* **C** *shed some of the bound fluorophore onto the coverslip, which is seen as a bright area between the two arrowheads. The body of the worm extends across the micrograph horizontally (***A** *and* **C:** *bar = 40 μm;* **B:** *bar = 20 μm).*

### *Specificity*

When schistosomula were incubated in DiI-LDL in the presence or absence of a fivefold excess of purified apoB or a 50-fold excess of unlabeled LDL, the binding of DiI-LDL was reduced by 85% and 78%, respectively, from control worms (Figure 4). A 50-fold excess of HDL reduced DiI-LDL binding by 33%, a statistically significant difference (Student's paired two-tailed *t*-test; $P < 0.05$). However a fivefold excess of either purified apoA1 or heterospecific hIgG, or a 50-fold excess of BSA, did not significantly block DiI-LDL binding (Figure 4). In one experiment, a fivefold excess of apoprotein E, a protein

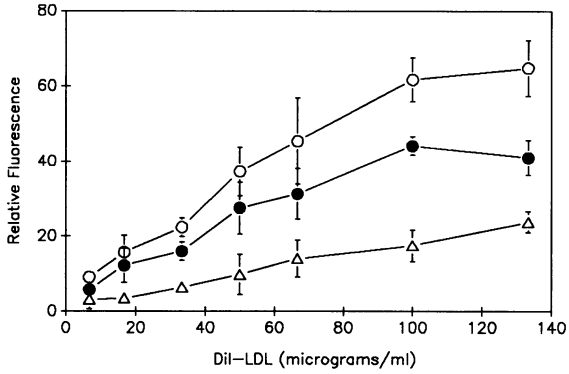


**Figure 2.** *Concentration dependence of DiI-LDL binding. Schistosomula were incubated for 3 hours at 37°C with the indicated amounts of DiI-LDL in the presence (open triangles) or absence (open circles) of 100 μg unlabeled LDL in a total volume of 150 μl, washed three times, and the RF of a 200 $\mu m^2$ area on the parasite measured. Specific binding (filled circles) was calculated by subtracting the RF in the presence of unlabeled LDL from that measured in the absence of unlabeled LDL. Each point represents the average and standard deviation of three separate experiments in which readings were taken from 15 worms. Where error bars are not visible, they are smaller than the symbols themselves.*

that binds to the human LDL receptor, did not inhibit DiI-LDL binding to the worm. Thus both LDL and apoB, but not apoA1, BSA or hIgG, strongly inhibit the binding of DiI-LDL, suggesting that DiI-LDL binds through the apoB moiety of the lipoprotein.

The ability of HDL to inhibit DiI-LDL binding partially was unexpected because of our previous failure to demonstrate HDL binding to the parasite.[5] To study HDL binding to schistosomula further, antibodies specific for either apoA1 or apoB were used to compare the level of binding of HDL and LDL to the parasite and to test the HDL and LDL for cross-contamination. Worms cultured in LDL bound not only anti-apoB antibodies (47 times above background) but also anti-apoA1 antibodies (five

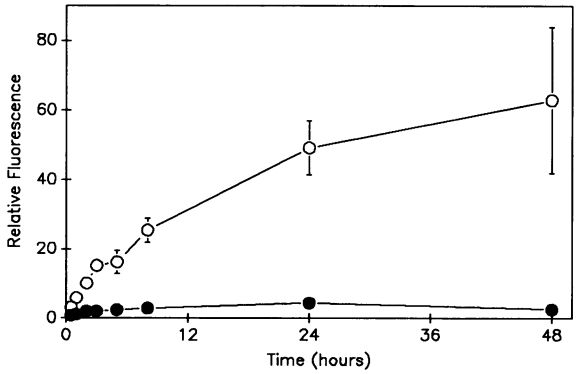


**Figure 3.** *Time dependence of DiI-LDL binding. Schistosomula were incubated with 67 μg/ml DiI-LDL in the presence (filled circles) or absence (open circles) of a 50-fold excess of unlabeled LDL at 37°C. At the times indicated, aliquots were removed, washed three times, and the fluorescence from 15 schistosomula was measured. Each point represents the mean and standard deviation of three separate experiments. Where error bars are absent, they are smaller than the diameter of the symbols themselves.*

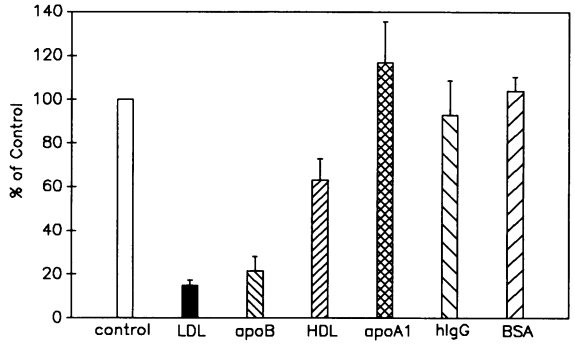


**Figure 4.** *Inhibition of DiI-LDL binding. Schistosomula were labeled with 67 μg/ml (20 μg in 300 μl) DiI-LDL in the presence or absence of an excess of LDL (1 mg), apo B (100 μg), HDL (1 mg), apoA1 (100 μg), hIgG (100 μg), or BSA (1 mg), in a total volume of 300 μl RPMI-PS. After 3 hours at 37°C, worms were washed three times and 15 parasites were measured in the photometer. The data is presented as a percentage of the control (100 × $RF_{inhibitor}/RF_{control}$) and represents the mean and standard deviation of three separate experiments.*

times more than background) (Figure 5). Worms cultured in HDL bound anti-apoA1 antibody 10 times more than background fluorescence (Figure 5), demonstrating a low level of binding of HDL to the worm. Schistosomula cultured in HDL also bound anti-apoB antibodies (seven times more than background). The small amount of apoB antigen in the HDL preparation probably is due to the presence of Lp(a) particles. Thus the 33% inhibition of DiI-LDL binding by HDL can be due to either the binding of Lp(a) or the binding of HDL itself.

### *Displacement by Unlabeled LDL*

To test whether DiI-LDL binding to the schistosomular tegument was reversible, we examined the ability of un-

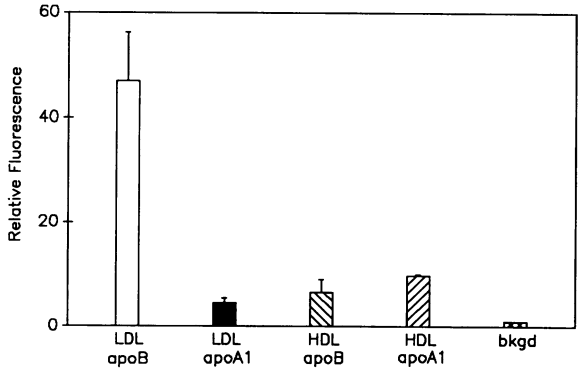


**Figure 5.** *LDL and HDL binding. Schistosomula were incubated for 3 hours at 37°C with LDL (60 μg) or HDL (60 μg) in a total volume of 300 μl, washed three times, and the binding of LDL and HDL to the worm detected with antibodies to either apoA or apoB (400 μl of a 1:200 dilution), washed three times, followed by rhodamine-conjugated secondary antibody (200 μl of a 1:40 dilution), washed again, and the RF measured from 15 worms. The labels under the bars indicate the type of lipoprotein in which the parasites were incubated in capital letters and the primary antibody in lower case. The level of background (bkgd) fluorescence in worms incubated in primary and secondary antibodies, or in secondary antibody alone, did not exceed 1.0. Each bar represents the mean and standard deviation of two separate experiments.*

labeled LDL to displace bound DiI-LDL. The addition of excess LDL displaced bound DiI-LDL by 23% after 0.5 hours (Figure 6). The extent of DiI-LDL displacement by excess LDL was statistically significant compared to control worms after 0.5 hours ($P < 0.05$). Excess LDL displaced up to 49% of DiI-LDL after 2 hours.

## *Distribution and Fate of Bound DiI-LDL*

### *Distribution at Short Times*

The parasites bound DiI-LDL on their surfaces (Figures 1A to C). The surface fluorescence was brightest toward the head, at the acetabulum (ventral sucker), and on the spines. The brightness was caused by the increased surface area of the tegumental membrane in these regions due either to folds in the membrane, to high regional density of the spines, or to the spines themselves. DiI-LDL also aggregated on the worm surface (Figure 1B). After a 3-hour incubation in DiI-LDL, essentially all schistosomula had ingested LDL, so the fluorescence could be seen in the esophagus and cecum as well as on the surface (Figure 1A). The intensity of the ingested fluorophore was generally greater in the esophagus than in the cecum. When examined under the microscope, the esophagus contracted and the fluorophore moved toward the cecal lumen. In the cecum, the DiI-LDL surrounded a nonfluorescent spherical structure (Figure 1A).

Even after an overnight incubation in DiI-LDL at 37°C, no fluorophore could be seen outside the gut in the worm tissues. Neither the gut epithelium nor the tegument endocytosed DiI-LDL. As the schistosomula moved under the coverslip, or if the coverslip was slid over the worms, the parasites lost sheets and fragments of the bound fluorophore onto the glass (Figure 1C). This shedding of bound DiI-LDL was probably an artefact because the surface fluorescence did not decrease in 24 hours (see below). Thus DiI-LDL was localized to the surface and in the gut for the first 18 to 24 hours.

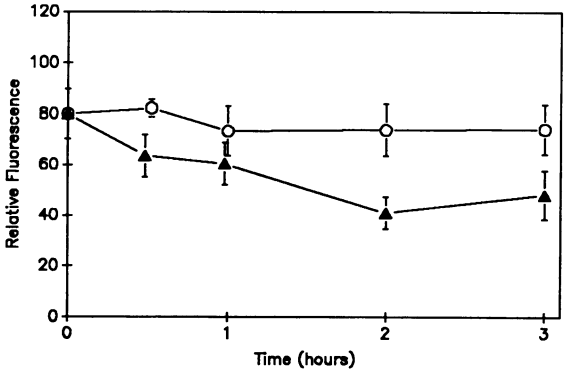


**Figure 6.** *DiI-LDL displacement with unlabeled LDL. Schistosomula were incubated for 3 hours with 67 μg/ml DiI-LDL at 37°C, washed three times, then incubated with an excess of unlabeled LDL (500 μg/150 μl; filled triangles) or in culture medium alone (open circles). At the times indicated, aliquots were washed three times and the RF of 15 parasites was measured. Each point represents the mean and standard deviation of three separate experiments. Some points are slightly offset to show the error bars. Error bars are absent when they are smaller than the diameter of the symbols themselves.*

### *Temperature Dependence of DiI-LDL Binding and Ingestion*

The RF of the surface bound DiI-LDL measured from worms labeled in 5°C was approximately 75% to 80% lower than that of worms labeled in 37°C, demonstrating that DiI-LDL binding to the worm surface was a temperature-dependent process (Figure 7). Furthermore the internal distribution of fluorophore differed in worm labeled at 5°C and 37°C. At 37°C, 80% to 94% of worms ingested DiI-LDL within 1 hour. At 5°C, the fluorophore sometimes was seen in the outer third of the esophagus but not in the cecum. However, when worms were labeled at 5°C, washed free of unbound fluorophore, and then incubated at 37°C, the labeled appeared in the cecum in most of the worms after 3 hours (Figure 8). The DiI-LDL in the cecal lumen must have come from either the outer tegumental membrane or the outer esophagus, or both. In either case, the movement of DiI-LDL into the cecum was temperature dependent. The movement also may indicate that the parasite can sweep molecules bound to the surface into the gut as a potential mechanism of clearance.

### *Long-term Fate and Distribution of DiI-LDL*

Schistosomula labeled in DiI-LDL for 3 hours at either 37°C or 5°C did not lose fluorophore from the surface in

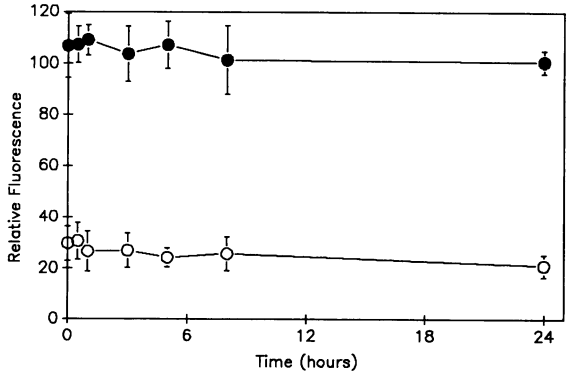


**Figure 7.** *DiI-LDL clearance from labeled schistosomula up to 24 hours after pulse. Parasites were incubated for 3 hours with 67 μg/ml DiI-LDL at 37°C (filled circles) or at 5°C (open circles), washed three times, then incubated in culture medium at 37°C. At the times indicated, aliquots were removed and the RF of the worms was measured. Each point represents the mean and standard deviation of three separate experiments. Error bars are absent when they are smaller than the diameter of the symbols themselves.*

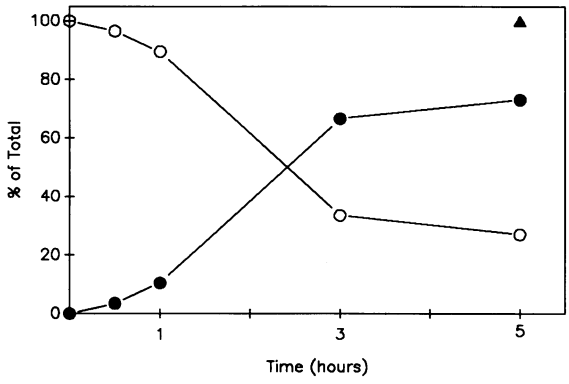


**Figure 8.** *Temperature dependence of ingestion. Schistosomula were incubated in 67 μg/ml DiI-LDL for 3 hours at 5°C, washed three times, and cultured at 37°C. At various times, the number of worms observed ingesting the fluorophore were scored as a percentage of the total number of worms counted (n = 50 to 60). The open circles represent worms with DiI-LDL on the surface and outer esophagus. The filled circles represent worms with DiI-LDL in the cecum as well as on the surface and in the esophagus. The closed triangle represents worms cultured for 5 hours in 5°C after labeling where the DiI-LDL was seen on the surface and in the outer esophagus but not in the cecum. Each point represents the mean of two separate experiments.*

the first 24 hours in culture (Figure 7). Such measurements were difficult to interpret beyond this time because the DiI-LDL was seen in internal structures of the worm, although fluorophore remained on the surface and in the gut (Figure 1A). Parasites pulsed for 18 hours in DiI-LDL, washed, and examined 3 days later had fluorophore throughout the body as well as on the surface and in the cecum (Figure 9A). After 3 days, the ceca were brighter than the ceca of worms incubated for 18 hours in DiI-LDL (compare Figures 1A and 9A). The distribution of the fluorophore in internal structures varied from worm to worm. The complexity of the worm body, which contains many organ systems (ie, kidneys, nervous system, muscles, germ cells, and primordia of adult tissues), precluded identification of the cells containing fluorophore in the absence of a second label. However the internalized fluorophore often appeared in discrete areas under the tegument (Figure 9A) that may be the cytons that synthesize the tegument and its membranes.

### *Bodipy-LDL*

The second fluorescent LDL, Bodipy-LDL, labeled the parasites slightly differently than DiI-LDL. The intensity of Bodipy-LDL on the worms was not as bright as that of DiI-LDL after short incubations, so overnight incubations were necessary to visualize the label. Schistosomula bound Bodipy-LDL on their surfaces and ingested the label. Unlike DiI-LDL, fluorophore could be seen throughout the body after an overnight incubation (Figure 9B). These observations suggest that either the insertion of Bodipy into the LDL particle differed from the insertion of DiI, or the parasite processed the two fluorophores differently. DiI is more hydrophobic and should be more tightly associated with LDL particles than Bodipy (see Via and Smith[14] for a comparison of fluorescently labeled LDLs). In summary, both types of fluorescently labeled LDL were similar in their distribution and fate, with the exception that Bodipy was more rapidly distributed throughout the worm than DiI.

## *Discussion*

These studies demonstrate that DiI-LDL binds to the surface of schistosomula with high specificity because the binding is largely inhibited by unlabeled LDL. DiI-LDL remains bound to the surface for more than 24 hours in culture. Specificity also is indicated by the inhibition of binding by apoB but not by apoA1, nonspecific hIgG, or BSA, and by the partial displacement of bound DiI-LDL by unlabeled LDL. The binding of DiI-LDL to the worm is concentration, time, and temperature dependent. DiI-LDL probably binds to a receptor on the worm *via* apoB. DiI-LDL and Bodipy-LDL are ingested rapidly into the parasite gut. Over time, the fluorophores are seen throughout the parasite body, suggesting that the LDL particle may be broken down and the worm is using lipids contained within the LDL.

The results obtained with DiI-LDL correlate well with previous work measuring bound LDL by indirect immunocytochemistry.[5,6] Saturation was reached at 100 μg/ml of DiI-LDL compared with 67 μg/ml measured by immunocytochemistry. The time course of binding, ie, a sharp increase in binding in the first 3 hours and a slower increase up to 48 hours in culture, is similar with both types of measurement. Furthermore both types of localization indicate that the LDL is aggregated partially on the surface. However experiments with DiI-LDL provide information not obtained previously, namely specific inhibition of DiI-LDL binding, displacement of bound DiI-LDL by unlabeled LDL, ingestion, and long-term distribution.

Rumjanek et al[9] examined whole worm extracts and found a 45-kd doublet that bound to an LDL affinity column. Although they did not determine the doublet's distribution in the worm, they were able to iodinate it by surface labeling techniques. They also reported inhibition (16% and 60%; n = 2) of the binding of $^{125}$I-labeled LDL to worm extracts by unlabeled LDL. Rogers et al[17] identified a protein of similar molecular weight, 43 kd, that binds LDL on Western blots and is present in both the tegument and the gut of adult *Schistosoma japonicum*. In contrast to the results here with *Schistosoma mansoni*, human LDL binding to the 43-kd protein on blots is inhibited strongly by HDL as well as by unlabeled LDL. Although the schistosome LDL receptors or binding proteins remain to be described further, clearly they will

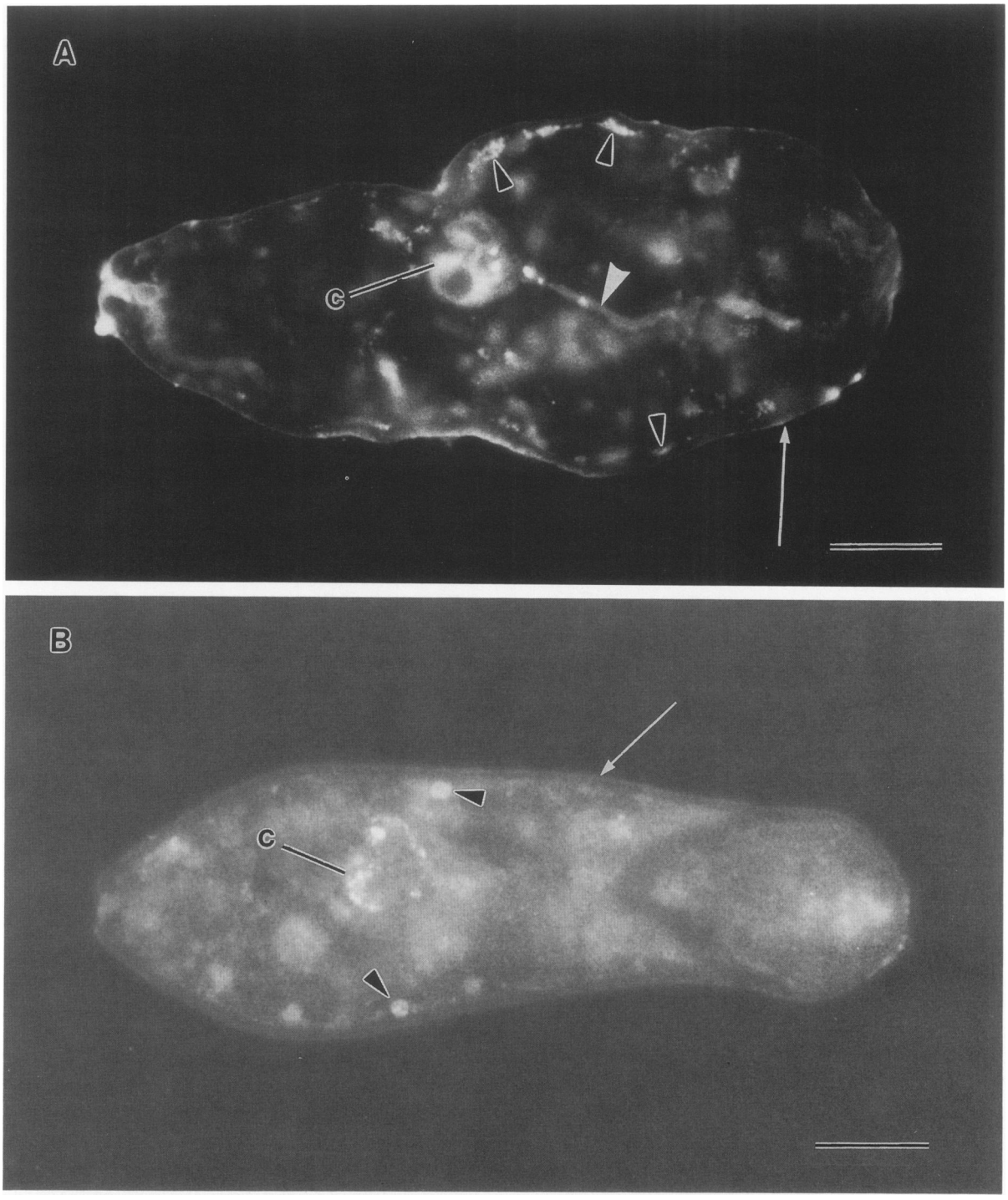


**Figure 9.** *Incorporation of fluorescently labeled LDL into worm tissues after 3 days or 18 hours in culture. Parasites were incubated in 67 μg/ml DiI-LDL for 18 hours, washed, and cultured for 3 days before examination* **(A)**, *or incubated in 130 μg/ml Bodipy-LDL for 18 hours and observed immediately after washing* **(B)**. *Note the appearance of label in discrete areas that may correspond to the subtegumental cytons (black arrowheads). Fluorophore is also seen on the surface (arrows), in the esophagus (white arrowhead) and cecum (c), and throughout the body (Bar = 20 μm).*

differ functionally from the human LDL receptor[18] because they do not promote endocytosis and do not appear to regulate 3-hydroxy-3-methyl-glutaryl coenzyme A reductase.[19]

The failure of nonspecific IgG to inhibit the binding of DiI-LDL is somewhat surprising because Kemp et al[20] reported that heterospecific mouse antibody binds to the surface of schistosomula. However the binding was less

at 37°C than 4°C and the immunoglobulin was lost from the surface in 5 to 10 minutes after binding at 37°C. Torpier et al[21] reported that freshly transformed but not 5-day-old schistosomula or adult worms bound nonspecific human IgG *via* the Fc portion of the immunoglobulin. The parasites used here were 1 day old and may not bind nonspecific human immunoglobulin. In contrast, antisera from patients with schistosomiasis block the binding of LDL to schistosomula[5] and produce antibodies that recognize the 45-kd doublet,[7] suggesting the LDL binding protein is antigenic.

The observation that DiI-LDL is not lost from the parasite surface within 24 hours and is still present after 3 days is in partial conflict with studies that measured turnover of other exogenous ligands or labeled components of the tegumental surface. Such experiments can be divided roughly into two groups, 1) a group with rapid turnover with a half-life less than 1 day; and 2) a group with slow or negligible turnover with no loss of the surface marker in 1 day (Table 1). The observed differences in the two groups are difficult to reconcile into a single model at this time because the labeling procedures in the different studies may have influenced the results. For example, polyvalent ligands may have stabilized or frozen certain molecules in the membrane, or iodine may have labeled the inner as well as the outer membrane. Alternatively labeling could induce a more rapid clearance of the labeled species than the unlabeled. Furthermore the rate of clearance of some molecules from the membrane can be increased by the presence of serum in the medium[22–24] or by placing the worms *in vivo*.[25–29] Thus the retention of a particular population of LDL on the parasite surface for days may or may not occur *in vivo*.

**Table 1.** *Turnover of Ligands and Surface Components on Schistosomula*

| Ligand/component | Half-life (hours) | Reference |
|---|---|---|
| Heterospecific IgG | <2.5 | 20 |
| Cationized ferritin | 3.5 | 3 |
| Parasite antigens | 6 | 32 |
| | 3 | 25 |
| Parasite proteins | 3.2 | 33 |
| Complement | 4 | 34 |
| Parasite-specific IgG | 5–8 | 34 |
| | | 35 |
| Concanavalin A | 8–10 | 36 |
| | | Caulfield et al, |
| Fl-PE | 11.7 | submitted |
| Parasite glycoprotein | 10–12 | 37 |
| Parasite glycolipid | 10–15 | 37 |
| Murine MHC | 15–39 | 38 |
| Parasite-specific serum | >20 | 39 |
| | | 40 |
| Host antigens | >45 | 27 |
| DNP-Cap-PE | >21 | 23 |
| DiI-LDL | >24 | This report |

DNP-Cap-PE, N-dinitrophenyl-ε-aminocaproyl-phosphatidylethanolamine; Fl-PE, fluorescein-phosphatidylethanolamine.

The sloughing of bound fluorophore onto the microscope slide glass observed here was noted also by Brink et al,[30] who followed the time course of binding of fluorescein-conjugated parasite-specific antibody. These authors saw sloughing only with higher concentrations of antibody. Thus sloughing may occur primarily when there are large amounts of ligand on the parasite surface. Here sloughing is probably an artefact of the worms being trapped beneath the slide because DiI-LDL is not lost from the whole population over time (Figure 7). On the other hand, such massive sloughing may be the *in vitro* correlate of the rapid loss of surface moieties induced by placing the parasites *in vivo*.

The presence of both DiI and Bodipy in the internal organs of the parasite over time suggests that the fluorophore containing LDL is broken down on the surface or in the gut and lipids are transported into the worm body. Because endocytosis of LDL is not observed and is consistent with the repeated failure to demonstrate endocytosis in schistosomula, the breakdown must occur either on the surface itself or in the lumen of the gut, rather than in lysosomes. We believe that the surface is the less likely of the two possibilities because DiI-LDL surface binding is very stable over time. Furthermore there is no change in the diffusion coefficient or the rate of loss of a phospholipid analogue inserted into the outer membrane as a consequence of LDL binding, which suggests that LDL lipids are not directly inserted into the tegumental membrane (Caulfield, Chiang, Yacono, Smith, and Golan, manuscript submitted for publication). The gut lumen, on the other hand, contains proteolytic enzymes[31] that could cleave apoB. The delay in the release of DiI from the labeled LDL may be related to the delayed expression of proteases in the gut after transformation (see Bogitch[31] for review). After transport across the gut epithelium, the fluorescent labels, and presumably sterols or phospholipids, could diffuse through the extracellular matrix to various organs, including the subtegumental cell bodies that synthesize the tegumental membranes.[1,3] Although it must be tested, this model of parasite lipid use and membrane biogenesis suggests that the parasite is highly dependent on its host for survival.

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