ecting forward from the elongated optic nerve head of the bird's eye [see K. G. Wingstrand and O. Munk, *Biol. Skr. K. Dan. Vidensk. Selsk.* **14**, 1 (1965) for a comprehensive introduction]. Retinal whole mounts were stained with cresyl violet to define the area of highest concentration of ganglion cells [A. Hughes, *J. Comp. Neurol.* **163**, 197 (1975)]. Since the angular subtense of the base of the pecten was determined with a reversible ophthalmoscope during each experiment, it was possible to establish the angular separation of the pecten and area centralis by comparing their linear separation with the length of the pecten's base on the retinal whole mount. This separation, together with the asymmetrical shape of the pecten's projection, gave the position of the area centralis on the tangent screen.

9. Stepping motors rotated the variable-slit aperture on the projector and a Risley variable biprism in front of one eye. Tuning curves were obtained by interleaving single sweeps of the stimulus slit at a variety of slit orientations or prism settings in quasi-random order until the mean response at each setting could be estimated.
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11. Simple cells have receptive fields that can be easily mapped by flashing a small spot into adjacent antagonistic regions with straight-line boundaries; fields in the thalamic relay can also be mapped into antagonistic regions but have concentric boundaries [D. H. Hubel and T. N. Wiesel, *J. Physiol. (London)* **160**, 106 (1962)].
12. Since the retinal disparities involved in binocular depth discrimination are small (*1*), one could predict technical problems in the study of disparity-selective binocular neurons in a paralyzed preparation. Of crucial importance are residual eye movements that cannot be eliminated completely because of the slight pulsations accompanying respiration and heartbeat [R. W. Rodieck, J. D. Pettigrew, P. O. Bishop, T. Nikara, *Vision Res.* 7, 107 (1966)]. In the monkey, in which disparity detection is accurate to tens of seconds of arc (J. Lott-Brown, personal communication), optical stabilization of the image may be necessary to overcome the noise introduced by these residual pulsations. In the cat, the range of neuron-to-neuron variation in preferred disparity appears to be large enough to be useful in comparison to the narrow disparity-tuning curves, particularly those recorded at some distance from the area centralis, where the variation is greater (*4*). This is disputed for area 17 [D. H. Hubel and T. N. Wiesel, *J. Physiol. (London)* **232**, 29P (1973); but note also H. B. Barlow, C. Blakemore, R. C. Van Sluyters, *ibid.* **242**, 38P (1974)]. These technical difficulties introduced by residual eye movement can be largely overcome by monitoring two binocular neurons simultaneously [see (*7*) and Fig. 3].
13. The OPT of the owl is a large and complex group of cell nuclei; we recorded from the two external laminae.
14. This picture is complicated by the presence in the cat LGN of subtle, binocular inhibitory effects [K. J. Sanderson, P. O. Bishop, I. Darian-Smith, *Exp. Brain Res.* **13**, 178 (1971)] for which we did not test.
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Multiplication of a Human Parasite (*Leishmania donovani*) in Phagolysosomes of Hamster Macrophages in vitro

Abstract. Leishmania donovani, *the etiological agent of human visceral leishmaniasis, was grown in hamster peritoneal macrophages in vitro. By electron microscopy, using a lysosomal marker, these parasitic protozoa were seen to multiply within host cell phagolysosomes. The survival mechanism of this intracellular parasite is based apparently upon resistance to macrophage lysosomal enzymic digestion.*

Most refractory to host immunity and chemotherapy are perhaps some intracellular parasites whose survival often depends on a delicate balance of host-parasite interplay (*1*). These parasites frequently live in different ecological niches within the host. For instance, nowhere else but in a living red blood cell can the erythrocytic stage of malaria grow well (*1*), whereas *Microsporidia* (*2*) and *Trichinella* (*3*) often invade muscle cells. Still others even parasitize the mononuclear cell series of the host reticuloendothelial system, phagocytic cells that constitute the first line of host cellular defense against invading microorganisms. The success of this type of intracellular parasitism hinges on subtle mechanisms whereby the parasites plainly resist lysosomal enzymes (*4*) or avoid exposure to lysosomes by residing in separate vacuoles (*5*, *6*). One typical example of such a parasite is *Leishmania donovani*, a trypanosomatid protozoan that is the causative agent of kala azar, a debilitating and often fatal disease of man. Although it has long been recognized that the amastigote stage of this parasite infects host macrophages, the mechanism of its survival within these cells remains open to speculation (*1*). This mechanism undoubtedly bears directly on the pathogenicity of this parasite and the failure of susceptible hosts to develop effective immunity against it. In this report, we present evidence indicating that, despite the fusion of lysosomes with parasite-containing or parasitophorous vacuoles, the *L. donovani* amastigotes not only persist but multiply within the phagolysosomes of cultured macrophages.

We used an in vitro system consisting of hamster peritoneal macrophages and amastigotes of *L. donovani* (*7*). Amastigotes of *L. donovani* were isolated from infected hamster spleen by differential centrifugation. Macrophages were collected from Syrian golden hamsters and cultured with 5 percent CO_2 in air, at 37°C, in an enriched medium 199 with the flying cover slip method. Details of the culture technique will be described elsewhere (*8*). It suffices to mention here that relatively homogeneous populations of macrophages can be maintained under satisfactory conditions regarding their adhesion, spreading, and survival on a long-term basis, by coating cover slips with polylysine and the use of lactobumin hydrolysate.

To study the *Leishmania*-macrophage interactions, 1- to 3-day-old cell monolayers on cover slips were covered with 0.2 to 0.3 ml of medium containing appropriate numbers of parasites to make a parasite to cell ratio of 2 to 1. Infection was allowed to occur for 4 hours, after which time samples were washed thoroughly to remove free parasites and then incubated for up to 7 days; experimental conditions were as those for the maintenance of macrophages. At different intervals, samples were removed and Giemsa stained for light microscopy to evaluate the infectivity and multiplication of the parasites within the macrophages. Infectivity and multiplication were assessed by counting the numbers of parasitized cells per 200 macrophages, and the number of parasites per 100 infected cells, respectively. The results from more than ten experiments clearly showed that the parasites attained a severalfold increase in number in 1 week. A typical example is presented in Table 1. Using an identical parasite–host cell system but handled in a different way, Herman (*9*) obtained somewhat similar, but much less consistent results. During the entire period of our study, no extensive cell detachment from cover slips and no cell lysis due to infection were apparent. Moreover, the increase in parasite numbers was well correlated with the frequency of dividing amastigotes observed, while the percentage of infected cells remained constant (Table 1). These results thus provide clear evidence indicating intracellular growth of *L. donovani* amas-

Table 1. Infectivity and multiplication of *Leishmania donovani* amastigotes in in vitro cultured hamster peritoneal macrophages.

Days after infection	Infected cells (%)	Number of parasites per cell
1/6	50*	1.9†
1	45	1.7
3	50	3.3
7	52	7.7

*Obtained from examining at least 200 cells per sample. †Obtained by counting the number of parasites in at least 100 infected macrophages per sample.

tigotes in hamster macrophages cultured in vitro. In similar experiments, however, glutaraldehyde-killed parasites were digested and rapidly cleared by the macrophages within 1 day. These results indicate that living parasites are either insusceptible to lysosome enzymic digestion or avoid fusion with these macrophage organelles.

To clarify this point, the subcellular events of parasite-macrophage interactions were studied by electron microscopy with particular emphasis on the occurrence of lysosome-phagosome fusion. Secondary lysosomes of cultured macrophages were prelabeled with an electron-dense marker (Thorotrast) according to the method of Jones and Hirsch (*6*). The cultures were subsequently infected with *L. donovani* amastigotes as described above and processed at different intervals for electron microscopy as described for another system (*10*). During the first 4-hour infection period, amastigotes became lodged in phagosomes, loosely surrounded by host membrane. Also taken into these parasite-containing vacuoles were some host tissue debris, including the original host membrane or membranes adhering to the parasites, and other membrane components derived from cell organelles of host spleen tissues. These parasitophorous vacuoles evidently became fused with Thorotrast-filled vacuoles, that is, the secondary lysosomes (Fig. 1a). From 1 to 4 hours during the infection period, vacuoles concomitantly containing both parasites and Thorotrast increased from approximately 50 to 80 percent of the total vacuoles examined. During postinfection periods from day 1 to 7, Thorotrast granules were present in more than 60 percent of all parasitophorous vacuoles (Fig. 1b), thus indicating that amastigotes persist within phagosome-lysosomal complexes of these macrophages.

The apparent absence of Thorotrast granules in some parasitophorous vacuole profiles probably reflects the uneven distribution of the label within these vacuoles, in conjunction with and compounded by the inherent problems associated with the use of ultrathin sections for electron microscopy, rather than an indication of the lack of phagosome-lysosome fusion. On the day after infection in vitro, dilution of Thorotrast granules with newly formed phagosomes or pinosomes evidently began to occur, and the parasite became tightly apposed, often only partially, against host vacuolar membranes (Fig. 1b). These factors also further reduced the chance of locating the label in parasitophorous vacuoles, accounting for the slight apparent decrease in number of labeled vacuoles after longer periods of incubation. That amastigotes are exposed to lysosomal contents is supported by the additional observation that parasites maintain their structural integrity in Thorotrast-laden vacuoles, whereas the extraneous host tissue debris coming to lie with them in the same vacuoles invariably disappears after 1 day (Fig. 1b). Experiments were also undertaken to investigate whether an externally administered marker for secondary lysosomes would become sequestered in preexisting parasitophorous vacuoles of macrophages. Thorotrast was fed to macrophages already infected in vitro with amastigotes for different time periods, as well as to explants of hamster spleen infected in vivo. The dense label became readily accumulated in vacuoles including those harboring the parasites, regardless of the length of the postinfection period or the origin of macrophages from the peritoneal cavity or the spleen. These findings further demonstrated the continuity of secondary lysosomes with the parasitophorous vacuoles, thereby strengthening our conviction that these

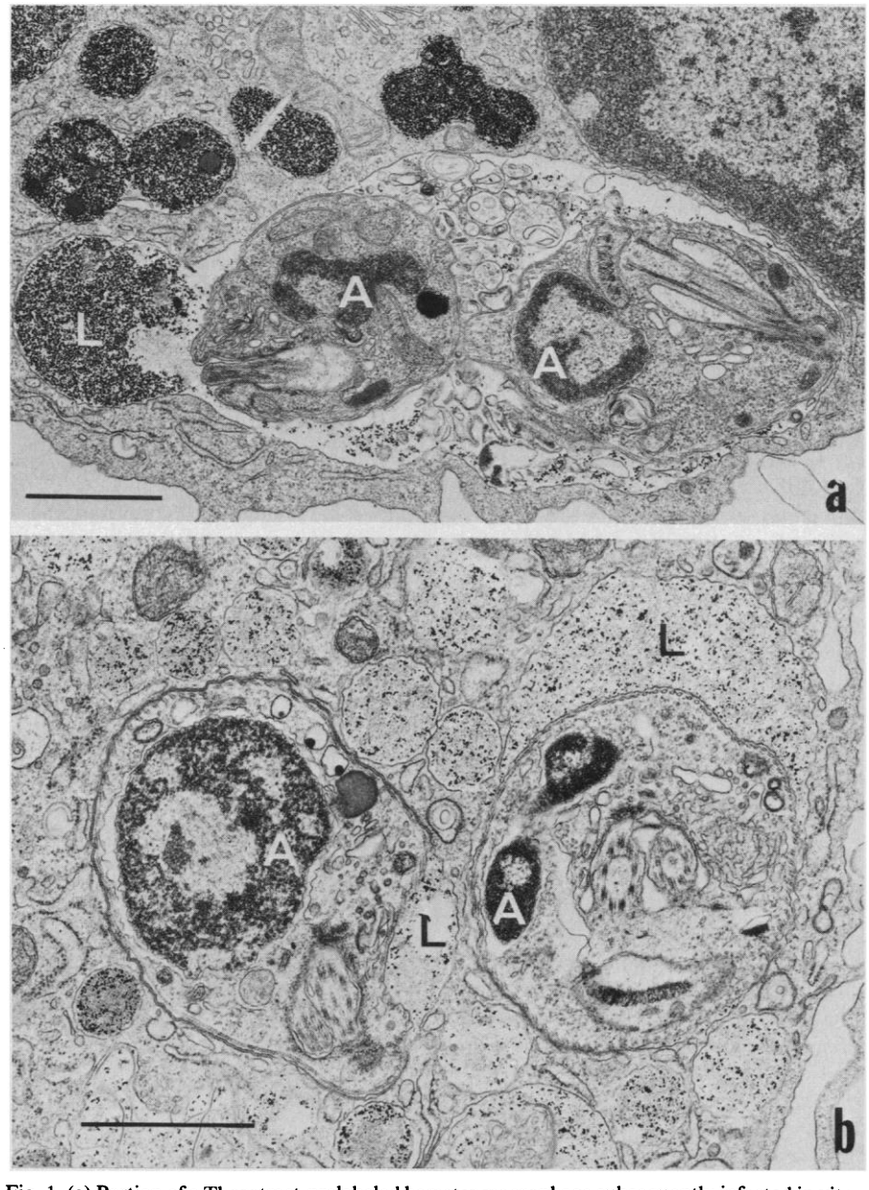

Fig. 1. (a) Portion of a Thorotrast-prelabeled hamster macrophage subsequently infected in vitro with *Leishmania donovani* for 1 hour, showing the fusion of a secondary lysosome (*L*) and a parasitophorous vacuole containing two amastigotes (*A*). Note the membrane debris lodged with the parasites in the same vacuole. Bar represents 1 μm. (b) Portion of a Thorotrast-prelabeled hamster macrophage infected with *Leishmania donovani* for 3 days, showing two dividing amastigotes (*A*) lying within Thorotrast-containing vacuoles, that is, secondary lysosomes (*L*). Parasite division is indicated by the presence of two flagella or two nuclei or both. Note the absence of any extraneous membrane materials within the parasitophorous vacuole. Bar represents 1 μm.

two subcellular compartments are the same. In parallel to our light microscope study, electron microscopic observations also revealed dividing amastigotes in macrophages after 3 days in vitro. Of particular significance was that not only were these forms dividing but they were present in Thorotrast-containing vacuoles (Fig. 1b), that is, secondary lysosomes. From all of the results obtained, we conclude that *L. donovani* amastigotes not only survive but multiply within phagosome-lysosome vacuoles of hamster macrophages.

Using a *Leishmania mexicana*–mouse macrophage system, Alexander and Vickerman (*11*) also observed apparent lysosome-phagosome fusion, which may, however, account at least in part for spontaneous curing in such cutaneous forms of leishmaniasis in contrast to the mostly fatal consequences in the *L. donovani*–hamster model. Additional significance attached to the use of this latter system is the close resemblance between the course of *L. donovani* infection in the hamster and human (*12*). The present study provides much stronger evidence in support of several proposals regarding the fundamental aspects of intracellular parasitism in leishmaniasis than offered previously (*11*). We concur with the interpretation that *Leishmania* resembles *Mycobacterium lepraemurium* (*4*), but differs from *M. tuberculosis* and *Toxoplasma gondii* (*5, 6*) (the latter lie in parasitophorous vacuoles that do not fuse with macrophage lysosomes). We favor the thesis that the surface properties of *Leishmania* (*13*) might render the parasites resistant to lysosomal enzymes and thus enable them to survive and multiply within phagolysosomal vacuoles of host cells. Inhibition of host lysosomal enzymes by *Leishmania* to account for its survival in macrophages (*11*) seems unlikely, as the host membrane debris adhering to the parasite evidently becomes digested. It is tempting to speculate that inherent differences in macrophage lysosomal constituent enzymes might possibly account for the considerable variation in susceptibility of different host species of *L. donovani* infection (*14*). Moreover, results of this study call attention to the use of lysosomotrophic drugs for the more effective chemotherapy of leishmaniasis, in accordance with the broad concepts advanced by De Duve (*15*) and by Jacques and Demoulin-Brahy (*16*).

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Bioconcentration of Xenobiotics in Trout Bile: A Proposed Monitoring Aid for Some Waterborne Chemicals

Abstract. *A technique is proposed for the monitoring of certain xenobiotic pollutants in suspect aquatic environments by fish bile analysis. Bile removed from rainbow trout* (Salmo gairdneri) *exposed to nine different radioactive compounds in vivo contained concentrations of radioactivity greater than those in the surrounding water. Bile-to-water radioactivity ratios as high as 10,000 : 1 were found after 24-hour exposures. The results of these experiments suggest that analysis of bile of wild or caged fish from a suspect site may be useful as a qualitative monitoring aid for certain types of xenobiotics in water.*

Studies of rainbow trout in this laboratory have established that several foreign compounds can be conjugated with glucuronic acid and excreted into bile in high concentrations (*1*). More recently, the results of other investigators have indicated that other fish species are able to conjugate certain phenols, such as pentachlorophenol, with sulfate (*2*). Although the biliary concentration of a variety of organic anions including conjugates of foreign compounds is thought to occur through a specific transport mechanism in mammals (*3*), very few studies have dealt with this process in fish (*4*). We have recently reported on the biliary concentration of several xenobiotic substances in rainbow trout (*5*).

Table 1. Biliary concentration of various xenobiotics by rainbow trout (*Salmo gairdneri*). Exposures were made at 12°C for 24 hours. Water hardness was 134 parts per million, measured by the $CaCO_3$ method, and *p*H was 7.2. Radioactivities are expressed as disintegrations per minute (dpm) per milliliter; each value of the 24-hour bile radioactivity is the mean of a minimum of five animals from at least two separate exposures. Abbreviation: UL, uniformly labeled.

Compound	Concentration in H_2O (mg/liter)	Radioactivity (dpm/ml)		Ratio (bile ^{14}C)/(H_2O ^{14}C)	Metabolites
		H_2O (0 hours)	Bile (24 hours)		
2′,5-Dichloro-4′-nitrosalicylanilide (Bayer 73; chlorosalicylic acid; ring-UL-^{14}C)	0.05	3,010	30,500,000	10,100	1
Di-2-ethylhexylphthalate (DEHP; carboxyl-^{14}C)	0.5	1,070	265,000	247	5?
Methylnaphthalene (ring-UL-^{14}C)	0.005	310	796,000	2,570	?
1-Naphthyl-*N*-methylcarbamate (carbaryl; naphthyl-1-^{14}C)	0.25	1,030	975,000	947	3
Naphthalene (ring-UL-^{14}C)	0.005	305	127,000	414	2
Pentachlorophenol (PCP; ring-UL-^{14}C)	0.1	4,070	21,800,000	5,360	2?
2,5,2′,5′-Tetrachlorobiphenyl (TCB; ring-UL-^{14}C)	0.5	3,640	39,000	11	2?
1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p*,*p*′-DDT; ring-UL-^{14}C)	0.1	180	22,500	124	1
3-Trifluoromethyl-4-nitrophenol (TFM; ring-UL-^{14}C)	0.5	2,020	2,150,000	1,064	1