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

This report deals with the biliary concentration of a structurally diverse group of chemical compounds and indicates that the sampling of bile may be of potential use as an aid in monitoring water quality or as a diagnostic tool in the investigation of chemically related fish kills.

Rainbow trout (usually 10 g of biomass per liter) were placed in a glass tank that contained 50 liters of dechlorinated water (*p*H 7.2) and the ^{14}C-labeled compound or compounds. The tanks were aerated and kept at 12°C and the system was allowed to remain undisturbed for 24 hours. The concentrations of the compounds in the exposures were below the level of acute toxicity for the times indicated and had no observable effects on the fish during the 24-hour exposures. The concentrations were chosen for convenience in metabolite detection rather than to simulate environmental levels. The amount of ^{14}C in the tank water was determined by counting suitable portions in 15 ml of ACS scintillation mixture (Amersham/Searle) in a model 6872 (Searle Analytic) liquid scintillation counter. After exposure, the fish were killed by cervical dislocation and the bile was collected by gallbladder puncture. Portions of crude bile were then placed in the scintillation mixture for counting, and the remainder was pooled for metabolite identification. The pooled bile was diluted with water and passed over a bed (5 by 15 cm) of XAD-2 resin in a glass column and washed with two bed-volumes of distilled water. The radioactive materials were eluted from the columns with three bed-volumes of methanol. The methanol was then concentrated to 30 ml. Thin-layer chromatography was performed on 0.25-mm silica gel plates. The plates were scanned for radioactivity by scraping segments (1 by 2 cm) of silica gel from them from the origin to the solvent front and counting the gel in ACS scintillation mixture.

The data shown in Table 1 indicate that the ratios of ^{14}C in bile to ^{14}C in water after the 24-hour exposures to the indicated compounds range from a low value of 11 for 2,5,2′,5′-tetrachlorobiphenyl (TCB) to 10,000 for 2′,5-dichloro-4′-nitrosalicylanilide (Bayer 73). In most cases the ^{14}C in bile was associated with metabolites of the parent compounds and some of these biliary metabolites have been characterized (*1*, *2*). It is evident that the lowest bile-to-water ratios were associated with compounds (DDT and TCB) that have a high lipid solubility, and this may be due to a low rate of metabolism or conjugation related to the sequestration of these compounds by tissue lipids. Of great interest are the high bile-to-water ratios of the compounds that have comparatively lower lipid solubilities, since from a monitoring point of view, more polar compounds may have a low bioaccumulation potential (*6*). Although much attention has been given in the past several years to monitoring for chemicals that tend to accumulate in the food chain, there are few innovations in the area of monitoring for potentially hazardous chemicals that have lower bioaccumulation potentials, such as phenols and certain components of petroleum products. A recent report has suggested the use of liver benzopyrene hydroxylase activity as a monitor for petroleum pollution (*7*). The data in Table 1 concerning naphthalene and methylnaphthalene appear to be relevant, since both of these compounds are constituents of crude oil (*8*), and the appearance of metabolites of these compounds in bile in high concentrations suggests the possibility of using this technique as a tool in the monitoring of petroleum pollution.

Although more work needs to be done concerning the qualitative and quantitative aspects of the biliary concentrating system in the diverse species of fish and in the development of specific identification techniques for xenobiotic compounds and their metabolites, the value of capitalizing on this process is apparent. The careful design of monitoring methods based on bile collection from either captured fish or caged fish placed at a suspect site may well serve to provide increasingly needed environmental indices (*9*).

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Asparaginase Entrapped in Red Blood Cells: Action and Survival

Abstract. *As a strategy to avoid serious allergic reactions to the antitumor agent asparaginase, this enzyme was entrapped in autologous red blood cells before intravenous injection into monkeys. Additional advantages of this approach are prolonged enzyme half-life and targeting of this agent into the reticuloendothelial system.*

Enzymes are potential therapeutic agents. After isolation and purification, enzymes can be injected to replace a missing deficient enzyme (*1*) or to digest a thrombus (*2*). An injected enzyme may have antitumor action if it destroys a tissue nutrient that is essential for tumor growth. For example, the cancer cell of childhood leukemia characteristically is unable to synthesize asparagine (*3*). Intravenous injection of the enzyme asparaginase destroys this essential tumor nutrient and causes regression of the cancer tissue. Remission of this type of leukemia in response to asparaginase therapy has been demonstrated (*4*). Unfortunately, the remission is usually short-lived or incomplete. Further therapy with this enzyme is complicated by life-threatening allergic reactions (*5*), immunosuppressive activity of the enzyme (*6*), and occasional toxicity against normal tissues, primarily liver and pancreas.

As a strategy to counteract the problems of antigenicity and host proteolytic degradation of therapeutic enzymes and, more specifically, to achieve a safer, more effective method of injecting asparaginase, we entrapped this enzyme in resealed red blood cell (RBC) ghosts and studied the fate and enzymatic function of these modified cells after injection in the monkey. The asparaginase used (MSD E.C.2 lot L594269-0-51, a gift