Table 1. Infectivity to mice of fecal forms of *Toxoplasma*. The cat was free of *Toxocara* eggs before and after the experiment. It was fed three mice with chronic toxoplasmosis on days 0, 1, and 2. Abbreviations: T, *Toxoplasma* in sections; t, typical lesions only. Each letter refers to one mouse.

Day	Mice dead/ inoculated	Serologic test	Histopathology	Organ passage
5	1:3	+	t	+
6	3:3		tTT	+
7	2:3	+	tTT	+
8	3:3	+	ttT	+
9	3:3	+	TT	+
10	3:3	+	TTT	
11	3:3	+	TT	
12	2:3	+	TT	
13	2:3	+	TT	
14	0:3	+		

However, if placed in formalin on the day that they were shed, they did not develop infectivity, although the accompanying *Toxocara* eggs embryonated, in agreement with a previous report (*3*). Fecal forms of *Toxoplasma* were not infectious after drying on glass for 24 hours.

That we could separate *Toxoplasma* from *Toxocara* eggs by washing is contrary to Hutchison's experience. However, our findings indicate that fecal forms of *Toxoplasma* cling to remaining fecal debris and fungal masses and that considerable washing may be necessary to remove them. After we removed mycelia and large debris in an 88-μ filter, it was easier to separate *Toxoplasma* from *Toxocara* eggs in a 44-μ filter.

We recovered fecal forms of *Toxoplasma* from cats freed of *Toxocara* by piperazine. Jacobs obtained *Toxoplasma* from six fecal specimens devoid of visible *Toxocara* eggs (*2*). Dubey also observed transmission of *Toxoplasma* in 2 of 109 fecal samples free of *Toxocara* eggs (*3, 4*). But these were isolated findings in a mass of data where *Toxoplasma* was associated with *Toxocara* eggs. Furthermore, resistance to tap water, formalin, and sodium hypochlorite was incompatible with characteristics of known forms of *Toxoplasma* and suggested that the infectious form was inside the nematode eggs (*5*). We transmitted a compatible strain of *Toxoplasma* (M-7741) in association with *Toxocara* in only seven of 18 attempts and in only three of eight attempts in *Toxocara*-free cats; these results indicate that Hutchison's failure to transmit *Toxoplasma* in five of five *Toxocara*-free cats (*1*) is statistically not too improbable. At least two of Hutchison's cats were used twice. In this connection, we have observed transmission only once in each of seven cats fed repeatedly. Transmission occurred five of nine times on first attempt, twice in nine second attempts, and in none of two on third attempt. The presence of low antibody titers did not seem to interfere with fecal transmission; of all 15 cats used, four of five transmitted without antibody in undiluted serum, and six of ten transmitted even though they had titers of 1:64, 1:32 (twice), and 1:16 (three times), at, or close to the time of transmission. The five cats that did not transmit had titers of 0, 1:4, 1:16, 1:128, and 1:256 (original serum dilutions). None of the cats became sick.

Reinvestigation not only failed to confirm Hutchison's hypothesis (*1*) but provided a simpler theory of fecal transmission, confirmed independently in the accompanying report by Sheffield and Melton (*6*). By eliminating the role of *Toxocara*, as shown conclusively in our washing experiments, we can now refocus our investigations on identifying and characterizing *Toxoplasma* in the gut rather than the nematode egg.

Removing the worm from consideration should simplify analysis of the epidemiology of toxoplasmosis and might more easily provide clues to the variable incidence of infection. Among U.S. military recruits, this ranges from below 5 percent in the Pacific mountain states to over 25 percent in some eastern and southern states (*7*), and up to 100 percent in the population over the age of 30 in the lowlands of Guatemala (*8*). Transplacental transmission appears to account for only a minute proportion of human infections (*9*). Carnivorism could be important in infecting cats, and in their potential for fecal dissemination of *Toxoplasma*. Ingestion of raw or undercooked meat can infect humans (*10*). The relative importance of this mode of infection in humans remains to be assessed.

Note added in proof: Hutchison has now reported fecal transmission of *Toxoplasma* by 2 of 21 worm-free cats (*11*).

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Mycoplasma Membrane Lipids: Variations in Fatty Acid Composition

Abstract. *The fatty acid composition of the membrane polar lipids of* Mycoplasma laidlawii *B can be dramatically altered. These variations result in characteristic morphological changes, and in most cases the cells remain viable. This organism should provide a useful system for clarifying the role of fatty acyl chains in biological membranes.*

Chemical analysis of biological membranes derived from a wide variety of organisms has shown that the fatty acid composition of any particular membrane tends to be fairly specific under a variety of conditions (*1*). Changes in age, temperature, and chemical environment can cause alterations in the composition of a particular membrane but, in general, these alterations in fatty acid composition are not great (*1*). However, the lipid composition of the membrane of *Mycoplasma laidlawii* B can be regulated to an appreciable extent by the components of the growth medium (*2*). We now report that, by addition of high concentrations of exogenous fatty acids to a growth medium low in lipid content, *M. laidlawii* B cells which show striking variations in the fatty acid composition of the membrane polar lipids can be obtained. These variations are accompanied by characteristic changes in cell morphology. In this organism the polar lipids, which are localized almost exclusively in the cell membrane (*3*), consist of glucose phosphatides and

Table 1. The fatty acid composition of *Mycoplasma laidlawii* B cells grown in various fatty acids. The fatty acids are designated by the number of carbon atoms, followed by the number of double bonds; *c* and *t* indicate the *cis* and *trans* configurations of double bonds; *i* indicates a methyl branch attached to the penultimate carbon atom, and *cp* indicates the presence of a cyclopropane ring.

Added fatty acid	Incorporation of fatty acids in polar lipids (moles per 100 moles)							
	12:0	14:0	16:0	18:0	18:1	18:2	16:0 *i*	19:0 *cp*
None	7.5	24.8	53.5	3.0	6.6	4.4	0.0	0.0
Palmitic (16:0)	13.5	3.9	68.3	2.0	6.8	5.4	.0	.0
Stearic (18:0)	5.0	5.0	8.0	65.0	10.3	6.7	.0	.0
Oleic (18:1 *c*)	3.5	6.4	20.2	0.9	68.9	trace	.0	.0
Elaidic (18:1 *t*)	3.9	4.7	16.5	.7	73.7	0.5	.0	.0
Isopalmitic (16:0 *i*)	6.3	3.1	3.3	.5	5.6	2.5	78.8	.0
Dihydrosterculic (19:0 *cp*)	1.1	5.5	15.4	1.8	15.3	trace	0.0	60.0

glycerol phosphatides as well as mono- and diglucosyl diglycerides (*4*). Together the phospho- and glycolipids account for 90 percent (dry weight) of the total membrane lipid (*4*).

Cells were grown and collected, the lipids were extracted, and the fatty acid compositions of the polar lipids were determined as described (*2*), except that a tryptose medium low in lipid content, prepared by multiple extractions with chloroform at acidic *p*H, was utilized.

When cells are grown in media to which various fatty acids are added, the exogenous fatty acid is incorporated in high amounts, representing between 60 and 80 mole percent of the total (Table 1) (*5*). Elaidic and isopalmitic acids, which are not normally present in this organism, are incorporated in highest quantities while dihydrosterculic acid is incorporated to a lesser extent. Palmitic, stearic, and oleic acids also show great increases over their "normal" concentrations. These variations in fatty acid composition are among the most striking to be reported for any biological membrane.

It should be noted, when considering the fatty acid compositions reported in Table 1, that *M. laidlawii* B is capable of *de novo* synthesis of certain even-numbered saturated fatty acids from acetate (*6*); thus, lauric, myristic, palmitic, and stearic acids are present in all cases as a biosynthetic background over which fatty acid incorporation is superimposed. Odd-numbered saturated fatty acids are not synthesized to any appreciable extent, while branched chain, cyclopropane-type, and unsaturated fatty acids are never synthesized. The low concentrations of oleic and linoleic acids appearing in cells to which these acids were not added are derived from residual amounts of these acids in the growth medium.

The morphology of cells was dependent on the fatty acid added to the growth medium. Cells grown in palmitic or stearic acids exist primarily as single spheres and short, swollen filaments; cells grown in stearic acid eventually undergo swelling and lysis. Cells grown in oleic, dihydrosterculic, or isopalmitic acid exist primarily as long, fine filaments at time of harvest. When viewed as wet mounts in the phase microscope, filamentous cells grown in palmitic or stearic acids appeared rigid whereas the oleic, dihydrosterculic, and isopalmitic filaments appeared fluid. The morphology and wet mount behavior of elaidic acid cells was intermediate between these two extremes.

A lipoprotein subunit membrane model has been proposed in which the fatty acid hydrocarbon chains of the membrane lipids specifically complement certain hydrophobic amino acid sequences in the membrane protein (*7*). This model implies that the various complex lipids of a given membrane should possess a limited and specific group of fatty acids. We have noted, however, that with the exception of stearic acid, *M. laidlawii* B cells can be successfully passed in medium supplemented with any of the above-mentioned fatty acids. The fact that drastic alterations in the structures of the fatty acyl groups of the membrane lipids do not appear to adversely affect the viability of this organism suggests that membrane structure and function are not critically dependent on a narrow and highly specific composition of the lipid hydrocarbon tails. A similar lack of fatty acid specificity has recently been reported for another species of *Mycoplasma* (*8*). Although some experiments indicate that *M. laidlawii* B will grow well on a wide variety of fatty acids, including saturated, unsaturated, branched-chain, and cyclopropane types, and will incorporate these acids into membrane lipids in high amounts, the range of acceptable fatty acids does have limits. The introduction of increasing numbers of double bonds or methyl branches on a fatty acid lowers and eventually eliminates its incorporation into the membrane lipids of these cells. Also, long-chain saturated fatty acids (22 carbons and higher) are not incorporated in significant quantities.

The lack of a cell wall or an internal membrane system, the ease of isolation of nearly pure cell membranes, and the localization of essentially all cellular lipid in the membrane make *M. laidlawii* B an excellent model system for the investigation of membrane structure and function (*2*). The ability to alter the fatty acid composition of the membrane lipids in a predictable fashion by incorporation of high amounts of a wide range of fatty acids further enhances the suitability of this organism for studies of the role of fatty acyl groups in biological membranes.

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