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Cultivation of Leptospires: Fatty Acid Requirements

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Both the parasitic and the saprophytic leptospires grow well on a pair of fatty acids (one saturated, the other unsaturated) if they contain at least 15 carbon atoms.

The fatty acid requirements of serotypes of *Leptospira interrogans* were previously investigated utilizing a medium which contained "fatty acid-poor" fraction V bovine albumin (Pentex, Inc., Kankakee, Ill.) (3). We recently became aware that, although this albumin contained only trace amounts of free fatty acids, it was contaminated with 2.25 mg of lipid per g of albumin. These lipids markedly affected the lipid composition of leptospires (4). Accordingly, the fatty acid requirements were reinvestigated using a "lipid-poor" albumin which contained <50 μg of lipid per g of albumin (4). The medium used in this study is the same as that previously described except for the albumin component (3).

Leptospires used in this investigation were from cultures in the logarithmic or early stationary phase of growth. Unless stated otherwise, a 1% (v/v) inoculum which yielded approximately 3×10^6 cells per ml was used. The results presented in this report were obtained from the third transfer in the test medium. In the absence of added fatty acids, none of the leptospires could be subcultured in the medium. Growth was measured daily with a Coleman (model 7) photonephelometer calibrated with an arbitrary standard. The relationship between nephelometer reading and number of organisms was verified by periodic counts with a Petroff-Hausser counting chamber. All incubations were conducted at 30 C for 5 to 9 days.

In the designation of fatty acids, when two numbers are used the first indicates fatty acid chain length, and the second indicates the number of double bonds. When three numbers are used, the first indicates position of double bond, the second, the fatty acid chain length, and the third, the number of double bonds. Both parasitic (eight serotypes) and saprophytic (eight serotypes) leptospires required fatty acids containing at least 15 carbon atoms. These results are in contrast to our earlier finding which indicated that the saprophytes could grow on fatty acids containing less than 15 carbon atoms (3), whereas the parasites required the longer chain fatty acids. The basis for this discrepancy was found to be the ability of the saprophytes to utilize short-chain fatty acids when very low levels of long-chain fatty acids were present. The saprophyte *patoc* grew on 4×10^{-4} M 12:0 (lauric acid) if as little as 0.5×10^{-5} to 10^{-5} M 16:0 (palmitic acid) was provided. The concentration of long-chain fatty acids associated with contaminating lipid of the fatty acid-poor albumin was calculated to be 10^{-5} to 5×10^{-5} M. The parasite *canicola*, on the other hand, required 1×10^{-4} to 2×10^{-4} M 16:0 to grow in the presence of 4×10^{-4} M 12:0.

Other studies carried out in the lipid-poor albumin medium further elucidated fatty acid requirements of the leptospires. The unsaturated fatty acid *cis*-9-18:1 (oleic acid) was generally a poor substrate, especially for the parasites (Table 1), whereas the combination of *cis*-9-18:1 and 16:0 or 16:0 alone were good substrates. The saturated fatty acid 18:0 (stearic acid) supported good growth of the saprophytes, but only a few of the parasites grew on this acid (Table 1). The two parasites *ballum* and *hardjo* required a combination of a saturated and a *cis*-unsaturated fatty acid (16:0 + cis-9-18:1) for growth (Table 1). The *trans* form of 9-18:1 (4×10^{-4} M) was found to substitute for the above pair of acids with an equivalent level of growth resulting (40×10^7 leptospires/ml). Similar results with *trans*-9-18:1 have been reported for the Kazan and Reiter strains of *Treponema pallidum* (1) and the goat mycoplasma strain γ (5), microorganisms which also require a pair of fatty acids for growth.

TABLE 1. *Growth of leptospires on various fatty acids*[a]

Serotypes tested	Palmitic acid (16:0)	Stearic acid 18:0	Oleic acid (*cis*-9-18:1)	16:0 +*cis*-9-18:1
Parasites				
canicola Hond Utrecht IV	35 (37)[b]	52	<2 (<2)[c]	40
copenhageni M20	32	<2	<2	45
coxi Cox	34	<2	<2	39
ballum S102	<2	<2	<2	42
grippotyphosa Mal 1540	30	45	10	47
hardjo Nebraska	<2	<2	<2	44
pomona Pomona	47	<2	<2	59
wolffi 3705	59	39	4	57
Saprophytes				
andamana CH_{11}	34	43	23	32
andamana correo	50	55	39	44
biflexa Lt 430	50	50	37	52
biflexa Lt 965	41	44	<2	44
biflexa Gent	49	45	<2	50
patoc Patoc I	64 (54)[b]	51	31 (16)[c]	62
sao-paulo Sao Paulo	63	54	54	57
semaranga Veldrat S173	36	43	34	58

[a] Concentration of fatty acids, 4×10^{-4} M; the 16:0 + 9-18:1 combination contains 2×10^{-4} M concentration of each acid. Results are expressed as number of leptospires $\times 10^{7}$ per milliliter.

[b] Growth on 3×10^{-4} M palmityl alcohol.

[c] Growth on 3×10^{-4} M oleyl alcohol.

Fatty acids (2) and fatty alcohols (T. Auran and R. C. Johnson, Bacteriol. Proc., p. 27, 1968) are the only two readily utilizable major carbon and energy sources known for the leptospires. Since the fatty alcohols were previously tested in a medium which was not lipid-poor, they were reinvestigated using the lipid-poor albumin medium. The results obtained with palmityl alcohol and oleyl alcohol were similar to those observed with the corresponding fatty acids (Table 1). In addition, a lipid analysis was conducted on *patoc* cells cultivated on palmityl alcohol. The fatty acid composition of the phosphatidyl ethanolamine of these cells was found to be the same as that of cells cultivated on palmitic acid (4), indicating that the fatty alcohols and fatty acids are metabolized in a similar manner.

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