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Fatty Acid Requirements of the Kazan 5 and Reiter Strains of *Treponema pallidum*

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The fatty acid requirements of two avirulent treponemes were investigated by using a "lipid-poor" albumin-thioglycolate medium. The Kazan 5 and the Reiter stains of *Treponema pallidum* required a pair of fatty acids for growth. One member of the pair was saturated and the other was unsaturated. The saturated fatty acids could contain either an odd or even number of carbon atoms, but a chain length of at least 14 carbon atoms was necessary. Unsaturated fatty acids with one, two, or three double bonds were satisfactory if they contained 15 or more carbon atoms. The pair of fatty acids could be replaced with a single 18-carbon monounsaturated fatty acid if it was in the *trans* configuration rather than the naturally occurring *cis* form.

Fatty acids are required for the growth of the Reiter strain of *Treponema pallidum* (6). Power and Pelczar (7) reported that the addition of long-chain saturated and unsaturated fatty acids to a 10% sheep serum medium improved the cell yield of the Reiter treponeme. The maximum level of growth was obtained with a mixture of 16:0, 18:0, and 9-18:1.

The fatty acid requirements of this treponeme were studied in detail by Oyama et al. (6). They found that the serum requirement could be replaced by defatted bovine serum albumin plus certain long-chain fatty acids. A large number of fatty acids were assayed for growth-supporting activity. These investigators reported that only a few of the saturated fatty acids possessed activity, whereas almost all of the unsaturated fatty acids tested promoted good growth of the Reiter treponeme. No specific combination of fatty acids was found to be necessary for growth (6).

The lipid composition of the treponemes can be markedly influenced by lipid contaminants present in the albumin component of the culture medium (3). The possibility that these contaminating lipids might influence the fatty acid requirements of the treponemes was investigated by utilizing a "lipid-poor" albumin (4). The results of this study are presented in this report.

MATERIALS AND METHODS

Treponeme cultures were obtained from the Venereal Disease Research Laboratory of the Center for Disease Control, Atlanta, Ga. The Kazan 5 and the Reiter strains of *T. pallidum* were cultivated in a modified Fluid Thioglycollate Medium (BBL, Cockeysville, Md.; reference 3) containing 2% "lipid-poor" bovine serum albumin (4) and the appropriate fatty acids. Stock cultures were maintained in fluid thioglycolate medium containing 10% rabbit serum.

Fatty acids were obtained from the Hormel Institute, Austin, Minn., and were of high purity grade. Bovine serum albumin (fraction V) was purchased from Pentex, Inc., Kankakee, Ill.

Cultivation was at 35 C, and the organisms used for the nutritional studies were from cultures in the logarithmic or early stationary phase of growth. A 10% (v/v) inoculum was used, and at least three transfers were made in the test medium. Growth was measured daily with a Coleman (model 7) photonephelometer calibrated with an arbitrary standard (9). The relationship between nephelometer reading and number of organisms was verified by periodic direct counts with a Petroff-Hausser counting chamber.

RESULTS

A thioglycolate medium containing 2% "fatty acid-poor" bovine albumin was originally used in our nutritional studies. The albumin component was found to be unsatisfactory because of its lipid content. Although the albumin contained only trace amounts of free fatty acids, it was contaminated with 2.25 mg of lipid per g of albumin. Phosphatidylcholine was the major lipid component with smaller amounts of cholesterol and cholesterol esters (4). The long-chain fatty acids associated with these lipids were utilized by the treponemes, making definitive studies of their fatty acid requirements impossible. Extraction of the albumin with chloroform and methanol (2:1) resulted in a "lipid-poor" albumin (<0.05 mg of lipid per g of albumin; reference 4) which was satisfactory for investigating the fatty

acid requirements of the Kazan 5 and Reiter strains of *T. pallidum*.

A pair of fatty acids was required for the growth of the Kazan 5 and Reiter strains. Neither the saturated fatty acid 16:0 nor the unsaturated fatty acid 9–18:1 alone supported the growth of the treponemes. However, the combination of the long-chain saturated and unsaturated fatty acids resulted in good growth (Table 1). The cell yield in the fatty acid-albumin-thioglycolate medium was approximately one-half to three-quarters that obtained in the 10% rabbit serum-thioglycolate medium.

Saturated fatty acids containing from 18 to 4 carbon atoms were tested for their growth-supporting properties when in combination with 9–18:1. Fatty acids containing 14 to 18 carbon atoms promoted growth, and both odd and even numbered fatty acids functioned in this capacity for Kazan 5. Similar results were obtained with 14:0 and 16:0 with the Reiter strain (Table 2). Saturated fatty acids did not support growth of either treponeme strain if they contained 13 or fewer carbon atoms (Table 2).

The unsaturated fatty acid requirements of the two treponemes were investigated next. The saturated fatty acid provided in the test medium was 16:0. In this medium, the addition of unsaturated fatty acids containing 18 or 16 carbon atoms supported growth (Table 3). No significant difference was observed in the activities of the 11 and 9 isomers of 18:1. Fatty acids containing one to three double bonds satisfied the unsaturated fatty acid requirement of these treponemes. Neither the shorter chain unsaturated fatty acid, 14:1, nor the 20-carbon polyunsaturated acid, 20:4, possessed growth-supporting activity in this medium (Table 3).

The Kazan 5 and the Reiter treponemes required a pair of fatty acids (one saturated, the other unsaturated) for growth (Table 1). The saturated fatty acids, 18:0 through 4:0, and the *cis*-unsaturated fatty acids 5, 8, 11, 14–20:4; 6, 9, 12–18:3; 9, 12–18:2; 9–18:1; 11–18:1; 9–16:1; and 9–14:1 when tested separately were found to be lacking growth-supporting activity.

In comparison, good growth occurred with certain single fatty acids. The *trans* form of either 9–18:1 or 11–18:1 supported growth of these two treponemes. The shorter fatty acid, *trans*-9–16:1, and the 18-carbon fatty acid containing two double bonds in the *trans* position, *trans*-9, *trans*-12–18:2, were lacking this activity (Table 4).

The influence of various chain lengths of saturated fatty acids in combination with *trans*-9–18:1 on the growth of Kazan 5 was also investigated. In contrast to the results obtained with *cis*-9–18:1 (Table 2), growth was inhibited when the longer chain saturated fatty acids (15:0, 16:0, 17:0, and 18:0) were combined with

TABLE 1. *Growth of Kazan 5 and Reiter treponemes on various fatty acids*[a]

Additions to test medium	Kazan 5	Reiter
16:0	0	0
cis-9-18:1	0	0
16:0 + *cis*-9-18:1	31	22

[a] Growth expressed as number of treponemes × 10^7/ml. Incubation was at 35 C for 5 to 7 days. Data derived from third transfer in test medium. Concentration of each fatty acid was 0.2 mM.

TABLE 2. *Effect of saturated fatty acid chain length on growth of treponemes on oleic acid*[a]

Additions to test medium[b]	Kazan 5	Reiter
None	0	0
4:0–13:0	0	0
14:0	37	15
15:0	37	ND[c]
16:0	26	35
17:0	35	ND[c]
18:0	22	ND[c]

[a] See footnote *a* of Table 1.
[b] Containing 0.2 mM *cis* 9-18:1.
[c] Not done.

TABLE 3. *Effect of unsaturated fatty acid chain length on growth of treponemes on palmitic acid*[a]

Additions to test medium[b]	Kazan 5	Reiter
cis-9-14:1	0	0
cis-9-16:1	32	16
cis-9-18:1	49	47
cis-11-18:1	50	48
cis-9, 12-18:2	26	35
cis-9, 12, 15-18:3	24	25
cis-5, 8, 11, 14-20:4	0	0

[a] See footnote *a* to Table 1.
[b] Containing 0.2 mM 16:0.

TABLE 4. *Effect of trans-unsaturated fatty acids on the growth of treponemes*[a]

Additions to test medium	Kazan 5	Reiter
trans-9-16:1	0	0
trans-9-18:1	28	18
trans-11-18:1	43	24
trans-9, trans-12-18:2	0	0

[a] See footnote *a* to Table 1.

TABLE 5. *Effect of various fatty acids in combination with trans-9-18:1 on the growth of Kazan 5*[a]

Additions to medium[b]	Kazan 5
None	25
8:0	17
10:0	21
12:0	23
14:0	22
15:0	0
16:0	0
17:0	0
18:0	0
cis-9-18:1	20

[a] See footnote *a* to Table 1.
[b] Containing 0.2 mM *trans*-9-18:1.

trans-9–18:1. Moreover, the shorter chain saturated fatty acids and *cis*-9–18:1 were compatible with growth when combined with *trans*-9–18:1 (Table 5).

DISCUSSION

Our initial results obtained by using fatty acid-poor albumin were similar to those reported by Oyama et al. (6). A single unsaturated fatty acid appeared to satisfy the fatty acid requirements of the Kazan 5 and the Reiter strains of *T. pallidum*. However, upon extraction of the albumin component of the medium with chloroform and methanol, a requirement for a pair of fatty acids (one saturated and the other unsaturated) was manifested by these treponemes. Compatible with growth were saturated fatty acids of 14 to 18 carbon atoms when combined with unsaturated fatty acids which contained 16 or 18 carbon atoms. Shorter fatty acids were without growth-promoting activity. The Kazan 5 and the Reiter strains appear to have identical fatty acid requirements.

The results of a study of the lipid composition of the Kazan 5 strain (3) provide an explanation for the fatty acid requirements of these treponemes. The Kazan 5 treponemes cannot synthesize, modify the chain length of, desaturate, or reduce fatty acids (3). Thus, the membrane lipid requirements dictate the fatty acids which must be supplied in the culture medium, and these fatty acids are incorporated unaltered into the lipids. The combination of a saturated and an unsaturated fatty acid is probably necessary to provide the correct "fluidity" for the treponeme membrane (8). The physical properties of the *trans* form of the monounsaturated 18-carbon fatty acids must approximate those of the pair of fatty acids (one saturated, the other *cis*-unsaturated), since the treponemes can grow in a medium containing either *trans*-9–18:1 or *trans*-11–18:1 as the only fatty acid. If the 18-carbon fatty acid has two double bonds in the *trans* position, this unique property is lost. Also, the *trans* form of the 16-carbon monounsaturated acid is devoid of this activity. Analysis of the lipids of Kazan 5 cells grown on *trans*-9–18:1 revealed that this fatty acid comprised 95% of the cellular fatty acids (3). These results are similar to those reported by Rodwell (10), who studied the fatty acid requirements of the goat mycoplasma strain Y.

The fatty acid composition of membrane lipids may affect membrane permeability and elasticity. Studies with phospholipid films have demonstrated that unsaturated or short-chain fatty acids keep the lipid layer from becoming too tightly packed (12). One possible interpretation of the results presented in Table 5 is that the lipid layer of membranes containing *trans*-9–18:1 may be quite condensed. The combination of this fatty acid with long-chain saturated fatty acids, which would increase the extent of packing of lipid molecules in the membrane, is incompatible with growth. Growth did occur when the shorter chain saturated fatty acids or *cis*-9–18:1 were in combination with *trans*-9–18:1.

The aerobic spirochetes of the genus *Leptospira* are similar to the Kazan 5 and Reiter treponemes in that they cannot synthesize fatty acids de novo and accordingly must have them supplied in the culture medium (2). However, the leptospires can desaturate and modify the chain length of fatty acids (4, 11) as well as use them as their major source of carbon and energy (2). This is in contrast to the Kazan 5 and Reiter treponemes which cannot beta-oxidize or modify the structure of the fatty acids (3). Other anaerobic spirochetes are not as biosynthetically deficient. *T. zuelzerae*, a free-living spirochete which has recently been renamed *Spirochaeta zuelzerae* (1), can synthesize its fatty acids de novo (5).

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