# Synthesis of unsaturated fatty acids in the slime mold *Physarum polycephalum* and the zooflagellates *Leishmania tarentolae*, *Trypanosoma lewisi*, and *Crithidia sp.*: a comparative study

EDWARD D. KORN, CHARLES L. GREENBLATT, and ANN M. LEES

Laboratory of Biochemistry, Section on Cellular Physiology, National Heart Institute, and Laboratory of Physical Biology, Section on Molecular Biophysics, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

SUMMARY On the basis of the unsaturated fatty acids which they synthesize, protists can be grouped in a meaningful way. Thus, the unsaturated fatty acids of the true slime mold are identical with the unsaturated fatty acids of the soil amoebae *Acanthamoeba* and *Hartmannella* but have no resemblance to those of the cellular slime mold *Dictyostelium*. Among the zooflagellates, *Leishmania enriettii* and *Leishmania tarentolae* synthesize unsaturated fatty acids that are very similar to those of the phytoflagellates *Euglena*, *Ochromonas*, and *Chlamydomonas*, while *Trypanosoma lewisi* and especially *Crithidia* have a different pattern of unsaturated fatty acids which more closely resembles that of the ciliated protazoa.

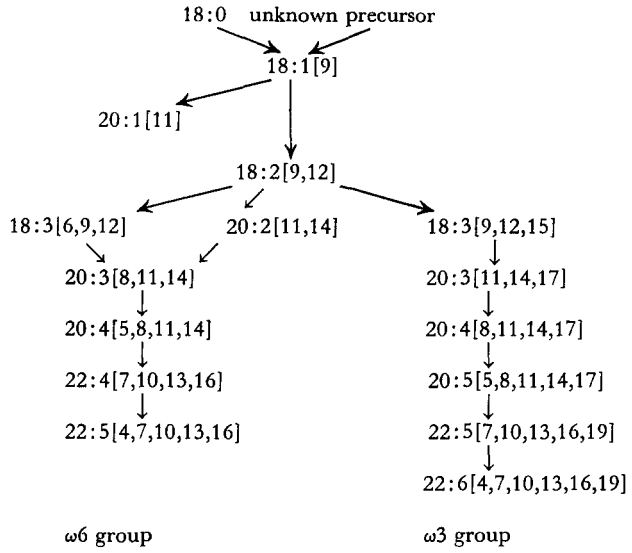
The unsaturated fatty acids of the true slime mold *Physarum polycephalum* include oleate, linoleate, 11-eicosenoate, 11,14-eicosadienoate, 8,11,14-eicosatrienoate, and arachidonate. *L. tarentolae* synthesizes 6,9,12-octadecatrienoate and 9,12,15-octadecatrienoate as well as polyunsaturated 20- and 22-carbon fatty acids derived from both of these 18-carbon acids. *T. lewisi* synthesizes 6,9,12-octadecatrienoate. *Crithidia* synthesizes 6,9,12-octadecatrienoate, 8,11,14-eicosatrienoate, and 4,7,10,13,16-docosapentaenoate but not 9,12,15-octadecatrienoate or any acids derived from it. *Leishmania* and *Trypanosoma* have been shown to convert stearate directly to oleate.

KEY WORDS protozoal fatty acids · unsaturated fatty acids · fatty acid biosynthesis · *Crithidia sp.* · *Leishmania tarentolae* · *Physarum polycephalum* · *Trypanosoma lewisi*

THE MAJOR POLYUNSATURATED fatty acids may conveniently be divided into two groups: those in which the double bond furthest from the carboxyl group is three carbon atoms from the methyl end of the molecule (ω3 group), and those in which the terminal double bond is six carbon atoms from the methyl end of the molecule (ω6 group). In mammals, a number of polyunsaturated 20- and 22-carbon acids of both the ω3 and ω6 groups are synthesized from dietary linolenic acid and linoleic acid, respectively, by repeated chain elongation and introduction of double bonds in a divinylmethane pattern toward the carboxyl side of existing double bonds (1). The ω6 group serves some as yet undefined essential function (2). Higher animals are dependent on the exogenous precursors because they are unable to introduce double bonds on the methyl terminal side of existing double bonds and, therefore, cannot synthesize linoleic acid from oleic acid, or linolenic acid from linoleic acid (3). These latter reactions are typical of higher plants (4) which do not, however, convert the 18-carbon acids to longer-chain fatty acids, or introduce more double bonds towards the carboxyl group.

Protists are not so limited. *Acanthamoeba*, for example, synthesizes linoleic acid from oleic acid, and also converts linoleic acid to arachidonic acid by a pathway differing in one step (5) from that previously described in the rat (1). *Euglena*, a chlorophyll-containing phytoflagellate thought to be similar to those organisms that were the common origin of higher plants and animals, synthesizes at least 59 fatty acids (6, 7) including those polyunsaturated fatty acids[1] typical of higher plants,

[1] The first number refers to the number of carbon atoms in the fatty acid chain, the second number to the number of double bonds in the molecule, and the numbers in brackets to the positions of the double bonds counting from the carboxyl end of the molecule.




SCHEME 1. Pathways of Synthesis of Unsaturated Fatty Acids in Protists.

higher animals, and algae (i.e., 16:3[7,10,13] and 16:4[4,7,10,13]).

Previous reports from this laboratory (5–9) and by Erwin and Bloch (10–13) have emphasized the potential usefulness of protists in delineating the mechanisms of biosynthesis of unsaturated fatty acids and, conversely, the possibility of applying this information to the taxonomy and phylogeny of the protists. We now add data on four more organisms including three zooflagellates of the order *Protomonadina*.

Scheme 1 summarizes the reactions of polyunsaturated fatty acid synthesis (except for the synthesis of the polyunsaturated 16-carbon acids) known to occur in the protists. In some instances the reactions have been demonstrated directly by addition of radioactive precursors to growing cells. In other cases, the presence or absence of reactions are inferred from the structures of the fatty acids found in the cells in detectable quantities. All but one or two of these steps occur in some organisms while one entire sequence may be missing from others. All protists so far examined are able to synthesize linoleic acid.[2] They differ, however, in many other respects and it is these differences which one must consider in grouping the protists according to their abilities to synthesize polyunsaturated fatty acids.

The reactions present in different combinations in each of the organisms studied are:

(*a*) The mechanism of synthesis of oleic acid. Many protists synthesize oleic acid by direct desaturation of stearic acid while others use the uncharacterized, indirect pathway of higher plants (16, 17). The third pathway to oleic acid present in many bacteria (12) has not been demonstrated in protozoa.

(*b*) The ability to convert linoleic acid to 18:3[9,12,15]. If this reaction is missing, the entire ω3 group of polyunsaturated fatty acids will not be synthesized.

(*c*) The ability to convert linoleic acid and 18:3[9,12,15] to longer-chain fatty acids by chain elongation and further desaturations.

(*d*) The utilization of 18:3[6,9,12] or 20:2[11,14] as the intermediate between linoleic acid and 20:3[8,11,14].

(*e*) The ability to synthesize 16:3[7,10,13] and 16:4[4,7,10,13].

By use of these criteria, the protists on which data are available can be grouped in a meaningful way.

## EXPERIMENTAL PROCEDURES

### PROTISTS

#### *Physarum polycephalum*

Sclerotia, kindly provided by Dr. R. D. Allen, Princeton University, Princeton, N.J., were initially cultured on large pans of wet oatmeal. The fatty acids extracted from such plasmodia were similar to those of the oatmeal. Consequently, it was necessary to grow the plasmodia on a medium free of significant quantities of fatty acids. To do this, plasmodia were first freed of contaminating organisms by permitting them to migrate on sterile, non-nutrient agar (18). Advancing portions of the plasmodia were excised and transferred to a second petri dish of non-nutrient agar. This process was repeated until no bacterial colonies formed when the plasmodium was transferred onto nutrient agar. Nutrient agar contained a modification of the tryptone–glucose–yeast medium described by Daniel and Rusch (18) in which the chick embryo extract was replaced by hemin (19). Submersion cultures were maintained at room temperature on the same medium with vigorous aeration, essentially as described by Daniel and Rusch (18). The medium was found not to contain significant concentration of fatty acids. Cultures were repeatedly monitored for contaminants by microscopic examination and by plating onto nutrient agars.

#### *Leishmania tarentolae*

*L. tarentolae* was obtained from Dr. W. Trager, The Rockefeller Institute, New York, N.Y. Analyses were originally made on cultures grown in Locke's solution over the blood–agar base of Senekjie (20). For more definitive analyses, the *Leishmania* were grown on the completely defined Trager's C medium (21) which contains only traces of fatty acids. All cultures were at room temperature without aeration.

[2] The one exception are those organisms in the class Acrasia, the cellular slime molds. These synthesize only diunsaturated fatty acids of unique structure (14, 15).

*Trypanosoma lewisi*

Lincicome's original strain of *T. lewisi* was grown in the rat and adapted to blood–agar culture by Mrs. E. J. Tobie, National Institutes of Health, Bethesda, Md. Two batches of organisms were analyzed. One was collected from the blood of infected rats, the other was grown on Locke's solution over a 30% rabbit blood–agar base. In no case were the trypanosomes grown on a fatty acid-free medium.

*Crithidia sp.*

*Crithidia sp.* (strain ReF-1) was obtained from Dr. L. Diamond, National Institutes of Health. These organisms were grown exactly as the *Leishmania*; originally on Locke's solution over blood–agar, and then on Trager's C medium at room temperature. In contrast to our experience with *L. tarentolae* and *T. lewisi*, luxuriant growth was readily obtained.

### Preparation and Analysis of Fatty Acids

The procedures used have all been described in detail elsewhere (5–8) and will be presented only in outline here. Protozoa were harvested by centrifugation, washed, sometimes lyophilized, and extracted with 19 volumes of chloroform–methanol 2:1 (v/v). The mixture was filtered and the solvent was evaporated under reduced pressure. The residue was dissolved in petroleum ether. For the analysis of total fatty acids, the samples were either methanolyzed with 0.5 N NaOH in methanol, or saponified in 0.5 N NaOH in 50% methanol, followed by esterification by the $BF_3$–methanol reagent. In some instances the phospholipids were separated from the neutral lipids and free fatty acids by chromatography on silicic acid, and the neutral lipids and free fatty acids separated by partitioning between alkaline aqueous ethanol and petroleum ether. Samples were then esterified as above.

The pattern of fatty acids was initially determined by gas–liquid chromatography (GLC) on a column of 17% ethylene glycol succinate polyester on Gas Chrom P, usually at an inlet pressure of 10 psi and at 195°. An argon ionization detector was used. Complete identification was achieved after isolation of the individual fatty acids. Fatty acids were first separated into groups according to their degree of unsaturation by chromatography of their mercuric acetate adducts on silicic acid (10, 22). Usually, saturated fatty acids were eluted in benzene, monounsaturated fatty acids in ether, diunsaturated fatty acids in methanol, triunsaturated fatty acids in 0.5% acetic acid in methanol, tetra- and pentaunsaturated fatty acids in 1% acetic acid in methanol, and hexaunsaturated fatty acids in 5% acetic acid in methanol. After removal of the mercuric acetate, the fatty acid esters were analyzed by GLC. When more than one peak was present in a fraction, the components were separated by preparative GLC (23).

Each fatty acid was hydrogenated and the saturated fatty acid identified by GLC. In most instances, the unsaturated fatty acid was subjected to oxidative degradation (24), and the dicarboxylic and monocarboxylic products identified by GLC. Thus, the fatty acids have been characterized by the retention times of their methyl esters, their chromatographic behavior on silicic acid as mercuric acetate adducts, the identification by GLC of their hydrogenation products and, in most cases, the identification by GLC of the products of oxidative degradation.

### Radioactive Experiments

In several experiments, *Leishmania* or trypanosomes were grown or incubated in the presence of sodium stearate uniformly labeled with $C^{14}$ as the albumin complex, or sodium acetate-1-$C^{14}$. Cells were harvested and the fatty acid methyl esters prepared as described above. A small aliquot was analyzed qualitatively and quantitatively by GLC for its fatty acid composition. Another aliquot was chromatographed on a preparative GLC column together with carrier fatty acid methyl esters and the emerging fractions were collected onto anthracene and counted in a scintillation counter (23). Fractions were collected between, and corresponding to, the peaks. Only the fractions containing fatty acids had significant radioactivity. Recovery of radioactivity was approximately 95%. The distribution of radioactivity among the fatty acids was thus directly obtained. The relative specific activity of each fatty acid was calculated by dividing the radioactivity thus determined by the area of the corresponding peak on the analytical chromatogram. Because of the complexity of the fatty acid composition of most protozoa, it is difficult to resolve the components completely by GLC and some overlap of peaks is unavoidable. The following procedure eliminated the possibility of significant cross-contamination.

The remainder of the fatty acid methyl esters were separated as their mercuric acetate adducts as described before. Recovery of radioactivity was over 90%. These fractions were then analyzed by GLC, and the distribution of radioactivity among the fatty acids was determined as above by preparative GLC in the presence of carrier compounds. Since each sample now contained only a few fatty acids, chromatography could be carried out under optimal conditions so that there was wide separation of peaks and essentially no contamination of one fatty acid by another. Fractionation of the mercuric acetate adducts was especially helpful because the highly radioactive fatty acids such as stearic, oleic, and linoleic were separated from the other fatty acids before GLC, thus reducing to insignificant levels the contamination

TABLE 1 COMPARISON OF THE FATTY ACID COMPOSITION OF *Physarum Polycephalum* AND *Acanthamoeba sp.*

| Fatty Acid | *P. polycephalum* Phospholipids† | *P. polycephalum* Neutral Lipids† | *P. polycephalum* Total Lipids† | *Acanthamoeba** Total Lipids |
|---|---|---|---|---|
| | *weight %* | | | |
| A‡ | 0.6 | 0 | 2.9 | 0 |
| B‡ | 0 | 0 | 1.3 | 0 |
| 14:0 | 0.4 | 1.7 | 0.3 | 9.2 |
| C‡ | 6 | 3.7 | 6.8 | 0 |
| 16:0 | 5.7 | 31.0 | 7.8 | 9.4 |
| 16:1[9] | 2.3 | 2.2 | 2.5 | 3.5 |
| 18:0 | 1.0 | 4.1 | 1.8 | 6.2 |
| 18:1[9] | 37.3 | 21.5 | 35.0 | 45.4 |
| 18:2[9,12] | 36.2 | 27.2 | 34.2 | 5.3 |
| 20:1[11] | 1.8 | 1.2 | 1.3 | 1.5 |
| 20:2[11,14] | 3.4 | 1.9 | 2.5 | 5.7 |
| 20:3[8,11,14] | 1.4 | 1.2 | 1.0 | 5.6 |
| 20:4[5,8,11,14] | 3.1 | 4.2 | 2.5 | 7.6 |

* From reference 8.

† The phospholipids accounted for approximately 82% and the neutral lipids (including free fatty acid) for 18% of the total lipids. The total lipids were from a different batch of cells.

‡ Fatty acids A, B, and C are saturated, probably branched, but otherwise uncharacterized.

of the more unsaturated or longer-chain fatty acids by radioactivity from the 18-carbon fatty acids. The data reported were obtained by the second procedure and were in good agreement with the preliminary data obtained by the first method.

## RESULTS

### *Physarum polycephalum*

The fatty acid composition of the *true* slime mold is of particular interest in comparison to the fatty acids of the *cellular* slime mold *D. discoideum* (14), and the soil amoeba *Acanthamoeba sp.* (8). *P. polycephalum* has qualitatively the same pattern of unsaturated fatty acids as *Acanthamoeba* (Table 1); that is, shows the presence of 20:1[11], 20:2[11,14], 20:3[8,11,14], and 20:4[5,8,11,14], and the absence of 18:3[9,12,15] and its metabolic derivatives. *P. polycephalum* does not contain the unique diunsaturated fatty acids 16:2[5,9], 18:2[5,9], and 18:2[5,11] characteristic of *D. discoideum*.[3]

Thus, the fatty acid composition supports the view that the true slime mold is protozoal, closely related to the soil amoebae, rather than fungal, as has also been suggested because of certain morphological characteristics (25).

[3] These acids are present in *D. discoideum* at all morphological stages; myxamoebae, migrating slugs (pseudoplasmodia) and mature fruiting bodies (M. Krichevsky, L. Love, A. Lees and E. D. Korn, unpublished observation). They are also synthesized by *Polysphondylium pallidum*, the only other species of Acrasia examined (F. Davidoff and M. Sussman, personal communication).

TABLE 2 THE FATTY ACID COMPOSITION OF TOTAL LIPIDS OF *Leishmania Tarentolae* UNDER DIFFERENT CONDITIONS OF GROWTH

| Fatty Acid* | Growth Medium: Locke's Blood–Agar | Trager's C† | Trager's C + Human Serum Albumin‡: −Arachidonate | Trager's C + Human Serum Albumin‡: +Arachidonate |
|---|---|---|---|---|
| | *weight %* | | | |
| 12:0 | 0 | 0.1 | 1.8 | 1.1 |
| 14:0 | 1.1 | 2.1 | 4.8 | 1.7 |
| A | 0 | 0.3 | 0 | 0 |
| 16:0 | 2.7 | 0.8 | 2.1 | 2.2 |
| 16:1[9] | 0 | 0.1 | 0 | 0 |
| B | 1.1 | 0.5 | 0.6 | 0.6 |
| 18:0 | 14.2 | 17 | 25.3 | 22.8 |
| 18:1[9] | 35.1 | 29.8 | 42.2 | 21.3 |
| 18:2[9,12] | 18.6 | 23.2 | 20.4 | 15.0 |
| 18:3[6,9,12] | 0.5 | 0.4 | 0 | 0 |
| 18:3[9,12,15] | 13.0 | 17.6 | 3.2 | 2.8 |
| 18:4[6,9,12,15] | 0 | 1.4 | 0 | 0 |
| 20:2[11,14] | 1.6 | 0.4 | Tr. | 0 |
| 20:3[8,11,14] | 0 | 1.3 | Tr. | 2.0 |
| 20:3[11,14,17] | 0 | 0.6 | Tr. | 0 |
| 20:4[5,8,11,14] | 0 | 0 | 0 | 2.2 |
| 20:4[8,11,14,17] | 0 | 1.7 | Tr. | 0 |
| 22:4[7,10,13,16] | 0.7 | 0.3 | Tr. | 18.0 |
| 22:5[4,7,10,13,16] | 4.5 | 0.9 | Tr. | 7.6 |
| 22:5[7,10,13,16,19] | 0 | 0.1 | Tr. | 0.5 |
| 22:6[4,7,10,13,16,19] | 7.8 | 1.8 | Tr. | 1.7 |

* The fatty acids from the cells grown on Trager's C medium were completely characterized as described under Experimental Procedures. The others are identified by retention time only. Fatty acids A and B are saturated, probably branched, but otherwise unidentified.

† Minimal dilution from blood–agar was $6 \times 10^7$.

‡ To 1 liter of culture media 2.5 g of albumin was added. In one culture 34 mg of sodium arachidonate was bound to the albumin. The albumin was not freed of its fatty acid.

### *Leishmania tarentolae*

We have reported (9) that *L. enriettii* converts stearic acid to 18:3[9,12,15].[4] In that earlier study we found no evidence for the synthesis of either 18:3[6,9,12] or 20-carbon and 22-carbon fatty acids of the ω3 or ω6 groups. We have now extended these studies to the related species *L. tarentolae*, which has provided more information on the synthesis of the longer-chain fatty acids.

When grown on Locke's blood–agar medium, the fatty acid composition of *L. tarentolae* was similar to that previously found for *L. enriettii*, except for a somewhat higher percentage of 22:5 and 22:6 (Table 2), and the presence of 18:3[6,9,12]. However, as before, the concentrations of the 20- and 22-carbon acids were no greater than *could* have been derived from the fatty acids in the medium. Fortunately, *L. tarentolae*, unlike *L.*

[4] Also, acetate-1-$C^{14}$ is converted to stearic, oleic, linoleic, and linolenic acids by *L. enriettii* (C. L. Greenblatt and E. D. Korn, unpublished observation).

*enriettii*, can be grown on a defined medium (Trager's C). In this case, all the polyunsaturated fatty acids were definitely synthesized by the protozoa, none being detectable in the growth medium.

Because of the rather low percentage of 20-carbon and 22-carbon acids and the low yield of cells, we wished to gain additional proof of the synthetic abilities of the organism. Therefore, two batches of cells were grown on Trager's C medium to which had been added sodium stearate-$C^{14}$ as a complex with sterile bovine serum albumin freed of fatty acids (26). One batch of cells was harvested after 3 days, the other after 12 days. Individual fatty acids were isolated and their radioactivities determined as described under Experimental Procedures. In both experiments, the stearic acid was converted to 18:1[9], 18:2[9,12], and 18:3[9,12,15] (Table 3), and in the 12 day experiment there was unequivocal synthesis of 18:3[6,9,12], 18:4[6,9,12,15], and 20- and 22-carbon fatty acids of both the ω3 and ω6 groups. In a reinvestigation of the fatty acids of *L. enriettii* we were still unable to detect any 18:3[6,9,12].

The length of time required for the conversion of radioactive stearic acid to the longer-chain polyunsaturated acids, and the relatively low concentration of those acids, suggested that *Leishmania* might be relatively unable (compared to *Euglena* or *Acanthamoeba*, for example) to elongate 18-carbon fatty acids. In one experiment, *L. tarentolae* were grown in the presence of arachidonic acid added as a complex with human serum albumin. It is interesting that the presence of serum albumin (from which fatty acids had not been removed) appeared to change the fatty acid composition of the *Leishmania* in the control flask. Most of the arachidonate taken up was converted to 22:4[7,10,13,16] and 22:5-[4,7,10,13,16] (Table 2). These three acids accounted for about 28% of the total fatty acids compared to approximately 1% in the control. One effect of this change in fatty acids was that the $R_F$ of the major phospholipid (uncharacterized) was reduced by about 50% when analyzed by thin-layer chromatography in chloroform–methanol–6M ammonia, 65:30:5. Thus, *L. tarentolae* appears to be able to metabolize 20-carbon acids well.

### *Trypanosoma lewisi*

The inability to grow *T. lewisi* on defined medium makes the data for this organism less definitive. Two types of cells were analyzed. One was harvested from the blood of infected rats, the other was from culture on blood–agar. Both types of cells contained 20- and 22-carbon polyunsaturated fatty acids of the ω3 and ω6 groups (Table 4), but it is not possible to conclude from these data to what extent the fatty acids were synthesized by the trypanosomes. The presence of stearic, oleic, and linoleic acids in another species of trypanosomes, *T. cruzi*, grown on blood–agar has been shown by von Brand (27).

To gain more information on the biosynthetic abilities of the trypanosomes, cells were grown on blood–agar medium in the presence of radioactive stearic acid for 19 days. The distribution of the radioactivity among the fatty acids (Table 5) demonstrates unequivocally the ability of *T. lewisi* to convert stearic acid to oleic and linoleic acids and also to 18:3[6,9,12]. There was no detectable radioactivity in any other fatty acids, including 18:3[9,12,15]. In another experiment, trypanosomes harvested from infected rats were incubated for 20 hr with acetate-$C^{14}$. Significant radioactivity was found only in 20:5, 22:5, and 22:6.

TABLE 3 Distribution of Radioactivity Among Fatty Acids of *L. Tarentolae* Grown in the Presence of Stearic Acid-$C^{14}$

| | 3-day experiment | | 12-day experiment | |
|---|---|---|---|---|
| Cpm Added to Culture* | $5 \times 10^6$ | | $10 \times 10^6$ | |
| Cpm Recovered in Fatty Acids | $1.3 \times 10^6$ | | $2.4 \times 10^6$ | |
| Fatty Acid | Cpm | Relative Specific Activity | Cpm | Relative Specific Activity |
| 12:0–16:1[9] | 0 | 0 | 0 | 0 |
| 18:0 | 338,000 | 19,850 | 680,700 | 40,400 |
| 18:1[9] | 333,500 | 11,250 | 410,000 | 13,750 |
| 18:2[9,12] | 287,000 | 11,900 | 330,000 | 14,200 |
| 18:3[6,9,12] | 70,000 | 3,880 | 87,300 | 87,000† |
| 18:3[9,12,15] | | | 230,000 | 13,000 |
| 18:4[6,9,12,15] | 0 | 0 | 21,000 | 15,000 |
| 20:3[8,11,14] | 920 | 700 | 26,000 | 20,000 |
| 20:3[11,14,17] | 950 | 1,400 | 18,100 | 30,000 |
| 20:4[8,11,14,17] | 780 | 1,000 | 52,000 | 30,600 |
| 22:5[7,10,13,16,19] | 0 | 0 | 18,500 | 20,600 |
| 22:6[4,7,10,13,16,19] | 0 | 0 | 41,400 | 23,000 |

* Stearic acid uniformly labeled with $C^{14}$ was added as a complex with fatty acid-free bovine serum albumin.
† This may be too high because of the difficulty in determining accurately the area of the very small peak due to 18:3[6,9,12].

TABLE 4 FATTY ACID COMPOSITION OF TOTAL LIPIDS OF *Trypanosoma Lewisi*

| Fatty Acid* | Growth Medium | |
|---|---|---|
| | Rat-Grown | Blood |
| | *weight %* | |
| 14:0 | 1.0 | 0.2 |
| A | 1.7 | 0.7 |
| 16:0 | 15.1 | 10.2 |
| 16:1[9] | 2.1 | 1.4 |
| 18:0 | 20.0 | 13.3 |
| 18:1[9] | 18.3 | 36.2 |
| 18:2[9,12] | 20.2 | 30.3 |
| 18:3[6,9,12] | 0 | 3.5 |
| 18:3[9,12,15] | 1.0 | 1.1 |
| 20:2[11,14] | 0.5 | 0 |
| 20:3[8,11,14] | 0.5 | 0 |
| 20:4[5,8,11,14] | 3.5 | 0 |
| 20:4[8,11,14,17] | 0 | 0.4 |
| 20:5[5,8,11,14,17] | 1.0 | 2.0 |
| 22:4[7,10,13,16] | 2.1 | 0 |
| 22:5[4,7,10,13,16] | 0.8 | 1.2 |
| 22:5[7,10,13,16,19] | 1.0 | 0 |
| 22:6[4,7,10,13,16,19] | 11.0 | 0 |

* Fatty acids identified only by retention time, silicic acid chromatography of mercuric acetate adducts, and hydrogenation.

The implications of these results are that trypanosomes synthesize fatty acids very poorly, if at all, de novo from acetate; that they can convert stearic acid to other 18-carbon acids, not including 18:3[9,12,15]; and that they elongate 18-carbon fatty acids only at a rather slow rate.

*Crithidia sp.*

The fatty acid composition of *Crithidia* (Table 6) is strikingly different from that of the *Leishmania* and *Trypanosoma*. *Crithidia* synthesizes large amounts of 18:3[6,9,12] and no detectable 18:3[9,12,15]. It also contains a high concentration of other fatty acids of the ω6 group, particularly 22:5[4,7,10,13,16]. *Crithidia* contains no fatty acids of the ω3 group, presumably because of its inability to synthesize 18:3[9,12,15].

TABLE 5 DISTRIBUTION OF RADIOACTIVITY AMONG FATTY ACIDS OF *Trypanosoma Lewisi* GROWN FOR 19 DAYS IN THE PRESENCE OF SODIUM STEARATE-$C^{14}$

| | |
|---|---|
| Cpm Added to Culture* | $5 \times 10^6$ |
| Cpm Recovered in Fatty Acids | 490,000 |

| Fatty Acid† | Cpm | Relative Specific Activity |
|---|---|---|
| 16:0 | 0 | 0 |
| 18:0 | 153,500 | 11,800 |
| 18:1[9] | 127,500 | 3,540 |
| 18:2[9,12] | 103,000 | 3,400 |
| 18:3[6,9,12] | 11,250 | 3,200 |

* Stearic acid uniformly labeled with $C^{14}$ was added as a complex with fatty acid-free bovine serum albumin.

† No radioactivity was found in any other fatty acids.

## DISCUSSION

All of the protists whose unsaturated fatty acids have been systematically studied are listed in Table 7 according to their abilities to perform the distinguishing reactions discussed in the introduction. Certain aspects of these data deserve elaboration.

(*a*) With respect to their unsaturated fatty acids the amoebae *Acanthamoeba*, *Hatrmannella*, and *Physarum* are closely related. All synthesize arachidonic acid apparently via 20:2[11,14], none synthesizes 18:3[9,12,15] or any ω3 group fatty acids, and none synthesizes detectable quantities of 22-carbon ω6 fatty acids. These data throw no light on phylogeny of the *cellular* slime molds. Neither soil amoebae, true slime molds, nor fungi have been found which contain even detectable quantities of the unique diunsaturated fatty acids that are the *only* polyunsaturated fatty acids of *D. discoideum* and comprise approximately 30% of its total fatty acids.

(*b*) Only the definitely protozoal organisms synthesize oleic acid by the direct desaturation of stearic acid. The euglenoid *Euglena*, the phytomonad *Chlamydomonas*, and the alga *Scenedesmus* are all incapable of that reaction (12). In addition, only the protophytae and algae synthesize the polyunsaturated 16-carbon acids (12).

(*c*) It deserves emphasis that two pathways seem to have evolved for the synthesis of arachidonic acid. *Euglena* and amoebae convert linoleic acid to 20:2[11,14],

TABLE 6 THE FATTY ACID COMPOSITION OF *Crithidia sp.*

| Fatty Acid* | Growth Medium | | | |
|---|---|---|---|---|
| | Locke's Blood–Agar | Trager's C† | | |
| | Total Lipids | Phospholipids‡ | Neutral Lipids‡ | Free Fatty Acid‡ |
| | *weight %* | | | |
| A | 2.1 | 2.6 | 5.6 | 7 |
| 14:0 | 0.6 | 1.5 | 2.6 | 0 |
| 16:0 | 2.4 | 1.4 | 1.7 | 9 |
| B | 1.0 | 0.6 | 1.7 | 0 |
| C | 1.0 | 0.6 | 0.9 | 0 |
| 18:0 | 5.9 | 18.8 | 9.4 | 12 |
| 18:1[9] | 18.2 | 20.7 | 32.2 | 9 |
| D | 16.0 | 9.4 | 3.0 | 10 |
| 18:2[9,12] | 16.1 | 19.9 | 4.7 | 42 |
| 18:3[6,9,12] | 21.3 | 19.2 | 29.1 | 7 |
| 20:3[8,11,14] | 0.7 | 1.0 | 0.9 | 2 |
| 20:4[5,8,11,14] | 0 | 0.1 | 0 | 0 |
| 22:4[7,10,13,16] | 0.7 | 0.9 | 1.7 | 0 |
| 22:5[4,7,10,13,16] | 13.9 | 12.6 | 6.4 | 3 |

* A, B, C, and D are saturated acids, probably branched, but not further characterized.

† Minimal dilution from blood–agar was $1 \times 10^8$.

‡ The phospholipids accounted for approximately 79%, the neutral lipids for 18%, and the free fatty acids for 3% of the total lipids.

TABLE 7 TENTATIVE SUMMARY OF SYNTHESIS OF UNSATURATED FATTY ACIDS BY PROTISTS

| | Oleic acid from | | Ability to Synthesize | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Stearate | Alternative Pathway | 18:3 [9,12,15] | 18:3 [6,9,12] | 20:2 [11,14] | $C_{20}$ & $C_{22}$ ω3 | $C_{22}$ ω6 | Polyunsaturated C16 |
| Euglenids: | | | | | | | | |
| *Euglena** | − | + | + | − | + | + | + | + |
| Phytomonads | | | | | | | | |
| *Chlamydomonas*† | − | + | + | + | ? | ? | ? | + |
| Algae: | | | | | | | | |
| *Scenedesmus*† | − | + | + | − | − | − | − | + |
| Chrysomonads: | | | | | | | | |
| *Ochromonas*‡ | + | ? | + | + | ? | + | + | ? |
| Protomonads: | | | | | | | | |
| *Leishmania enriettii* | + | ? | + | − | ? | ? | ? | − |
| *Leishmania tarentolae* | + | ? | + | (+) | (+) | (+) | (+) | − |
| *Trypanosoma lewisi* | + | ? | − | (+) | ? | ? | ? | − |
| *Crithidia* | + | ? | − | + | − | − | + | − |
| Ciliates: | | | | | | | | |
| *Tetrahymena*† | + | ? | − | + | − | − | ? | − |
| Amoebae: | | | | | | | | |
| *Acanthamoeba*§ | + | ? | − | − | + | − | + | − |
| *Hartmannella*‖ | + | ? | − | − | + | − | + | − |
| *Physarum* | ? | ? | − | − | + | − | + | − |

+ indicates fatty acid is synthesized; (+) indicates fatty acid is synthesized in small amount; − indicates no detectable synthesis; ? indicates the absence of data.

* Reference 7. † Reference 12. ‡ Reference 28. § Reference 8. ‖ Reference 13.

while in certain of the zooflagellates 18:3[6,9,12] appears to be the intermediate. With regard to the synthesis of 18:3[6,9,12], it should be noted that the zooflagellates may resemble more closely the phytomonad *Chlamydomonas*, and especially the chrysomonad *Ochromonas* (which also converts stearate to oleate), than they do *Euglena*. A more thorough characterization of the polyunsaturated fatty acids of *Chlamydomonas* and *Ochromonas* might be useful in establishing their relationships to the zooflagellates and to each other.

(*d*) The zooflagellate *Leishmania enriettii* resembles the phytoflagellate *Euglena* in that both synthesize only one octadecatrienoic acid, 18:3[9,12,15]. *L. tarentolae*, however, synthesizes both octadecatrienoic acids, 18:3[9,12,15] and 18:3[6,9,12]. This development is even more pronounced in *T. lewisi*, which appears to synthesize only 18:3[6,9,12], and in *Crithidia sp.*, in which 18:3[6,9,12] is a major component and 18:3[9,12,15] is totally absent. In this respect, *Crithidia* resembles the ciliated protozoa *Tetrahymena*.

Since the studies of Yorke et al. (29), it has been noted by many investigators that macromolecules, especially those supplied by serum, are an important factor for maintaining or culturing the parasitic flagellates in vitro. As has often been the case (30,31), this ostensibly macromolecular requirement may eventually be resolved as a necessity for fatty acids (provided as lipoproteins, or bound to albumin). Lipids, including stearic acid, have been reported to be essential for growth of *T. cruzi* (32, 33). It seems likely, however, that this requirement is not absolute since Tobie and Rees (34) have grown *T. cruzi* in dialysis culture under conditions where stearic acid would not be available to the cells.[5] It seems more probable to us that the parasitic flagellates synthesize only very small amounts of polyunsaturated fatty acids and that the concentrations of these acids in the cell may vary with conditions of growth. Thus, the 20- and 22-carbon fatty acids comprised only 3.6% of the fatty acids of *T. lewisi* grown on blood–agar at room temperature but accounted for 20% of the fatty acids of cells parasitizing the rat (Table 4). Similarly, the fatty acid composition of *L. tarentolae* was profoundly influenced by the addition of arachidonic acid to the growth medium and even, apparently, by the presence of serum albumin (Table 2).

It is tempting to speculate that the very low concentration of the ω6 fatty acids in *Leishmania* and trypanosomes is related to certain salient features of their physiology and to their parasitic mode of life. Greenblatt and Glaser (20) have reported that profound leakage of *L. enriettii* occurs at temperatures above 30°. A role for the ω6 group of polyunsaturated fatty acids has been postulated in membrane permeability (35).

In contrast to *Leishmania* and *Trypanosoma*, *Crithidia*, which contains large amounts of ω6 fatty acids (especially

[5] Unpublished observation, C. L. Greenblatt, D. Nelson, and P. Glaser.

if 18:3[6,9,12] is included) is very easy to grow in culture, and, indeed, grows in the gut of the host animal, a less controlled environment than that of the intracellular and blood parasites. A defective membrane would lead to great difficulties for a free-living organism, but might be a useful adaptation to parasitism. Increased membrane permeability would make the metabolic products of the host more readily available to the parasite, while the constancy of the environment would eliminate the osmotic stress to which a free-living cell is exposed.

*Manuscript received June 10, 1964; accepted August 3, 1964.*

## References


1. Mead, J. F. *Federation Proc.* **20:** 952, 1961.
2. Thomasson, H. J. *Intern. Z. Vitaminforsch.* **25:** 62, 1953.
3. Bernhard, K., and R. Schoenheimer. *J. Biol. Chem.* **133:** 707, 1940.
4. Shorland, F. B. In *Comparative Biochemistry*, edited by M. Florkin and H. S. Mason. Academic Press, Inc., New York, 1962, Vol. 3, p. 1.
5. Korn, E. D. *J. Biol. Chem.* **239:** 396, 1964.
6. Korn, E.D. *Biochem. Biophys. Research Commun.* **14:** 1, 1964.
7. Korn, E. D. *J. Lipid Res.* **5:** 352, 1964.
8. Korn, E. D. *J. Biol. Chem.* **238:** 3584, 1963.
9. Korn, E.D., and C. L. Greenblatt. *Science* **142:** 1301, 1963.
10. Erwin, J., and K. Bloch. *J. Biol. Chem.* **238:** 1618, 1963.
11. Erwin, J., and K. Bloch. *Biochem. Z.* **338:** 496, 1963.
12. Erwin, J., and K. Bloch. *Science* **143:** 1006, 1964.
13. Erwin, J., A. D. Hulanicka, and K. Bloch. *Comp. Biochem. Physiol.* **12:** 191, 1964.
14. Davidoff, F., and E. D. Korn. *J. Biol. Chem.* **238:** 3199, 1963.
15. Davidoff, F., and E. D. Korn. *J. Biol. Chem.* **238:** 3210, 1963.
16. Stumpf, P. K. *Nature* **194:** 1158, 1962.
17. James, A. T. *Biochim. Biophys. Acta* **70:** 9, 1963.
18. Daniel, J. W., and H. P. Rusch. *J. Gen. Microbiol.* **25:** 47, 1961.
19. Daniel, J. W., K. L. Babcock, A. H. Sievert, and H. P. Rusch. *J. Bacteriol.* **86:** 324, 1963.
20. Greenblatt, C. L., and P. Glaser. *Exptl. Parasitol.*, in press.
21. Trager, W. *J. Protozool.* **4:** 269, 1957.
22. Mangold, H. K. *J. Am. Oil Chemists' Soc.* **38:** 708, 1961.
23. Karmen, A., L. Giuffrida, and R. L. Bowman. *J. Lipid Res.* **3:** 44, 1962.
24. Rudloff, E. von. *Can. J. Chem.* **34:** 1413, 1956.
25. Stafford, H. A. In *Comparative Biochemistry*, edited by M. Florkin and H. S. Mason. Academic Press, Inc., New York, 1960, Vol. 1, p. xvii.
26. Goodman, D. S. *Science* **125:** 1296, 1957.
27. von Brand, T. *Rev. Inst. Med. Trop. Sao Paulo* **4:** 53, 1962.
28. Haines, T. H., S. Aaronson, J. Gellerman, and H. Schlenk. *Nature* **194:** 1282, 1962.
29. Yorke, W., A. R. D. Adams, and F. Murgatroyd. *Ann. Trop. Med. Parasitol.* **23:** 501, 1929.
30. Ham, R. G. *Science* **140:** 802, 1963.
31. Oyama, V. I., H. G. Steinman, and H. Eagle. *J. Bacteriol.* **65:** 609, 1953.
32. Boné, G. J., and G. Parent. *J. Gen. Microbiol.* **31:** 261, 1963.
33. Citri, N., and N. Grossowicz. *Nature* **173:** 1100, 1954.
34. Tobie, E. J., and C. W. Rees. *J. Parasitol.* **34:** 162, 1948.
35. Ramalingaswami, V., and H. M. Sinclair. *Brit. J. Dermatol.* **65:** 1, 1953.