The physiology of Leishmania

by

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Members of the genus Leishmania are like most, if not all, trypanosomids essentially aerobic organisms, but endowed with the faculty of enduring for certain periods experimentally imposed anoxic conditions. In nature they probably do not encounter severe oxygen deficiencies either in the intermediate host, or in the final host, the probability being that at least in the latter the parasites have as ready access to oxygen as the host cells. Nevertheless a word of caution may be indicated. The oxygen supply in the deeper layers of skin lesions, for example, may differ materially from those characteristic of normal tissues; experimental determinations would seem desirable and feasible.

Despite their aerobic type of life all members of the genus must be classified as aerobic fermenters, that is, even in the presence of ample oxygen they excrete organic acids, indicating incomplete oxidation of food material. This is known since Chang's (6) and Fulton and Joyner's (13) papers with the newer data of Crowther et al. (11) and Chatterjee and Ghosh (9) supplementing the older findings. It was thus shown that Leishmania donovani forms aerobically lactic and succinic acids, as well as acetic and pyruvic acids, while anaerobically only the excretion of lactic and succinic acids has been found. These endproducts are undoubtedly derived from the utilization of exogenous carbohydrates. There is no indication that leishmanias store any larger amount of a genuine reserve carbohydrate such as glycogen. In fact, the only polysaccharide so far described from Leishmania donovani yielded upon acid hydrolysis glucose, galactose and arabinose (Chatterjee and Ghosh, 9). It may be a structural

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polysaccharide or allied to the antigenic polysaccharide described for *Trypanosoma cruzi* by Gonçalves and Yamaha (15).

The spectrum of utilizable exogenous carbohydrates, on the other hand, is fairly broad. *Leishmania enrietti*, for instance, shows a definitely increased rate of oxygen consumption when glucose, fructose, mannose, galactose, maltose, sucrose or raffinose are available as substrate (Zeledón, 32) (Table 1.). In view of the apparent ready utilization of some disaccharides, it is not surprising that Chatterjee and Ghosh (9) detected a sucrase in *Leishmania donovani*. They found the enzyme to be of a purely hydrolytic type with an optimal pH of 7.1 to 8.0, a Km of $5 \times 10^{-3}$M and a $Q_{10}$ of approximately 2 with full activity maintained up to a temperature of 44°C.

It is interesting to note that the spectrum of carbohydrates available to *Leishmania* is broader than that reported utilized by some trypanosomids, such as *Trypanosoma lewisi* (Riley, 21). On the other hand glycerol which is readily consumed by *Trypanosoma rhodesiense*, in fact preferentially to glucose (Riley, 23), has been shown by quantitative chemical procedure to be utilized only in small amounts by the human leishmanias (von Brand et al., 4). The oxygen consumption of *Leishmania enrietti*, however, is definitely stimulated in the presence of glycerol (Zeledón, 32). Such observations suggest rather profound differences in permeability, or perhaps in the transport mechanisms between various trypanosomids.

The pathways by which the endoproducts of carbohydrate metabolism are formed have hardly been investigated in *Leishmania* as yet. In view of what is known from other protozoa, it may be reasonable to assume that the initial sequence is the conventional Embden-Meyerhof scheme. But so far only a single enzyme, hexokinase, has been described from the flagellates under consideration. Chatterjee et al. (10) showed that cell-free extracts of *Leishmania donovani* rapidly phosphorylated glucose, fructose, mannose, galactose, and d-glucosamine, with only very weak activity against ribose and glycerol. Apparently only a single enzyme is involved in hexose phosphorylation, as the mutual inhibition exerted by the various sugars indicates. The enzyme seems not to be dependent markedly on functional SH-groups. It should incidentally be noted that the weak activity against ribose does not necessarily indicate it is not being used. Indeed, Medina et al. (20) showed a very pronounced increase in oxygen consumption of *Leishmania brasiliensis* under the influence of this sugar.

The terminal sequence of carbohydrate utilization has not yet been studied in detail for *Leishmania*. In fact not a single relevant enzyme has been characterized, but the occurrence of isocitric dehydrogenase activity has been demonstrated (Ghosh and Chatterjee, 14). It has been shown (von Brand and Agosín, 3, Zeledón, 33) (Table 2) that at an appropriately low pH the organisms are able to utilize practically all intermediates of the Krebs cycle. It has furthermore been found that malonate inhibits succinate oxidation by *Leishmania brasiliensis* (Medina et al., 20) and that fluoroacetate inhibits the respiration of *Leishmania enrietti* (Zeledón, 34). These observations may indicate the existence of a
### TABLE 1

**Influence of some carbohydrates on the oxygen consumption of leishmanias.**

*Oxygen consumption expressed in percent of endogenous rate.*

<table>
<thead>
<tr>
<th>Species</th>
<th>Glucose</th>
<th>Fructose</th>
<th>Galactose</th>
<th>Mannose</th>
<th>Rhamnose</th>
<th>Maltose</th>
<th>Cellobiose</th>
<th>Sucrose</th>
<th>Melibiose</th>
<th>Raffinose</th>
<th>Glycerol</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leishmania donovani</em></td>
<td>340</td>
<td>440</td>
<td>186</td>
<td>296</td>
<td>116</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fulton and Joyner (13)</td>
</tr>
<tr>
<td><em>Leishmania enrietti</em></td>
<td>718</td>
<td>590</td>
<td>418</td>
<td>781</td>
<td>148</td>
<td>246</td>
<td>152</td>
<td>600</td>
<td>127</td>
<td>781</td>
<td>409</td>
<td>Zeledón (32)</td>
</tr>
</tbody>
</table>

### TABLE 2

**Influence of some Krebs cycle intermediates on the oxygen consumption of leishmanias.**

*Oxygen consumption expressed in percent of endogenous rate.*

<table>
<thead>
<tr>
<th>Species</th>
<th>pH</th>
<th>Pyruvate</th>
<th>Citrate</th>
<th>α-Ketoglutarate</th>
<th>Succinate</th>
<th>Fumarate</th>
<th>Malate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leishmania tropica</em></td>
<td>5.6</td>
<td>100</td>
<td>159</td>
<td>162</td>
<td>177</td>
<td>136</td>
<td>180</td>
</tr>
<tr>
<td><em>Leishmania enrietti</em></td>
<td>5.0</td>
<td>125</td>
<td>90</td>
<td>125</td>
<td>107</td>
<td>191</td>
<td>171</td>
</tr>
</tbody>
</table>
functional tricarboxylic acid cycle, but the available evidence is not strong enough to represent a final proof. It should be emphasized that the demonstration of substrate utilization, or the demonstration of characteristic enzymes, does not necessarily prove the presence of a full cycle, since enzymes usually ascribed to a functional Krebs cycle actually can be used for the synthesis of amino acids or other processes having no connection with the terminal respiration. To prove the presence of a Krebs cycle, it must be demonstrated that the reactions occur as an orderly sequence in accordance with the reactions of the cycle.

It must furthermore be recalled that *Leishmania* excretes under aerobic conditions succinic acid, a typical intermediate of the cycle. It is clear that the reactions of the tricarboxylic acid cycle would soon come to a complete standstill unless oxaloacetic acid, the motor of the sequence, were resynthesized by an auxiliary reaction and re-fed into the sequence. It is possible, granting that *Leishmania* may possess a Krebs cycle, that as in *Trypanosoma cruzi* (Bowman et al., 2) aerobic carbon dioxide fixation is involved. The problem has, however, not yet been studied for *Leishmania*.

Some interesting data on the lipid chemistry of *Leishmania* have become available in recent years. The question what type of sterol occurs in the flagellates is at present difficult to answer categorically. Hafevy (17) described ergosterol from *Leishmania tropica*, while Williamson (28) found cholesterol in *Leishmania donovani*. It was present as free cholesterol and in the form of rather numerous unidentified cholesterol esters (Williamson and Ginger, 30). In view of similar contradictory findings concerning the nature of the sterol deposited in, or formed by members of the genus *Trypanosoma* caution in evaluating the significance of the above reports is indicated. I am inclined to agree with the assumption expressed most recently by Williamson and Ginger (30) and Threlfall et al. (27) that the type of medium used in securing the flagellates largely determines the nature of the sterol deposited. It is assumed that in blood-containing media the cholesterol of the flagellates is directly derived from the sterol of the medium, while ergosterol encountered in the flagellates more likely was synthesized by the organisms. However, no critical experiments concerning the biosynthesis of sterols have been done as yet.

There is little indication that leishmanias utilize fatty acids for energetic purposes. The only suggestive observation has been reported by Medina et al. (20) who found that the respiration of *Leishmania brasiliensis* is about double the endogenous rate when acetic acid is offered as substrate.

Although no detailed information is as yet available concerning the various lipids occurring in leishmanias, relatively detailed data on the fatty acids of two species have been published recently by Korn and Greenblatt (18) and Korn et al. (19). Their analyses performed with the help of modern gas-chromatographic procedures emphasize the complexity of the fatty acids. In *Leishmania tarentola*, for instance, 21 fatty acids with chain length longer than C11 have been found. The nature and the relative amounts of the acids varied somewhat with the medium on which the flagellates had been developed. It is especially
significant that the organisms did contain polyunsaturated higher fatty acids when grown on Trager's C medium which does not contain them as ingredients, evidently indicating that the flagellates had synthesized them. While in this case the precursors of the acids are not known in detail, further experiments by the same authors proved that absorption and interconversion of higher fatty acids occur.

It was thus found that radio-active stearic acid added to the medium was taken up by the flagellates and transformed into 4 different unsaturated $C_{18}$, 3 unsaturated $C_{20}$, and 2 unsaturated $C_{22}$ acids. Of special significance is that in the case of *Leishmania tarentolae* one of the $C_{18}$ acids was unsaturated at the 6, 9, and 12 positions, because *Leishmania enrietti* appeared unable to synthesize this particular acid, indicating the possible existence of species differences in biosynthetic abilities.

Of great interest is also that the octadecatrienoic acid formed by *Leishmania enrietti* is $\alpha$-linolenic acid (KORN and GREENBLATT, 18) since this acid previously was believed to be characteristic for photosynthetic organisms only. In context with the fact mentioned previously that leishmanias may be able to synthesize ergosterol, another frequent constituent of plants, the finding of $\alpha$-linolenic acid may have phylogenetic significance, a point discussed by the above authors.

Stearic acid is not the only higher fatty acid available to *Leishmania tarentolae*. KORN et al. (19) have shown that the flagellate is able to readily absorb arachidonic acid and to transform it into polyunsaturated $C_{22}$ acids.

One more point related to fatty acid synthesis deserves mentioning. KORN and GREENBLATT (18) recovered significant amounts of radioactivity from stearaldehyde, stearic, oleic, linoleic, and $\alpha$-linolenic acids isolated from the lipids of *Leishmania enrietti*, when the latter was grown in the presence of radioactive stearate. However, no radioactivity was found in any acid with less than 18 C atoms, although the organisms also contain acids varying in chain length between $C_{10}$ and $C_{18}$. This finding is interpreted as indicating synthesis of unsaturated $C_{18}$ acids and stearaldehyde from stearic acid without previous degradation of the latter and without reutilization of 2-carbon units.

The nucleic acids of *Leishmania* have received but little attention up to the present time. It has been established by caesium chloride density gradient centrifugation that the base composition of the deoxyribonucleic acid of *Leishmania tarentolae* corresponds to 54 mole percent of guanine plus cytosine, a value well in the range of those found in other zooflagellates, but sharply differing from those reported for ciliates and rhizopods (SCHILDKRAUT et al., 25). Subsequently Du Buy et al. (12) established by means of a similar technique that the DNA extracted from *Leishmania enrietti* could be resolved into two bands. The major one had a guanosine plus cytosine content of 57 percent, the minor one of 36 percent. It was established that the DNA of the kinetoplast contained essentially only the minor component, with apparent little if any of the major component present. The latter was referrable to the DNA of the nucleus, but it could not be established with certainty whether or not the latter also contained some of the minor component. In view of the facts mentioned above concerning the sterols
and the nature of the linolenic acid stored by *Leishmania* it deserves to be men­tioned that the DNA of the chloroplast-containing algae *Chlamydomonas rein­hardi*, *Chlorella ellipsoïdes*, and *Euglena gracilis* contain major and minor bands of DNA quite similar to those described for *Leishmania*.

Compounds usually associated with the nucleic acids (guanosine, uracil, hypoxanthin, and ribose), in addition to phosphate, were found by GREENBLATT and GLASER (16) to leak out rapidly from *Leishmania enrietti* when the flagellar was maintained on a simple medium at elevated temperature, that is, above 30°C instead of the usual 22-23°C. It is probable that this leakage of metabolically significant compounds is due to a general increase in hydrolytic activity at higher temperatures, conceivably due to an activation of lysosome enzymes. It should especially be noted that this leakage is not limited to, but only greatly enhanced, by the elevated temperature. In the range 10 to 40°C leakage increased about 30 fold, while respiration only doubled (Fig. 1).

It should be noted incidentally that elevation of temperature is not the sole mechanism by which pronounced leakage can be induced. GHOSH and CHATTERJEE (14) found the antibiotic nystatin, as well as the detergent CTAB to induce in *Leishmania donovani* heavy leakage of materials absorbing at 260 mμ and free amino acids, but also of proteins and nucleic acids. They interpret this as indicating profound alteration in permeability of the cell membrane.

The chemical constitution of the proteins of *Leishmania* has not yet been studied, but it can be inferred from the nature of the 13 amino acids leaking from *Leishmania enrietti*, (GREENBLATT and GLASER, 16) (Table 3), that they probably contain the usual array of amino acids. This is also indicated by the fact that TRAGER (26) found it neccessary to add 17 amino acids to his defined

| Amino acids leaked by Leishmania enrietti, after GREENBLATT and GLASER (16). |
|---------------------------------|-----------------|
| Aspartic acid                  | Valine          |
| Threonine                      | Leucine         |
| Serine                         | Isoleucine      |
| Glutamic acid                  | Tyrosine        |
| Glycine                        | Arginine        |
| Alanine                        | Lysine          |
| Phenylalanine                  |                 |
medium used in the cultivation of Leishmania tarentolae. Most of them were shown to be essential ingredients of the medium. It should be recalled at this point that Williamson and Desowitz (29) did not find really significant differences in amino acids in the case of 10 trypanosomids studied. It is, however, very likely that the leishmanias can metabolize proteins. It has been known for a long time, since Salle and Schmidt's (24) studies, that ammonia accumulates in cultures of Leishmania tropica. A turnover of amino acids is also indicated by the fact that Leishmania donovani (Chatterjee and Ghosh, 7) as well as Leishmania enrietti (Zeledón, 35) contain active transaminase systems. It appears that the former parasite has a broader range of amino group donors than the latter both in the a-ketoglutaric → glutamic acid system, as in the pyruvic acid → alanine system.

The last topic to be considered briefly concerns the respiratory activities of Leishmania. Their endogenous respiration is apparently relatively low, but sufficient endogenous reserves are available to maintain the flagellates viable and motile for several hours as we have repeatedly observed in my laboratory. In the presence of a utilizable substrate the rate of oxygen consumption, or the rate of anaerobic CO₂ production can rise rather sharply. With glucose as substrate, for instance, the oxygen consumption of Leishmania donovani and L. enrietti is raised approximately 3 to 8 times above the endogenous rate (Fulton and Joyner, 13; Zeledón, 31; Chatterjee and Ghosh, 9) while the anaerobic carbon dioxide production rose even more (Ghosh and Chatterjee, 14) (Fig. 2). Of course, many substrates besides glucose have been tested; reliable data will be found in the papers by Fulton and Joyner, (13); Medina et al. (20), and Zeledón (32, 33).

The aerobic respiration of various Leishmania species is strongly inhibited by cyanide (von Brand and Johnson, 5; Zeledón, 35) while the data for azide are somewhat contradictory. Leishmania enrietti appears not to be susceptible to azide inhibition (Zeledón, 35), while some inhibition has been reported from Leishmania donovani (Fulton and Joyner, 13). While it does seem likely that heavy metal catalysis characterizes the respiration of the flagellates, one can, at the present time, not be certain that they possess a functional cytochrome system. The only positive finding reported is the occurrence of cytochrome oxidase in cell-free extracts of Leishmania donovani (Ghosh and Chatterjee, 14). This, of course, is insufficient evidence for the assumption of a complete conventional cytochrome system. It is well to remember that the cytochrome picture is complicated in the genus Trypanosoma. T. cruzi thus contains an incomplete system (Baernstein and Tobie, 1), T. lewisi, a complete one (Ryley, 21, 22), while in T. rhodesiense the bloodstream form contains no cytochromes at all, but the culture form does (Ryley, 23).

In view of the fact that the leishmanias alternate in their life cycle between Leishman-Donovan bodies, and motile leptomonad forms, the question should be raised whether the metabolism of these stages differs to a great extent. Little information on this point is available. All the data summarized so far are derived from studies with the leptomonads, for the simple reason that they are easily
cultivated, while the isolation of Leishman-Donovan bodies is a difficult and time-consuming task. The only comparative study is due to Fulton and Joyner (13). They found in some respects, such as the ability to consume oxygen or sugar, general similarities between both stages, but also definite differences. The oxygen consumption of the leptomonad stage was thus more susceptible to cyanide and amidine inhibition than that of the Leishman-Donovan bodies. It also appeared probable that the latter contain relatively more oxidizable reserve substances than the former. This seems indicated by the relatively high endogenous respiration of the Leishman-Donovan bodies and by the fact that their respiration is percentage wise less stimulated by glucose than that of the leptomonads (Fulton and Joyner, 13). A more detailed comparative study of both the chemical composition and the metabolism of the main stages in the life cycles of Leishmania would appear to be one of the most pressing problems for the future. But of no less importance is the elucidation of the many unsolved problems already indicated in my presentation for the motile stages and a detailed comparison of the metabolic activities of various species of Leishmania. The comparative physiologist and biochemist has a field here before him that in the future should open up more and more.

SUMMARY

The metabolism of all members of the genus Leishmania is characterized by aerobic fermentations, which lead to the excretion of various partly oxidized metabolic endproducts. The flagellates utilize several carbohydrates freely, but hardly anything is known about the intermediate carbohydrate metabolism. At suitably low pH they are able to consume intermediates of the Krebs cycle, but the presence of a functional tricarboxylic acid cycle has not yet been demonstrated conclusively. Recent data on lipid metabolism and biosynthesis have revealed interesting parallels to phytoflagellates, as have newer studies on nucleic acids. The composition of the proteins of Leishmania is essentially unknown, but the fact that under certain experimental conditions numerous amino acids are leaked into the medium, indicates that probably all common amino acids are present. The respiration of the flagellates is probably characterized by heavy metal catalysis, but it is uncertain whether or not they contain a fully functional cytochrome system.

RESUMEN

El metabolismo de los organismos del género Leishmania, se caracteriza por presentar fermentaciones aeróbicas con la excreción de productos finales parcialmente oxidados. Las formas flageladas utilizan fácilmente varios carbohidratos pero se sabe poco de su metabolismo intermedio. A un pH bajo son capaces de utilizar sustancias intermedias del ciclo de Krebs pero la presencia de un ciclo funcional en estos flagelados no ha sido demostrada en forma definitiva. Da-
tos recientes sobre síntesis y metabolismo lipídico y sobre ácidos nucleicos, han demostrado un paralelismo interesante entre estos flagelados y aquellos que poseen clorofila. La composición protéifica de las leishmanias es prácticamente desconocida; sin embargo, bajo ciertas condiciones, se obtiene la salida de la célula de numerosos aminoácidos, sugiriendo que los aminoácidos más comunes forman parte de las proteínas. La respiración es probablemente catalizada por metales pesados pero no se sabe con certeza si poseen un sistema funcional completo de tipo citocromo.

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31. **Zeledón, R.**

32. **Zeledón, R.**

33. **Zeledón, R.**

34. **Zeledón, R.**

35. **Zeledón, R.**
Fig. 1. Influence of temperature on leakage and respiration of *Leishmania enrietti*, after Greenblatt and Glaser (16).

Fig. 2. Stimulation of endogenous oxygen consumption of *Leishmania donovani* by glucose, after Chatterjee and Ghosh (9).
A linear plot of the slopes of respiration and leakage, both set at 1.0 at 10°C.

Effect of glucose addition on the anaerobic metabolism of L. donovani.
- Suspensions only: × - × suspensions plus glucose