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Autor: Janovy, J.
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School of Life Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, USA

Problems in the comparative physiology of some trypanosomatid flagellates[1]

J. JANOVY jr.

Summary

There is considerable evidence that trypanosomatid species vary in their metabolic characteristics, that they vary in the mechanisms by which they control these characteristics, and that a single species may vary metabolically without varying structurally. Studies to date from a number of laboratories also indicate there is still reason to believe that metabolic characteristics of trypanosomatid flagellates, as manifest in culture, are at least correlated (in some cases) with the behavior of the flagellate species in the metazoan host. It appears that our major tasks for the next several years are 1. to discover the extent to which these correlations are manifestations of characteristics required for life in the metazoan host, 2. to discover the extent to which these correlations are manifestations of characteristics which determine infection site within the metazoan host, 3. to discover which if any metabolic control mechanisms contribute to the multiplicity of clinical infections often seen in this group of protozoa, and 4. to discover the critical links between energy metabolism, the control of that metabolism, and life inside a host or host cell.

Key words: Leishmania, Herpetomonas, Crithidia, Trypanosomatidae, infectivity, virulence, macrophage, lysosome.

Culture forms of trypanosomatid flagellates are no different from other eukaryotic cell types available in culture, in the sense that they do provide the worker with easily-grown material for physiological, biochemical and structural

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Correspondence: John Janovy jr., School of Life Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 68588, USA

studies. However, these cells are able to perform one major function which separates them qualitatively from many other eukaryotic cells, viz., they are able to invade a host animal, often a specific cell within that animal, multiply within the host or host cell, and may produce a species-dependent course of infection (Schnur et al., 1973; Trager, 1974). The morphological form of the parasite within the host or host cell may differ markedly from that in culture. Furthermore, congeneric organisms within the family may be virtually indistinguishable, even at the ultrastructural level, while at the same time producing very different clinical infections in the same host species (see Gardener, 1974, especially Fig. 21; Janovy, Daggett and Lee, 1974). Although physiological and biochemical differences between life cycle stages of a single trypanosomatid species have long been recognized (von Brand, 1966), recent work has shown "infectivity" to be a function which not only varies with life cycle stage (Keithly, 1976), but which can also be manipulated by culture techniques to produce relatively infective or relatively non-infective organisms within a single life cycle stage (Giannini, 1974, 1974a). Thus, although the trypanosomatids are, as eukaryotic cells, vulnerable to any of the wide variety of detailed physiological and biochemical studies typically performed by the scientific community, these studies must rightfully be placed within the context of the overall biology of the protozoans. That context includes the ability to form a lasting association with a host, or host cell, usually of another phylum.

A useful approach to the problem of context has been to perform comparative studies of related organisms which produce predictably different types of infections in the mammalian host. Table 1 gives a short list of attributes which have been shown to vary within a single genus, and in some cases between strains of a single species, although it should be noted that to date not one of these attributes has been shown to be *the* one responsible for infectivity, virulence, or differences in clinical types of infection.

Recent work from this laboratory suggests that not only should comparative functional studies be continued, but that physiological control mechanisms within these organisms should also be examined. We have concentrated on two species of the genus *Leishmania*, *L. donovani* which produces a generally fatal visceral infection in humans and animal models, and *L. mexicana* which produces a self-limiting cutaneous infection in humans. One major difference in the environments of these two species is the local temperature. In addition, the events of transmission involve a major thermal shock in which the parasites are exposed to homeotherm body temperatures following multiplication in an insect. Consequently, some of our work has concerned the effects of homeotherm body temperatures on certain physiological features of the two parasite species. Generally it has been demonstrated that the species differ in their temperature of maximum respiratory activity, in their temperature of minimum stimulation of respiratory activity by exogenous glucose, and in the temperature at which their glucose consumption ceases to be stimulated by anaerobiasis (Janovy,

Table 1. Some attributes or characteristics demonstrated to vary with *Leishmania* species, isolates, strains or growth phases

Attribute	Reference
Oxygen consumption rates	Janovy and Poorman, 1969; Simpson, 1968; Zeledón and de Monge, 1967
Growth rates in culture	Zeledón and de Monge, 1967
Extent of respiratory stimulation by exogenous carbohydrates	Zeledón and de Monge, 1967
Growth rates in vivo	Stauber, 1966
Specific activity of a number of enzymes	Janovy, 1972
Nutritional requirements	Simpson, 1968
Infectivity and virulence	Stauber, 1966; Giannini, 1974, 1974a
Antigenicity	Simpson, 1968; Maekelt, 1972
High molecular weight materials released into incubation medium	Schnur et al., 1972; Decker and Janovy, 1974
Substrate affinities of homologous enzymes	Poorman and Janovy, 1969
Thermal inactivation of homologous enzymes	Janovy, 1972
Clinical symptoms in the human	Maekelt, 1972
Range of susceptible host species	Stauber, 1963
Malate dehydrogenase isozyme patterns	Gardener et al., 1974
Pellicular microtubule arrangements	Janovy, Daggett and Lee, 1974
Control of CO_2 fixation	Janovy, Greiner and Decker, 1976
Presence and activity of cytochromes	Krassner, 1966
Glucose utilization rates	Mukkada et al., 1974

Greiner and Decker, in press; Janovy and Poorman, 1969; Poorman and Janovy, 1969). However, an even more intriguing difference between the species is also manifest under homeotherm body temperatures. In work now in press (Janovy, Greiner and Decker, in press) it has been shown that in *L. donovani,* exogenous glucose stimulates CO_2 fixation while in *L. mexicana* exogenous glucose inhibits CO_2 fixation, as measured manometrically over the 25–37° C range. One immediately wonders the extent to which metabolic control mechanisms contribute to the tissue localizations exhibited by the two species.

A major contextual tool has also been recently provided, for comparative physiological studies, by the work of Giannini (1974, 1974a), who demonstrated that in *Leishmania donovani* exponential and stationary phase culture flagellates have different infectivities. It appears from this work that the organisms acquire infectivity as they enter the stationary phase of culture growth. Thus the flagellates may differentiate functionally without differentiating structurally. Preliminary studies in our laboratory, conducted according to the methods of Janovy, Lee and Brumbaugh (1974), show no ultrastructural differences be-

tween exponential and stationary phase organisms, except in the proportion of "degenerating" forms. It remains to be discovered which physiological attributes of this species are acquired during stationary phase and which of those that are acquired are responsible for or contribute materially to the enhanced infectivity.

If one considers the trypanosomatid flagellates a group of similar species, exhibiting general *kinds* of metabolic features (e.g. aerobic-anaerobic shifts) but perhaps specific manifestations of those features, then the works of Marr (1974) and Mukkada et al. (1974) combine in an interesting way with the above mentioned work of Giannini (1974, 1974a). Both laboratories have made the point that certain trypanosomatid flagellates exhibit delayed glucose utilization in culture. According to their observations, neither *Crithidia fasciculata* nor *Leishmania tropica* utilize exogenous glucose to any great extent until quite late in the exponential or early in the stationary growth phase. Preliminary work in our laboratory indicates glucose utilization in *Leishmania donovani* follows a similar pattern. However, in the case of *Leishmania donovani,* culture medium glucose concentration, as measured by the Glucostat reaction with deproteinized medium, goes virtually to zero at least 24 h prior to the onset of true stationary culture phase. Culture-dilution assays of viability (Janovy and Poorman, 1969) indicate stationary phase organisms are as capable of initiating a subculture as are exponential phase organisms, and thus are as "viable". We have not yet reconciled this observation with the ultrastructural results mentioned above. With their source of exogenous glucose depleted, stationary phase organisms should be in a different functional state than exponential phase flagellates. One might conclude from Giannini's (1974, 1974a) work that such a functional state would prepare the fagellate for survival in a mammalian macrophage.

A contextual framework within which to place comparative physiological studies can often be enlarged through the use of models, and recent studies in this laboratory have explored differentiations and parasite-host cell relationships of some model (i.e. not medically important!) trypanosomatids. For example, Daggett and Decker (1973) demonstrated that a virulence series exists, within the so-called "lower" trypanosomatid species, and that within that series there exists an additional series of species of increasing ability to lengthen their survival in a mammalian host. The series consists of *Leishmania donovani, Crithidia harmosa, Herpetomonas megaseliae, Herpetomonas samuelpessoai, Herpetomonas muscarum, Crithidia luciliae, Leptomonas costoris,* and *Blastocrithidia culicis. Leishmania donovani,* 2S strain, survives readily in CF 1 mice for two weeks and multiplies in that host. *Crithidia harmosa, H. megaseliae* and *H. samuelpessoai* survive one to six days in mice and with a proper adaptation regimen can double the survival time. *Herpetomonas muscarum* and *Crithidia luciliae* can survive 24 h in mice. The remainder cannot survive 24 h. Lest one think that the observed survival of these species in the mouse is too far outside

the realm of trypanosomatid reality, it should be pointed out that at least two of the species, *Herpetomonas megaseliae* and *Crithidia harmosa* actually undergo morphological transformations within host macrophages and assume an intracellular amastigote form ultrastructurally indistinguishable from that of *Leishmania donovani* (see Janovy, Daggett and Lee, 1974). Thus within the family *Trypanosomatidae* a series exists not unlike the mutant series which have proven so fruitful in attempts to dissect the genome and order the series of genetic expressions of some other microorganisms. One would like to think we are now in a position to use this trypanosomatid series to correlate certain physiological characteristics with infectivity and adaptive ability.

To date we have studied only one of the above lower trypanosomatid species physiologically, but results suggest that this species, *Herpetomonas megaseliae,* exhibits metabolic shift similar to those already being investigated in the more virulent forms (Janovy et al., 1975). Glycolytic activity decreases with onset of stationary phase, anaerobic stimulation of glucose uptake increases with onset of stationary phase, and cyanide sensitivity increases with the onset of stationary phase. The shift to aerobiasis is accompanied by some of the same kinds of ultrastructural changes, e.g. in mitochondrial morphology (Janovy, Lee and Brumbaugh, 1974), that accompany parallel metabolic shifts in *Leishmania donovani. Herpetomonas megaseliae* does have one characteristic, however, which makes it a particularly attractive experimental animal. It undergoes an easily-assayed one-step structural differentiation in which the kinetoplast and flagellar base migrate to the posterior end of the cell. Thus, unlike many of the trypanosomatids now being studied, particularly members of the genera *Leishmania* and *Crithidia,* the differentiation state of the animals can quickly be determined from a stained smear. Our present studies, conducted primarily by Mr. Stephen Knight, concern the very basic characteristics of differentiation in the animal and while they are not directly in the area of metabolic control, nevertheless point to certain metabolic questions which can ultimately be answered using model organisms such as *H. megaseliae.* For example, *H. megaseliae* must structurally de-differentiate prior to multiplication (Janovy et al., 1975) and multiplication of the two DNA-containing organelles, the nucleus and kinetoplast, can be separated by use of inhibitors (Knight, 1976). Differentiation, however, i.e. the posterior migration of the kinetoplast, is not dependent upon the structural integrity of the mitochondrion, as shown by ultrastructural studies of acriflavin-treated organisms. The recent demonstrations of metabolic shifts accompanying entry into stationary growth phases in certain *Trypanosomatidae,* of relatively high virulence as a feature of stationary phase organisms, and of variations in enzymatic activities associated with growth phases (Marr, 1974; Mukkada et al., 1974) all point to a continuing need for comparative studies of the metabolic changes which accompany the life of these protozoa in culture. Dyskinetoplastic trypanosomatids have been shown to differ metabolically from their parent stocks (Hill et al., 1968), thus *H.*

megaseliae provides a model in which structurally differentiated but metabolically dissimilar flagellate preparations, from the same parent stock, can theoretically be studied. Such studies will aid our understanding of not only the relationship between metabolic state and infectivity, but of the organismic-level role of the kinetoplast, specifically its contribution, if any, to the metabolic state of a stationary phase, differentiated, trypanosomatid.

In addition to the above problems, questions and tools, one must consider the evidence that some trypanosomatid species can resist the actions of host cell lysosomes. The works of Alexander and Vickerman (1975) as well as that of the Rockefeller group (Chang and Dwyer, 1976) suggest the parasites are resistant to host cell lysosomal enzymes, since electron microscopy reveals that host cell secondary lysosomes do fuse with parasitophorous vacuoles and parasites multiply within these vacuoles. The most obvious, evolutionarily economical, and straightforward conclusions from these observations are 1. that the parasite surface is itself resistant to lysosomal enzymes, and 2. that parasite products either directly inactivate the lysosomal enzymes or alter the intravacuolar environment so as to render them inactive. In either case, future studies of trypanosomatid metabolism must take these conclusions into account. The relationships between energy-yielding pathways and synthetic pathways responsible for surface coat integrity must be established. In addition, at some point the effects of parasite metabolic products, including the by-products of energy metabolism, upon host cell lysosomal enzymes in situ must be determined. Many studies of a variety of trypanosomatid flagellates have shown that a large array of molecular species is released from these organisms in vitro and that the total package of these products varies according to species, according to available exogenous substrates, and with the presence of anti-leishmanial drugs (Decker and Janovy, 1974; Schnur et al., 1972). Evidence reported in Bhattacharya and Janovy (1975) suggests that at least some of these materials also are released in vivo and incorporated by host cells. Should it eventually be shown that parasite metabolic products alter the intravacuolar environment so as to inhibit host cell lysosomal enzymes, then comparative metabolic studies will indeed show why some trypanosomatid species can establish an infection while others cannot. Researchers in this area should be forewarned: in an age where nothing in biological research is simple, it might be like kissing one's sister to discover that a trypanosomatid species is infective because of some fortuitous (albeit genetically determined!) composition of its waste products package. In any event, the infectivity series mentioned above cannot help but play a significant role in these future studies.

Alternatively, should the trypanosomatid surface coat be shown necessary for survival within a host phagocytic cell, i.e. it is resistant to lysosomal enzymes, then the metabolic support of this coat must be explored if for no other reason than as a potential site of chemotherapeutic action. To date, several trypanosomatid species have been reported to have lectin-binding surface coats (e.g.

Dwyer, 1974). The turnover rates of coat components, species-specific glycoproteins, and adjustments in coat components with differentiation and transitions between life cycle stages, must all be determined. Again, a series of trypanosomatid species, of varying infectivity, should be considered a powerful tool for use in these comparative studies.

Finally, the comparison of physiological attributes of "adapted" and "nonadapted" trypanosomatid stocks may reveal functional characteristics typical of relatively invasive organisms. For example, there have been several attempts to cultivate, or experimentally produce, the amastigote stage of *Leishmania donovani.* Some of these attempts have been partially successful (Lemma and Schiller, 1964) but for some reason the cultivation techniques have not been widely applied. Recently a possibly more fruitful approach has been reported by Gillig and Honigberg (1973), who attempted "adaptation" of two *Leishmania donovani* strains to culture growth at 37° C. The 3S strain, usually considered relatively infective, adapted to 37° C while the less infective Khartoum strain did not. These observations must take their place in the long list of ancillary observations on physiological or biochemical differences between organisms of different infective properties. However, unlike many previous studies, they do suggest research in which *L. donovani* can be a useful model organism. Studies of membrane composition, surface coat properties, and metabolic pathways of the two strains will inevitably reveal differences between adaptable and non-adaptable stocks of the *same* species. Furthermore, the model is particularly attractive in that its ability to establish the lasting relationship with the host can easily be determined (Keithly, 1976).

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1 Alexander J., Vickerman K.: Fusion of host cell secondary lysosomes with the parasitophorous vacuoles of *Leishmania mexicana*-infected macrophages. J. Protozool. *22,* 502–508 (1975).
2 Bhattacharya A., Janovy J. jr.: *Leishmania donovani*: Autoradiographic evidence for molecular exchanges between parasites and host cells. Exp. Parasit. *37,* 353–360 (1975).
3 Chang K.-P., Dwyer D. M.: Multiplication of a human parasite (*Leishmania donovani*) in phagolysosomes of hamster macrophages *in vitro.* Science *193,* 678–680 (1976).
4 Daggett P.-M., Decker J. E.: Insect trypanosomatids: alteration of secretion with increased survival in a mammalian host. J. Protozool. *20,* 512 (1973).
5 Decker Joan E., Janovy J. jr.: *Leishmania donovani* and *Leishmania mexicana*: Production of the excretion factor. Comp. Biochem. Physiol. *49B,* 513–523 (1974).
6 Dwyer D. M.: Lectin binding saccharides on a parasitic protozoan. Science *184,* 471–473 (1974).
7 Gardener P. J.: Pellicle-associated structures in the amastigote stage of *Trypanosoma cruzi* and *Leishmania* species. Ann. trop. Med. Parasit. *68,* 167–176 (1974).
8 Gardener P. J., Chance M. L., Peters W.: Biochemical taxonomy of *Leishmania* II. Electrophoretic variation of malate dehydrogenase. Ann. trop. Med. Parasit. *68,* 317–325 (1974).
9 Giannini S. M.: *Leishmania donovani* promastigotes infectivity for hamsters is correlated with frequency of subculture. J. Protozool. *21,* 421 (1974).

10 Giannini S. M.: Effects of promastigote growth phase, frequency of subculture, and host age on promastigote-initiated infections with *Leishmania donovani* in the golden hamster. J. Protozool. *21*, 521–527 (1974a).
10a Gillig C. J., Honigberg B. M.: Adaptation of *Leishmania donovani* promastigotes to cultivation at 37°. J. Protozool. *20*, 504 (1973).
11 Hill G. C., Brown C. A., Clark M. V.: Structure and function of mitochondria in *Crithidia fasciculata*. J. Protozool. *15*, 102–109 (1968).
12 Janovy J. jr.: Temperature and metabolism in *Leishmania*. III. Some dehydrogenases of *L. donovani*, *L. mexicana* and *L. tarentolae*. Exp. Parasit. *32*, 196–205 (1972).
13 Janovy J. jr., Daggett P.-M., Knight S. A., Gunderson J.: Differentiation of *Herpetomonas megaseliae*: Population and physiological changes. Proc. Oklahoma Acad. Sci. *55*, 130–135 (1975).
14 Janovy J. jr., Daggett P. M., Lee K. W.: *Herpetomonas megaseliae*: Architectural rearrangements during amastigote formation. J. Parasit. *64*, 716–718 (1974).
15 Janovy J. jr., Greiner E. C., Decker J. E.: Temperature and metabolism in *Leishmania*. IV. The effects of anaerobiasis. Exp. Parasit. (in press).
16 Janovy J. jr., Poorman A. E.: Temperature and metabolism in *Leishmania*. I. Respiration in *L. donovani*, *L. mexicana* and *L. tarentolae*. Exp. Parasit. *25*, 276–282 (1969).
17 Janovy J. jr., Lee K. W., Brumbaugh J. A.: Differentiation of *Herpetomonas megaseliae:* Ultrastructural observations. J. Protozool. *21*, 53–59 (1974).
18 Keithly J.: Infectivity of *Leishmania donovani* amastigotes and promastigotes for golden hamsters. J. Protozool. *23*, 244–245 (1976).
19 Knight S. A.: *Herpetomonas megaseliae*: Effects of hydroxyurea on morphology and growth. J. Parasit. *62*, 512–522 (1976).
20 Krassner S. M.: Cytochromes, lactic dehydrogenase and transformation in *Leishmania*. J. Protozool. *13*, 286–290 (1966).
21 Lemma A., Schiller E. L.: Extracellular cultivation of the leishmanial bodies of a species belonging to the protozoan genus *Leishmania*. Exp. Parasit. *15*, 503–513 (1964).
22 Maekelt G. A.: Immune responses to intracellular parasites. I. Leishmania. In: Immunity to parasitic animals ed. by E. J. L. Soulsby, p. 425. Academic Press, New York 1972.
23 Marr J. J.: Regulation of aerobic fermentation in protozoans. III. Apparent unimportance of pyruvate kinase in carbohydrate energy metabolism. Comp. Biochem. Physiol. *49B*, 531–545 (1974).
24 Mukkada A. J., Schaefer F. W. III, Simon M. W., Neu C.: Delayed *in vitro* utilization of glucose by *Leishmania tropica* promastigotes. J. Protozool. *21*, 393–397 (1974).
25 Poorman A. E., Janovy J. jr.: Temperature and metabolism in *Leishmania*. II. Aldolase in *L. adleri*, *L. donovani*, *L. mexicana*, and *L. tarentolae*. Exp. Parasit. *26*, 329–335 (1969).
26 Schnur L. F., Zuckerman A., Greenblatt C. L.: Leishmanial serotypes as distinguished by gel diffusion of factors excreted *in vitro* and *in vivo*. Israel med. J. *8*, 932–942 (1972).
27 Schnur L. F., Zuckerman A., Montilio B.: Dissemination of leishmanias to the organs of Syrian hamsters following intrasplenic inoculation of promastigotes. Exp. Parasit. *34*, 432–447 (1973).
28 Simpson L.: The leishmania-leptomonad transformation of *Leishmania donovani:* nutritional requirements, respiration changes and antigenic changes. J. Protozool *15*, 201–207 (1968).
29 Stauber L. A.: Immunity to leishmania. Ann. N. Y. Acad. Sci. *113*, 409–417 (1963).
30 Stauber L. A.: Characterization of strains of *Leishmania donovani*. Exp. Parasit. *18*, 1–11 (1966).
31 Trager W.: Some aspects of intracellular parasitism. Science *183*, 269–273 (1974).
32 von Brand T.: Biochemistry of parasites. Academic Press, New York 1966.
33 Zeledón R., de Monge E.: Physiological studies on the culture form of four strains of *Leishmania brasiliensis*. I. Nitrogen content, substrate utilization, and effect of metabolic inhibitors on respiration and its relation to infectivity. J. Parasit. *53*, 937–945 (1967).