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Entamoeba histolytica-like Amoebae Occurring in Man

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Six Entamoeba histolytica-*like amoebae of the Laredo-type are compared with classical* E. histolytica *strains with regard to morphology, temperature-tolerance, response to hypotonic solutions, antigenic make-up, pathogenicity, sensitivity to drugs, and biochemistry. Sharp discontinuities are shown to exist between the 2 groups in some parameters, sufficient, under certain conditions, to justify creation of a new species designation for the Laredo-type amoebae. Nevertheless, this action is not recommended at the present time because of the small number of "aberrant" strains so far isolated, and because of the limited comparative studies so far carried out.*

Entamoeba moshkovskii *is compared with the Laredo-type* E. histolytica. *No consistent distinction can be made between the 2 groups except for the sources of the strains—the former is free-living and the latter parasitic. It is therefore suggested that* E. moshkovskii *may actually be a parasite of the Laredo type, only incidentally encountered in free-living environments because of its wide temperature and tonicity tolerances. Since, here too, only limited studies are so far available, it is suggested that the term "* moshkovskii-*like* E. histolytica *" be used until sufficient data are accumulated to assign these amoebae to a more precise location in the taxonomic scheme.*

In 1956, Dr F. H. Connel became aware that a strain of *Entamoeba histolytica* he had isolated (the "Laredo" strain) possessed the unusual capability of living and multiplying at room temperature as well as at body temperature. Between 1964 and 1966 5 more such isolations from human faeces were reported (Nelson & Jones, 1964 (no strain name); Entner & Most, 1965 (JA and AG strains); Richards, Goldman & Cannon, 1966 (strains 403 and Huff). It is the purpose of this review to attempt to answer several questions. In what ways and by how much do these "aberrant" amoebae differ from classical *E. histolytica*? Do the differences justify raising these strains to the status of a separate species? What is the relationship of these strains to *Entamoeba moshkovskii*,[2] the free-living amoeba which has identical morphology to *Entamoeba histolytica*?

DIFFERENCES BETWEEN LAREDO-TYPE AND CLASSICAL *E. HISTOLYTICA* [3]

Temperature tolerance

Table 1 shows that, whereas the range of temperature under which *E. histolytica* trophozoites can survive in certain specified conditions is 20°C–43°C, the range for Laredo-type amoebae is 0°C–41°C (Siddiqui, 1963). The minimum temperature for growth of *E. histolytica* in continuous culture is around 30°C (Cabrera, 1958; Cabrera & Porter, 1958) whereas for Laredo-type amoebae it is 10°C (Richards, Goldman & Cannon, 1966). The normal or optimum temperature for *E. histolytica* is around 37°C, but is 25°C–30°C for the Laredo-type strains. As results of this nature have now been replicated by several different workers over a period of more than

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[2] My thoughts on the status of *E. moshkovskii* have benefited from an exchange of views with Dr R. A. Neal but I assume sole responsibility for the opinions expressed.

[3] Very few, if any, of the studies to be cited are precisely comparable in respect of culture media, experimental techniques or strains used. Nevertheless, there is, by and large, an impressive similarity in results obtained in different laboratories. For this reason techniques will be cited only incidentally in the following analyses except when they appear to have had a significant influence on results.

TABLE 1

TEMPERATURE PREFERENCES OF CLASSICAL AND LAREDO-TYPE *E. HISTOLYTICA*

Reference	Range of temperature tolerated or which supported proliferation [a]		Normal or optimum temperature [a]		Remarks
	E. histolytica	Laredo-type	*E. histolytica*	Laredo-type	
Cabrera (1958)	29°C-42.5°C	ND	37°C	ND	Temperature shifted gradually above and below optimum
Cabrera & Porter (1958)	31.7°C-41.3°C	ND	37°C	ND	Temperature shifted in a single step above and below optimum
Dreyer (1961)	ND	10°-35°C	ND	ND	Clone culture tested
Siddiqui (1963)	20°C-43°C	0°C-41°C	37°C	30°C	Amoebae kept at given temperature 72 h and subcultured back at optimum temperature
Nelson & Jones (1964)	37°C	" room temperature "	37°C	" room temperature "	Limits of temperature tolerance not studied
Entner & Most (1965)	37°C-43°C	20°C-<43°C	37°C	37°C	
Rosas & Najarian (1965)	ND	22°C-40°C	ND	30°C	
Albach, Schaffer & Watson (1966)	ND	No higher than 32°C	37°C	25°C	
Richards, Goldman & Cannon (1966)	>27°C-35°C	5°C-35°C	35°C	27°C	Temperatures shifted gradually and in single steps

[a] ND = not done.

a decade, there can be no doubt that these differences in temperature tolerance represent stable, valid, and rather simple characteristics for distinguishing classical *E. histolytica* from Laredo-type amoebae.

Morphology and response to hypotonic solutions

The morphology of Laredo-type amoebae is known only for cultured organisms since in no instance have they been recognized as different from classical *E. histolytica* before being grown in the laboratory. Goldman, Gleason & Carver (1962) and Entner & Most (1965) have called attention to the fact that nuclear chromatin in the Laredo JA and AG strains is sometimes distributed unevenly in the form of one or more masses applied to the nuclear membrane. On the other hand, in a detailed study of size and nuclear morphology in a classical strain of *E. histolytica* (K9) and in a Laredo-type strain (Huff), before the latter was recognized for what it was, no significant differences in size or morphology were noted (Gleason, Goldman & Carver, 1963). In view of the fact that at least 4 of the 6 known strains of Laredo-type amoebae (Laredo, Huff, 403 and Nelson's strain) were initially carried in the laboratories of experienced workers as if they were typical *E. histolytica* strains, and since undoubted classical *E. histolytica* also sometimes shows uneven massing of nuclear chromatin on the nuclear membrane (Goldman, Gleason & Carver, 1962), it may be concluded that at present there are no consistent morphological criteria that can differentiate *E. histolytica* trophozoites from the Laredo-type amoebae in culture.

Cultured Laredo-type trophozoites were capable of completing cycles of division, encystment and excystment in extremely hypotonic solutions, such as culture medium diluted 1 : 64 with distilled water (Richards, Goldman & Cannon, 1966). Upon transfer to such solutions the amoebae were first immobile but typical motility was resumed within a few hours and functioning contractile vacuoles were developed. By contrast, *E. histolytica* did not survive even a 1 : 2 dilution of culture medium for any length of time (Richards, Goldman & Cannon, 1966; Gordeeva, 1966). Such results have been reported so far for 14 strains of *E. histolytica* and for 5 strains of Laredo-type amoebae. In an unpublished study conducted by myself and co-workers in India, some 50 more strains of *E. histolytica* have also failed to grow in diluted medium.

Thus, a simple culture characteristic, in addition to temperature tolerance, that serves to distinguish classical *E. histolytica* from Laredo-type amoebae is the reaction to hypotonic solutions. Although it is desirable that more strains be tested, this appears to be a stable and dependable differentiating characteristic.

Antigenic make-up

Analyses of the antigenic relationships between classical *E. histolytica* and Laredo-type amoebae have been carried out by the techniques of fluorescent antibody, precipitation in agar gel, and haemagglutination. Details are presented below.

Fluorescent antibody reactions. The fluorescence work of Goldman and co-workers in the period 1960–67 was based upon quantitative measurements of the fluorescence imparted to individual amoebae after exposing them to various fluorescein-labelled antisera. Fluorescence of amoebae was measured with specially designed microfluorimeters, and the brightness figures were taken as representing the degree to which antibody had been bound by antigens in the different amoebae studied. Details of methodology and controls must be sought in the original publications.

The Huff strain, which was recognized several years later as being a Laredo-type amoeba, reacted less intensely with anti-*histolytica* sera than 3 strains of classical *E. histolytica* (Goldman, Carver & Gleason, 1960). In experiments involving cross-absorption and cross-staining, fluorescence results were interpreted as indicating that Huff was deficient in some antigens present in a classical *E. histolytica* strain (Goldman & Gleason, 1962). The Huff strain was also compared with *E. histolytica* K9 with regard to its suitability as an antigen in a fluorescent antibody test for amoebiasis (Goldman, 1966). Huff antigen yielded positive results in only 33% of proven acute cases, compared with 87.5% positives obtained with K9 antigen. In addition, the serum titres obtained with Huff organisms were lower than with K9.

An early comparison of the type strain itself, Laredo, with K9 showed differences in antigenicity with 5 anti-*histolytica* sera when Laredo was grown at 25°C (Goldman et al., 1962). With one antiserum a clear-cut difference was also apparent when the amoebae were cultured at 37°C.

In the most complete study thus far, 5 Laredo-type strains (Laredo, Huff, JA, AG and 403) were compared with 8 strains of classical *E. histolytica*, all 13 strains being grown at 35°C (Goldman & Cannon, 1967). The fluorimetric results indicated a distinct superiority in the ability of the *E. histolytica* group to react with the test reagents—a human antiserum derived from a patient with amoebic liver abscess, and a rabbit antiserum prepared against classical *E. histolytica*.

Finally, Siddiqui & Balamuth (1965) reported that an anti-*histolytica* serum stained the corresponding *E. histolytica* strain somewhat brighter than the Laredo strain (2+ *versus* 1+).

Precipitation in agar gel. Krupp (1966) compared Huff and Laredo with 8 strains of classical *E. histolytica*. All strains were grown at 37°C in the same all-liquid medium with the same single bacterial associate. Extracts of amoebae were subjected to electrophoresis in relatively thick layers of agar and then allowed to react with anti-*histolytica* sera. *E. histolytica* antigens showed 3–8 precipitin bands, Huff showed 2–3 bands, while Laredo showed 3–4 bands: 1 or 2 bands were also obtained with the horse serum component of the culture medium.

Lunde & Diamond (1969) investigated 4 strains of *E. histolytica* growing at 35.5°C and the Laredo strain at 25°C, all from axenic cultures. As in Krupp's work, amoebic extracts were subjected to electrophoresis and allowed to react with anti-*histolytica* sera. At least 6–8 distinct bands were found in all the *E. histolytica* extracts, but only 2 were found in the Laredo antigen. Characteristically, a prominent slow-moving component present in all *E. histolytica* antigens was absent in Laredo.

Siddiqui & Balamuth (1965) obtained a weak reaction between anti-*histolytica* serum and Laredo antigen in double-diffusion experiments. The reaction with *E. histolytica* antigen was considerably stronger.

Haemagglutination. Only a single comparison has so far been carried out with this technique. Lunde & Diamond (1969) sensitized red blood cells with extracts of either axenic *E. histolytica* (35.5°C) or axenic Laredo amoebae (25°C). In testing a small group of human sera they obtained significantly lower haemagglutination titres with the Laredo antigens than with *E. histolytica*.

Summary of antigenic studies. All except 2 of the studies cited above suffer from 2 important deficiencies: no antiserum against Laredo-type amoebae was used to investigate cross-reactions from both directions, and no comparisons were run of Laredo-type organisms maintained at more than a single

temperature. Thus the precise extent of antigenic dissimilarity between classical and Laredo-type *E. histolytica* cannot yet be detailed. These lacunae in our knowledge, however, must not be allowed to distort the otherwise clear picture that has been emerging—that while the classical and Laredo-type organisms share certain antigenic configurations they are certainly not identical. On the contrary, 3 very different immunological techniques have all shown distinct antigenic differences between the 2 groups.

Unfortunately, there is no immediate likelihood of extensive antigenic studies with large numbers of strains to clarify relationships further. Studies using extracts of amoebae are almost impossible unless axenic or, at least, monoxenic cultures can be employed, but such cultures are available in only a limited number of laboratories. Fluorescent antibody methods can cope with crude cultures but the differences encountered thus far have been so subtle as to tax the most experienced eye, and thus microfluorimetric methods and equipment will probably continue to be necessary. Even fewer laboratories are equipped for such studies. Thus from the practical standpoint, rapid identification and differentiation of Laredo-type strains on the basis of antigenicity is not yet feasible.

Pathogenicity

Information concerning the clinical status of the individuals from whom the 6 known Laredo-type amoebae were isolated is presented in Table 2. In 3 instances (Huff, Nelson's strain, and 403) the original hosts are described unequivocally as asymptomatic or cyst-passing carriers. The clinical status of the other 3 hosts is more difficult to assess either because the original descriptions are incomplete (JA and AG), or because the patient's symptoms could be assigned as readily to the benign tumour from which he was suffering as to the amoebic infection (Laredo). At any rate, none of this latter group can be considered as showing clear-cut symptoms of amoebic invasion of tissue or of amoebic colitis. Beaver et al. (1956a) carried out experimental infections of human volunteers with the freshly isolated Huff strain. In 81 out of 130 attempts, infections were established, but no symptoms characteristic of clinical amoebiasis were found in any of the infected volunteers (Table 3). Thus, there is an over-all impression of benignity for these strains in the human host. This in itself, however, does not provide much of a distinction from classical *E. histolytica* since the latter are also frequently found in asymptomatic hosts living apparently as commensals in the lumen of the bowel.

There are 5 published accounts of attempts to infect experimental animals with 5 of the Laredo-type amoebae (all except Nelson's strain). Table 3 shows that in 4 of these experiments, involving rats, hamsters and guinea-pigs, only 12 infections were obtained out of a total of 245 animals inoculated. Furthermore, no signs of tissue invasion were seen in any instance. In contrast to this record of low infectivity and non-invasiveness is the report of Beaver et al. (1956a) which cites 17 infections out of 38 rats, guinea-pigs and dogs inoculated with the Huff strain. Of these 11 showed mild to severe

TABLE 2

CLINICAL STATUS OF ORIGINAL HOSTS OF LAREDO-TYPE STRAINS

Reference	Strain	Clinical description
Dreyer (1961); Goldman, Gleason & Carver, 1962) [a]	Laredo	History of diarrhoea for several years; weight loss; epigastric pain; polypoid tumour of transverse colon (adenomatous polyp) removed surgically, followed by complete recovery.
Beaver et al. (1956b)	"H" = Huff	Consistent cyst-passer without symptoms of amoebiasis for 2 years.
Entner & Most (1965)	AG	"... patient with a recurring case of mild, chronic amoebiasis which did not respond to drug therapy".
	JA	"... isolated in May 1963 from a young male in an institution for mental illness".
Nelson & Jones (1964)	No name	"... established ... from a cyst-bearing stool from a carrier".
Richards, Goldman & Cannon (1966)	403	Isolated from an asymptomatic carrier.

[a] From unpublished data supplied by F. H. Connel.

TABLE 3
INFECTIVITY AND VIRULENCE OF LAREDO-TYPE STRAINS IN EXPERIMENTAL HOSTS

Reference	Laredo-type strain used	Experimental host	Route of inoculation [a]	No. infected/ no. inoculated	Remarks
Beaver et al. (1956a)	"H" = Huff, cultured at 37°C	Rats	IC (t)	10/10	Mild lesions in 4, no lesions in 6 animals
		Guinea-pigs	IC (t)	5/18	Severe lesions in 1, mild in 4 animals
		Dogs	Oral (c)	2/10	Severe lesions in 1, mild in 1 animal
		Humans	Oral (c)	81/130	No symptoms in all 81 infections
Rosas & Najarian (1965)	Laredo, cultured at 22°C and 30°C	Rats Hamsters	IC (t) IH (t)	0/25 0/63	
Healy & Gleason (1966)	Huff and Laredo, cultured at 37°C	Rats	IC (t)	1/21	No lesions in the single infection
Goldman & Cannon (1967)	Huff, Laredo, AG, JA, 403 cultured at 35°C	Guinea-pigs	IC (t)	0/51	
Neal & Johnson (1968)	Huff, Laredo, AG, JA, 403 cultured at 25°C	Rats	IC (t)	11/85	No lesion found in any animals

[a] IC = intracaecal; IH = intrahepatic; t = trophozoite; c = cysts.

lesions of the intestinal tract. This difference in results of infectivity experiments is due, perhaps, to the fact that Beaver's work was performed with a relatively newly isolated strain, while the others dealt with strains maintained *in vitro* for a few to many years.

It seems fair to conclude from these limited reports that the Laredo-type organisms are of low or even no pathogenicity to the human host, and of low or restricted pathogenicity to the common laboratory animals that are susceptible to classical *E. histolytica*. Nevertheless, since there is ample evidence in the literature of the existence of *E. histolytica* strains that are equally non-virulent in experimental and human hosts, the factor of pathogenicity in experimental animals is of limited value in differentiating the 2 groups of amoebae. It is clear that more strains of Laredo-type organisms need to be studied as soon as they are isolated before we can be certain that complete non-pathogenicity characterizes the group as a whole.

Sensitivity to drugs

Entner and his co-workers have compared the sensitivity to drugs of 3 Laredo-type strains (Laredo, AG and JA) and 5 classical *E. histolytica* (Entner, Evans & Gonzalez, 1962; Entner & Most, 1965). Determinations were made on amoebae growing in all-liquid medium at a temperature of 37°C. The 3 Laredo-type strains were all more resistant than the other strains to the following degrees: emetine—10-fold; cycloheximide (Actidione)—20- to 50-fold; fumagillin—20- to 100-fold; carbarsone (N-carbamylarsanilic acid)—2-fold; and chlorbetamide (Mantomide)—2-fold.

Albach, Schaffer & Watson (1966) made similar determinations on the Laredo strain *versus* 4 classical *E. histolytica* strains. In this case, however, Laredo was tested at 25°C while the others were tested at 37°C. The results showed Laredo to be more resistant to emetine, about 30- to 50-fold, and to oxytetracycline (Terramycin), about 5-fold. Susceptibility to paromomycin (Humatin) was about the same as the classical *E. histolytica* strains.

Inasmuch as in these studies each type of amoeba demonstrated reasonable uniformity of susceptibility to drugs within their respective types, and a consistent difference in susceptibility between the 2 types, we may conclude that the differences represent real and characteristic attributes of the 2 types of amoebae. Unfortunately, however, drug testing of the precision shown in the studies cited above

requires special culture techniques to avoid the complications associated with indirect influences on bacteria associated with the amoebae. This imposes serious limitations on the possibility of screening large numbers of new strains in order to identify and characterize Laredo-type organisms.

Biochemistry

The Laredo, AG and JA strains all utilized only glucose, maltose, and galactose out of 11 sugars tested. In this respect they showed no differences from 5 strains of classical *E. histolytica* (Entner, Evans & Gonzalez, 1962; Entner & Most, 1965). On the other hand, analysis of the free amino-acid composition of Laredo compared with 3 strains of classical *E. histolytica* revealed quantitative differences in 6 of the amino-acids, alanine, serine and threonine being more abundant in Laredo, while leucine-isoleucine, arginine and valine were more abundant in the other amoebae (Albach & Shaffer, 1965). Differences were also found in filtrates of the medium in which the amoebae had grown for either 72 h at 37°C (classical strains) or for 6 days at room temperature (Laredo).

Nine strains of *E. histolytica* were compared with the Laredo and Huff strains with regard to the presence and character of the enzyme glucokinase (Reeves, Montalvo & Sillero, 1967). Each of the classical strains revealed the same 2 electrophoretically distinct isoenzymes, but Laredo and Huff showed only a single band comparable in its migration to the faster of the 2 isoenzymes.

The biochemical evaluation of Laredo-type strains is as yet extremely sparse. However, so far it appears that the Laredo group does indeed possess at least some biochemical attributes clearly distinct from those characterizing classical *E. histolytica* strains.

NOMENCLATURE OF LAREDO-TYPE AMOEBAE

It is fashionable, in discussions of the intestinal amoebae, to refer to the "*E. histolytica* complex" and to emphasize the fact that this species appears to include strains showing a continuous and wide spectrum of characteristics. For this reason, the first point to be made with regard to the Laredo-type organisms is that they do not represent simply one extreme of a continuous spectrum of classical *E. histolytica* strains; instead they demonstrate a clear discontinuity in several attributes from the strains comprising the classical *E. histolytica* group. This is exemplified most obviously with regard to temperature and tonicity tolerances, since there are no known strains of *E. histolytica* showing characteristics intermediate between the Laredo and classical types.

A second point is that the Laredo-type amoebae possess a cluster of common characteristics. That is, strains showing certain temperature tolerances also show certain tonicity tolerances, drug sensitivities, antigenic reactions, and biochemical characteristics. So far there are no known strains of classical *E. histolytica* (judged by temperature requirements) which show, for example, the resistance to drugs characteristic of Laredo.

The third point to consider is that in spite of morphological identity with the classical strains, Laredo-type strains are rather easily distinguishable in culture by the simple tests of temperature and tonicity tolerances. Thus, the practical needs of the taxonomist or diagnostician are not too difficult to meet, especially in comparison with the over-all difficulties of working with the intestinal amoebae.

It would appear then, from the above considerations, that good justification exists for acknowledging the Laredo-type amoebae as members of a species separate from *E. histolytica*. In fact, apart from the morphology of the mature cyst, there may already be more precisely known differences between the Laredo-type strains and *E. histolytica* than between the latter and *Entamoeba coli*. That morphological identity is not an overriding factor in retaining a single species designation when dealing with the intestinal amoebae is shown by the accepted use of separate species names to identify the morphologically similar amoebae isolated from man, snakes, frogs and other hosts.

When all the above is said, it is my opinion that, at present, a decisive reason for not setting up a new species to include the Laredo-type group is that too few strains have as yet been isolated, and too few laboratories have so far performed comparative studies. It is perfectly conceivable that strains may yet be located that will not show the sharp discontinuities we see at present; or it may be that, as studies are performed by new and different workers with new or modified techniques, distinctions may be found even among the classical *E. histolytica* strains of a type to parallel those shown by the Laredo-type. For these reasons I consider that the designation *E. histolytica* should be retained to include all strains showing the well-known morphology and isolated from human sources. The qualifying

term " Laredo-type " or something equivalent should be used to describe strains with characteristics similar to those of the Laredo strain of *E. histolytica*.

I think it important to emphasize that the subject of nomenclature should remain open and flexible. Should several more Laredo-type strains be isolated, and should they all continue to show the same family of attributes we now ascribe to the Laredo strain, the case for setting up a new species will be greatly enhanced. Emile Brumpt (1949) believed in the existence of a *histolytica*-like species which he called *Entamoeba dispar*. This species, he claimed, was identical in appearance to non-invasive *E. histolytica* (which he called *E. dysenteriae*), was never pathogenic in man, and was only slightly infective to animals but never invasive. We should be impressed by the fact that this is an accurate, if incomplete, description of the Laredo-type strains now known.

RELATIONSHIP OF *ENTAMOEBA MOSHKOVSKII* TO *E. HISTOLYTICA*

Entamoeba moshkovskii has been isolated in various parts of the world from sewage treatment plants and from streams receiving the effluent of sewage plants (see Neal, 1966, for bibliography). Tshalaia (1941), who first described the amoeba, recognized that, in spite of its similarity in appearance to *E. histolytica*, it possessed 2 distinctive characteristics: it multiplied at temperatures from 10°C to 37°C, and it survived exposure to hypotonic solutions in which it formed contractile vacuoles. Thus, the species was clearly distinct from any strains of *E. histolytica* known at the time.

With the isolation of Laredo-type strains directly from human sources, and the demonstration by Richards, Goldman & Cannon (1966) that such strains show the same temperature and tonicity tolerances as *E. moshkovskii*, it is clear that the status of the latter as a separate species should be re-examined.

Table 4 tabulates data concerning *E. moshkovskii* and various Laredo-type organisms. Unlike the situation with regard to the latter and classical *E. histolytica*, no clear-cut distinction can be made between strains of *E. moshkovskii* and the Laredo group. Both are entirely similar with regard to temperature and tonicity tolerance, and to the nature of the enzyme glucokinase that both possess. With regard to pathogenicity, no laboratory infections have been obtained with *E. moshkovskii*, and, except for Beaver's work discussed above, only a few isolated successes have been achieved with Laredo-type strains. Antigenic studies reveal similarities and dissimilarities consistent with the distribution of reactions among different strains of a single group (see in particular Goldman & Cannon, 1967). Thus, from the standpoint of known characteristics, there is less basis for separating *E. moshkovskii* from the Laredo group than for separating the latter from classical *E. histolytica*.

There remains the consideration that the amoeba called *E. moshkovskii* has always been isolated from the free-living state rather than directly from some animal host. However, as Neal (1953) has pointed out, the fact that he and others found the amoeba in crude sewage entering disposal plants implies that the organism was continually pouring in from outside sources. Furthermore, in Stewart & Beck's (1967) study of 67 species of protozoans, *E. moshkovskii* fell unequivocally into the parasitic group on the basis of the absence of nuclear DNA-histone. This is striking and unusual confirmation of the concept already voiced by de Carneri (1963), Neal (1966), and Goldman & Cannon (1967) that *E. moshkovskii* might actually be a parasitic species incidentally encountered in free-living environments in which it can survive because of a wide temperature and tonicity tolerance.

In my opinion, the most important reasons for not at present including *E. moshkovskii* under the umbrella designation of " Laredo-type *E. histolytica* " are that too few strains have been studied in sufficient detail, and that the *moshkovskii* group may turn out to be a conglomerate of parasitic forms with *E. histolytica*-like morphology whose hosts may possibly include other species besides man. On the basis of all the above considerations, I think the designation " *moshkovskii*-type *E. histolytica* " would accurately describe and place these particular organisms in the framework of our thinking about amoebae that look like *E. histolytica* and form quadrinucleated cysts. With the accumulation of more data, a more precise assignment of these amoebae in the taxonomic scheme should become possible.

POSTSCRIPT

Since this review was written in early 1968, 3 more papers have appeared which bear directly on the subject. Antigenic studies by Ali Khan & Meerovitch (1968), using the techniques of haemag-

TABLE 4

COMPARATIVE DATA FOR *E.MOSHKOVSKII* AND LAREDO-TYPE *E. HISTOLYTICA* IN VARIOUS PARAMETERS

Parameter	Reference [a]	Findings	
		E. moshkovskii [a]	Laredo-type *E. histolytica* [a]
Temperature at which multiplication occurs	Lachance (1963) reviews previous literature	10°C-37°C (several reports of inferior growth at 37°C)	
	Richards, Goldman & Cannon (1966)	ND	10°C-35°C
	Various authors (see Table 1)	ND	10°C-37°C
Response to hypotonic solutions	Neal (1953) reviews previous literature	Survival for several days with formation of contractile vacuoles	ND
	Gordeeva (1967)	Permanent cultures in 1:64 dilution of ordinary growth medium, with development of contractile vacuoles	ND
	Richards, Goldman & Cannon (1966)	ND	Complete life cycle in 1:64 dilution of ordinary growth medium, with development of contractile vacuoles
Antigenic relationships	Zaman (1960) (immobilization techniques)	No cross-reaction with *E. histolytica*; strong cross-reaction with *E. ranarum* from frogs	ND
	Goldman, Carver, Gleason (1960) (FA)	No specific reaction with a single anti-*histolytica* serum	Reduced specific reaction with 3 anti-*histolytica* sera
	Goldman, Gleason & Carver (1962) (FA)	Reduced specific reaction with 4 anti-*histolytica* sera; no specific reaction with same serum mentioned above	Specific reactions with 4 anti-*histolytica* sera; reduced specific reaction with single serum negative for *E. moshkovskii*
	Goldman & Cannon (1967) (FA)	Specific reactions with 2 anti-*histolytica* sera	Specific reactions with 2 anti-*histolytica* sera
	Siddiqui & Balamuth (1965)	Reduced reaction with anti-*histolytica* serum by FA, none by gel diffusion	Reduced reactions with anti-*histolytica* serum by both FA and gel diffusion
Pathogenicity	Neal (1967) reviews previous literature	No experimental infections obtained with kittens, guinea-pigs, rats, hamsters, or man	ND
	Goldman & Cannon (1967)	No infections obtained with guinea-pigs	No infections obtained with guinea-pigs
	Various authors (see Table 3)	ND	No infections or mild infections in laboratory animals and man; see text above for details
Biochemistry	Reeves, Montalvo & Sillero (1967)	Electrophoresis of glucokinase shows only one band, in contrast to two for classical *E. histolytica*	A single glucokinase band in same position as the one in *E. moshkovskii*
	Stewart & Beck (1967)	Negative for nuclear DNA-histone, like all other *parasitic* protozoans tested. All *free-living* protozoans were positive; 67 species tested	ND

[a] ND = not done; FA = Fluorescent antibody.

glutination and precipitation in gel, have confirmed the greater reactivity with anti-*E. histolytica* sera of 1 strain of *E. histolytica* over 2 Laredo-type strains and 1 of *E. moshkovskii*. Reeves & Bischoff (1968) studied electrophoretic mobilities of 5 enzymes derived from 10 strains of *E. histolytica*, 5 Laredo-type strains, and 2 of *E. moshkovskii*. All Laredo-type strains and *E. moshkovskii* were similar to each other and distinguishable from *E. histolytica* in 4 or 5 out of 8 characterized bands. De Carneri (1968) compared 3 strains of *E. histolytica*, 2 of Laredo-type and 1 of *E. moshkovskii* with regard to susceptibility to 8 drugs. In contrast to work reported earlier in this paper, variability within the *E. histolytica* group often encompassed that found in the other amoebae. This was probably due to the fact that each culture appears to have been grown with different bacterial associates, rendering these results of limited value for characterizing the amoebae. The over-all effect of these additional studies is, thus, to confirm the earlier findings reported in the body of this paper.

RÉSUMÉ

AMIBES SEMBLABLES À *ENTAMOEBA HISTOLYTICA* ISOLÉES CHEZ L'HOMME

L'auteur a comparé six amibes semblables à *Entamoeba histolytica*, du type Laredo, isolées chez l'homme, avec des souches classiques d'*E. histolytica* sous les rapports suivants: morphologie, tolérance aux températures, comportement en solutions hypotoniques, structure antigénique, pathogénicité, sensibilité aux médicaments et biochimie. Pour certains de ces caractères, il existe entre les deux groupes des différences marquées qui, dans certaines conditions, sont suffisantes pour justifier une nouvelle désignation d'espèce pour les amibes du type Laredo. Toutefois, cela n'est pas recommandé actuellement car les souches « aberrantes » qui ont été isolées jusqu'à présent sont trop peu nombreuses et les études comparatives qui ont été faites trop limitées.

On a également comparé *Entamoeba moshkovskii* avec *E. histolytica* du type Laredo. Il n'est pas possible de distinguer nettement ces deux groupes si ce n'est par l'origine des souches, les premières étant libres et les secondes parasites. Il se pourrait que *E. moshkovskii* soit en fait un parasite du type Laredo que l'on rencontrerait occasionnellement à l'état libre puisque cette amibe peut supporter des températures et des pressions osmotiques très variables. Cependant, cet aspect de la question n'a pas non plus été suffisamment étudié et l'on pourrait peut-être utiliser le terme « *E. histolytica* de type *moshkovskii* » jusqu'à ce que l'on ait réuni assez de données pour assigner à ces amibes une position taxonomique plus précise.

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