LEISHMANIA TROPICA IN ORIENTAL SORE
(Ultrastructure Study)

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Summary

Ultrastructure of Leishmania tropica was studied in 22 patients with oriental sore.

Seventy biopsies were taken from these patients. The whole structure and internal organelles of parasite were studied. When parasite enters the skin it is ingested inside phagocytes and by degeneration of phagocyte it is expelled.

The interesting points observed in our studies were : 1. Presence of ribosomes in flagellar structure (between flagellar fibers) 2. The complex of mitochondria and kinetoplast which have been well developed. 3. Presence of indented cup like stuctures which seem to be the part where the parasite is ingested. 4. Division of the parasite in multiple stages.

Cutaneous Leishmaniasis is an endemic disease in Iran. Since June 1971 we have studied the ultrastructure of Leishmania Tropica in the Department of Dermatology of Razi Medical School and Taj Pahlavi Cancer Institute, Tehran University.

History

In 1956, the ultra structure of Leishmania organism was first studied. Since then research in this field was carried on by many other workers. In 1966 Leishmania Mexicana (LM) was studied by Jadin and Creemers. [1,2,3,4] Sanyal, Chatterjee and Sen Gupta [5,6,7] studied the Human L. D. and also L. D. in cutaneous nodules. In 1969, Djaceznko[8] studied the flagellum form of L.D.

There are only two reports about the fine structure of L.T., the etiological agent of oriental sore. The first article was published by Pham[9] in 1970 and the second by Rondanelli[10] in 1971.

General Consideration

We studied 22 patients with cutaneous Leishmaniasis. Our total number of biopsies was 70. Out of these only 29 specimens were examined. The age of our patients varied between 4 and 65 years. Eleven were males and eleven females. Number of lesions in a single individual varied between 1 and 9 and the duration of lesions varied between 10 days and 9 months.

The diagnosis of oriental sore was established on the basis of smears and biopsies. Intradermal tests were not performed.

Material and Methods

Small pieces of skin were fixed by immersing in glutar aldehyde (GA) 3%

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and osmium tetroxide (OSO_4) 2% (PH : 7/2, 7/4). They were cut into 1 mm cubes and fixed in G.A. and OSO_4 2% for one hour at 4°C. The tissues were rapidly dehydrated in graded alcohols, embedded in Epon 812 and sectioned by porter Blum - Ultra Microtom - MT1 with a glass knife suitable for this section (300 - 500A°). They were mounted on specimen girde, stained with uranyl acetate for 10 minutes and for further 10 minutes with lead citrate. The specimens were examined with Siemens Elmescope A - 1 electron Microscope (220V, 180 KW).

Ultrastructure

The Leishmania bodies were seen in histiocytes near the nucleous and golgi bodies in low magnification (less than 15000). The host cell did not show any change in the early stages of the infection but gradually some microvesicles, microvacuoles and cytoplasmic homogenisation appeared. In some areas of the cytoplasm the mitochondrial crests disappeared eventually, the cell membrane ruptured and the parasite got released.

Morphology of Parasite

Leishmania tropica (L.T.) appears in oval or round shapes in longitudinal or transverse sections. Its length varies between 2.8 - 2.6 microns and its diameter is 2-1.2 micron. The elongated form may reach 4 microns[10].

1. *Periplast*: The L.T. cell membrane consists of two layers. The thickness of the outer layer which itself consists of two layers, is about 80-120A°. The outer membrane is similar to the outer membrane of host cell, but the inner one is like the membrane of a parasite (Protozoa). The inner layer's thickness is about 250 A^0 and consists of about 80-90 microtubules[10]. The LT has fewer microtubules than L.D. These microtubules are arranged linearly so as to form an inner membrane parallel to the exterior membrane and play a protective role. The distance between the two membranes varies. The microtubules around the flagellum are larger and we may ask whether the flagellum is in fact a developed microtubule. Periplast is an elastic organ and some authors believe that it is made from myonemlike fibres. Other investigators believe that it may play some role in the parasite's movement. However, others believe that the periplast is only a protective structure[11].

2. *Nucleus*: The spherical nucleus is often in the centre of the parasite and its diameter is about 0/8-1 micron. It has a double membrane with pores. The nucleus contains some dense chromatin particles and one or two nucleolii.

3. *Flagellum*: The intracellular flagellum in longitudinal sections appears as fibre like structures. Its average length is about 1/5 micron and its average diameter 0/2 micron. There are some thin fibrils in the flagella which are between 100-160 A° long. The flagellum is encircled by the flagellum pocket. In transverse section the structure is encircled by the F. P. The flagellum itself consists of a central pair of fibres surrounded by nine peripheral pairs. In each pair the fibres are together with a single fibre. Sometimes there are two flagella inside the FP. Inside the FP small protrusions may develop which sometimes contain particles of food material. The flagellum plays a major role in the movement of the parasite.

4. *Blepharoplast*: The basal body or Blepharoplast is below the flagellum in a transverse position. The blepharoplast has the same structure as the flagellum but lacks the central fibre pair and contains many ribosomes. The lower surface of the blepharoplast is in close proximity to the kinetoplast, but there is no actual contact.

5. *Kinetoplast*: Using an optical microscope the kinetoplast can be seen

as a black spot, but under an electron microscope it is seen to have a sausage-like shape with a double outer layer. Inside the kinetoplast there is a spiral consisting of thin fibres which themselves contain D. N. A. the length of this spiral being between 0/1 to 1/2 micron. Also within the kinetoplast there may be one or more mitochondrial crests. The kinetoplast and blepharoplast acting together through the flagellum, play a part in the movement of the Parasite.

6. *Mitochondria*: Inside L. T. we have observed a few mitochondria which appear as discs with two outer membranes their diameter averaging 0/5 micron. The inner membrane clear crests grow inwardly. In some sections the crest appear unconnected to the inner membrane. In one of the L. T. cases we have observed well developed kinetoplast and mictochondria.

7. *Golgi Body*: This structure is partialy developed in L. T and in the anterior portion of the parasite it appears as dark and dense fibre.

8. *Phagosome*: Phagosomes are digestive vacuoles and they appear in the region where phagocytosis is taking place.

9. *Lysosomes*: Lysosomes are particles within the parasite which may be either dispersed or clustered in groups. Their diameter is 0/5 micron and they are sometimes surrounded by a halo. Lysosome may be detected by special chemical reactions.

10. *Ribosomes*: L. T contains a large number of dark particles as ribosomes with diameters of about 150 A°. Groups of ribosomes are observed which are interconnected by R. N. A. messengers. Such groups are known as POLYSOMES. We have observed ribosomes within the flagellum.

11. *Vacuoles*: Within L. T we have observed vacuoles with various sizes, shapes and distributions. These vacuoles possibly play a role in the nutrition of the parasite.

12. *Cup like formation*: Pham[9] and et al have described a cup like formation at the lower pole of the parasite. They believe that pinocytosis takes place here. Neither Rondanelli[10] nor we have observed such a complete formation but we have observed several indentations in the anterior part of the parasite. We believe this is where pinocytosis takes place.

Discussion

We have studied the shape of the L. T by examining a large number of sections. (Figures 1-10). Although the question of the effect of the chemical reagents used in the preparation of the sections on the actual shape of the parasite remains unanswered, we may nevertheless draw the following conclusions.

After the parasite is innoculated through the skin it enters the histiocytes by phagocytosis. (Fig. 1). At a later stage the host cell is destroyed and the parasite is released. Some workers believe that there is a degree of adaption between the parasite and the host cell. Under the electron Microscope L. T has the same size as L. D. Measurements have been made by Chatterjee using a micro-meter eye-piece. In one of the medical publications it has been stated that no ribosomes have been observed in the flagellum but we have observed this.

We have also observed L. T in the process of division (Fig. 10). The division of L. T is probably by mitosis and we have seen the nucleus of L. T in the prophase stage. Jadin and Creemers have reported that in the non flagella form of L D the mitochondria is not fully developed; but we

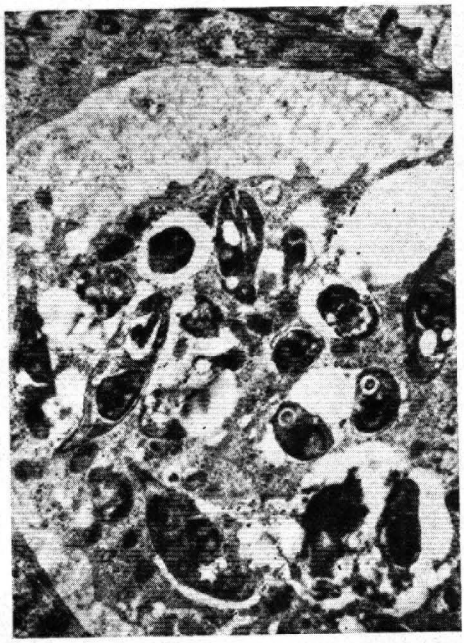

Fig. 1 A host cell, inside the protoplasm, large numbers of L.T. organisms can be seen. The degeneration of protoplasm is noticed.

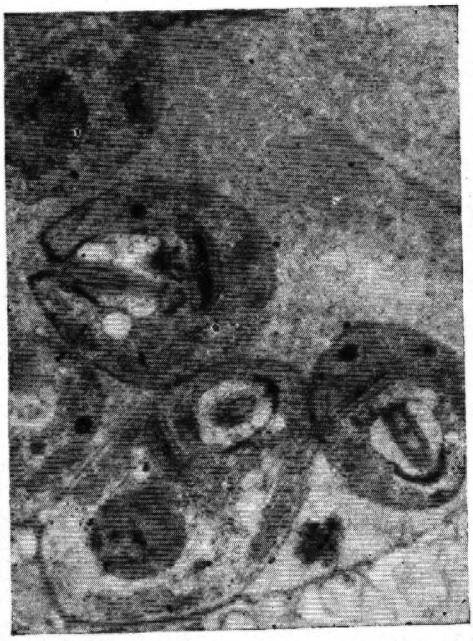

Fig. 2 Four LT : In the centre flagellum and flagellar pockets are noticed. The structure of Kinetoplast is noticed in three of LT.

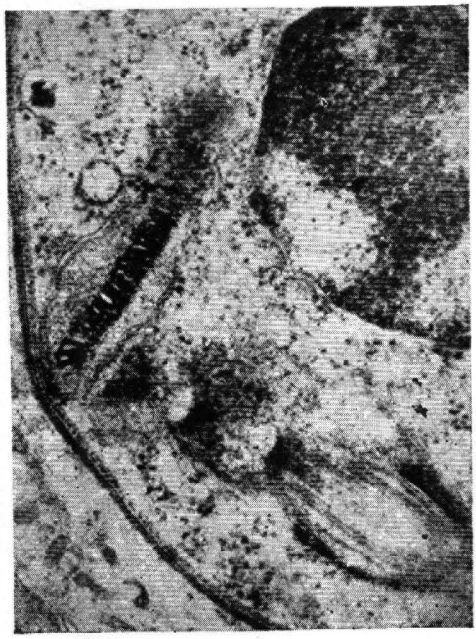

Fig. 3 Double membrane periplast. Note the large nucleus; a kinetoplast and intra cellular flagella.

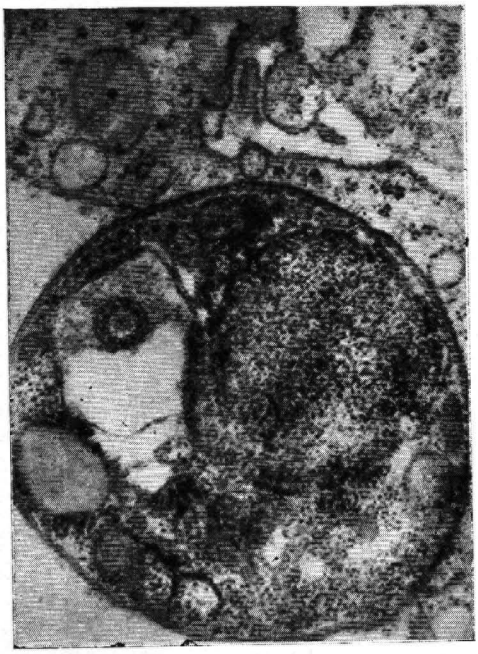

Fig. 4 A large nucleus and transverse section of a flagella. The nucleus is surrounded by a double porous membrane.

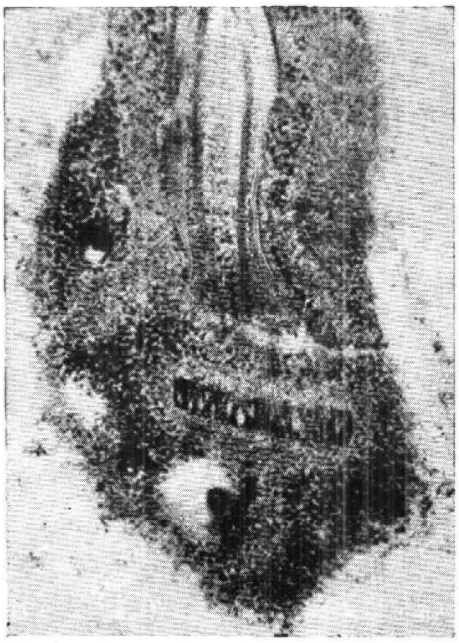

Fig. 5 A longitudinal section of a flagella and a kinetoplast. The dark section between flagellum and kinetoplast is the basal body or blepharoplast.

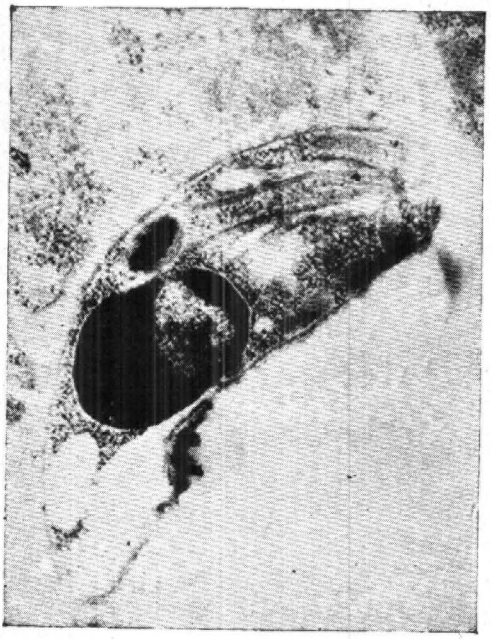

Fig. 7 The presence of ribosomes between flagellar fibres. Few free vacuoles are noticed.

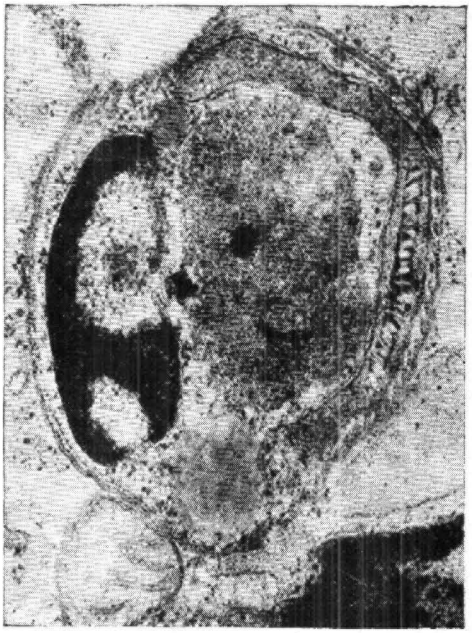

Fig. 6 The well developed mitochondia and kinetoplast complex.

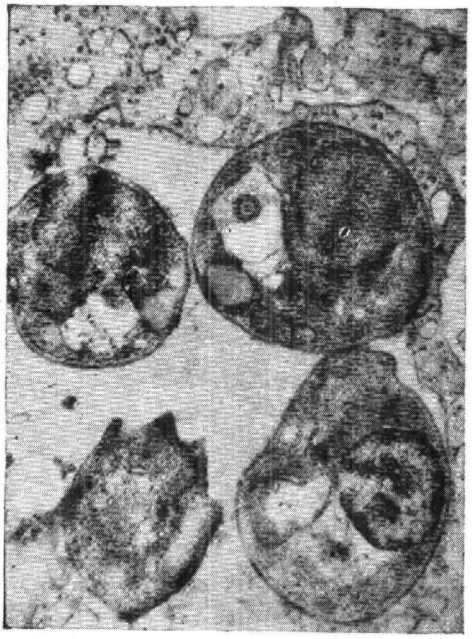

Fig. 8 Four LT. In one of them the indented cup like formation is noticed

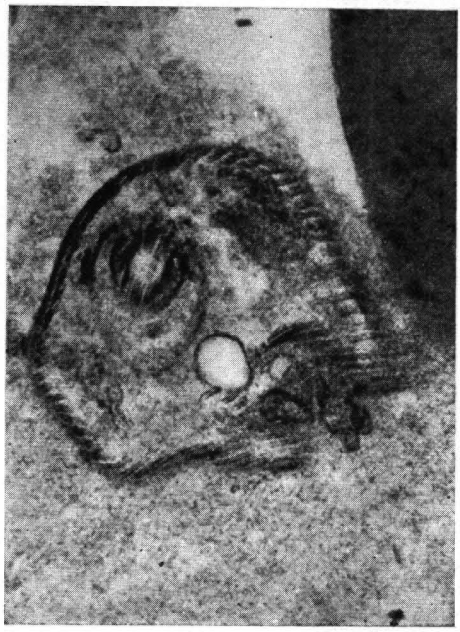

Fig. 9 A cup like formation with food particles. A vacuole is also present.

have seen a fully developed mitochondria and kinetoplast in one of our specimens (Fig. 6). In the case of the golgi body we have not seen a fully developed one. Pham has observed a cup like formation at the lower pole of the parasite but we have seen only a series of indentations in the anterior part (Fig. 8). There is no general agreement on the site of pinocytosis. Jadin[1,2,3] Creemers[4], and Aleman Cesa[12] believe that pinocytosis takes place in the FP flagellum packet. We intend to use radioactive tracers to locate the true site or sites of pinocytosis.

Finally, we present some distinguishing features of Leishmanias :

1. The size of L. T is the same as that of L. D. but both are smaller than L. M.
2. The number of perioplast microtubules in LT is less than in LD. The shape and position of these perioplast microtubules is different, and this may be related to the antigenicity (Manukian 1968).
3. The number of mitochondria in LT is greater than that in LD.
4. LT has no reticulum endothelial.
5. The site of pinocytosis cannot be used to identify different species of Leishmania.

However, these indicators are more related to acquired properties and ecological factors and are therefore not sufficient in themselves to identify different species of Leishmanias.

Acknowledgement

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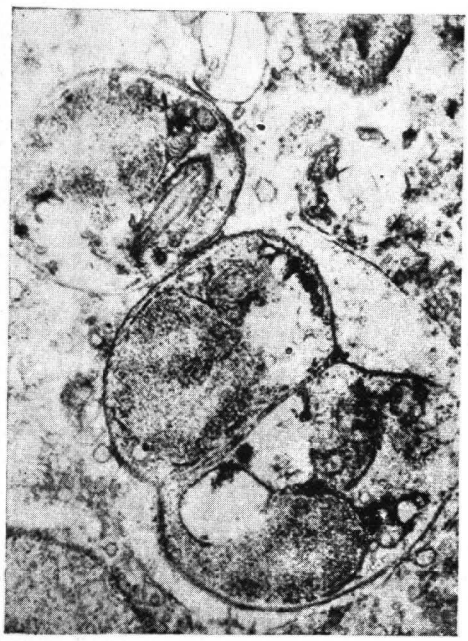

Fig. 10 A division of a parasite.

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TRUE or FALSE ?

Human epidermal growth factor (hEGF) and human β - urogastrone are the same molecule which possess seemingly unrelated biological activities.

(Answer page No. 102)