evident only after a very prolonged period of incubation (4–10 weeks). The strains were variable in their morphology and physiological reactions: these characters, being unstable, were not considered safe to use for the purpose of distinguishing types from different human subjects or clinical conditions.

The fact that the cocci and the diphtheroids took a very long time to grow in the primary haemocultures and grew rapidly in the subcultures, is compatible with our and other authors' previous data indicative of an evolution starting from MRU and CWD forms carried within the erythrocytes and platelets.

Our present data concerning the percentage of growths of cocci and diphtheroids within the haemocultures from subjects in various pathological situations, may be compared with the following data concerning blood cultures made in duplicate from 70 normal subjects in Birmingham (England) and 56 in Ancona (Italy) and incubated contemporaneously in the Department of Bacteriology of the University of Birmingham and in our own laboratory[1]: growth of cocci: 15%; growth of diphtheroids: 25%.

Such comparison indicates that, as regards the presence and evolution of the bacterial forms under consideration, no significant difference has been detected between normal subjects and subjects suffering from various types of pathological situations. It has to be noted that the cultures from patients suffering from rheumatoid arthritis behaved in a way opposite to that which one would have expected on the basis of other authors' points of view. The same may be said for 14 cases of neoplastic diseases, which in the table are distributed within groups 3, 5, 6, 7: 1 growth of *P. mirabilis*, 1 of *E. coli* and 1 only of a diphtheroid have been observed.

From the results here described, it may be concluded that the presence within the circulating blood of CWD bacterial forms evoluting in the haemocultures towards conventional forms of cocci and diphtheroids, seems not to be particularly related to any of the states of illness which have been examined, but to be rather the consequence of an already recognized generalized crypto-infection.

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Intracellular behaviour of *Leishmania enriettii* within murine macrophages

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Summary. Both promastigotes and amastigotes of *Leishmania enriettii* were readily ingested by mouse peritoneal macrophages (MPM). Promastigotes after their entry within MPM were rapidly immobilized and their multiplication was never observed. Microscopic examination revealed that ingested promastigotes were degraded within MPM. Nonmotile amastigotes of *L. enriettii* taken up by MPM, on the other hand, multiplied intracellularly and eventually destroyed the infected cells.

The role of host macrophages in which *L. enriettii*, an obligatory intracellular protozoan parasite, normally resides as amastigotes, in resistance and/or immunity against in vitro infection, has received some attention. Thus, it has been reported that macrophages from normal, allergized or immune guinea-pigs, when infected in vitro with amastigotes, are unable to restrict their intracellular multiplication[1-3]. In addition, it has also been shown that macrophages from normal mice when infected with amastigotes of *L. enriettii* could support their growth, whereas macrophages 'activated' to kill *Listeria monocytogenes* could destroy the amastigotes[4]. It was the purpose of the present experiments, to investigate the in vitro fate of *L. enriettii* promastigotes and amastigotes in macrophages obtained from the peritoneal cavities of normal (non-immune) mice.

Materials and methods. Cultures of mouse peritoneal macrophages (MPM) obtained from unelicited peritoneal cavities of normal (nonimmune) DBA/2 or C3H mice were prepared by conventional procedure[5]. The culture of *L. enriettii* was maintained as promastigotes at room temperature on conventional NNN-medium slopes[6]. The parasites were maintained as amastigotes in adult guinea-pigs by s.c. inoculation of promastigotes into the shaven dorsal surface of the right ear. Amastigotes were obtained by aseptic homogenization of excised leishmanial nodules in cold phosphate buffered saline (PBS). 24-h-old MPM cultures were infected with 10^6 viable promastigotes or 10^7 amastigotes to give a parasite to cell ratio of around 10 to 1. The infected cultures were incubated at 37 °C in a CO_2 air atmosphere for 1 h and then washed thoroughly to remove extracellular parasites. Thereafter, fresh growth medium without parasites was added to each culture and reincubated at 37 °C. Infected coverslip cultures were taken out periodically and examined under the phase contrast microscope, or stained with May-Grünwald-Giemsa, and then examined. At appropriate time intervals, the total number of intracellular parasites and the total number of macrophages were counted in at least 15 microscopic fields (×40) per coverslip. From this data the number of parasites per macrophage was calculated for each time interval sample.

Results and discussion. Under the experimental conditions used, a high proportion (70–90%) of MPM of both C3H and DBA/2 origin became infected with promastigotes or amastigotes of *L. enriettii*. Entry of majority of the promastigotes within MPM proceeded via the body of the parasites (figure 1). Phase contrast microscopy on living cultures revealed that the promastigotes subsequent to their entry within MPM maintained their motility for a period of 20–

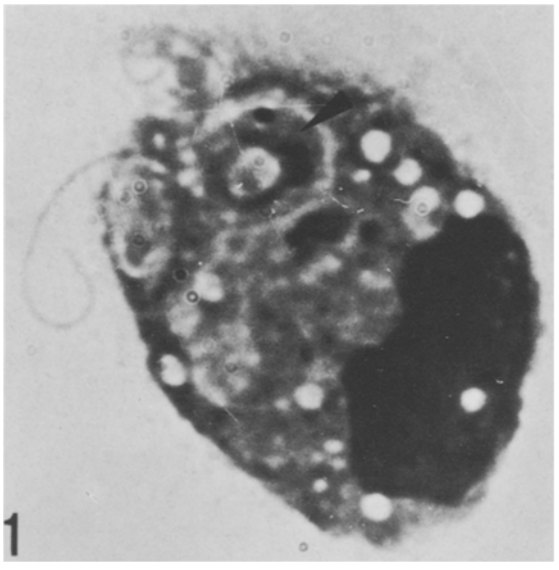

Fig. 1. Photomicrograph of May-Grünwald-Giemsa stained preparation (×2700) showing promastigote (arrow) in the process of being ingested within vacuole with the body entering first. Note that the flagella is still outside the macrophage.

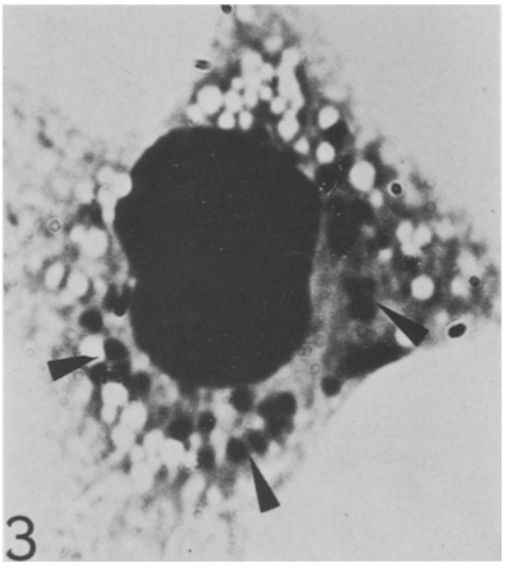

Fig. 3. Photomicrograph of C3H MPM infected with amastigotes showing intracellular parasites (arrows) 36 h after infection (May-Grünwald-Giemsa stain. ×2700).

35 min. Thus, it can be assumed that promastigotes were alive immediately after entry within MPM. Microscopically no evidence of promastigote multiplication within MPM could be obtained. By 4th–5th post-infection day virtually all the parasites had disappeared and the MPM in which the parasites were destroyed displayed no signs in loss of their morphological integrity. As illustrated in figure 2, the extent of macrophage loss among MPM cultures infected with promastigotes was not significantly different when compared with uninfected (control) cultures during the experimental period. In contrast to promastigotes, the behaviour of amastigotes within MPM was different. Unelicited MPM were unable to restrict the multiplication of ingested amastigotes (figure 3). As shown in figure 2, infected MPM population declined slightly after 36 h incubation period; however, prolonged incubation periods resulted in considerable loss of MPM, presumably as a result of the disintegration of infected MPM due to heavy parasitization. This observation is in accord with the report of Behin et al.[4] who found that, whereas normal MPM infected with amastigotes of *L. enriettii* could support their multiplication, MPM 'activated' nonspecifically could kill the ingested parasites. A second assay procedure involving uptake of ^{3}H-thymidine by infected MPM culture has also provided data suggesting killing of promastigotes and proliferation of amastigotes in MPM cultures (in preparation). The present results have established that, for the killing of *L. enriettii* promastigotes, MPM do not require the type of 'activation' which is required to kill the amastigotes of *L. enriettii* or *Listeria monocytogenes*[4]. The observed distinctness in the behaviour of *L. enriettii* promastigotes and amastigotes within MPM recall the recently reported findings of Nogueira and Cohn[7] with MPM and *Trypanosoma cruzi*. These investigators have shown that in normal MPM, epimastigotes of *T. cruzi* are killed within phagolysosomes, whereas trypomastigotes and amastigotes are capable of escaping from the phagocytic vacuole and multiply in the cytoplasmic matrix.

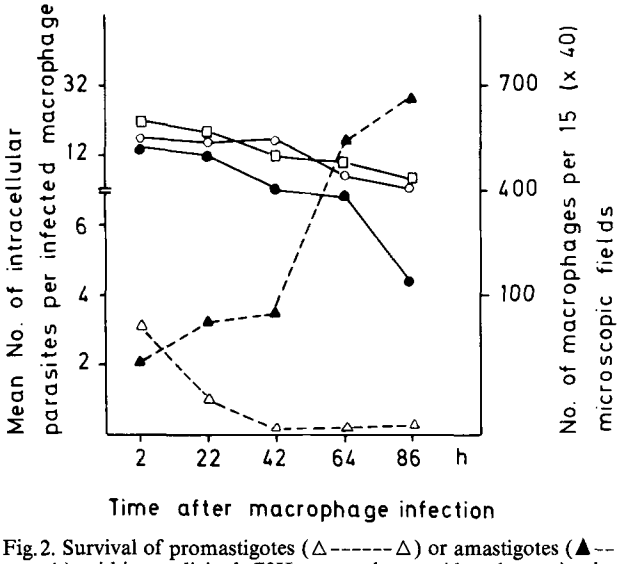

Fig. 2. Survival of promastigotes (△------△) or amastigotes (▲------▲) within unelicited C3H macrophages. Also shown in the graph are the number of macrophages in cultures which were uninfected (□———□), infected with promastigotes (○———○) or those infected with amastigotes (●———●) at particular time intervals.

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