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Laboratory Demonstrations and Posters

Scanning electron micrographs showing the early behaviour of macrophages and promastigotes of *Leishmania*

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Monolayered macrophage preparations from four common wild rodents in Kuwait (*Gerbillus cheesmani, Jaculus jaculus, Meriones crassus* and *Mus musculus*) were exposed *in vitro* for various periods of time to promastigotes of a Kuwaiti human isolate of *Leishmania tropica*. The behaviour of promastigotes and cells was then studied by SEM. A sequential picture of invasion was presented by selected SE micrographs taken from various preparations. Host cell differences, if any, are neglected in the present report.

A proportion of the promastigotes soon become attached by the tips of their flagella to cells, mostly at the latter's ectoplasmic active extensions. Stretched flagella at this stage suggest attempts by parasites to free themselves from a strong holdfast. A small tubular pseudopodium is produced at the point of attachment and grows gradually enveloping the flagellum to contain it completely within 4 to 10 min (or more). In most cases the tube is cylindrical and narrow, fitting rather closely around the flagellum; sometimes it is wider, possibly a response to an actively moving flagellum. The ingestion tube may now undergo lesser or greater retraction before it starts growing wider over the parasite's body. The earliest complete ingestion of promastigotes observed was within about 30 min of exposure in *Mus musculus* and *J. jaculus*. Previous records were: 2 to 5 min in a phase-contrast study of *L. donovani* and hamster macrophages (MILLER, H. C. & TWOHY, D. W., 1967; *J. Protozool.*, **14,** 781) and one hour in an SEM study of *L. tropica* and macrophages of laboratory mice (ZENIAN, A. *et al.*, 1979; *J. Cell. Sci.*, **39,** 187). Newly ingested parasites may remain within a bulge outside the main body of the cell for some time. Filopodial projections or microspikes at the tip of the growing ingestive tube, as seen by ZENIAN *et al.*, were not observed.

Concerning whether the anterior or posterior end is ingested first it seems now that anterior end first is the rule (confirming ZENIAN *et al.*, and some other authors). As to the penetration/engulfment controversy, it seems that the parasite plays an active role if at all, only very briefly, in initial attraction towards the macrophage; the rest of the ingestive process is actively taken by the cell. The greater part of ingestion may be described as an enveloping or enclosing process (a special modification of classical amoebic "circumfluence") rather than the commonly used term "engulfment".

Finally, it may be added that other, but much less common, methods of ingestion seem to occur, and that the behaviour of promastigotes with peritoneal macrophages *in vitro* is not necessarily the same as their behaviour with macrophages at dermal lesions.

Cultivation of bloodstream forms of pleomorphic *Trypanosoma brucei* stocks

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An *in vitro* system for the continuous cultivation of vertebrate-infective bloodstream forms of *Trypanosoma brucei* has been developed (BRUN *et al.*, 1979; *Acta trop.*, **36,** 387-390). This allows the propagation of pleomorphic, tsetse fly transmissible stocks at 37°C. A feeder layer of rabbit embryo fibroblast-like cells was used in combination with a modified Minimum Essential Medium (MEM) and 15% rabbit serum. MEM (Eagle) with Earle's salts, sodium bicarbonate and L-glutamine was supplemented with HEPES (25 mM), glucose (2·0 g/l), Na-pyruvate (1 mM) and 1% MEM non-essential amino acids. The rabbit serum was freshly obtained from New Zealand Whites and could be stored for several months at − 20°C. Commercially available rabbit sera have so far given disappointing results.

The rabbit embryo fibroblast-like cell line was isolated from 15-day-old New Zealand White embryos. The embryos were aseptically removed, washed with Earle's BSS, finely chopped and incubated in 0·5% trypsin 1 : 250 (Difco) in Earle's BSS (without $Ca^{++}/^{+}Mg^{++}$) at 37°C for 5 min. The remaining tissue pieces were disintegrated by passing them several times through a Pasteur pipette. The cells were then removed from the trypsin by centrifugation, counted and seeded into T-25 tissue culture flasks. After two subcultures the cells were ready to be used as feeder layer.

Cultures were initiated in 24-well tissue culture plates or T-25 flasks either with bloodstream forms from mice or metacyclic forms from infected *Glossina m. morsitans*. The metacyclic forms transformed to slender-like bloodstream forms and started to divide, leading to an established culture. Two trypanosome populations could be found in our culture system. The first one grew in between the feeder layer cells mainly as monomorphic slender-like forms and the second one in the supernatant where variable pleomorphism could be observed. Growth in culture was similar to that

in the mouse, with a GDT (generation doubling time) of 8 to 9 hours. So far, one *T. rhodesiense* and four *T. brucei* stocks have been tested (all pleomorphic and tsetse fly transmissible) and all could be continuously grown as bloodstream forms *in vitro*.

Different manual and automated methods in Chagas's serology

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Screening for Chagas's antibodies in rural areas requires a simple and rapid method needing no special laboratory equipment. The rapid slide flocculation test (RSFT) fulfils these conditions. The lyophilized antigen is very stable and ready to use after reconstitution of original volume by adding distilled water. After mixing one drop of reagent and one drop of serum on a plate and rocking for five minutes, the result is read visually.

Compared with the indirect immunofluorescence test, an IF titre of 1 : 50 or higher reveals a clearly positive reaction (medium to strong flocculation). Sera with negative IF reaction show no flocculation.

In blood donor centres a screening test for Chagas's antibodies is very important to avoid infections by blood transfusion.

The rapid haemagglutination test (RHT) adapted to GROUPAMATIC "ROCHE", an automatic blood grouping machine, is an ideal tool to screen for Chagas's antibodies. The reagent is prepared by coupling the reconstituted lyophilized soluble antigen to tanned human red cells of group O, Rh negative. Results are automatically recorded. Specificity and sensitivity are comparable to the flocculation test.

Sera revealing positive results with the flocculation or rapid haemagglutination tests are subsequently analysed by indirect immunofluorescence tests (IFT). Determination of titre and differentiation of IgG and IgM type Chagas's antibodies are a very helpful tool for precise diagnosis and facilitate decisions on chemotherapy.

The combination of the three methods RSFT, RHT and IFT permits a rapid, safe and reliable serological diagnosis of Chagas's disease. These tests are sensitive, specific and well adapted to meet all requirements of routine serology in different fields of application.

Dietary suppression of rodent malaria

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The relationship between the level of dietary protein and the morbidity of *Plasmodium berghei* infection in rats has not been clearly shown. This may be due to the use of diets of uncertain composition such as those made to simulate human diets.

We have examined the situation by using synthetic diets of precise composition to show the relationship of parasitaemia to the protein level and the protein to energy ratio of the diet.

Our results show that the morbidity associated with *P. berghei* infection in rats can be related to the protein content of the diet, but it is not related simply to the protein intake. The results are better explained by the relationship of the protein to energy consumption. Thus, while a low protein intake is effective in restraining the development of high parasitaemias, it will only do so if there is a low protein to energy ratio in the diet. Consequently an unrestricted intake of a low protein diet is more effective in controlling the degree of parasitaemia than a limited intake of a high protein diet.

There are several possible mechanisms: (i) an enhanced immunological response, (ii) a decrease in reticulocytosis related to protein deprivation or (iii) some nutritional factors other than p-ABA.

We are not yet in a position to comment on the possible importance of altered immunological responsiveness. Reticulocytosis appears not to be important as all rats showed normal or above normal levels of reticulocytes. The nutritional aspect would seem to be significant; recent studies that we have made with aminoacid mixtures indicate that there is competition for essential aminoacids between parasite and host.

Correlation of clinical, parasitological and immunological data of patients with *Schistosoma haematobium* infections

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Clinical, parasitological and immunological findings in 35 patients with urogenital schistosomiasis, admitted to the Clinical Department between October 1978 and December 1979, were evaluated. The egg output was quantitatively assessed by the filtration trypan blue staining technique (FELDMEIER *et al.*, 1979; *Tropenmed. Parasit.*, **30,** 417). Nearly all patients originated from an area where urogenital schistosomiasis is endemic. 28 patients were infested with *Schistosoma haematobium*, whereas seven patients had a mixed infection of *S. haematobium* and *S. mansoni*. 23 patients complained of a typical symptom, e.g., dysuria or abdominal pain or haematuria or a combination, whereas in 12 patients no symptoms were observed. Eosinophil counts varied considerably but only 13 patients had values above 500 per μl. The demonstration of antibodies against the heterologous antigen (*S. mansoni*) assessed by indirect haemagglutination test (IHA) proved unreliable as a diagnostic method. In only 13 sera titres suggesting of *Schistosoma* infection were found. Endoscopic and histopathological findings characteristic of urogenital schistosomiasis were seen in only 15 cases out of 20, whereas negative findings were