body inhibits transformation from epimastigotes to trypomastigotes. Control of differentiation might be mediated by interaction between GP72 and lectins in the triatomine alimentary tract.

Stability, Amplification and Selection

The main features of T. cruzi isozyme profiles remain stable over long periods in clonal populations. There are minor but consistent differences between different stages of the life cycle (epimastigote, trypomastigote, amastigote). Differences in GPI band intensities have also been noted when clones are grown at different temperatures (G. Widmer et al., unpublished). It is therefore essential that the same life cycle stages and growth conditions are used when T. cruzi stocks are prepared for isozyme comparisons. The only exception to the evidence that isozymes provide stable markers of intrinsic genetic differences is a report by A. Tanuri and D.F. de Almeida (unpublished) who observed radical changes in isozyme profiles when clones were grown on enriched or depleted culture media. Cloning by colony selection on agar was used, however, and the results might be explained as selection of sub-populations.

Laboratory strains of T. cruzi or stocks newly isolated from single mammals or vectors may consist of heterogeneous mixtures of zymodemes. Some T. cruzi stocks show mixed isozyme profiles (Refs 31, 32 and J. Alencar and M.A. Miles, unpublished), and repeated isolation from the same host can yield stocks with different profiles. Clones derived from a single stock can also have different profiles. Zymodeme growth rates can differ markedly and selection of sub-populations readily occurs in vitro or in vivo as stocks are amplified. Rapid clonal selection during growth of mixed populations can be predicted from computer models of growth rates (J.A. Dvorak, unpublished). Thus any method for characterizing T. cruzi that requires prior amplification of populations will fail to detect the full range of heterogeneity. This has been well illustrated by schizodeme analysis which provides a more sensitive means of detecting mixed populations in amplified stocks (see below). Nevertheless, during surveys the same isozyme profile has generally arisen independently of isolation procedure and the consistency of the geographical and host associations described above suggest that isozyme data reflect phenomena of fundamental importance.

The decision as to whether T. cruzi is a species-complex or a single polytypic species will benefit from a more complete understanding of T. cruzi genomic structure and function. This will emerge from molecular karyotyping by pulsed field gradient gel electrophoresis and recombinant DNA techniques. It is already known that T. cruzi has around 20 chromosomes, with no detectable mini-chromosomes: gene distribution and copy number indicate that the organism is diploid for at least some genes (W.C. Gibson and M.A. Miles, unpublished). Zymodeme analysis of T. cruzi has achieved much, and its discriminate use will become apparent once the T. cruzi genome is understood in more detail.

The Complexity of Trypanosoma cruzi Populations revealed by Schizodeme Analysis

C. M. Morel*, M. P. Deane** and A. M. Gonçalves*

Stocks, strains and clones of the haemoflagellate parasite Trypanosoma cruzi can be characterized at the genotype level by means of schizodeme analysis. This

Acknowledgements:
Our studies are supported by grants from the Wellcome Trust, and Wolfson Foundation. M.A.M. is a Wellcome Trust Senior Lecturer.

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Fig. 3. Zymodeme associated expression of the GP72 carbohydrate epitope.

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technique is based on the electrophoretic separation of restriction-endonuclease generated fragments of kinetoplast DNA—the bizarre mitochondrial DNA that characterizes the Kinetoplastida. The electrophoretic profiles, also known as ‘restriction fingerprints’, are stable biochemical markers which can be efficiently used to differentiate closely related parasite populations.25,40-44.

Fig. 4 shows the schizodeme analysis of several T. cruzi strains, on a gel stained by ethidium bromide (left) or silver (right). Each profile is formed by sharp bands which differ in molecular weight as well as in intensity. These bands are derived mostly from the minicircles of kinetoplast DNA. As these molecules are present in large amounts and have some degree of heterogeneity, fragments are abundant and not in stoichiometric amounts. Therefore the restriction profiles are complex and highly informative, allowing the discrimination of closely related organisms. These characteristics of schizodeme analysis allow us to investigate more efficiently the complexity and the dynamics of T. cruzi populations. Our results demonstrate that T. cruzi is a very complex collection of organisms which frequently exist as heterogeneous populations in a dynamic equilibrium.

How homogeneous are the isolates of T. cruzi obtained from the vertebrate and invertebrate hosts? Schizodeme analysis of cloned cultures has previously shown that one laboratory strain (CL) isolated originally from an invertebrate host, was really a mixture of at least two subpopulations of quite distinct biological behaviour.25. Fig. 5 (left) and also our previous data41 show that mixtures are sometimes found in isolates from human chagasic patients. Other isolates seem to be quite homogeneous, all the clones displaying absolutely identical profiles even after different schemes of passages (not shown). In Fig. 5 (right) we see another commonly observed pattern: the clones display restriction fingerprints that are quite similar but present minor differences among them.

Thus T. cruzi appears to circulate in nature as heterogeneous populations of varying complexity. How can this affect our laboratory ‘strains’? What are the consequences in clinical, diagnostic and epidemiological studies?

In order to see what occurs when a given ‘strain’ is maintained for long periods in the laboratory, we investigated what had happened with one of the most frequently used T. cruzi strains—the Y-strain isolated in 195345. For this purpose we asked for samples of this strain from several laboratories and analysed them side by side. Our partial results are shown in Fig. 6 (Gonçalves et al., unpublished). Although most of these samples displayed the profile shown at the extreme left (the ‘canonical’ Y-strain restriction fingerprint), we detected at least four other different profiles. This heterogeneity could be due either to selection of subpopulations or to laboratory mix ups. (Errors due to mishandling or mislabelling of trypanosomatid strains are probably not uncommon. One example has been reported in the literature46 and another is illustrated in Fig. 6 (right) where we received two cultures, one labelled Leishmania mexicana, the other Leishmania
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Brazilian, but schizodeme analysis showed both to be L. mexicana.

Heterogeneous Populations

We have previously studied the behaviour of heterogeneous populations of T. cruzi in the laboratory using both natural and artificial mixtures of parasites. Dvorak and collaborators have also studied this subject using different approaches, including computer simulation of the behaviour of mixtures of different strains. Fig. 7 shows some of our experiments. The two lanes at the extreme left display the profiles of the two frequently used strains (Y and F). The next four lanes represent the analysis of the populations present in an opossum at different intervals after infection with a mixture of the two strains. The Y-strain, distinguished by a characteristic band, was only detectable at the second time point.

Fig. 7 (right) shows what can happen when T. cruzi populations are isolated from human patients and brought to the laboratory. In this experiment, strains isolated by haemoculture were inoculated into mice and followed during two years by schizodeme analysis. The first two lanes show an example where we could not detect any modification of the strain, but the next two lanes show that another isolate underwent a complete change in the restriction profile, indicating that the passages in mice had selected a subpopulation of the parasite.

These results illustrate the complexity of natural populations of T. cruzi and how dynamic is the equilibrium among the various types of organisms in any given sample. The study of T. cruzi relies on methods for the amplification of the initial parasite population, but these methods can induce selection of subpopulations. We cannot always be sure that the strain isolated from a human patient is responsible for the clinical form, and not a minor component of the parasite population that was over-amplified during isolation. Thus, how can we preserve the original complexity of the isolated populations during laboratory manipulations?

We still do not have answers to these problems. T. cruzi seems to be a universe of organisms with quite different biological characteristics which often co-exist in mixed populations. The development of new methods for parasite characterization, in particular those using DNA probes, will probably bring new light in the study of these problems, once the need for multiple steps for population amplification has been minimized. But we should never forget that the real complexity and dynamics of T. cruzi in nature will always be much richer than those we have inside the laboratory.
Following the example of the 'leishmaniacs', researchers on T. cruzi met in Panama City in January 1985 to select international reference strains and discuss characterization methods. Reference strains (see Table) were selected on the basis of their zymodeme and schizodeme profile, behaviour in mice, growth rate, DNA content, reactivity to monoclonal antibodies, or susceptibility to drugs. They are to be assembled and amplified at the Gorgas Memorial Laboratory (Panama) and will be distributed, as stablate sets, to collaborating laboratories (13 national centres have been suggested, see right).

A data sheet was designed that could also be used as a request form for the characterization of newly isolated strains. The coding system and terms (isolate, stock, clone etc.) were derived from donor populations. As illustrated in the preceding section-related research with new vigor in a sound and systematic fashion.

It is notable that 13 of the T. cruzi reference strains are derived from clonal populations. As illustrated in the preceding article it was emphasised at the meeting that uncloned T. cruzi populations could radically change their composition during isolation or subsequent growth in vivo or in vitro. The uncloned reference strains might therefore be unstable in their behaviour. In this context, the latest progress with characterization methods was considered, encompassing morphological, behavioural, biochemical, antigenic and lectin binding properties. Papers presented on these topics are published as a special issue of the Revista da Sociedade Brasileira de Medicina Tropical.

Unlike the leishmanias, T. cruzi is not formally separated into distinct taxa. Nevertheless, it is abundantly clear that there is an extraordinary heterogeneity among T. cruzi strains. The Panama meeting and report are a significant contribution to unifying the T. cruzi research effort.

They will encourage researchers to tackle the difficult areas of strain-related research with new vigor in a sound and systematic fashion.

†With profound regret, we have recently learned of the death of Dr Hugo Lumbreras in Peru.

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References

c WHO (1985) TDR/EPICHA-TCC-85.3

Table: Trypanosoma cruzi Reference Strains

<table>
<thead>
<tr>
<th>M/HOM/PE/00/Peru</th>
<th>M/HOM/BR/00/12 SF</th>
<th>M/HOM/CO/00/Colombia</th>
<th>M/HOM/BR/00/Y strain</th>
<th>M/HOM/BR/00/CL strain</th>
<th>M/HOM/CH/00/Tulahuen</th>
<th>M/HOM/AR/74/CA-1</th>
<th>M/HOM/AR/74/CA-1/72</th>
<th>M/HOM/AR/00/CA-I/78</th>
<th>M/HOM/AR/00/Miranda 83</th>
<th>M/HOM/AR/00/Miranda 88</th>
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*Derived from clonal populations.
Liposomes as Drug Carriers in Leishmaniasis and Malaria

C.R. Alving

Experimental studies suggest that liposomes could substantially improve the performance of antileishmanial drugs in the chemotherapy of visceral leishmaniasis. In this article, Carl Alving discusses the potential for overcoming resistance to antimonial drugs; for 'buffering' the toxicity of drugs; and for drug delivery under conditions where hospitalization is impossible or inconvenient. Liposomes can also be used experimentally to reduce the toxicity and increase the efficacy of parenterally-administered primaquine in the treatment of sporozoite-induced murine malaria.

Liposomes are synthetic lipid spheres that can encapsulate a wide variety of water-soluble or water-insoluble drugs (see Box 1); they have been proposed as drug carriers in a broad range of clinical disorders, including visceral leishmaniasis and malaria1-4. The ability to synthesize liposomes according to specific requirements has led to several 'targeting' strategies to increase the precision of liposomal drug delivery4.

For most types of drug targeting it is desirable to concentrate a drug in a particular body compartment, to enter a compartment normally forbidden to the drug, or to avoid a particular body compartment4. Few, if any, drugs or drug carriers completely satisfy all these requirements. In the case of liposomes (or other particles) containing antitumour drugs, a major difficulty has been the propensity of parenterally administered particles to concentrate in reticuloendothelial (RE) cells, particularly Kupffer cells, rather than being delivered to solid tumours5,6. But in the case of leishmaniasis, the RE cell is the very location in which the parasite organism spends most of its normal life span in the mammalian host. Several laboratories have exploited this to show that liposome-encapsulated drugs can be delivered efficiently and safely in the chemotherapy of leishmaniasis.

Another cell to which parenterally-administered liposomes travel in the liver is the parenchymal cell (hepatocyte)6-9 — the same cell type that harbours the hepatic tissue stage (exoerythrocytic stage) of malaria parasites. Liposomes have therefore been used to deliver drugs to interrupt the malaria life cycle at this exoerythrocytic stage.

Visceral Leishmaniasis

Three groups of investigators independently, and almost simultaneously, reviewed liposomal drug delivery in a broad range of clinical disorders, including visceral leishmaniasis and malaria. Liposomes have therefore been used to deliver drugs to interrupt the malaria life cycle at this exoerythrocytic stage.

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