Further radioactive substance being given, emission computed tomography of the kidneys was then performed using the same gamma camera connected to an image analysis computer (Gamma-11, Digital Equipment Co) in its rotation mode. Data were collected for 20 s at each of 64 views equally spaced through 360° around the patient. Sagittal sections were then computed (fig 2d,e), which clearly showed that in the left kidney the functioning cortical tissue extended across the middle of the kidney. The radiographs were then reviewed and another ultrasound investigation done, after which it was concluded that the left kidney showed partial reduplication of the collecting system with interposed solid tissue having the characteristics of normal renal parenchyma. The renal abnormality was therefore a hypertrophied column of Bertin.

**Discussion**

The column of Bertin may be a source of considerable confusion in radiographic investigation of the kidney since it may simulate a renal tumour on an excretion urogram and may require angiography for diagnosis. Ultrasonography may be unhelpful, not only if the patient yields poor-quality images owing to, for example, obesity but also because the calyceal appearance in healthy kidneys varies considerably and a small amount of distortion is therefore easily missed. Conventional radionuclide images may also, in this case, be unhelpful. The usual procedure to determine the nature of the suspected lesion would then be to proceed to selective renal angiography. In this case the necessity for this invasive investigation of a healthy kidney was obviated by the clear tomographic radionuclide images obtained. In other patients we have found that emission tomographic imaging of the kidney can be a useful adjunct to other non-invasive studies.

We are grateful to Dr P G Rose, consultant radiologist, for his advice and opinion on this case.

**References**


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**Cryptic stage of sleeping-sickness trypanosome developing in choroid plexus epithelial cells**

**M O ABOLARIN, D A EVANS, D G TOVEY, W E ORMEROD**

**Abstract**

Electronmicrographs of the choroid plexus from rats infected with *Trypanosoma brucei* rhodesiense showed that trypomastigotes from the perivascular spaces may penetrate and undergo multiple division in the ependymal cells which locally constitute the blood–brain barrier. Progressive degeneration of the ependymal cell liberates trypomastigotes back into the perivascular space, from which re-entry into the blood may occur. Re-entry to the blood does not take place from any tissues other than the brain and its membranes.

These findings suggest that the ependymal cells of the choroid plexus are the site of the cryptic stage of the sleeping-sickness trypanosome.

**Introduction**

African sleeping sickness is regarded as one of the most enigmatic of diseases because of its tendency to relapse after apparently successful treatment with chemotherapy. Similarly, when spontaneous remission occurs parasitemia may be re-established months or even years after the initial infection. This occurs particularly with the Gambian form. Such behaviour suggests the existence of a cryptic stage somewhere in the tissues from which relapses may be generated. Such a cryptic stage occurs in *Plasmodium vivax* malaria. Hitherto, however, only indirect evidence of a cryptic stage has been documented, and one study suggested the choroid plexus as a site where cryptic forms of the sleeping-sickness parasite might be expected to develop.

Recent work on the relapse of *Trypanosoma brucei* infections...
in rodents indicated that during remission a site of infection remains in the region of the brain. Such remissions induced by treatment with salicylhydroxamic acid in combination with glycerol and associated with complete removal of parasites from the blood are invariably followed by relapse parasitaemias, presumably due to reinvasion of the blood by trypanomastigotes from extravascular sites.

We present direct evidence for the existence of a cryptic stage of the sleeping-sickness trypanosome developing in the ependymal (ependymal cells) covering the choroid plexus.

Materials and methods

Two strains of T. brucei were used: T. brucei (TREU 667), a strain derived from an animal but of uncertain origin and known to produce a chronic infection in mice, and T. brucei rhodesiense (LSHTM 180), isolated from man in Botswana and passed seven times. Both strains were preserved by freezing below −80°C. All inoculations were made in phosphate buffer (pH 7.4) with 1% dextrose added.

Donor mice inoculated with TREU 667 were allowed to develop infection for three, 10, or 21 days and then treated with salicylhydroxamic acid 500 mg/kg and glycerol 4 g/kg (see Table 1). Once the blood was shown to be clear of trypanosomes (DEAE cellulose concentration method) clean mice were inoculated with donor tissue, in some cases blood, in others brain homogenate. Table 1 shows the numbers of clean mice that became infected together with the first days of patent parasitaemia after inoculation.

In other experiments one mouse was injected with TREU 667 and another with LSHTM 180. Salicylhydroxamic acid and glycerol were given as before and clearance of parasites from the blood verified in the

<table>
<thead>
<tr>
<th>Day of treatment of</th>
<th>No of clean mice parasites/mice inoculated with blood</th>
<th>Mice inoculated with brain</th>
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<tr>
<td>donor mice after</td>
<td>No of donor mice</td>
<td></td>
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<tr>
<td>infection</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>7</td>
<td>0/12</td>
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<tr>
<td>10</td>
<td>8</td>
<td>1/11 (10)</td>
</tr>
<tr>
<td>21</td>
<td>8</td>
<td>1/11 (16)</td>
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* Donor mice infected with TREU 667 treated with salicylhydroxamic acid and glycerol on day stated. Clean mice then inoculated with donor blood or brain homogenate. Evidently parasites persisted in brain, which they entered after about 10 days.

![Fig 1](image1.png) Trypomastigote (T) in intact ependymal cell. **Fig 2**—Multiple-division form (M) in intact ependymal cell (N=nucleus of ependymal cell) from rat treated with salicylhydroxamic acid and glycerol. Flagellar profiles arrowed. **Fig 3**—Multiple-division form (M) and trypomastigotes (T) in degenerating ependymal cell (N=nucleus of ependymal cell). Limiting membranes of adjoining cells arrowed. **Fig 4**—Liberation of trypomastigotes after destruction of ependymal host cell.
same way. The mice were killed on day 13 and their organs homogenised and injected into clean mice (see table II). Groups of five mice were injected with extract from each homogenised organ and the experiment repeated twice.

<table>
<thead>
<tr>
<th>TABLE II—Distribution of persistent forms of sleeping-sickness parasite in tissue. Figures are first days of parasitaemia after injection of donor tissue*</th>
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<tbody>
<tr>
<td>TREP 667</td>
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<td>LSHTM 180</td>
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* 0 = No parasitaemia. — Not injected. Homogenised organs from two infected donor mice treated with salicylhydroxamic acid and glycerol injected into groups of five clean mice. Only mice injected with brain became parasitaemic.

Four rats were killed with ether 24-27 days after inoculation with LSHTM 180 and their brains dissected. One had been treated with salicylhydroxamic acid and glycerol, with the clearance of parasites from the brain verified as above. Choroidal pleures of the lateral ventricle were fixed in situ with 5% glutaraldehyde, extracted, and post-fixed with 1% osmium tetroxide. Microsections were cut from Araldite and examined in an AEI 801 electron microscope.

Results

The inoculation experiments showed that when the blood had been cleared by salicylhydroxamic acid and glycerol, with the clearance of parasites from the brain verified as above, choroidal pleures of the lateral ventricle were fixed in situ with 5% glutaraldehyde, extracted, and post-fixed with 1% osmium tetroxide. Microsections were cut from Araldite and examined in an AEI 801 electron microscope.

Discussion

The choroidal pleures is of particular interest both because of the large numbers of trypanomastigotes which accumulate in its peripheral spaces and because it forms part of the blood-brain barrier, which is of particular relevance in the treatment of sleeping sickness. The ependymal cells which cover the ventricular surface of the choroidal pleure, together with the tight junctions between them, form a barrier to the passage of trypan blue. Suramin, which is close in chemical structure and in pharmacological and chemotherapeutic activity to trypan blue, is one of the most important drugs used in treating sleeping sickness, but it is often ineffective in the later stages. Presumably this is because it cannot reach trypanosomes that have crossed the blood-brain barrier or have entered other closed sites. If the ependymal cells were invaded by the trypanosome these cells might act as such a closed site and protect the trypanosome not only against drugs but also against other large molecules of the host's protective mechanisms.

Altematively, other sites in the brain and its membranes may be available for the development of intracerebral forms, and we have evidence (as yet incomplete) that development of intracellular stages may also take place in ependymal cells which line the ventricles. Our present aim, however, is to present evidence of intracellular development of T brucei in the choroidal pleura which has not hitherto been described and to relate this finding to the presence of extracellular trypanomastigotes in the perivascular region of the choroidal pleura. Perivascular trypanomastigotes in the choroidal pleura are already well known, and Van Mark et al16 quoted some nine reports of their existence.

Some of these perivascular trypanomastigotes entered the space by penetrating the vessel wall, as previous workers have postulated, but others, we consider, entered it when the cells in which they were reproducing had been destroyed.

Intracellular trypanomastigotes are not seen as often in the choroidal pleura as those that are extracellular. We have examined some 20-30 such forms, and because of partial or total destruction of the cell, the cell wall is not always been easy to decide whether they were intracellular or not. Now that we recognise the existence of intracellular trypanomastigotes in the choroidal pleura, however, we have little difficulty in finding them by transmission electron microscopy in ependymal cells of rats and mice.

We believe that our finding is important in understanding sleeping sickness, particularly in relation to the persistence of the infecting organism in the brain and its failure to respond to chemotherapy if this has been delayed beyond a certain interval. Invasion of the cerebrospinal fluid and secondary meningitis which we have observed in experimental sleeping sickness may occur via the choroidal pleura; and this may act as a useful model for understanding other meningoencephalitic diseases in which the pathogen may also develop in ependymal cells and be distributed via the choroidal pleura.

We thank C A J Brightman and Senait Assefa for determining the infectivity of tissue homogenates.

References

10 Goldman EE (1910). Quoted by Bradbury.13

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