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# The pathogenesis of trypanosomiasis of the CNS

## Studies on parasitological and neurohistological findings in trypanosoma rhodesiense infected vervet monkeys*

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**Summary.** Parasitological examinations of the cerebrospinal fluid of 20 vervet monkeys (*Cercopithecus aethiops*), that had been infected with Trypanosoma rhodesiense, revealed that the CSF was regularly infested with trypanosomes in the early phase of the disease, at the earliest on the 13th day, in most of the animals in the 3rd or 4th week, after infection. Follow-up examinations of the CSF during the further course of the disease also regularly proved positive for trypanosomes. Histological studies in the animals that died at a mean of 65 days after infection (range 35 to 107 days) revealed encephalitis in the animal with the longest course of the disease . In all the other animals, meningitis alone was found. This was accompanied by a modified early encephalitic reaction, characterized by lympho-plasma-cellular infiltrates exclusively in the adventitial sheaths of those blood vessels passing into the brain from the leptomeninges affected by inflammatory infiltration. The early encephalitic reaction is interpreted as the morphological manifestation of an infestation of the perivascular spaces (Virchow-Robin spaces) with parasites. It indicates that CSF parasitosis in the early phase represents the point of departure for the encephalitis that develops in the late phase of the disease, and that the encephalitis presumably develops as a result of the migration of the trypanosomes out of the subarachnoid space into the perivascular spaces, and from there into the brain.

**Key words:** African trypanosomiasis – Involvement of CNS – Pathogenesis

The pathogenesis of the late stage of human trypanosomiasis is unknown. Although it appears that encephalitis as the morphological expression of the late stage, is a consequence of parasitic infiltration of brain tissue, the question of how the trypanosomes get into the brain, which

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is so important for therapy, has not been answered. Haematogenous infiltration of the majority of organs by parasites in the early phase of the disease does not occur in the brain so that delayed invasion of brain tissue by haematogenic pathways does not seem probable.

In this paper the pathogenesis of cerebral trypanosomiasis is discussed on the basis of parasitological and neurohistological findings which were compiled from examination of vervet monkeys infected with Trypanosoma rhodesiense. The findings are part of the results of experiments in the course of a research programme carried out at the Kenyan Trypanosomiasis Research Institute, Muguga, Kenya.

## Material and methods

Twenty vervet monkeys (*Cercopithecus aethiops*) were used. They had been infected intravenously with Trypanosoma rhodesiense (EATRO 1989): one "host" animal with the stabilate of the strain, the other 19 animals with inocula containing $10^2$ to $10^6$ parasites, obtained from the blood of the animal infected with the stabilate. The animals remained untreated until death. In vivo examinations included daily quantitation of the parasitemia by wet film, examination of the CSF for parasites by wet film and infectivity test in white mice at intervals of, on average, two weeks. Weekly control of the blood status (leukocytes, erythrocytes, haemoglobin, PCV), of weight and temperature was carried out and in some of the animals, the CSF cell count and IgG and IgM in blood and CSF were determined (by radioimmunodiffusion with Tripartigen and LC-Partigen immunodiffusion plates, Behring-Werke, Marburg FRG). Post mortem examination included histological work-up of the brain, fixed in formalin and embedded in paraffin: hemisphere sections from the cerebrum and cerebellum, including parts of the brain stem and the region of the ventricles were stained using Giemsa, haematoxylin and eosin, and cresyl violet. Furthermore, microscopic examination of the heart, lungs, liver, kidneys, adrenals, spleen, lymph nodes, intestine and of striated muscles were carried out.

**Table 1.** Parasitosis of the CSF in 20 monkeys infected with Trypanosoma rhodesiense (results of CSF-infectivity tests)

| animal | inoculum | duration of disease in days | parasitosis CSF (day of test) | later CSF-controls |
|---|---|---|---|---|
| L 11 | $10^5$ | 35 | + (19) | + + |
| L 3 | $10^3$ | 39 | + (19) | + |
| L 6 | $10^3$ | 40 | + (19) | + |
| L 4 | $10^2$ | 46 | + (19) | + |
| L 10 | $10^5$ | 46 | + (19) | + |
| T 17 | $10^4$ | 55 | + (37) | + + |
| L 5 | $10^3$ | 56 | + (26) | + |
| T 20 | $10^5$ | 58 | + (13) | + + + |
| L 20 | $10^3$ | 59 | + (19) | + |
| L 13 | $10^3$ | 62 | + (19) | + |
| T 19 | $10^5$ | 62 | + (37) | + + |
| T 10 | $10^4$ | 63 | + (27) | + |
| L 14 | $10^3$ | 64 | + (19) | + |
| L 19 | $10^3$ | 64 | + (19) | + |
| T 18 | $10^4$ | 76 | + (37) | + + |
| T 5 | $10^4$ | 77 | + (37) | + + + |
| T 6 | $10^5$ | 90 | + (37) | + + |
| T 1 | $10^2$ | 92 | + (37) | + + + |
| T 8 | $10^6$ | 95 | + (37) | + + + |
| T 4 | Stabilat | 107 | + (41) | + + + |

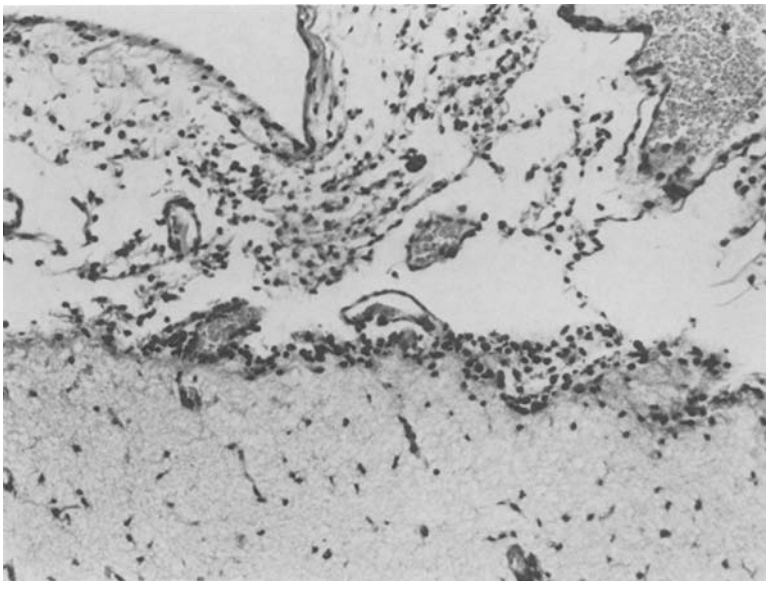

**Fig. 1.** Meningitis, 92 days after infection: moderate lympho-plasma-cellular infiltrates in the subarachnoid and pial connective tissue of the convexity of the brain. Giemsa ×125

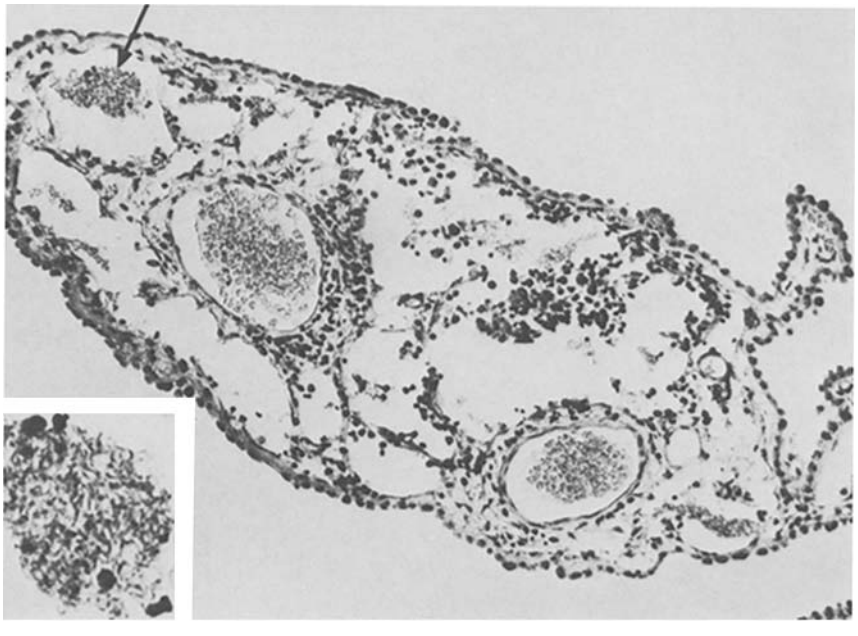

**Fig. 2.** Choriomeningitis and parasitosis of the chorioid plexus of a lateral ventricle, 76 days after infection: enlarged plexus villus with inflammatory infiltrates as well as accumulations of trypanosomes, especially in dilated lymph spaces (*arrow*); Giemsa ×30. *Inset*: the accumulation of trypanosomes, marked by arrow, magnified (×400)

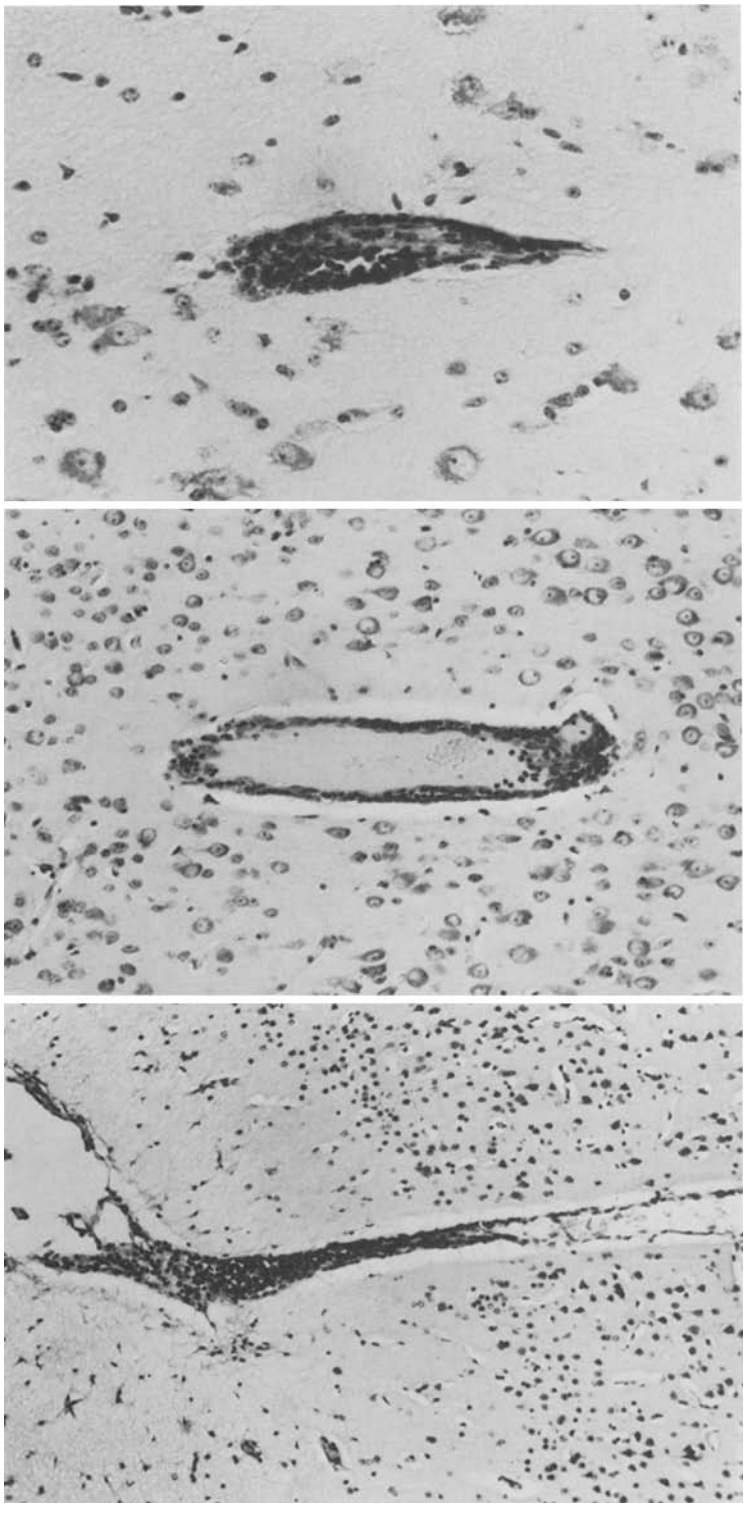

**Fig. 3.** Modified early encephalitic reaction of various brain vessels in the phase of meningitis, 64 and 92 days after infection: lympho-plasma-cellular infiltrates affecting exclusively the adventitial sheaths of the vessels (*Left:* a vessel entering the cortex, Giemsa ×120; *middle:* a vessel in the cortex, Giemsa ×125; *right:* a vessel in the thalamus, Giemsa ×250)

**Table 2.** Modified early encephalitic reaction: frequency of adventitial round cell infiltrates of cerebral vessels in the phase of meningitis, appointed in 5 histological brain sections

| animal | inoculum | duration of disease (days) | number of infiltrates |
|---|---|---|---|
| L 11 | $10^5$ | 35 | 3 |
| L 3 | $10^3$ | 39 | - |
| L 6 | $10^3$ | 40 | 5 |
| L 4 | $10^2$ | 46 | 4 |
| L 10 | $10^5$ | 46 | 5 |
| T 17 | $10^4$ | 55 | 10 |
| L 5 | $10^3$ | 56 | 18 |
| T 20 | $10^5$ | 58 | 3 |
| L 20 | $10^3$ | 59 | 1 |
| L 13 | $10^3$ | 62 | - |
| T 19 | $10^5$ | 62 | 4 |
| T 10 | $10^4$ | 63 | 10 |
| L 14 | $10^3$ | 64 | 2 |
| L 19 | $10^3$ | 64 | 2 |
| T 18 | $10^4$ | 76 | 5 |
| T 5 | $10^4$ | 77 | 4 |
| T 6 | $10^5$ | 90 | - |
| T 1 | $10^2$ | 92 | 12 |
| T 8 | $10^6$ | 95 | 5 |

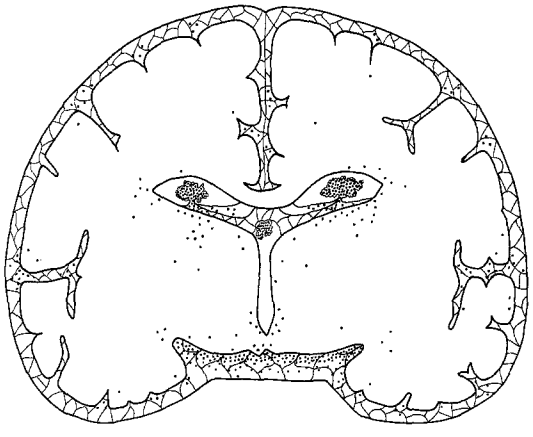

**Fig. 4.** Meningitis and modified early encephalitic reaction: predilection of the meningitic infiltrates (*dots*) for the choriomeninges and the basilar meninges, predilection of the infiltrates of the encephalitic early reaction (*dots*) for brain regions near the meninigitic sites of predilection. Drawing from histological preparations

## Results

### *In vivo data*

Following infection, all the animals showed progressive debility, loss of weight, apathy and anaemia. In the days before death, there were transient states of somnolence, but at no time were clear neurological symptoms observed. Death took place at the earliest after 35 days, at the latest after 107 days (average survival time 65 days).

Parasites were found in the blood from the 4th or 5th day onwards, almost daily, in fluctuating numbers. In the CSF, parasites were found for the first time on the 13th day and were present in the majority of

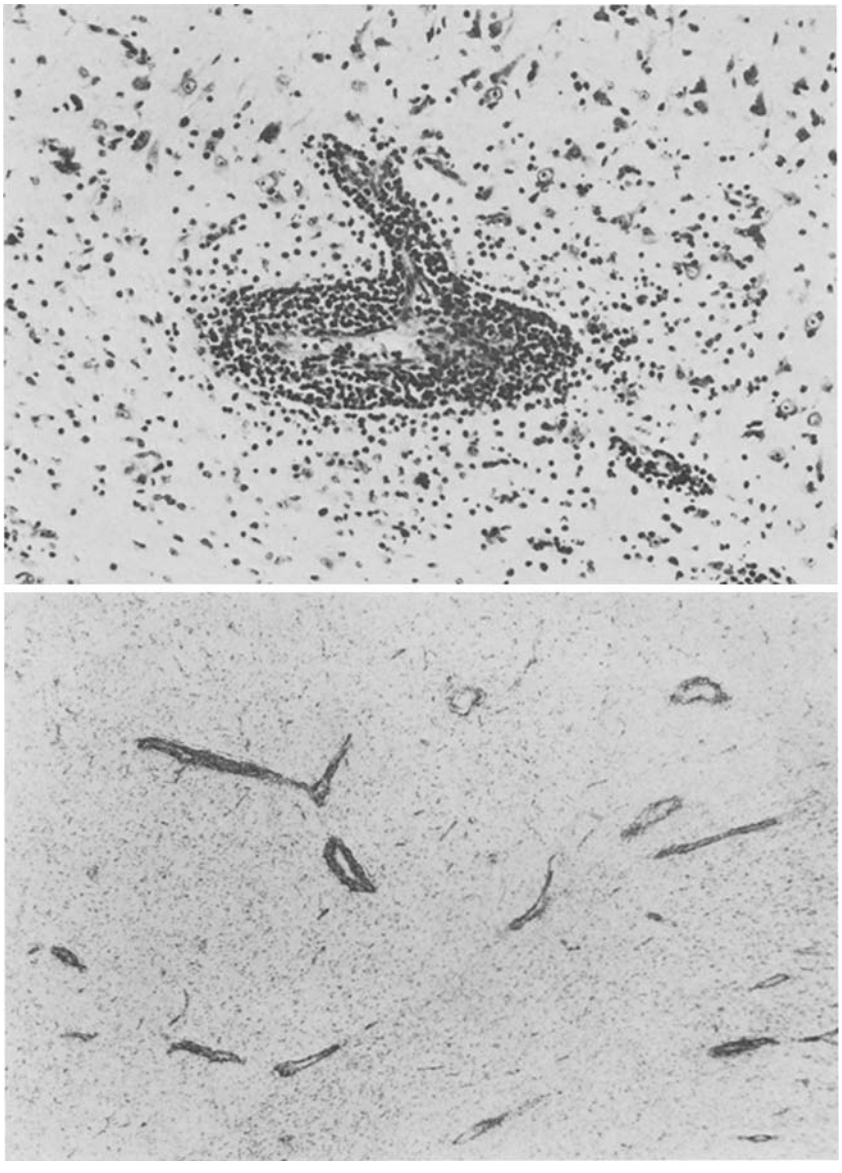

**Fig. 5.** Encephalitis, 107 days after infection: perivascular inflammatory infiltration particularly around largecalibre blood vessels (*left*), brain stem, Giemsa ×25; inflammatory infiltrates comprising lymphocytes and plasma cells, perivascularly and freely located within the brain tissue, besides proliferation of microglia cells in the brain tissue surrounding the vessel (*right*), hippocampus, Giemsa ×125

the animals during the 3rd and 4th week; subsequent follow-up examinations of the CSF were always positive for the parasites (Table 1). This parasitisation of the CSF was accompanied by an increase in the cell count (more than 50/3 from the 4th week onwards) and the appearance of IgM (to 6.1 mg/dl). With regard to the in vivo observations, the disease faithfully imitated human T. rhodesiense infection.

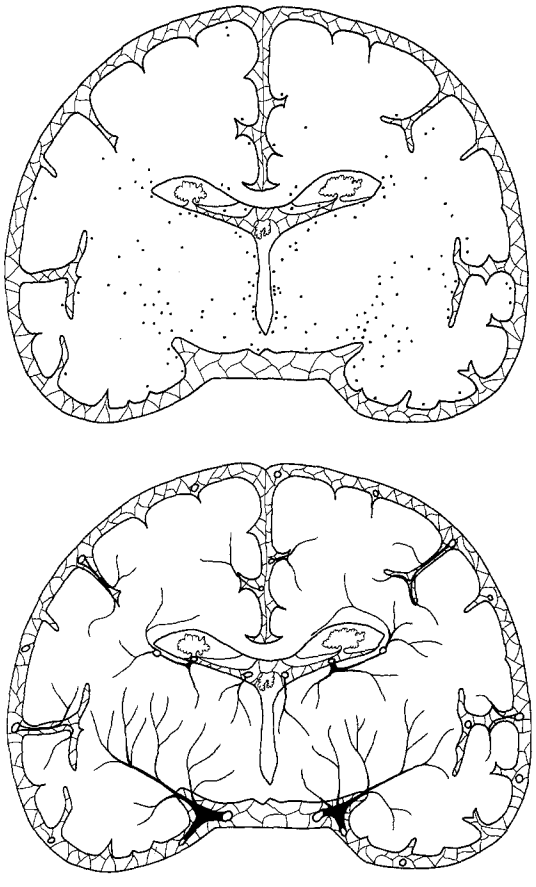

**Fig. 6.** Localization of the inflammatory infiltrates in the case of encephalitis (*above*) in comparison with the ramification of the vessels entering the brain from the basal meninges and the choriomeninges (*below*): evident relation of the infiltrates (*dots*) to the rami of the basal cerebral arteries and the rami of the inner cerebral veins

*Neurohistological findings*

Histologically, all the monkeys revealed a chronic meningitis with infiltrates of lymphocytes, plasmocytes and histiocytes, not only in the subarachnoid connective tissue but also in that of the pia (Fig. 1). The meningitis showed a significant preference for the chorioid plexus (Fig. 2) and for the cisterns at the base of the brain. In other parts meningitis was poorly or minimally developed.

In addition to the meningitis, most of the animals (16 out of 19) also revealed sporadic, usually lowgrade, infiltrates of lymphocytes and plasma cells, exclusively in the adventitial sheaths of cerebral blood vessels (Fig. 3), especially those vessels entering the brain. In individual animals, the infiltrates were found at varying frequency: in a total of 5 brain sections per animal, up to 18 were present (Table 2). In terms of localisation, preference was shown for the periventricular and basal parts of the brain stem in the neighbourhood of the predilection sites for meningitis (Fig. 4). In a single animal – that with the longest survival (107 days) – fully developed

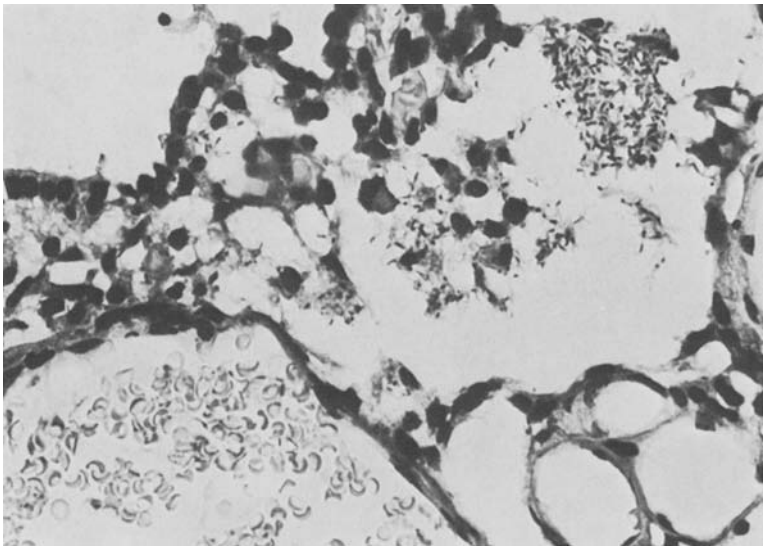

**Fig. 7.** Accumulations of trypanosomes in dilated perivascular spaces of subependymal vessels in the wall of a lateral ventricle below the chorioidal plexus (not in the figure). Giemsa × 400

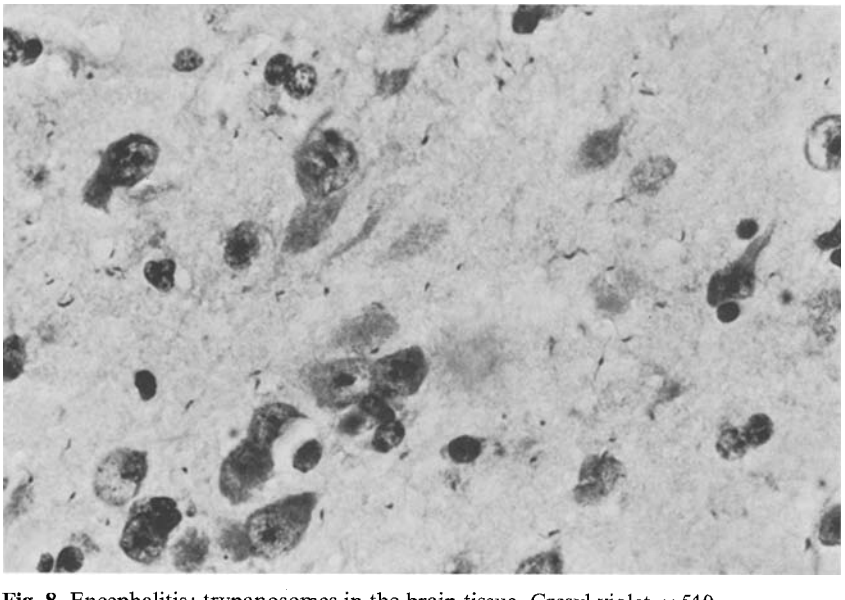

**Fig. 8.** Encephalitis: trypanosomes in the brain tissue. Cresyl violet × 510

encephalitis was found in addition to meningitis. The encephalitis was characterized by (i) varyingly broad perivascular infiltrates consisting of lymphocytes, plasma cells and histiocytes, particularly associated with arteries and veins (Fig. 5), (ii) by collections of lymphocytes and plasma cells within the brain tissue, and (iii) by the proliferation of microglial cells, particularly

in the neighbourhood of vessels showing inflammatory infiltration (Fig. 5). The infiltrates showed predilection for the brain stem and the periventricular white matter (Fig. 6).

In all the animals, trypanosomes were found in large numbers in the connective tissue of the chorioid plexus (see Fig. 2). In the connective tissue of the CSF-containing leptomeninges, however, trypanosomes were to be found in small groups or singly, only in the animals with encephalitis and in 3 further animals with meningitis. Further small groups of trypanosomes were also observed in dilated perivascular spaces of subependymal veins in four animals with meningitis (Fig. 7). In the brain tissue, trypanosomes were observed only in the animal with encephalitis (Fig. 8).

## Discussion

On the basis of these findings it is reasonable to conclud that infection of the CNS originates in infestation of the cerebrospinal fluid with parasites. The earliest time for this infestation (2 weeks after infection) corresponds with the time of the first parasitic invasion of the organs (with the exception of the brain). The tissue response to the parasitisation of the CSF is chronic meningitis. In common with the findings in man (v. Bogaert 1962; Manuelidis et al. 1965; Poltera et al. 1977), the meningitis is characterized by infiltrates of lymphocytes, plasma cells and macrophages, and also by particular predilection sites (choriomeninges and leptomeninges of the cisterns at the base of the brain). In the choriomeninges, the meningitis is associated with an accumulation of very large numbers of trypanosomes; outside this area, in the CSF-containing leptomeninges, trypanosomes can only rarely be demonstrated histologically. The meningitis is accompanied by an increase in the cell count, and the appearance of IgM in the CSF from the 4th week onwards.

The meningitis is accompanied by inflammatory involvement of cerebral vessels, especially vessels entering the brain. This is characterised by sporadic, usually low-grade, lympho-plasma-cellular infiltrates limited to the adventitial sheaths of the vessels, and can be considered to be a modified early encephalitic reaction following the meningitis. In our material this condition was found in 16 out of 19 animals, which suggests that it is a regular companion of the meningitis.

Fully developed encephalitis, that is encephalitis with inflammatory affection of the cerebral parenchyma itself, was seen in only one animal. However, fully developed encephalitis of the same kind was observed in two further monkeys (from another series of experiments) after ineffective treatment and relapse of the infection (Schmidt and Sayer 1982). In comparison with human Rhodesian trypanosomiasis (van Bogaert 1962, Manuelidis et al. 1965, Poltera et al. 1977), encephalitis in these cases showed no discrepancies with regard to quality of the inflammatory changes and their predilection for the brain stem and the cerebral medulla.

With regard to the early parasitisation of the CSF, the neurohistological findings indicate that the trypanosomiasis of the CNS is a continuous chron-

ic inflammatory process whose development and course is characterized by three phases:

Phase 1: development of a chronic meningitis after parasitisation of the CSF.

Phase 2: further development of a modified early encephalitic reaction by progression from the leptomeninges to the vessels entering the brain and finally.

Phase 3: involvement of the brain with development of an encephalitis by spread from the brain vessels into the adjacent brain parenchyma.

This course of inflammation indicates that we have to classify this disease as a spreading meningoencephalitis (Spatz 1930). This is in agreement with van Bogaert (1962) and Manuelidis et al. (1965) who have already described the encephalitis of sleeping sickness as meningoencephalitic in type on the basis of neurohistological examinations in human african trypanosomiasis.

The development of an encephalitis following primary meningitis permits us to assume that the causal pathogens are able to invade from the subarachnoid space into the perivascular spaces of the vessels of the brain, and thence into the substance of the brain itself. In our opinion, such a mechanism is responsible for the encephalitis that develops in sleeping sickness. An invasion of trypanosomes into the perivascular spaces of the cerebral vessels is made possible by the communication of the perivascular spaces with the subarachnoid space. The perivascular spaces (Virchow-Robin spaces) have long been a subject of controversy. From electronmicroscopical examinations it is now agreed (Cervós-Navarro 1980, among others) that these spaces communicate with the subarachnoid space, and can thus be considered to be perivascular "prolongations" of the latter into the brain substance; the electron microscopic investigations have also shown that the perivascular spaces are present even in small cerebral arteries and veins and thus extend a considerable distance into the brain. Trypanosomes were observed in perivascular spaces by Goodwin (1970). In our monkeys there were trypanosomes in dilated perivascular spaces of subependymal veins, this observation may be evidence for infestation of the perivascular spaces by parasites. However, there are also aspects of the tissue change which point to this, in particular the modified early encephalitic reaction. Perivascular cellular infiltrates can be understood to be the morphological expression of inflammatory reactions to trypanosomes which invade perivascular spaces; that is suggested by the restriction of the infiltrates to the vascular adventitia "ensheathing" the perivascular spaces. A further piece of evidence supporting the development of encephalitis via dissemination from the subarachnoid space is the localization of the inflammatory infiltrate of the modified early encephalitic reaction and of the fully developed encephalitis. They both reveal a predilection for the periventricular cerebral white matter and the brain stem in the neighbourhood of the ventricles and the basal cisterns. These are regions in proximity to the predilection sites of the meningitis i.e. the choriomeninges and the basal leptomeninges.

Here, trypanosomes are to be found in large masses. From these places large-calibre blood vessels with correspondingly large perivascular spaces "radiate" into the brain: from the choriomeninges branches of the internal cerebral veins and from the basal leptomeninges branches of the arteries of the base of the brain (Fig. 6). Thus, it may be supposed that trypanosomes are most likely to get into the brain via the perivascular spaces of vessels entering it. This supposition is supported by the fact, that the localization of the inflammatory infiltrates of the modified early encephalitic reaction and of the encephalitis itself have evident relation to the ramification of the blood vessels entering the brain from the choriomeninges and the basal meninges (Fig. 6). In addition, there is the fact that these blood vessels are particularly affected by inflammatory cellular infiltrations, not only in the case of modified early encephalitic reactions, but also in encephalitis (Fig. 5). In our opinion, these findings make it apparent that migration of trypanosomes out of the subarachnoid space into the perivascular spaces of the brain vessels should be regarded as the decisive factor in the pathogenesis of the encephalitis. In addition, they indicate that the modified early encephalitic reaction represents the pathogenetic link between the primary meningitis and the secondary encephalitis, and thus may be considered the percursor of the encephalitis.

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