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LIGHT MICROSCOPIC NEUROPATHOLOGY OF LONG-TERM EXPERIMENTAL *TRYPANOSOMA BRUCEI GAMBIENSE* INFECTION IN THE RAT

by

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Summary — A detailed neuropathological description of the brain lesions in rats with terminal *Trypanosoma brucei gambiense* infection is presented. A generalised meningo-encephalitis was found which is comparable to the lesions encountered in human cases of sleeping sickness. The main lesion is a perivasculitis while demyelinisation areas and neuronal lesions are absent or very scarce.

KEYWORDS: *Trypanosoma brucei gambiense;* Neuropathology; Experimental; Rat.

Introduction

The elaboration of an experimental model for chronic African trypanosomiasis in small rodents has long been difficult, due to the great susceptibility of these animals to the infection and to the diffuse pathology induced by inoculation of parasites of the brucei group (13). In recent years, however, models have been proposed in the mouse (17, 27, 25).

Wéry and co-workers (33) have introduced models employing rats and mice infected with *Trypanosoma brucei gambiense* stocks isolated in Zaïre. With these models, serological, hematological and parasitological studies (3) and a general histopathological study (31) have already been performed.

There are several studies dealing with the histopathology of the central nervous system involvement in trypanosomiasis (1, 11, 21, 22, 24, 26, 32), but a detailed study in small rodents has not yet been performed.

The present paper reports the light microscopic neuropathology of long term *T. b. gambiense* infection in the rat using an isolate from Zaïre (33).

Material and methods

Animals and parasites

Twenty young female outbred Wistar rats (K.U.L. Leuven, Belgium) weighing approximately 200 g were used. Nineteen rats were inoculated by

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intraperitoneal injection of 10,000 to 20,000 *T. b. gambiense* parasites, stock Mongo, originally isolated in Zaïre (stabilate Mongo-Bemba 68/ITMAP/1832, 6th passage in rats). This trypanosome stock produces a chronic infection with a survival rate of 50 % for more than 300 days. The parasitological kinetics of this model have been studied in detail and need not be recalled here (3, 18, 19).

Sixteen animals were sacrified between 4 and $6^{3/4}$ months after infection, when their clinical status was poor and appeared irreversible. Three animals died during the observation period and were not utilised for pathological examination.

One uninfected rat served as control.

Preparation of tissue specimens

After pentothal anesthesia, the animals were perfused, according to the technique of Baleydier *et al.* (2) with saline containing 1 % procain for one minute, via a catheter inserted in the aortic arch while the thoracic aorta was clamped. This perfusion was immediately followed by perfusion with a solution containing paraformaldehyde 1 % and glutaraldehyde 1 % in cacodylate buffer 0.36 M pH 7.3, for 15 min.

The fixed cerebrum was then removed and divided with a mid-sagittal transsection. One half was used for light microscopy in this study, while the other half was processed through for electron microscopy (unpublished results).

The following sections were studied: a section through the frontal lobe, one through the thalamus, one passing posteriorly of the thalamus, one through the mesencephalon, one through the pons and cerebellum, and one through the medulla oblongata. Paraffin sections were studied after staining of 10 μm thick sections with cresyl violet, luxol fast blue, trichrome Masson and of 5 μm thick sections stained with Giemsa. In addition, some sections were studied after Bodian (20 μm thick) or after Perl's iron stain.

Results

Macroscopy

The control rat was entirely normal.

The infected animals had a very poor general condition. There was paresia of the hind limbs in 7 animals sacrificed 5 months post infection.

Light microscopy

The control rat showed an entirely normal cerebrum.

One infected rat, sacrificed 5 months post-infection, had almost normal cerebral structures. The tissue of the pituitary stalk was, however, filled with many extravascular trypanosomes and with inflammatory cells, including iron

laden macrophages (fig. 1). There was a slight microglial reaction in the floor of the 3rd ventricle. In the surrounding leptomeninx, there were numerous inflammatory cells and trypanosomes.

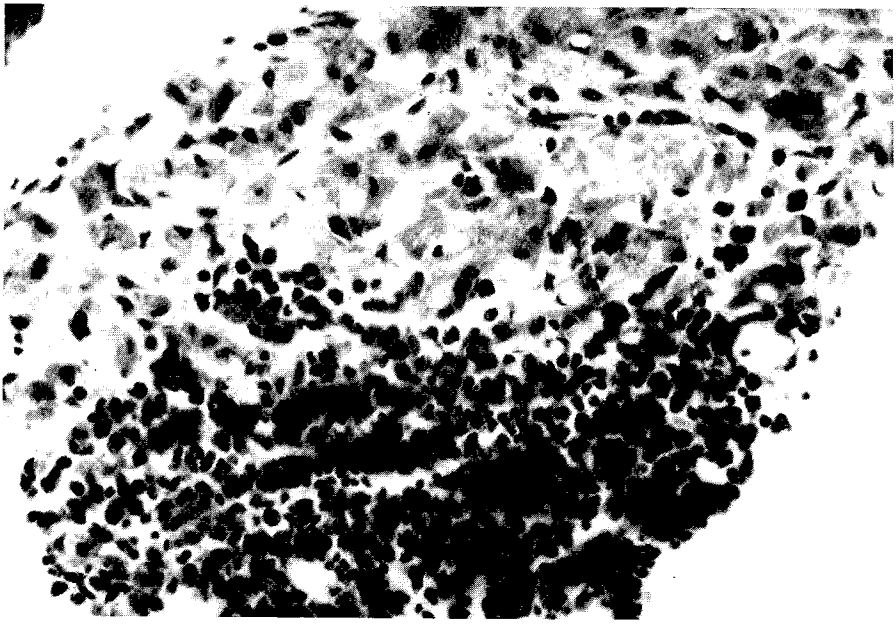

Figure 1
Pituitary stalk: many inflammatory cells (bottom) intermingled with trypanosomes (not visible at this magnification). Five months post-infection. Klüver-Barrera. Magnification 324 ×.

Fifteen out of 16 infected rats showed severe cerebral lesions. In these animals, there was a striking leptomeningitis, most intense at the base of the brain, in the perichiasmatic zone and in the areas facing the mesencephalon and the brain stem. This leptomeningitis extended towards the lower aspect of the frontal lobe and laterally in the lateral fissure surrounding the mesencephalon and the cerebellum. In the lateral fissure, the leptomeningitis could be followed till the pia mater at the insertion base of the choroid plexuses of the lateral ventricles. In few cases there were leptomeningeal inflammatory foci at the superior aspects of the cerebral convexities. The inflammatory infiltrate was of a lymphoplasmocytic type, with also numerous macrophages and Mott cells. Subpial gliosis predominated at the base. Trypanosomes were ubiquitous but abunded in the lateral fissure and in the pia mater at the insertion of the choroid plexus. The leptomeningitis extended in the parenchyma as a perivasculitis (fig. 2), following the course of the great vessels, the arterioles and the venules.

In the *cerebral cortex,* there were no major lesions, except some perivasculitis of variable intensity in some animals in the lower aspects of the frontal and the temporal lobes. Sometimes, there were perivasculitis lesions in the more profound areas of the cerebral cortex, contiguous with alterations in the white matter. The neurons had a normal appearance. In

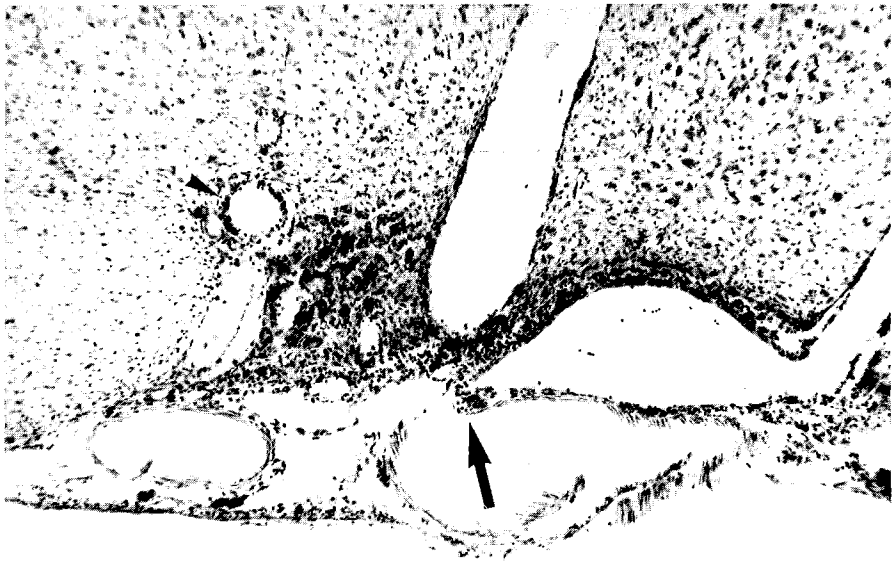

Figure 2

Perichiasmatic region of hypothalamus. Leptomeningitis (arrow) and intraparenchymal perivasculitis (arrowhead). Six months post-infection. Cresyl-violet. Magnification 80 ×.

two animals, which were severely affected, there were microglial nodules. The Ammons' horn presented some slight perivasculitis with microglial reaction. In some cases, neuronophagy was seen.

In the *white matter* and in the *mesencephalon,* there was, with decreasing intensity, inflammatory infiltration of the cerebral peduncles, the optic tract, the internal capsule, the corpus callosum, the white matter of the temporal lobe, the white matter of the parietal lobe and of the frontal lobe. The mesencephalon was infiltrated in its totality. The neurons appeared to be normal. There was generalised gliosis with both microglial and astrocytic components. The inflammation was sometimes very extensive, forming diffuse zones or inflammatory granulomas, the latter being rare. Granulomas were composed of lymphocytes, plasma cells, macrophages and some astrocytes. We have not seen demyelinisation, except in the inflammatory granulomas.

The *central gray nuclei* showed perivasculitis. In the thalamus, the perivasculitis was localised in the lower and lateral aspects and was contiguous with infiltration of the white matter of the internal capsule. In the hypothalamus, the infundibulum and the periventricular areas were affected. The striatum was most affected in its medial and posterior thirds. The globus pallidus was affected in continuity with the inferior aspects of the frontal lobe.

In one rat, sacrificed 4 months post infection, there were many extravascular trypanosomes in the infundibulum and in the leptomeninx at the brain base.

Brain stem. The pons and the medulla oblongata were infiltrated by the surrounding meningeal inflammatory reaction. The inflammatory reaction was also conspicuous at the floor of the 4th ventricle. The neurons of the gray nuclei and those of the substantia reticularis were well preserved, in spite of the heavy inflammatory infiltration. Myelin sheaths surrounding perivasculitis areas were somewhat paler than normal.

Cerebellum. Alterations were most pronounced in the white matter but extended also towards the granular layer (fig. 3). The appearance of the inflammatory infiltrates were similar to those of the cerebral white matter. The neurons of the nucleus dentatus were normal, even in the presence of an inflammatory infiltrate. The same was true for the Purkinje cells except for the areas with an inflammatory infiltrate. Sometimes, a severe perivasculitis originating in the leptomeninx could be seen running through the cortex. Inflammatory granulomas had the same appearance as in the white matter of the cerebrum, and were always rare, but were more frequent in the cerebellum than in the cerebrum.

Figure 3
Cerebellum: perivasculitis (arrows) in the white matter near the dentate nucleus. Four months post-infection. Cresyl-violet. Magnification 80 ×.

Choroid plexuses. Except for the infiltration of the pia mater at the insertion of the plexuses, the choroid plexuses were normal. In three cases, however, the fibrovascular axis of the choroid plexus was filled with many trypanosomes (fig. 4), accompanied by inflammatory cells, which were very numerous in one of these three cases. The three animals presented the most violent meningo-encephalitis.

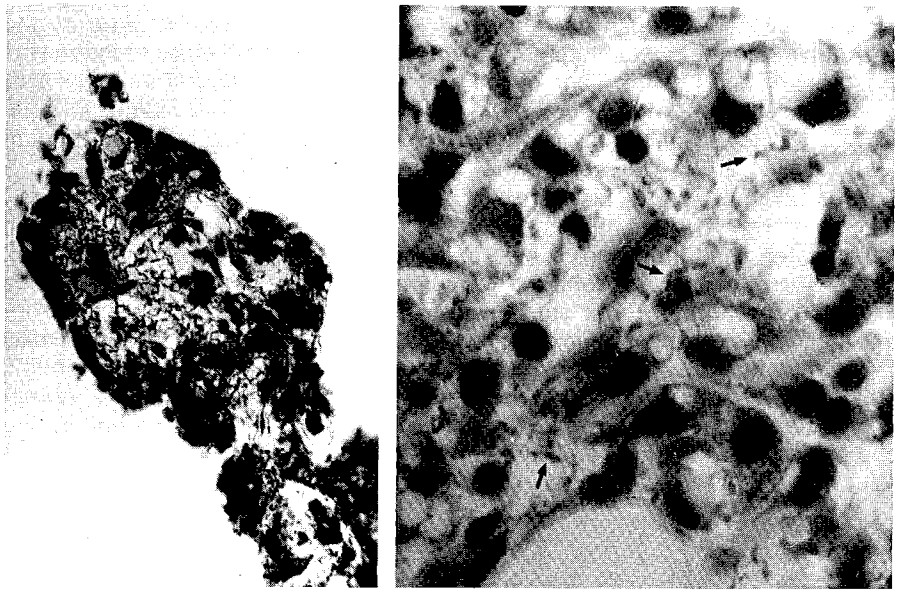

Figure 4

Choroid plexus of lateral ventricle. Inflammatory cells and trypanosomes in the matrix of a choroid plexus villus. Seven months post-infection, Klüver-Barrera. a) Magnification 324 ×. b) Trypanosomes are clearly visible (arrows). Magnification 800 ×.

The pineal gland was recovered only once. It showed infiltration of the connective tissue septa by numerous mononuclear cells and trypanosomes.

Presence of trypanosomes. Trypanosomes were easily found in the leptomeninx and in the Virchow-Robin spaces. They were absent in inflammatory granulomas but abundant in the parenchyma, in relation with inflammatory foci or apart from them (fig. 5).

Discussion

Our results are in agreement with those reported in the literature since Mott (16) on the trypanosomal meningo-encephalitis both in humans and in experimental animals, with respect to the cellular composition of the inflammatory lesions and to the topography of the same lesions.

Except for studies by Bourguignon *et al.* (5, 29) in which infected monkeys were employed, a study of Van Bogaert (28) dealing with infected dogs, and a study by Van Bogaert and Janssen (30) on human material, the myelin sheaths, using specific staining methods, have rarely been studied. In our material, except for paleness of the myelin sheaths surrounding perivasculitis lesions, the only demyelinisation areas were encountered in inflammatory nodules and in fact these areas were localised in areas of necrosis. The few reports on demyelinisation distant from inflammatory foci (5, 10, 30) have not received confirmation in other papers (4, 14, 23).

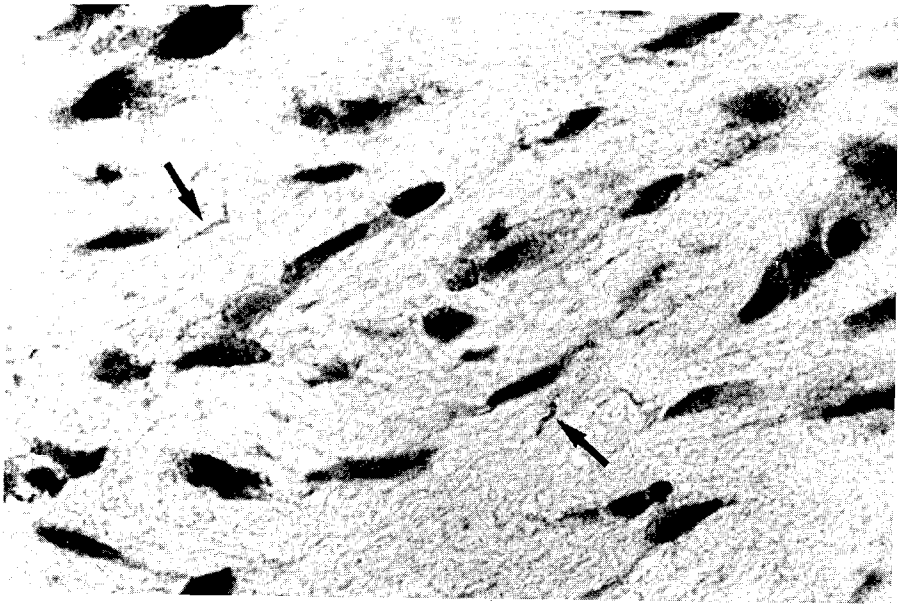

Figure 5
Extravascular trypanosomes in the white matter (arrows). Six months post-infection. Giemsa. Magnification 800 ×.

Neurons, in both human and experimental infections, are generally remarkably preserved (7, 16, 23, 27, 28, 30), except for neuronophagy within the inflammatory foci.

Mott (16) and other authors (9, 23, 26, 27, 28, 29, 30) have noticed that the cerebral lesions predominated at the brain base, especially around the brain stem. We have found, in the hypophyseal stalk of one of our rats, numerous trypanosomes and inflammatory cells, while the rest of the brain and the choroid plexuses were still normal. This finding could mean that the pituitary itself is responsible for the invasion of the brain. The pituitary, and especially the neurophypophysis, is not protected by a blood brain barrier at the level of its vessels (6, 8, 20). Hypophyseal lesions, with presence of many trypanosomes, have been described in some experimental studies (12, 15) but the relation with the brain lesions is not yet settled.

In summary, we have reported morphological findings in a rodent model with *T. b. gambiense* infection which are very similar to those described in human trypanosomiasis.

In a forthcoming paper, we will deal with the progression of these lesions with the advent of time.

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Neuropathologie en microscopie optique de l'infection chronique à *Trypanosoma brucei gambiense* chez le rat.

Résumé. — Une description neuropathologique détaillée des lésions cervicales observées chez le rat avec une infection à *T. b. gambiense* est présentée. Il existe une méningo-encéphalite généralisée comparable à celle de l'homme atteint de la maladie du sommeil. La lésion principale est une périvasculite tandis que les foyers de démyélinisation ou de lésions neuronales sont absents ou très rares.

Lichtmicroscopische neuropathologische bevindingen bij ratten met een langdurige *Trypanosoma brucei gambiense* besmetting.

Samenvatting. — Een gedetailleerde neuropathologische beschrijving van hersenletsels bij ratten met een terminale *T. b. gambiense* infectie wordt weergegeven. De letsels zijn gekenmerkt door een veralgemeende meningo-encefalitis vergelijkbaar met deze die bij patiënten met slaapziekte wordt aangetroffen. Het grondletsel is een perivasculitis, terwijl demyelinisatiezones en neuronale letsels afwezig zijn of slechts zelden voorkomen.

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