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THE ISOLATION OF *TOXOPLASMA GONDII* IN THE BLOOD OF A POSITIVE H.I.V. PATIENT

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The frequency of cerebral biopsies which are performed in order to diagnose the infection in AIDS patients with a compatible clinical aspect is justifying our intentions concerning the utilization and evaluation of reliable and non invasive new diagnostic techniques for the parasite identification in these risk populations. Consequently, the isolation of protozoa in organic samples means an unanswerable diagnosis of the infection in spite of the poor references about this aspect and its development may be considered as a priority in this group of patients.

There are some references concerning the isolation of protozoa from blood, cerebrospinal fluid and brain in animal inoculation and in tissular cultures with fribroblasts (J.C. Mason et al., 1987, *Transplantation, 44*: 588-5910), being the first of these techniques the more sensible (B.J. Luft & J.S. Remington, 1988, *J. Infect. Dis., 157*: 1-6).

There are few studies about the diagnostic methodology in these patients due to the negative serology in a great number of cases, even in the acute phase of the infection.

The infection survey in white mice after their intraperitoneal inoculation of biological samples is actually a good model of the diagnosis of the toxoplasmosis in these patients.

Within a protocol in thirty H.I.V. positive patients, in order to put in evidence the infection by *Toxoplasma gondii*, sets of mice were inoculated intraperitoneally with patients' buffy coat and ganglionar homogenate.

The patient from which the isolation was performed showed the clinical-epidemiological card as follows:

Risk factor: parenteral drug abuser; contact with animals: negative; age/sex: 21 years/man; clinical aspects: fever, lymphadenopathy and hepatomegaly; analytic: normal; microbiology: germs absence; ganglionic biopsy: follicular lymphoid hiperplasia; C.D.C. classification: IIIrd group.

The immunological study of seroimmunoglobulins show the values of the Ig G, Ig A and Ig M of 1790, 280 and 160 mg/dl respectively.

The study of the T4/T8 quotient and the response against mitogens (A-Concanavalin, Phytohemagglutinin and Phytolaca antigens) showed values of 0,80 and normal.

The mentioned patient was investigated with three conventional serological tests: Dye test, Direct agglutination and Indirect immunofluorescence. The diagnostic title for the Dye test was of 1:8. For the Direct agglutination and I.F.I. we used the commercial kits of Bio-Merieux with the considered diagnostic titles of 1:32 and higher for the Direct agglutination and 1:50 or higher for the I.F.I.

Parasite isolation — A set of three mice was inoculated with the buffy coat from 10 cc of the patient's blood. After an observation of 6 weeks, the mice were sacrified in order to obtain serum and to extract the brain for histological sections of 7 μm. The sections were stained with hematoxilin/eosine. A set of the brain histological sections was submitted to an immunological study through the I.F.I.

Serological study of toxoplasmosis — The above mentioned patients presented negative titles in the Dye test and I.F.I., but in the Direct agglutination the title was of 1:32 (80 U.I./ml).

Parasite isolation — The mice sacrified six weeks after the intraperitoneally inoculation of the buffy coat did no reflect presence of antibodies according to the three serological tests we used.

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The observation of the brain sections demonstrated the abundant presence of free forms (endozoites) in the encephalic mass (Figs 1 and 2).

The histoimmunological study of the brain revealed the presence of an amount of fluorescent proliferative forms in the tissue.

The toxoplasmosis is one of the main causes of morbi-mortality in the patients affected by AIDS. In our environment it is the fourth opportunist infection in the mentioned patients, preceded by the infections caused by ***Candida albicans***, Pneumonia by ***Pneumocystis carinii*** and mycobacteriosis (J. Mallolas et al., 1988, Comunicación 22-01 - III Congreso SEIMC, Granada, Libro de comunicaciones: 278; J. M. Mostaza et al., 1988, Comunicación 22-04 - III Congreso SEIMC, Granada, Libro de comunicaciones: 280.

There are important gaps of knowledge concerning the pathogeny of this infection, its peculiar neurotropism in the immunosuppressed patients and its unusual frequency in the patient affected by AIDS (B. J. Luft et al., 1984, ***JAMA, 252***: 913-917; M. J. D. Post et al., 1983, ***Am. J. Roentogenol. Radium. Ther Nucl. Med., 140***: 861-868).

The serological diagnosis in these patients is charged with important difficulties. The certain diagnosis depends on the protozoa isolation or its visualization in tissular samples (R. E. McCabe & J. S. Remington, 1985, p. 1540-1549. In ***Principles and practice of infectious diseases***. New York: Wiley and Sons; J. Ruskin & J. Remington, 1976, ***Ann. Int. Med., 84***: 193-194).

In the whole of the studied patients in our protocol (30) 56.6% of them presented histoimmunological evidence of *T. gondii* mouse. We also consider as a relevant data that 53% of the patients presenting an evident infection did not show serological positivity.

In comparison with the relatively frequent isolation of the parasite in mouse through ganglionic homogenate there are no important findings concerning the parasite isolation in this patient's blood and we must emphasize that the above mentioned patients did not present any clinical evidence of the disease.

The majority of the authors have accepted that once adquired the infection by *T. gondii* it remains in a latent stage as cystozoite form preferently at the level of the central nervous system and muscle (J.S. Remington, 1958, ***Am. J. Ophthalmol., 46***: 261-267).

Usually, there is a situation of dynamic balance between the protozoa and the host, consisting in the break of cysts, the release of free forms and the early formation of new tissular cysts. In presence of a normal immunity this process is responsible for the maintenance of the antibodies (J.K. Frenkel, 1973, p. 343. In ***The Coccidia***. Baltimore. Hammond D.M.). But this situation may be radically different in the immunosuppressed patient due to the presence of parasitaemia and the absence of specific antibodies that are able to produce a critical pattern of encephalopathy due to the tropism of the protozoa.

Moreover, in these patients, the application of the histological patterns of Piringer-Kuchinka and Dorffman for the diagnosis of toxoplasmic lymphadenitis seems to be poorly adequated (R. F Dorffman & J. S. Remington, 1973, ***New Engl. J. Med., 289***: 878-881).

These new diagnostic techniques, when applied to patients affected by AIDS gave good results in the diagnosis of toxoplasmosis in highly complex populations.

A very interesting aspect was the permanence of the proliferative forms (endozoites) six weeks after the infection. Other authors (D. J. P Ferguson & W. M. Hutchinson, 1987, ***Parasitol. Res., 73***: 483-491) have met early forms after the experimental inoculation in mice in the eleventh day and have also observed the disappearance of proliferative forms twenty one days after the inoculation.

This finding poses an interesting question: how much time will be necessary to diagnose a toxoplasmosis by this procedure in these patients?

If future investigations are following this line, we have the possibility to obtain a clinical weapon of great value due to its specificity, sensitivity and quickness, concerning the diagnosis of toxoplasmosis in this group of patients.

Finally, the close correlation between the conventional histology and the histoimmunology demonstrates the high sensitivity of the white mouse when compared with *T. gondii* isolates undetectable through other procedures.

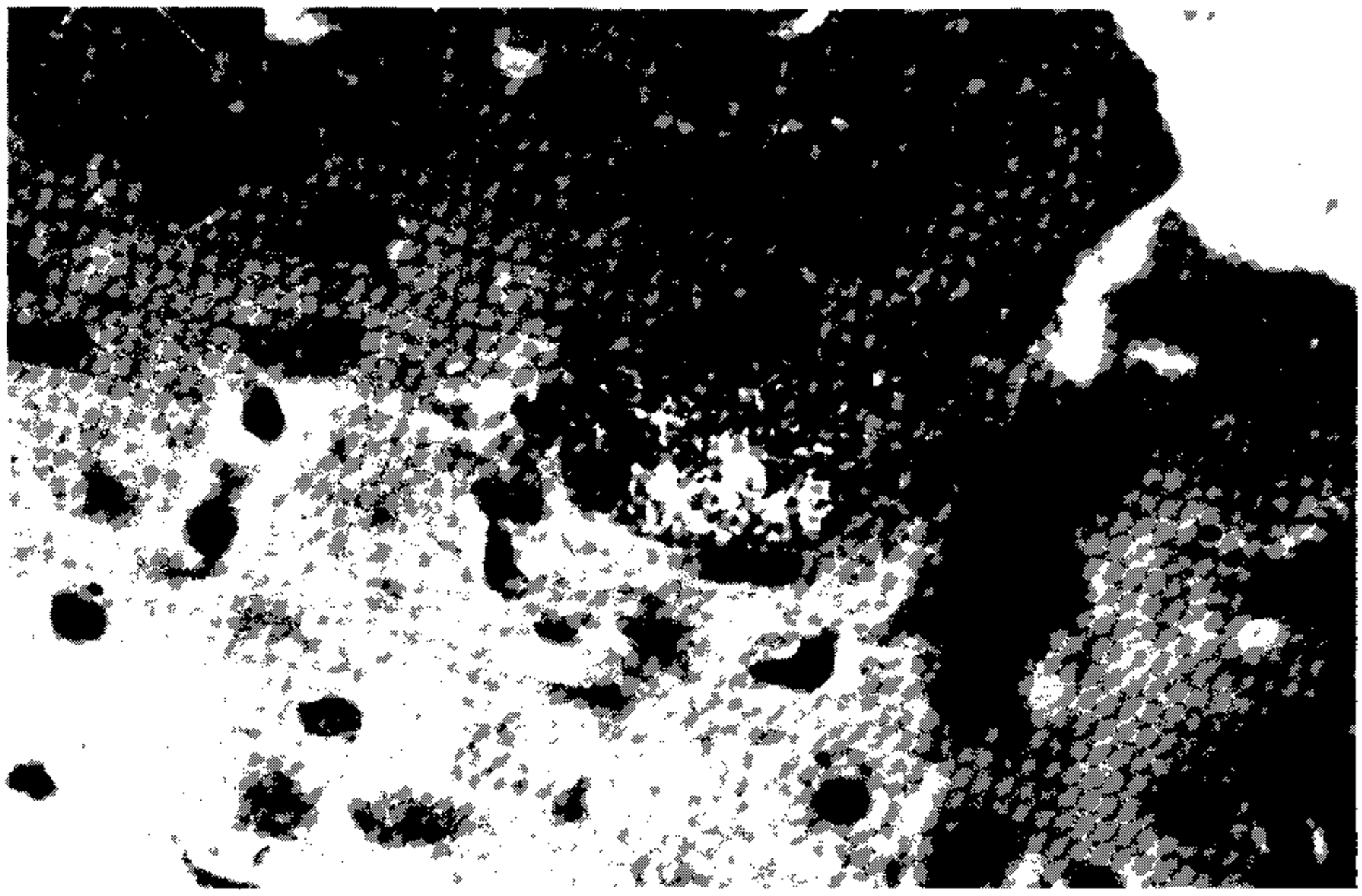

Fig. 1: accumulation of endozoites of *Toxoplasma gondii* in the mouse brain after the inoculation of the buffy coat of a H.I.V. positive patient. X 400 H/E.

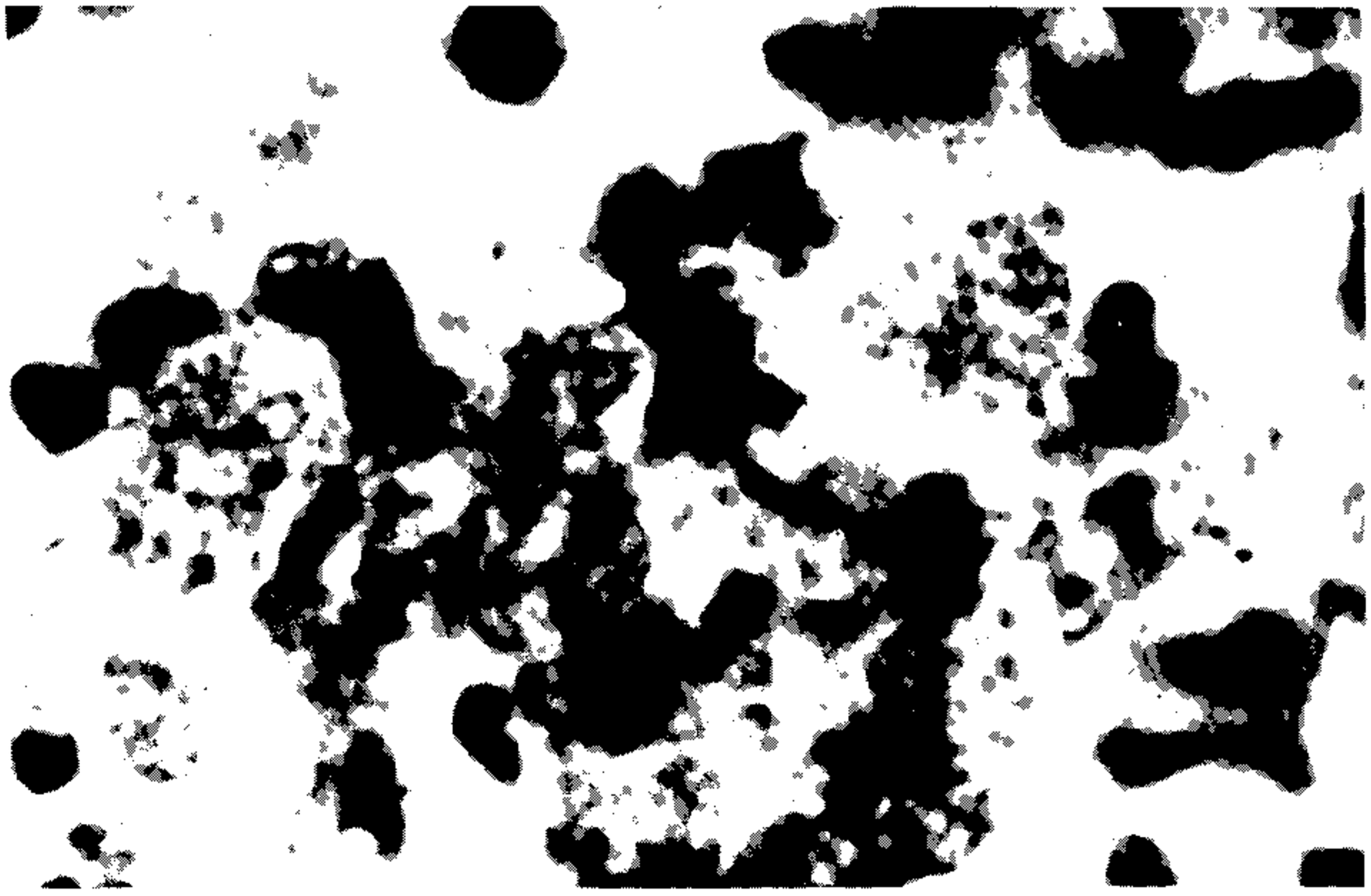

Fig. 2: proliferative forms (endozoites) of *Toxoplasma gondii* in the brain of intraperitoneally inoculated mouse with the buffy coat of a positive H.I.V. patient. X 1000. H/E.