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A SIMPLE TECHNIQUE FOR THE RAPID DIAGNOSIS OF RABIES IN FORMALIN-PRESERVED BRAIN

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ABSTRACT

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A simple technique is described for the rapid diagnosis of rabies in formalin-preserved brain. Brain tissue was emulsified, washed with phosphate-buffered saline and digested with trypsin. The digested material was stained according to conventional immunofluorescent procedures. Digestion with trypsin markedly enhanced the staining of rabies inclusions and eliminated non-specific staining of formalin-preserved brain. The method seems to be more accurate than histological examination.

INTRODUCTION

In rabies successful post-exposure treatment depends on rapid diagnosis and early treatment. Failures often occur because treatment was not started soon enough after exposure (Anderson, Daly & Kidd, 1966). At least 98% of rabies-infected brain specimens are identified as such by the fluorescent antibody test for rabies (FATR). Very early stages of infection, however, cannot be diagnosed by the FATR (Kissling, 1975). Such very early cases, where brains give false negative results, do not present any danger of infection even though they may eventually be found to be positive by mouse inoculation.

The FATR requires fresh, frozen or glycerine-preserved tissue (Lennette, Woodie & Magoffin, 1965). Accurate identification of rabies in formalin-fixed, paraffin-embedded brain section was recently shown to be feasible (Johnson, Swoveland & Emmons, 1980). The preparation of histological sections, however, is time consuming and requires sophisticated apparatus. In this study a simple technique is described for the rapid diagnosis of rabies in formalin-preserved brain.

MATERIALS AND METHODS

Specimens

Brain specimens presented for the routine diagnosis of rabies were used in this investigation. The specimens usually consisted of a portion of brain in 50% aqueous glycerine for the FATR and another piece in 10% formalin for histological examination. Occasionally brain, preserved in formalin only, was available. The specimens were examined within 5–15 days of collection.

Preparation of specimens

Brains preserved in glycerine were processed according to standard immunofluorescence techniques. Impression smears were made of different portions of brain and fixed in acetone for 15 minutes at −20 °C.

Approximately 2–3 g of formalin-preserved brain, including portions of hippocampus, brain stem and cerebellum, was emulsified in 15 mℓ of phosphate-buffered saline (PBS). The emulsion was centrifuged at 1 000 × g for 5 min and the supernatant discarded. The pellet was resuspended in 15 mℓ of 0,25% trypsin solution (m/v) containing 0,3% sodium citrate (m/v) and 0,6% sodium chloride (m/v). The pH was adjusted to 7,8. Following trypsin digestion for 30 minutes at 37 °C, the suspension was centrifuged and the pellet washed once in PBS. Smears were made of some specimens before digestion and of all the specimens after digestion. The smears were air-dried and fixed in acetone for 45–60 minutes at −20 °C.

Staining

Smears were stained according to standard immunofluorescent procedures (Lennett *et al.*, 1965). Hyperimmune antirabies gamma globulin, produced in a guinea-pig and conjugated with fluorescin isothyocyanate[1], was used for staining. Stained smears were examined with a Zeiss microscope, equipped with an HBO 200 watt mercury vapour lamp and a BG 12 exiting filter.

Evaluation

The immunofluorescence obtained with formaline-preserved, trypsin-digested specimens was compared with the fluorescence obtained with undigested and glycerine-preserved specimens. In Table 1 the results are ranked according to the ease with which a diagnosis was made. Ease of diagnosis was judged by the number of cells with inclusions, the brilliance of specific fluorescence and the absence of non-specific fluorescence.

TABLE 1 Comparison of the efficacy of immunofluorescence in the diagnosis of rabies in formalin-preserved, trypsin-treated and glycerine-preserved brain specimens respectively

Specimen No.	Days preserved	Species	Formalin-preserved	Trypsin-treated	Glycerine-preserved
1	5	Goat	D[1]	++	+++
2	5	Ox	+	++	+++
3	6	Ox	+	+++	+++
4	7	Mongoose	D	+++	+++
5	8	Mongoose	D	+++	+++
6	9	Ox	D	+++	+++
7	9	Ox	D	+++	+++
8	10	Kudu	−	++	+++
9	11	Ox	−	++	++
10	12	Goat	−	+	++
11	12	Otocyon	D	+++	+++
12	15	Otocyon	D	+++	+++

D = Doubtful
− = Negative
+ to +++ = Ease with which a diagnosis was made according to the number of inclusions, brightness of fluorescence and absence of non-specific fluorescence

The results in Table 2 give a comparison between the diagnosis made on formalin-preserved, trypsin-digested specimens and that by conventional diagnostic methods. Histological examination was done only when glycerine-preserved specimens were not available.

TABLE 2 The effect of trypsin treatment on immunofluorescence observed in known rabies positive and negative, formalin-preserved, brain specimens

Initial diagnosis[1]	No. of specimen	Diagnosis after trypsin treatment	
		Positive	Negative
FATR positive	38	37	1
FATR negative	13	0	13
Histologically positive[2]	3	3	0
Histologically negative	11	2	9

[1] Diagnosis based on duplicate brain specimens preserved in glycerine
[2] Diagnosis based on histopathology only, in absence of glycerine-preserved specimens

[1] E. Merck, Darmstadt, West Germany

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RESULTS

The preparation of smears, including trypsin digestion and staining of formalin-preserved brain specimens, was usually completed within 2 hours.

In a small percentage of trypsin-digested specimens fluorescence was slightly less brilliant than the fluorescence obtained with glycerine-preserved specimens. However, they yielded positive results typical of rabies. Antigen was observed as multiple cytoplasmic inclusions. Both large inclusions and diffuse, finely granular inclusions, sometimes outlining the entire cytoplasma, were observed. While it was sometimes possible to identify inclusions in undigested, formalin-preserved specimens, identification was markedly enhanced by trypsin digestion (Table 1). Very dull fluorescence and non-specific fluorescence of undigested specimens resulted in a doubtful diagnosis in 7 out of 12 specimens. No difference in fluorescence was observed in brains preserved in formalin for 5–15 days or in the brains of the 6 different species examined.

Out of the 41 specimens found to be positive with conventional tests 40 yielded a positive result after trypsin digestion of the formalin-preserved part of the brain (Table 2). Thirteen specimens preserved in glycerine and negative with the conventional FATR also gave negative results with digested, formalin-preserved brain. Two out of 11 histologically negative, formalin-preserved specimens gave a positive result after trypsin digestion.

DISCUSSION

The histological identification of Negri bodies is the least effective test for rabies (Atanasiu, 1975) and the one most likely to produce false positive results (Derakhshan, Buhmanyar, Noorsalehi, Fayaz, Mohamad & Ahouraii, 1978). The FATR is highly specific and gives a positive diagnosis in over 98% of cases. The test, however, requires fresh, frozen or glycerine-preserved specimens (Lennette *et al.*, 1965), and these are not always available.

The results obtained in this study clearly show that a rapid and specific diagnosis of rabies can also be made with formalin-preserved specimens (Tables 1 & 2). The slightly less brilliant fluorescence observed in some specimens does not in any way detract from the accuracy of the method. Out of the 41 cases found to be positive by conventional methods 40 were also found to be positive after trypsin digestion of formalin-preserved brain tissues. The sensitivity of the FATR on trypsin-digested rain is clearly illustrated (Table 2). Out of 11 cases proved to be negative on histological examination, 2 were found to be positive after trypsin digestion and examination by the fluorescent antibody technique.

With a fatal, zoonotic disease such as rabies highly specific diagnostic methods are essential. The examination of trypsin-digested, formalin-preserved brain seems to be more specific and more rapid than histological examination. However, the well-tested methods on fresh, frozen or glycerine-preserved specimens for the FATR and/or mouse inoculation should remain the foundation of rabies diagnosis. Where only formalin-preserved specimens are available or where adequate preservation of fresh material is not possible, the method currently described provides a rapid and accurate method for the diagnosis of rabies.

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