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LIGHT-MICROSCOPICAL DIAGNOSIS OF RABIES (*)

A Reappraisal

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Summary

The validity of reports suggesting that Lyssa and Negri bodies are non-specific in the light-microscopical diagnosis of rabies was investigated. With Seller's impression technique, a substantial proportion of specimens found to be non-rabid with the fluorescent-antibody technique showed structures indistinguishable from Lyssa or Negri bodies. Neither histological examination nor inoculation of animals with nonrabid Seller-positive material explained the nature of these rabies-like structures. The simplicity and reliability of the fluorescent-antibody technique and the occasional serious complications of prophylactic anti-rabies measures make the diagnostic use of Seller's method at best undesirable and at worst dangerous.

Introduction

Of the various diagnostic staining methods for rabies, Seller's procedure has been the most widely recommended "because of its accuracy and simplicity", (1). The use of these methods is based on the conviction that the presence of Negri

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bodies means rabies and nothing else. This conviction is so strongly held that when a Seller-positive specimen fails to stain positively with rabies fluorescent-antibody (F.A.) staining, it is described as "false negative",(2–4). The F.A. technique is now routinely used for the diagnosis of rabies in countries with the necessary technical and financial resources. However, in 20 of the 64 countries in which rabies is present, diagnosis is still based solely on light-microscopical criteria,(5). Furthermore, all recent editions of standard pathology textbooks maintain that the Negri body (light microscope) is specific to rabies, (6).

A Negri or Lyssa body is recognised as "characteristically acidophilic in staining reaction and takes on the pink to purplish-pink color in differential stains that use basic fuchsin or eosin with methylene blue as their base ... The most characteristic feature of a Negri body is its internal structure... the matrix of the Negri body has an acidophilic staining reaction, and contained within this magenta-red structure are small inner bodies (inner Korperchen), basophilic granules that stain dark blue to black. The size of these inner granules generally varies from 0.2 to 0.5 micrometers",(1).

In 1975 one of us (I.D.) reported the occurrence of Negri bodies in the brain of a patient who had died from Reye's syndrome. On the basis of this observation and a review of the literature, it was argued that the confident diagnosis of rabies requires the use of F.A. or electron-microscopical methods, or both. This conclusion was based on clinical similarities between variola and rabies and on the similarity in light-microscopical brain histology in the two diseases,(2). We have now re-evaluated the precision of Seller's method in the definitive diagnosis of rabies.

Methods

All specimens from animals suspected of having rabies that were delivered to the W.H.O. Collaborating Centre for Reference and Research on Rabies at the Pasteur Institute of Iran were included in this study. 233 specimens were examined between March 21, 1976, and March 20, 1977. Specimens from the following animals were examined: 68 dogs, 18 cows, 5 wolves, 3 cats, 3 foxes, 1 goat, 1 hyena, 1 jackal, and 1 human being.

Seller's and F.A. Staining

4 or more slides were prepared for the Seller's and F.A. staining techniflues (at least 2 for each staining method), according to the guidelines recommended by W.H.O. (1). The definitive diagnosis was based entirely on the result of the routine F.A. staining on the original material or on the material obtained from the brain of laboratory animals after intracerebral inoculation of material from the suspected specimen (brain or salivary gland). From all samples a sufficient amount was preserved in a sterile petri dish at–20°C.

Histological Evaluation

A piece of cerebellum or hippocampus (or both) of all samples was fixed in 10% formaldehyde for staining with haematoxylin and eosin (H. &E.) and Mann's stain. At least two 5 μm sections were made–more in cases which showed Negri or Lyssa bodies with Seller's method but were negative with rabies F.A. staining. The sections were examined for intracellular inclusions as well as other signs of viral encephalitis.

Mouse-inoculation Test

All F.A.-negative specimens and those considered positive or equivocal by Seller's staining but negative by F.A. technique were inoculated into mice. A 10% suspension of the suspect specimen was prepared in physiological saline containing 10% inactivated normal rabbit serum and antibiotics (500 I.U. penicillin and 2 mg streptomycin per ml). Ten mice 4–6 weeks old were inoculated intracerebrally with 0.03 ml of the supernatant of the suspension after they had stood at laboratory temperature for 30 min. The inoculated mice were observed for 20 days.

Results

Of the 233 specimens, 104 were positive in the F.A. examination. Another 4 were diagnosed as definitely positive after F.A. confirmation by intacerebral inoculation of mice with the suspected material. Thus, the percentage of definite rabies among the specimens was 44%. Routine evaluation of Seller's slides showed that 48 (20.6%) were positive for Negri or Lyssa bodies. There were 29 specimens which were considered "suspicious or positive" in the primary evaluation of Seller's slides. 14 of these were from brains which were liquefying by the time they reached the laboratory, and they were excluded from further statistical analysis.

The slides were re-evaluated by another observer, who had no knowledge of the results of the F.A. or Seller's staining or the animals' behaviour. Seller's preparations from 178 of the 233 specimens were still available for re-evaluation. 86 were positive by Seller's technique, but only 65 of these had definite rabies by the F.A. test. Thus, about 11.7% of the re-evaluated specimens were found to be positive for rabies by the Seller's method in the presence of negative F.A. results.

In both evaluations of the Seller's slides, the proportion of Negri to Lyssa bodies was very small, irrespective of the results of F.A. examination. H. &E. and Mann's staining of the formalin-fixed rabid material (hippocampus and/or cerebellum) showed clear evidence of encephalitis in 20.7% and the presence

of intracytoplasmic Negri or Lyssa bodies in 10.3% of the specimens. In only 6 specimens was the presence of rabies inclusion bodies demonstrated with all staining techniques used (F.A., Seller's, H. &E., and Mann's).

Discussion

In some instances of Seller-positive non-rabid samples the examiners were so confident of the rabid nature of the examined material that the bitten persons were called for interview with a view to starting prophylactic therapy. Only 2 hours later the negative result obtained with the F.A. method reassured the examiners and the patients that the animal was not rabid.

Neither the tissue sections nor the animal passage of Seller-positive F.A. negative specimens provided an insight into the nature of the non-rabid eosinophilic structures. The inclusion bodies found in the vast majority of the F.A. confirmed Seller-positive specimens had the form of a Lyssa body. This has also been noted by previous observers,(6). These inclusions were morphologically indistinguishable from the structures seen in the Seller-positive F.A.-negative samples.

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