Rabies Diagnosis

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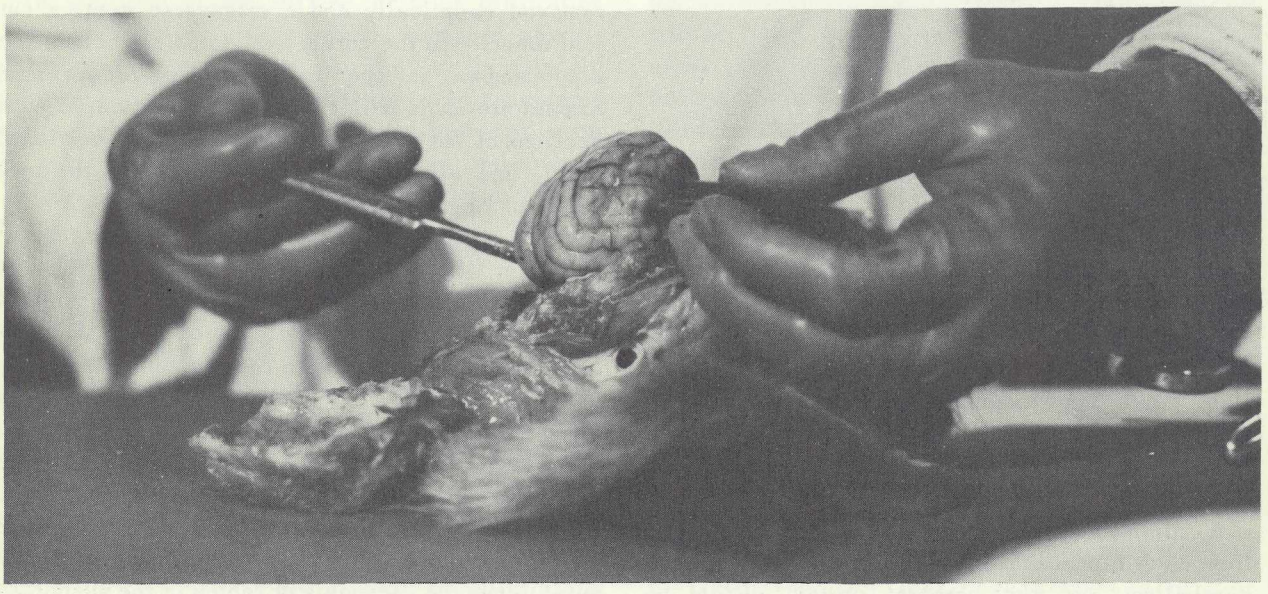
The laboratory diagnosis of rabies is primarily dependent upon the demonstration of Negri bodies, the pathognomonic inclusion of rabies, in central nervous tissue. This may be done on the original material or after passage through susceptible animals.

Heads of animals suspected of rabies should receive immediate attention upon arrival at the laboratory, so that, if indicated, treatment of exposed persons may be initiated. The brain should be removed aseptically so that animal inoculations may be performed. The skin is incised along the midline over the cranium and laid back. After removal of the temporal muscles, the skull is painted with tincture of iodine. Using sterile instruments, the calvarium is removed by sawing through the skull from each side of the *foramen magnum* to the frontal bones. These two cuts are united by a transverse cut through the frontal bones. The calvarium then is lifted off. After removal of the meninges and cutting the *tentorium cerebelli*, the brain is lifted out onto a petri dish or paper plate.

Negri bodies, if present, are most numerous in the hippocampus. This structure is exposed by a longitudinal incision into the lateral ventricles. In the case of the dog, this cut is made on the dorsal surface of the cerebral hemispheres parallel to and about one-half inch on either side of the midline of the brain. The hippocampus is recognized as a glistening white cylindrical structure.

Small transverse sections are cut from the hippocampus, and also from the cerebral and cerebellar cortices. These portions are placed, cut surface up, on a paper towel or wooden tongue depressor. A clean microslide then is pressed gently against the cut surface, using enough pressure to produce a thin impression of brain tissue on the slide. At least three impressions may be made on one slide. While still moist, the tissue is stained by pouring Sellers' stain from a drop bottle onto the slide, letting it remain for about 10 seconds, then washing under running tap water. The slide is allowed to air dry and is then ready for microscopic examination.



After removing the calvarium and cutting away the meninges, the entire brain is lifted out and placed onto a petri dish or paper plate.

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Sellers' stain has the advantage of fixing and staining in one operation. It is prepared as follows:

Stock solutions:

2 grams methylene blue dissolved in 100 milliliters absolute methyl alcohol (acetone free)

4 grams basic fuchsin dissolved in 100 milliliters absolute methyl alcohol (acetone free)

Working stain:

25 milliliters absolute methyl alcohol (acetone free)

15 milliliters stock solution methylene blue

2-4 milliliters stock solution basic fuchsin

The methyl alcohol and methylene blue solution are mixed in a drop bottle, and 2 milliliters of the basic fuchsin solution are added. A trial stain is made using methyl alcohol-fixed brain smears, which may be kept for this purpose. If, in the trial stain, the thicker portions appear bluish, basic fuchsin is added in 0.5 milliliter amounts until the properly stained smear appears reddish violet in the thinner areas, shading into purplish blue in the thicker portions.

Mixing the working solution of Sellers' stain.

Examination is carried out under oil immersion. With Sellers' stain, Negri bodies appear purplish red to purplish pink, in contrast to the light blue staining of neuron cytoplasm, dark blue nuclei, and copper-colored red blood cells. The matrix is light pink. Negri bodies contain basophilic inner granules which stain dark blue, and are the distinguishing staining characteristics of the Negri body.

A minimum of six brain smears, including one from each hippocampus, one from each side of the cerebellum and the cerebral cortex, should be examined. If Negri bodies can be demonstrated, the diagnosis of rabies is established.

If no Negri bodies are found upon microscopic examination, the possibility of rabies is not excluded, since at least 10 percent of animal brains examined microscopically in the laboratories and found Negri negative have been proved to be positive for rabies upon animal inoculation.

Small portions of hippocampus, cerebral cortex, and cerebellum saved at the time of brain dissection for staining, plus the medulla oblongata, are pooled and ground in a sterile mortar and pestle into a homogeneous paste. To this, enough sterile saline is added slowly to make a 10 percent brain suspension, which is centrifuged at 2,000 r.p.m. for 10 minutes. If evidence of bacterial contamination is found upon microscopic examination, or if the animal has been dead for quite a while, an antibiotic is added to the suspension before intracerebral inoculation into mice, to prevent death of mice in 2 or 3 days from bacterial contamination. Addition of 500 units of penicillin G (sodium salt) and 2 milligrams of streptomycin to 1 milliliter of the supernate of the centrifuged brain suspension and allowing to stand at room temperature for 30 minutes before mouse inoculation is sufficient to prevent the death of the mice from bacteria. With a ¼-milliliter syringe and a 27-gauge needle, .03 milliliter of the supernate is injected intracerebrally into at least four mice. Observations are made on these mice daily for at least 21 days, and symptoms of tremors, paralysis, humping, weakness of hindlegs, and death are recorded. As soon as the mouse is found dead, its brain is removed aseptically and a transverse section cut just anterior to the cerebellum so as to include the hippocampus in the cross section. Impression smears are made with this cut section just as with sections of the original brain, stained with Sellers' stain, and examined microscopically for the presence of Negri bodies.

Even though no Negri bodies were present in the brain of the suspected animal, if the virus of rabies were present in that animal, intracerebral injection of this brain will produce clinical symptoms of rabies in mice, with the constant occurrence of Negri bodies. The incubation period of rabies after the intracerebral inoculation of mice varies, with deaths occurring from the sixth day until the twentieth day, and in rare cases even longer. Finding of Negri bodies in the brains of mice dying after showing the above-mentioned symptoms establishes the diagnosis of rabies in the suspected animal. If mice remain normal for a period of 21 days after inoculation, the suspected brain is considered negative for rabies virus.