litis. Using the intracerebral route, the disease may be transferred across a strain barrier (Lewis to Wistar rat) in addition to transfer within an inbred rat strain (Lewis to Lewis rat). Evidence is presented that intracerebral implantation of lymphoid cells from donors sensitized to adjuvant only or from normal rats may result in injury of a type seen in allergic encephalomyelitis and believed to have an immunological basis.

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A Rapid Procedure for Staining Nissl Granules in Brain Tissue, to Be Used for Photomicrography.* (30156)

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Although the sectioning of frozen brain tissue is generally regarded as a useful technique for inspecting certain topographic and gross morphologic changes in brains after experimental surgery, several shortcomings of this technique have led many experimental neurologists to utilize more time-consuming methods for preparing their brain specimens. For example, it is widely held that frozen sections stain rather poorly and, while they may be adequate for gross microscopic inspection, they are rarely of such quality as to provide a useful material for photomicrographic illustrations. In experiments which involve the study of various changes of the nerve cell body, the use of celloidin or paraffin embedded material has been regarded as imperative, because some artifacts, such as vacuolation of normal cells, usually observed in frozen material, make interpretations of the results difficult, if not impossible.

In this laboratory it has been found, however, that frozen sections can be prepared in such a way that they are of comparable quality to celloidin embedded material in every respect. This involves dehydration of individual sections in various grades of alcohols *before* they are mounted. Such a dehydration

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shrinks the brain tissue rather uniformly. Mounting the sections in ethanol-gelatin mixture, although it introduces some hydration, does not cancel the effect of the previous dehydration. This procedure provides for a greater contrast among cells of various nuclear groups and, therefore, the material becomes adequate for a detailed microscopic analysis as well as photographic presentation. Vacuolation of normal cells can be usually avoided by soaking the brain specimen in 20% ethanol overnight before sectioning. The procedure is a rapid one; approximately a 3½-hour period is sufficient for making the first section of a series available for microscopic study. In contrast to this, more than a month is usually required for preparing celloidin embedded brain tissue.

The procedure described in the staining schedule should provide no difficulties. Some steps in this schedule, however, require special care. The ethanol-gelatin solution should be stirred well before sections are placed in it and a gentle stirring is advisable even during the time the sections float in this mixture. Any clumping of gelatin on the sec-

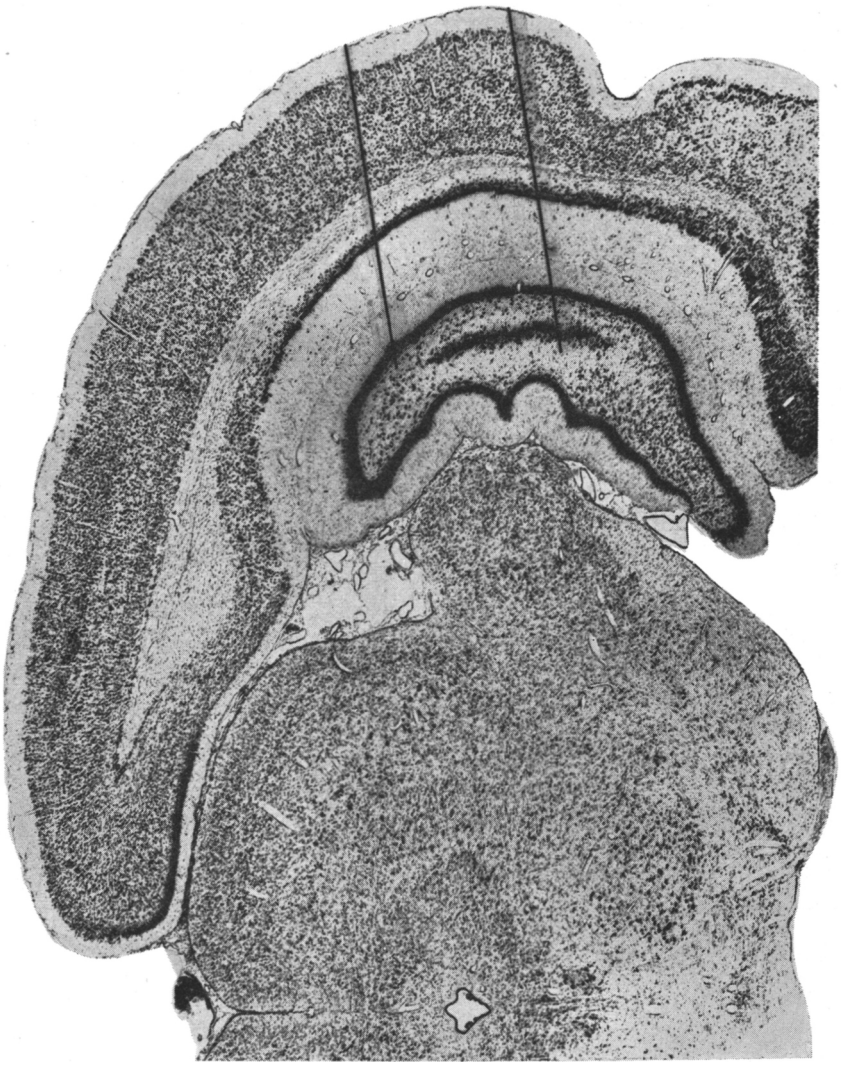

FIG. 1. Portion of a Nissl stained coronal section of a rat brain. Celloidin embedded material. × 24, 25 μ.

tions may result in spotty staining. Mounting of the sections is accomplished according to Albrecht's method(1).

Staining of the section used for Fig. 2 was rather light. If a greater contrast is desired, it is advisable to overstain and then control the speed of differentiation by immersing the mounted sections from time to time in ethanol-acetic acid solution (pH between 5.3 and 6.0) while differentiating them in 95% ethanol.

Staining schedule

1. After fixating the brain by using a 10% formalin in 1.25% saline,
2. cut frozen sections 20 to 40 μ thick.
3. Dehydrate individual sections in 70% ethanol for 15 to 30 minutes, and
4. in 95% ethanol for 60 to 90 minutes.
5. Place sections in a mixture of equal parts of 1% gelatin and 80% ethanol for 3 to 5 minutes at 45°C.
6. Mount by blotting the sections with filter paper.
7. Continue dehydration in
   95% ethanol for 5 to 10 minutes,
   normal butanol for 15-20 minutes, and
   methyl salicylate for 15 to 20 minutes.

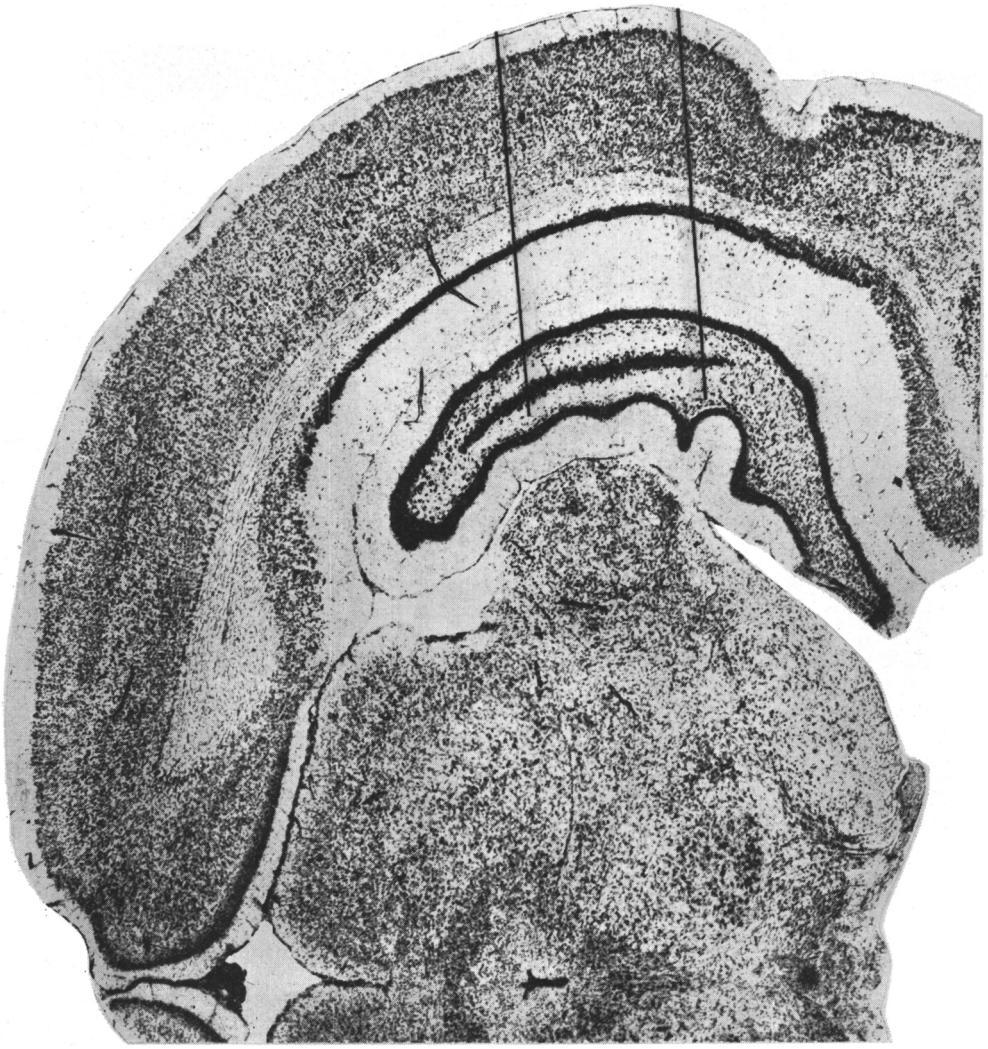

FIG. 2. Portion of a Nissl stained coronal section of a rat brain prepared to match the section shown in Fig. 1. Frozen material. × 22, 25 μ.

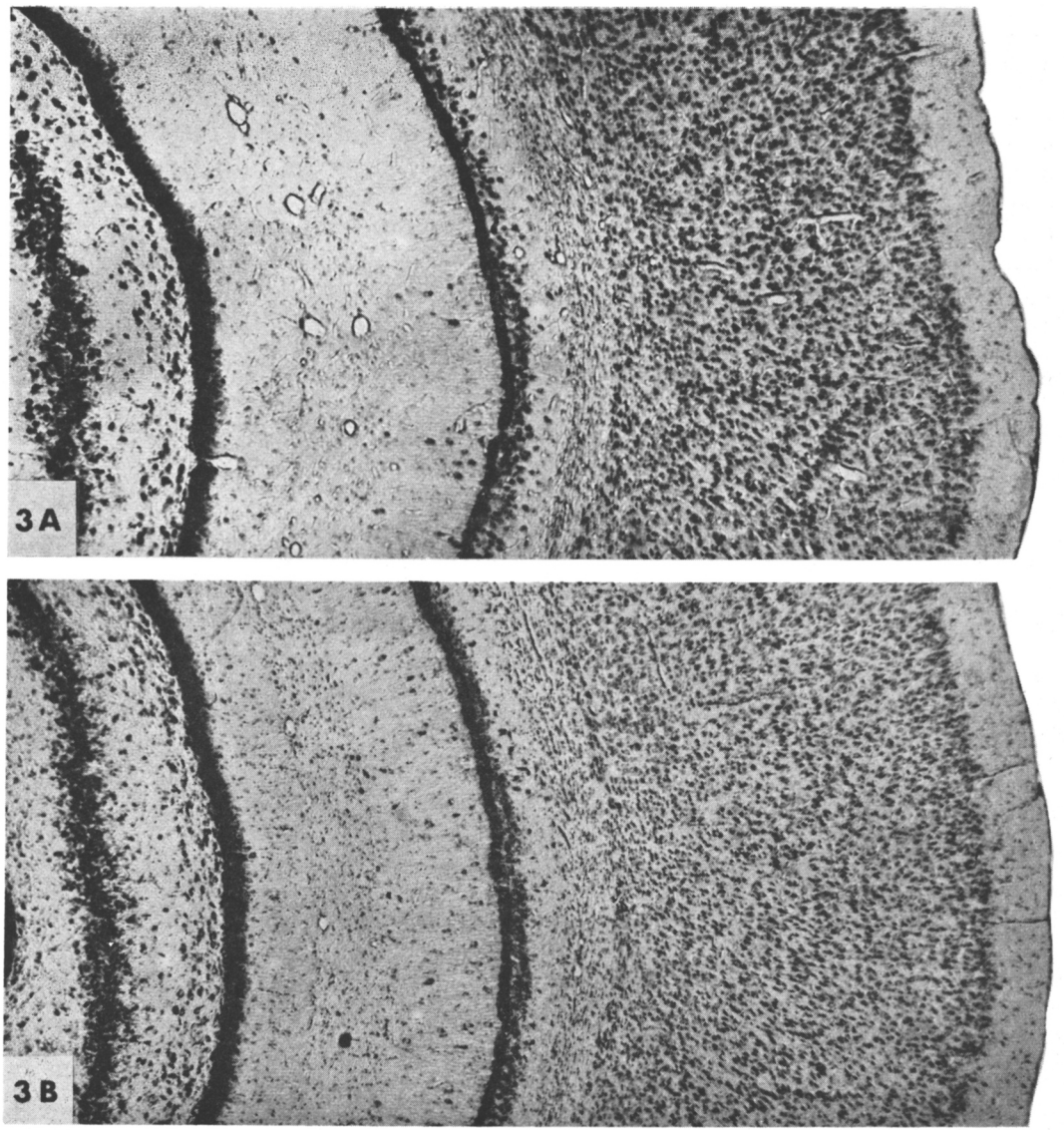

FIG. 3. A. Detail of area outlined in Fig. 1. × 72. B. Detail of area outlined in Fig. 2. × 70.

8. Hydrate in
   95% ethanol for 3 to 5 minutes,
   70% ethanol for 5 to 10 minutes, and
   distilled water for 30 to 40 minutes.
9. Stain in a 0.25% cresylecht violet solution with its pH lowered to 5.3 by adding glacial acetic acid. Depending on thickness, staining time between 8 and 12 minutes is adequate.
10. Wash in distilled water for 1 to 3 minutes, and
11. in 70% ethanol for 5 to 10 minutes.
12. Differentiate in 95% ethanol for 20 to 40 minutes.
13. Dehydrate in normal butyl alcohol for 5 to 10 minutes.
14. Clear in xylene for 5 to 10 minutes.
15. Cover in synthetic resin.

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