

achromotrichia factor could not be demonstrated, when the same ration without added crystalline vitamin B₆ was used.

It is to be expected that the new vitamin K-deficient diet will be found a useful tool in studies of some of the biological problems involved in vitamin K metabolism, since the diet apparently does not permit a bacterial K-vitamin synthesis.

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A Method for Staining of Carious Lesions in Teeth.*

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In the study of experimental rat caries large numbers of animals must be employed to obtain significant statistical results. As a result the methods for examination of the molar teeth become very important. Some investigators have employed the very tedious method of preparing stained thin-sections by the ordinary technics. Others have attempted to reduce the time and expense by resorting to either gross inspection of the carious teeth or to rapid grinding and examination of ground sections. At the suggestion of Dr. B. F. Miller of the University of Chicago the author has developed a rapid, simple and precise method for the staining of carious areas in rat molars (and also in human teeth). Previously,¹ the author had developed a method for the demonstration of insoluble calcium salts in the tissues. It was found that this method cannot be applied to the study of teeth because the silver solution used in the technic will not penetrate the dense dental tissues. Dentin will get a very superficial black coating but enamel is entirely unstained. However, the surprising observation was made that carious areas stained deep black. This can be explained by the greater permeability to the silver solution of the rarefied carious tissue. That the action of acid actually increases the permeability of enamel and dentin was proved by the following experiment: into healthy, extracted human teeth symmetrical holes were drilled, two into each. One of these

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¹ Gomori, G., *Am. J. Path.*, 1933, **9**, 253.

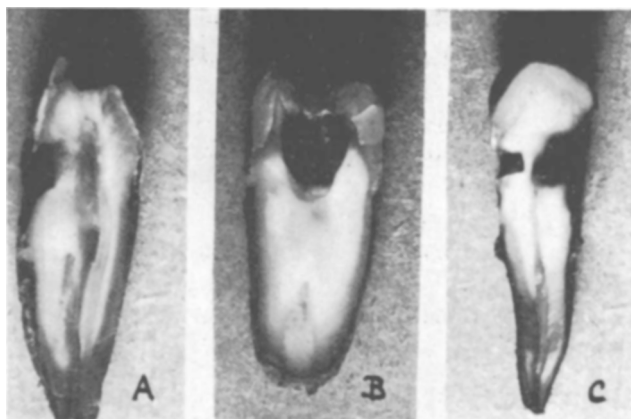


FIG. 1.
Ground Surfaces of Human Teeth.

- A. Proximal caries.
- B. Secondary caries under a filling.
- C. Two holes drilled into a tooth. The left hole, treated with distilled water; the right hole, treated with lactic acid.

holes was packed with cotton soaked in distilled water, the other with cotton soaked in 5 to 10% lactic acid. The holes were sealed with paraffin. The cotton plugs were changed twice daily. After 3 days the plugs were removed, the teeth washed in distilled water and stained according to the technic mentioned. The holes that were treated with distilled water only did not show any staining by silver, whereas the holes packed with lactic acid were surrounded by a black area up to 2 mm in width (Fig. 1).

The silver impregnation method is very suitable for the examination of large numbers of rat jaws for carious lesions. The stained jaws may be directly examined under the surface microscope, or decalcified, embedded in celloidin and made into microscopic sections.

The method is as follows: 1. Fix in neutralized 80 to 95% alcohol or in neutral formalin. 2. Wash tissues in repeated changes of distilled water. 3. Impregnate with a 0.25 to 0.5% solution of silver nitrate for 12 to 24 hours. 4. Wash in many changes of distilled water for at least 24 hours. 5. Reduce in a 5% solution of sodium hypophosphite for 24 hours. 6. Wash under the tap for several hours. 7. Fix in a 2% solution of sodium thiosulfate (hypo) for 12 hours. 8. Wash under the tap for several hours.

The stained jaws may be either dehydrated in alcohol, cleared in cedar oil and examined directly under the dissecting microscope,†

† This variant of the technic has been used by Dr. B. F. Miller (Proc. Soc. Exp. Biol. and Med., 1938, **39**, 389).

or they may be decalcified and embedded in celloidin. Six or more jaws may be included in a single celloidin block. The sections can be counterstained with any stain desired. Sections of several to many dozens of rat jaws depending on the number of sections required, can be cut, stained and mounted in a few hours.

In the finished sections the carious areas are stained deep black (Figs. 2 and 3). A superficial layer of bone and dentin, up to about 20 micra in thickness, will usually be stained black or dark brown, and, in addition, some superficial osteoblasts with their processes. Calcium-containing debris in the fissures will show up as a black, finely granular mass. Healthy enamel is unstained, the exposed



FIG. 2.
Large destructive lesion in a rat molar. $\times 17.5$.

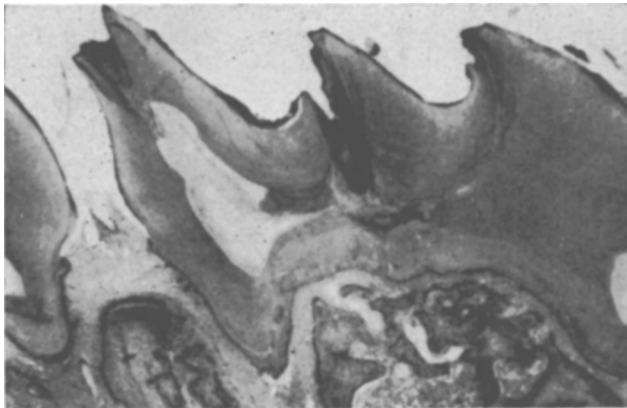


FIG. 3.
Small, deep lesion in a rat molar, starting at the side of a cusp. $\times 35$.

dentin at the tips of the cusps however, often shows a more or less superficial staining. Examination of the sections for lesions is very easy because the carious areas take such an intense, conspicuous black stain.

For decalcification one should employ a 5 to 10% solution of sulfosalicylic acid instead of mineral acids which attack the silver deposits.

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Susceptibility of Field Mice and Meadow Mice to St. Louis Encephalitis.

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In the vicinity of St. Louis the 3 most common species of wild mice are the field mouse, *Reithrodontomys megalotis*, the house mouse *Mus musculus* and the meadow mouse, *Microtus ochrogaster*. As long ago as the epidemic of 1933 efforts were made to trap mice in the homes of encephalitic patients. Several field mice were captured but no representative of either of the other species were obtained in such homes. Beginning in 1934 we tested the susceptibility of field mice to the virus of St. Louis encephalitis and found that they can be infected both by intracerebral and intranasal inoculation. Harford, Sulkin and Bronfenbrenner¹ have reported that the house mouse, *Mus musculus*, is also susceptible to this infection.

More recently we have captured a large number of field mice and also have been able to capture a number of meadow mice. Tests for the susceptibility of these strains of mice to the encephalitic virus have been carried out using simultaneous tests on white Swiss mice for comparison.

¹ Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1939, 41, 331.