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Colloidal carbon as a multilevel marker for experimental lesions

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Summary. The use of colloidal carbon for the anatomical marking of experimental lesions is proposed. Visualization of the lesion site may be readily performed through this procedure at the macroscopic, light microscopic, and ultrastructural levels in the same specimen. The chemical inertness of the marker and its relative permanency greatly add to its usefulness.
Key words. Carbon; central nervous system; demyelinating diseases; electron microscopy; injections; light microscopy; lysophosphatidylcholines.

There is a modern trend in neuroscience to place minute lesions, or microinjections, into the nervous system to serve a variety of experimental goals. There is often a need to identify those foci sequentially at macroscopic, light and electron microscopic levels. A difficulty in localizing the lesion while preparing tissues for histopathology may emerge from the small size of the locus and the lack of inflammatory reaction.

This note reports the use of colloidal carbon as a morphological marker for multilevel identification of such injection sites, using as an example the injection of lysolecithin into the spinal cord of experimental animals which has proven to be an extremely useful model for studying demyelination and remyelination in the central nervous system[1,2].

Materials and methods. The colloidal carbon suspension was prepared as follows: Three to 5 ml of India ink ('Pelikan' special ink, batch C11/1431A) were dialyzed against distilled water for 48–72 h through a Spectrapor semipermeable membrane (Spectrum Medical Industries, Inc., Los Angeles, CA 90054) with 12,000 MW cut-off. The suspension could be stored in the refrigerator for many months. To mark the site of lysolecithin injections, one drop of dialyzed ink was added to 1 ml of 1% lysophosphatidylcholine solution. The surgical operation has been described elsewhere[2]. This procedure produces well-localized, reproducible lesions of known age.The size of the lesion may vary according to the amount of lysolecithin injected. Injection of 2 µl of a 1% lysolecithin solution into the lower thoracic or upper lumbar region of the rat spinal cord produces a focal area of primary demyelination of approximately 0.4 mm diameter. At different postoperative times, intracardiac perfusion fixation was performed and spinal cords were dissected and inspected macroscopically to locate the injection sites. Subsequently, tissue blocks containing the lesions were processed and embedded in Epon. One micrometer thick sections were stained with Stevenel blue for light microscopy[3] or reacted with antiserum to myelin basic protein (MBP) for immunocytochemistry[4]. Thin sections were stained with lead citrate for electron microscopy[5].

Results. The injection tracks in lysolecithin-injected animals appeared macroscopically as straight black lines in the ventrolateral white matter of the spinal cord (fig. 1). The lesions remained labeled for at least 70 postoperative days, the longest survival time allowed in this series.

Light microscopy of 1 µm thick sections reacted with anti-MBP serum revealed reaction in the healthy, but not in the demyelinated, area. The presence of carbon could be readily detected within the affected tissue (fig. 2). At later stages, when remyelination took place (fig. 3), carbon tattoo remained as an indicator of the original site of lysolecithin injection.

The removal of carbon took place slowly, primarily by the action of invading phagocytes. The electron micrograph of figure 4 depicts part of a macrophage actively involved in the phagocytosis of both myelin debris and colloidal carbon.

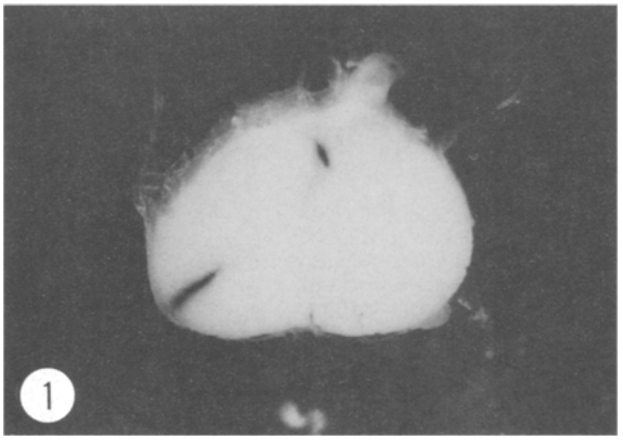

Figure 1. Cross section of an unstained spinal cord block, with the site of lysolecithin injection marked with carbon. × 15.

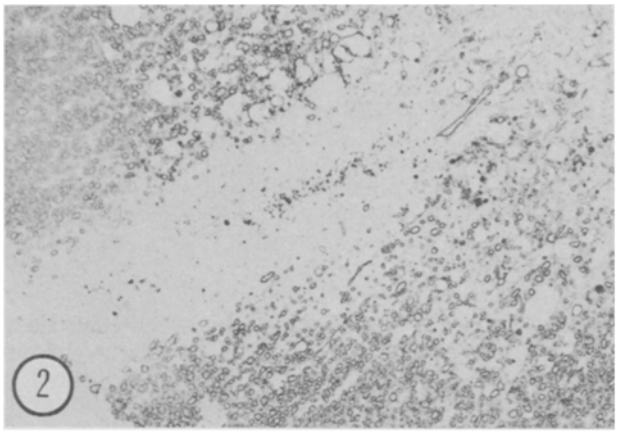

Figure 2. Low power micrograph of a lesion track at 28 days postoperatively. Immunocytochemistry with anti-MBP serum. Positive reaction is seen in healthy, but not in damaged, tissue. Marking of the lesion by carbon is evident. × 128.

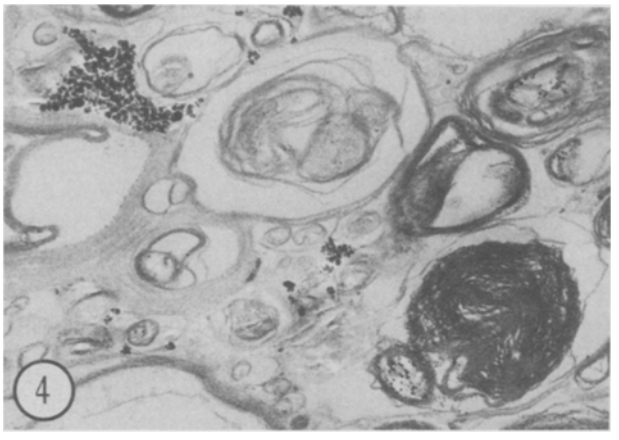

Figure 4. Electron micrograph demonstrating active ingestion of carbon particles and tissue debris by a macrophage. Only part of the cytoplasm is shown. × 9200.

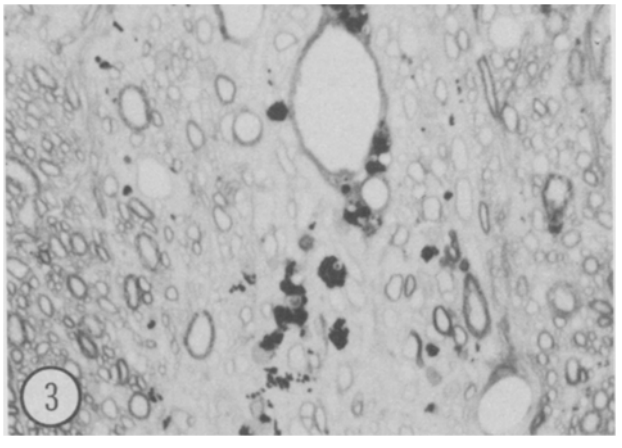

Figure 3. Optical micrograph at 60 days postoperatively. Remyelination has occurred, but the tracer is still present indicating the site of the original injection. Stevenel blue staining. × 550.

Discussion. Colloidal carbon has been used in experimental histopathology for a variety of purposes since the mid-nineteenth century[6]. In our laboratory, it was used for establishing the hematogenous origin of brain macrophages[7] and for correlating ophthalmoscopic with histopathologic findings in an experimental retinal immunopathy[8]. In the present report, it served as a marker of the lesion site in the lysolecithin model of demyelination.

There are several advantages in using colloidal carbon as a marker. In many instances, lesions placed experimentally in the nervous system or in other tissues are invisible to the naked eye or even to observation with a low power dissecting microscope. A substance injected in small amounts into the neural parenchyma may or may not produce a lasting histological change, and even less likely a macroscopic lesion. The method used by us permits a sequential visual location of the injection site a) *macroscopically,* without the need for microtomy, b) *microscopically,* without the need for histological staining, and c) *ultrastructurally,* without resorting to histochemical reactions. All of the above steps may be easily followed in the same specimen. Compared to other tracing substances like horseradish peroxidase, colloidal carbon displays longer permanency, as it is easily ingested, but not digested, by macrophages and other cells[9].

Colloidal carbon is a particulate foreign substance which, when released into the circulation, is cleared up by the reticuloendothelial system. In large doses, it may actually block this system[10]. However, the amount used for local tracing purposes is infinitesimal compared to that needed for effective blockade. Furthermore, the ingestion of amorphous carbon particles, or even diamond dust, by macrophages does not seem to cause the harmful effects produced by other particles of comparable size such as silica, which leads to cell lysis[11]. Therefore, colloidal carbon may then be considered practically inert and serving merely to tattoo the lesions.

The applicability of the method is certainly not limited to neurobiology. Colloidal carbon seems to be a very useful tool in tracing lesions placed in other tissues as well (del Cerro et al., work in progress). Thus, it may be effectively used as a multilevel marker in the general field of experimental pathology.

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