A New Anaesthetic Technique for Slugs

During recent studies on the in vitro culture of organs of the Grey Field Slug, *Agriolimax reticulatus*, it was found necessary to use anaesthesia. Since organs were to be explanted from these anaesthetized slugs, it was necessary that the anaesthetic to be used should give 100% recovery and it would also be advantageous if the anaesthetic left no residue in the animals' tissues.

A survey was made of the methods for anaesthetizing and narcotizing gastropods[1]. The methods which were used for slugs gave inconsistent recovery rates varying from 0.92%, and often involved the use of complex chemicals.

Since carbon dioxide is widely used as an anaesthetic, especially with insects, it was decided to study its effect as an anaesthetic for slugs. Little mention is made in the literature of the use of carbon dioxide as an anaesthetic for gastropods. It has been used in conjunction with Sevin (1-naphthyl N-methyl-carbamate; Union Carbide)[2] to relax muricids and also to relax *Nucella lapillus* prior to fixation[1]. Carbon dioxide has been used in conjunction with Nembutal and MS 222 (Sandoz: meta-amino-benzoic acid ethyl ester methansulphonate)[3] as an anaesthetic for *Lymnea stagnalis*. Recently[4] gaseous carbon dioxide has been used for anaesthetizing slugs but no method is described.

A plastic container, 6 cm deep with an outlet in the base, was used as the anaesthetic chamber. A layer of cotton wool, about 3 cm thick, was placed in the bottom of the container and moist filter paper placed upon this. Gaseous carbon dioxide, saturated with water, was passed into the chamber via the basal inlet. The slug was placed upon this moist filter paper and a perforated lid placed on the plastic container. The slug usually remained fairly inactive and the time taken for it to succumb was very variable, sometimes in excess of 1 h.

Using the same chamber, but with no basal inlet, this technique was modified for use with solid carbon dioxide. Crushed solid carbon dioxide (Drikold, I.C.I.) was placed under the cotton wool, in a layer 1 cm deep, and the gas given off by this was used as the anaesthetic. When the slugs were placed in this chamber they were very active and secreted mucus, but not excessively. They moved about 3 times their length before succumbing to the effects of the anaesthetic. When anaesthetized the animals were invariably in a fully extended state, often with the penial sac everted.

Using this technique anaesthesia is rapid and, even after 5 min, sufficient for minor operations. 10–15 min has been shown to be the best exposure time. Strong mechanical stimulation of the anterior end of the slug during the first 2–3 min after removal from the chamber produces no response. The slugs slowly recover from the anaesthetic and appear normal after 20–30 min. Extending the time in the anaesthetic beyond 15 min does not delay the animals recovery time to any extent. However operating time can be extended indefinitely by working in an atmosphere of carbon dioxide. Recovery is 100% unless the exposure to the carbon dioxide is well in excess of 60 min.

This anaesthetic technique has been used in this laboratory for the past 2 years. During this time it has been found to be extremely reliable[5].

Résumé. On a étudié l'effet anesthésique de l'anhydride carbonique sur les limaces. La technique décrite permet une anesthésie totale pendant une heure avec une guérison de 100%.

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Observation of Nerve Fibers in Incident Light

In previous publications we have described the development and application of a new type of microscope employing tandem scanning[1,2]. This microscope was designed expressly for in vivo observation of brain cells. A major limitation in the first versions of this microscope was the great light loss entailed by the scanning system, resulting in such low image brightnesses that the only satisfactory source for observation of nervous tissue was the reflected light of the sun. Recently one of us (M. D. E.) designed a scanning system compatible with the original microscope, but having a much greater coefficient of light transmission, thus making possible observation of unstained nervous tissue with a conventional artificial light source.

The scanning system is based on the wheel illustrated in Figure 1, silvered except for 800 symmetrically placed radial slits of 40 μ width. Light from a 200-watt mercury short arc lamp (PEK) was focused on the wheel which was rotated at 200 rpm. The radially symmetrical slits on opposite sides of the wheel were made optically congruent by inverting prism systems as in the original tandem scanning microscope[2].

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The scanning system of the original tandem-scanning microscope was based on a Nipkow wheel with 26,400 holes of 90 μ diameter arranged in 80 interlocking Archimedean spirals. The coefficient of light transmission for a single transit through this wheel was 0.08. The corresponding coefficient of light transmission through the new, slit wheel is 0.15.

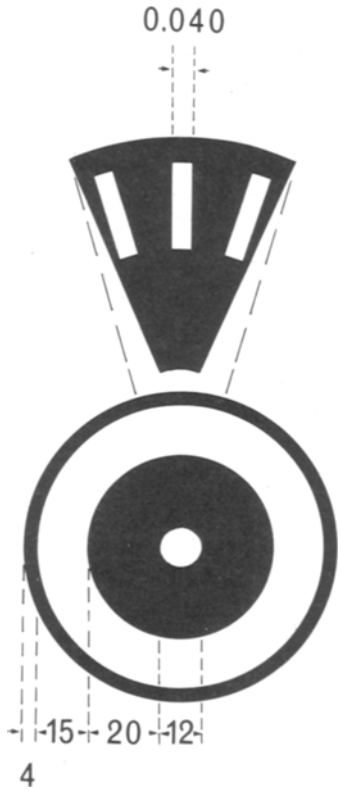

Fig. 1. Schematic of scanning wheel. The width of the slits on the exploded segment in the upper part of the drawing is greatly exaggerated. All dimensions indicated in mm.

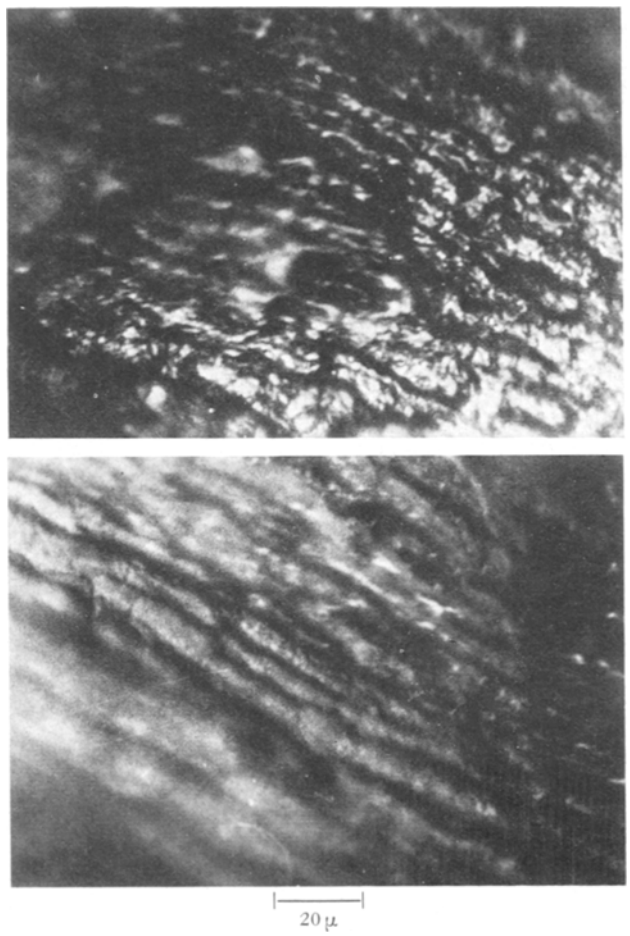

Fig. 2. Photographs of unstained fibers of cat lumbar dorsal rootlet. The photograph on the bottom was taken with the scanning system operating. The photograph of the same rootlet on the top was taken with the scanning system removed. The photographs were made on Tri-X film developed in Diafine. The light source was a 200-watt mercury arc lamp.

With the new scanning system, we observed and photographed individual fibers of unstained nerves in thick bundles. Figure 2 shows such fibers in a lumbar dorsal rootlet of a cat, fixed but unstained. The picture at the bottom of the figure was taken with the scanning microscope. The depth of focus in the tandem-scanning microscope is very shallow. Only a few fibers are in focus at any given focal plane. A photograph of the same rootlet without the scanning system is shown on the top, with the focal plane of the objective at a position comparable to the photograph on the bottom. Without the scanning system there is no way of screening out the scattered and flare light from the entire nerve bundle.

For most applications within the CNS itself, even the new system does not achieve sufficient image brightness. We are investigating 2 further innovations: (1) redesigning the scanning system so as to make the inverting system unnecessary; this should decrease problems of internal light loss and scattered light from the surfaces of the inverting systems, and (2) adapting the microscope for use with a laser light source. Preliminary investigations using a 5 mwatt He-Ne continuous wave laser (Spectra-Physics Model 120) with spatial and diffusion filters have resulted in photographs of quality approaching those with the mercury arc lamps, but of greater image brightness[3].

Résumé. On décrit une version améliorée d'un microscope «tandem scanning», à lumière réfléchie. Ce microscope utilise un système plus efficace de scanning, qui produit une image plus lumineuse que celle qu'on a pu obtenir jusqu'à présent. Avec ce microscope des fibres de nerfs ont pu être photographiées sans coloration.

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