SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD OF PREPARING PARAFFIN SECTIONS OF BONE

THE following method, while having no claim to originality, has given such satisfactory results that it seems worth while to suggest it to others.

Pieces of bone as large as $2 \times 2\frac{1}{2}$ cm by 2 mm have been used with excellent results. Decalcification may be carried out before or after the piece for sectioning is selected. For decalcifying, the writer uses 5 per cent. or 10 per cent. nitric acid, followed by a 5 per cent. solution of sodium sulfate (24 hours) and running water (24 hours). After dehydration, specimens are cleared with equal parts of absolute alcohol and chloroform, two changes of chloroform, and are then passed through several changes of paraffin (melting point 47° to 49°), and are finally embedded in harder paraffin (56° to 58°). Beginning in the morning, specimens can be dehydrated during the day, left over night in chloroform and infiltrated in paraffin and embedded the following day. It is possible to embed small pieces of bone within 14 hours after removing them from 70 per cent. alcohol. For rapid embedding it is essential that the solutions and specimens are kept in tightly stoppered bottles and that the atmosphere is fairly dry; occasional gentle shaking hastens diffusion. Relatively large amounts of solutions are desirable, and these may be used repeatedly if kept well stoppered.

After embedding, the side of the bone to be sectioned is exposed to water,[1] and best results are obtained for most bony structures only when the entire surface to be sectioned is exposed. After remaining in water until the tissue shows a noticeable swelling, usually from 2 to 10 days, the exposed surface is dried and dipped in melted paraffin, which is then more uniformly mixed with the original paraffin block by means of a hot needle. If cutting becomes more difficult after a few sections are removed, the block should be returned to water for a longer period.

Serial sections (10 μ) are cut and mounted with water-glass fixative: 100 cc of water, 1 cc standard water-glass solution and 1 cc concentrated ammonia water.[2] (Treating of sections with 70 per cent. alcohol slightly acidified as recommended by Ullrich has been unnecessary for our work.) Clean slides are flooded with the solution, sections added, the whole warmed slightly and the excess fluid drained. Slides should be dried at least 48 hours. The use of water-glass fixative has eliminated one of the most serious difficulties previously encountered in making paraffin sections of bone; namely, the loss of sections in the staining process. This loss, of course, can be avoided by treating the slides with a thin solution of celloidin, but this is more trouble than the use of water-glass fixative. The fixative containing gum arabic and potassium bichromate mentioned by McClung[3] adheres bone sections perfectly, but interferes with staining.

Good results have been obtained both from solid bone of homogeneous structure (hyperostosis in chickens) and from bone containing regions of varying hardness and different structures (for example, entire tibia-fibula of chick embryos near hatching, single specimens containing both epiphysis and diaphysis of bones from adult chickens, and cervical vertebrae). Excellent longitudinal sections through three vertebrae and the included spinal cord have also been obtained; larger pieces have not been attempted.

Haemalum and eosin give good staining results following this technique.

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APPARATUS FOR HEAVY WATER STUDIES IN SMALL ANIMALS

WHEN heavy water is given to animals, it becomes important both to follow its content in the living organism as well as (for economic reasons) to recover the material after it has left the body.

These ends have both been served in the employment of simple modifications of the Haldane metabolism train, which we shall briefly describe. The apparatus shown in the figure was used in establishing the depressant effects of heavy water on the mammalian metabolism, as recently reported by one of us[1] to the Society for Experimental Biology and Medicine.

The mouse in chamber E receives a stream of air free from carbon dioxide and water. The CO_2 is removed as it passes through A and B, while concentrated sulfuric acid in C (bubbling flask) and D (pumice granules saturated with H_2SO_4) removes the water. The size of condenser A protects the train against the large amount of CO_2 given off by dry ice in close proximity.

[1] Elizabeth C. and Eugene Cutuly, SCIENCE, 80: 564–565, December 14, 1934.
[2] H. Ullrich, "Aufkleben von Paraffinschnitten mit ammoniakhaltiger Wasserglaslösung," Planta 10, 1930.
[3] C. E. McClung, "Handbook of Microscopical Technique," p. 129. Paul B. Hoeber, Inc., New York, 1929.
[1] H. G. Barbour, *Proc. Soc. Exp. Biol. and Med.*, 32: 8, 1935.