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.

LORNA THIGPEN DAVID
STORRS AGRICULTURAL EXPERIMENT STATION
STORRS, CONN.

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.

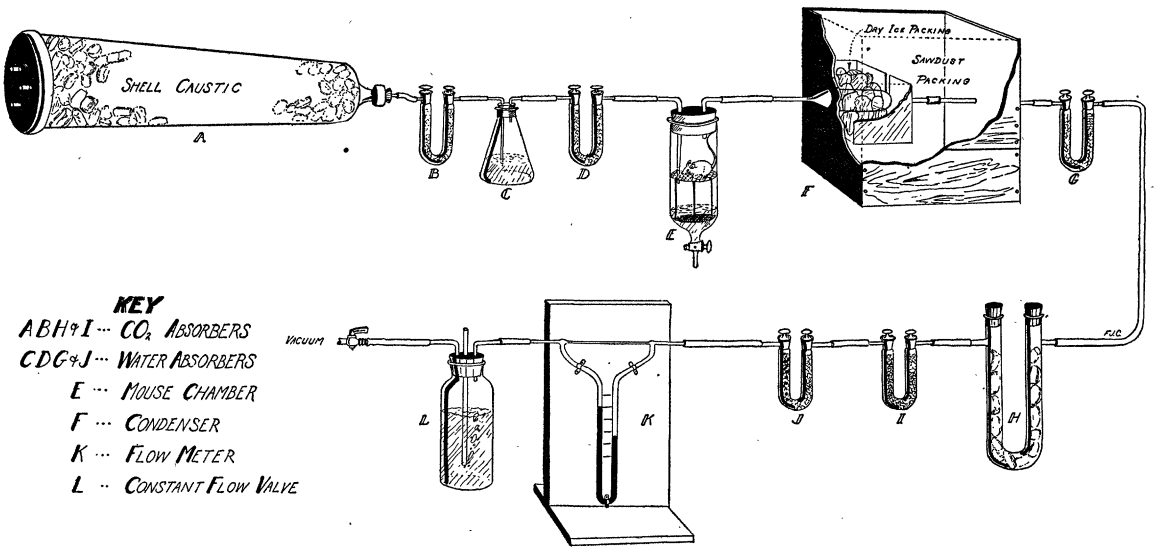

FIG. 1.

The mouse chamber itself contains mineral oil enough to cover the feces, which rest on the lower mesh platform. The urine falls to the neck of the chamber and can be drawn off by the stopcock, free of oil. A coarser mesh platform supports the mouse at a level high enough to prevent the tail touching the oil.

From the chamber the air passes into the condenser (F) consisting of an ordinary 100 cc pipette, the end of which is seen projecting from the box. The pipette is enclosed in dry ice well insulated with sawdust.

The "insensible perspiration," consisting of all the water vapor from the animal's lungs and skin, accumulates as snow in the pipette. Within one day the apparent size of the snow pile approaches that of the mouse itself. In a series of fifty-one determinations, it was found that 97.9 per cent. of the total water vapor was thus caught in the pipette, the sulfuric acid in G trapping the remainder.

Expired CO_2 is caught in absorbers H and I (shell caustic moistened by three and one drops of water, respectively). Absorber J (H_2SO_4 and pumice) is weighed together with H and I.

The air flow measured by the venturi meter (K) is kept at a constant rate of 200 cc per minute by means of the constant-flow water valve (L).

The specific gravity of the insensibly lost water or (if oil be omitted from the chamber) of the total water lost by the mouse is quickly determined from 0.01 cc of the melted snow by the falling drop method of Barbour and Hamilton.[2] The D_2O percentage of the mixture is calculated from the formula $\frac{G-1}{.1079}$, where G is the specific gravity. The denominator is the fractional part of the specific gravity of pure deuterium oxide (Urey and Teal[3]).

This simple calculation of the D_2O content from a linear specific gravity curve leads to an error in the D_2O volume of not over 0.05 per cent. (Lewis and Luten[4]).

Some idea of the value of the insensibly lost water as an index to the actual D_2O/H_2O ratio prevailing in the living body may be gained by the following comparisons in the two mice, from the carcasses of which about two thirds of the water was regained by distillation:

DEUTERIUM OXID (PER CENT. OF TOTAL WATER)

	Mouse No. 12	Mouse No. 16
Found in insensibly lost water last 12 hours before death	3.8	11.6
Found in carcass (redistilled from acid K_2MnO_4 by courtesy of Dr. Paul K. Smith)	3.6	10.1

HENRY G. BARBOUR
F. J. COCHRAN
YALE UNIVERSITY

[2] *Jour. Biol. Chem.*, 49: 625, 1926.

[3] *Rev. of Mod. Physics*, 7: 34, 1935.

[4] *Jour. Am. Chem. Soc.*, 55: 5061, 1933.

BOOKS RECEIVED

Bulletin of the National Research Council, No. 97. Pp. 51. National Academy of Sciences, Washington. $1.00.

DAVIS, H. T. and W. F. C. NELSON. *Elements of Statistics.* Pp. xi + 424. Principia Press.

JENNINGS, H. S. *Genetics.* Pp. xi + 373. 70 figures. Norton. $4.00.

KRONIG, R. DE L. *The Optical Basis of the Theory of Valency.* Pp. x + 237. 67 figures. Cambridge University Press, Macmillan. $4.50.