Onderstepoort Journal of Veterinary Science and Animal Industry, Volume 15, *Numbers* 1 *and* 2, *July and October*, 1940.

Printed in the Union of South Africa by the
Government Printer, Pretoria.

A Method for Preparing Sections of Bone Without Decalcification.

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It is usual in histological technique to soften bone by subjecting it to some form of decalcification prior to embedding and cutting. The resulting preparation, while suitable for most histological purposes, offers some disadvantages when the degree of ossification or calcification, as well as the structure and growth of bone, have to be studied in relation to each other.

Depending on the method employed, the loss of mineral salts by such decalcification can be quite considerable as is apparent from the few figures, below, and it is also more than probable that the prolonged treatment with acids does not tend to improve the staining properties of the cells. This loss of salts was shown in a small expermient in which transverse rings, about 3 mm. thick, were sawn from the compact shaft of a metacarpus of a horse. The pieces were weighed the first time in the dry state (i.e. fat and water free) and then subjected to the most common methods of "softening", the material being deemed soft when a needle could be pushed through the thickness of the ring with ease.

Medium.	Length of time treated.	Loss of weight dry.
Nitric acid 5 per cent.....	3 days (soft).....	79 per cent. loss.
Formic acid and Sod. citrate.....	18 days (soft).....	73 per cent. loss.
Müller's fluid.....	3 months (still hard)..	25 per cent. loss.
Celloidin cedar wood oil.....	3 months (hard).....	5 per cent. gain in weight.

The gain in the latter case was evidently due to the celloidin with which the bone had become impregnated, as no attempts were made to dissolve and wash this out before drying and weighing the pieces of bone the second time.

It will be appreciated, therefore, that in deficiency disease such as osteoporosis, rickets, etc. and even in normal histology of growing bone, where the degree and mode of calcification is such a variable quantity, the ability to study and compare bone in sufficiently thin but uniform sections, without any previous interference with the calcification process, is of considerable importance.

The possibilities and disadvantages of preparing section from non-decalcified bone were mentioned in an earlier publication (Thomas and v. d. Wath, 1937) using the freezing microtome.

Fairly good sections may be obtained in this way, especially if the material is from a young animal or soft from the effects of advanced disease. When the bone is harder, the delicate lamellae tend to crumble before the knife, even when support is given in the form of a preliminary embedding in gelatine. The more brittle the material, therefore, the greater is the thickness at which it has to be cut. It is this uneven thickness of sections which makes comparison of the process so difficult and which has led to endeavours being made to find means of embedding and cutting bone, which would give more uniform and satisfactory sections.

The embedding method outlined below was evolved largely thanks to the untiring efforts of our technical assistant, Miss Moolman, to whom the greatest credit is due. The method certainly does not solve all the difficulties, nor does it allow of the cutting of the harder types of bone such as the compact substance of the shaft of a long bone. However, with care and some dexterity, excellent section can be obtained of the softer and moderately hard bones, especially the costo-chondral junctions of the ribs. Such sections can be cut to a uniform thickness 5-10 μ and stained by most of the more useful methods, Von Kossa's silver impregnation, Haemalumeosin, Von Gieson, etc. Strange as it may seem, good sharp microtome knives do not seem to suffer any damage when cutting even fairly hard bone embedded in this way, provided care is taken in handling the microtome. The process is essentially a double embedding method (celloidin-paraffin) for which nothing original is claimed except its adaptation to the cutting of non-decalcified bone.

Briefly it consists of the following operations:—

Selected pieces of fresh bone are sawn into small slices about 3 mm. thick or less so as to include a portion of the cartilaginous line of growth. Biopsy material e.g. the costo-chondral junction of ribs including a few mm. of the cartilage may be divided into slices with a sharp knife with the piece of rib lying on its flat surface. Such pieces are fixed in formalin for a day or more and are then ready for the embedding process which comprises the following steps:—

I. Wash in running water for 1-2 hours to remove all traces of formalin as this adversely affects the silver nitrate staining.

II. Dehydrate in 70, 80, 90, and 100 per cent. alcohol in two days changing overday and overnight.

III. Ether-alcohol $\overline{aa}$ one day.

IV. Celloidin 2 per cent. one or two days.

V. Celloidin 4 per cent. one or two days.

VI. Wipe off excess celloidin and place piece in small quantity of chloroform.

VII. When material sinks in chloroform, about an equal amount of cedar wood oil is added gradually during the day, the vessel being left open for the chloroform to evaporate and left therein overnight.

VIII. Change to fresh cedar wood oil and leave for 2-3 days or indefinitely until ready to embed.

IX. Place in benzine bath in oven at 58° C. for 10-15 minutes to remove excess cedar wood oil only.

X. Embed and fix firmly to microtome block. (Wax of M.P. 50° C. is used here, but higher M.P. may be found more suitable elsewhere.) Cut in the usual way on sliding knife type microtome with a fairly heavy knife set at a good slant. Cooling of the embedded piece with ice prior to cutting is essential with this low M.P. wax. Normal porcine and bovine rib material is usually easier to cut than ovine and caprine which is harder. Sections from 5-10 μ thick for small pieces and up to 15 μ for larger ones can be obtained in this way. It must be stressed, however, that a person attempting this for the first time must be prepared to spend a considerable amount of patience and time until the knack of handling this hard material is acquired.

Once cut the sections are fixed to the slide with glycerin-albumen, allowed to dry in an oven at 37° C. for 2-3 days, deparaffinated, and stained in the usual way. For storage the pieces should preferably be returned to cedar wood oil, or failing this be re-embedded in paraffin to prevent the drying out of the cedarwood oil, as this renders the material difficult or impossible to cut.

It will be seen thus, that impregnation with cedar wood oil undoubtedly has an indirect "softening" effect, which, however, is insufficient for cutting the more compact types of bone. This effect can possibly be explained as a "lubrication" of the path of the knife in its passage through the calcareous matrix. This together with good support afforded by the double embedding can be said to constitute the essentials of this method.

REFERENCE.

THOMAS, A. D., AND VAN DER WATH, J. G. (1937). Bone Biopsy as an Aid to the Study and Diagnosis of Deficiency Diseases. *Onderstepoort Jnl. of Vet. Sci.*, and *An. Ind.*, Vol. 8, No. 2, pp. 431-439.