

ON A METHOD OF PREPARING THE MEMBRANOUS
LABYRINTH. By ALBERT A. GRAY, M.D., F.R.S.E.

OUR anatomical knowledge of the soft parts of the internal ear has been obtained not by observations made upon the organ as a whole, but by piecing together the appearances seen in fragments. It is nothing short of astonishing how anatomists have been able to do so with such accuracy. Nevertheless such a state of matters is in the highest degree unsatisfactory, for it means that few anatomists will be willing to spend the requisite time to acquire their knowledge from nature, and no preparations, so far as I know, exist from which students can learn the subject in the same way. Furthermore, it is in human nature to desire the view of any object in its entirety, not as broken fragments.

Several methods have been described with the object of obtaining the labyrinth as a whole, but they seem to have failed, and in my experience of them they failed completely.

At intervals during the past two years I have made many attempts in various directions, but only recently have I found out a method which can be depended on to give certain results.

The general principle of my method is to embed the whole pyramid of the temporal bone so thoroughly that no acid can affect the soft parts, then to decalcify and *disintegrate* the bone so completely that no force whatever is required to remove the destroyed tissue surrounding the labyrinth, and finally to remove the embedding material in such a way that the membranous labyrinth is left uninjured.

The pyramid of the human temporal bone is removed from the base of the skull in the post-mortem room. The superfluous bone is removed with a saw; the stapes is carefully extracted from the oval window, and a small hole is filed in the superior semicircular canal. The structure is then immersed in 90 per cent. alcohol for at least a fortnight, the alcohol being frequently changed. It is then transferred to absolute alcohol, where it remains at least a fortnight, this alcohol also being frequently

changed. During this period it must be kept in a glass stoppered jar. The alcohol obtained from the chemist is not really absolute; it may be made so, however, by putting an ounce of anhydrous sulphate of copper to every pound of alcohol. When wanted for use, the clear alcohol at the top is poured off as desired, care being taken that the sulphate of copper does not pour out along with it.

From absolute alcohol the bone is removed quickly to xylol, where it again remains at least a fortnight, the xylol being frequently changed. I find that if a vacuum, more or less complete, be made now and then in the jar above the level of the xylol, diffusion occurs much more rapidly and completely. I do this by fixing an india-rubber cork, with a glass tube through it, into the mouth of the jar, and extracting the air and gases through a rubber tube connected with a small air-pump.

From the xylol the bone is removed to melted paraffin of a melting-point of 52° C. or 54° C. The paraffin must be changed two or three times during the fortnight which must be allowed for the embedding. This is important to the success of the method; the paraffin must permeate the soft parts far more completely than is necessary in the case of embedding for microscopic section purposes. I frequently make use of the air-pump to produce a vacuum above the melted paraffin, but sometimes the latter solidifies during the process; this, however, does not appear to affect the ultimate result. Of course the temperature of the paraffin stove must not be allowed to rise above 55° or 56° C.

After the bone has been in the paraffin bath for a period ranging from two to three weeks, the paraffin is cooled as quickly as possible and the bone cut out from the block. The superfluous paraffin is then carefully scraped off the bone and decalcification is proceeded with.

For this purpose neither nitric nor hydrochloric acid by itself is suitable; they should be mixed. The solution I use consists of 2 parts pure nitric acid, 2 to 3 parts pure hydrochloric acid, and 6 to 18 parts water; the nitric acid should be mixed with the water first, and the hydrochloric acid added.

The bone is put into a large quantity of this mixture and suspended near the top by fine twine. The mixture should be

frequently changed, and in about three weeks or a month decalcification and disintegration will be complete. It will be found that while the cancellous portions of the bone are still firm, being supported by the paraffin, the dense bone which surrounds the labyrinth will have become quite pulpy; indeed the labyrinth should lie almost loose in the pulp; if this is not so, the preparation should be put back into the acid and left longer, and the solution should be made stronger by adding a little more hydrochloric acid.

After decalcification the mass should be thoroughly washed for twenty-four hours in gently running water, care being taken that it does not get roughly handled during the process.

Some of the cancellous portions of the bone may now be picked very carefully away with the point of a sharp knife, and then the mass is carefully removed to absolute alcohol, where it remains for about ten days, the alcohol being changed several times. From the alcohol it is transferred rapidly to xylol, which slowly dissolves out the paraffin and leaves the membranous labyrinth transparent. Surrounding portions of cancellous tissue may be slightly adherent, but they can be separated by fine sharp scissors as the structure lies in the xylol. If the dehydration has been thorough, the extremely delicate structures do not collapse or shrink when the paraffin is removed. The specimen is preserved in xylol in the glass jar when the paraffin has been melted out. The courses of the various portions of the nerves stand out very plainly, and may be shown still more clearly if the specimen has previously been stained and fixed with osmic acid.

Very pretty specimens may also be made by injecting the endolymph spaces (before hardening) with carmine gelatine, the injection being done through the aqueductus vestibuli.

The blood-vessels may be injected through the internal auditory artery, also of course before hardening.