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HISTOLOGY

Demineralization of Bone

Demineralization of specimens for the preparation of histological sections is a time-consuming process. Mineral acids demineralize the specimens faster than chelating agents or organic acids, but the resultant distortion in cellular morphology is greater when mineral acids are used. The rate of demineralization depends mainly on the temperature and concentration of the acid, but since cellular definition and enzymes are adversely affected if the temperature is raised, or when the concentration of the acid is increased, demineralization is usually carried out below or at room temperature and a concentration of mineral acid above 5 per cent of commercial nitric acid (55 per cent) is seldom used. Under these conditions even small specimens of bone must be immersed in acid for days before they are completely demineralized. For teeth, the period of time runs into weeks.

The whole process of demineralization can be reduced to hours, even minutes, if ultrasonic energy is propagated through the demineralizing solution. Subsequent sectioning of the specimen with a freezing microtome, in a cryostat, or as paraffin-imbedded sections all produce preparations in which the cellular detail is superior to those seen in similar specimens treated with the same mineral acid for longer periods of time. All soft tissue detail is improved, but the most significant improvement in cellular morphology is seen in the chondrocytes and osteocytes.

In these preliminary experiments a 'DiSONtegrator system 80' with a frequency of 90,000 per sec was used. The temperature of the water in the tank was controlled by a constant exchange of the tank water from the cold-water supply. The best results were obtained when the demineralizing fluid and fixed specimens were placed in a large beaker immersed in the partially filled tank directly over the crystal. To prevent damage to the tank, sodium bicarbonate was added to the water in it and the beaker was covered to prevent spattering of the agitated acid.

Nitric acid at a concentration of 2·5, 5 or 10 per cent was used in these experiments and the acid solutions were changed every half-hour. Although it is not possible to indicate an optimum concentration of the nitric acid at this stage, the more rapid decalcification in the 10 per cent solution of nitric acid seemed to produce less cellular distortion in the sections.

The time taken to demineralize a specimen is affected not only by the degree of mineralization, but also to a marked degree by the amount of investing soft tissue. When an adult rat femur is divided into three segments and 10 per cent acid is used, decalcification can be accomplished in less than 2 h. Without the investing soft tissue demineralization is complete within 1 h.

When haematoxylin and eosin are used the time taken to stain sections decalcified in this manner is considerably reduced and the differential staining of the various tissues is more selective.

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Histology of the Posterior Chamber of the Swimbladder of *Argentina*

Argentina was the first genus among the salmonoid fishes in which the swimbladder was reported to lack an open communication with the oesophagus, typical for the sub-order Salmonoidei[1,2]. This has since been found to apply also to the families Microstomidae and Opisthoproctidae[3]. A more detailed description of the structure of the swimbladder of *Argentina silus*[4] revealed the occurrence of a large number of flat bundles of blood vessels, which constitute a counter-current system. The name micro-retia mirabilia[3] has been established for this type of rete mirabile. In *Argentina* a gas gland tissue was also detected[4], being in contact with capillary loops from the micro-retia. From these findings it was concluded that the swimbladder of *Argentina silus* has a capacity for gas secretion comparable to the swimbladder with a rete mirabile of the compact type.

A thin sac at the caudal end of the swimbladder of *Argentina sphyraena* has been briefly described and suggested to be a posterior chamber[3,5]. Observations also argue that it might have a resorbent function[3]. The draining vessels were seen to be a part of the hepatic portal system[5].

The present investigation was intended to produce histological evidence for a supposed resorbent function of the posterior chamber of the swimbladder. Several specimens of *Argentina silus* and *A. sphyraena* were caught off the west coast of Norway at the Biological Station of the University of Bergen. The animals were brought to the surface (200 metres depth to surface in 20 min). The swimbladder in most of them had not burst—an occurrence often reported in captured specimens of *Argentina*. The animals could be kept in aquaria for 6–10 h after capture. The posterior sac of the swimbladder was observed. Longitudinal and transverse sections, fixed in Bouin's fluid, were stained with Azan, and the morphology and histology of the posterior part of the main chamber and the posterior thin-walled sac were investigated. Swimbladders from some specimens were, immediately after killing, freeze-dried, treated with formaldehyde gas for 1 or 3 h at 80° C, and then infiltrated with paraffin *in vacuo* in order to condense catecholamines and 5-hydroxytryptamine to fluorescent products[6]. They were then investigated under a fluorescent microscope in dark-field illumination.

It was confirmed that the posterior sac constitutes a posterior chamber of the swimbladder in communication with the anterior part of the organ. It is not separated from the anterior chamber by a membrane with a central opening and possessing a radial muscle, as is normal in