A Method for the Preparation of Dry Skeletal Material

J. P. Scott, Wabash College

The preparation of transparent specimens by the Spalteholz method depends upon the fact that flesh can be decolorized with 1% potassium hydroxide. While following this method the author allowed a specimen to clear for too long a time and thus discovered that the hydroxide solution will destroy flesh, ligaments, and bones, in the order named. But, if maceration is stopped at the right time, ligaments and bones can be perfectly preserved. Detailed methods for preparing skeletal material with hydroxide are given below.

Skulls and Disarticulated Bones. Short method.—Skin and clean the animal. Fix in 95% alcohol for at least 24 hours. Place in 1% potassium hydroxide at 40° C. until bones are cleaned and disarticulated, changing fluid once or twice. Remove, wash, and dry. Rat skeleton requires 3 days. **Long method.**—Proceed as above, but carry on the entire process at room temperature. A safer method, but it requires several weeks.

Ligamentous Skeletons.—Skin animal except for nose and feet. Remove viscera posterior to the diaphragm. Arrange in natural position and fix in 95% alcohol for at least 24 hours. Soak in 1% potassium hydroxide at room temperature until the flesh becomes colorless, changing fluids two or three times. Place in fresh solution and dissolve flesh at 40° C. Remove when the first signs of disarticulation appear (lower jaw usually falls off first). Wash in a fine jet of water, mount, and dry. Rat skeleton requires two weeks or more for clearing, three hours for dissolving flesh.

Cautions.—Hydroxide solution must be thoroughly mixed, as the hydroxide sinks and will act unevenly if concentrated in the bottom of the dish. Do not cut away flesh, as this exposes bones to the action of the hydroxide. Do not let cleaned bones remain in the hydroxide, as they will eventually dissolve. Keep vessel covered, so that the solution is not concentrated by evaporation. On the whole, the slower the action the better the preparation. The highest safe temperature for clearing is about 25° C. The above directions apply specifically to the skeletons of small mammals.

The superiority of the hydroxide method over the older ones lies in the fact that the macerating agent is allowed to penetrate all parts of the muscles so thoroughly that its action is almost uniform. The 95% alcohol dehydrates the specimen and thus facilitates penetration by the hydroxide.

Attempts to speed up the clearing process have met with little success. Increasing the strength of the hydroxide (to 3%) produces a fairly clean skeleton but swells the periosteum to a thick jelly, weakens the ligaments, and weakens lightly ossified bones such as the

ribs. Increasing the temperature during clearing (as in the short method given above) will produce maceration in a short time, but the limb bones fall apart before the thicker muscles are dissolved. The results indicate that heat is injurious to any but the strongest bones.

Formalin specimens may be used, but clearing and maceration are extremely slow. The entire process may be carried on at 40° C. with such specimens.

The hydroxide method has so far been applied chiefly to small mammals. Preliminary experiments with amphibia indicate that the 1% solution of hydroxide is too strong.

Prolonged action of the hydroxide not only decolorizes the bones but also converts all fat to soap and leaves them perfectly clean. There is no necessity to brush the skeleton or to use bleaching agents, either of which processes may damage the skeleton. There are no unpleasant odors of decomposition during clearing or maceration following fixation in alcohol.

The ligamentous skeletons are wonderfully strong and elastic when wet and, in small mammals at least, require practically no support when mounted. Every bone except the hyoid remains articulated. Even the penial bone of the male rat is preserved.

The method is one which involves a very small amount of trouble. Excluding the labor of mounting, a completely cleaned rat skeleton may be prepared with approximately 1½ hours' actual attention. Furthermore, even totally inexperienced workers are able to produce Grade A preparations on the first trial.