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Special Staining Technique in Histochemistry

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The process of development of bones is called osteogenesis. It is achieved by endochondral and intramembranous ossification. The bones of appendicular skeleton develop by endochondral ossification except scapula, which is developed by intramembranous ossification. All facial bones are flat bone and develop through intramembranous ossification. The long bones develop from at least three centres of ossification. Endochondral ossification process carried out by primary and secondary centres ossification in different long bones during prenatal life. A primary ossification center is the first area of bone to start ossification. In long bones the primary centres occur in the diaphysis/shaft and in irregular bones the primary centers occur usually in the body of the bone. A secondary ossification center is the area of ossification that appears after the primary ossification center has already appeared – most of which appear during the postnatal and adolescent years. Most bones have more than one secondary ossification center. In long bones, the secondary centres appear in the epiphyses. The shape of the bone is influenced by the number of secondary centres of ossification and their fusion in a particular bone.

Several techniques are used to study the appearance of centre of ossification and extent of calcification. The potassium hydroxide clearing and alizarin staining method for the study of ossification center has proved very useful for the study of bones of embryos and small animals. The advantages of this method of study are:

1. Losing the small bones is very less.
2. All bones are retained in their position.
3. Identification of similar bones becomes easy and accurate.
4. The bones may be disarticulated after identification and examined from all angles, equally as well as in dried preparations and
5. Many animals may be processed together without danger of mixing their bones.

The method was originally developed by Schultze (1897) and has subsequently been modified by a number of investigators including Mall (1906), Dawson (1926), Lipman

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(1935), Chumley, Crow and Griffen (1939), Gamble (1945), True (1947), Humason (1962) and Daina and Karen (2014). The steps usually employed include :

1. Fixation in formalin or alcohol.
2. Bleaching with hydrogen peroxide.
3. Incomplete maceration in potassium hydroxide
4. Staining with alizarin red S. and
5. Clearing in graded concentrations of glycerine

Various authors have suggested the omission of one or more of these steps. It has been found possible to omit fixation and bleaching and to transfer the specimens directly from the staining solution to undiluted glycerine without the intermediate steps.

Solutions needed: 1 percent potassium hydroxide, 0.5 percent alizarin red S in 1 percent potassium hydroxide, and glycerine. A schedule suitable for goat foetus is as follows.

1. Wash the fixed specimen in running tap water overnight (8 hrs) to remove all fixatives.
2. Remove skin and eviscerate.
3. Place in 3 percent potassium hydroxide for 3-5 days for maceration and gently remove the macerated soft tissues.
4. Pour off solution and replace with fresh 1 percent potassium hydroxide. Add to this a few drops of 0.5 percent alizarin red S (0.003% Alizarin red - S solution) in 1 percent potassium hydroxide to make a medium pink solution. Leave the specimen in this for 3-4 days.
5. Pour off solution and replace with clearing solution (Glycerine + 1% KOH + benzene) for 2-4 days (as per the size of the specimen).
6. Gradually increase the amount of glycerine and after complete clearing of the specimen store it in the 100% glycerine solution till examination.

Result : Centre of ossification is indicated by the staining of the bone deep red while soft tissue remains unstained.

Reference

Diane Trueman, Stuart W. Jackson, Brian Trueman. (1999) An Automated Technique for Double Staining Rat and Rabbit Fetal Skeletal Specimens to Differentiate Bone and Cartilage. *Biotechnic & Histochemistry* **74**:2, pp 98-104.

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Gerald N. Webb, Richard A. Byrd. (1994) Simultaneous Differential Staining of Cartilage and Bone in Rodent Fetuses: an Alcian Blue and Alizarin Red S Procedure without Glacial Acetic Acid. *Biotechnic & Histochemistry* **69**:4, pp 181-185.

Menegola, E., Broccia, M. L., &Giavini, E. (2001). Atlas of rat fetal skeleton double stained for bone and cartilage. Teratology, **64** (3), pp 125-133.

Noback, C. R. (1944). The developmental anatomy of the human osseous skeleton during the embryonic, fetal and circumnatal periods. The anatomical record, **88** (1), pp 91-125.

Pramod, K. L., & Vaswani, V. R. (2011). Museum preservation of skeleton of fetus & small vertebrates. Recent Research in Science and Technology, **3** (2).

Rasweiler, J. J., Cretekos, C. J., & Behringer, R. R. (2009). Alcian Blue/Alizarin Red staining of cartilage and bone of short-tailed fruit bat (Carolliaperspicillata). Cold Spring Harbor Protocols, (3), pdb-prot 5166.

Y Yamazaki, M Yuguchi, S Kubota, K Isokawa. (2011) Whole-mount bone and cartilage staining of chick embryos with minimal decalcification. *Biotechnic & Histochemistry* **86**:5, ppps 351-358.