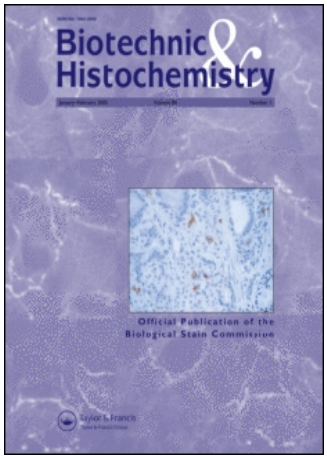
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Examining the microanatomy of various fetal body parts using the orcein-picroindigocarmine staining method

K. Demirtaş [a]; N. Gülekon [b]; T. Peker [b]; S. Ömeroğlu [c]; A. Anil [b]; Hb Turgut [b]

[a] Faculty of Medicine, Department of Anatomy, Yeditepe University, İstanbul
[b] Faculty of Medicine, Department of Anatomy, Gazi University, BeŞevler, Ankara
[c] Faculty of Medicine, Department of Histology and Embryology, Gazi University, BeŞevler, Ankara, Turkey

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Examining the microanatomy of various fetal body parts using the orcein-picroindigocarmine staining method

K Demirtaş[1], N Gülekon[2], T Peker[2], S Ömeroğlu[3], A Anıl[2], HB Turgut[2]

[1]Yeditepe University, Faculty of Medicine, Department of Anatomy, İstanbul, [2]Gazi University, Faculty of Medicine, Department of Anatomy, Beşevler, Ankara, [3]Gazi University, Faculty of Medicine, Department of Histology and Embryology, Beşevler, Ankara, Turkey

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Abstract

It is difficult to distinguish muscle, vessel, nerve, fascia, tendon and many other structures in fetal sections. The aim of the study reported here was to research the practicability of orcein-picroindigocarmine staining for distinguishing anatomic structures in histological sections containing complex structures. Histological serial sections of hand, foot, head and neck of four 18- to 20-week old fetuses were used. Bone matrix was stained bright blue, collagen fibers tones of green to blue, elastic fibers brown, cartilage matrix tones of pink, and chondroblasts blue. Muscles and erythrocytes were stained yellow to green, epidermis reddish brown, skin appendices including hair and nail stained light yellow to green.

Key words: fetuses, orcein-picroindigocarmine stain, sectional anatomy

Sectional anatomy is one method for determining the interrelations among anatomic structures (Spitzer and Whitlock 1998, Bergman et al. 2004). This type of topographic anatomy is becoming increasingly important for improving body imaging techniques and computerized technologies (Ellis et al. 1991, Bergman et al. 2004). Sectional imaging not only provides a new viewpoint for anatomists, but also contributes to development of multidisciplinary skills through interaction among specialists from different disciplines (Lester and Olds 2001). It is difficult to distinguish structures such as muscle, vessel, nerve, fascia, and tendon in sections obtained from cadavers (McMinn and Hutchings 1992). Therefore, multiple tissue staining methods have been used, and still are used, to identify various structures and to determine their relations with each other (Bancroft and Stevens 1990, Arend and Kolts 2002). There are few sectional anatomical studies of fetuses in the literature.

Materials and methods

Four 18- to 20-week-old fetuses were used in the present study. All fetuses were therapeutically aborted after the parents' permission was obtained and the study was approved by the local ethical committee. Crown-rump length, biparietal diameter and femur length for each fetus were measured sonographically to estimate the ages just before the abortion procedure. The fetuses were fixed in 10% formalin. The forearms of all fetuses were cut off at the elbow joints, the legs at the knee joints, and the neck at the level of the seventh cervical and first thoracic intervertebral disk. The excised fetal body parts were decalcified in 5% formic acid for 2–6 weeks. After decalcification, all samples were dehydrated. After dehydration, all material was embedded in paraffin. Serial 5 μm sections were cut using a microtome (Leica SM 2000 R, Germany). Deparaffinized

Correspondence to: Nadir Gülekon, M.D., Ph.D. Gazi Üniversitesi Tıp Fakültesi, Anatomi Anabilim Dalı, Beşevler, 06500 Ankara, Turkey, Phone: +90 312 2024617, Fax: +90 312 2124647. E-mail: inadir@gazi.edu.tr

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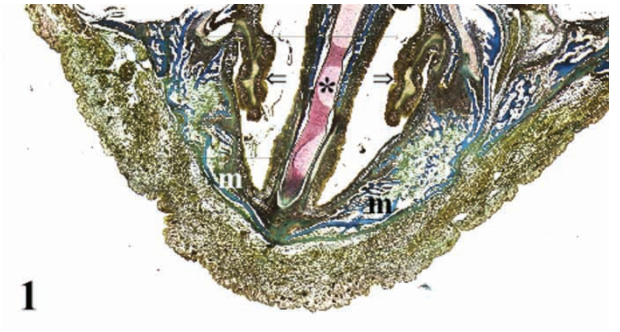


Fig. 1. Transverse section through anterior nasal spine to posterior nasal spine. The nasal septum was not ossified and is stained (*). The nasal crest of the maxilla at the posterior part of the section is ossified (m). The inferior nasal concha (open arrows) and meatus are located on the lateral wall of the nasal cavity. The skin and its appendices are green. The facial artery courses under the zygomaticus major. ×12.5.

sections were stained first with orcein (ICN Biomedicals Inc., Aurora, OH), then with picroindigocarmine (Merck, Darmstad, Germany; C.I. no. 73015). We used a staining technique modified from Steven et al. (2000).

Staining solutions

Orcein
Dissolve 1 g orcein in 100 ml 70% ethyl alcohol; add 1 ml HCl.

Picroindigocarmine
Dissolve 0.25 g indigocarmine in 70 ml saturated aqueous picric acid.



Fig. 3. Transverse section through the cricoid cartilage and an upper cervical vertebra. Inset (×40) shows the carotid sheet surrounding the common carotid artery (c), vagus nerve (v), jugular vein (j), and a lymph node (l). The vagus nerve is surrounded by a perineurium stained green in front of the common carotid artery and a lymph node composed of lymphoid tissue whose cells are stained brown. The thyroid gland (t) is stained yellowish-brown and viewed in front of the common carotid artery. ×40.

Staining procedures

Orcein
Hold all deparaffinized sections in distilled water for 5 min before staining with orcein. Cover

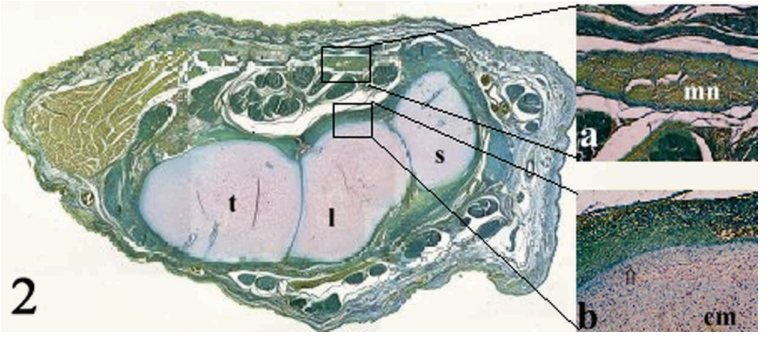


Fig. 2. Transverse section through the proximal row of carpal bones (s, scaphoid; l, lunate; t, triquetrum) in a 20-week-old fetus. Connective tissue surrounding the median nerve (mn) in the carpal tunnel is stained bright green. Tendons of the flexor digitorum superficialis and flexor digitorum profundus are stained dark green (inset a, ×100). Section through the lunate bone (inset b, ×100). The ligamentous structures are stained blue-green, perichondrium (open arrow) blue, and chondral matrix (cm) light pink. ×100.

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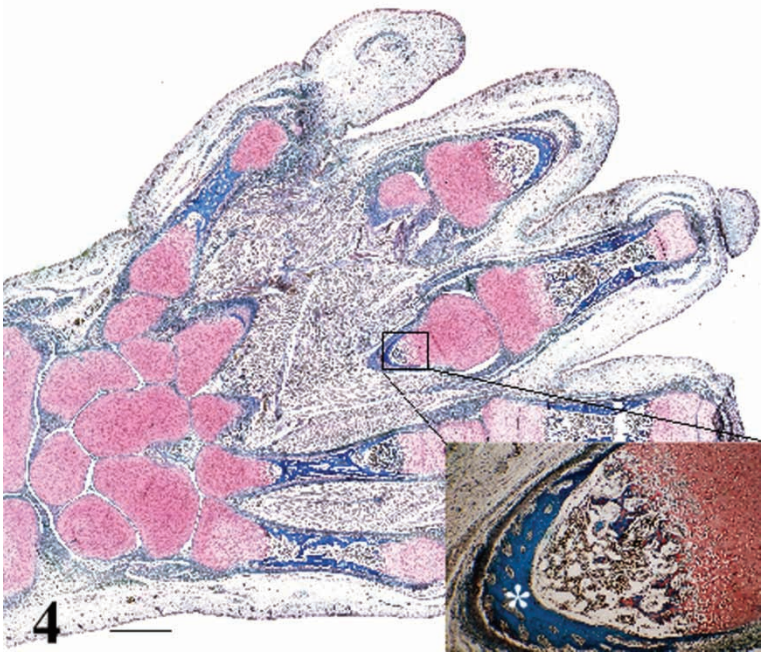


Fig. 4. Coronal section of the hand. Inset (×40) shows endochondral ossification in the body of the third metacarpal bone (*). Chondrocytes appeared separately or as groups in reserve cartilage. Cartilage cell columns were formed by fast multiplying and hypertrophied chondrocytes. ×40.

sections with the staining solution for 40–45 min at room temperature. Rinse in distilled water 1 min. Fix in 96% ethyl alcohol.

Picroindigocarmine

Stain sections in picroindigocarmine solution 24 min. Keep in 70% ethyl alcohol 2–4 min. Differentiate in 96% ethyl alcohol 2 min. Clear in xylene 18–20 h and mount in Entellan.

All stained serial sections were examined under a photomicroscope (Leica DM 4000B, Wetzlar, Germany). Photographs were taken at 12.5, 40 and 100 × magnification.

Results

We used orcein and picroindigocarmine stains for the study reported here. Bone matrix was stained dark blue, cartilage matrix pink, chondroblasts blue and muscle from yellowish brown to yellowish green. Collagen fibers appeared greenish blue, erythrocytes yellowish green, epidermis reddish brown, skin appendices including hair and nails light yellow to green, and elastic fibers brown. Consequently, these structures could be distinguished easily (Figs. 1–5).

Discussion

Many techniques are available for differential demonstration of tissue components; one of these is the Van Gieson method. This technique stains collagen fibers red, nuclear structures blue to black, and muscle, fibrin, elastic and reticular fibers are stained yellow. Cartilage may be stained various colors. Another method is the Masson trichrome

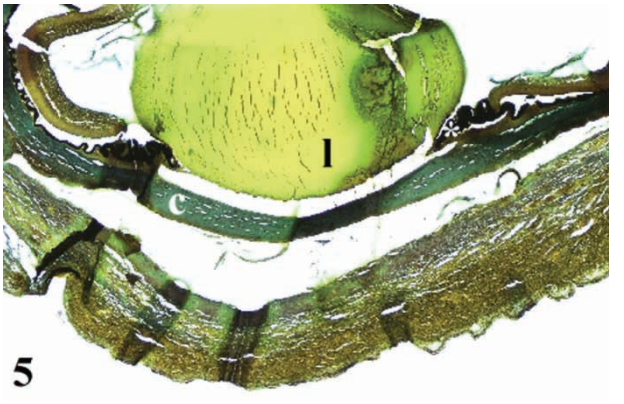


Fig. 5. Transverse section through upper eyelid and eyeball. The cornea (c) is stained blue-green, the lens (l) yellow-green and the iris (*) brown-green. Sclera formed especially by collagen and some elastic fibers is stained dark green, vascular layer just below the sclera dark brown and retina light brown. ×100.

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technique. Collagen and reticular fibers are stained blue to green with this method. Muscle cells and erythrocyte cytoplasm are stained red, nuclear structures are blue to black and elastic fibers are stained light red.

Various stains including Orcein, Verhoeff, Weigert's resorcin fuchsin and Gomori's aldehyde fuchsin stains are used for staining elastic fibers. Elastic fibers stain black with Verhoeff's method, brown-purple with Weigert's resorcin fuchsin method, blue-purple with Gomori's aldehyde fuchsin stain, and dark brown with Orcein techniques. Although elastic fibers are demonstrated clearly by these methods, other tissue components cannot be distinguished (Horobin and Fleming 1980, Bancroft and Stevens 1990). To show collagen and elastic fibers in a single section simultaneously, these stains must be combined. These combined staining techniques must be selected with care because of their overlapping color ranges. For example, Weigert's resorcin-fuchsin cannot be used with azan or Masson trichrome stains because these stains color both collagen and elastic fibers blue so that it is impossible to distinguish them (Steven et al. 2000).

Gudiene et al. (2003) investigated human basilar artery using different staining methods. Orcein stained fibers of the internal elastic membrane dark brown and collagen fibers of the tunica adventitia light brown. Other structures of the tunica adventitia, however, could not be demonstrated. Gomori's method stained cell nuclei of the tunica intima dark blue and the internal elastic membrane dark red. These investigators reported that muscle cells and elastic fibers in the tunica media layer could not be distinguished because of their similar colors. In the study reported here, histological sections of the common carotid arteries of human fetuses were stained with orcein and picroindigocarmine; elastic fibers of the internal elastic membrane were stained reddish brown and elastic fibers of the tunica media light brown. Both layers could be distinguished easily. We believe that this result was due to the interaction of orcein with picroindigocarmine.

Many factors affect staining procedures. The fixative, embedding material, section thickness, concentration, pH and chemical characteristics, and molecular weight of the stain are only a few of these factors (Sampson 1979, Horobin and Flemming 1988, The Internet Pathology Laboratory for Medical Education, 2007). For example, trichrome staining techniques provide good staining of specimens fixed in formalin (Bancroft and Stevens 1990). The molecular weight of the dye has important effects on staining. The dyes used in our study have a small molecular weight compared to other multiple tissue stains; therefore, it was possible to stain both intracellular and extracellular structures. The permeability of the tissue is a related factor. Collagen fibers are more permeable than muscle fibers; therefore, collagen fibers can be stained with a high molecular weight dye, whereas muscle fibers can be stained only by a low molecular weight dye (Horobin 1980, Horobin 2002). The pH of a stain also is important. Many stains with a low pH provide better results (Bancroft and Stevens 1990). The staining solutions used in our study were acidic (orcein, pH 2.03; picroindigocarmine, pH 2.55).

There are few studies of fetal sectional anatomy in the literature. The identification and relations of anatomical structures in fetuses is difficult because fetal tissues have not completely developed. In our study, orcein-picroindigocarmine staining provided color contrast in different tissues so they could be distinguished clearly. We believe this staining method will be useful broadly for histological observations owing to its specificity, sensitivity, and clarity.

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