A method for studying the skeleton of human fetuses

It is at times desirable to examine closely the human fetal skeleton. Radiographs are simple and can be made of quite small fetuses, but the detail provided is not very good. The following method, adapted from procedures used in animal teratology, was devised during an unpublished study of malformations in 1000 human fetuses.

1. It is essential that the fetus does not come into contact with formaldehyde. If storage is necessary, use alcohol.

2. The contents of the abdomen and thorax are removed, as is the anterior abdominal wall. It is helpful but not essential for the thoracic cage to remain intact. Removal of the brain is optional, but if it is not removed in fetuses over 14 to 16 weeks' gestation then the calvarium tends to cave in. The eyes, tongue, trachea, and oesophagus are removed.

3. Fix in 95% alcohol or methylated spirit 74 over-proof for about 4 weeks. Longer will do no harm. With large fetuses it is worth changing the alcohol once.

4. Place in acetone for about 4 weeks, changing the acetone once for larger specimens as before. Longer will do no harm.

5. Transfer to alcohol for a further 1 to 2 weeks.

6. The fetus is placed in a solution of potassium hydroxide. If in any doubt use a 0·5% solution, but if the fetus is 16 weeks or over then 1·0% will be better. If the solution is too strong or too warm, the specimen will disintegrate. Leaving the specimen in the sunlight for a few hours can be enough to ruin it. It is best to experiment with a few unimportant human specimens first (animal fetuses are no substitute). If clearing is incomplete then the tissues remain opaque and the bones are poorly displayed (another cause of this is inadequate use of acetone). I prefer weak solutions, the clearing process taking 1 to 2 weeks, but it can be done faster with stronger solutions (animal experiments can be repeated, human ones cannot). Avoid handling the specimen.

7. When the bones are clearly visible, replace the clearing solution with a 0·5% solution of potassium hydroxide containing 2 to 3 drops of a saturated aqueous solution of alizarin red and leave for 8 to 12 hours.

8. Replace the stain with 50% glycerine and leave for 2 weeks. Use 80% glycerine for a further 2 weeks, and finally use 100% glycerine. Store in 100% glycerine, and add a crystal of thymol to prevent bacterial growth.

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