KLINGERS TECHNIQUE OF BRAIN FIXATION
ALAZARIN STAINING OF FETAL BONES

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Klingers Technique/Background

- In 1931 at the University Of Basel, Josef Klinger developed a new method of dissection based on a fixation technique for brain tissue.
- He developed a "freezing "technique that eloquently revealed the white matter tracts.
- Klinger worked with anatomists, surgeons and other scientists and his models and dissections remain the most elegant ever created.

Fixation Of Brain

- Brain tissue will begin to deteriorate as soon as its blood supply is interrupted .
- The deterioration is rapid, and its essential to arrest the deterioration as quickly as possible.
- The process of preserving the brain is called fixation.
- 2 common ways of fixing the brain are freezing and the use of fixing solutions.

Freezing

- Quicker way of fixing brain tissue and preserves more of the biochemistry of the tissue.
- This is more intricate to deal with as it must remain frozen until usage.
- Hence there must be a combination of freezing and usage of fixing solutions for optimal fixation.

Fixing Solutions

- Most common fixing solution used is Formalin.
- 10% Formalin solution is adequate for most purposes.
- Fixing the brain with Formalin takes longer but the brain is more resilient and easier to handle.
- Immersion of brain in Formalin will achieve this process.

Method

- The brain is immersed in 10% Formalin by means of ligature around the Basilar Artery.
- The formalin is replaced after 24 hours
- Thereafter the formalin is replaced again after 2 weeks.
- Remove and wash the brain in cold running water for several days.
- Place the brain in formalin and store in deep freeze for 8 days.

- The brain is then thawed in running water for 24 hours.
- This process of freezing and thawing is repeated 3 times.
- After the third freezing procedure the brain can be kept in 5 % Formalin indefinetely.
- This process of freezing and immersion in formalin creates a brain specimen that is firm and well fixed forever.
- This technique is ideal for " flaking out" threads of grey and white matter demonstrating the fibre tracts on a whole brain.

Alizarin Technique of staining of foetal bones

- This technique, a modification of Davis and Gore(1936) can be used for staining fetal bones and small vertebrates.
- This method renders the muscle transparent and the skeleton a clear brilliant red (Alizarin Red).
- This is an effective method of bone evaluation in small embryos and fetuses.

Principle of Staining

- The principle used here is the affinity of Alizarin Red S to bind with the calcium of bones.
- This dye stains only the ossified areas of bone.
- When completed there is a red skeleton structure in the transparent muscle.

Method

- Remove thoracic and abdominal viscera.
- Fix the fetus in methylated spirits for 7 days.
- Replace spirits after 7 days with new methylated spirits.
- Then fix in alcohol and leave for 7 days leaving the specimen in a "relaxed state".
- Place in 2% KOH till the bones become visible and this takes 2-4 weeks.
- KOH digests the specimen facilitating dyeing with the Alizarin Red S.

- Transfer fetus into 0.0001% Alizarin Red S made in 1% KOH till bones have stained .
- Remove access stain by immersing in 1% HCl
- Leave the fetus in solution made of
  a) Glycerine – 2 parts
  b) 70% Alcohol- 2 parts
  c) Benzyl Alcohol – 1 part
- This should be for 24 hours.

- The specimen should be immersed in 50% Glycerine till it sinks to the bottom of container.
- Thereafter immersion should be 75% Glycerine until the specimen sinks to the bottom.
- Finally the fetus is immersed in 100% Glycerine and also wait till it sinks.
- The specimen now can be stored in 100% Glycerine indefintely.

Summary

- Hence, the specimen is digested in KOH, stained with Alizarin Red S and finally cleared by adding Glycerine to the muscle of the fetus.
- The addition of Glycerine to the specimen has toughened it and it can now be handled.
- This allows for minimal distortion and shrinkage of the specimen rendering it morphologically intact.