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MLS 202

Assignment questions:

1) Write on the purpose of fixation.

2) List 5 compound fixatives and composition.

1) fixatives denature proteins by coagulation, by forming additive compounds, or by a combination of coagulation and additive processes. A compound that adds chemically to macromolecules stabilizes structure most effectively if it is able to combine with parts of two different macromolecules, an effect known as cross-linking. Fixation of tissue is done for several reasons. One reason is to kill the tissue so that postmortem decay (autolysis and putrefaction) is prevented.Fixation preserves biological material (tissue or cells) as close to its natural state as possible in the process of preparing tissue for examination. To achieve this, several conditions usually must be met.

First, a fixative usually acts to disable intrinsic biomolecules—particularly proteolytic enzymes—which otherwise digest or damage the sample.

Second, a fixative typically protects a sample from extrinsic damage. Fixatives are toxic to most common microorganisms (bacteria in particular) that might exist in a tissue sample or which might otherwise colonize the fixed tissue. In addition, many fixatives chemically alter the fixed material to make it less palatable (either indigestible or toxic) to opportunistic microorganisms.

Finally, fixatives often alter the cells or tissues on a molecular level to increase their mechanical strength or stability. This increased strength and rigidity can help preserve the morphology (shape and structure) of the sample as it is processed for further analysis.

Even the most careful fixation does alter the sample and introduce artifacts that can interfere with interpretation of cellular ultrastructure. A prominent example is the bacterial mesosome, which was thought to be an organelle in gram-positive bacteria in the 1970s, but was later shown by new techniques developed for electron microscopy to be simply an artifact of chemical fixation.Standardization of fixation and other tissue processing procedures takes this introduction of artifacts into account, by establishing what procedures introduce which kinds of artifacts. Researchers who know what types of artifacts to expect with each tissue type and processing technique can accurately interpret sections with artifacts, or choose techniques that minimize artifacts in areas of interest.

2) I) Formalin solution (10%, unbuffered):

formaldehyde (37-40%)- 10ml

distilled water- 90 ml

mix well.

ii) Formalin solution (10%, buffered neutral):

formaldehyde (37-40%) -100ml

distilled water - 900ml

NaH2PO4-4.0g

Na2HPO4 (anhydrous)- 6.5g

mix to dissolve.

iii) Carnoy's fluid- fixation time 12-24hours.

methanol, absolute- 60.0ml

chloroform- 30.0ml

glacial acetic acid- 10.0ml

iv) Bousin's fluid-fixation time 6 hours.

saturated aqueous solution of picric acid- 75ml

formalin (-40% aqueous solution of formaldehyde) -25ml

glacial acetic acid- 5ml

fixed tissues should be transferred to 70% alcohol.

v) zenker's solution- fixation time 4-24hours

distilled water - 950ml

potassium dichromate- 25g

Mercuric chloride - 50g

glacial acetic acid- 50g

fixed tissue should be washed overnight in running tap water before processing.