INTRODUCTION

Histopathology is a science completely dependent upon microscopic examination and interpretation. Basic requirements for arriving at a conclusive diagnosis include correct biopsy procedure, proper fixation and processing techniques, adequate sectioning and staining. Identification of structural and morphological details of tissue components is very important for arriving at a conclusive diagnosis. However, sometimes the presence of a foreign substance or alteration in tissue details can create confusion and lead to an incorrect or inconclusive interpretation. Such entities or changes are broadly termed as “artefacts”.

An artefact can be defined as an artificial structure or tissue alteration on a prepared microscopic slide as a result of an extraneous factor.

CAUSES OF ARTEFACTS

1. Clinical application of chemicals
2. Local injection of anesthetics
3. Surgical suctioning
4. Excessive heat
5. Freezing
6. Surgical mishandling of specimen

7. Inadequate tissue fixation
8. Improper fixation medium
9. Faulty tissue processing
10. Embedded sponges
11. Improper staining.

CLASSIFICATION OF ARTEFACTS

1. During surgery
   a. Injection artefacts
   b. Forceps artefacts
   c. Fulguration artefacts.

2. During fixation and transport
   a. Fixation artefacts
   b. Freezing artefacts
   c. Artefacts during transportation.

3. Tissue processing artefacts
4. Other artefacts
   I. During Surgery.

A. Injection Artefacts
   Injection of large amounts of anesthetic solution into the area to be biopsied can produce two major tissue changes:\(^1,2\)
   i. Needle insertion may produce hemorrhage with extravasation that masks the cellular architecture
   ii. Separation of connective tissue bands with vacuolization can occur.

REMEDIY

Local anesthetic infiltration is acceptable if the field is wide enough in relation to the lesion. Direct injection into the lesion is best avoided.
B. Forceps Artefacts: When the teeth of the instrument penetrate the specimen, it results in voids or tears and compression of the surrounding tissue. The surface epithelium may be forced through the connective tissue producing small “pseudocysts”. Compression of the specimen results in loss of cytological details. Nuclear dimensions are especially affected.

Crush artefact: Crush artefact occurs due to tissue distortion resulting from even the most minimal compression of the tissue. It occurs most commonly from mutilation of tissues with forceps during removal, but can be produced by dull scalpel blades that tear the tissue instead of incising it. Crushing produces a destructive and dangerous type of artefact that rearranges tissue morphology and squeezes the chromatin out of nuclei. Inflammatory and tumor cells are most susceptible.

**REMEDY**

1. Use small atraumatic forceps or Adson’s forceps without teeth
2. A suture should be placed in one edge of the specimen (substitute for forceps for tissue immobilization).

C. Fulguration Artefacts: These artefacts are produced as a result of electrocautery. Heat generated causes marked alteration in both the epithelium and connective tissue. The epithelium and connective tissue get detached and the nuclei assume a spindled, palisading configuration. Separation of epithelium from basement membrane has been observed. The fibrous connective tissue, fat and muscle have an opaque, amorphous appearance. When heat is applied, tissue fluids boil and precipitate protein. Such tissue on microscopic examination shows a coagulated and torn appearance. Alternating zones of coagulation that impart a “Herring bone” appearance can also be seen.

**REMEDY**

1. The use of electrocautery is contra-indicated as biopsy technique for it is deleterious to interpretation of the tissue although it is desirable for surgery because it aids hemostasis
2. Care must be exercised to use the cutting and not the coagulation electrode when obtaining a biopsy specimen so that low milliampere current will be produced that will allow efficient cutting and liberation of specimen
3. The incision margin should be adequately away from the interface of the lesion with normal tissue to prevent thermal changes
4. One should avoid accidental contact of cutting tip with the metallic instrument used for holding the specimen
5. Scalpel should be used for initial incision around or into the lesion to be biopsied and followed by electrosurgery to complete the removal of tissue.

II. Fixation Artefacts: During fixation, tissues commonly change in volume. The mechanisms involved are ill-understood, but various factors have been suggested, including inhibition of respiration, changes in membrane permeability and changes in ion transport through the membranes. Some intercellular structures such as collagen swell when fixed. Tissues fixed in formaldehyde and embedded in paraffin wax shrink by 33%. The nuclei in frozen sections are usually bigger that those of the same tissue which has been subjected to conventional preparation. Prolonged fixation in formalin can give rise to secondary shrinkage. Hypertonic solutions give rise to cell shrinkage; isotonic and hypotonic fixatives to cellular swelling and poor fixation. The best results, using electron microscopy as the criterion, have been obtained using slightly hypertonic solution (400-450 mOsm; isotonic solutions are 340 mOsm) for fixation.

1. Artefacts related to diffusion of unfixed material: Diffusion of unfixed material may produce false localization due to movement to some place other than its original location. For example, false localization occurring with glycogen is known as streaming artefact
2. Diffusion of materials out of tissue: Examples include inorganic ions, cofactors for enzymes, biogenic amines. These have implications in techniques like enzyme histochemistry
3. False fixation of extraneous material to tissue: This may occur in autoradiography with (H³) labeled amino acids, sugars, thymidine and uridine. Tissues may incorporate these substances into themselves by active metabolism resulting in too high localization of various radioactively labeled substances
4. Improper Fixation: Delay in fixation or inadequate fixation produces changes like [Figure 1]:
   1. Altered staining quality of cells
   2. Cells appear shrunken and show cytoplasmic clumping

![Figure 1: Photomicrograph showing tissue autolysis due to improper fixation (×10)](image-url)
3. Indistinct nuclear chromatin with nucleoli not always seen
4. Vascular structures, nerves and glands exhibit loss of detail
5. Impression of scar formation or loss of cellularity
6. Use of improper fixative: Fixation in alcohol results in poor staining of the epithelium and improper fixation of the connective tissue. Collagen bundles have an amorphous appearance that is not a result of scar formation but rather result of artefact. Alcohol fixes tissues but causes shrinkage. Therefore, alcohol is not recommended as a substitute for formalin except in extreme emergencies. It also makes the tissue brittle, resulting in microtome sectioning artefacts with chattering and a Venetian blind appearance [Figures 2 and 3]. Currently, 10% neutral buffered formalin is highly recommended for routine fixation purposes. One excellent indicator of poor fixation is the loss of detail of extravasated RBC’s.

**Fixation artefact simulating acantholytic disease**

Tissue fixed in rehydrated formalin exhibits a prominent acantholysis of superficial epithelium with preservation and attachment of the basal cell layer to the underlying tissue. This acantholytic artefact simulates Pemphigus, Hailey-Hailey disease or Darier’s disease. A similar artefact is produced by “fixing” the specimen in tap water. Tissues allowed to air-dry will dehydrate, particularly if placed on an adsorbent surface such as gauze sponge. Such tissue cannot be reconstituted and will show dehydration artefact. Other solutions such as saline/saliva are totally inadequate as these substances do not fix tissues and autolysis continues. Curling of tissue during fixation can be prevented if the tissue is laid out on a piece of glazed paper and then fixed (especially applicable to thin mucosal tissues).

**Artefacts introduced during specimen transport**

I. Freezing Artefacts: These are characterized by formation of interstitial and intracytoplasmic vacuoles resulting from ice-crystal formation taking place as 10% formalin freezes at -11°C. The tissue sections exhibit “Swiss cheese holes” in epithelium and tissue spaces representing areas where forming ice crystals rupture tissue. Sometimes, a granular paranuclear condensation of cytoplasm caused by dehydration of the cells as a result of freezing combined with fixation process is seen.

**Remedy**

1. Use of Lillie’s acetic acid, alcohol, formalin (AAF): A fixative containing 40% formaldehyde: 10 parts; glacial acetic acid: 15 parts and absolute ethyl alcohol: 85 parts. This solution’s freezing point is below –30°C (-22°F). The preservation of morphological details compares favorably with that of tissue fixed in 10% formalin.
2. Acetic acid causes cellular constituents to swell, thereby, counteracting the adverse effects of alcohol. However, it causes good nuclear preservation.

**II. Improper specimen transport, processing and sectioning**

During transport, proper measures should be taken for preventing curling of tissue.

Remedy: If the biopsy specimen consists of a small piece of tissue, it is imperative to maintain the epithelium in correct alignment by securing the specimen to a rigid cardboard. Deeper elliptical biopsies usually do not constitute a problem as the various tissue components can be easily identified.

**III. Processing artefacts**

A. Processing floaters or cutting board metastasis: These are extraneous pieces of tissue contaminating small biopsies. This type of section contamination can take place-
1. When a biopsy is handled in the laboratory, it may become adulterated with small fragments of other tissues being processed in the same batch.

2. When the tissue sections are being floated out on a water bath they may pick up residual fragments of other sections previously floated out on same water bath. Floater artefact may be suspected if:
   1. A tissue fragment looks different from others by virtue of section thickness and/or staining intensity.
   2. A tissue fragment is on a slightly different plane from others, especially if superimposed.
   3. A tissue fragment showing pathologic changes totally different from others and of a type that one would not have expected at all under the clinical circumstances of the particular case.

Remedy: Use clean cassettes, cutting boards, instruments etc.

B. Sponge artefacts: These are seen in tissues placed in cassettes sandwiched between sponges. Angulated holes in the tissue, often triangular with smooth edges are seen at the perimeter. When paired, the angulated holes are occasionally connected by a narrow intervening channel bridging the individual defects. The degree of sponge penetration is related to tissue consistency and is dependent on stromal matrix. For example, soft and lightly fixed specimens generally exhibit greater penetration of sponge bristles than harder well-fixed tissue.

Remedy: Tissue or lens paper is recommended as a substitute.

C. Artefacts of Chemical Treatment:
   a. Most common artefact of this type is “shrinkage”. This may be due to dehydration by alcohol. All types of tissue components may appear smaller than they are and clear spaces may appear around such cells and between tissues.
   b. Loss of fat: Fat containing cells appear “empty”.

IV. Artefacts introduced by microtome:
   a. Curved ribbons and consecutive sections

| Causes                                      | Remedies                                      |
|---------------------------------------------|-----------------------------------------------|
| Leading and trailing edges of block not parallel | Trim with sharp scalpel until parallel         |
| Knife blunt in one area                     | Sharpen or use different part of knife        |
| Surplus area of wax at one side             | Trim away excess wax                          |
| Tissue varying in consistency               | Re-orient block by turning through 90°        |
|                                             | Cool block with ice                           |
|                                             | Mount individual sections                     |

b. Alternate thick and thin sections [Figure 2]

| Causes                                      | Remedies                                      |
|---------------------------------------------|-----------------------------------------------|
| Wax too soft for tissue or conditions       | Cool block with ice or re-embed with wax of higher melting point |
| Block or knife loose                        | Tighten                                       |
| Insufficient clearance angle                | Slightly increase clearance angle             |
| Mechanism of microtome faulty               | Check for any obvious faults. e.g., Pawls may be worn |

c. Thick and thin zones parallel to knife

| Causes                                      | Remedies                                      |
|---------------------------------------------|-----------------------------------------------|
| Knife or block loose in holder              | Tighten                                       |
| Excessively steep knife angle               | Reduce clearance angle to minimum             |
| Tissue or wax too hard for sectioning conditions | Use sharp heavy duty knife                |
|                                             | Reduce slant angle of knife                    |
|                                             | Use heavy duty microtome                       |
|                                             | Use softening fluid on tissue                  |
| Calcified areas in tissue                   | Rehydrate and decalcify                       |
|                                             |                                               |

d. Scoring or splitting of sections at right angles to knife edge

| Causes                                      | Remedies                                      |
|---------------------------------------------|-----------------------------------------------|
| Nick in knife edge                          | Use different part of knife or re-sharpen the knife |
| Hard particles in tissue                    | If calcium deposits are present, then decalcify |
|                                             | If mineral or other particles are present, remove with fine sharp pointed scalpel |
| Hard particles in wax                       | Re-embed in fresh filtered wax                |


e. Sections will not join to form a ribbon

| Causes                                      | Remedies                                      |
|---------------------------------------------|-----------------------------------------------|
| Wax too hard for sectioning conditions      | Breathe on the block to warm or re-embed in lower melting point wax |
| Debris on knife edge                        | Clean with xylene-moistened cloth             |
| Knife angle too steep or too shallow        | Adjust to optimal angle                       |

f. Sections become attached to block on return stroke

| Causes                                      | Remedies                                      |
|---------------------------------------------|-----------------------------------------------|
| Insufficient clearance angle                | Increase clearance angle                      |
| Wax debris on knife edge                    | Clean with xylene-moistened cloth             |
| Debris on block edge                        | Trim edge with sharp scalpel                  |
| Static electrical charge on ribbon          | Earth microtome to water tap                   |
|                                             | Humidify air with moist bath near the knife   |
|                                             | Place Bunsen burner near knife to ionize the air |


g. Areas of tissue in block not present in sections

| Causes                                | Remedies                                |
|---------------------------------------|-----------------------------------------|
| Incomplete impregnation of tissue     | Return tissue to vacuum impregnating bath for a few hours or reprocess if fault is excessive |
| Wax block becoming detached from wood | Re-attach with hot spatula               |

h. Excessive Compression of Sections

| Causes                                | Remedies                                |
|---------------------------------------|-----------------------------------------|
| Blunt knife                           | Sharpen                                 |
| Bevel of knife too wide               | Re-sharpen to produce secondary narrow bends or have reground |
| Wax too soft for tissue or sectioning conditions | Cool block with ice or use high melting point wax |

i. Sections expand and disintegrate on water surface

| Causes                                | Remedies                                |
|---------------------------------------|-----------------------------------------|
| Poor impregnation of tissue           | Return tissue to vacuum impregnating bath for a few hours |
| Water temperature too high            | Cool                                    |

j. Sections roll into a tight coil instead of remaining flat on knife

| Causes                                | Remedies                                |
|---------------------------------------|-----------------------------------------|
| Knife blunt                           | Sharpen                                 |
| Too little rake angle                 | Re-sharpen to produce shallow cutting angle or reduce knife tilt if clearance angle is excessive |
| Section thickness too great for wax in use | Reduce section thickness or use higher melting point wax |
|                                       | Breathe on block from which sections are being cut |

V. Artefacts of staining and mounting:

A. Stain Particles: Incorporation of stain particles occurs when staining chemicals are not dissolved properly or the stain is not fresh, leading to precipitation. Remedy: Filter the stain

B. Air Bubbles: These get trapped when cover slip is placed over stained section. These appear as tiny spherical features.

Remedy
1. Use adequate amount of mounting media
2. If the mounting media has attained greater viscosity, xylene can be used as a thinning agent.

VI. Miscellaneous:

a. Folds in sections: Sometimes wrinkles on the wax sections cannot be straightened resulting in folds or pleats. This is a common artefact in tissues with hard tissue component and is difficult to avoid even with greatest care

b. Cloudy appearance of sections: Faulty dehydration technique results in cloudiness in sections. Remedy: Rehydrate the tissue section and repeat the processing

c. Dirt or Dust: Usually the dirt on the slides is “out of focus” when the section is in sharp focus because the cover slip thickness separates the two

d. Foreign bodies including starch from glove powder entrapped in fibrin and wood fragments from the surface on which tissue is handled and dissected. It is easy to identify these particles of foreign material as artefactual because there will be no associated tissue reaction like foreign body giant cells.

3. Pollen grains contaminating the mountant used (DPX); especially in summer months. These may be misinterpreted as pathogenic fungi but critical examination will reveal that the grains lie outside the plane of section

e. Artefactual pigments

1. Formalin Pigment: Acid formaldehyde hematin is formed in the tissues by the action of acid formaldehyde solution on hemoglobin. It is therefore found in association with blood and is most commonly seen in spleen, liver, lung, bone marrow and in association with areas of hemorrhage and blood vessels. The pigment appears dark brown in color and is composed of small birefringent crystals. Remedy: Formation of pigment can be limited by fixing in non-acid formaldehyde. For example, neutral buffered formalin. However, formation of formalin pigment is not always prevented by the use of buffered formalin especially, prolonged fixation. Formalin pigment may be removed by treating the section with alcoholic ammonia or alcoholic sodium or potassium hydroxide. These methods, however, have the tendency of removing the sections from the slide. Therefore, the method of choice is use of saturated alcoholic solution of picric acid before the staining procedure. The time required for removal varies from 15 minutes to overnight. Malarial and bilharzial pigments are similar to formalin pigment in all their reactions but may be differentiated from the latter by their intracellular distribution

2. Mercury pigment: Tissues fixed in solutions containing mercuric chloride contain a varying amount of a dark brown or grey deposit of granules or irregular masses. The deposits are found throughout the tissue. Remedy: It is easily removed from the section by oxidation with iodine to mercuric iodide which can be subsequently removed with sodium thiosulfate

3. Chromate Deposits: If tissues are not thoroughly washed in water after fixation in chromate-containing fixatives, the chrome salts will react with the dehydrating alcohol forming a yellow-brown to black precipitate within the
4. Stain Deposits: They may be amorphous or crystalline in appearance and are usually highly colored. They are most likely to occur if concentrated solutions are allowed to evaporate on the sections. Remedy: Surface layers of precipitated stain are removed from the staining bath by filtration otherwise the precipitate will be deposited on the surface of section as it is withdrawn from the staining bath.

- Paraffin Wax: Wax is sometimes retained in the section, particularly in nuclei. It can be clearly recognized by its position, refractile appearance and its birefringence. Remedy: It can be removed by treating the section in xylene at 60°C.

Water: Water droplets in a section may occasionally simulate a light brown pigment. A similar artefact is produced by “fixing” the specimen in tap water. Other pigments encountered in histological sections are hematoidin (Bright yellow), hemosiderin (light brown), melanin (dark brown) among others.

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