THE THEORY OF FIXATION

In this article, all data for which no reference is given is derived from the previously unpublished work of the author.

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Fixation is a process which converts a biological entity in the form of living tissue to a dead mass of proteins,fats,cellulose,minerals,etc., with the fond hope that the morphology of this dead mass corresponds fairly closely to that of the living material. This presumptive hope is valid in those cases where it has been possible to compare the morphology of fixed material with that of the living. This comparison can only rarely be made at all, and then only with extreme difficulty, for available methods of microscopic and phiycco-chemical analysis are so crude that close studies can not ordinarily be made without the rapidly ensunng death of the living material.

Chemically considered, fixation is mostly an irreversible coagulation and denaturation of proteinaceous material. To the biologists' regret, the organic chemists have really established very little about the chemical changes that constitute coagulation.Indeed,until a much clearer picture than we now have is made available on these chemical precesses, most discussion on the chemistry of fixation, and therefore its theoretical basis, is worse than academic.I have,however,attempted to stumble in the direction of a few vague hypotheses on the basis of such meagre information as is now available. However it might be said that the work I have done has raised far more questions than it has settled. The only theoretical basis on which any chemical-fixation work can be analyzed is on that of the reactions of proteins.

Zirkle (1928, et.seq.,) has probably done the most important recent work on fixation. He has shown (1928, '29) that the fixation images, which are the appearances exhibited by stained,mounted sections fixed in a particular amnner, vary according to the pH of the fixative solution when a whole series of metallic chromates are used for fixatives. When the fixative pH is below a point that is approximately 4.4, a so-called acid image is produced. In this case,the cytoplasm of the cell (Zea root-tips, Iron-hematoxylin stain were used) is disrupted to give a stringy or reticular appearance to the truly homogeneous living cytoplasm in which distinct vacuoles are embedded. In contrast with the false picture that is given to the cytoplasm, the components of the nucleus and its derivatives is probably the truest that can be obtained. Chromosomes,spindle fibers,nucleoli,and the nuclear reticulum are fixed in such a way that subsequent staining gives high contrast and that study of these elements is aided;furthermore,there is a fairly accurate preservtaion of these elements,as shown by comparison with living material.A controversy still exists over the spindle fibers,which is not quite settled.In the cytoplasm,however, mitochondria are dissolved out.

On the other hand,a basic fixation,where the pH is above 4.4, gives the following image: Cytoplasm is accurately fixed as an homogeneous substratum in which vacuoles,with distinct bounding membranes, and mitochondria, are dispersed. In the nucleus however, the chromatic reticulum has disappeared, and is replaced

by a homogeneous mass, which Zirkle defines as fixed 'karyolymph. In contrast to a fixed reticulum, which frequently shrinks from the nucleolus to leave an emty place or 'halo', the karyloymph is always in direct contact with the nucleolus. Chromosomes are only faintly distinguishable and then only in metaphase plates. Prophases and telophases are indistinguishable.

This conception of acid and basic fixation can be extended to other fixative and mixtures. Chart I is a summary of Zirkle's data on a large variety of chemicals.

CHART ONE

Fixative	Nucl Fix	Cytopl Fix	Mitochondria	Nucleoli	Note
Chromates, ph 4.4 or less	A	A	A	stained	type acid
$LiCr_2O_7$, pH 4.6	z A	A	A	unst	see (1)
Dicarboxyl acids (2)	A	A	A	st	
Oxalic acid	A	A	P??	st	
Dicarboxyl & Formalin	B	B	P	st	
Aliphatic acids (3)	A	A	A	unst	
Trichloroacetic ac	B	B	P	st	
Aliphatic ac & Formalin (4)	BA	BA	PA	st	see 4b
Cu salts, acids (3)	A	A	A	st	
Formic acid & formalin	A	A	A	unst	
Aldehydes (5)	A	A	P	st	
Aldehydes & picric ac 5a	A	A	P	st	
Fomalin & picric ac	B	B	P	st	
Amines & Chromic ac at 5.0	B	B	P	st	6
Formamide " " "	A	A	A	st	

1. This is an anamolous case. The nucleoli were fixed as very large irregular bodies. No explanation is available.
2. Acetic, Propionic, Malonic, Succinic, Glutaric, malic, tartaric acids
3. Acetic, Propionic, Butyric, Valeric, Glycollic, Glyceric, Gluconic, Lactic and Formic acids
4. Except formic acids 4b. Acetic acid & Formaldehyde mordants only central nucleoli
5. Formaldehyde, Acetaldehyde, Propional, Chloral e. Except formaldehyde
6. Ethylene diamine, Ethyl amine, Diethylamine, Triethylamine, Dimethylamine, Trimethylamine, Pyridine, Diisoamylamine.