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The Nucleolus

By definition and etymology, a nucleolus is a "small nucleus", or any "diminutive body found within the nucleus of a cell", plant or animal. This general definition is qualified in so far as many cells in many widely separated phyla of plant or animal organization contain particular bodies, morphologically similar, in their nuclei, so that the term nucleolus has come to be largely restricted to this particular cell organ. Other types of bodies may be found within the nucleus, having varying degrees of morphological relationship to 'true' nucleoli: such may be chromosomes, chromocenters, chromatin net nets, various fixation-coagulation artefacts, intra-nuclear division centers, and perhaps others. We shall, however, restrict our use of the term nucleolus to bodies which do not directly and obviously participate in the mechanics of karyokinesis, which typically disappear before or during the early metaphase of the division of the cell, which do not react positively with the Feulgen chromatin reaction. Various modifications may have to be made, for which see below, particularly in the case of protozoid animals. However, the nucleolus appears so generally and characteristically in the cells, or rather nuclei, of higher animals and plants, and similar perhaps more primitive forms appear in the lower animals, that the nucleolus can be characterized as a definite and specific cell organ. In view of this obvious fact, it is surprising that the field of nucleolus research has been so neglected, particularly in relation to the immense amount of work on chromosome cytology. This paper is a brief resume of the work that has been done on nucleolus, and contains some suggestions as to future investigation in the area.

Very little of the chemical morphology and physiology is known. Likewise, our conception of the physico-chemical processes of biological staining is fragmentary; however, the stain reactions of the nucleoli, and the modifications of these by fixation effects, may give us a clue to their nature and serve as a tool for the investigation of the history and development. With respect to fixation reactions, Zirkle has investigated Zea mays root-tips, staining with Iron-hematoxylin. It should be emphasized with respect to any such studies that the

later treatment of the fixed material is of great importance. Zirkle used alcohol dehydration and paraffin embedding. (Zirkle, 1928a, 1929a, 1931, 1933, 1934, 1935)

Acetic acid, 5%, fixes the nucleoli so that they do not stain
Formalin and acetic acid, 25% & 5% fix so that some nucleoli in the central region stain
Formalin, 25% causes stained nucleoli but imparts a totally different fixation image to the root-tip cells, i.e., mitochondria are fixed and stained. Furthermore, many of the nucleoli are abnormally large, occupying a large proportion of the cell volume (author, unpub.)

Inasmuch as the Formalin-Acetic combination gives the acid image (dissolution of mitochondria, fixation of the nucleolar reticulum, acetic acid must be the faster penetrant, while the effect of the formalin is more or less of a mordanting one. To confirm this conclusion, one might fix for a short time in acetic acid alone, and then transfer to formalin. The physical chemistry of these observations is of course quite obscure. The author, in *Allium cepa*, has found that after formalin-acetic fixation, some nucleoli in the central region are stained. However, many nuclei which have two or more nucleoli, in this area, may exhibit one stained, one unstained nucleolus. This illustrates the erratic manner of the fixative. The author is at present conducting an investigation to determine what influence a change in the composition of the fixative may have upon the number and distribution of such nuclei. In the preparations already made, the distribution is entirely random in the entire central area.

Most other fixative components commonly used, (Chromic acid, Peric acid, osmic oxide-as in Flemming, Potassium Bichromate, and the heavy metals in combination with bichromates,) fix the nucleolus for staining, regardless of their variegated effect on other nuclear and cytoplasmic components. This effect applies only to the Iron-Hematoxylin stain. For example, the Iodine-Methyl Violet method (see Johanssen, DA, 1940) which stains chromosomes and nucleoli very nicely after Bouin's or Navashin's (Belling modification) fails to give a differential stain to these at all after formalin. Safranin, differentiated with HCl gives an intermediate image. (Lederberg, unpub.) After acetic acid, safranin will stain nucleoli nicely, as Iron-Hematoxylin will not.

Other acids have been used in a comparison of their effects with that of acetic. (Zirkle, '33, '34, '35) In this respect, in combination with formalin, the following results have been obtained:

Formic acid, causes no mordanting (staining) of nucleoli,
Acetic Acid, a few nucleoli are stained (v. supra)
Propionic Acid, identical with Acetic
Valeric acid, causes a universal stain of nucleoli

Trichloroacetic acid, fixes nucleoli for stain, but the image is that of the formalin present so that this effect may be presumed as one of slow penetration. The action is quite anomalous inasmuch as it is by far the strongest acid of those mentioned, but gives nevertheless the basic image in combination with neutral formalin. The butyric and dicarboxylic acids act on accordance with their fat solubility. Zirkle ('28b, '31) has used certain special fixatives to investigate nucleolar history in *Zea* and in *Pinus strobus* (V infra.)

Nucleoli (true nucleoli, plasmosomes) are often described as being "oxychromatic" or staining preferentially with acid dyes in contrast with chromosomes, or, more generally, chromatin, which is 'basichromatic'. This distinction is not entirely valid, and can never be used as a critical test for distinction between nucleolar and chromatin derivatives. There is, however, one stain reaction which generally can be used for such a distinction, namely the Feulgen Nucleal-Farbung. This stain procedure, similar to the Schiff reagent for the detection of aldehydes in Organic Chemical Analysis consists of the application of fuchsin-sulfurous acid (i.e., Basic fuchsin which has been decolorized by NaHSO_3)

in acid solution) to sections that have been hydrolysed in hot HCL. This hydrolysis presumably leads to the liberation of aldehydes from the ribose component of the nuclear reticulum or chromosome nucleic acids, which reacts with the reagent to form an addition compound which has a deep purple color. No case has yet been clearly established where nucleoli (plasmosomes) have reacted positively to the Feulgen stain. There is a possibility in the case of growing oocytes. Chromosomes and their derivatives (as the interkinetic, nuclear reticulum) invariably react positively.