Ethyl Alcohol as a Fixative for Smear Materials *

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Recent smear methods have been developed by BELLING (1923), HEITZ (1926), and others in connection with aceto-carmine fixation, and by TAYLOR (1924), NEWTON (1926), BELLING (1928), LA COUR (1931) and others in connection with fixation either by modified FLEMMING’s fluid or by NAVASHIN’s fluid.

All of them have proved very useful in the general chromosome study, especially owing to the rapidity with which preparations can be made ready for observations and also due to the fact that a whole nucleus or a chromosome set can be investigated at one time instead of a section of either.

As far as the study of the structural details of chromosomes is concerned however and also even for a general study especially when permanent preparations are required, the aceto-carmine smears are not quite satisfactory, especially for the early prophase, on account of the weak affinity of the chromatic substance to carmine at this phase. Some of the other smear methods are too complicated, while some are expensive. These conditions prevent a universal application of smear methods.

To overcome these shortcomings the writer would like to recommend the smear method in connection with ethyl alcohol fixation.

Alcohol is, probably the oldest fixative as was stated by MANN (1902). However, among botanists STRASBURGER is probably the pioneer who used alcohol to a considerable extent in cytological work and with great success.

But the opinions of various authors as to the value of alcohol as a fixative are diverse. LEE and MAYER (1910) admit its usefulness for

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small objects, while many authors, such as Baranetzky (1880), Tellyesniczky (1898), Chamberlain (1924), Kardasewitsch (1925), Kissé (1926), etc. disregarded alcohol as a fixative, especially for the study of the nuclear substance or of cytological details. For instance Kissé (1926) states: "Zum Fixieren zytologischer Details kommt er [Alkohol] kaum in Betracht,..."; and Baker (1933) asserts: "It is clear that alcohol is not a fixative for chromatin". The fixation studied by these authors, however, was not "smear fixation", it being mostly the so-called "mass fixation".

The writer, who has been engaged in staining chromosomes in smear material by Feulgen's nucleal reaction, and was attempting to simplify the fixation process, tried ethyl alcohol fixation at the suggestion of Dr. Fujii. The results were quite satisfactory, not only for the Feulgen's nucleal staining but also for various other stainings so far as the general chromosome morphology was concerned.

The material used for this investigation was chiefly the PMC of Fritillaria verticillata var. Thunbergii, but the PMC of Lilium Henryi and Tradescantia, and the somatic tissue of Drosophila melanogaster were also served.

Taylor's procedure of fixation was generally followed, but here, the fixative being ethyl alcohol, there was no need to wash the material in running water, nor to bleach it after fixation; and the slides with smears were left in the alcohol until they were about to be stained.

30, 40, 50, 75, 96, and 100 per cent ethyl alcohol was tried.

Among them a concentration of less than 40 per cent was found unsuitable for PMC smears, due to an insufficient coagulating effect upon the viscous mass (tapetal cells) squeezed out from the pollen sac, which resulted in detachment of the smear material from the slide. 40 and 50 per cent alcohol fixed the outer part of the chromosome well, but some deformation occurred in the internal part probably due to insufficient fixation. 75 and 96 per cent alcohol generally gave a satisfactory result; and especially the latter concentration showed excellent results and was most suitable for Feulgen's nucleal staining, because there were no need of further treatment with alcohol to remove plasmal from the material, previous to the application of the nucleal test.

Fixation by absolute alcohol gave the same results as with 96 per cent alcohol.

Text-fig. 1, a shows 2 nuclei of the PMC of Fritillaria verticillata var. Thunbergii, at a pachytene stage, which were fixed with 96 per
cent ethyl alcohol and stained after Feulgen's nucleolar reaction, in which the syndetic chromonemata are well fixed and individual chromomeres (of some authors) are clearly shown as black dots in the photograph. Text-fig. 1, b, is a reproduction from Belling (1933, Fig. 1) of a similar stage in a PMC of Lilium regale, which was fixed with Navashin's fluid and stained with brazilin. A comparison of these two figures, offers convincing evidence that 96 per cent ethyl alcohol and Navashin's fluid show an equally good results, and shows that fixation by Navashin's fluid can in no way be preferred to alcohol fixation, so far as the general morphology of chromosomes is concerned.

Ethyl alcohol as a fixative for smear material does not produce such a crescent or hemispherical conglomeration in the nuclei as was stated by Tellyesniczy. The formation of such a conglomeration may be due to slow penetration of fixatives in a "mass fixation". In the case of smears, the layer of material on the slide is very thin, so that the fixative penetrates into the material very quickly without producing a one-sided effect in the direction of penetration into the nucleus.

The chromatic substance in the nucleus or in the chromonema of the chromosome is imbedded in the hyalonema. The chromatic struc-

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1) The term "Hyalonema" as a structural integral part (matrix, or a part of it, of many authors) of a chromosome was first used by Fujii in his report (Japanese) of the research carried out in 1932, presented to the Imperial Academy, Japan, which was printed in April 1933.
ture imbedded in hyalonema, when treated with alcohol and washed with running water for 24 hours or more, even for as much as 48 hours, or immersed in 10 per cent aqueous solution of NaCl for 2 days, in no case showed any difference in FEULGEN's nuclear reaction as compared with similar material which was fixed with BENDA's, NAVASHIN's or CARNOY's fluid and subsequently treated with 10 per cent NaCl solution, the FEULGEN's reaction being positive in both cases. For FEULGEN's nuclear tests, SHINKE and SHIGENAGA also used microtome sections of alcohol fixation material, and obtained a positive result. The alcohol fixation material and a non-fixation material however showed a remarkable difference after treatment with 10 per cent NaCl solution for 2 days, the former showing a normal positive reaction to FEULGEN's test, while the latter a very weak reaction or a positive reaction in certain limited portions of a chromonema, as the chromatic substance dissolved out irregularly from the latter. From these data it seems to me that the results of FISCHER's (1899) and BAKER's (1933) experiments for testing the fixing power of ethyl alcohol upon aqueous solutions of nucleoprotein as conducted in test tubes, are not applicable to the case in which such a chromatic substance forms a constituent part of a chromosome in the cell nucleus, though a detailed comparison of the individual parts of the chromonemata in alcohol fixation and other fixations are reserved for future study. Certainly however it must be admitted that whenever lipoid components of any cell structure are to be preserved in fixation, alcohol fixative is not suitable, as is also the case with many other that are well-recognized.

The facts mentioned above show that ethyl alcohol can be used as a fixative for smear cells equally with such well-known fixatives as BENDA's, NAVASHIN's and CARNOY's solutions. It may even be preferred to any one of them for fixation of PMC, provided that FEULGEN's nuclear reaction is to follow; because BENDA's and NAVASHIN's solutions preserve a substance in the callose membrane of the meiotic stage of PMC (NAVASHIN's solution does not retain the substance so much as BENDA's fluid), which gives a reddish violet reaction strongly, owing presumably to the presence of an aldehyde, making the observation of FEULGEN's test on thymonucleic acid difficult, while ethyl alcohol dissolves out the substance in question and consequently the FEULGEN's nuclear test may be applied without hindrance.

It may also be added that FEULGEN's nuclear reaction can be applied to the smear material of PMC without any previous fixation, though one may sometimes find a certain disadvantage due to the
presence of plasmal, as in the case reported by FUJII (1927) on the application of FEULGEN's nucleal reaction on the somatic cells of higher plants; in this case N/1 HCl seems to serve as a coagulator of the viscous plasm of the smears so that the material is kept adhering fast to the slide.

**Summary**

Ethyl alcohol fixation of smear materials for the study of general morphology of chromosomes has been tested and found quite suitable for staining with various dyes, and especially suitable for FEULGEN's nucleal method.

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**Bibliography**


