branching[5]. Consequently, hyphae growing on cellophane should possess a high degree of lytic activity. Flooding them with solutions disturbs the delicate balance between wall-lytic and wall-synthetic enzymes in the hyphae leading to the violent disintegration of apices. The augmentation of branching and lytic propensity by growing the fungi on cellophane strongly suggests that autolytic enzymes play a role in hyphal branching.

The authors thank Prof. S. Bartnicki-Garcia, Department of Plant Pathology, University of California, Riverside, USA for critically reading the manuscript and for his suggestions.

21 Feburary 1984

1. Bartnicki-Garcia, S. and Lippman, E., *J. Gen. Microbiol.*, 1972, **73**, 487.
2. Mahadevan, P. R. and Mahadkar, U. R., *J. Bacteriol.*, 1970, **101**, 941.
3. Mahadevan, P. R. and Rao, S. R., *Indian J. Exp. Biol.*, 1970, **8**, 293.
4. Thomas, D., Das, J. and Mullins, J. T., *Science*, 1967, **156**, 84.
5. Suryanarayanan, T. S. and Swamy, R. N., *Proc. Indian. Acad. Sci.*, 1981, **B90**, 137.
6. Mehta, N. M. and Mahadevan, P. R., *Indian J. Exp. Biol.*, 1975, **13**, 131.
7. Sukumaran, C. P. and Mahadevan, P. R., *Indian J. Exp. Biol.*, 1975, **13**, 127.

THE USE OF ACETOCARMINE AS A SPECIFIC STAIN FOR NUCLEOLUS

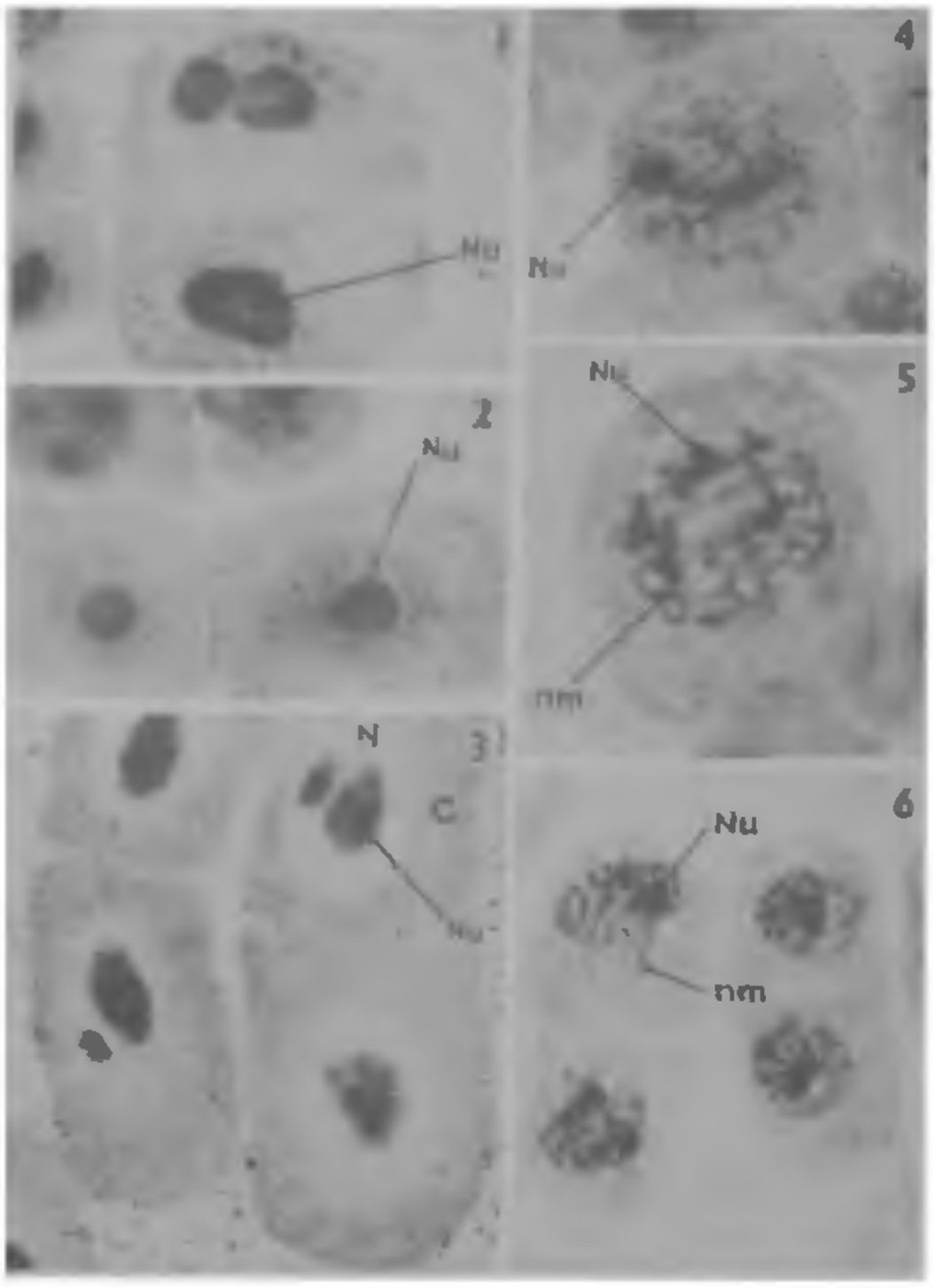
K. GEORGE and C. PRAKASH
Department of Botany, University of Kerala, Kariavattom 695 581, Trivandrum, India.

THE importance of the study of nucleolar structure and function in cytology and cell physiology calls for the development of a proper and easy method of staining this organelle at various stages of cell cycle. The property of the nucleolus reducing silver salts has been originally employed for its specific staining[1–7]. The different fixatives used in these methods modify the affinity of cell structures in a marked way[8,9] and the methods have proved to be unsatisfactory because of artefact produced by metallic inpregnation or poorly differentiated internal cell structures in addition to the fact that these methods are laborious, time and resource consuming[5–7]. Acetocarmine method of staining chromosomes[10] has been modified by Rattenbury[11] for specific staining of nucleoli. But other investigators[12,13] have expressed concern over the inadequacy of acetocarmine to distinguish between nucleoli and chromatin. Therefore, attempts have been made to modify and improve the acetocarmine staining technique to give a satisfactory differentiation between nucleus and nucleolus.

Root tips of *Allium cepa*, *Vicia faba* and *Limnocharis flava* were used in the present study. One set of root tips of these plants were stained with acetocarmine according to the method of Rattenbury[11]. A second set of root tips without any pretreatment in formalin or fixation in Carnoy were directly hydrolysed in 1 N HCl at 60°C for 6 min and washed with distilled water. Hydrolysed root tip is squashed with two drops of 2% acetocarmine on a slide and covered with a coverslip for observation under the microscope. Sufficient staining of the nucleus is ensured by carefully introducing a little more acetocarmine through one edge of the coverslip and drawing it out from the other edge with the help of blotting paper. The slide is then kept at 60°C for 2 min. The edges of the coverslip are sealed with paraffin wax and kept at room temperature (27 ± 5°C) for a minimum period of 48 hr. With complete sealing the prepared slide could be kept for several weeks for observation.

In the first set of squash preparations the nucleoli were not sufficiently differentiating as regards the nucleolar area and the chromatin containing nuclear region. Visibility of the intranuclear and nucleolar structures was obscure probably due to hardening by the formalin used for pretreatment or modification and distortion by the chemicals in the fixative. Destaining of the nuclear chromatin was incomplete and inadequate. On the other hand, in the second set of squash preparations the nucleoli were well stained with optimum intensity at various stages of cell cycle in all the plants alike (figures 1–6). The destaining of the nuclear chromatin was adequate showing distinct differentiation from the nucleolar region. There was even a difference in colour when viewed in transmitted light; nuclear region being light violet and nucleolar region pink. A survey of the interphase cells revealed the existence of mono, di and trinucleolate nuclei (figures 1–3).

In this method the nucleoli as well as nuclear chromatin are simultaneously stained first but on keeping for no less than 48 hr the chromatin gets destained leaving the nucleoli well stained more or less permanently. This indicates that acetocarmine in good



Figures 1–6. 1–3. Differentially stained nucleolus (Nu), nucleus (N) and cytoplasm (C) in *V. faba*, *L. flava* and *A. cepa*. **4.** Nu disappearing in prophase (*L. flava*), **5.** Nucleolar material (nm) deposited on chromosomes in prophase (*V. faba*) **6.** nm disappearing from chromosomes and moving to NOR in telophase with reappearance of Nu (*V. faba*) × 1100.

time is a more specific stain for nucleoli than for chromosomes. Best results in the visualisation of nucleoli are achieved by this method of differential staining of RNA containing nucleolar body without staining simultaneously the DNA containing chromatin structures. Cyclic behaviour of the nucleolar material could be followed by the present method. It is observed that at prophase the stained regions of the chromosomes getting increased as the division cycle progresses along with supercoiling, folding and condensation of chromatin fibers[14,15] together with a corresponding reduction of the nucleolar size (figures 4, 5) and at telophase reappearance of the nucleolar material takes place (figure 6) first as a layer deposited on all the chromosomes and later transferred to the nucleolus organizing regions with a corresponding reduction of the stained regions of the rest of the chromosome parts. This is suggestive of a reversible transference of nucleolar material from and to the chromosomes during division. It agrees with the earlier findings[16–20] that the nucleolar materials originate from all the chromosomes of the complement or that nucleolus contributes to the chromosome matrix during mitosis.

The present method of acetocarmine staining removes the disadvantages of pretreatment with formalin and the consequent hardening, contraction and artefacts of cell structures and also of the fixatives which modify the internal architecture of the cell, nucleus and nucleolus. Further, it has the advantage of being a simpler class room technique requiring no special equipment and achieving good results of specific nucleolar staining with only a slight variation in the usual procedures of acetocarmine squash preparation for chromosomes.

Thanks to Dr C. A. Ninan and the Kerala University for facilities and to the UGC for a fellowship to CP.

1 December 1983

1. Montgomery, T. H., *J. Morphol.*, 1898, **15**, 265.
2. Estable, C. and Sotelo, J. R., *Stain Technol.*, 1952, **27**, 307.
3. Tandler, C. J., *Exp. Cell Res.*, 1959, **17**, 560.
4. Das, N. K., *Exp. Cell Res.*, 1962, **26**, 428.
5. Vincent, W. S., *Int. Rev. Cytol.*, 1955, **5**, 269.
6. Busch, H. and Smetana, K., *The nucleolus*, Academic Press, New York, 1970.
7. Sibdas Ghosh, *Int. Rev. Cytol.*, 1976, **44**, 1.
8. Raju, C. R., *Stain Technol.*, 1983, **57**, 55.
9. Stockert, J. C., Fernandez Gomez, M. E. and Lopez Saez, J. F., *Stain Technol.*, 1969, **44**, 239.
10. Belling, J., *Biol. Bull.*, 1926, **50**, 160.
11. Rattenbury, J. A., *Stain Technol.*, 1952, **27**, 113.
12. Henderson, S. A. and Lu, B. C., *Stain Technol.*, 1968, **43**, 233.
13. Franklin, A. L. and Gary Filion, W., *Stain Technol.*, 1981, **56**, 343.
14. George, K., *J. Sci. Ind. Res.*, 1974, **33**, 406.
15. George, K., *Perspect. Cytol. Cenet.*, 1983, **4**, 15.
16. Zircle, C., *Bot. Gaz.*, 1928, **86**, 402.
17. Heitz, E., *Planta Med.*, 1931, **15**, 495.
18. McClintock, B., *Z. Zellforsch. Mikrosk. Anat.*, 1934, **21**, 294.
19. Estable, C. and Sotelo, J. R., *Publ. Inst. Invest. Cien. Biol.*, 1951, **1**, 105.
20. Belyaeva, E. S. and Volkova, L. V., *Tsitologiya*, 1964, **6**, 286.