# DIFFERENTIAL STAINING OF THE CELL ORGANELLES OF *ALLIUM CEPA* USING A NEW FIXATIVE AND A NEW STAIN

By S SUBRAMANYAM
(*Cytogenetics Laboratory, Department of Biochemistry, Indian Institute of Science, Bangalore-12*)

Received on April 13, 1960

ABSTRACT

The reactions of the root-tip cells of *Allium cepa*, fixed in Iodine-formaldehyde-acetic acid (I.F A ), Acetic alcohol and Navashin's fluid to Giemsa stain are compared with those obtained after staining with Methyl green—Pyronin I. F. A gives optimal preservation of the cell organelles but differs from Acetic alcohol in that it accentuates the blue colour of Giemsa and the red of Methyl green—Pyronin The red colour of Giemsa is transient in material fixed in Navashin for one hour and absent in those fixed for twenty-four hours The differential staining with Methyl green—Pyronin was observed only when the time of fixation in Navashin was limited to one hour

The nucleus and the chromosomes in prophase and late telophases are stained purple red by Giemsa and bluish green by Methyl green—Pyronin. The chromosomes at other phases of division have a slightly darker shade The nuclear membrane, the nucleoli, the cytoplasm, the achromatic figure and especially the phragmoplast are blue in Giemsa and red in Methyl green—Pyronin The cell plate which is unstained in early stages of formation stains in late telophase

## INTRODUCTION

During an analysis of the reaction of the nucleus of living yeast cells to various fixatives and stains it was discovered that Iodine-formaldehyde-acetic acid (I F.A.) solution alone gave a life-like preservation (Royan, 1956, 1958 *a*; Thyagarajan and Subramaniam, 1957; Aswathanarayana and Subramaniam, 1958). On staining the fixed but unhydrolysed smears with Giemsa's solution the yeast nucleus was shown to have a blue nuclear membrane, red chromocentres and blue nucleolar equivalents (Royan, 1958 *b*; Subramaniam *et al*, 1959). Further, the fixatives commonly employed for the study of plant nuclei did not give a life-like preservation of the organelles of the yeast nucleus (Subramaniam, 1960).

Till the demonstration of the nucleus in living yeast cells, the selection of fixatives was arbitrary. Moreover, the Giemsa stain now in vogue in yeast cytology had rarely been tested on plant material. The life-like preservation of the yeast nucleus, by I. F. A., a fixative not tried on plant material, and the facility with which the Giemsa stain could be used to stain differentially the nuclear organelles necessitated confirmation by tests on plant material

While the utility of I. F. A. as a fixative could be evaluated in yeasts by studying the reaction of living cells with visible nuclei (Royan, 1956, 1958 *a*; Thyagarajan and Subramaniam, 1957; Aswathanarayana and Subramaniam, 1958) the same procedure would not be possible in the case of the root tips composed of several layers of cells.

A historical account of the Giemsa stain is given by Conn (1940) and its suitability for cytological investigations is indicated by Gatenby and Cowdry (1928) and Gatenby and Painter (1937). Its use in Microbial cytology is reviewed by Robinow (1944, 1956), Marshak (1955), Vendrely (1955) and Royan (1958 *a*).

Jacobson and Webb (1952) stained cells from tissue cultures in May-Grunwald followed by Giemsa and observed that the deoxyribonucleoproteins were purple red while the ribonucleoproteins were blue. Hartman and Payne (1954) obtained comparable results in *Escherichia coli* stained directly in Giemsa.

Immers (1957) employed Giemsa in his investigations on fertilization and cleavage of the sea-urchin eggs. The deoxyribonucleic acid in the pronucleus of the unfertilized sea-urchin egg was located by Agrell (1958) using Giemsa stain and enzymatic digestion. According to Kamahora, Inamori, Furusawa and Mori (1953) the constituents of the Giemsa stain have differing affinities for DNA, RNA and the proteins. The pink purple of the chromatin is said to be a mixture of the dark blue colour imparted to the DNA by Azure I and the pink colouration, by eosin, of the histone and non-histone proteins. The affinity of Methylene blue for RNA would explain the blue colour of the nucleolus and the cytoplasm.

Giemsa stain followed by a 2% alcoholic solution of Safranin was used by Wright and Skoric (1928) to locate symbiotic bacteria in plants. In sections, the bacteria were deep blue, the cytoplasm of the plant cells light blue, the nuclei pink, the nucleoli blue and the cell walls red. This is perhaps one of the rare instances of the use of Giemsa in plant cytology. The observations embodied in this paper attempt an evaluation of the action of I. F. A. as compared to other fixatives on the root tips of *Allium cepa* and of the Giemsa stain with conventional staining methods.

## MATERIAL AND METHODS

The roots of freshly germinated bulbs of *Allium cepa* were washed under the tap, excised and then transferred to the three fixatives, *viz.*, Acetic alcohol, Navashin's fluid and Iodine-formaldehyde-acetic acid. The vials were kept under an exhaust pump to ensure quick penetration and rapid fixation. Good preservation was obtained by keeping the root tips for two hours in Acetic alcohol (1 : 3) and for only an hour in Iodine-formaldehyde-acetic acid {Gram's Iodine [diluting 1 part of Lugol's iodine (1% $I_2$ in 2% aqueous KI) with 2 parts of water] 8.8 c.c.; Formaldehyde (B. D. H. Sample, 37-41%) 1.2 c.c.; and

Glacial acetic acid (Analar quality) 0.5 c c.] Material fixed in Navashin (Darlington and La Cour, 1950, p 115) for a short period of one hour and for a longer one of 24 hours exhibited differences in their reaction to the stains. The Acetic-alcohol fixed material was down-graded while that from I.F A. was passed through two changes of 70% alcohol extending for a period of 24 hours to remove the iodine The Navashin material was washed under the tap for 24 to 48 hours. After removal of traces of the fixatives the root tips were either stored in 70% alcohol or immediately dehydrated, cleared in mixtures of absolute alcohol and chloroform followed by pure chloroform and then impregnated and embedded in paraffin. The blocks were sectioned at 6 $\mu$ and 10 $\mu$.

### Staining with Giemsa's Solution

The sections washed well under the tap for 15-20 minutes and kept in distilled water for five minutes were stored in Sorensen's phosphate buffer of pH 7 0 for 10 minutes. They were then transferred to the Giemsa stain (2.5 ml. of Michrome brand (Gurr) stock solution and 47.5 ml of the buffer of pH 7.0) and examined periodically up to 24 hours directly in the stain. To get the best preparations it was desirable to overstain the sections and then de-stain them carefully in 50% alcohol prepared in buffer. After rinsing in 20% alcohol and in buffer they were quickly dehydrated through alcohol grades, passed through alcohol-xylol (1 : 1) mixture and then through two changes of xylol before being mounted in Canada balsam. There is occasionally a shrinkage on transfer of slides from alcohol to xylol. The stain has a tendency to fade in permanent preparations. Observations were made on buffer mounts as well as from permanent preparations The former was considered necessary to evaluate the changes that occur during dehydration.

### Staining with Methyl Green—Pyronin Solution

A 1% stock solution of Methyl green—Pyronin (Gurr sample, Special for Nucleic acids) in 0 25% Phenol in distilled water was prepared and was diluted with three times its volume of water just before use. Slides rinsed in distilled water were stored in it for 15 to 30 min. They were then rinsed in distilled water, blotted to remove the excess of water and passed through absolute alcohol, absolute alcohol-xylol mixture (1 : 1), and three changes of xylol before mounting in Canada balsam. The overstaining, if any, was removed by absolute alcohol.

Observations were made with a Bausch and Lomb Research Microscope using a $\times$ 90 objective and a $\times$ 10 eye-piece and the selected stages were photographed at 1/3 their magnification on Kodak Microfile film using a Leica attachment. Colour transparencies of some of the stages were prepared on Kodak Ektachrome film.

## OBSERVATIONS

1. *Acetic alcohol—Giemsa*:—The progress of staining on transfer of sections to Giemsa solution was followed. After 5 to 15 minutes in the stain, the nuclei and chromosomes were greenish blue, while the other organelles were blue. On continued stay the blue began to be more prominent and the nuclei and chromosomes slowly stood out as deep purple structures.

Attention is invited to the fact that the red component of the Giemsa stain begins to have an affinity for the structures only when the cytoplasm has become deep blue.

When the sections were examined after removal from the bath and without rinsing, the blue colour dominated. The nuclei were purplish red. Differentiation in alcohol removes the excess of blue and leaves the nuclear matrix purple red. If this process is continued the purple red staining is also removed by alcohol leaving the nucleus greenish blue (Subramanyam and Subramaniam, 1957, Photo 1) Direct examination in the stain does not reveal the nuclear envelope owing to the intense staining of the nucleus. It is only when the nucleus is purple red after differentiation, that the blue nuclear membrane could be recognised (Photo 1). The slides were therefore differentiated in alcohol to give the desired grade. When on prolonged differentiation in alcohol the nucleus is greenish blue, the nuclear membrane still retained the blue staining (Photo 23, Subramaniam *et al*, 1959). The nucleoli not discernible in overstained nuclei became clear on differentiation in alcohol. They appeared as bluish organelles (Photos 1 and 2) Over-differentiation improved the clarity of the nucleoli which appeared as if they had stained borders. The cytoplasm was generally blue.

During prophase and late telophase the chromosomes were purple red (Photos 2 and 8). In prometa-(Photo 3), meta-(Photo 4) and anaphases (Photos 5 and 6) the chromosomes were dark owing to a coupling of the purplish red with blue In early telophase (Photo 7) the chromosomes were deep purplish red especially when they were contracting at the poles. The polar caps in prophase (Photo 2), the developing (Photo 3), and the fully formed spindle at metaphase (Photo 4), the interzonal fibres and other regions of the spindle in anaphase (Photos 5 and 6) and the phragmoplast in early telophase (Photo 7) were stained blue. This was particularly prominent in the phragmoplast. The achromatic figure, though of the same colour as the cytoplasm, stood out owing to its greater affinity for the blue component. The cell plate seen in early telophase as an unstained streak (Photo 7) began to stain blue in late telophase along with the two knob-like remnants of the phragmoplast at its end (Photo 8).

In using Giemsa as a regressive stain the time of stay in the staining bath necessary to give good preparations was also explored. Acetic alcohol material stained for one hour gave good preparations on differentiation. A longer stay in the staining bath naturally necessitated a longer time of de-staining.

Unlike the earlier workers who appear to have used Giemsa stain as a progressive one (Jacobson and Webb, 1952) the observations reported above are based on the results obtained by differentiating overstained sections. It became interesting therefore to evaluate the utility of Giemsa as compared to Methyl green-Pyronin.

2. *Acetic alcohol—Methyl green-Pyronin* —Shimamura and Ota (1956) offer evidence for the presence of pentose nucleic acid in the achromatic figure. They record the absence of pentose nucleic acid in the cell plate, which appeared as an unstained line in the middle of the phragmoplast. Their conclusions are drawn from sections differentially stained with Toluidine blue, Thionin and Methyl green-Pyronin. The fixatives used were Acetic alcohol and Telyesniczky's fluid

On staining sections of *Allium cepa*, fixed in Acetic alcohol, the nuclear membrane (Photo 16) and the nucleoli (Photos 16 and 17) were red, while the nuclear matrix showed bluish green areas (Photo 16). The chromosomes in prophase (Photo 17), as well as late telophase (Photo 22) were bluish green, while in prometa-(Photo 18), meta-(Photo 19), ana-(Photo 20) and early telophases (Photo 21) they tended to be more bluish The polar caps (Photo 17), the developing spindle in prometaphase (Photo 18), the spindle at metaphase (Photo 19), the poleward regions of the spindle (Photo 20), the phragmoplast (Photo 21) and its knob-like remnants and the cell plate (Photo 22) were all stained red. It is only during the early telophase (Photo 21) that the cell plate remained unstained, reminiscent of a similar experience with the Giemsa stain (Photo 7).

3. *Iodine-formaldehyde-acetic acid—Giemsa* :—The reaction of the cell organelles to Giemsa in material fixed in I F.A. was comparable to that observed after Acetic alcohol fixation. Whereas after Acetic alcohol the chromosomes and nuclei assumed a purplish red tinge after a stay of 30 to 45 minutes in the stain, in I F A. material the identical organelles became purplish red only after a lapse of one to three hours. The blue colour of the cytoplasm appeared more intense when the sections were examined directly from the stain. But this could be removed easily during differentiation. In permanent preparations the organelles having an affinity for the blue component had a more intense colour as compared to those of cells fixed in Acetic alcohol

When material over-stained in Giemsa was carefully differentiated in 50% alcohol, the nuclear matrix was purplish red, the nucleoli and the nuclear membrane blue (Photo 9) The chromosomes in prophase (Photo 10) and and late telophase (Photo 15) were purple red. But this colour appeared deeper in prometa-(Photo 11), meta-(Photo 12), ana-(Photo 13) and early telophases (Photo 14) The cytoplasm and the achromatic figure (Photos 11 to 13) were stained in shades of blue The cell plate (Photo 14) appeared unstained in early telophase The spindle fibres and especially the interzonal

ones were clear in Acetic alcohol as well as in I F. A. Giemsa preparations (Photos 6 and 13).

4 *Iodine-formaldehyde-acetic acid—Methyl green-Pyronin*.—The nuclear membrane and the nucleoli were stained red (Photos 23 and 24). The chromatin of the resting nuclei (Photo 23) and the chromosomes at prophase (Photo 24) and late telophase (Photo 29) were stained bluish green. The chromosomes during prometa- (Photo 25), meta- (Photo 26), ana- (Photo 27) and early telophases (Photo 28) tended more towards blue. The polar caps (Photo 24), the spindle (Photos 25 and 26), the poleward and the interzonal regions of the spindle at anaphase (Photo 27) and the phragmoplast (Photo 28) were stained red. The cell plate seen as an unstained area in early telophase (Photo 28) appeared as a red line in late telophase (Photo 29) along with the remnants of the phragmoplast at its sides. Material fixed in I. F. A. had a greater affinity for the red colour of Methyl green-Pyronin. Attention was more concentrated on the contrast of the cell organelles in order to evaluate whether Giemsa gave as specific a distinction between the chromosomes and the achromatic figure as Methyl green-Pyronin.

5. *Navashin—Giemsa*:—Unlike the two previous fixatives the cell organelles were not stained in differential colours by Giemsa in material fixed for 24 hours in Navashin's fluid. All the organelles though clear appeared in different shades of blue. The cell plate was seen only as a stained line on the equator of the phragmoplast. The characteristic red or purplish red staining of the chromosomes was not observed either when over-stained slides were examined directly in the stain or during de-staining in 50% alcohol.

Such an experience led to a doubt whether the absence of affinity for the red component of the Giemsa stain may not be due to over-fixation. Therefore, the reaction of the cells to Giemsa after fixation for an hour in Navashin's fluid was tested. Direct examination in the stain showed the dark blue nuclei and the chromosomes being overlaid by a slight reddish tinge after a stay in Giemsa's solution for one to three hours. The red tinge was very transient even in wet preparations and disappeared completely during the quick dehydration necessary for making the slides permanent.

6. *Navashin—Methyl green-Pyronin*:—Contrary to the experience with Giemsa where the red colour was transient, a positive differential staining with Methyl green-Pyronin was obtained when the fixation time in Navashin had been limited to one hour.

The chromatin in Photo 30 was bluish green. The nuclear boundary, the nucleoli (Photos 30 and 31), the cytoplasm, the polar caps (Photo 31), the spindle (Photos 32 to 34), the phragmoplast (Photos 35 and 36) and the cell plate with the remnants of the phragmoplast (Photo 37) were all stained in a red colour. The cell plate was seen as an unstained line in early telophase

(Photo 35) only in a few instances In many, the cell plate was stained red both in early as well as late telophases (Photos 36 and 37).

The difficulty experienced in getting a differential staining of the cell organelles with Giemsa in material fixed in Navashin for 24 hours was parallelled by the experience in attempts to stain such sections with Methyl green-Pyronin. Even after storage for 24 hours the nuclei and chromosomes were only red. The bluish green colour assumed by the chromatin when Acetic alcohol or I. F. A. fixed material were stained with Methyl green-Pyronin was absent in Navashin material fixed for 24 hours.

The reaction of the various organelles to staining with Giemsa and Methyl green-Pyronin solutions are summarized in Table I.

TABLE I

STAINING REACTIONS

| *Organelles* | *Giemsa* | *Methyl green-Pyronin* |
|---|---|---|
| I. *Resting cells* | | |
| (i) Chromatin | Purple red | Bluish green |
| (ii) Nuclear membrane<br>(iii) Nucleolus<br>(iv) Cytoplasm | Blue | Red |
| II. *Dividing cells* | | |
| (i) Chromosomes | | |
| (*a*) Prophase and late telophase | Purple red | Bluish green |
| (*b*) Meta-, ana-, and early telophases | Dark Purplish red (Purple red + blue) | Dark Bluish green |
| (ii) Polar caps<br>(iii) Spindle<br>(iv) Poleward regions of the spindle<br>(v) Phragmoplast | Blue | Red |
| (vi) Cell Plate | | |
| (*a*) Early telophase | Unstained | Unstained |
| (*b*) Late telophase | Stained | Stained |

## DISCUSSION

Iodine-formaldehyde-acetic acid gave optimal preservation of the cell organelles. Material fixed in it can be sectioned with ease unlike those in Acetic alcohol which show shrinkage as well as brittleness. The difference between I. F. A and Acetic alcohol is that there is an accentuation of the blue colour of Giemsa and the red of Methyl green-Pyronin in the former fixative.

Immers (1957) recorded his inability to stain the resting nuclei during the first mitosis of the fertilized sea-urchin eggs The nuclei of the root tip cells of *Allium cepa*, however, show a clear differential staining (Photos 1 and 9). Jacobson and Webb (1952) described the nuclear membrane as colourless while Agrell (1958) reported it as faint red in the pronucleus of sea-urchin eggs In *Allium cepa* the nuclear membrane has the same colour as the cytoplasm and the nucleolus (Photos 1 and 9; *c f* Subramanyam and Subramaniam, 1957, Photo 1). This is reminiscent of the staining of nuclear membrane red (Photos 16, 23 and 30) by Methyl green-Pyronin and would conform to the presence of a distinct membrane reported from electron micrographs of plant (De, 1957) and animal cells (Callan and Tomlin, 1950; Watson, 1954; Kautz and De Marsh, 1955; and Barer *et al*, 1959)

The slight variations in the colour of the chromosomes during the various phases of division in *Allium cepa* (Table 1), parallel a similar observation by Jacobson and Webb (1952) and disagree with those of Immers (1957) who records a uniform magenta tint during all the phases.

Jacobson and Webb (1952) suggested that the deoxyribonucleoproteins were stained purple red by May-Grunwald-Giemsa and the ribonucleoproteins blue (*c f.* Kamahora *et al*, 1953). Confirmation for the above was offered by them by staining isolated nucleoproteins before and after treatment with enzymes The nucleoli and the cytoplasm of *Allium* which are blue in Giemsa (Photos 1, 2, 9 and 10) are red in Methyl green-Pyronin (Photos 16, 17, 23, 24, 30 and 31; *See* Table I) It should be emphasized, however, that the composition of the fixative and the time of fixation are major factors in obtaining a polychromatic staining This is evident from the transient nature of the red colour of Giemsa in Navashin material and an accentuation of the blue colour of Giemsa and the red of Methyl green-Pyronin in I. F. A. material.

Plant cells fixed in Acetic alcohol and stained with Giemsa showed the same differential staining of the nuclear organelles as in yeast cells fixed in I F. A. (Subramaniam *et al.* 1959). Evidence is presented in this paper to show that this similarity in the structure of yeast and plant nuclei could be demonstrated in material fixed in I. F. A and stained with Giemsa

## ACKNOWLEDGEMENT

Grateful acknowledgement is made to Dr. M K. Subramaniam for his guidance and encouragement and to the authorities of the Indian Institute of Science, Bangalore, for the award of a Scholarship.

## References


1 Agrell, I .. *Arkiv for Zoologi*, 1958, **11**, 457-459
2. Aswathanarayana, N V and Subramaniam, M. K *Proc Indian Acad Sci*, 1958, **47 B**, 263-273.
3 Barer, R, Joseph, S and Meek, G.A. . *Exptl Cell. Res*, 1959, **18**, 179-182
4. Callan, H. G and Tomlin, S. G. *Proc Roy. Soc Lond*, 1950, **137 B**, 367-378
5. Conn, H J. .. "Biological Stains". (Geneva, N Y Biotech Pubs) 1940
6. Darlington, C D and La Cour, L F. . "The Handling of Chromosomes" (London Allen and Unwin), 1950.
7 De, D N. . *Exptl Cell Res*, 1957, **12**, 181-184
8. Gatenby, J B. and Cowdry, E V. . "Bolles Lee's Microtomist's Vade-Mecum" (London Churchill), 1928,
9 ———, J B and Painter, T S "The Microtomist's Vade-Mecum (Bolles Lee)" (London Churchill), 1937
10 Hartman, P. E and Payne, J I *J Bact.*, 1954, **68**, 237-242
11 Immers, J .. *Exptl Cell Res*, 1957, **12**, 145-153
12. Jacobson, W and Webb, M *Ibid*, 1952, **3**, 163-183
13 Kamahora, J, Inamori, K, Furasawa, E and Mori, T *Med Jour. Osaka Univ*, 1953, **4**, 235-240
14 Kautz, J and De Marsh, Q B *Exptl Cell Res*, 1955, **8**, 394-396
15 Marshak, A . *Internat Rev Cytol.*, 1955, **4**, 103-114
16. Robinow, C F *Jour Hygiene*, 1944, **43**. 413-423
17. ——— *Bact Revs* 1956, **20**, 207-242
18 Royan, S *Proc Indian Acad Sci.*, 1956, **44 B**, 47-55
19. ——— . *Ibid*, 1958 *a*, **47 B**, 31-42
20 ——— *Curr Sci.*, 1958 *b*, **27**, 400-401.
21 Shimamura, T and Ota, T *Exptl Cell Res*, 1956, **11**, 346-361
22 Subramaniam, M K Royan, S, Thyagarajan, T R., Aswathanarayana, N V and Subramanyam, S *Indian Inst Sci., Golden Jub. Res Vol*, 1959, 96-102
23 Subramaniam, M K . *Proc Indian Acad Sci*, 1960, **51 B**, 66-73
24 Subramanyam, S and Subramaniam, M K. . *Curr Sci*, 1957, **26**, 360
25 Thyagarajan, T R and Subramaniam, M K *Proc Indian Acad. Sci*, 1957, **45 B**, 187-195

26 Vendrely, R . *Internat. Rev Cytol*, 1955, 4, 115-[illegible]

27 Watson, M L. *Biochim et Biophys. Acta*, 19[illegible], 15, [illegible]

28 Wright, W H. and Skoric, V *Phytopath*, 1928, 18, 803-807


## DESCRIPTION OF PHOTOMICROGRAPHS

### PLATE I

Photos 1-8 Sections of roots fixed in Acetic alcohol and stained with Giemsa × ca., 2,000.

1 The nuclear membrane is continuous

2 Prophase The polar caps merge with the nuclear membrane

3 Prometaphase The fibrous nature of the spindle is clear

4 Metaphase The prominently stained chromosomes lie on the equator of the spindle

5 Anaphase The poleward regions of the spindle are very clear

6 Anaphase The interzonal fibres are blue

7 Early telophase The phragmoplast is seen with the clear unstained cell plate.

8 Late telophase The cell plate shows the remnants of the phragmoplast at its ends

9-12 Sections of the root tips fixed in Iodine-formaldehyde-acetic acid and stained with Giemsa × ca , 2,000

9 The nuclear membrane is clear

10 Prophase. The polar caps and the nuclear membrane are discernible

11 Prometaphase The fibrous spindle is very distinct

12 Metaphase The spindle and the chromosomes are clear

### PLATE II

13-15 Sections of roots fixed in Iodine-formaldehyde-acetic acid and stained with Giemsa × ca., 2,000

13 Anaphase with the poleward and interzonal regions of the spindle

14 Early telophase. The phragmoplast is prominent. The cell plate is unstained

15 Late telophase with the stained cell plate and the knob-like remnants of the phragmoplast

16-22 Sections of root tips fixed in Acetic alcohol and stained with Methyl green-Pyronin. × ca , 2,000

16 Resting nucleus The two nucleoli and the nuclear membrane are stained red.

17 Prophase with polar caps imperceptibly merging with the nuclear membrane.

18 The fibrous spindle stained red is clear

19 Metaphase with the spindle.

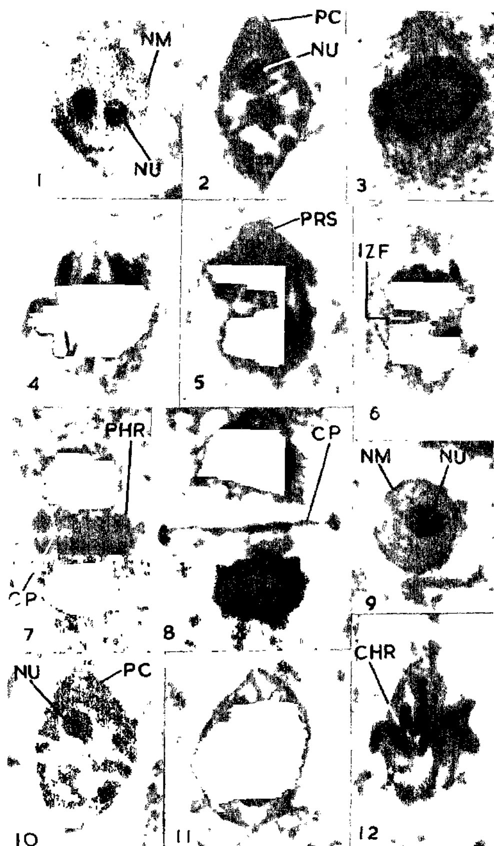


PLATE I

Differential staining of the cell organelles of *Allium cepa* using a new fixative and a new stain

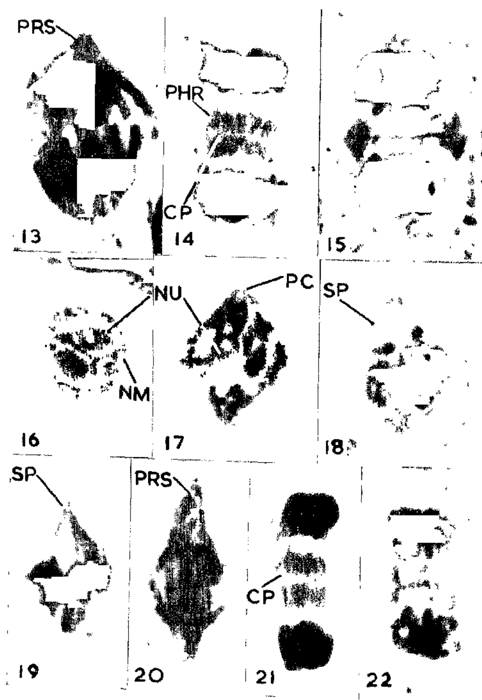


PLATE II
Differential staining of the cell organelles of *Allium cepa* using a new fixative and a new stain

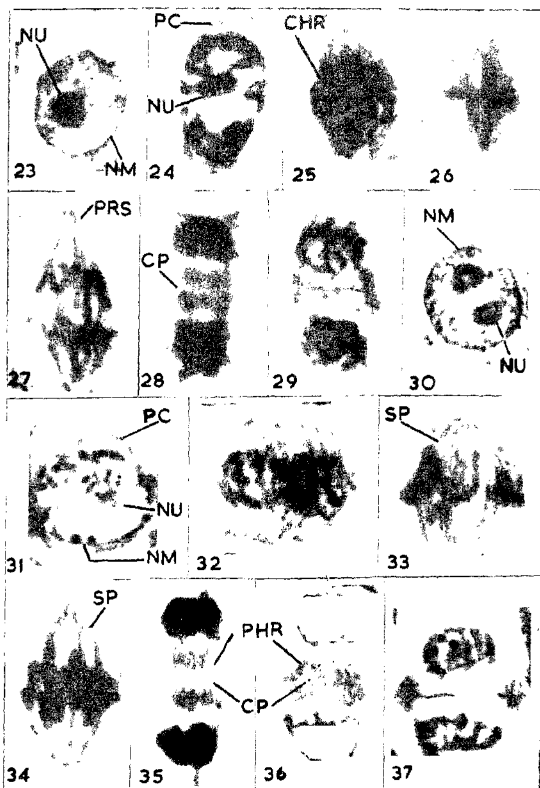


PLATE III

Differential staining of the cell organelles of *Allium cepa* using a new fixative and a new stain

20 The poleward regions and the interzonal part are distinct

21 Early telophase The unstained cell plate is seen along the middle of the phragmoplast

22 Late telophase showing the stained cell plate

### PLATE III

23-29 Sections of roots fixed in Iodine-formaldehyde-acetic acid and stained with Methyl green-Pyronin × ca , 2,000

23 The nuclear membrane is as thick as in Giemsa preparations

24 Note the contrast between the polar caps and the chromosomes

25 Prometaphase The chromosomes are stained darker

26 Metaphase The spindle is obscuring the chromosomes

27 Anaphase The interzonal and the poleward regions are stained in almost the same intensity

28 Early telophase Note the unstained cell plate on the equator of the phragmoplast

29 Late telophase The cell plate and the remnants of the phragm oplast are stained

30-37 Sections of roots fixed in Navashin's fluid and stained with Methyl green-Pyronin × ca , 2,000

30 Note the nuclear membrane.

31 Prophase The polar cap is outside the nuclear membrane

32 Prometaphase The fibrous spindle can be seen distinctly

33 Metaphase Note the spindle fibres converging at the poles.

34 Early anaphase with the bipolar spindle converging at both the poles

35 Early telophase. Phragmoplast with the unstained cell plate

36 Early telophase Phragmoplast with the stained cell plate

37 Late telophase The remnants of the phrgmoplast are prominent

### KEY TO LETTERING

CP , Cell Plate, CHR , Chromosomes , IZF., Interzonal fibres ; NM , Nuclear membrane; NU , Nucleolus ; PHR., Phragmoplast , PC , Polar cap ; PRS , Poleward regions of the spindle ; SP , Spindle