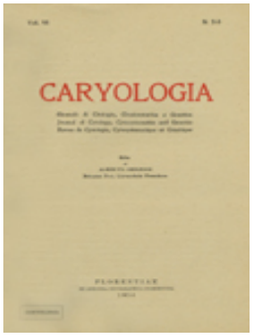

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(with Plate VII and 6 text-figures)

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INDUCTION OF DIVISION THROUGH NUCLEIC ACID TREATMENT

by

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(with Plate VII and 6 text-figures)

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INTRODUCTION

The problem of differentiation in plant tissues is a long debated subject, and various speculations have uptil-now been made for a solution of this problem. HUSKINS and his associates in his series of papers (1947, 1948*a*, 1948*b*) explained the phenomenon on the basis of his well known « Endopolyploidy » concept. He suggested that the socalled « Resting cells » of the differentiated tissue are in a highly metabolic condition. These go on dividing endomitotically evidenced by the presence of a large number of prochromosomes in these tissues, and thus a condition comparable to the polytene thread of Drosophila. Their non-visibility due to their physical and chemical nature is mainly responsible for the development of the general belief of their non division. As the formation of gene products seems to be of absolute necessity for differentiation, this theory of HUSKINS has created considerable interest in different quarters.

The vigorous researches initiated thereby have opened up new possibilities in this line of investigation, and innumerable side issues of fundamental importance have gradually emerged. Since the publication of pioneer works of Huskins on induction of chromosome division in differentiated tissue by Indole 3 Acetic acid (HUSKINS & STEINITZ, 1948*b*), several other chemicals (D'AMATO, 1950) had been on trial, some of which have already yielded quite satisfactory results. Here in this laboratory, this is one of the main problems in which we are at present engaged on a large scale. In addition to effects noted through the application of different chemicals (unpublished observations), the present report deals with the results of nucleic acid treatment on resting nuclei.

This was undertaken to find out the principle underlying the endopolyploidy of differentiated cells and their division through various agents.

Methods followed

The technique that has been employed for this scheme is quite simple and is the same as applied to the *Allium test* of Levan and his co-workers (Levan & Tjio, 1948; Levan, 1949, Levan & Lotfy, 1949 etc.). Bulbs of onion were kept for different intervals of time in 1% nucleic acid solution viz. for 2, 3 or 4 hrs. and then observed immediately following oxyquinoline, coumarin fixation and orcein staining or Feulgen procedure preceded by fixation in Acetic-alcohol. Recovery experiments in Knop's standard solution whenever necessary to detect the delayed effects were carried out for 24, 48 or 72 hrs. in jars at a temperature of 12-16°C. and observed as usual following Aceto-orcein or Feulgen technique. The yeast nucleic acids were obtained in Crystalline form from B.D.H. products.

Observations

Treatments for varying periods viz. 3, 4, 6, 7 and 8 hrs. in 1% nucleic acid solution revealed different degrees of effect on chromosome structure. Extreme clumping of chromosome mass could be observed following 4 hrs. of treatment, whereas in 6 hrs. treatment comparative straightening of chromosomes were noted. 7 & 8 hrs. of treatment proved to be extremely toxic and resulted in the immediate killing of cells. Thus no significant deviation in results became apparent after treatment in 1% nucleic acid solution for varying periods, ultimate result being the death of the cells either sooner or later.

Remarkable results were recorded from experiments involving treatment of root tips in .01% solution of nucleic acid. In this case, even 4 hrs. treatment revealed a high percentage of cells containing tetraploid number of chromosomes. The cell size too was comparatively much larger than the diploid ones (Fig. 4). Under normal conditions, as revealed in control, these remain in a resting state. The possibility that these cells were normal diploids which underwent colchicine mitosis due to treatment was remote as these revealed the clear double chromatid

appearance of each chromosome in metaphase. Thus the evidences show that there are normal polyploid cells induced to divide in solution of nucleic acid. Similarly a number of nuclei in prophase stage were observed containing high number of chromosomes (Fig. 6). Fragments were noted further in certain percentage of polyploid prophase nuclei (Microphoto N. 1).

In addition to induction of division in polyploid nuclei, another important peculiarity noted in such slides is the occurrence of somatic reduction as noted by several authors in *Allium* and *Trillium*. (HUSKINS, 1948; WILSON & CHENG, 1949; HUSKINS & CHENG, 1950; PATAU, 1950, THERMAN, 1951; SHARMA & BHATTACHARJEE, 1953). Treatment in .01% nucleic acid solution for 4 hrs. revealed the presence of chromosomes in paired state in some of the nuclei. In all cases, however, not all the 16 chromosomes have been found to form clear 8 pairs, rather in some of them, in addition to paired ones unpaired chromosomes too could be noted. Prometaphase stage has been recorded in certain cells with 8 chromosomes indicating their origin through somatic reduction (Fig. 5). A certain percentage of cells showed the presence of 2 groups of 8 chromosomes in side view metaphase within a common wall (microphoto N. 3). As this effect is observable even after 4 hrs. of treatment, the possibility of their origin through somatic reduction in the previous division seems excluded in view of the time period necessary for the completion of a division cycle in root tip cells of *Allium*. On the other hand, reasonably their origin may be claimed through reduction occurring in the preceding prophase. The 16 chromosomes thus separated in two groups of 8 in prophase indicated their further behaviour in metaphase. The behaviour of such cells in anaphase are also quite marked in the appearance of two anaphase groups of 8 chromosomes separating into 8 chromatids each in each of the 4 poles (microphoto N. 4). Somatic reduction in tetraploid nucleus was also observed in early anaphase, whereby 16 chromosomes moving towards each pole show their distinct double chromatid constitution (Fig. 4).

Certain percentage of cells indicates the presence of micronucleus in addition to their normal nuclei (Fig. 1). These are reflections of the irregular behaviour of chromosomes during the divisional stage. Tripolar condition was marked in certain cells, the chromatin content in 3 different poles indicating the possible normal constitution in one and reduced contents in the other two (Fig. 2). Formation of two large telophase nuclei suggesting their polyploid condition could also be observed in certain cells (Fig. 3).

For a study of the fate of the cells induced to divide in nucleic acid solution, the treated roots were allowed to recover for 24, 48 and 72 hrs. respectively in Knop's solution and observed following aceto orcein technique. Polyploidy here to could be detected with a slight decrease in frequency in longer periods of recovery. Presence of a high number of fragments in prophase and metaphase further were recorded. The persistence of pairing behaviour even after 72 hrs. of recovery in the polyploid nucleus is noteworthy after treatment in .01% nucleic acid solution for 6 hrs. (microphoto N. 2).

DISCUSSION

Differentiation, as interpreted by Huskins (l. c.), involves the metabolic nucleus in a highly active condition, the manifestation of which does not permit of a microscopic observation. The endomitotically dividing nuclei of differentiated cells — their activity, can be compared with the polytene nature of the salivary gland chromosome of Drosophila. He has thus encompassed under the same heading gene reduplication, an essential step in differentiation and also differences in the chromosome number in different tissue.

The interpretation of Huskins received support from other centres too, where division was induced and manifestation of polyploid nuclei noted by the use of sodium dichlorophenoxyacetate. Continuous treatments in such cases had to be applied for consecutive days, to induce division in the differentiated cells of somatic tissue. Considerable irregularities in their behaviour are also met with.

Unpublished works from this laboratory reveal that some other groups of chemicals are also endowed with the same property. These include a variety of growth promoting hormones and vitamins. Inspite of the demonstration of this important phenomenon responsible for differentiation causes bringing about such unnatural state of nuclei

Fig. 1. Cell showing a micronucleus in addition to a normal nucleus.

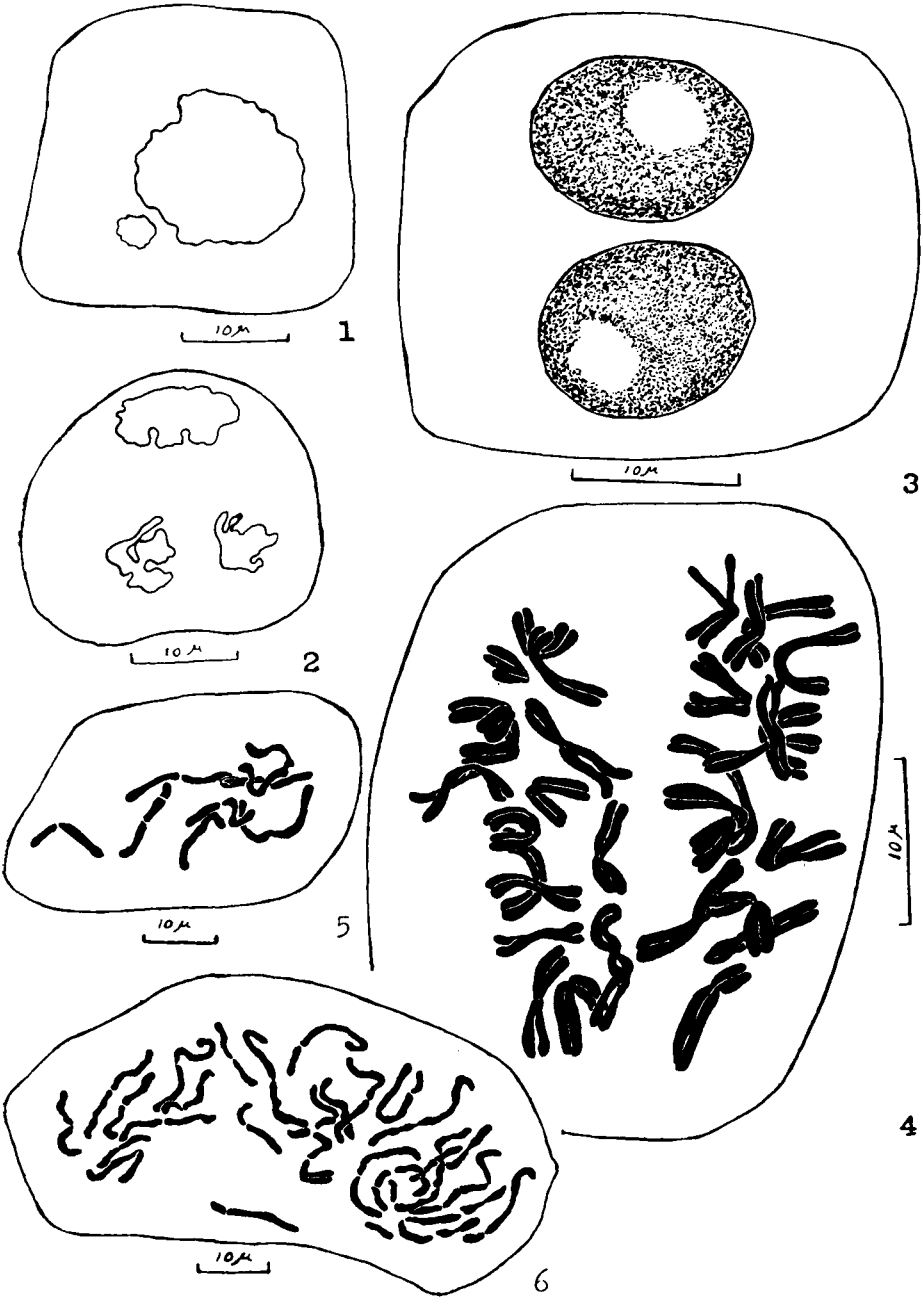
Fig. 2. Late telophase showing 3 poles.

Fig. 3. Binucleate polyploid nucleus showing reduction.

Fig. 4. Early anaphase in polyploid nucleus showing reduction.

Fig. 5. Reduced number of chromosomes in prometaphase.

Fig. 6. Prophase showing high number of chromosomes.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in the differentiated cells and the initiation of their division has uptil now been obscure.

The present report may be claimed to have provided certain clues towards the solution of this issue. The property of nucleic acid to induce division in the permanent cell even in a very dilute concentration has been elucidated. The polyploid nature of these cells has further been brought out. That the cases observed are not due to C-mitotic effect is evidenced by the nature of the individual chromosomes included within the 4n nucleus. Each and every member shows double chromatid appearance and a thickness comparable to that of normal metaphase chromosome of *Allium*. As the case is noted even only after 2½ to 3 hrs. of treatment, the possibility of C-mitosis in the previous division and their subsequent manifestation as normal chromosome in the next division is excluded. The completion of two full division cycles in case of *Allium* require much more time than applied in the present set of experiments. It has been pointed out that polyploidy is apparent in prophase as well as in metaphase. In addition to normal polyploid types, cases are also on record where a large number of fragments are present in prophase and onwards.

Besides polyploidy, reduction in chromosome number is another feature coming out after nucleic acid treatment. The reduction could be noted in prophase, metaphase as well as in anaphase. The tendency of formation of nuclei with 8 chromosomes is, therefore, a feature apparent in all the stages of division in a few cells.

The problem that is posed thus is, « What is the causal factor responsible for this induction »? A solution of the above issue would consequently cover the cause leading to apparent cessation of division, or more precisely, endomitotic division in differentiated cells. An understanding of this problem necessarily require a deeper approach to the process of chromosome duplication and cell division. In endomitotically dividing cells, as the theory goes, the nuclear division proceeds at a slower rate than chromosome division, thus resulting in polyploid nuclei.

Chromosome division and the separation of two chromatids are known to be absolutely related to their nucleic acid charge. The gene must accumulate its maximum and specific amount of nucleic acid charge for its duplication and separation. The giant nuclei of the differentiated region show very little basophily indicating the absence in them of the requisite amount of nucleic acid. This deficiency in the amount of DNA, we consider, is responsible for abnormal nuclear division of differentiated cells. The various growth promoting substances viz. the

hormones, the vitamins are active due possibly to their capability of affecting nucleic acid synthesis. This seems more evident as nearly 3 hrs. time is required for the manifestation of the effect, the time which seems is reasonable enough for influencing the process. In what way, or to be more precise, through what chemical steps they influence this synthesis is yet obscure. But that the change in nucleic acid synthesis is the basic cause of the phenomenon is evidenced by the initiation of division in root tips grown in nucleic acid medium as done in the present scheme of work. The deficiency in the amount of nucleic acid responsible to some extent for endomitosis is thus met directly by the nucleic acid of the medium. Equational and reductional division too, apparently are correlated with the presence of adequate amount of DNA and RNA in the cell. Reduction of chromosome number accompanying meiotic division is naturally the outcome of vigorous synthesis of nucleic acid in the germ cell. In the present work, similarly the reduction division noted in root-tip cells is possibly the result of incorporation of nucleic acid in the medium. The time required for the appearance of the effect might be visualized as nearly the period necessary for the conversion of incorporated RNA to DNA.

The continued addition of nucleic acid consequently leads to division not coinciding exactly with the normal divisional process. Reduction division is simulated to some extent in the sense that some of the chromosomes with their two chromatid members intact go to one pole and others to the other. This cannot be fully compared with reduction division as interchange of segments are not involved. Further, the number of chromosomes going to two different poles is not always equal. Huskins claimed that even if the number in the two opposing poles is not the same, equality is maintained by the amount of chromatin matter present in the two. This idea finds no support in our recent observations where the members in two different poles in some cases not only vary in number, but also differ grossly in amount. This is not withstanding the fact that the number of chromosomes moving to two opposite nuclei is in most of the cases the same.

Even though this reduction is brought about through an abnormal process, some of these daughter nuclei at least survive and enter into the following division. This is clear in certain cases where 8 chromosomes could be distinctly counted in the metaphase stage. During prometaphase and late prophase stages where a tendency to form 8 paired groups are marked, a semblance with meiosis can be visualized as emphasized before. It has further been elucidated that the pairing

does not always involve all the chromosomes of the set, but rather scattered univalent structures too are found. This peculiar behaviour of the chromosomes is of profound significance as it occurs under strictly limited condition, resulting to certain degree an important event in the life cycle of the plant. How far the causal factor is common in the two is yet to be investigated. In general, it may be inferred that a change in the nucleic acid metabolism bringing about reduction division is also caused after treatment with nucleic acid. But our knowledge as to the way through which this change is facilitated is still nebulous.

The present report may be claimed to have provided an additional clue to the problem of induction of division in permanent tissue as well as somatic reduction brought about through other chemicals. The addition of nucleic acid amounts to direct change in a particular metabolism of the cell which is indirectly affected by other growth promoting substances.

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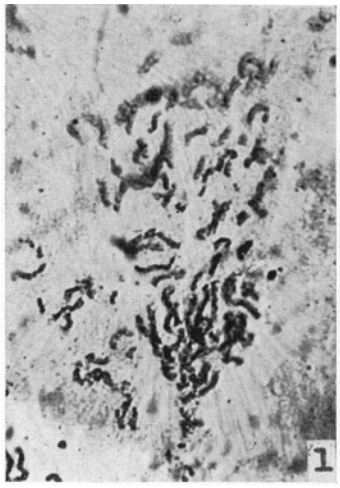

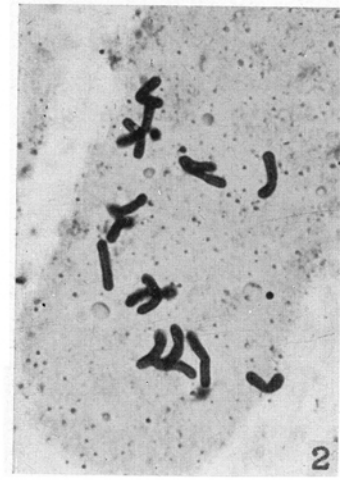

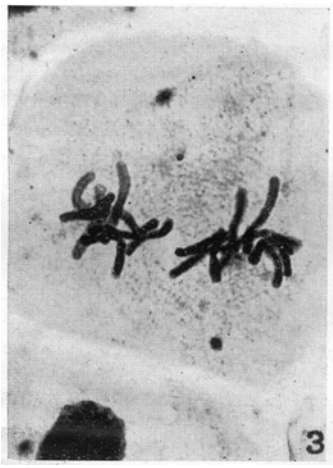

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EXPLANATION OF PLATE VII.

Fig. 1. Prophase showing high number of fragments.

Fig. 2. Metaphase showing paired chromosomes.

Fig. 3. Side view metaphase showing reduced number in two sets.

Fig. 4. Side view anaphase showing reduced number in two sets.

SUMMARY

1. Root tips of *Allium cepa* were treated for varying periods of time in nucleic acid solution and the effects noted.

2. When necessary, recovery experiments were performed in Knop's solution and observed after 24, 48 and 72 hrs. interval

3. .01% Nucleic acid solution cause the induction of division in differentiated cells. Their polyploid condition too has been brought out.

4. In addition to induction of division, somatic reduction too in certain cases have been noted.

5. The interpretation of Huskins as regards the problem of differentiation has been discussed.

6. Induction of division and somatic reduction, their origin have been considered through changes brought about in the nucleic acid metabolism of the cell.

RIASSUNTO

Apici radicali di *Allium cepa* furono trattati con soluzioni di acido nucleinico per periodi di tempo variabili con riprese in soluzioni di KNOP.

La soluzione di acido nucleinico allo 0,01% induce la mitosi in cellule differenziate di natura poliploide. In certi casi può indurre anche la riduzione del numero somatico. Tanto l'induzione della mitosi quanto l'induzione della meiosi sono considerate per la loro origine in rapporto a cambiamenti nel metabolismo dell'acido nucleinico. Viene discussa al riguardo l'interpretazione data in proposito da HUSKINS.