CHROMOSOME COILING: ABNORMALITIES INDUCED BY POLYAMINES

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Received May 16, 1960

PUTRESCINE has been found to induce chromosome aberrations in cells undergoing meiosis in *Oenothera* [8] but not in cells undergoing mitosis in roots of *Vicia faba* [9]. In re-examining the action of putrescine and cadaverine in less toxic concentrations [cf. 9], we have tested the ability of these polyamines either to induce chromosome breakage or to upset the process of chromosome condensation. Our results confirm that the diamines, putrescine and cadaverine, do not induce chromosome breakage in cells of *Vicia faba* roots, and a similar result has been found for a tetramine, spermine. They also show that polyamines do induce chromosome abnormalities, which seem to be attributable to disturbances in the cycle of chromosome coiling and uncoiling occurring during mitosis.

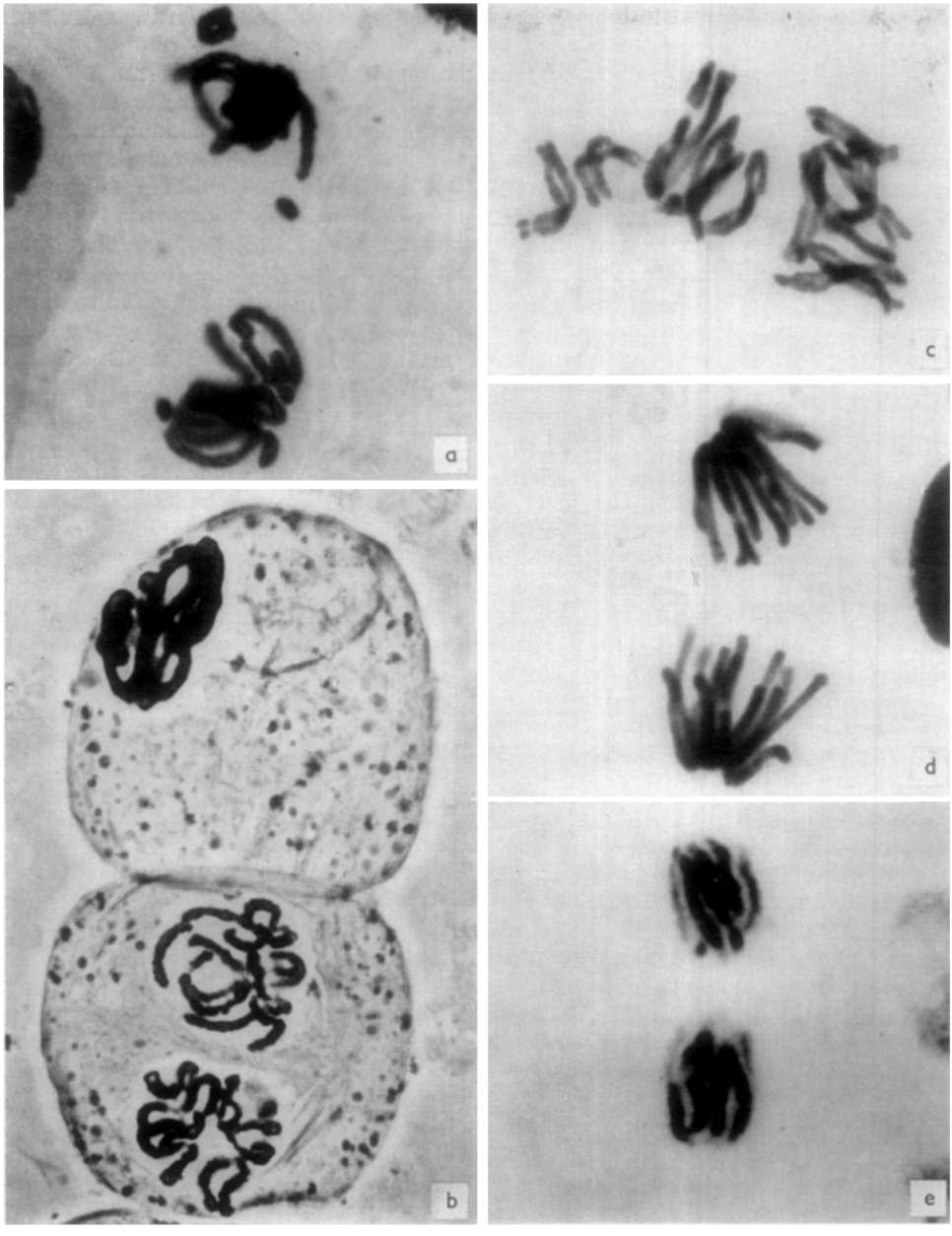
The view that chromosomal condensation is a reflection of a change in configuration of DNA molecules has been previously presented [2, 3]. The polyanionic DNA molecules appear to be bound end-to-end by protein to form long strands [5] that are loosely cross-linked in the nucleus to form an open gelwork. It has been proposed [4] that polycations such as putrescine, spermidine, and spermine, which would have a profound condensing effect on such a system [7], may function in this capacity during prophase. These compounds are widely distributed in animal and plant cells [6] and have now been found in association with viral DNA [1].

The observations to be reported here indicate that polyamines present from late interphase to telophase affect the processes of chromosome coiling and uncoiling.

Materials and methods.—Beans (*Vicia faba*) were grown in aerated distilled water until lateral roots had formed. They were placed in aerated solutions of putrescine–2 HCl, cadaverine–2 HCl and spermine–4 HCl, 0.5 m*M*, for 3, 6, or 24 hours. After treatment, the beans were returned to distilled water. Roots were fixed in La Cour's 2BD at the end of the treatment and after a 24-hour period of recovery. They were

[1] Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

Fig. 1.—*Vicia faba.* (*a*) Abnormal anaphase–telophase. The chromosomes have not formed a compact group at each pole and there appear to be chromosome fragments lagging in the cytoplasm. 0.5 m*M* putrescine 3 hours. (*b*) Two abnormal cells, the upper cell showing a metaphase with chromosome fusion and the lower a telophase in which the chromosomes have failed to uncoil. 0.5 m*M* spermine 6 hours. (*c*) Normal metaphase, slightly squashed. The two chromatids of each chromosome can be distinguished (cf. upper cell *b*). (*d*) Normal anaphase. (*e*) Normal late anaphase–early telophase (cf. *a*, which appears to have stopped at a stage between *d* and *e*.) Photograph (*b*) was taken using phase contrast. All photographs unretouched. ×2500 (approximately).


a
b
c
d
e

macerated and bleached in ammonium oxalate and H_2O_2 (after Ford), hydrolyzed in 1 *N* HCl and prepared as Feulgen squashes.

Results.—The original failure to find chromosome breakage in *Vicia* [9] could have been the result of differential death of injured cells before the metaphase at which the breaks would be observed. To avoid such differential elimination, treatments of short duration were used. No chromosome breakage was found; however, chromosome and nuclear abnormalities occur even after 3-and 6-hour treatments with solutions of all three compounds. They were similar in all treatments.

Abnormalities appear first at anaphase and telophase. The chromosomes do not complete their movement to the poles; they form a loose rather than a compact group. After the cessation of chromosome movement, the chromosomes fail to uncoil and swell as they would under normal conditions. As a consequence, the chromosomes remain separate and distinguishable and late anaphase and telophase nuclei are palmate, rather than round; and chromosomes, or parts, especially the satellites of the two long chromosomes, may lie away from the main group of chromosomes and eventually form micronuclei (Fig. 1 *a*).

The effect on metaphase chromosomes is even more extreme and it is seen after a 6-hour treatment, i.e., after the effects found at anaphase and telophase. The metaphase chromosomes condense into a single amorphous structure in which individual chromosomes cannot be distinguished (Fig. 1 *b*). This structure is Feulgen positive.

These various abnormalities are observed immediately after treatments of 3 and 6 hours and are also seen after 24 hours. In the later fixation, degenerating nuclei are observed; some have lost almost all ability to react with Feulgen stain and are represented by small hollow bodies similar to nucleoli.

The abnormalities found after treatment were absent from control roots at all times. The presence, in the first fixations, of normal metaphases along with abnormal anaphases indicates that the effects are not the result of poor fixation and overhydrolysis. The combination of an osmic acid fixative and the hydrolysis time used here normally gives excellent results.

Discussion.—The distortions of chromosome structure in the presence of polyamines can occur within 3 hours. This indicates that the processes disturbed involve spiralization and appear to be those that, in late interphase and prophase, complete the condensation of the highly dispersed interphase chromosome. The earliest detectable effect of polyamines, seen at anaphase–telophase, is the prevention of chromosome uncoiling. Later, metaphase chromosomes are found to have condensed together into a single unit, rather than into a number of separate chromosomes. These results indicate that polyamines act on post- and premetaphase chromosomes; they suggest that in fact the same mechanisms are responsible for the changes at both stages.

We would attribute the action of the polyamines in affecting chromosome condensation to their ability to form stable complexes with DNA-protein. This leads us to suggests that compounds of similar properties, which do occur naturally [6], may play some part in the normal cell control of chromosome coiling and uncoiling. We may suspect that naturally occurring polyamines are involved in the formation of the chromosome abnormalities, for example, point unions, induced by irradiation while prophase changes are taking place [4].

Summary.—Lateral roots of *Vicia faba* have been treated with 0.5 m*M* solutions of putrescine, cadaverine, and spermine. Chromosome uncoiling at late anaphase and

early telophase was inhibited; prophase contraction occurred but the metaphase chromosomes were fused into a single mass. These results are evidence that polyamines are capable of upsetting the normal processes of chromosome coiling and uncoiling.

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CHROMOSOME PREPARATIONS OF LEUKOCYTES CULTURED FROM HUMAN PERIPHERAL BLOOD[1]

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Received May 18, 1960

SINCE the introduction of the hypotonic pretreatment of mitotic cells by Hsu and Pomerat [1] and by Hughes [2] mammalian chromosomes from tissue-cultured material have been extensively studied. Published studies concerning the human karyotype and somatic chromosome anomalies are increasing in frequency. Most of the work on humans has depended upon bone-marrow puncture or skin biopsy for material, and upon classic squashing techniques. The techniques described here represent the successful attempt to combine the convenience of Nowell's [3] method for the cultivation of peripheral blood leukocytes with the advantages inherent in the air-drying method of Rothfels and Siminovitch [4]. Air-drying of metaphase cells cultured on glass provides well-spread chromosomes in one focal plane with a minimum of overlay

[1] This investigation was supported in part by the United States Public Health Service Grants C-4534, C-1659 (National Cancer Institute), SF-4 (Senior Research Fellowship), A-2231 (National Institute of Arthritis and Metabolic Diseases), CF-11,168 (Pre-Doctoral Fellowship, National Cancer Institute), and by an Institutional Grant from the American Cancer Society.

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