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The Effects of Ultracentrifuging Germinating Seeds of Onion and Rye

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THE EFFECTS OF ULTRACENTRIFUGING GERMINATING SEEDS OF ONION AND RYE

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Normally, the process of mitosis is an orderly, integrated series of events, that is, karyokinesis and cytokinesis are usually linked together and function as one continuous process. Occasionally, however, either one or both of these processes may be interfered with in some way so that a dissociation takes place between the two. This gives rise to an abnormal situation so that each process may proceed independently of the other or one may be inhibited while the other goes on uninterrupted. The most common abnormal condition of this type is the inhibition of cell cleavage while nuclear division continues, resulting in the production of polyploids. This occurs rarely as a natural phenomenon, but more frequently as the result of disease, or quite commonly under experimental conditions. A few of the experimental agents used for inducing modification or inhibition of cell division are injury, heat, cold, ultraviolet and X-radiations, narcotics, anaesthetics, hypertonic and hypotonic solutions, colchicine, and high centrifugal forces.

Plant tissues have been centrifuged at various speeds by several investigators, namely: Mottier (1899) Andrews (1915) Nemec (1915, 29) Weber (1924) Schaede (1930) Luyet and Ernst (1934) Luyet (1935) Kostoff (1935, 37, 38) Beams and King (1935, 36, 37, 38, 39) and others.

The present experiments were conducted to try to demonstrate cytologically the effects of high centrifugal force upon the tissues of germinating seeds of onion and rye.

The seeds employed for the experiments were White Portugal onion (*Allium cepa*) and American fall rye (*Secale cereale*). Seeds were placed in a damp chamber and left in the dark for 20 to 48 hours. By this time they had germinated and grown sufficiently to withstand the experimental conditions. Those seeds of most uniform size and growth were placed in the rotor of the air turbine ultra-centrifuge of Beams, Weed and Pickles (1933). Tap water or starch solution was put into the rotor with the seeds to prevent crushing. Centrifuging was carried on at 50,000 to 150,000 times gravity for 10 to 90 minutes; the longer time and slower speed were found to give better results for this material.

Plate I

The figures are photomicrographs of the cells of onions and rye, 3200X and stained with Heidenhain's hematoxylin.

1. Rye "giant cell." Material was centrifuged for 20 minutes at 100,000 times gravity and allowed 7 hours recovery. The large, dark area is the nucleus.

2. Onion a. large binucleate cell.
   b. non-nucleate cell.
   c. small binucleate cell.
   d. "giant cell."

   Material centrifuged at 50,000 times gravity for 90 minutes. (See Fig. 14.)

3 and 4. Onion binucleate cells.

This is the same material as noted for Fig. 2. (See Figures 15 and 16.)

5, 6 and 7. Onion binucleate cells.

This is the same material as mentioned for Fig. 2. (See Figures 17, 18, and 19.)

8 and 9. Onion "giant cells."

This is the same material as mentioned for Fig. 2. (See Figures 24 and 25.)

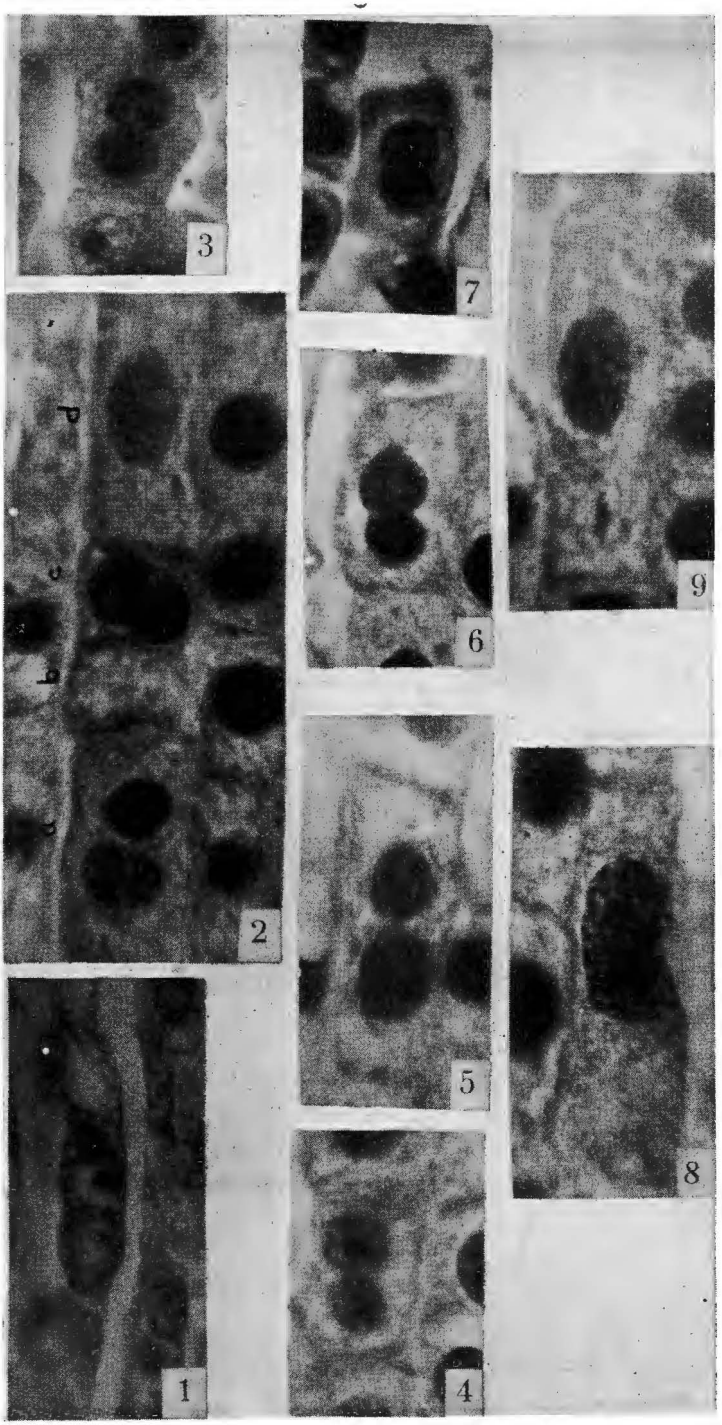
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Plate II

All of the drawings in this plate are camera lucida drawings of selected areas in the Onion preparations, many of which are the same as the photomicrographs of Plate I. Figure 10 is from control material. Figures 11, 12, 13, 22, and 23 are from material which was centrifuged for 20 minutes at 100,000 times gravity and allowed to recover for 7 hours. Figures 14, 15, 16, 17, 18, 19, 20, 21, 24, and 25 are from material which was centrifuged for 90 minutes at 50,000 times gravity and allowed to recover for 11 hours. All figures are 1000X, except as noted.

10. Normal mitosis in which 2n equals 16.

11. Abnormal mitosis. The spindle is absent and the metaphase plate is incompact. There are 16 double chromosomes which are loosely intertwined.

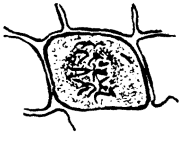
12. Abnormal mitosis. The spindle is absent and the metaphase plate is incompact. This cell contains 32 chromosomes which are single.

13. Abnormal mitosis. This anaphase cell is tetraploid on each side, due to a fusion of the mitotic figures of a binucleate cell or the mitosis of a "giant cell."

14. A row of abnormal cells. In this figure are shown examples of the result of disturbing mitosis at different stages. In *a*, a binucleate cell has formed. In *b* and *c*, cell division has taken place, but both nuclei have been isolated in one cell, leaving the other non-nucleate. In *d*, the two nuclei have fused, forming a "giant cell" having approximately double the normal size of nucleus and cell body.

15, 16, 17, 18, 19, 20, 22, and 23. All are binucleate cells.

24 and 25. "Giant cells." (2000X)



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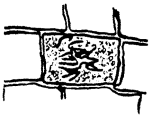

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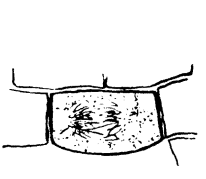

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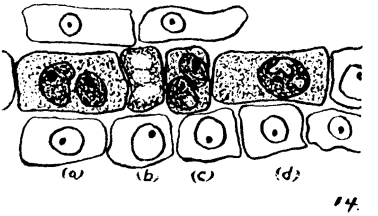

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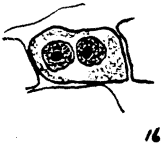

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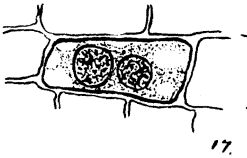

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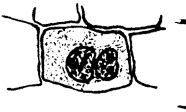

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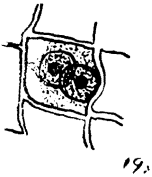

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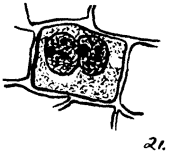

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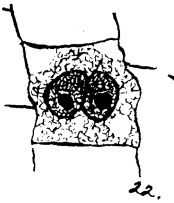

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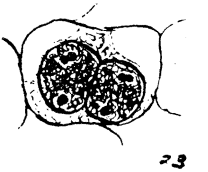

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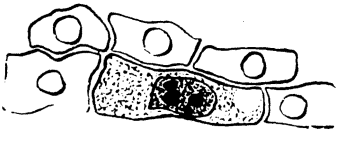

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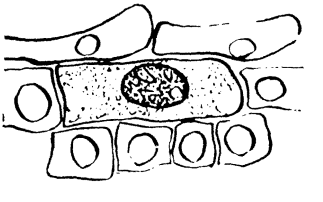

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After centrifuging, the seeds were again placed in a damp chamber (dark) and allowed to recover for periods of 7 to 22 hours. The tips were then cut off and put into Allen's B_3 fixative. Sections were cut at 6 microns and stained with Heidenhain's iron-hematoxylin.

The general effect of centrifugal force upon the cytoplasm and nucleus of plant cells is to cause a stratification of their components in order of their relative specific gravity. This is mentioned by Luyet (1935), Kostoff (1937), and Beams and King (1935). The nucleus or chromatin material generally becomes concentrated at the centrifugal pole, and the fatty material along with the spindle materials becomes concentrated at the centripetal pole. Kostoff (1938) mentions an exception to this general rule. He found certain cells to have their nuclei toward the centripetal pole after centrifugation. In my material, the resting nuclei (which contained diffuse chromatin) were those least effected by the centrifugal force. This may be due to the chemical condition of the nucleoproteins. The deeply staining chromatin of the prophase nucleus which has condensed must be composed of heavier nucleoproteins because of a more complex molecular arrangement. Furthermore, a difference in the viscosity of the cell may alter to a certain extent the stratification of the various materials.

The application of a force of approximately 50,000 times gravity for 90 minutes produced changes in the cell that interfered with normal mitosis. This force is insufficient to cause a permanent tissue injury but is sufficient to cause marked cytological changes in the cells. The time mentioned (90 minutes) is long enough to centrifuge the root tip, for in that time a number of dividing cells will be effected.

Onion (*Allium cepa*)

During the prophase the chromosomes are displaced by ultracentrifuging to the centrifugal pole of the nucleus and occasionally are fragmented. However, cells thus effected will recover and apparently divide normally.

Application of the centrifugal force in metaphase usually causes the disappearance of the spindle and produces a loosely arranged metaphase plate. If the chromosomes have divided, the tetraploid number is present (Fig. 12), and in the absence of the spindle they do not separate and no cell plate is formed. (Beams and King, 1938; Kostoff, 1938). The chromosomes eventually produce a restitution nucleus that is twice the normal size and, since the cell has grown without dividing, it also is much larger than the

normal cell. (Such "giant cells" may be seen in Figures 2d, 8, 9, 14d, 24, and 25). Subsequent mitoses contain many more than double the normal somatic number of chromosomes (Fig. 13). If the spindle does not disappear it becomes greatly distorted and the entire mitotic figure may be thrown to the centrifugal end of the cell. Here a more or less normal anaphase and telophase may ensue, but because of the position of the spindle, the resulting daughter cells are of unequal size.

The spindle is harder to displace and seems to be more rigidly situated in the cell in the anaphase than in the metaphase. This substantiates the work of Heilbrunn, (1928), and of Kostoff, (1938), that the viscosity of the cell is higher during anaphase than at any other time. If the force is applied in anaphase before the cell plate has started to form, the spindle (phragmoplast) is displaced to the centripetal end of the cell and no cell plate forms. The two groups of chromsomes then clump separately and become resting nuclei. As no cell plate is formed, a binucleate cell is produced (as in figures 2a, 3, 4, 5, 6, 7, 14a, 15 through 23). These are similar to those found by Beams and King (1938). This is the most abundant type of abnormality found, probably because of the relatively long duration of the anaphase stage in mitosis.

If the pragmoplast has started to differentiate a cell plate, cytokinesis will undoubtedly take place, because the newly forming cell plate is quite stable and apparently not displaced. However, the nuclei will be displaced to the centrifugal pole of the cell and a binucleate cell and a non-nucleate one are formed; both are of normal size (Figures 2b, 2c, 14b, and 14c). The non-nucleate cell apparently dies and disappears from the root tissues.

RYE (*Secale cereale*)

Observations on the rye were confined to an investigation of the "giant cells" because of the extremely small size of its normal cells. Here, apparently, a different mechanism is disturbed as a result of the centrifuging. If the force is applied in the metaphase, the spindle is destroyed and chromosome division proceeds as in the onion. In later divisions, however, cytokinesis is not restored, and the resulting cell and nucleus are approximately five or six times as large as normal at 17 hours after centrifuging (Fig. 1.).

Discussion

As previously mentioned, both plant and animal cells have been centrifuged by several investigators. (See Beams and King, 1938 and Kostoff, 1938 for a recent review of the literature). In general it has been shown that the cell materials stratify in the order of their relative specific gravities. In most cases the chromosomes, particularly during mitosis, are displaced to the centrifugal end of the cell. Kostoff (1938), has been able to induce alterations in somatic chromosome sets of various plants. After centrifuging in an ordinary laboratory centrifuge he found that various results had taken place, such as the failure of the chromosomes to separate, of the cell to divide, and injury to the spindle to give rise to various types of polyploid cells. Beams and King, (1938), were able to displace chromosomes or the whole spindle to the centrifugal end of the cell or to cause the disappearance of the spindle in metaphase, in wheat. These conditions give rise to abnormal daughter cells. They were also able to produce binucleate cells by applying centrifugal force in anaphase or telophase before the cell plate had begun to form. The cell plate forming substance or organizer in this case was displaced to the centripetal end of the cell where it was inactivated. They point out that the typical spindle of cytokinesis normally induces cell plate formation. This conforms with the work of Jungers (1931). He worked with the endosperm of Iris. This is a multinucleate and after the last nuclear division, secondary spindles arise alongside of the spindles connecting daughter nuclei. These secondary spindles connect the nuclei with neighboring spindles and meet in the secondary spindles, thus bringing about the inclusion of single nuclei within cell walls. In my material, if the spindle was displaced early enough in the cycle no cell membrane or wall appeared and the spindle and phragmoplast eventually disintegrated.

In general, my work confirms that of Kostoff (1938) and Beams and King (1938).

Conclusions

1. Centrifuging at high speeds stratifies the cell materials in the order of their relative specific gravity. Cells may recover from such treatment.

2. In many cells at metaphase the spindle disappears but the chromosomes divide, giving rise to a tetraploid cell. In other cells the spindle and chromosomes are displaced and the subsequent division results in unequal daughter cells.

3. Cells centrifuged while in anaphase or telophase before the cell plate has begun to form, have the chromosomes or developing nuclei displaced to the centrifugal end of the cell, and the spindle to the centripetal end of the cell, where it seems finally to disintegrate. This condition usually gives rise to a binucleate cell.

4. The cell plate forming substance may be displaced, resulting in a failure of normal cell plate formation.

5. Extremely large "giant cells" are formed in rye as a result of more prolonged inhibition of cytokinesis.

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BIBLIOGRAPHY

Andrews, F. M. 1915 Die Wirkung der Zentrifugalkraft auf Pflanzen. Jahrb. Wiss. Bot., *56,* 211.

Beams, H. W. and R. L. King. 1935 The effect of utracentrifuging on the cells of the root tip of the bean (Phaseolus vulgaris). Proc. Roy. Soc. B., *118,* 264.

————1936 The effect of ultracentrifuging upon chick embryonic cells, with special reference to the "resting" nucleus and the mitotic spindle. Biol. Bull. *71,* 188.

————1937 The suppression of cleavage in Ascaris eggs by ultracentrifuging. Biol. Bull., *73,* 99.

————1938 An experimental study on mitosis in the somatic cells of wheat. Biol. Bull., *75,* 189.

Beams, J. W., Weed and Pickels 1933 The ultracentrifuge. Science, *78,* 338.

Carothers, E. E. 1936. Components of the mitotic spindle with special reference to the chromosomal and interzonal fibers in the Acrididae. Biol. Bull., *71,* 469.

Darlington, C. D. 1936 The external mechanics of the chromosomes. Proc. Roy. Soc. B., *119,* 264.

Fry, H. J. 1937 Studies on the mitotic figure. VI. Midbodies and their significance for the central body problems. Biol. Bull., *73,* 565.

Gross, F. 1936 Cleavage of blastomeres in the absence of nuclei. Quart. Journ. Mic. Sci., *79,* 57.

Harvey, E. B. 1936 Parthenogenetic merogeny or cleavage without nuclei in Arbacia punctata. Biol. Bull., *71,* 101.

Heilbrunn, L. V. 1928 The colloid chemistry of protoplasm. Berlin.

Kostoff, D. 1935 Chromosome alteration by centrifuging. Zeitschr. ind. Absto. u. ver., *69,* 301.

————1937 Chromosome alterations by centrifuging. Science, *86,* 101.

————————1938 The effect of centrifuging the germinating seeds. Cytologia, *8,* 420.

Lindstom, E. W. and K. Koos 1931 Cytogenetic investigations of a haploid tomato and the diploid and tetraploid progeny. Am. Journ. Bot., *18,* 398.

Luyet, B. J. 1935 Behavior of the spindle fibers in centrifuged cells. Proc. Soc. Exper. Biol. & Med., *33,* 163.

Luyet, B. J. and R. J. Ernst 1934 On the comparative specific gravity of some cell components. Biodynamica, *2,* 1.

Mottier, D. M. 1899 The effect of centrifugal force upon the cell. Ann. Bot., *13,* 325.

Nemec, B. 1915 Eineges ueber Zentrifugiente Pflantzellen. Bull. Intunst. Acad. Sci. Boheme, *20,* 1.

Schaede, R. 1930 Zentrifugalversuche mit Kernteilungen. Planta, *11,* 243.

Schrader, F. 1934 On the reality of spindle fibers. Biol. Bull., *67,* 519.

Sharp, L. W. 1934 Introduction to cytology. McGraw Hill.

Timberlake, H. G. 1900 The development and function of the cell plate. Bot. Gaz., *30,* 73-154.

Weber, F. 1924 Methoden der Viskositatsbestimmung des Lebenden Protoplasma. Abderhalden's Handb. d. biol. Arbeitsmethoden. Abt. XI, *2,* 665-718.

Wilson, E. B. 1925 The cell in development and heredity. Macmillan.