Studies on the Male Gametophyte in Angiosperms

V. Colchicine treatment as a proof of the essential function of the spindle mechanism in karyokinesis in the pollen tube

By

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Received February 15, 1939

The mechanism of division of the generative nucleus in pollen tubes has been discussed by many investigators over a considerable period of time. It was pointed out by the writer (1938) that the problem was unnecessarily complicated by artifacts in fixation. The writer was able to establish the presence of the spindle and sometimes of the spindle fibres in the course of the generative nucleus division of both *Crinum latifolium* and *Hippeastrum vittatum* in fixed and stained materials (Suita, 1936). He also found the same thing in the case of living materials (Suita, 1938, 1939), and after repeated, successive vital observations was able moreover to clarify the fact that, 1), the chromosomes are arranged, in many cases, near the equatorial plane at the metaphase of the division, 2), the spindle appears in the course of the division, and the daughter chromosomes diverge to the opposite poles, though the velocity of migration of each chromosome is not always the same, 3), the nuclear division is generally followed by the cell plate formation, although it is markedly delayed in a considerable number of cases, 4), separation of the two daughter cells, the male cells, from each other seems to take place at certain intervals after karyokinesis, 5), the cytosome, the so-called droplets-sheath, of each male cell never disappears for at least 25 hours after karyokinesis, that is, until the death of the pollen tube, and 6), no stable and inflexible membranes are formed throughout the life course described above (Suita, 1939).

On the other hand, the spindle mechanism and the spindle substance in other somatic and reproductive tissues have been investigated by processes of experimental treatment, in particular by means of chemical and physical stimulations (Nemec 1904, 1910, Sakamura 1920, Yamaha 1927, Wada 1932, 1937, 1938, Shigenaga 1937), which suppress the nuclear division through disturbances of the spindle mechanism. If the division of the generative nucleus in the pollen tube is performed through the spindle mechanism, it may be expected that the division will be arrested and an irregular mitosis

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1) Contributions from the divisions of Genetics and of Plant-Morphology, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 221.
will take place when the pollen tubes are treated with such spindle inhibitors. Of all such spindle inhibitors, however, colchicine seems to have the least harmful effect on the other physiological functions of plant cells. In the present investigation, therefore, colchicine was employed for the spindle inhibition test.\(^1\)

Pollen tubes of *Hippeastrum vittatum* were cultivated on a thin hanging drop of colchicine-sugar-agar in a moist chamber, which enabled vital observations to be made with high power objectives (cf. Suita, 1937). To prepare the colchicine-sugar-agar, varying amounts of crystal colchicine (Merck) were added to sugar (5%, 7.5%, 10%) -agar (1%) solution (saccharose puriss., Merck, and agar, Merck, in distilled water). Three different degrees of sugar concentration were prepared for the present investigation, because higher concentrations of colchicine seemed to cause rupture of the pollen tube before the nuclear and cell division took place. Thus, the combinations of 0.00004%, 0.0004%, 0.004%, 0.01% or 0.04% of colchicine, with 5%, 7.5% or 10% of sugar and 1% of agar were employed. A 0.04%–10%–1% colchicine-sugar-agar solution, for instance, means here one that is prepared by the addition of 0.04 gr. of colchicine and 10 gr. of sugar to 90 c.c. of 1% agar solution. This notation is not strictly scientific

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\(^1\) According to the abstract of the paper presented before the general section of the Botanical Society of America (Amer. Journ. Bot. Suppl. to Vol. 25, No. 10), Eigsti seems to have tried a similar experiment as the present one.
but is used only for convenience' sake. Another method of investigation, also employed with effective results, was one in which the pollen tubes were cultivated on 5%–1% sugar-agar and immersed and observed in colchicine-sugar solutions of various concentrations.

Preparations were fixed and stained with acetocarmine at 12, 18, 30, 40, or 47 hours after pollen dusting. Their cytological and physiological conditions were tested by vital observation before fixation. And the mode of karyokinesis was examined and compared with that of the normal course.

Upon examination of the configurations of karyokinesis, the effect of colchicine could be recognized in concentrations of more than 0.0004%. Similar features of abnormal karyokinesis as in the colchicine-treated somatic divisions in the root tip cells, stamen hair cells and others (Dustin, Havas et Lits 1937, Nebel and Ruttle 1938, Levan 1938) were also observed, as seen in figures 16–24. In particular, it was noticed that in the course at development from the late prophase to the anaphase, the nuclear cavity tended to be much more elongated in the colchicine-treated mitosis than in the normal course of division, and that several chromosomes became considerably separated from the rest of the chromosome group (figs. 17 and 21), divided there (fig. 22) and entered into the resting stage, thus forming three or more nuclei in a pollen tube (fig. 23). Other features also indicate that the daughter chromosomes do not diverge to the opposite poles.

These features can not be seen in cultures without colchicine. As shown in figures 7–15, the daughter chromosomes diverge to the opposite poles and the two daughter nuclei are formed there in the normal course of development, although the tendency of the chromosomes in the pre-
metaphasic stage to gather in one (equatorial) plane is somewhat weaker in narrow tubes than in larger ones or in pollen grains having room enough for the division (Suita, 1936).

These results alone, however, do not seem enough to warrant any conclusion as to the prevalence of the spindle mechanism in the course of the nuclear division in the pollen tube. It may be premature to discuss the spindle mechanism on the basis only of the results obtained by colchicine treatment, because the colchicine seems to have not only a simple suppressing action on the spindle function, but also to have other physiological effects on the cytoplasm. These results described above, however, can support, at least, the conclusion obtained from the observations and experiments on fresh and fixed materials that the generative nucleus division is performed through the spindle mechanism in the pollen tube (Suita, 1936, 1939).

No one has hitherto discussed the effects of colchicine on the cytoplasmic constituents and cyto-physiological properties of plant cells. It may not be necessary for the mere induction experiments of polyploid plants to test the physiological properties of the colchicine-treated cells. To study and discuss the mechanism of the nuclear and cell division, however, such tests are of prime necessity. In the present investigation, the writer was able to ascertain that colchicine inhibited the smooth growth of the pollen tube in some cases, caused the rupture of the tube cell, and caused marked delay of the beginning of karyokinesis. The details of these results will be re-
ported in a subsequent paper entitled “the physiological effects of colchicine on the pollen tube.”

In conclusion the writer wishes to express his cordial thanks to Prof. SINOTO who has supplied him with helpful suggestions and valuable criticism throughout the course of this investigation.

A part of the expenses of this investigation was defrayed out of a grant from the Japan Society for the Promotion of Scientific Research.

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Literature


和文要約

吹田信英：被子植物に於ける雄性配偶體の研究 V. コルヒチン處理による花粉管内核分裂の機構の検討

花粉管内核分裂は、これ迄数々正常な有絲分裂ではないものとして取扱われて来たが、筆者の固定染色の方法及び生際連続観察の方法による研究の結果、少なくとも Crinum 及び Hippostrum の培養花粉管では、通常の細胞分裂と同様紡錘体機構により核分裂が行われ、後、細胞板が形成されて遂には 2 個細胞の分離が起こる事が明にされた。紡錘体機構を慣乱して有絲核分裂に異常を與へるものと称せられるコルヒチンを作用させた結果によれば、花粉管中の核分裂はやはり阻害されて、紡錘体機構の破壊された様な外観を呈する。この事実は前記の花粉管核分裂の紡錘体機構説を支持するものである。但しコルヒチンの作用は単一なものではなく、種々の他の生理的影響を含むものなのであるから、慎重な検討、特に細胞生理学的な方面からの検討を必要とする。