Cellular Growth

The senior author observed unusual "spearlike" tips forming on *Allium* roots immersed in a 0.01 per cent solution of colchicine. After 24 hours startling changes in the roots were noted[35] (cf. Chapter 2). *Colchicine-tumor*,[79] the name given to this growth, is appropriately descriptive. Similar anomalies were observed earlier by Nemec and others.[35] This growth pattern can also be reproduced with chemicals other than colchicine or by certain physical treatments.[79, 44] Although the c-tumors were not new to biology, the revival of interest in colchicine brought them to the attention of many experimenters.[34, 33, 93, 86, 44, 37, 59, 55, 133, 115, 111, 90, 88, 62, 154, 128, 18, 4, 10, 8, 21]

Roots with c-tumors may have some cells with many chromosomes within the single cells, because polyploidy is a consequence of c-mitosis. The correlation between larger leaves, stems, seeds, and flowers, and increasing numbers of chromosomes is well established.[135, 152] This concept influenced the first conclusion that c-tumors were directly correlated with the polyploid cells. On the contrary, an enlargement of root tips is not the result of polyploid cells induced by the drug, even though polyploid cells may be created at the same time the c-tumor is formed.[82] The c-mitosis and c-tumor are independent processes.[82]

Now we know that in similar manner, enlarged cells may be induced in various parts of plants.[106] All these anomalous formations induced by colchicine are the result of changing the growth pattern.[62, 90] Such structures as pollen tubes,[35, 60, 137] stylar cells of the flower,[148] hair cells of stem and root,[53, 55, 115] hypocotyl, and other somatic cells all show particular enlargements after treatment with colchicine. They are in contrast to the untreated or normal cells that enlarge by a cell tension that shows distinct polarity. By a broad interpretation, all deviations expressed as growth patterns and appearing as a response to colchicine will be classified as c-tumors, in spite of the fact that this name originally designated a specific kind of root tip enlargement after treatment with colchicine.

The processes of meiosis and gametophytic development are changed by colchicine.[2, 9, 29, 79, 114, 124, 122, 148] Response depends upon the concentration, stage of development when colchicine reaches the cell, length of exposure, and, of course, concentration. As might be expected, the spindle is inhibited, but there are also other changes that accompany the colchicine-effect.[1] For that reason the problem of a "colchicine-meiosis"[79] is included in this chapter along with the action upon embryo sac development[48] and pollen tube studies.[35]

Colchicine acts upon cells during their differentiation processes. One noticeable change is found in the cell walls.[53] Their chemical composition is altered also, and various physical marks show that action of colchicine is not limited to the mitotic spindle or upon certain cytoplasmic constituents.[53] Enough data are at hand to prove that differentiation processes in plants are modified by colchicine.[35, 53, 156, 151]

Among unicellular organisms, processes of division, enlargement, and differentiation, are closely integrated within one cell. For that reason one would expect to find the results from a colchicine exposure difficult to interpret. Conceivably, all three processes go on within one cell at the same time; hence, colchicine may act upon each phase in a specific manner, yet simultaneously. If this interpretation is correct, the confusing picture drawn from the literature dealing with colchicine and microbiological materials may be partly explained by the inability to distinguish the specific process being studied, whether a cell division, cell enlargement, or differentiation and maturation. There is general agreement that the actions reported in this research are contradictory. Under some conditions, however, colchicine is effective if introduced to specific microbiological cultures within certain concentrations.

A mechanism for action of colchicine upon processes of growth and differentiation is difficult to visualize. Nevertheless, there should be some aspects of metabolism that might help toward the solution of this problem.[103, 16, 89, 77, 155, 142, 130, 99, 56, 46, 47, 48, 45] Generally, the work with physiology[101, 99] has been done with such isolated processes as enzyme reactions[127] or respiration[110] under a restricted set of conditions for experimental material. At least a start has been made in this direction, but more can be done in the future.

4.1: Colchicine Tumors in Roots, Hypocotyl, and Stems

The root tumor forms at the region of elongation, a section between the meristematic area and the differentiated cells of a root[90, 35, 79, 82, 62] (Fig. 2.1). Normally cells elongate linearly to the axis of the root. They seem to show a polarity in this respect. When colchicine is present, an enlargement of the cell takes place in all directions. That is, an isodiametric expansion occurs, rather than a polarwise

elongation. The volumes of cells from a c-tumor are about the same as the volumes of elongated cells in untreated roots.[62] Therefore, the direction of growth is modified, but not necessarily the total amount of expansion.[62]

Cells of the cortex become inflated.[79] This leads to a swelling at the particular place along the root. Longitudinal and cross sections of treated and untreated roots within five or six layers of cells show where the change occurs, and reveal particularly the difference in the shape of individual cells. These comparative studies confirm the opinion that direction of growth is altered when colchicine is present. The action is not unique for colchicine. Growth-promoting substances, as naphthaleneacetic acid (NAA) and indolebutyric acid, induce tumors.[97, 90, 91, 42, 27, 7, 34, 44, 79, 81, 59, 61] Acenaphthene, another compound that has a c-mitotic potential, may cause tumors on roots.[108] Not all compounds that create tumors arrest mitosis. In fact, certain phytohormones that do not stop mitosis may induce root tip enlargements. The idea of an autonomy of c-mitosis and c-tumors gains support from these general observations with several chemicals.[82]

Specific thresholds below which no tumors form, are demonstrable for colchicine. Concentration specificity is shown also by NAA.[81] If two solutions, colchicine and NAA, are combined, the threshold concentration does not change.[81] There is no evidence that two solutions, each capable of inducing tumors alone, will in combination lower the threshold value. Thus, the mechanism for creating the tumor may be different for these particular substances.[81] The threshold changed, however, when sulfonamide (2 per cent prontosil) was added to colchicine.[4, 69]

The combined solutions of *meso*-inositol and colchicine prevented the usual production of a c-tumor with roots of *Allium*.[18] Apparently this antagonism by *meso*-inositol operates at 19°C. since a repetition at 26°C. did not reveal such antagonism.[26] The critical role of temperature is seen in pollen tube enlargements, where the maximum width induced by colchicine occurs only at a specific temperature.[127] Above or below that optimum the pollen tubes are close to normal dimension in spite of the same concentration of colchicine present in each test.

Venom from bees was demonstrated to have an antagonistic action upon the formation of root tumors by colchicine.[59] The specific differences between kinds of plants was also shown. Tomatoes were more sensitive than wheat seedlings. A 69 per cent reduction of tumors was obtained for tomatoes and 47 per cent with wheat.[59, 61]

Ethyl alcohol changes the c-mitotic threshold for *Allium* root cells from 0.006 per cent, when colchicine alone is used, to 0.01 per cent if alcohol (0.5 per cent) is added. If the concentration of alcohol is

increased to 2 per cent, other poisonous actions occur. Alcohol acts as an antidote with respect to c-mitosis and the c-tumor.

When two chemicals work together to accelerate an activity beyond the effect obtainable by each chemical independently, the response is known as a synergism. Colchicine and numerous other chemicals have been tried for their synergistic action.[78] Some give accelerated response and others do not. Phenylurethane along with colchicine increases the action of drug upon roots of *Allium*.[30]

Tissue cultures of *Helianthus tuberosus* were handled by combined treatments of heteroauxin (10^{-9}) and colchicine (10^{-6}). Small doses of colchicine enhance the action of heteroauxin because the tissues seem to divide more actively and huge cells with many chromosomes develop as a result. A stimulating action seems evident from these experiments. Increasing the concentration of colchicine leads to repetitive c-mitoses and an inhibition of cellular multiplication among the tissues.[91]

Generally, favorable conditions for growth increase the promotion of a tumor from a specific treatment.[84] The range in concentration is fairly broad, but there are limits marked by minimum and maximum concentrations. The formation of tumors within certain limits is proportional to concentration. Finally, the thresholds for c-mitosis and c-tumors are close to each other with some indication that the threshold for the latter process is lower than that for c-mitosis.[82]

As soon as the independence of c-mitosis and c-tumor was suspected, a specific experiment was designed to test autonomy.[79] Root primordia of *Allium fistulosum* were subjected to intense X-ray treatment. Consequently, the mitotic capacity of meristematic cells was destroyed. Following X-irradiation, bulbs were placed over colchicine, and typical c-tumors formed with no evidence for several days thereafter of c-mitoses in these roots. We may conclude, therefore, that enlargement occurs without a simultaneous division of cells. Polyploidy following a c-mitosis is not necessary for tumor formation.[79]

Swelling at the hypocotyl when seedlings were soaked in colchicine gave the first evidence that tumors were in no way related to c-mitosis or induced polyploidy. Although cells in the hypocotyl are not meristematic, they are capable of elongating or expanding. Colchicine causes an isodiametric expansion of cells much the same as among cortical cells in roots.[62]

The tumor formation is proportional to concentration within certain limits.[55] Different species show different degrees of response to the same concentration. Another factor is the specific moment when seedlings are placed in colchicine.[115] If the seedling has not yet elongated, there is swelling throughout the entire hypocotyl. But the seed-

ling that has already elongated, let us say to 23 mm. before treatment begins, shows practically no swelling at the hypocotyl.[115] All these points fall in line with the proposition that tumor formation is basically a growth response to colchicine (Fig. 4.1).

Stems of *Tradescantia* cut from the plant and placed in colchicine show extreme swelling at the node where leaves are attached.[148] The nodal enlargements are in every respect comparable to root and hypocotyl tumors. A petiolar swelling also may occur if expanding leaves are placed in colchicine.

The growth responses observed for roots and stems raised the question of a possible hormone action. However, the standard tests for measuring phytohormone potency gave negative results.[34, 90, 59] No
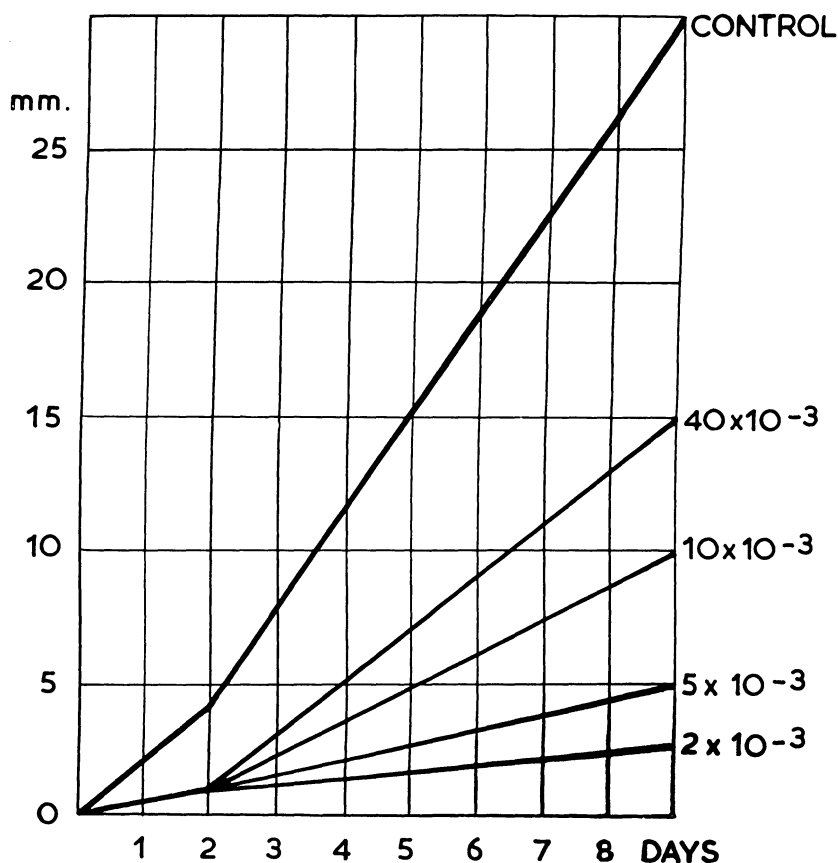
Fig. 4.1—Elongation of hypocotyl of **Lepidium** seedlings. Reduction in length is proportional to concentration of colchicine. (Adapted from Gremling)

responses were obtained from colchicine applied to the *Avena, Helianthus,* and *Pisum* tests.[115] Colchicine is not a phytohormone, but the basic relation between growth responses shown by tumors and the reactions noted for phytohormones in causing cell enlargement is not understood. There are numerous cases reported where colchicine changed growth rates.

Resistance to colchicine by cells of *Colchicum* was demonstrated under the section dealing with c-mitoses. A similar resistance can be proved with colchicine and tumor formation. Enough species of *Colchicum* were tried to give conclusive proof of a resistance.[88, 79, 9, 84] Not all plants supposedly containing colchicine are resistant as tested by the tumor test.[88] The resistance shown by tumor experiments is not proof of a c-mitotic resistance, and vice versa. This point was not always appreciated because the independence of the two processes was not understood until specific tests were finished.

Golden hamsters showed resistance to colchicine under laboratory conditions.[107] This specific resistance may be explained in the following way: Animals inhabiting regions where *Colchicum* is found would come in contact with seeds, fruits, leaves, and corms of the plant and would consume amounts of varying strength. Enough colchicine would be present to kill susceptible individuals, while others might survive. Therefore, by selection in nature the hamster may have acquired this specific resistance.

4.2: Effects of Colchicine on Pollen Tubes, Hair Cells, and Other Parts of Plants

The number of chromosomes per pollen tube does not increase after c-mitosis in the generative cell.[35, 137] An enlarged pollen tube is independent of the action of colchicine upon the nucleus. When a pollen grain germinates in artificial media, a tube grows out and away from the grain (Fig. 4.2). Such filaments are very narrow and elongation of the tube is polarwise. Colchicine decreases the length and increases the width of a tube. An enlargement even greater than the grain itself may occur (Fig. 4.2). These are the pollen tube tumors. A stimulation has been reported when hormones are added to cultures with colchicine.[76, 150]

A lateral expansion is comparable to the isodiametric extension of root or hypocotyl cells. The tubes seem to "bloat" or inflate like balloons (Fig. 4.2*F*). Since there is no bursting, the increase must take place by an orderly deposition of cell wall material forming the tube.[35] Colchicine causes these pollen tube enlargements. When the concentrations are of low dosage, a stimulation is observed.[150, 76]

An interaction between concentration and temperature condition was expressed in measurements with calculated averages of pollen
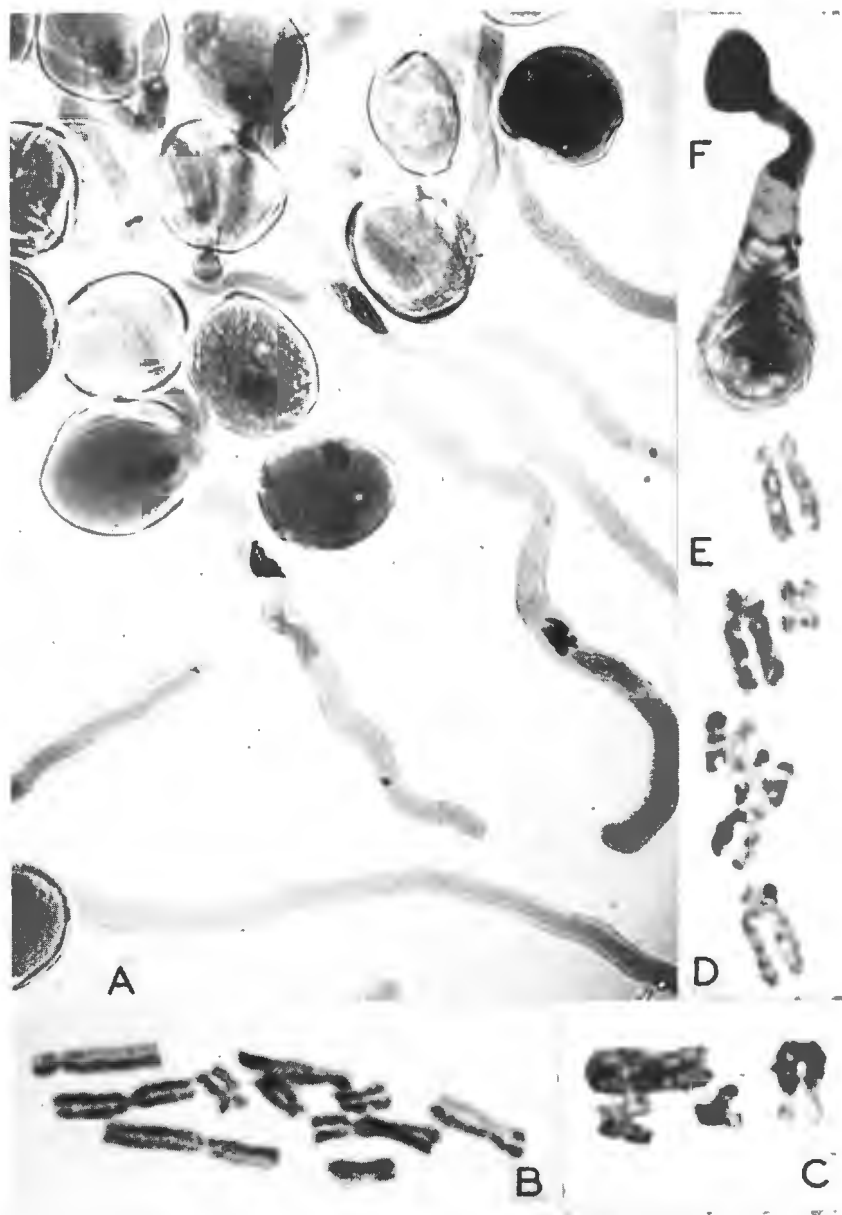
Fig. 4.2—Pollen tubes of **Polygonatum pubescens** from cultures in sucrose agar, treated with colchicine and untreated. **A.** Control culture, pollen tube with generative cell in metaphase, stained with iron acetocarmine. **B.** Colchicine mitosis of a diploid species, n-10, to be compared with Figure 2.4D of Chapter 2, the tetraploid species, n-20. **C, D, E.** Reversion to interphase; c-pairs are not separated completely at centromeric region. **F.** Pollen tube c-tumor that is a response to colchicine independent of any polyploidy. Tube wall staining shows depositions not commonly observed in control. Stained with iron alum haemotoxylin. (Eigsti, 1940)

tube widths.[127] Five-and-one-half-hour cultures at 25°C. had tubes with a 30 per cent increase in width over the control. No such significant differences in width were found at 20°C. or 30°C. Although the mean tube length was less than control for all temperature levels, only at the optimum, 25°C., was maximum width obtained.[127] The concentration of drug, 0.01 per cent, remained the same for all tests. No similar increase in width was found upon adding 3-indoleacetic acid, vitamin B_1, or NAA to the culturing medium.

Pollen from *Colchicum autumnale* L. was tested for response to colchicine. Germination was depressed by concentrations ranging from 1.0 to 0.1 per cent.[60] Tumors were observed comparable to those in pollen samples from species not known to produce colchicine, and thus a resistance such as was shown to c-mitosis and c-tumor has not been demonstrated for the case of the pollen tube tumors. The response from these tests is of further interest in light of the report that bees carrying pollen from flowers of *Colchicum* yield honey that is poisonous due to a high colchicine content.[146] From this indirect evidence it would thus seem that the pollen contains the drug. The quantities of colchicine which are carried in the flowers are described in Chapter 5.

Epidermal protuberances on roots, the root hairs, involve no mitotic stages.[53] These cells are suitable for testing the action of colchicine upon enlargement of root hairs. Eight species of plants were included in a study to measure differences in root hair development between control and treated cases.[53]

Bulbous tips appeared in contrast to the normal long, thin filamentous root hairs. The polyploid condition is not involved since the nucleus does not divide. Here again is evidence for an independence between the c-tumor and c-mitosis. Sometimes the end of a particular hair becomes forked.[53]

Other plant parts, the stem, leaf, and flowers, have hairlike cells. For *Helianthus,* a protuberance quite different from the normal is produced following treatment with colchicine.[73]

Staminal hair cells of *Rhoeo discolor* form a chain of cells like beads.[29] Colchicine causes the distal cell to enlarge considerably beyond the normal size. Each cell successively from the tip to base is enlarged, but the size decreases progressively from the tip to the basal cell. The largest cell, an end cell, is also the youngest. Maximum increase is then proportional to the age of the cell; younger cells expand more than older ones.[29]

The stylar portion of a pistil is elongate and is composed of elongated cells. Flowers of *Tradescantia* treated with colchicine before the pistil develops, show modification of these floral parts.[148] Short, stubby pistillate structures replace the long filamentous styles. The number of cells does not change, but the manner in which elongation

proceeds becomes considerably altered. Cross sections as well as longi-tudinal views are very instructive.[148]

Floral parts from *Carthamus tinctorius* follow similar patterns of induced changes when treated with colchicine before the flowers mature. Blunt, wrinkled petals and short, single gynoecia with woolly hairs replace the pointed, elongate petals, double gynoecium, and stiff, pointed hairs of normal flowers.[73]

Enough data have been collected to confirm the fact that colchi-cine alters the way in which cells enlarge.[147] Growth by increase in volume is modified under specific conditions, and this may be related to changes in viscosity of cytoplasm caused by colchicine.[28, 32, 68, 37, 39, 126, 88, 98, 103]

To explain the mechanism for a c-tumor, certain parallels were drawn between viscosity changes in the cytoplasm and dissociation of the cytoplasmic proteins.[103] Colchicine caused a decrease in viscosity that was correlated with the formation of the c-tumor in *Allium*. In this explanation, a dissociation was the primary causal factor. A similar mechanism was described in connection with the c-mitosis.[103]

The idea of a narcosis was also introduced to account for a c-tumor, but instead of there occurring a narcotized cell division, it is the growth process by cell enlargement that is influenced by colchicine.[108] In regard to this hypothesis and the preceding one, much additional information is needed for a full explanation of the action of the drug during cell enlargement.

4.3: Colchicine-Meiosis and Gametophytic Development

In pollen mother cells or megaspore mother cells that are in con-tact with colchicine at the time of reduction division, the meiotic stages are converted into a "colchicine-meiosis."[79] Only at this time can such a process as c-meiosis take place (Fig. 4.3). Earlier, that is, during divisions in the archesporium, and in later cycles, when micro-spores or generative cells divide, the processes become true c-mitoses.[79] Since the c-meiosis represents a special case, primarily because meiosis is a particular kind of division, it is discussed in this chapter with other aspects of growth and reproduction. Obviously the spindle inhibition is common to both c-mitosis and c-meiosis; so also are the c-pairing phenomena (Table 4.1), a secondary action of the suppressed spindle, and the "c-bivalents" accompanying c-meiosis. These and related characteristics of c-meiosis occur only during a certain time in the reproductive cycle (Figs. 4.3 and 4.4; Tables 4.1 and 4.2).[79, 29, 124, 148]

To help visualize how essential a timing sequence is in producing the c-meiosis, a survey of the particular cell, treated stage, and ex-pected results are given in Table 4.2. From this outline one can see
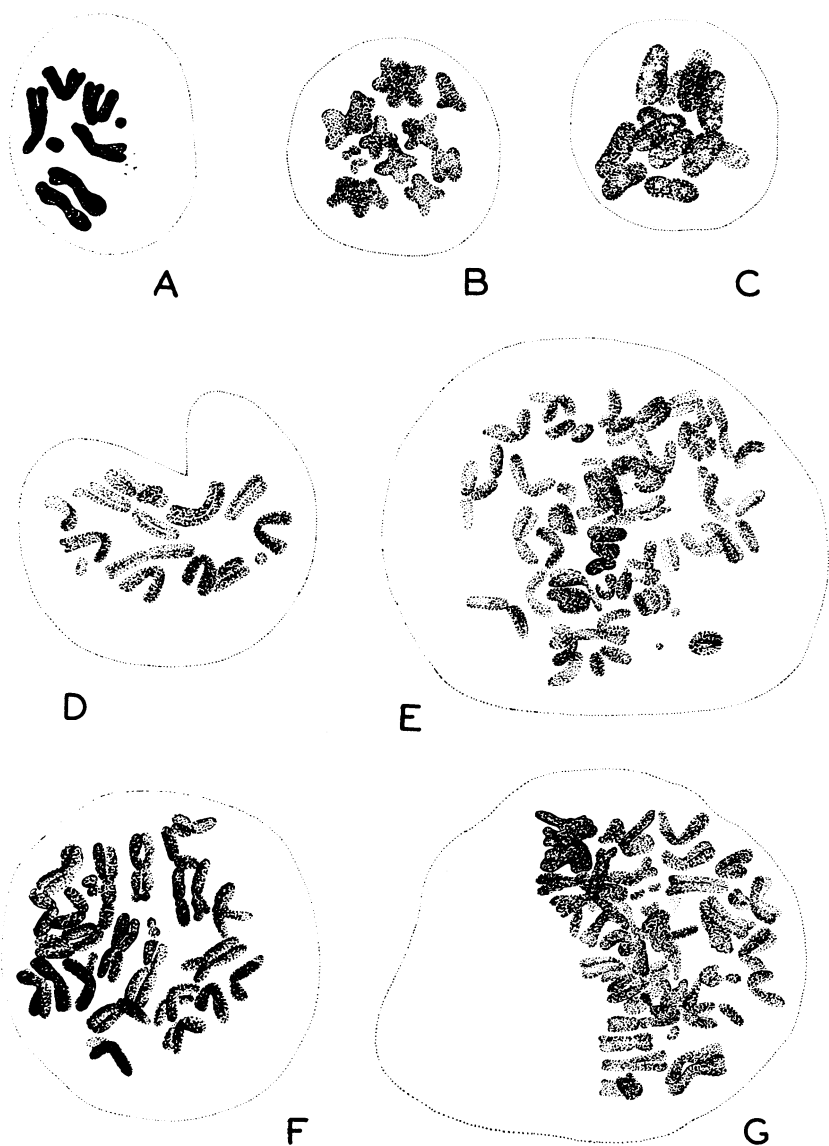
Fig. 4.3—Pollen mother cells of **Tradescantia palludosa**. Control and treated cu'tures. **A.** Untreated microspore. **B.** Univalents induced by colchicine. **C.** Desynaptic metaphases, four days after treatment was made. **D.** Diploid microspore from a treatment that became effective at the second meiotic division. **E.** Octoploid microspore 21 days after treatment; time of treatment 48 hours, then time allowed for recovery, two meiotic divisions inhibited, and one premeiotic c-mitosis. **F.** Tetraploid microspore, 12 days after treatment. **G.** Hexaploid microspore, an unequal division that is similar to a distributed c-mitosis. (After Walker)

that action during division leading up to meiosis creates octoploid or tetraploid pollen mother cells.[79] In contrast, activity during meiotic divisions I and II creates tetraploid monads, and activity at division II only, diploid monads. Monadal formation is a special feature of the c-meiosis. The monads replace the usual tetrads of microspores forming at the close of a meiosis.[29, 148, 122, 79]

Since archesporial divisions become regular c-mitoses, these are not described in great detail here, except to say that one c-mitosis in this
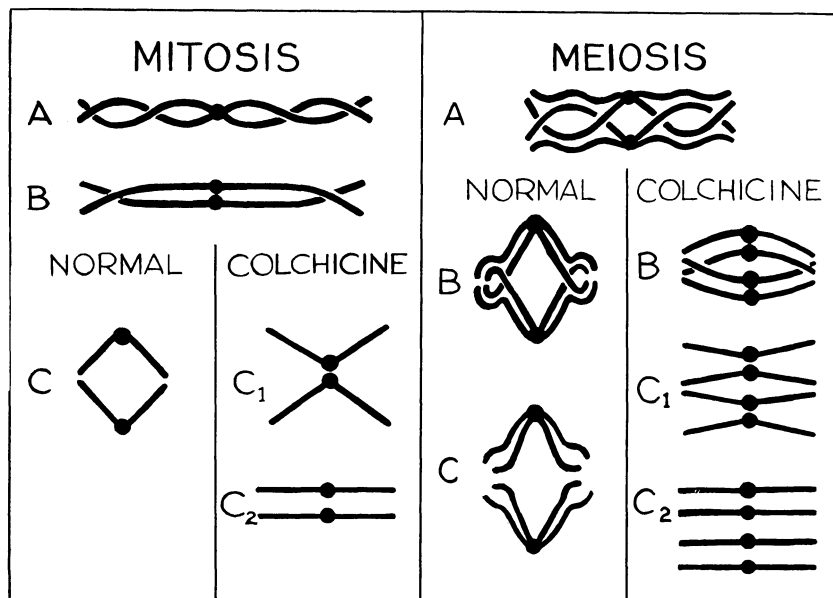

Fig. 4.4—Comparison of a c-meiosis and c-mitosis. The stage reached when colchicine becomes effective determines the action in meiosis. (After Levan)

tissue gives rise to tetraploid pollen mother cells, and that two c-mitoses bring about the octoploid condition. Beyond this degree of polyploidy the meiotic processes are so upset that no further action of colchicine can be obtained at meiosis. The premeiotic stages of *Allium cernuum* with diploid, tetraploid, and octoploid numbers 7, 14, and 28 pairs, respectively, were observed and followed up to the first meiosis.[79] Already at tetraploid stages, the polarities of meiotic spindles were irregular. The multiple spindle aspects during recovery from a c-mitosis were noticed at meiosis if the previous c-mitotic cycles of archesporial cells caused polyploidy.

Pairing of homologous chromosomes and chiasmatal formation formed during prophase are decisive functions before a regular meiosis

or a c-meiosis begins. Colchicine reduces the pairing as shown by the reduction in chiasmata and increased frequency of univalents.[2] The calculations from several independent studies confirm the action on pairing. *Allium cernuum* rarely showed univalents in controls, but among treated cases, 8 cells out of 31 had no bivalents. Moreover, no cell among 31 pollen mother cells studied had more than 5 bivalents when the total with full pairing could have been 7.[79] Among *Tradescantia*, 12 univalents (Fig. 4.3C) were produced by a full c-meiosis. Similar cases are reported with other species.

The terminalization of chiasmata is different when colchicine is present; therefore, there is reduction in chiasmata as well as change in the kind of chiasmata (Table 4.3).[2] Whether crossing-over is changed has not been tested genetically, but the cytological picture seems to warrant a conclusion that cross-overs would occur in places they are not generally expected.

If recovery sets in while the univalents are distributed through the cell, there is no congregation into the equatorial plate. But the

TABLE 4.1
RELATION BETWEEN TREATMENT AND STAGE
(After Levan)

Developmental Stage	Stage Treated	Results Obse ved
Archesporium................	division I division II	tetraploid pollen octoploid pollen
Pollen mother cell............	resting stage prophase	no effect abnormal asynapsis irregular bivalents
Pollen mother cell............	meiosis I meiosis II	tetraploid monad diploid monad
Pollen.....................	resting stage first division	no effect diploid pollen

univalents collect at poles where the particular chromosomes happen to lie. On the other hand, bivalents, if they have persisted, upon recovery orient in the equator.

Unlike the tendency toward supercontraction at the metaphase of a c-mitosis, the c-meiotic chromosomes do not show the usual contraction.[79] In fact, they are less contracted; this is a very striking action induced by colchicine. Such lack of contraction is correlated with a decrease in the frequency of chiasmata. These are the major effects noted when colchicine acts during premeiotic stages. Full action up-

sets the meiosis so that abnormal metaphase I and irregularities occur in subsequent stages.

If prophases have proceeded normally, pairing is regular, but colchicine introduced at the metaphase stage reduces spindle fibers. Under these conditions, the bivalents remain scattered in the cytoplasm, and the separation of two homologous chromosomes proceeds

TABLE 4.2
RELATION BETWEEN TIME OF TREATMENT AND RESULTS
(After Dermen)

Days After Treatment	Results
4	meiotic chromosomes in short broken chains; reduction of chromosomes not noticed
5 or 6	diploid and tetraploid pollen mother cells
8	tetraploid and octoploid pollen mother cells
11	polyploid microspores
12	failure at meiosis I and II; haploid, diploid, tetraploid microspores

where each pair happens to lie. Since each homologous chromosome of the pair is cleft and clearly separated, except at the region of the centromere, a colchicine-anaphase I is characterized by two cruciform "c-pairs" lying close to each other. The straight, cruciform anaphase I chromosomes are a contrast to normal ones at this stage.

As the first telophase begins, chromosomes lose their staining capacity, the chromatids remain connected at the centromere, and the usual transformation to interphase between the meiosis I and II takes place.[114] The outlines of chromosomes are difficult to trace at this stage and can be overlooked, making it appear that division II begins without an intervening interphase, a prophase II, or a metaphase II.

When the second c-meiotic division begins, chromosomes condense and assume a prophase appearance. The contraction of the chromatid proceeds in a prophase II. During this time the relic spiral disappears and a chromosome of c-metaphase II comes into the picture. These chromosomes are held together at the centromere up to late prophase; then they are straightened, and as fairly long chromosomes they separate from each other completely. The second c-meta-

phase II merges with the second c-anaphase II. All the chromosomes remain within one cell, so that instead of a tetrad of 4 cells, a monad results with all 4 sets of chromosomes contained within one cell (Fig. 4.3). The monad is tetraploid. C-telophase II concludes the c-meiosis with unraveling and loss of the stainable structure.[114]

The full c-meiosis has been sketched briefly without taking into consideration deviations and abnormalities caused by different concentrations, exposure, and stage at which the drug acts. Abnormal diploid, tetraploid, hexaploid, and octoploid microspores may be found, as was noticed for *Tradescantia* and *Rhoeo* (Fig. 4.3).[29] Polynucleate cells were produced from certain members of the *Aloinae*[122] and these cases arose from a treatment that probably began in prophase of meiosis.

Reduction divisions in *Carthamus tinctorius* L. were treated by a special technique in which the entire inflorescence was treated.[73] Under these conditions 10 to 17 pollen grains appeared within a single pollen mother cell (Fig. 4.5). Most grains had a nucleus, except for the very small grains. In view of the fact that this species is dicotyledonous, while the major descriptions of c-meiosis were made from monocotyledonous types, these differences may be in order. The simultaneous formation of tetrads within a pollen grain of the dicotyledons may account for the variations. *Carthamus* and *Allium* show certain fundamental differences.

The aftereffects of colchicine point out a possible influence upon pairing at meiosis in *Antirrhinum* as long as 6 weeks and possibly

TABLE 4.3
ACTION OF COLCHICINE ON CHIASMATA IN *Fritillaria*
(After Barber, 1940)

Treatment	Total Number	Percentage Proximal Locations	Percentage Medium Locations	Percentage Distal Locations
Control	215	92	6.9	1.1
0.5%	127	62	25.5	12.5
0.25%	80	70	17.5	12.5

up to 15 weeks after treatment of the plant.[129] An increase in univalents was 37 per cent among the treated plants compared with control.[129] A time lapse of such long duration between treatment and the colchicine-effect is of particular interest. Whether the colchicine is retained in the plant or the chromosomal mechanism is specifically affected was not determined. Similar meiotic irregularities were found

in treated plants of *Ribes* that remained diploid, and thus meiotic irregularities induced by colchicine would seem to be carried along, not entirely explainable by tetraploidy.[143]

Colchicum autumnale L. is a sterile plant in middle and southern Japan. Cytological analysis showed many irregularities during meiosis of these plants.[138] In contrast to these figures, the root tip mitoses
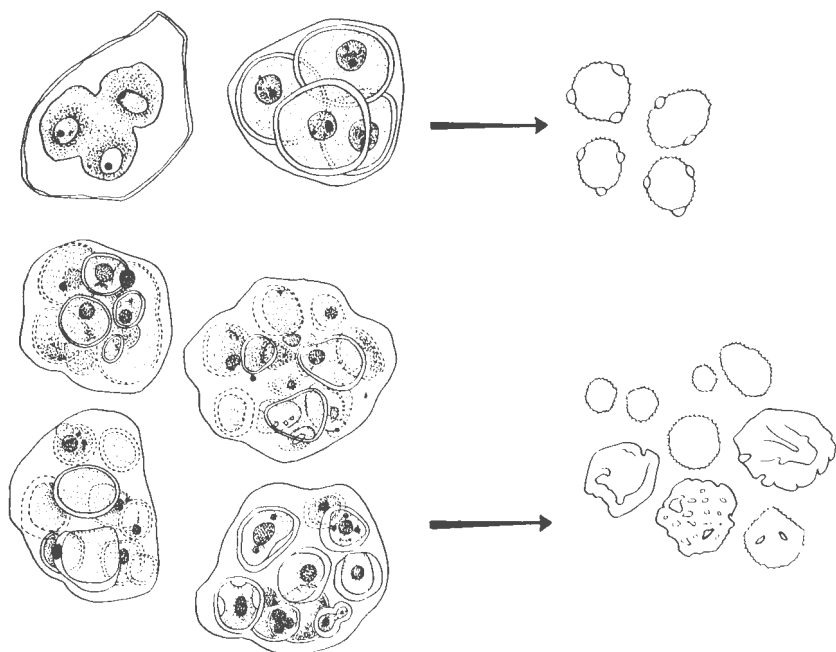

Fig. 4.5—**Above.** Untreated pollen mother cells and pollen. **Below.** The large multicellular pollen mother cells and abnormal pollen grains of **Carthamus tinctorius.** Flowers treated in an early stage of development. (After Krythe)

were regular. The pollen grains from *Colchicum* were irregular, being monosporic, disporic, trisporic, or tetrasporic. Many grains carried fragments. The interpretation made from these studies was to the effect that colchicine contained in the cells of *Colchicum* created an autotoxicosis that led to sterility in this species.

Irregular pollen and poor germination were not reported for a European representative of *C. autumnale* L. used for pollen tube germination.[60] In this instance the pollen tubes that formed did not show a resistance to the presence of colchicine added to the medium. There was no evidence that the pollen of *Colchicum* carried the drug within the protoplasm of the grains since responses obtained were reportedly the same as pollen tubes of other species not known to produce colchicine, e.g., *Polygonatum*[35] and *Antirrhinum*.[127]

If the microspore nucleus is treated with colchicine, a typical c-mitosis appears. Since the haploid numbers prevail, an otherwise precise picture of the c-mitosis can be obtained. A diploid uninucleate pollen grain is formed after the c-mitosis (Fig. 4.3).

When monad microspores with numbers higher than haploid divide without colchicine, some interesting cells are formed. These may be regarded as an aftereffect of colchicine. Multipolar divisions are common, and in particular, a tripolar division gives rise to a huge grain, with two vegetative cells appressed close to the wall, and one generative cell. On occasion, two generative cells are formed.[79] These conditions are similar to the recovery phases described in earlier chapters.

Pollen grains of *Polygonatum* with one generative cell, a haploid, and a tube cell were tested for c-mitotic characteristics (Fig. 4.2).[35] The method of testing is described in detail in Chapter 16. In Chapters 2 and 3, illustrative material was drawn from pollen tube c-mitosis, but here it is pertinent to point out that the c-mitosis in this structure never exceeds the diploid number. Very rarely do the c-pairs become completely separated, so reversion to the interphase goes from an arrested metaphase rather than through c-anaphase. Enough tests have been run to report conclusively that there is a termination to c-mitosis and, unlike the divisions in root tips that continue to build high numbers, multiple-ploidy has never been found in pollen tubes with *Polygonatum* or reported from other sources. Then the microgametophyte never exceeds diploidy.

In the case of embryo sac development in *Tradescantia,* the nuclei that regularly divide during the process of gametophyte formation seem to build up the amount of chromatin, although as is expected, no spindle forms with colchicine. Therefore, the chromosomes remain together. The size of the large nucleus, the size of the embryo sac, and a tendency toward cell formation lead one to infer that c-mitoses proceed to but do not go beyond the eight-cell condition, normal for an embryo sac in *Tradescantia* (Fig. 4.6). Aside from the c-mitotic aspect, the unusual increase in the embryo sac beyond that for the control is of interest in light of our discussion about the action of colchicine on growth processes involving increase in volume.[148]

The ovules of *Carthamus tinctorius* did not develop into seeds, and no descriptive cytology accompanied the successive stages that must have taken place when colchicine acted while the embryo sac stages were in formation. This would be of interest for a comparison with *Tradescantia.*[73, 95]

4.3–1: Gametophytes of mosses, liverworts, and ferns. In 1908, a series of experiments with mosses demonstrated that polyploidy could be induced artificially. The Marchals used regenerative tissues to isolate polyploid races. Three decades elapsed between the first work

early in the twentieth century and the next significant colchicine experiments.[64] Colchicine has been tried recently for a number of mosses, using protonemata and propagula, treating the tissues in special culturing media. Size differences between colchicine-treated and untreated cells have been used as criteria for the changes in number of chromosomes (Table 4.4).

Diploid gametophytes of the male and female thalli from *Marchantia polymorpha* were made by colchicine.[9] Chromosomal check showed that the numbers were increased. Another hepatic, *Pallavacinia* spp., was subjected to colchicine.[157] Again new patterns of growth showed that changes were induced. One may assume that the number of chromosomes was increased, although the modification in cellular form without a corresponding increase in chromosomes makes
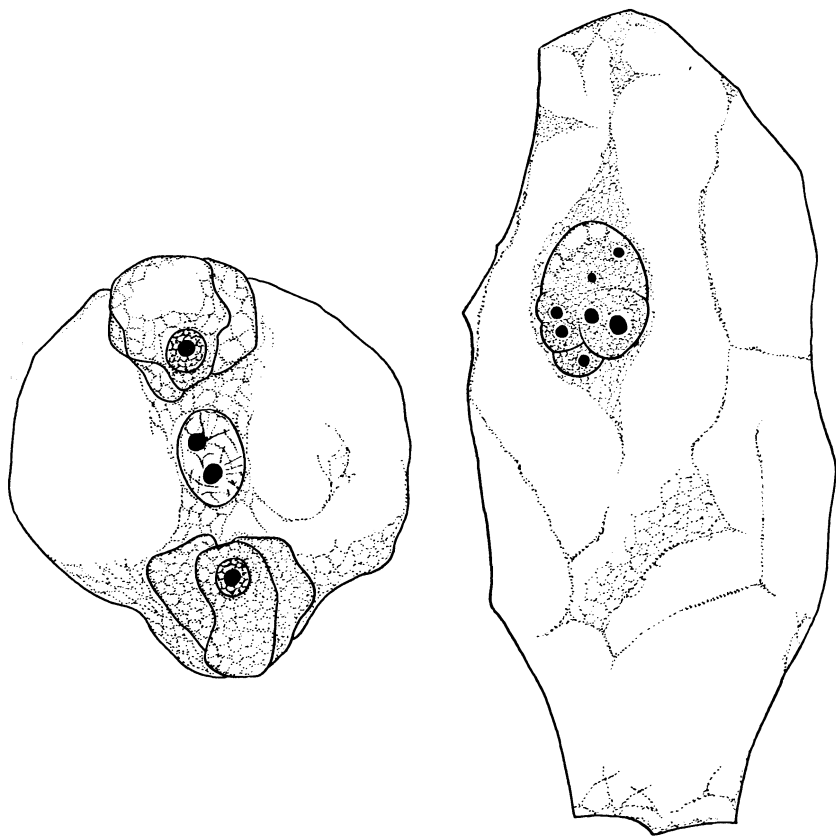

Fig. 4.6—Embryo-sac stages of **Tradescantia**. Untreated stage with cells distributed in the sac and a smaller cavity. Treated stage with all nuclear material grouped in the center of sac. The size is not a response to polyploidy. (After Walker)

TABLE 4.4

ACTION OF COLCHICINE ON ALGAE AND GAMETOPHYTES OF MOSSES,
LIVERWORTS, AND FERNS

Species	Results	Reference
Aulacomnium androgynum	morphological changes	4–64
Cladophora spp.	cross wall thickened	4–53
Closterium spp.	temporary inhibition	4–80
Dryopteris felix-mas	morphological changes	4–117
D. subpubescens	abnormal sperms	4–94
Gonium spp.	temporary inhibition	4–80
Goniopteris prolifera	abnormal sperms	4–94
Hormidium spp.	leukophytic isolate	4–125
Hydrodictyon spp.	cellular changes	4–53
Marchantia polymorpha	diploid gametophytes	4–9
Micrasterias thomasianas	no c-mitosis	4–67
Nitella mucronata		cf.4–88
Nostoc commune		cf.4–88
Oedogonium spp.	polyploids	4–140
Oedogonium	cellular wall changes	4–53
Pallavacinia	morphological changes	4–157
Polystoma	temporary inhibition	4–80
Spirogyra spp.	plastid changes	4–158
		4–68
Ulva spp.	temporary inhibition	4–80

it less certain than previously believed possible for chromosomal num-
bers to be increased as cell form changed.

Fern prothalli and sporogenous tissues were tested for the induc-
tion of polyploidy following colchicine.[117] Evidences of changes in
numbers were obtained for several species of ferns. In another applica-
tion of colchicine to growing prothallia regularly producing sper-
matozoids, some unusually large sperms were obtained. Also some
changes in the shape of cells were noticed along with the increases
in size. Dilute solutions were used for early stages of germination of
the prothalli.

Information at hand shows that the gametophyte stages of green plants can be doubled in manner similar to the sporophytic cells, notably among the seed plants.

4.4: Microbiological Data

Controlled cultures using unicellular organisms are admirably suited for experiments with colchicine. A wide concentration range may be used because the strongest dosages show a minimum toxicity. Furthermore, the experimental subjects are numerous considering the bacteria, yeasts, filamentous fungi, algae, and protozoa. Considerable preliminary work has been started, but contradictory conclusions and no small amount of confusion still exist.

In some cases the methods are not clearly described, nor are they carefully planned. Modifications such as concentration, media, and exposure would prove helpful. The interpretations have been very narrow, and patterned generally after the known action of colchicine upon the nucleus of vascular plants and multicellular organisms. As an illustration, the doubling of chromosomes is a remarkable action with vascular plants, and it would be helpful to know more about the hereditary materials in bacteria, but colchicine can hardly resolve the problem of chromosomes in bacteria when cytologists have had such great difficulties in demonstrating structures in untreated cultures.

Yeast cells that have an advantage over bacteria in size of internal structures have been tested with colchicine. The results can not be considered decisive. Even among the algae where chromosome numbers for species have been established, there are no clear cytological data to prove that the number of chromosomes can be doubled by colchicine. There is discussion of haploids, diploids, and tetraploids among fungi, but present work with colchicine does not provide answers either through demonstration of chromosomes or by genetic evidence.

Changes in the sizes of cells within a culture and direct action upon the growing organism indicate that the drug has some influence upon growth processes related to increase in size. Of course, these changes are not transmitted to succeeding generations. The mechanism of growth by cellular enlargement can not be analyzed from such tests. Metabolism of bacteria in relation to colchicine represents an unexplored field. Preliminary work has been done. In 1907, interesting work was done on temperature and toxicity using cultures of *Paramecium*.[58] Otherwise, this field of experimentation has been overlooked.

Finally the processes of differentiation and cellular structure are influenced by colchicine. Fungi and algae show evidence that during

the process of cell wall formation the action of colchicine modifies structure.[53] These aspects are treated in a subsequent section of this chapter.

4.4–1: Bacteria. Tests with colchicine have included a range of species.[19, 149, 17, 159, 37, 113, 66, 51, 134, 63, 41, 109, 35, 25, 13, 14, 43, 144, 104] Some report no reaction and others claim that colchicine acts upon growth by inhibition. Toxicity was also noted (Table 4.5).

Certain species of bacteria tolerate high concentrations of colchicine in the medium. One source of powdered colchicine had bacteria present in the material; small quantities of powder added to sterile solutions of colchicine showed species of *Agrobacterium*.[35] For a number of species of microorganisms, colchicine without any additional nutrient supported bacterial growth. It was a habitat for bacteria. Undoubtedly these forms were able to use colchicine as a food.

The bacteria growing in a medium of strong dosage (1 per cent) produced aberrant cells larger than the initial culture, but no continuation of these types has been possible. An increase in size may represent a condition similar to the cell enlargements for vascular plants. These are not hereditary changes. Single cell isolations have not been reported. It would be of interest to know more about these types. They should be singled out for subculture, since mass transfer for isolating the particular deviates has objections. Some morphological alteration temporary for a specific culture undoubtedly has been obtained. Increases amounting to 40 per cent were measured for *Bacillus mesentericus*.[113]

Polynuclear cells in *Escherichia coli* cultures were reported but no follow-up of this work has been discovered.[134] Apparently a repetition has not been accomplished.

In a metabolism test, respiration was inhibited in *Micrococcus aureus*. A growth stimulation was obtained for *Photobacterium phosphoreum*.[104] No changes were observed in the desoxyribose nucleic acid and the ribose nucleic acid when cultures of *Micrococcus aureus* were used.[17] This is a sample of the fragments of information; more are tabulated elsewhere (Table 4.5).

4.4–2: Yeasts and other fungi. The common brewers' yeast, *Saccharomyces cerevisiae*, has been tested by more independent workers than any other of the microorganisms. A variety of concentrations of colchicine were used and different techniques for culture, as well as staining to determine cytological changes were tried.[116, 5, 4, 83, 41, 126, 54, 39, 144, 75, 9, 6, 119, 52, 132, 145]

A wide choice of responses is at hand, ranging from reports of no action to those citing definite cytological change demonstrated by special staining methods. Dumbbell-shaped nuclei were seen after a 96-hour treatment with 0.1 per cent colchicine. Other workers were unable to obtain these same results (Table 4.6).

TABLE 4.5
ACTION OF COLCHICINE ON BACTERIA

Species	Results	Reference
Agrobacterium spp.	growth not inhibited	4-35
Bacillus mesentericus	size increase 40%, growth changes	4-113
Bacterium megatherium	negative results	4-149
Bacterium spp.	no action	4-66
Bacterium spp.	indecisive results	4-43
"Bacteria"	no action	4-144
"Coliform bacteria"	mutations	4-109
Escherichia coli	polynuclear cells	4-134
E. coli phage		4-25
Micrococcus spp.	inactive	4-19
M. aureus	negative results	4-19
Micrococcus spp.	morphological changes	4-149
M. aureus	respiration inhibited	4-17 4-159
Mycobacterium tuberculosis	stimulates cells, prevents variants	4-63
Photobacterium phosphoreum	growth increases	4-104
Proteus vulgaris	inhibition	4-37
Streptococcus catarrhalis	toxic action	4-149
S. hemolyticus	inhibition	4-37

Camphor induced giantlike cells now called the "camphor forms." In old cultures these appear with low frequency. A few were found after treatment with colchicine, but their frequency was not high enough to warrant the conclusion that colchicine had the same capacity as camphor to produce giant forms.[4]

In light of the known antagonistic action of ethanol as discovered for cells of *Allium,* the production of alcohol by the yeast cell itself may serve as a kind of antidote or protection against colchicine.[82] These facts have not been verified with experimental data.

Brewing tests did not bring out specific differences between treated and control cultures of *Saccharomyces cerevisiae.*[82] The usual sedimentation, foam head, and other comparative values revealed no

changes induced by colchicine. Methylene blue was decolorized more rapidly as evidence of some basic metabolic change.

There is a possibility that colchicine may serve as a source of energy. Another conclusion led to the idea that the drug serves as a buffer against the toxic substances accumulating in an active culture. Filamentous fungi from a variety of families[9] have been tested for possible induction of polyploidy. A polyploid strain of *Penicillium notatum* was isolated in one laboratory.[52] This new strain was supposed to yield more penicillin than the original strain. The polyploids were obtained by another group who rechecked these specific types. Polyploidy and increased penicillin was not confirmed (Table 4.6) .[119]

TABLE 4.6

ACTION OF COLCHICINE ON YEASTS AND OTHER FUNGI

Species	Results	Reference
Allomyces javanicus	changes induced	4–6
Aspergillus spp.	mutants	4–132
Botrytis cinerea	hypertrophy of hyphae	4–145
Coprinus radians	conidia influenced	4–144
Diaporthe perniciosa	no conidial formation	4–145
Mucor sp.	no change	4–9
Penicillium notatum	polyploids	4–52
P. notatum	no polyploids	4–119
Psilocybe semilanceolata	conidia changed	4–144
Saccharomyces cerevisiae	no changes noted	4–4
		4–83
		4–144
		4–75
		4–5
S. cerevisiae	cytological changes	4–126
	cells enlarge	4–39
	methylene blue decolorized more rapidly	4–41
	stimulation	4–116
	inhibition	4–54
Stropharia merderia	conidia changed	4–144
Verticillium dahliae	no conidial formation	4–145
"Wide range of families"	no change	4–9

Hypertrophy of the hyphae and failure to form conidia were regularly noted among several species of fungi, but doubling of chromosomes or evidence of polyploidy was never demonstrated. Possible mutagenesis[123] was reported for *Streptomyces griseus*. Concentrations ranging from 0.5 to 1.0 per cent introduce changes in growth patterns that resemble the tumors previously reviewed. No better specific information is at hand for the yeasts and fungi than for bacteria. That mycelial growth may be influenced is probable, but polyploidy or induction of mutations is extremely doubtful (Table 4.6).

Colchicine increases the frequency with which resistant sporangia of *Allomyces javanicus* developed mixed thalli from the sporophytic generation. When germinating zygotes were treated, some nuclei were thought to have been converted into polyploids. The cytological records of chromosomes were not available to confirm the polyploidy.[6] A series of treatments involved the use of colchicine and sodium nucleate, so the specific action of colchicine may be in some way related to the use of the sodium nucleate.

4.4–3: Algae. The first artificially induced polyploid among plants might well be credited to Gerassimov who treated *Spirogyra* by temperature shock and apparently succeeded in increasing the volume of the nucleus. This was done in 1901. A confirmation made some years later strongly supports the thesis that *Spirogyra* cells were doubled. One might hope that colchicine would be useful in repeating this classical experiment by chemical means, or at least demonstrate that the drug is not effective. The results with algae and colchicine are not any farther along than those with the other specimens of fungi.[140, 158, 125, 65, 67, 180, 9, 88] The treatment of *Spirogyra* with colchicine should be tried with a wide range of concentrations and cytological control.

A polyploid strain of *Oedogonium* was said to be obtained from treatment with colchicine, but no exact cytological data went with the report to prove the doubling of chromosomes had taken place.[140]

Temporary inhibition of mitosis in cells of *Micrasterias thomasianas* was recorded in cultures. The general conclusion was reached that colchicine was ineffective except for some temporary changes in plastid structure.[67] Unfortunately, only limited ranges of concentrations of colchicine were employed for the *Micrasterias* work. Some dosages may be more effective than others.

Leukophytic variants were isolated from colonies of *Hormidium* sp. treated with colchicine.[125] Several generations of subculture brought a return to the chlorophyllous type. If a change was induced, the weakness of a non-green variant did not permit a survival in competition with unchanged chlorophyllous types.

Plastid changes are to be expected in the treated generation. Whether or not changes are retained upon transfer to culture without colchicine remains unconfirmed. Supposedly the elasticity of plastids in *Spirogyra* changes under the influence of colchicine.[158]

Inhibitions at higher concentrations were secured with *Gonium* and *Polystoma*. Upon recovery the cells remained diploid as far as the investigators were able to judge. Some action seems to have been registered upon the zoospores and zygotes of the green alga *Ulva*.[80]

Studies dealing with the cell wall and colchicine are of interest from the view of differentiation. Cell structure and composition of the wall are modified by colchicine (Table 4.4).

4.4–4: Protozoa. A number of investigations[3, 11, 20, 24, 49, 57, 58, 71, 118, 136, 144] on various aspects of colchicine and the protozoa, as well as regenerative studies[136] have been published since 1938. As long ago as 1907, the action of colchicine on *Paramecium* was studied in relation to toxicity and temperature changes.[58] Increasing toxicity with raising the temperature was demonstrated by this early work. No one has repeated these studies in the modern period, but most have been concerned with cell division and problems of polyploidy. Undoubtedly the influence of cytology and genetics preconditioned much of the experimentation since 1937.

The species of protozoa tried for response to colchicine show that strong solutions can be tolerated at 22° to 24°C. Fission occurs for a number of species.[71] The microinjections of colchicine give further information on the penetrability of the drug that may influence the reaction. Failure of the drug to penetrate the cell may be one key in explaining the resistance to colchicine of protozoa as a group.

Some retardation in growth and changes in new cells developing within a culture containing colchicine have been recorded. As a general rule, the direct action of the chemical upon the cell or nucleus has not been demonstrated. Some increases in "radio-sensitivity" accompanied the pretreatment by colchicine.[57] In this case the cells appeared to be more sensitive to action of the X-ray after a treatment.[57]

Table 4.7 may be used as a reference for a survey of work completed upon the protozoa as a group.

4.5: Differentiation Processes

After a treatment with colchicine the new leaves, developing when growth is resumed, appear wrinkled and distorted. Apparently the drug has directly or indirectly caused these new types. Some changes are a result of chimeras which are discussed in connection with polyploidy, yet other very similar anomalies cannot be correlated directly with an increase in the number of chromosomes. These cellular and

TABLE 4.7

ACTION OF COLCHICINE ON PROTOZOA

Species	Results	Reference
Amoeba proteus	fission not inhibited with 2% solution	4–71
A. sphaeronucleus	microinjection inhibits division of nucleus	4–20
Chilomonas spp.	fission not inhibited	4–71
Chlamydomonas spp.	not effective on division	4–49
		4–83
		4–144
Chlamydomonas spp.	growth retarded	4–24
Euglena spp.	ineffective	4–71
		4–144
		4–83
Oxytricha spp.	no action	4–71
Paramecium spp.	raising temperature increases toxic action of colchicine	4–58
P. caudatum	fission not retarded	4–71
P. caudatum	growth retarded	4–3
P. caudatum	radiosensitivity increased	4–57
P. multimicronucleatum	no action	4–71
Peranema	fission	4–71
Plasmodium relictum	no retarding action	4–11
P. vivax	no action	4–118

anatomical variations are probably a direct action from the drug by other means than nuclear changes.[153] As an example, the c-tumor response occurs from contact with colchicine. Yet more difficult to explain are the changes that persist into several generations of propagation.[40] Vegetative propagations that continue the anatomical variations are not as difficult to explain as variations that reportedly persist or occur after several generations of seed propagation.

Not so much attention has been directed to the cell wall and related problems of differentiation as to nuclear aspects, i.e., c-mitosis.[35] Colchicine causes modification of cytoplasmic and cellular processes.[131] Sufficient evidence is at hand to make this assumption. The actions of c-mitosis, the c-tumor, and differentiation are independent although very closely related to each other. For example, the nearly

simultaneous action upon division, enlargement, and differentiation can conceivably take place when unicellulars are subjected to colchicine. At least the processes may merge into each other so closely that separating the actions becomes difficult or nearly impossible.

Analysis and reports from widely different sources are brought together in this section that treats the microscopic, microchemical, and gross anatomical changes in plants.[22, 121, 50, 53, 151, 105, 112, 135]

4.5–1: Microscopic and microchemical data. The cell walls of treated plants show different types of depositions which form striations.[53] These are regularly observed for pollen tubes growing in media containing colchicine. When stained, their distinction becomes more clear. The submicroscopic structure of pollen tube walls has not been studied. Data are accumulating from other sources that point up the possibilities in this field.[73]

Excellent photomicrographs showed that the cells of algae were changed after growing in media carrying colchicine.[53] The newly formed portions of cells in *Oedogonium* showed swelling and local thickenings inside the cell (Fig. 4.7). These were scattered without regular order along the wall. Inner cell walls of *Cladophora* became thicker than controls, showing that unusual depositions had occurred (Fig. 4.8). Finally, the regular network characteristic for *Hydrodictyon* became distorted through swelling of the middle parts of connecting cells (Fig. 4.9). Also the points of contact were enlarged. These three cases comparing treated and untreated cells leave no doubt that colchicine exerts a strong influence during cellular differentiation.[53]

The root hairs grown in cultures containing colchicine (0.25 to 0.5 per cent) offer a comparable source for analysis of cell wall structure. Earlier we described the tumors that were formed on root hairs. Now microscopic and microchemical study has correlated the cell structure with the form taken under treatment. After the cell walls were stained with chloro-zinc-iodide and these structures viewed with polarized light, the irregularly deposited micelles were in distinct contrast to regular arrangements viewed in untreated root hairs. Photomicrographs with polarized light are instructive for these comparisons.[53]

Pollen mother cells developing in colchicine (*Carthamus tinctorius* L.) were protoplasmically interconnected at the points where cells touched each other.[73] Later, as pollen grains formed, one large cell was composed of numerous pollen grains within a common wall (Fig. 4.10). Another developmental feature was the wall intrusion which was essentially an excessive deposition of a callous-like material on the inner wall (Fig. 4.10). The origin and nature of these developments are unknown, but the change is an effect of colchicine.
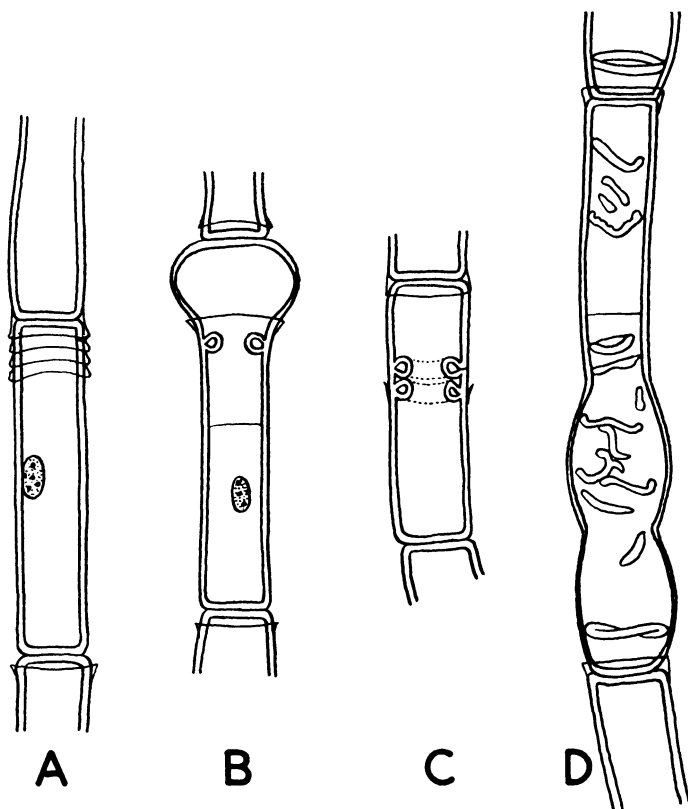
Fig. 4.7—**Oedogonium** cultures, treated and untreated. **A.** Untreated cell showing the usual ring and cellular striations. **B.** Enlargement caused by colchicine, indentation of cellular layers a result of treatment. **C.** Inner cell thickening, and depositions. **D.** Enlargement of the cell from treatment and irregular depositions. (After Gorter)

An interesting vascularization following recovery from colchicine has been described for the huge cells in *Allium* roots that form in the differentiated pericycle at points where lateral roots originate. Scalariform vessels developed and a unique tumor was left buried in the root.[156] Nuclear contents that were estimated to contain over 1000 chromosomes as a result of 6 or more c-mitoses disappeared during the differentiation process. A complex series of pretreatment with NAA (0.0002 per cent) and colchicine (0.25 per cent) interspersed with recovery periods preceded this development. No one can doubt that an interesting problem of differentiation is presented by this work.

Stomatal development regularly proceeds from an embryonic mother cell and eventually forms the guard cells,[139, 151, 88] with as-

sociated subsidary components. Independently, several investigations have shown that colchicine interferes with this differentiating process.[74] These stomatal anomalies, brought into focus by reports from such cases as pollen tube walls, root hairs, algal and fungal cell walls, as well as other differentiating cells, afford added evidence that colchicine acts in some way upon cells that are differentiating. This is the first time that so many diverse instances of the action of colchicine have been brought together under one discussion. These problems deserve attention. We have not exhausted the list of instances that may have further bearing on this aspect.

4.5—2: Gross anatomical variations. When the outer layer of cells, the epidermis, has a different number of chromosomes from those of cells deeper in the leaf, some distortions become evident. These cases are well documented and belong to problems in polyploidy. Less known and understood are the cases that cannot be readily explained by chromosomal numbers.[153] A few of these instances are described here.

New shoots of *Ligustrum* arose after treatment with colchicine.[82] The leaves were darker green, appeared to be thicker, and answered the description of an induced polyploid. These characters were transferred several times by vegetative propagation. The chromosomal numbers did not correlate with these differences.
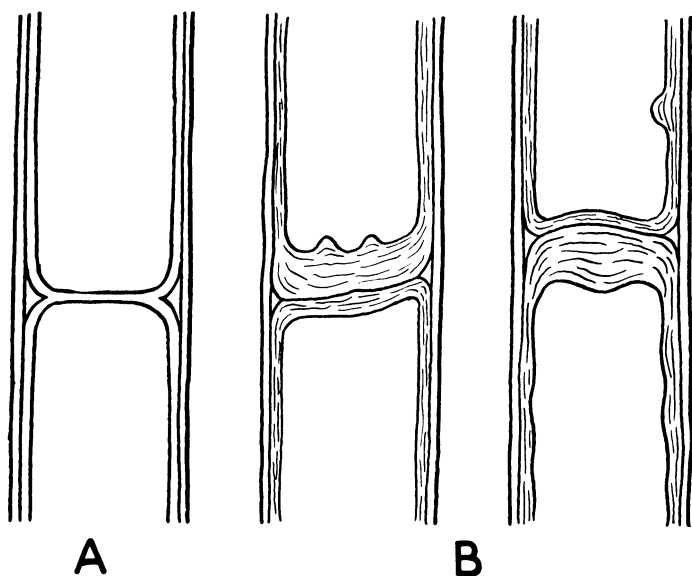

A   **B**

Fig. 4.8—The end walls of **Cladophora** with extra depositions in treated cases, **B**, compared with control, **A**. (After Gorter)
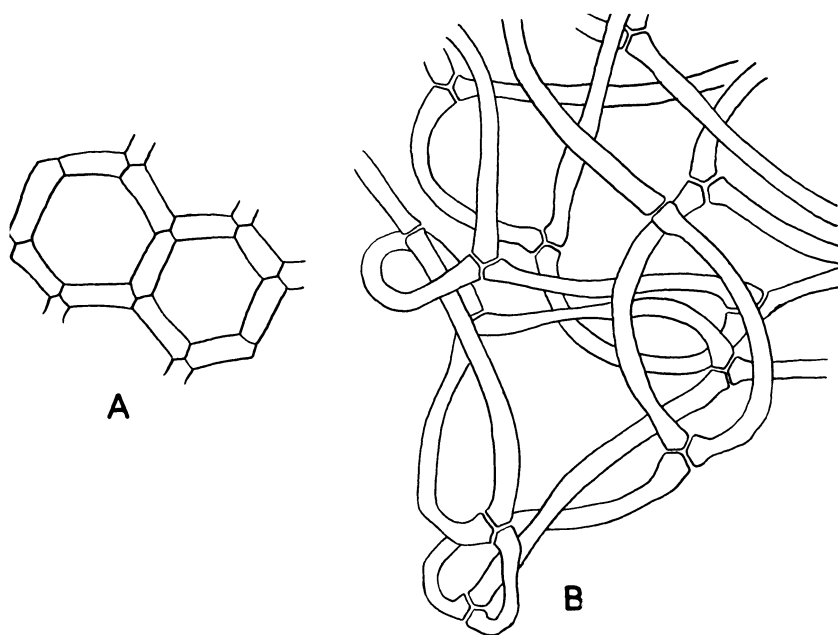
Fig. 4.9—The network of **Hydrodictyon** becomes distended and unorganized by treat-
ment with colchicine. A. Control cells. B. Treated cellular network. (After Gorter)

Sugar beets developed after a treatment showed consistent size
increase for roots, but polyploidy was not found with these particular
cases. Larger roots are regularly developed in known triploid and
tetraploid progenies.[79] Barring some error in method, the explanation
for larger beets falls outside the scope of polyploidy. Perplexing
variations appeared in subsequent progenies of sorghum plants that
were treated with colchicine. Chromosomal numbers were diploid,
so polyploidy was not correlated with these types. Additional proge-
nies from treated F_1 plants were significantly lacking in uniformity
as compared with untreated cases. These variants were not classified
with aberrants reported previously and described above, i.e., the
Ligustrum variations, because while the lack of uniformity followed
a segregation pattern, the control material did not show a similar
segregation.[82] Although no explanation was given, the hereditary
mechanism was not ruled out as a possible cause. The instance is
cited in this discussion primarily to emphasize that results from treat-
ing colchicine are not in every case quickly disposed of as the effect
of a c-mitosis, leading to polyploidy which in turn is the explanation
for new variants. That colchicine has caused a more basic deviation
not correlated with a doubling of chromosomes seems quite rea-
sonable even though the full explanation remains in question.

A survey of the literature[36] on colchicine hints that more examples could be obtained in which colchicine induces changes not directly correlated with a change in the number of chromosomes. Obviously hundreds of polyploids have been induced by colchicine. Yet, alongside these majority reports come the difficult cases that appear as anomalous anatomical and morphological deviations. These are certainly problems for future study.

4.6: Metabolism and Colchicine

Physiological studies with colchicine that had some relation to c-mitosis were touched upon briefly in Chapter 3. At the basis of cellular changes such as c-tumors and cell differentiation there must also be physiological processes involving action of colchicine. These are difficult to evaluate. However, tests have been run that show colchicine has a capacity to influence certain metabolic processes as understood by special tests.[141, 102, 92]

Enzymatic reactions performed *in vitro* proved that the transformation of starch by malt diastase was accelerated. The basis for stimulation of this order was not explained, although as a constituent of the reaction medium, colchicine favored the rate of enzymatic action. Increasing the concentration of colchicine increased the rate of reaction correspondingly.[127]

Diastase activity was scored by quantitative measurements of the increase in sugar (Benedict's solution). Control values were given at 100.0, and if the reaction time was accelerated, the value accordingly fell below 100.0. With each tenfold increase in concentration the rate was increased. Values of 84.0 ± 2.5, 78.9 ± 2.5, and 70.3 ± 1.7 were obtained for three concentrations, 10 p.p.m., 100 p.p.m., and
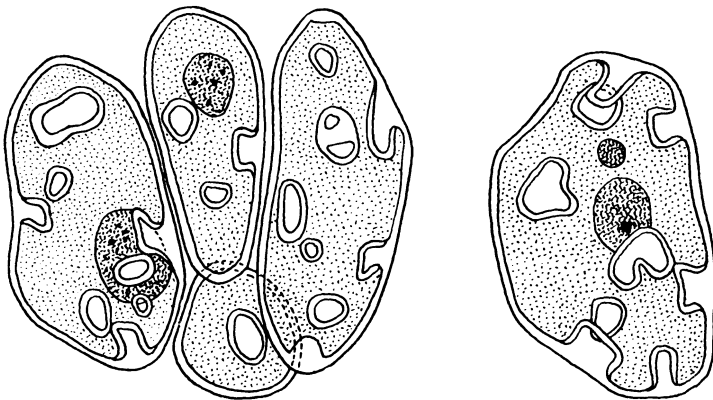

Fig. 4.10—Cellular intrusions among the pollen mother cells of Carthamus tinctorius caused by treatment with colchicine. (After Krythe)

1000 p.p.m., respectively. In other words, a control solution that reduced 25 cc. of Benedict's solution in a certain time was equal to 100 and the solution (1:1000) with colchicine showed a value of 70.3 ± 1.7 because the time taken to reduce the standard amount was shortened, as expressed by these values.[127]

These data are interesting when correlated with reports of stimulation in growth through seed and shoot treatments.[70] *Colchicine may act upon enzymes in such a way as to accelerate the transfer of starch to sugar, which processes may in turn stimulate growth.*

Excised roots of maize treated with colchicine showed lowered rates of respiration and dipeptidase response. Also, the elongation of individual roots was retarded. Since conditions vary from test to test the comparisons may not be wholly alike.[110]

Virus tumor tissues (Black's original R_1 strain from *Rumex acetosa* L.) were treated with a wide range of concentrations (0.00001 to 100.0 p.p.m.) of colchicine.[101] Growth was stimulated with concentrations of 0.02 to 0.2 p.p.m. with maximum acceleration at 0.1 p.p.m. Increasing the concentrations beyond a point of stimulation brought inhibition. The maximum uptake of oxygen occurred at 0.1 p.p.m. This value was estimated at 25 per cent above the control. Growth was measured over a period of 3 weeks and respiration tests ran for 3 hours. Curves were plotted to show the similarities and differences.[101]

Decreases in structural viscosity paralleled the formation of c-tumors in root tips of *Allium;* the decreases were most pronounced at 24 hours.[103] Changes in cytoplasmic proteins were correlated with changes that led to formation of tumors.

Rates of plasmolysis among *Elodea* were changed by a pretreatment with colchicine.[88] Not only the time for changing the form of cytoplasm but the shape of structures formed after plasmolysis was different in controls and treated cells.

REFERENCES

1. AMERIO, G., AND DALLA, N. Studi di citologia sperimentale: l'effeto di agenti chimici et fisici diversi sopra la struttura della cellule vegetale. Ist. Lombardo Rendic. III. 75:109–18. 1942.
2. BARBER, H. The experimental control of chromosome pairing in *Fritillaria.* Jour. Genet. 43:359–74. 1942.
3. BARROS, R. A colchicina eos Paramecios. Univ. São Paulo Bol. Fac. Filos. Cien. e Letras III Biol. Geral. 17:97–116. 1940.
4. BAUCH, R. Beziehungen zwischen polyploidisierenden, carcinogenen und phytohormonalen Substanzen. Auslösung von Gigas-Mutationen der Hefe durch pflanzliche Wuchsstoffe. Naturwiss. 30:420–21. 1942. Sulfonamides as antagonists to the polyploid effect of Colchicine. Naturwiss. 33:25–26. 1946. Sulfonamide und Colchicin. Ein botanischer Beitrag zum Sulfonamidproblem. Die Pharmazie. 4:1–7. 1949.
5. BEAMS, H., *et al.* Cytological studies on yeast cells with special reference to the budding process. Cytologia. 11:30–36. 1940.
6. BENEKE, E., AND WILSON, G. Treatment of *Allomyces javanicus* var. Japonesis Indoh with colchicine and sodium nucleate. Mycologia. 42:519–22. 1950.

7. BERGER, C., AND WITKUS, E. A cytological study of c-mitosis in the polysomatic plant *Spinacia oleracea,* with comparative observation on *Allium cepa.* Torrey Bot. Club Bull. 70:457–67. 1943. Further studies of the cytological effects of combined treatments with colchicine and naphthaleneacetic acid. Amer. Jour. Bot. 36:794–95. 1949.
8. BHADURI, P. Effects of different forms of colchicine on roots of *Vicia faba* L. Jour. Roy. Micr. Soc. 59:245–76. 1939.
9. BLAKESLEE, A. The present and potential service of chemistry to plant breeding. Amer. Jour. Bot. 26:163–72. 1939. Effect of induced polyploidy in plants. Amer. Nat. 75:117–35. 1941.
10. BOAS, F., AND GISTL, R. Über einige Colchicinwirkungen (Protoplasma. 33: 301–10. 1939). Abstr. Bot. Zentralbl. 33: (12) :82. 1940.
11. BOATNEY, R., AND YOUNG, M. The effect of colchicine on bird malaria. Jour. Parasit. 25:446–47. 1939.
12. BOND, L. Colchicine stimulation of seed germination in *Petunia axillaris.* Jour. Hered. 33:200–201. 1942.
13. BONNETTI, E., AND ILLENYI, A. Die Wirkung des Colchicins auf den Bakterienstoffwechsel. Zentralbl. Bakter. 2–3:114. 1941.
14. BOYD, J., AND BRADLEY, P. On the assessment of the influence of chemical compounds on bacteriophage multiplication. Brit. Jour. Exp. Path. 32: 397–407. 1951.
15. BOYLAND, E., AND BOYLAND, M. The action of colchicine and *B. typhosus* extract. Biochem. Jour. 31:454. 1937.
16. BRUMFIELD, R. Effect of colchicine pretreatment on the frequency of chromosomal aberrations induced by X-radiation. Proc. Nat. Acad. Sci. 29:190–93. 1943.
17. CAFIERO, M., AND ZAMBRUNO, D. Sul' azione della colchicina sulla moltiplicazione della *Staphylococcus aureus.* Boll. Soc. Ital. Biol. Sper. 24:1326–27. 1948.
18. CHARGAFF, E., *et al.* Inhibition of mitotic poisoning by meso-inositol. Science. 108:556–58. 1948.
19. CHODAT, F., AND MARTIN, G. Sur l'inefficacité de la colchicine dans un processus purement nucléaire. C. R. Soc. Phys. et Hist. Nat. Genève. 55:70–71. 1938.
20. COMMANDON, J., AND FONBRUNE, P. DE. Action de la colchicine sur *Amoeba sphaeronucleus.* Obtention de variétés géantes. C. R. Soc. Biol. Paris. 136:410–11, 423, 460, 746, 747, 763. 1942.
21. CONSTANTIN, T. Mitogenesis and colchicine in the onion root. Bull. Histol. Appl. Physiol. Path. Tech. Microscop. 17:97–108. 1940.
22. Cormack, R. Intercellular inclusions in white mustard roots ,induced by colchicine treatment. Nature. 163:362–63. 1949.
23. CORNIL, L. Action of colchicine in aqueous solution on germination of wheat. C. R. Soc. Biol. Paris. 138:932–33. 1944.
24. CORNMAN, I. Disruption of mitosis in *Colchicum* by means of colchicine. Biol. Bull. 81:297–98. 1941. Susceptibility of *Colchicum* and *Chlamydomonas* to colchicine. Bot. Gaz. 104:50–61. 1942. Alleviation of mitotic poisoning by glucose. Jour. Cell. and Comp. Physiol. 35:301–2. 1950.
25. CZEKALOWSKI, J., AND DOLBY, D. Effect of enzyme inhibitors on the genesis of phage. Nature. 163:719–20. 1949.
26. D'AMATO, F. The effect of M-inositol on c-mitosis and c-tumor reaction. Caryologia. Pisa. 1:358–61. 1949. The quantative study of mitotic poisons. by the *Allium cepa* test. Data and problems. Protoplasma. 39:423–33. 1950.
27. ————, AND AVANZI, M. Reazioni di natura auxinica ed effetti rizogeni in *Allium cepa* L. Studio cito-istologico sperimentale. Nuovo Giornale Botanico Italiana N. S. 55:161–213. 1948.
28. DANGEARD, P., *et al.* Étude relative à l'action de la colchicine sur la cyclose cytoplasmique. C. R. Soc. Biol. Paris. 141:1055–57. 1947.
29. DERMEN, H. Colchicine, polyploidy and technique. Bot. Rev. 6:599–635. 1940. A cytological analysis of polyploidy. Jour. Hered. 29:210–29. 1938.
30. DEYSSON, G. Action simultanée du phényluréthane et de la colchicine sur les méristèmes radiculaires d'*Allium cepa.* C. R. Acad. Sci. Paris. 220:367–69.

1945. Contribution à l'étude du "syndrome mitoclasique." Centre de Documentation Universitaire, Paris. 1948. Pharmacodynamic study of cellular division. Rev. Gen. Sci. 56:152–60. 1949.

31. DEYSSON, G., AND DEYSSON, M. Action of meso-inositol on growth and mitosis of higher plants. Bull. Soc. Chim. Biol. Paris. 32:268–75. 1950.

32. DOUTRE, L., AND BIRABEN, J. Pharmacodynamic activity of colchicine as a medicament for cancer. Action on cytoplasmic cyclosis. Jour. Méd. Bordeaux. 124:463–65. 1947.

33. DRAGOIU, I., AND CRISAN, C. The action of colchicine on the roots of the plants *(Allium cepa* and *Phaseolus vulgaris)* . Bull. Acad. Méd. Roumaine. 4:326–38. 1939.

34. DUHAMET, L. Recherches sur l'action de l'hetero-auxine et de la colchicine sur la croissance de racines isolées de *Lupinus albus.* Rev. Cytol. et Cytophysiol. Vég. 8:35–75. 1945.

35. EIGSTI, O. A cytological study of colchicine effects in the induction of polyploidy in plants. Proc. Nat. Acad. Sci. 24:56–63. 1938. The effects of colchicine upon the division of the generative cell in *Polygonatum, Tradescantia,* and *Lilium.* Amer. Jour. Bot. 27:512–24. 1940. Colchicine — a bacterial habitat. Amer. Jour. Bot. 33:4. 1946.

36. ———, AND DUSTIN, P., JR. Colchicine bibliography. Lloydia. 10:65–114. 1947. Colchicine bibliography III. Lloydia. 12:185–207. 1949.

37. EULER, H. Factors affecting the pyruvic acid content of rat blood. Arkiv. Kemi. Mineral. Geol. 20A. No. 12. 10 pp. 1945. Growth ergones and antiergones. Svensk. Kem. Tid. 58:180–87. 1946. Action of colchicine and X-rays. Influence of saponin and digitalis glycosides on seed germination and mitosis. Arkiv. Kemi. Mineral. Geol. 22A. No. 14. 10 pp. 1946.

38. ———, AND PERJE, M. Action of vitamins and antivitamins on seed germination and mitosis. Arkiv. Kemi. Mineral. Geol. 20A. No. 2. Chem. Abst. 41:2125. 1945.

39. ———, et al. Changes produced in yeast cells by Röntgen rays and chemical substances. Z. Physiol. Chem. 277:1–17, 18–25. 1942.

40. FRANZL, R., AND CHARGAFF, E. Bacterial enzyme preparations oxidizing inositol and their inhibition by colchicine. Nature. 168:955–57. 1951.

41. FREI, W., AND BRUNNER, T. Beziehungen zwischen Zellteilung und Oxydation bei Bakterien. Schädigung der aeroben und anaeroben Oxydation von Bakterien mit Cyankali, Pyridin, Colchicin und Ultraviolettstrahlen. Helv. Physiol. et Pharm. Acta. 5:252–64. 1947.

42. FUNKE, G. Growth of water plants in solutions of phytohormones and other substances. Proc. Ned. Akad. Wetensch. 45:937–43. 1942–43.

43. GAROFALO, F. L'azione della colchicina. Boll. Soc. Ital. Biol. Sper. 22:391–95. 1946.

44. GARRIGUES, R. Effects of colchicine and chloral on the roots of *Vicia faba.* Rev. Cytol. et Cytophysiol. Vég. 4:261–301. 1940. Colchicine. La Nature. 1945:244–46. 1945.

45. GAVAUDAN, P. Pharmacodynamie de l'inhibition de la caryocinèse. Librairie le François, Paris. 1947.

46. ———, AND BREBION, G. The scale of functional inhibitions in the plant cell. Mem. Services Chim. État. Paris. 35:No. 3, 73–78. 1950.

47. ———, AND GAVAUDAN, N. Mécanisme d'action de la colchicine sur la caryocinèse des végétaux. C. R. Soc. Biol. Paris. 128:714–16. 1938.

48. ———, et al. La toxicologie générale et la notion d'activité thermodynamique. Mem. Services Chim. État. Paris. 31:384–423. 1944. L'importance de la notion d'activité thermodynamique en toxicologie et en pharmacodynamie. Rec. Trav. Scient. Station d'Ess. Bouchet. 1:5–22. 1945.

49. ———, AND KOBOZIEFF, N. Action of colchicine on caryokinesis and cytodieresis in *Chlamydomonalineae.* C. R. Soc. Biol. Paris. 127:790–93. 1938.

50. GEISSLER, G. Knob formation on onion roots by mitosis poisons, growth and germination inhibitors. Naturwiss. 37:563–64. 1950.

51. GOHAR, M., AND MAKKAWI, M. Antibacterial action of colchicine and colchiceine. Jour. Pharm. and Pharmacol. 3:415–19. 1951.

52. GORDON, W., AND McKECHNIE, J. Autopolyploidia induced in *Penicillium notatum* by colchicine. Anales Farm. Bioquim. Buenos Aires. 17:12–17. 1946.
53. GORTER, C. De invloed van colchicine op den groei van den celwand van wortelharen. Proc. Kon. Nederl. Akad. Wetensch. 48:3–12. 1945.
54. GRACE, N. Note on sulfanilamide and other chemicals that act as plant growth-promoting substances. Can. Jour. Res. 16:C, No. 3, 143–44. 1938.
55. GREMLING, G. Troubles morphologiques determinés par la colchicine chez les végétaux. Assoc. France Avanc. Sci. 63rd Session, Liege. Pp. 595–607. 1939.
56. GUINOCHET, M. Modifications of the physiochemical reactions of the plant cell, caused by mitosis-inhibiting substances. C. R. Acad. Sci. Paris. 210:579–80. 1940.
57. HALBERSTAEDTER, L., AND BACK, A. Influence of colchicine alone and combined with X-rays on *Paramecium*. Nature. 152:275–76. 1943.
58. HAUSMANN, W., AND KOLMER, W. Über die Einwirkung kolloidaler Gifte auf Paramacien. Biochem. Z. 3:503–7. 1907.
59. HAVAS, L. Is colchicine a "phytohormone"? Growth. 2:257–60. 1938. Parthenocarpy and accompanying hormonal syndromes induced by unrelated chemicals. Nature. 157:629–30. 1946. Problems of growth studied with the aid of colchicine in plants and animals. Ann. Soc. Roy. Sci. Med. et Nat. Bruxelles. 1:57; cf. Chem. Abst. 42:4676c. 1948. Hormone-mimetic and growth effects of colchicine in plants. Exp. Cell Res. Suppl. 1:597–601. 1949. Effect of bee-venom on colchicine-induced tumors. Nature. 166:567–68. 1950.
60. ————, AND BALDENSPERGER, A. The oncological aspect of the "immunity" of *Colchicum* to colchicine. Science. 114:208–10. 1951.
61. ————, AND FELFOLDY, L. Fasciations and kindred teratisms induced in plants by polyploidogenic agents. Magyar. Biol. Kut. Muukai. 17:131–40. 1947.
62. HAWKES, J. Some effects of the drug colchicine on cell division. Jour. Genet. 44:11–22. 1942.
63. HEISE, F., AND STEENKEN, W. Growth and virulence of tubercle bacilli. A study of the effect of vitamin B_1, and B_6, riboflavine, colchicine, phthiocol and thyloquinone. Amer. Rev. Tuberc. 44:635–36. 1941.
64. HEITZ, E. Moosmutationen 1. Spontane und durch Colchicin ausgelöste polyploide Mutanten dei *Aulacomnium androgynum* (L.). Arch. Julius Klaus-Stift. 20:119–25. 1945. Über "Colchicinmuster." 8. Jahresber., Schweiz. Ges. Vererbungsforsch. Arch. Julius Klaus-Stift. (¾), pp. 497–99. 1948.
65. JAKOB, H. Influence de la colchicine sur le développement de certaines algues d'eau douce. C. R. Acad. Sci. Paris. 230:1203–5. 1950. Action de la colchicine sur certaines algues d'eau douce et comparison avec l'action des substances metaboliques en provenance d'algues. Rev. Gen. Bot. 58:348–52. 1951.
66. JENNISON, N. The inactivity of colchicine for bacteria. Jour. Bact. 39:20–21. 1940.
67. KALLIO, P. The significance of nuclear quantity in the genus *Micrasterias*. Ann. Bot. Soc. Zool. Bot. Fenn. 24:1–122. 1951.
68. KARTASHOVA, N. Treatment of vegetable cells with colchicine. C. R. Dokl. Acad. Sci. URSS 46:372–74. 1945.
69. KAYSER, F., AND BESSON, S. Action of sulfonamides and colchicine on development of pea roots. C. R. Soc. Biol. Paris. 142:84–86. 1948.
70. KING, C. Changes of carbohydrates of germinating wheat seeds in manganese sulfate, indoleacetic acid and colchicine media. Bot. Bull. Acad. Sinica. 1:9–24. 1947.
71. KING, R., AND BEAMS, H. Comparison of the effects of colchicine on division in protozoa and other cells. Jour. Cell. and Comp. Physiol. 15:252–54. 1940.
72. KOSTOFF, D. Induction of polyploidy by pulp and disintegrating tissues from *Colchicum* sp. Nature. 143:287–88. 1939.
73. KRYTHE, J. The effect of colchicine on the anthers of *Carthamus tinctorius* L. Proc. Acad. Sci. Amsterdam. 45:283–87. 1942.
74. LANG, K., *et al.* Über die Hemmung von Desoxyribonucleotide spaltenden Fermenten durch Colchicin. Experientia. 5:449. 1949.

75. LAUR, C. Experimental study of the action of colchicine on certain phases of cellular development. Ann. Anat. Path. 15:792–99. 1938.

76. LEE, T., AND HWANG, T. Growth stimulation by manganese sulfate, indole-3-acetic acid and colchicine in pollen tube growth. Acta Brev. Sinensia. 8:21–22. 1944.

77. LEFEVRE, J. Actions similaires sur les mitoses végétales de l'anéthol et des substances du groupe de la colchicine. C. R. Soc. Biol. Paris. 133:616–18. 1940.

78. LETTRÉ, H.Über Mitosegifte. Ergebn. Physiol. 46:379–452. 1950. Über Synergisten von Mitosegiften V. Mitt. Versuche zur Aufhebung der synergisti schen Wirkung durch Phosphagen. Naturwiss. 38:13. 1951.

79. LEVAN, A. The effect of colchicine on meiosis in *Allium*. Hereditas. 25:9–26. 1939. The effect of acenaphthene and colchicine on mitosis of *Allium* and *Colchicum*. Hereditas. 26:262–76. 1940. The macroscopic colchicine effect — a hormonic action? Hereditas. 28:244–45. 1942.

80. ————, AND LEVRING, T. Some experiments on c-mitotic reactions with *Chlorophyceae* and *Phaeophyceae*. Hereditas. 28:400–108. 1942.

81. ————, AND LOTFY, T. Naphthalene acetic acid in the *Allium test*. Hereditas. 35:337–74. 1949.

82. ————, AND ÖSTERGREN, G. The mechanism of c-mitotic action. Observations on the naphthalene series. Hereditas. 29:381–443. 1943.

83. ————, AND SANDWALL, C. Quantitative investigations on the reaction of yeast to certain biologically active substances. Hereditas. 29:164–78. 1943.

84. ————, AND STEINEGGAR, E. The resistance of *Colchicum* and *Bulbocodium* to the c-mitotic action of colchicine. Hereditas. 33:552–66. 1947.

85. LEVINE, M. The effect of colchicine and acenaphthene in combination with X-rays on plant tissue. Torrey Bot. Club Bull. 72:563–74. 1945. Action of colchicine on cell division in human cancer, animal and plant tissues. Ann. N. Y. Acad. Sci. 51:1365–1406. 1951.

86. ————, AND LEIN, J. The effect of various growth substances on the number and the length of roots of *Allium cepa*. Amer. Jour. Bot. 28:163–68. 1941.

87. LOO, T., AND TANG, Y. Growth stimulation by manganese sulphate, indole-3-acetic acid, and colchicine in the seed germination and early growth of several cultivated plants. Amer. Jour. Bot. 32:106–14. 1945.

88. MAIROLD, F. Studien an colchicinierten Pflanzen. Protoplasma. 37:445–521. 1943.

89. ————, AND WEBER, F. *Matricaria chamomilla* durch Colchizinierung ohne Strahlenblüten. Phyton. 2:271–75. 1950.

90. MANGENOT, G. Substances mitoclasiques et cellules végétales. État actuel de la question d'après les travaux publiés jusqu'à la fin de 1940. Rev. Cytol. et Cytophysiol. Vég. 5:169–264. 1941. Action de la colchicine sur les racines d'*Allium cepa*. Hermann and Cie., Paris. 120 pp. 1942.

91. MARTIN, G. Action de la colchicine sur les tissus de topinambour cultivé *in vitro*. Rev. Cytol. et Cytophysiol. Vég. 8:1–34. 1945.

92. MARTINEZ, L. Influence of various alkaloids on the growth of wheat seedlings. Farmacognosia. Madrid. 9:231–46. 1949.

93. MASCRE, M., AND DEYSSON, G. Les poisons mitotiques. Biol. Méd. 40:1–54. 1951.

94. MEHRA, P. Colchicine effect on the mitotic division of the body nucleus in the pollen grains of some *Ephedra* Spp. Proc. Nat. Inst. Sci. India. 12:333–40. 1946. Colchicine effect and the production of abnormal spermatozoids in the prothalli of *Dryopteris subpubescens* (Bl.) C. Chr. and *Goniopteris prolifera* Roxb. Ann. Bot. 16:49–56. 1952.

95. MOL, W. DE. Colchicine treatment of *Scilla* to produce polyploids. Papers Mich. Acad. Sci. 35:3–7. 1951.

96. MUNTZING, A., AND RUNQUIST, E. Note on some colchicine-induced polyploids. Hereditas. 25:491–95. 1939.

97. NAUNDORF, G., AND HAASE, E. The auxin metabolism of polyploid plants after colchicine treatment. Naturwiss. 31:570. 1943.

98. Nebel, B. Cytological observations on colchicine. Collecting Net. 12:130–31. 1937.
99. ————, and Ruttle, M. Action of colchicine on mitosis. Genetics. 23:161–62. 1938.
100. Newcomer, E. Colchicine as a growth stimulator. Science. 101:677–78. 1945.
101. Nickell, L. Effect of certain plant hormones and colchicine on the growth and respiration of virus tumor tissue in *Rumex acetosa*. Amer. Jour. Bot. 37:829–35. 1950.
102. Nihous, M. Toxic effect of aqueous solutions of colchicine on the germination of *Pisum sativum*. C. R. Soc. Biol. Paris. 138:128. 1944.
103. Northen, H. Alterations in the structural viscosity of protoplasm by colchicine and their relationship to c-mitosis and c-tumor formation. Amer. Jour. Bot. 37:705–11. 1950.
104. Obaton, F. Influence de la colchicine sur le développement de *Photobacterium phosphoreum*. C. R. Acad. Sci. Paris. 208:1536–38. 1939.
105. Ollivier, H. Étude cyto-toxicologique de l'influence de divers agents physiques et chimiques sur les plantules de blé. Rev. Can. Biol. 7:35–159. 1948.
106. O'Mara, J. Observations on the immediate effects of colchicine. Jour. Hered. 30:35–37. 1939.
107. Orsini, M., and Pansky, B. The natural resistance of the golden hamster to colchicine. Science. 115:88–89. 1952.
108. Östergren, G. Narcotized mitosis and the precipitation hypothesis of narcosis. Mécanisme de la Narcose. Colloques Internat. Centre Nat. Recherche Scient. 26:77–87.
109. Parr, L. A new "mutation" in the coliform group of bacteria. Jour. Hered. 29:381–84. 1938.
110. Patton, R., and Nebel, B. Preliminary observations on physiological and cytological effects of certain hydrocarbons on plant tissues. Amer. Jour. Bot. 27:609–13. 1940.
111. Piettre, L. Action of colchicine on plants. C. R. Soc. Biol. Paris. 131:1095–97. 1939.
112. Postma, W. Opermerkingen over de cytologie van normale en van met colchicine behandelde *Cannabis*-planten. Erfelijkheid in Praktijk. 4:171–73. 1939.
113. Pottz, G. Effects of colchicine on bacteria. Proc. Okla. Acad. Sci. 22:139–41. 1941.
114. Praaken, R., and Levan, A. Notes on the colchicine meiosis of *Allium cernuum*. Hereditas. 32:123–26.
115. Reese, G. Beiträge zur Wirkung des Colchicins bei der Samenbehandlung. Planta. 38:324–76. 1950.
116. Richards, O. Colchicine stimulation of yeast growth fails to reveal mitosis. Jour. Bact. 36:187–95. 1938.
117. Rosendahl, G. Versuche zur Erzeugung von Polyploidie bei Farnen durch Colchicin-behundlung sowie Beobachtungen an polyploiden Farnprothallien. Planta. 31:597–637. 1941.
118. Ruhe, D., et al. Studies in human malaria. XIV. The ineffectiveness of colchicine, S. N. 12,080, S. N. 7266 and S. N. 8557 as curative agents against St. Elisabeth strain vivax malaria. Amer. Jour. Hyg. 49:361. 1949.
119. Sansome, E., and Bannon, L. Colchicine ineffective in inducing polyploidy in *Fenicillium notatum*. Lancet. 251:828–29. 1946.
120. Santavy, F. Polarografie a spektrografie kolchicinu a jeho derivatu. Publ. Fac. Med. Brno. 19:149–72. 1945.
121. Sass, J., and Green, J. Cytohistology of the reaction of maize seedlings to colchicine. Bot. Gaz. 106:483–88. 1945.
122. Sato, D. The effect of colchicine on meiosis in *Aloinae*. Bot. Mag. Tokyo. 53:200–7. 1939.
123. Schuldt, E., and Gottlieb, D. Colchicine as a mutagenic agent for *Streptomyces griseus*. Ill. Acad. Sci. Trans. 43:51–52. 1950.
124. Shimamura, T. Effect of acenaphthene and colchicine on the pollen mother cells of *Fritillaria* wild var. *Thunbergie* Baker. Jap. Jour. Genet. 15:179–80. 1939. Studies on the effect of centrifugal force upon nuclear division. Cytologia. 10:186–216. 1940.

125. Siebenthal, R. A leucophytic clone of *Hormidium* derived from a culture treated with colchicine. C. R. Soc. Phys. et Hist. Nat. Genève. 58:187–92. 1941.

126. Sinoto, Y., and Yuasa, A. Karyological studies in *Saccharomyces cerevisiae*. Cytologia. 11:464–72. 1941.

127. Smith, P. Studies of the influence of colchicine and 3-indole acetic acid upon some enzymatic reactions. Proc. Okla. Acad. Sci. 21:105–8. 1941. Studies of the growth of pollen with respect to temperature, auxins, colchicine and vitamin B_1. Amer. Jour. Bot. 29:56–66. 1942.

128. Soyano, Y. The hypertrophy in roots induced by several chemicals. Bot. Mag. Tokyo. 34:185–95. 1940.

129. Sparrow, A. Colchicine-induced univalents in diploid *Antirrhinum majus* L. Science. 96:363–64. 1942.

130. Sreenivasan, A., and Wandrekar, S. Biosynthesis of vitamin C during germination. I. Effect of various environmental and cultural factors. Proc. Indian Acad. Sci. 32B:143–63. 1950.

131. Stalfelt, M. Effect of heteroauxin and colchicine on protoplasmic viscosity. Proc. 6th Internat. Congress Exp. Cytology (1947). Exp. Cell Res. Suppl. 1:63–78. 1949.

132. Steinberg, R., and Thom, C. Mutations and reversions in reproductivity of *Aspergilli* and nitrite, colchicine and d-lysine. Proc. Nat. Acad. Sci. 26 (6): 363–66. 1940.

133. Steineggar, E., and Levan, A. The cytological effect of chloroform and colchicine on *Allium*. Hereditas. 33:515–25. 1947. The c-mitotic qualities of colchicine, trimethyl colchicine acid and two phenanthrene derivatives. Hereditas. 34:193–203. 1948.

134. Sterzl, J. Morphological variability of the nuclear substance and genetic changes induced by colchicine in *"Escherichia coli."* Nature. 163:28. 1949.

135. Straub, J. Quantitative und qualitative Verschiedenheiten innerhalb von polyploiden Pflanzenreihen. Biol. Zentralbl. 60:659–69. 1940.

136. Sturtevant, F., et al. Effect of colchicine on regeneration in *Pelmatohydra oligactis*. Science. 114:241–42. 1951.

137. Suita, N. 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. Jap. Jour. Genet. 15:91–95. 1939.

138. Takenaka, Y. Notes on cytological observations in *Colchicum*, with reference to autotoxicosis and sterility. Cytologia. 16:95–99. 1950.

139. Tonzig, S., and Ott-Candela, A. L'azione della colchicina sullo sviluppo degli apparati stomatici. Nuovo Gior. Bot. Ital. 53:535–47. 1946.

140. Tschermak, E. Durch Colchicinbehandlung ausgelöste Polyploidie bei der Grünalge *Oedogonium*. Naturwiss. 30:638–84. 1942.

141. Ubatuba, F. Inhibition of growth of oat rootlets. Rev. Brasil Biol. 5:263–74. 1945.

142. Umrath, K., and Weber, F. Elektrische Potentiale an durch Colchicin oder Heteroauxin hervorgerufenen Keulenwurzeln. Protoplasma. 37:522–26. 1943.

143. Vaarama, A. Permanent effect of colchicine on *Ribes nigrum*. Hereditas. Suppl. Abst. 680–81. 1949.

144. Vandendries, R., and Gavaudan, P. Action de la colchicine sur quelques organismes inférieurs. C. R. Acad. Sci. Paris. 208:1675–77. 1939.

145. Vanderwalle, R. Observation sur l'action de la colchicine et autres substances mitoinhibitrices sur quelques champignons phytopathogènes. Bull. Soc. Roy. Bot. Belg. 72:63–67. 1939.

146. Vietez, E. Palynological observations on some Spanish honeys. Torrey Bot. Club Bull. 77:495–502. 1950.

147. Wada, B. Lebendbeobachtungen über die Einwirkung des Colchicins auf die Mitose, insbesondere über die Frage der Spindelfigur. Cytologia. 11:93–116. 1940.

148. Walker, R. The effect of colchicine on microspore mother cells and microspores of *Tradescantia paludosa*. Amer. Jour. Bot. 25:280–85. 1938. The

effect of colchicine on somatic cells of *Tradescantia paludosa.* Jour. Arnold Arb. 19:158–62. 1938. The effect of colchicine on the developing embryo sac of *Tradescantia paludosa.* Jour. Arnold Arb. 19:442–45. 1938.

149. WALKER, A., AND YOUMANS, G. Growth of bacteria in media containing colchicine. Proc. Soc. Exp. Biol. and Med. 44:271–73. 1940.

150. WANG, F. Effects of auxin, colchicine and certain amino acids on the germination of *Lotus corniculatus* pollen. Biochem. Bull. China. 38:1–3. 1944.

151. WEBER, F. Spaltöffnungsapparat-anomalien colchicinierter *Tradescantia*-blatter. Protoplasma. 37:556–65. 1943.

152. WEICHSEL, G. Polyploidie, veranlasst durch chemische Mittel, insbesondere Colchicinwirkung bei Lebuminosen. Zuchter. 12:25–32. 1940.

153. WEISSENBOCK, K. Studien an colchizinierten Pflanzen. I. Anatomische Untersuchungen. Phyton. 1:282–300. 1949.

154. WERNER, G. Untersuchungen über die Möglichkeit der Erzeugung polyploider Kulturpflanzen durch Colchicinbehandlung. Zuchter. 11:51–71. 1940.

155. WEYLAND, H. The action of chemicals on plants and its significance in medicine. Z. Krebsforsch. 56:148–64. 1948.

156. WITKUS, E., AND BERGER, C. Induced vascular differentiation. Torrey Bot. Club Bull. 77:301–5. 1950.

157. WOLCOTT, G. The effect of colchicine on a hepatic. Jour. Hered. 32:67–70. 1941.

158. YAMAHA, G., AND UEDA, R. Über die Wirkung des Kolchizins auf *Spirogyra.* Bot. and Zool. Syokubuta Oyobi Dobuta. 8:1709–14. 1940.

159. ZAMBRUNO, D. Azione della colchicina, della narcotina, e dell'androstendione sulla moltiplicazione delle *Staphylococcus aureus.* Giorn. Batt. Immul. 34:55–57. 1946.