

Techniques of Colchicine Treatment

A. In Animals

16A.1: Solutions

It has been explained in Chapter 5 that the substance which has been repeatedly called *colchicine* in this book may have differed from author to author. One reason for this discrepancy is the factor of crystallization. Whereas pure, amorphous colchicine is very soluble in water, crystallization from aqueous or chloroformic solutions yields complex crystals which are less soluble and may have other biological properties.⁵⁵ Colchicine may crystallize with $\frac{1}{2}$ molecule of water, with $\frac{1}{2}$ molecule or 1 molecule of chloroform. This last form of crystalline colchicine is only soluble in water in the proportion of 4 per cent.⁵⁵ It has often been used in experimental research. In botanical work, results may be modified by the presence of chloroform, which is itself a mitotic poison.⁵⁵ In experiments on animals, where the amounts of colchicine used are far smaller and the solutions much more dilute, the presence of chloroform does not appear to have any importance. But, for any quantitative estimation of the activity of the drug, it must not be forgotten that crystalline colchicine with 1 molecule of chloroform contains 25 per cent by weight of the solvent.⁵⁵ On the other hand, chemical work has demonstrated that the plant *Colchicum* contains many alkaloids closely related to colchicine, but with different pharmacological properties.^{51, 52} One of these, desmethylcolchicine, is found in the colchicine preparations of the U.S. Pharmacopeia.³⁶ In the most recent work on colchicine, care has been taken to purify the alkaloid before testing it.^{26, 9} This applies only to a very small number of the papers, and some results may differ because the injected drug differed in its mode of preparation from the plant.¹ While the above-mentioned differences are only

of importance for quantitative work, the changes that colchicine may undergo in solution are far more important, especially for work with warm-blooded animals or tissue cultures. Colchicine solutions should always be freshly prepared, or kept protected from the action of oxygen and light. For work on plants, where rather concentrated solutions are used and where no problems of general toxicity arise, this is not so important. In animal work, and especially for all work on birds or mammals, it is most important to use freshly prepared solutions.⁴³ Standing in the presence of air, colchicine appears to undergo a slow oxidation about which little is known (cf. Chapter 7). This decreases the spindle-inhibiting action, but may not affect similarly the general toxicity, which is increased in cold-blooded animals such as frogs.³⁷ These remarks apply to solutions, whether in water or fatty solvents. The latter have been mainly used for local applications in cancer chemotherapeutic tests.^{10, 8}

The important point is that each paper should mention clearly the origin of the colchicine, whether crystalline or not, whether purified and how, the method of preparing the solutions before the experiments, and the temperature at which these are conducted. It is only in this way that a valid comparison of results is possible.

16A.2: Temperature

In Chapter 7, several instances have been given of the effect of temperature on the action of colchicine. This has long been known, but has often been overlooked.²⁹ Most workers mention that the alkaloid does not influence cell division in unicellular organisms (cf. Chapter 4). However, while *Paramecium* is unaffected by colchicine solutions at a one per cent concentration at 15°C., the same solutions kill the paramecia in less than 4 hours at 33°C. Exposure to this temperature is in itself not harmful to the organisms.³⁵

These temperature effects are not yet understood properly. They explain the considerable differences between colchicine pharmacology in cold-blooded animals and in birds and mammals (cf. Chapter 7). For instance, colchicine-arrested metaphases remain intact for hours and even days (Fig. 2.2) in amphibia; in mammals, on the contrary, the nucleus of a cell arrested at metaphase by a spindle poison undergoes rapid destruction. In all *in vitro* work, the temperature should be constant and checked carefully.

16A.3: The Study of Mitosis

Colchicine may be utilized for many different purposes when analyzing mitotic growth, and techniques may considerably differ. For instance, in studies on the morphology of chromosomes or pseudo-spindle in arrested metaphases, quantitative data, except those about

effective colchicine concentration, may not be of paramount importance. The same may apply to some work where colchicine is mainly a tool for increasing the "visibility" of cellular division. When the topography of mitotic growth is the main purpose, several instances of which have been given in Chapter 9, precise data about the mitotic rate may not be important. On the contrary, when using colchicine to assess the importance of cellular proliferation, either in complex tissues or in tissue cultures, it is indispensable to understand the complex action on the mitotic count. This point will be considered further.

Special techniques for the production by colchicine of abnormal growth in embryos have been mentioned in Chapter 8. The experimental creation of polyploid animals has been one aim of colchicine research. The methods used and the results obtained merit some discussion, which will be found in the last paragraph of this chapter.

16A.3-1: In vivo studies. Many methods have been utilized in the study of c-mitosis in animal cells; they are all variants of two: viz., placing cells in contact with colchicine solutions, or injecting these by various routes into the cell or into the animal.

The intracellular injection is of great interest, for it was possible to demonstrate by this procedure that some cells were resistant to colchicine since the alkaloid did not penetrate into the cytoplasm. Such experiments have been performed only on one unicellular, *Amoeba sphaeronucleus*. Mitotic division of this species is not affected when it is grown in colchicine solutions. Very minute quantities of a one per cent solution of the alkaloid were introduced in the cytoplasm with a micropipette. Typical mitotic arrest, together with formation of polyploid nuclei, resulted when the timing of the injection was properly related to the mitotic cycle.²²

Many cold-blooded animals, invertebrates, fish, amphibians, have been studied after immersion in colchicine solutions. One important pathway of absorption is through the branchiae. In such experiments, care should be taken to avoid sunlight and to replace the colchicine solution which may lose its activity through chemical changes.

Injection is often the easiest way to administer colchicine to pluricellular animals. In the study of hematopoiesis in the chick, colchicine was simply injected into the egg yolk through the shell.³ In adult animals, subcutaneous or intraperitoneal injections are the most frequently used. One most important point, if a quantitative study of the number of mitoses is needed, is to inject all animals at the same hour of the day, so as not to be disturbed by the diurnal variations of mitotic rate.¹³ This is also influenced by feeding the animals, more precisely by the blood glucose level, and experimental animals should be kept under standard and specified dietetic conditions.¹⁴

In mammals, and especially the small rodents, which have been widely used for colchicine work, some tissues are most favorable for the study of mitosis and the influence of colchicine and similar poisons. The skin lends itself to repeated biopsies, for instance the ear of the mouse, from which small fragments may be punched out at hourly intervals.^{13, 14} However, the mitotic activity of the skin is low, and counting is long and tedious, even after colchicine. The number of mitoses is increased little by mitotic arrest, probably because under normal conditions they are of long duration, up to three hours. The influence of the sexual cycle is considerable (Chapter 9, Fig. 9.6) and must not be overlooked.¹³ The cornea may be studied by staining whole mounts and counting the number of mitoses per thousand cells; this method has only been utilized in mammals by one group of workers,¹⁸ though it appears to offer many advantages over the skin. Bone marrow and intestinal crypts are zones of maximal mitotic growth in mammals. They both provide excellent material for studying the action of colchicine. In bone marrow, comparative studies may be made between the white-cell- and the red-cell-forming tissues. In the intestine, quantitative estimation of mitotic growth is possible,⁴¹ though the counting of mitoses may be difficult because of their rapid destruction of pycnosis. The intestinal mitoses have been one of the best tools for the study of mitotic poisons at Brussels. Contrary to the mitoses of lymphoid tissue, which are strongly affected by hormonal influences such as those of the "alarm-reaction" or pituitary-adrenal stimulation,⁴¹ the intestine provides a tissue with uniform growth,⁵⁷ not affected by the adrenal cortical hormones.²⁸ Intestinal fragments should always be taken from the same location, for the mitotic activity is greater in the duodenum, and decreases gradually towards the large intestine, where few mitoses are seen. The gastric mucosa of the mouse has also been proposed;^{9, 56} it offers an interesting comparison between squamous-celled and glandular epithelium in a single organ. The regenerating liver is a favorable material in rats, and quantitative estimations of mitotic growth are possible.¹¹ However, it has been shown that the repartition of mitoses was not uniform throughout the remaining liver.⁴⁵

Local applications of colchicine have been most useful in the study of c-mitosis and regeneration in amphibians.¹⁷ The study of recovery after a prolonged colchicine impregnation (five days) has been discussed in Chapter 2 (cf. Fig. 2.7).⁴⁹ The inhibition of regeneration of the tail of *Xenopus* larvae has been illustrated in Chapter 9; the technique involved a local application of an aqueous solution of colchicine to the amputated tail.⁴⁴ Local application has also been found useful in studies on the mitotic activity of genital tissues in rodents⁵⁸ and of the human vagina before removal of a fragment by

biopsy;^{54, 50} this is one of the methods for treating human tumors with the alkaloid, prepared in a vaseline-lanoline paste (Chapter 10).^{10, 8} Local applications of colchicine-impregnated agar cut into small fragments have also proved useful in studying the origin of colchicine malformations in eggs;³⁰ this technique does not seem to have received the attention it deserves.

Another method by which colchicine is brought into direct contact with the cells is the use of the so-called "ascites-tumors" in mice. These are neoplasms freely growing in fluid gathered in the abdominal cavity. Colchicine is injected intraperitoneally, and repeated observations of the cells are possible by removing a small amount of the ascites fluid.⁴²

16A.3-2: *In vitro techniques.* For many studies, it is preferable to keep precise amounts of colchicine in contact with the cells which are studied. This enables the results not to be disturbed by general toxicity reactions and other pharmacological side-effects of colchicine (Chapter 7). More concentrated solutions may be tested, which, injected to whole animals, would have brought death through nervous and respiratory paralysis. These techniques apply especially to warm-blooded animals.

In invertebrates, however, some remarkable results, discussed in Chapters 2 and 3, have been obtained by the study at 38°C. of the isolated nervous system of the grasshopper, *Chortophaga viridifasciata* De Geer. Embryos, at an age equivalent to 14 days' development at 26°C., are removed from the egg in artificial culture medium. The maxillary and thoracic appendages, the head, and the posterior half of the abdomen are discarded, and the embryo is mounted with the ventral nervous system close to a cover slip, which is sealed. These hanging-drop preparations may be observed for several hours under oil-immersion objectives^{19, 31} (cf. Chapter 3, and Fig. 3.1). This has proved to be one of the most interesting techniques for the study of the spindle destruction by colchicine and of the mitotic cycle.¹⁹ Isolated eggs of invertebrates, for instance *Arbacia*,⁴ should also be mentioned here, although the techniques do not differ from those used in experimental embryology (cf. Fig. 3.3 and Chapter 8).

In mammals, two tissues have provided excellent material for the study of mitosis *in vitro*. Fragments of the ear of mice may be incubated in Warburg flasks, and the action of various chemicals on mitotic growth studied on the epithelium, the mitoses of which persist for several hours, provided that glucose is added to the medium.¹⁵ Bone marrow is readily available in many mammals, including man, and its mitoses may most simply be observed in cover-slip preparations at 37°C. Glucose does not appear to be as necessary as for epidermal cells.² This technique has provided most useful data on

the physiology of cellular division in bone marrow and on the actions of various substances on rate of cell multiplication (Chapter 9). The cells, which are suspended in homologous serum, are able to divide regularly for more than 24 hours after explantation.²

A method for *in vitro* cultivation of immature rat ovaries has been described⁷ and should be of great interest for endocrinological research.

Colchicine has been used with the main techniques of tissue culture, especially with hanging-drop preparations, which enable a continuous observation of growth.¹² Some estimation of the quantitative amount of newly formed cells may be made by planimetric measurement of the whole culture, but the influence of cell migration must not be neglected.¹² Tissue cultures are especially favorable for cinematographic methods.¹² A very thorough study of the action of colchicine on the rate of mitotic growth and on the repartition of the various types of abnormal or arrested mitoses has been made possible by this technique^{12, 42} (Chapter 9, Fig. 9.1). Tissue cultures are also most useful for comparing normal and neoplastic cells,²¹ for the study of synergists or antagonists of colchicine, and for testing other mitotic poisons⁴² (cf. Chapter 17). It should, however, be mentioned that cultures of chick fibroblasts will not always behave like fibroblasts from mammals.⁴⁸ For the study of colchicine derivatives or other spindle poisons, cultures of various types of cells from different animals should be compared.

16A.3-3: Mitotic counts. When colchicine is used as a tool for studying growth (Chapters 9 and 10), when the problem of mitotic stimulation by colchicine is considered (Chapter 9), or when substances acting synergically or as antagonists to the alkaloid are studied (Chapter 17), a precise estimation of the number of mitoses in controls and at various intervals after mitotic arrest is indispensable. Some of the methods outlined in the preceding subsection provide excellent material for counting cell divisions, but even with tissue cultures, the problem may be complicated because only the periphery of the explanted fragment grows rapidly. Precise counts of the total number of cells in mitosis are possible both with the ear-clip technique^{13, 14} and the methods of bone-marrow explantation.² In more complex tissues a reliable standard may be difficult to find. For instance, many authors define the "mitotic index" as the number of mitoses found in a given area, i.e., so many microscopic fields, of tissue. This is a good method when dealing with uniform and fairly simple tissues, for example, the regenerating liver,¹¹ but not when complex tissues are considered. In the small intestine of mammals, for instance, it is preferable to count the number of mitoses per

hundred glandular crypts. This method has been widely used by the junior author in studies of mitotic poisoning.²⁴

Many data obscuring the problem of possible mitotic stimulation by colchicine result from the difficulty of comparing tissues before and after the action of the alkaloid. To cite one instance, the great increase in mitotic activity in the crop-sac of pigeons injected with prolactine and colchicine has been mentioned (Chapter 9). Is it possible to compare quantitatively the mitotic counts in this tissue? From the figures which have been published one may conclude that it is not, for after prolactine and colchicine, there is not the same number of cells in a given area of tissue as in the same area of normal epithelium or of prolactine-thickened crop-sac.⁴⁰ A quantitative result could only be correct if it were possible to count a very large number of cells, and not only the mitoses in a given area. Such counts are not often reported in this type of work (Chapter 9). Another error is that of injecting a hormone at a too short interval before colchicine. Theoretically, the mitotic index should remain constant; that is to say, the numbers of cells entering prophase should not vary during the period of action of colchicine. It has been pointed out that this is not often so with hormone-stimulated growth.^{16, 23} Considerable errors may result from hasty interpretations of the significance of mitotic increases.

Any quantitative work supposes also that the exact number of cells arrested at metaphase by colchicine is known. In warm-blooded animals, and apparently also in amphibia,⁴⁴ this is never so, even with large doses. Increasing the dosage of alkaloid is never a good solution either, for it increases secondary, nonspecific toxic reactions and the percentage of destroyed arrested mitoses, and may also depress the number of prophases. It is often very difficult, especially in mammals, to know exactly how many metaphases with clumped chromosomes undergo degeneration, for this is rapid, and the nucleus breaks down to many small fragments. The data about the duration of c-mitosis in animals are scarce and widely divergent, as pointed out in Chapter 2.⁵³ It is also necessary, when planning an experiment with colchicine acting as a tool, to know how long after an injection of the alkaloid the animal should be killed. Many factors complicate this estimation: There may be a period of latency like that observed in tissue cultures (Fig. 9.1);¹² some anaphases may persist even with large doses. Recovery starts after an interval which is not always known. In some tissues this may be rather short, and in the study of epidermal mitosis it is recommended to kill the animals six hours after colchicine. This duration appears favorable for many experiments on mammals, but it is obviously too short in cold-blooded

animals. Here again, temperature may play a great part, but no quantitative work relating temperature to the duration of action of colchicine exists. In tissue cultures, colchicine may be left to act much longer, and 24 hours is often mentioned in work with bone marrow.²

This brings in another problem which we have not yet dealt with: the duration of interphase. It is evident that, if colchicine were acting longer than a normal interphase, no more new prophase would be available and the mitotic index would cease to rise. While most data on grasshoppers,¹⁹ tissue cultures,¹² and complex tissues indicate that interphase is far longer than mitosis, precise information is often lacking. It has been suggested that colchicine itself may provide a means for measuring the duration of interphase.³⁹ If new prophase were indefinitely provided by the tissues, i.e., if interphase duration did not interfere with mitotic counts, the number of arrested mitoses would increase until all the cells would be in a condition of c-mitosis. This is never observed, and even in the fastest growing tissues never many more than 50 per cent of the cells show c-mitoses. This is because after a certain time no more interphasic cells are ready for prophase. On the curve of the numbers of mitoses in function of time, the time which elapses between the beginning of mitotic arrest and the leveling of the number of mitoses is related to the duration of interphase. Theoretically, under ideal conditions, it is equal to interphase.³⁹ This is of interest for workers handling colchicine and certainly deserves further study. In the preceding chapters, enough has been said about the complexities of c-mitosis to prevent conclusions to be drawn hastily. One fact remains true: In colchicine experiments, the duration of the action of the alkaloid should be much shorter than the interphasic duration of the cells which are studied.

Considering the great variations in mitotic duration which are mentioned in the literature (from about 30 minutes to three hours in the mouse), our ignorance about the duration of interphase, the difficulties of accurately counting mitoses, and the complexities of colchicine's pharmacology, it is evident that quantitative conclusions are only possible in a few instances. The advantages of tissue cultures are obvious.

16A.4: Polyploidy

Polyloid animals have been produced experimentally,^{25, 27, 6} but colchicine has not yet proved very effective in doubling the chromosome number. This is probably only a question of technique, though cellular destruction, nondivision of the centromeres, and restitution during early development (Chapter 8) may be factors which prevent

colchicine from acting on animal cells as in plants. Under the heading of polyploidy should be considered only doubling or multiplying by 2, 3, 4, . . . the numbers of chromosomes (cf. Chapter 11). Most results obtained with colchicine are related to triploidy.

Any experimental change in the numbers of chromosomes should be checked by chromosome counts. This point may seem quite obvious, but in early reports of "polyploidy" in mammals, changes in cell volume alone were mentioned. It is known from previous experimental data, mainly on amphibians,²⁵ that the size of the polyploid animals remains the same, or is even smaller, than the diploid size, though individual cells become larger and larger with increasing numbers of chromosomes. However, to deduce from measurement of cell size alone the degree of -ploidy cannot be accepted as a valid scientific method.⁵ Considerable error may be involved; for instance, making smears of red blood cells and comparing the diameters is incorrect and cannot bring evidence of triploidy, as has been claimed.^{32, 33} The red blood cell volumes would be a better choice, but these were not measured, either by indirect calculation from the diameter, or by measuring the packed red blood cell volume in a hematocrit tube. Some "polyploid" mammals have been claimed to be larger and to grow faster than the euploid ones.^{32, 33} This is in contradiction with all data on amphibia, and as the numbers of colchicine-polyploid animals which have been studied is very small, and as they were not of pure breed, the data lack the necessary statistical significance.⁶

In the work on the unicellular *Amoeba sphaeronucleus*, polyploidy was assessed without counting the chromosomes, which are very numerous and small. Here, the action of the alkaloid injected intracellularly at metaphase could be followed under the microscope. A single nucleus resulted from the arrested metaphase, and its volume was roughly double that of normal amoebae. Checks were made possible by grafting these abnormal nuclei into normal amoebae, and vice versa.²² The cellular volume became proportional to the size of the nucleus. However, even in these experiments, mitotic abnormalities were observed in the "polyploid" species, and it is not possible to assert with certainty that a true doubling of the chromosome number and not aneuploidy had resulted from the injections of colchicine. Claims of colchicine-induced polyploidy in frogs, rabbits, and pigs have been repeatedly published.^{32, 33, 38} The females were artificially fertilized by sperm mixed with colchicine. The alkaloid is supposed to reach the egg at the time of the second maturation division, which would be arrested. The egg would thus remain diploid, and after fertilization with haploid sperm, triploid animals would be expected. Monstrous development in frogs treated similarly had pre-

viously been reported in a short note.²⁰ A frog sperm suspension with 2.6×10^{-4} M colchicine was most toxic to eggs, and only 8 per cent of these developed normally. It has been claimed that this did not result from a direct action of the alkaloid on the eggs at fertilization.³⁴

The production of triploidy deserves close attention.^{32, 33, 6} A surprising fact is that the rabbits and pigs were considered to have an abnormal growth with increased weight and size. In the first papers, triploidy was deduced from the increased size of red blood cells and spermatocyte heads. The accuracy and significance of these measures have been severely criticized.⁶ However, chromosome counts were later published. In frogs, tetraploid, but also diploid, triploid, and pentaploid cells were found.⁴⁶ In rabbits, a considerable variation of chromosome number was found. While the diploid one was the most frequent, it is clear from the results published that the animals were heteroploid.⁴⁶ The same applies to the single triploid pig. While in a preliminary note about this animal it was claimed that the mitotic count in the testicle was "certainly over 45 and not more than 48," and that the animal resulted from the fusion of a spermatozoon with 15 chromosomes ("Old Swedish" race) and an egg with a doubled chromosome complement of 32 (mixed race), the results of a later publication are by no means so clear.^{46, 33}

It is already evident that in producing artificial "polyploids" one should deal with animals with a well-known number of chromosomes and should not cross two varieties with different and imperfectly known numbers.⁵ The detailed study of the testicular mitoses of the abnormal pig shows chromosome numbers varying between 19 and 51, with an "average" of 49. It was assumed that the probable number of 49 was correct.³³ This should result from the fecundation of a diploid egg with 2×15 chromosomes by a spermatozoon with 19 chromosomes. Evidence for this is given from the chromosome count of a normal brother of this pig, which had 34 (19+15) chromosomes. However, one of the authors mentions as an interesting point that aneuploid cells could be observed in the so-called triploid.⁴⁶

From these descriptions it is apparent. (1) that colchicine may have altered the second meiotic division of the egg, but that only indirect evidence is produced, and that the concentration present when the sperm reached the eggs is unknown; (2) that no polyploid animals have been produced by colchicine, while other methods have proved quite efficient in amphibia; (3) that triploidy is not proven, and that aneuploidy is possible.

It remains possible that colchicine may prove as useful in polyploidy breeding in animals as in plants, but the premature claims of the Swedish authors do not rest on firm ground. The technique of insemination with colchicine is open to criticism, and even more, the

absence of repeated chromosome counts in various organs. It appears surprising that the bone marrow, the skin, or the cornea was not chosen for chromosome counts and that so many publications and claims rest on such meager technical data.

B. Techniques in Plants

16B.1: Solutions Used

Compared with warm-blooded animals, cells of plants tolerate relatively strong concentrations of colchicine. The substance diffuses rapidly through plant tissues and may be translocated in the plant through the vascular system. Active concentrations remain in contact with the cells for a longer time than is recorded by the total exposure to the drug. Apparently the effects of colchicine are retained for a long time. Penetrability, its low toxicity, and retention in the cell, along with the complete recovery through reversibility by the cell, are unique qualities of colchicine for doubling the number of chromosomes in plants.

Successful procedures have favored stronger solutions applied for shorter periods over the dilute ones applied during long exposure.^{3, 4, 9, 11, 13, 15, 18, 21, 22, 24, 25, 26, 27, 30, 17, 33} Schedules with specific concentrations advocated and exposure recommendations are given in the papers. If a universal concentration were selected for treating plants, the strength would be 0.2 per cent aqueous solution. This concentration, or one close to it, has been used more frequently than any other. Wide ranges are effective, but there is an optimum which produces the highest percentages of changed cells. Generally, one gram of colchicine is dissolved in 500 ml. water. The length of time for keeping cells in contact with the drug varies from 24 to 96 hours. In addition to concentration and exposure, the growing conditions of a particular tissue are important. Cells must be in a high state of cell division for maximum effective use of colchicine.¹²

A study of the action of colchicine upon mitosis requires the use of wide ranges in concentration in order to obtain minimum, optimum, and maximum effects. The objectives are somewhat different from using the drug as a tool for making polyploids.

The carrier used for colchicine in treating seed plants may be water, emulsions, agar, or lanolin. Wetting agents have been used effectively. Sometimes the addition of glycerine has been recommended.⁹ The emulsions are sprayed on to the plants or lanolin pastes applied, as suitable. Aqueous solutions are applied by drop-

ping, brushing, or total immersion of the plant in the solution. The latter method has been used effectively for root systems and seedlings.

16B.2: Seed and Seedlings

One of the most convenient ways to treat plants uses the germinating seed placed in solution. The seed may be presoaked or placed directly into the colchicine. Different lots may be removed after given intervals. Then some exposures will not cause doubling; others will prove lethal; and other lots will be at the optimum exposure. In this way the most effective concentration and time of exposure can be determined by the survival of treated seeds transplanted after treatment. Overexposures kill the seedlings, and underexposure does not lead to new polyploids.

Plants, when young, are well adapted to treatment. If only the plumule is treated, the roots remain unharmed, and plant growth is not so totally harmed. The growing point may be immersed in colchicine, or the solution applied to the plant by brush treatment. By sowing seeds in rows, and treating each row with different exposures, the differences between too much treatment and too little will show at the time seedlings are ready for transplanting. Selections for probable polyploids can be made at this time.

Seedlings of monocotyledonous plants are difficult to treat with colchicine. Special methods^{7, 18, 13, 5} had to be devised for these cases. Admitting the drug to the growing tissues that lie beneath a coleoptile sheath has been the chief problem.

16B.3: Root Systems and Special Structures

Soaking entire root systems has been effective for many species of the Gramineae.^{19, 18, 29} An alternate period of soaking in colchicine 12 hours and in water 12 hours has worked out with good success. The number of exposures depends upon the particular experiment, material, and concentration. Reference to specific schedules in the literature shows what directions have been most successful. The technique was developed for sterile species hybrids of grasses and specifically for wheat-rye sterile hybrids to make fertile amphiploids.³³

Scales of liliaceous plants,¹⁵ bulbs, corms, and rhizomes represent structures that call for modifications in method. Usually a large mass of meristematic tissues are present, and unless the whole group of cells responds, the production of mixoploids and chimeras becomes an inevitable result.

Expanding buds of woody stems require proper timing in order to introduce colchicine when the cells are in their peak of division. In this way mature woody plants can be treated when dormancy is

being broken. By grafting the changed sectors, the new polyploids can be propagated.⁹ Periclinal and sectorial chimeras are frequently produced in treating woody species. These chimeras may be propagated for generations through grafting. Their role in horticulture is being more fully appreciated from a breeding point of view.

16B.4: Special Techniques for Studying the Action of Colchicine

Pollen grains that can be used for artificial culturing work serve well for testing the action of colchicine upon mitosis and growth processes. The specific morphology of somatic chromosomes were studied in *Polygonatum*, and discovery of natural polyploidy was made directly from these observations. Another valuable feature is the small amount of chemical that can be tested. Other mitotic poisons soluble in water can be adapted for testing with the pollen tube methods.

Several modifications have been made in pollen tube studies since the original paper was published in 1931 by Trankowsky. The particular conditions for an experiment must be worked out and followed thereafter. In pollen tube studies the detail is not as important as a routine which, once successful for an operation, is always done in that way.⁶

Mitosis in the cells of staminal hairs of *Tradescantia* can be studied *in vivo*. Single cells may be followed through the stages of mitosis. When such cells are growing in agar containing colchicine, the total time required for a c-mitosis can be measured. Special chambers for keeping the cells alive for long periods were designed for these studies. While the general technique for observing mitosis in the living cell of *Tradescantia* has been known for many years, the adaptations for experimental cytology are new.³³

Colchicine was used so effectively with root tips of *Allium cepa* that the test has become known as a method for experimental work, the *Allium cepa* test. Threshold concentrations in relation to solubility are some of the contributions from this method. Standardization of procedures have been devised so that a variety of chemicals can be measured for properties of mitotic inhibition or chromosomal breakage. The time for exposure, for recovery, and for fixation after treatment are important parts of the routine method.

Allowing roots to germinate when suspended over a test solution is a modification of the *Allium cepa* method, and more specifically known as the onion root germination test.

Tissue cultures for excised roots, virus tumor tissue, proliferating cells, and regenerative tissues generally may be adapted for the use of colchicine. *In vitro* and *in vivo* studies are made by these methods.

16B.5: Chromosome Studies

The pollen mother cells stained by acetocarmine are universally a most important source for studying chromosomes in plants. The procedure for determining the number of chromosomes is rapid. More important than deciding what the number might be, are the pairing characteristics at meiotic metaphase, chiasmatal frequencies, lagging of chromosomes at meiotic anaphase, configurations due to translocations, and the irregularities of meiotic processes generally. These are the problems associated with polyploidy that must be studied at the pollen mother cell stage.

Root tips are used for a check of the somatic numbers of chromosomes. Pretreatment of roots before fixation with chemicals that arrest mitosis at metaphase facilitates the study.² Distributions of chromosomes in an arrested metaphase are easier to count and compare for size and morphology.^{16, 8, 14, 2}

Leaf cells in division combined with acetocarmine and Feulgen technics are another source for counting chromosomes in polyploids and related diploids. The longer period of time during which leaf cells provide material and the abundance and availability of material are favored in this test.

Pollen tube cells that undergo mitosis in the tube rather than inside the pollen grain can be treated with colchicine in sucrose-agar media. Scattered chromosomes are easily counted, and the morphology of somatic chromosomes in haploid sets can be measured.¹⁰

Causes of sterility in pollen and pollen mother cells may not be the same when viewed in the embryo-sac stages, or among megaspore mother cells. Frequently the polyploid may be pollen-sterile and female-fertile, or vice versa. Transmission of certain extra chromosomes occurs only through the female and not through the male gametophyte. Cytological methods to measure chromosomal variations in the female gametophyte are long and difficult procedures, but they are important to a full knowledge of why some strains are lower in fertility than others.

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