CHAPTER 17

# Mechanism of Colchicine-Mitosis

## 17.1: Introduction

While many activities of colchicine have been discussed in the previous chapters, it is evident that this alkaloid would be known merely as an effective treatment for gouty patients (Chapter 7) had it not been for its remarkable property of destroying the spindles of mitotic cells. The consequences of this, both in animal and botanical work, have been described. As a polyploidizing agent alone, colchicine has become of world-wide importance and has opened new vistas in experimental agriculture. The scope of the work which has been published since 1934 is so great that all its aspects cannot be covered in this book. More detailed information on some aspects of the colchicine problems may be found in several review papers to which the attention of the reader is directed.[14, 19, 25, 32, 43, 50, 57, 58, 69, 77, 81, 97, 102, 18, 111]

Many still unsolved problems have been mentioned in the text, and it would be useless to discuss again their various aspects. However, the main action of colchicine, as evidenced by microscopy and by the production of polyploids, is in changing the properties of the spindle. Other chemical or physical agents are also capable of destroying the spindle and preventing mitosis from proceeding. The uniqueness of colchicine appears with greater clarity when it is compared with the other "spindle poisons." While no attempt will be made to cover spindle poisoning, this great field of cellular pharmacology, it appears evident that the mechanisms of c-mitosis may be better understood from the study of other agents altering mitosis like colchicine. Many chemicals closely related to colchicine have been studied, and relations between their chemical structure and their spindle activity throw light on the possible action of colchicine.

*17.1–1: Historical.* Spindle poisons were known long before colchicine, and the fact that none of them was so successful is in itself

a demonstration of the singularity of colchicine. The action of narcotics on divisions of sea-urchin eggs was studied by Hertwig in 1887,[48] two years before the discovery of c-mitosis by Pernice;[95] inactivation of the spindle was conspicuous. Phenylurethane in "narcotic" doses was later used in experimental work to study the influence of mitosis on the respiration;[125] the latter was not modified when the spindle was inactivated. In plants, Nemec[86] studied another narcotic, chloral hydrate. Figure 17.1, which is from a later paper,[98] demonstrates how similar the arrested mitoses after chloral hydrate are to c-mitosis. The induction of polyploid plants was, however, never recorded, probably because of the too great toxicity of this narcotic. This points to one of the principal qualities of colchicine and explains most of its success in practical botanical work: its low toxicity and high efficiency.[92]

A classical monograph dealing with animal cells was written by Politzer,[97] who had done important work in the years 1920–1930. Several basic dyes appear to influence the spindle, but Politzer's work is mainly concerned with chromosome poisons, which act somewhat similarly to the ionizing radiations (so-called "radiomimetic" drugs), and he mentions only occasionally metaphase poisoning and spindle destruction.

In 1929, in A. P. Dustin's laboratory, Piton[96] demonstrated the action of various arsenical derivatives on mitoses in mice. These experiments were later extended to grafted tumors.[29] However, the concept of c-mitosis did not yet exist, and observing the gradual increase in the numbers of mitoses, it was thought that a mitotic stimulation was taking place. Actually, it was only after the study of colchicine that it was clearly realized that arsenicals were also spindle poisons, and much later, that they also influenced plant mitosis. Another curious observation is that of Rosenfeld,[99] who noted arrested metaphases in cells treated with ammonia.

On the other hand, it was demonstrated by Lewis[72] that heat alone could inactivate the spindle. Sax observed a similar behavior of plant mitoses in *Tradescantia*.[104] This research opened a way for the successful production of polyploid plants (cf. Chapter 11) and polyploid vertebrates (cf. Chapter 16A), but it was not linked to the other observations of what came to be called c-mitosis.[70] After the discovery of colchicine, and mainly after the observation of its action on plant cells, a host of new spindle poisons was described, and other chemical and physical means of arresting metaphases were found. None was more efficient than colchicine, with the exception of some derivatives closely related to colchicine.

*17.1–2: Colchicine and the spindle.* Before discussing further other mitotic poisons, it is important to stress the peculiar properties

of colchicine. These have been analyzed at length in Chapters 2, 3, and 4, and only a short summary is necessary at this point. Colchicine is a mitotic poison; that is to say, it belongs to the vast and rapidly increasing group of substances which act specifically on dividing cells. In Chapter 7 many other actions of the alkaloid on "resting" (intermitotic) cells were mentioned, but these are limited to

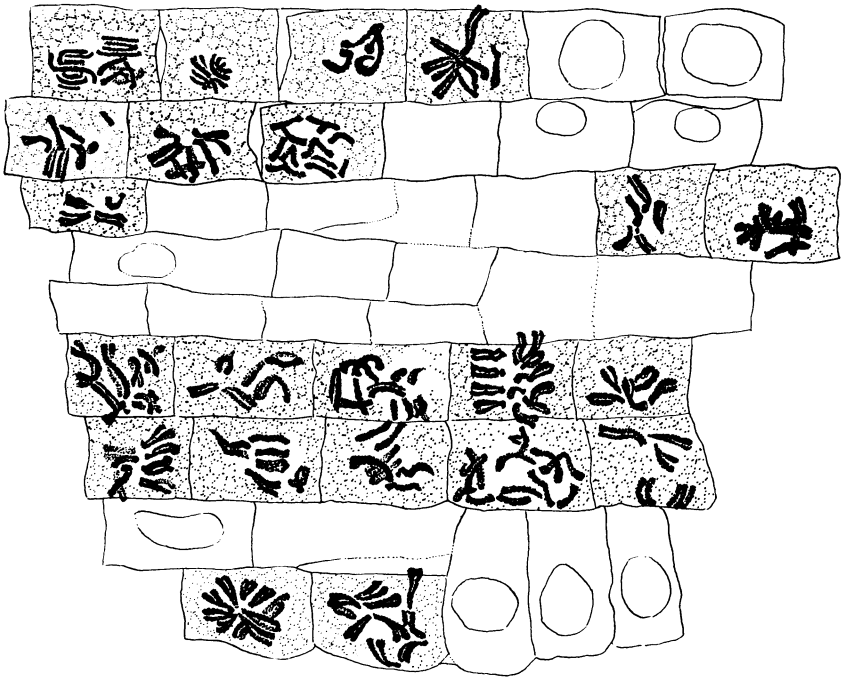


Fig. 17.1—Root tips of **Vicia faba** treated for three hours by a 1 per cent solution of chloral hydrate and replaced for 24 hours in water. Pseudo-metaphases and pseudo-anaphases. (After van Regemoorter,[98] Fig. 1)

some specialized tissues and to some groups of animals. Effects on cell-shape, apart from mitosis, have also been recorded in Chapter 4. These are most interesting for a proper understanding of the c-mitotic effect, but are mainly side-effects, usually brought about with strong concentrations of the alkaloid.

On the contrary, the spindle action is remarkably specific, and solutions of colchicine diluted to one part in one billion, may still exhibit spindle poisoning: colchicine has *high activity*. This is expressed as the inverse of the activity threshold. Colchicine is also of *great efficiency;* that is to say, it acts over a wide range of concentra-

tion. This is especially visible in plant cells, where the general toxic reactions of strong doses described in animals (Chapter 7) are avoided. No other spindle poison is at the same time so active and so efficient, though some of the colchicine derivatives may poison animal spindles at concentrations lower than colchicine.[71, 41, 43, 92, 66]

The changes of the mitotic spindles under the action of colchicine have been described at length in Chapter 3. Suffice it to recall here that the fibrous and polarized spindle is very rapidly changed into an amorphous "pseudo-spindle" or "hyaline globule," which is incapable of moving the chromosomes.[40, 51] Much evidence is at hand to demonstrate that the action of the alkaloid is proportional to its concentration and is totally *reversible,* two facts of great importance in the interpretation at a molecular level of spindle inactivation. Chromosome changes are usually only a consequence of the arrest of mitosis, especially in warm-blooded animals. In plants, the continuation of the normal chromosome-cycle in cells devoid of spindles is the basis of colchicine polyploidy. Cytoplasmic changes have been recorded in plants and animals, especially a decrease in the general viscosity, or rigidity, as evidenced by centrifugation.[88] This may be a consequence, and not the cause, of spindle inactivation.

Most of the other cellular changes are indirect consequences of the spindle inhibition. Short and thick chromosomes are frequently met in arrested metaphases. In plant cells, the cycle of chromosome reduplication is not disturbed by the alkaloid, while in animals, only a few instances of polyploid cells resulting from the multiplication of chromosomes in colchicine-treated cells have been recorded. Here, the prolongation of metaphase leads often to degenerative nuclear changes. Modifications in the shape of cells and in the growth of cell membranes have been recorded (cf. Chapter 4). These involve fibrous proteins, and may be of a similar nature to the spindle changes.

Considering the many data that have been gathered, it can be stated here that colchicine appears to be one of the most specific and least toxic of all the spindle poisons. Hence, any work which helps to solve the problem of spindle inactivation by this complex molecule may throw more light on the mechanism of cell division and on the physiology of the peculiar fibrous protein which constitutes the spindle. The importance of this cannot be underestimated, for all cellular growth in nucleated cells involves the separation of the two groups of chromosomes by the fibrous strands of the spindle.*

* Whether similar mechanisms exist in bacteria is still open to discussion, though nuclei have been recognized by many authors, and at least one group has tentatively identified a mitotic spindle.[24] It may be that the plurinucleated bacterial forms which arise under the influence of some antibiotics, e.g. penicillin, are true polyploid cells. Some antibiotics have been shown to be spindle poisons in warm-blooded animals,[1] and future work may lead to the extension of the concept of mitotic poisoning to microorganisms.

*17.1–3: Materials and methods.* While the problems of colchicine technique have been reviewed in Chapter 16, it is necessary to say something more about this subject in introducing a chapter on spindle poisons. The fundamental processes of mitosis are very similar in all nucleated cells, but it would be an error to think about cell division as an identical phenomenon in all nature from the unicellulars to higher plants and animals. Though the changes brought about by exposure to colchicine are nearly identical, it has been pointed out in previous chapters that *Amoeba* reacts only when the alkaloid is injected with a micropipette into the cytoplasm, that in plant cells, chromosome division proceeds for a long time in the absence of any spindle, and that in animals the hormones and other influences regulating cellular multiplication interfere with the action of colchicine (cf. Chapters 7, 8, and 9).

Spindle poisons have been studied by a small group of research workers, and each laboratory has used the cellular material which appeared the most convenient. It would be unwise to compare uncritically results obtained on *Allium* root tips or on sea-urchin eggs with those observed in fibroblast cultures or in mammals injected with colchicine, or to compare colchicine and spindle-poison effects in normal and neoplastic cells, in embryos or in adults, in slow-growing cells or in tissues stimulated to cellular multiplication by the action of hormones – both in plants and animals. These facts may seem evident from previous chapters. The great mass of data that has accumulated for twenty years about spindle poisons can only be discussed with caution. It is clear that the time is not yet ripe for a single theory covering all types of cells. This important point should be kept in mind when, in the next pages, different and apparently conflicting theories are considered. The only firm ground is that of the experimental facts, and this alone provides a varied and interesting insight into the action of spindle poisons.

*17.1–4: The problem.* The purpose of this chapter can now be defined more clearly. The fundamental problem is that of spindle inactivation by colchicine, a highly specific property of a complex molecule. Other spindle poisons will be considered as far as they help to understand colchicine, and also the modifications of the fibrous properties of the spindle, as evidenced by its structure and by submicroscopic evidence (polarized light) [105, 51] (Chapter 3).

The following points will be considered:

(1) Like most biological activities, spindle formation and modifications during mitosis may be under the control of enzymes. Most work on the effects of colchicine on enzyme systems does not bring much useful evidence, but should be pursued. Some of the latest theories, discussed in Subsections 17.5–2 and –4, point to enzymes as the targets inhibited by colchicine.

(2) A great amount of work on plant cells with a large series of chemicals has indicated that the destruction of the spindle was most closely related to physical properties such as solubility. In short, c-mitosis appeared as a "narcotized" mitosis, and the theories of narcosis explain many findings. It will be seen further whether colchicine fits into such a theory (Subsection 17.3–5).

(3) Work with a molecule as complex as colchicine benefits from experiments with related chemicals having simpler structures. These have clearly indicated which, in the molecule of colchicine, are the groups necessary for the production of c-mitosis. Other substances that inactivate spindles and have definite chemical properties which may explain their action, are of varied structure and range from the simple inorganic arsenic salts to complex molecules, alkaloids, or antibiotics. Though no chemical explanation of spindle destruction by all these substances can be given, the comparison of their structures and activities with that of colchicine throws some light on the singular properties of this alkaloid.

(4) Another approach to the problem of colchicine and the spindle is through the study of antagonists and synergists. Some of the work done in this field has given rise to controversies, but it cannot be ignored. It is evident that the discovery of a substance capable of preventing colchicine from destroying mitotic spindles might at least throw some more light on the biochemistry of the alkaloid and the spindle and on the complex reaction which apparently takes place between them.

From all these studies, however scattered and incomplete they may yet be, emerges an outline of a new cellular pharmacology which should ultimately not only explain why colchicine is a mitotic poison but help, by what can properly be named a "biochemical dissection of mitosis," to explain the mechanics of cell multiplication and of growth.

## 17.2: Metabolic Actions of Colchicine

We will consider under this heading only the facts which help to explain c-mitosis. Other properties of the alkaloid have been described in Chapters 4 and 7. The resistance of some plants and animals to colchicine will be mentioned. While the mechanism of resistance is very imperfectly understood, it may be related to the influence of the drug on cellular physiology.

*17.2–1: Enzymes.* The work done in this field has been conducted with quite different purposes, some authors being interested in mitosis, others in possible mechanisms of colchicine treatment of gout, the origin of hemorrhages observed in malignant growths (Chapter 10), or the formation of c-tumors in plants.

An over-all decrease in tumor respiration was one of the first biochemical observations on colchicine. Its relation with the inhibition of mitosis is not evident.[10, 106]

It has been demonstrated that a $1.2 \times 10^{-2} M$ solution of colchicine inhibits dephosphorylation and the deamination of desoxyribonucleotides. Desoxyribonuclease is also inhibited; however, the relation of these facts to mitosis is by no means clear, and the concentrations of colchicine are far greater than those effective in spindle poisoning.[60] In rats injected 0.2 mg. of the drug, a decrease of the alkaline phosphatase activity was recorded in liver tissue; there was no increased disintegration of ribonucleic acid (RNA).[31] The RNA content of fibroblasts growing *in vitro* was decreased by colchicine.[23] Pyrophosphatase, an enzyme which was found in great quantities in a benzopyrene-induced sarcoma in a rat, was inhibited after a colchicine injection, though no action on the enzyme could be detected *in vitro*.[9]

Other work on changes in purine metabolism, possibly linked with the curative effect of colchicine in gout, demonstrates that, while the nucleotidase of the intestine of calves was not affected, that of human serum was inhibited. Xanthine-dehydrase was also inhibited in guinea pigs, but the concentrations of colchicine (50 per cent and more) were far larger than those effective both in spindle poisoning and in therapeutics.[53]

Inhibition of dehydrogenase activity by colchicine and sodium cacodylate, another spindle poison, was reported in 1938,[38] but no further data on this subject have been published since. A strong decrease of liver dioxyphenylalanine-decarboxylase in rats, and of the pressor amines of the adrenals,[47] may be related to the general toxicity reactions of the alkaloid (Chapter 7). *In vitro* studies of rat liver slices demonstrated an inhibition of creatine synthesis, and blocking of the formation of *p*-aminohippuric acid from *p*-aminobenzoic acid. The methylation of nicotinamide was also inhibited. There appeared to be a relation between amount of drug and degree of inhibition. The formation of creatine from guanidoacetic acid and L-methionine was inhibited by 65 per cent by a $10^{-3} M$ solution of colchicine.[83]

In plant material, enzymatic reactions, *in vitro,* of malt diastase were accelerated by the addition of colchicine; however, the rates of conversion of sucrose by invertase were not influenced.[115] In the germinating grains of *Triticum aestivum* L., the activity of amylase was increased by $10^{-6} M$ colchicine. No significant changes of photosynthesis have been detected.[42]

Some further results will be considered in the paragraphs on the action of *meso*-inositol (17.5–2) and adenosinetriphosphoric acid (17.5–4). It is evident at this point that no significant relation between enzyme inactivation and spindle poisoning has been detected.

*17.2–2: Resistance in plants and animals.* Cells of *Colchicum autumnale* L. yield as much as four parts per thousand of alkaloid. Thus, some of the mitoses of the plant may be in close relation to large doses of colchicine, and the questions arose by what mechanism these mitoses are protected, and whether c-mitosis is possible in *Colchicum*. The first experimenters used as a test the bulbous enlargements of the root tips of *Colchicum* and concluded that large doses of colchicine were active. However, as mentioned in Chapter 4, this is only presumptive evidence, and c-tumors may arise without any mitoses taking place (Chapter 4). Cytological work was carried further on several species of *Colchicum* and with various concentrations of the alkaloid.[20] The results were compared to those of the spindle poison, *acenaphthene* (cf. Subsection 17.3–2). No true resistance in excised root tips grown on agar with strong concentrations of colchicine[20] was observed, though the concentration of alkaloid necessary to induce full c-mitosis was considerable (5 per cent in water). The possible influence of the chloroform present in crystalline colchicine has been ruled out; chloroform is only a weak spindle poison.[117] It is clear that mitoses in *Colchicum* are considerably more resistant than any other plant mitoses towards the alkaloid. This type of resistance appears somewhat similar to that of venomous animals towards their own venom, but in the case of the plant, the basic mechanism is not understood and further research would be useful. Evidently, this is linked with the other unsolved problems of the role and metabolism of colchicine in *Colchicum* sp. The glucoside found in the plant, *colchicoside,*[82] may be of some significance (cf. Subsection 17.4–1).

During routine laboratory tests the discovery was made that golden hamsters resist very large doses of colchicine,[90] considerably greater than the lethal doses for rabbits, guinea pigs, mice, and rats. The tests yielded no c-mitotic values, but only toxicity values which proved beyond doubt that natural resistance exists with the hamsters. Another similar case is the resistance of rabbits to aconite.

Hamsters are native to the region where species of *Colchicum* are abundant (cf. Chapter 1). Through a long period of evolution the hamsters may, by the processes of survival of those animals that lived after eating the *Colchicum,* have passed this resistance on to succeeding generations. Any part of the *Colchicum,* leaf, flower, seed, fruit, corm, would contribute generous portions of colchicine that would be lethal to an animal without resistance.

Such resistance displayed by the hamsters is of interest in connection with the evolutionary problems involved. Further work should be done with the mitotic processes to make comparison of the action of colchicine upon these features.

## 17.3: Physical Action

An inhibition of spindle function and the destruction of its fibrillar structure can be the consequence of physical agents acting on the cells during division. On the other hand, it appears most probable that many of the spindle poisons which have been described do not act by combining in the chemical sense of the word with the spindle proteins, but by altering some of the physical conditions necessary for the proper development of mitosis.

*17.3–1: Inhibition of the spindle by physical agents.* That modifications of the physical environment of the cell, without any mitotic poison being present, may induce c-mitosis is evidenced from the action of heat, cold, and high hydrostatic pressures.

The reversible changes of the mitotic spindle under the influence of an increased temperature were described in 1933.[72] Before colchicine, heat-shock was perhaps the most reliable method for producing polyploid plants (cf. Chapter 11).[104] It is also one of the most efficient methods of inducing polyploidy in mammals, as mentioned in Chapter 16A. In *Triton vulgaris,* on the contrary, larvae kept in water at 3°C. show a typical metaphase arrest, with chromosomes grouped in a single star. The only difference with colchicine is that the alkaloid does not depress prophases, and that ball metaphases (cf. Chapter 2) are more frequent.[4] The hypothesis that cold should mainly affect the centrosomes and centromeres and prevent the orientation of spindle fibers at their contact[4] is interesting and deserving of further study. Cold may have played a significant part in the evolution of polyploid species, especially during the periods of glaciation.

The action of high hydrostatic pressures, about 200 atmospheres, is similar to that of temperature changes in that it brings reversible changes of the spindle, which loses its fibrous appearance.[94] This has been demonstrated both in animal cells (*Urechis*) and in plants (pollen mother cells of *Tradescantia*). The exact significance of these results is far from being understood and need not be discussed here.

Evidently, the proper functioning of the spindle is only possible within a limited range of physico-chemical conditions. It is thus not surprising that changes induced by chemicals of various and unrelated structures may also arrest mitosis by inhibiting the spindle. Research in this field will now be discussed, and the "narcosis theory" of c-mitosis explained. Most of this work, for obvious experimental reasons, has been conducted on plant cells, mainly the *Allium* root tip, and on eggs of invertebrates or vertebrates. A few observations have been made on tissue cultures.

*17.3–2: Simple aromatic and aliphatic mitotic poisons.* A very extensive study on plant cells has been conducted by several groups of

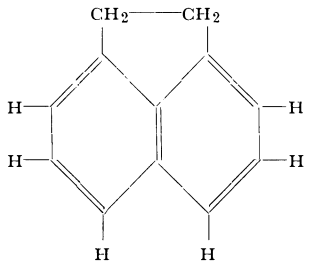
workers, that happened to be widely separated by the events of the second World War. The similar conclusions which were reached have thus an added significance. The names of Gavaudan (Marseille, France), [41, 43] Schmuck (U.S.S.R.), [107, 108, 109] and Levan and Östergren (Lund, Sweden) [70, 71, 91, 92] should be mentioned at this point. This work began with the search for some polyploidizing agent more effective than colchicine and led to an intensive study of chemicals and of the relation between their structure and their activity. One of the first substances demonstrated to be effective for the induction of polyploidy in plants was *acenapthene* (I). This was discovered in 1938,[107, 85, 119, 34] and the simplicity of its chemical structure, apparently without any relation to that of colchicine, quite naturally led other authors to investigate various aromatic derivatives.

In the following years, haloid derivatives of acenaphthene were also found to be effective c-mitotic poisons, as well as later haloid derivatives of other aromatic compounds,[71, 41, 113, 114] and various derivatives of benzene and naphthalene. All of these were soluble in lipids and, contrary to colchicine, had low water solubility. In France, many mono-substituted derivatives of benzene and naphthalene were tested by the Gavaudans on *Triticum*. This extensive work can only be briefly reviewed here. It appeared that, while benzene was only weakly active, it was necessary only to add some side-chains to obtain effective c-mitotic poisons. One exception was *hexamethylbenzene,* the inactivity of which was linked with its high degree of symmetry. Nitro- and halo-derivatives of benzene and naphthalene were studied, and many found to be mitotic poisons. However, total inactivation of the spindle was not always observed, and partial c-mitosis (mero-stathmokinesis) or abnormalities of spindle orientation (tropokinesis) were often the only cellular changes. C-mitosis was also observed under the influence of anesthetic drugs, such as phenylurethane, acetophenone, or anesthesine.[41, 43]

It soon became evident that no definite chemical structure was necessary, but that nearly all aromatic derivatives were c-mitotic poisons under proper experimental conditions, except those with a carboxyl, for instance, benzoic acid, or an amino-group. It was evident that an increased solubility in water was unfavorable for spindle poisoning. More recently, however, *amino-acenaphthene* was demonstrated to be a spindle poison for fibroblasts in tissue culture.[66, 69]

In 1944, the French authors linked their observations with Ferguson's notion of *thermodynamic activity,* which expressed the tendency of a given substance to escape from the phase in which it is dissolved. It can be measured by the relation between the lowest active concentrations of a substance and its highest solubility in water. The conclusion was reached that with only a few exceptions, all the chemicals which had proved to arrest spindle activity acted like chemically

indifferent poisons, and that their influence on mitosis was quite similar to the changes brought about in the nervous system by the so-called indifferent narcotics. Physical changes appeared prominent, and c-mitosis was called a "narcotized" mitosis. The substances listed as not following the rule included *aniline, phenol, hexanitrodiphenylamine,* and *colchicine.* The activity of phenol and aniline, two




**(I) Acenaphthene**

simple derivatives of benzene, demonstrated that in the series of benzene derivatives, the hypothesis that the substances with high thermodynamic potential and high solubility in lipids were the most active spindle poisons, could not be accepted without some corrections.[41, 43]

The Swedish authors,[70, 71, 91, 92] studying the *Allium* root tips, came to nearly identical conclusions, linking lipoid solubility with the mechanism of c-mitosis. They studied a large number of compounds, listed in the papers of Östergren, (cf. also [69]) who proposed a theoretical explanation of "narcotized mitosis" which will be discussed in Subsection 17.3–4. It should be pointed out here that all these experiments could easily be carried out on root tips, but that the conclusions cannot be too rapidly extended to animal cells, which would not resist treatments with strong concentrations of lipoid-soluble substances, often of high toxicity. It is however evident that some drugs known as narcotics in animals, do possess c-mitotic properties.

*17.3–3: Narcotics and indifferent inorganical substances.* Among the chemicals capable of inducing narcosis in animals, we have already mentioned chloral hydrate,[126, 98, 39] which is a spindle poison, as shown in Figure 17.1. Ethylcarbamate (ethylurethane) is a narcotic in animals and a spindle poison in the egg of *Paracentratus lividus* LK.,[93] in amphibians and in plant cells.[25] In other animal cells, e.g., the intestinal mucosa and the bone marrow of mammals, ethylcarbamate acts like a chromosome poison.[30] Chloroform[76] and ether are known to arrest cell division in plants and in some eggs of animals.[48, 97] In the corneal cells of *Salamandra,* ethyl alcohol, ether, and chlorethone also prevent the proper activity of the spindle.[97]

None of these substances, however, has an activity comparable to that of colchicine, and their mitotic effects are only visible in relatively concentrated solutions.

These facts, demonstrating that no evident relation exists between the chemical constitution and the c-mitotic action, and that lipoid solubility is always present, confirm the theory of c-mitosis as a narcotized mitosis. Lipoid solubility is one of the foundations of Overton's well-known theory of narcosis in animals. The wide use of gaseous narcosis in medical practice prompted some workers to study this group of narcotics on the root tips of *Allium cepa*. These were kept humid in a mixture of atmospheric air and the gases, which were under pressure. Propane, nitrogen, nitrous oxide, methane, argon (under a pressure of 75 atmospheres), and hydrogen (200 atmospheres) induced c-mitosis and typical c-tumors. However, only propane, nitrogen, and nitrous oxide induced polyploid cells, for the other gases depressed too much the number of new mitoses.[35] This observation of c-mitosis under the influence of an inert gas like argon definitely demonstrates that the chemical structure may be quite indifferent to the production of inactive spindles, and that physical changes play a great part. C-mitosis appears at this point to be a general reaction of the spindle under the most varied conditions. Work discussed further will show how far these results may explain the action of colchicine.

*17.3–4: Narcosis and colchicine.* The facts gathered so far point towards a close relation between metaphasic (spindle) poisoning and lipoid solubility or thermodynamic activity. The precise relation between lipoids and the function of the spindle is by no means clear, and narcotics appears to modify mitosis somewhat like cold[4] or high hydrostatic pressure.[94] It is not surprising that the problem appears complex, for very little is known about the main target of all these poisons, namely, the spindle. That it is fibrous and anisotropic is evident and is no longer discussed.[110, 50] How it functions is the subject of much controversy, for it is not yet demonstrated whether the fibers "pull" the chromosomes towards the poles (after gathering them at the equator of the cell), or if the chromosomes are "pushed" polewards by a "Stemmkörper" lying at anaphase in the center of the cell. The results of colchicine research indicate (Chapter 2) that traction must play an important role in the movements of the anaphase plates, but how this traction takes place and on what support the fibers are anchored are still unsolved problems. The shortening of the fibers involves most probably changes from fibrous to globular proteins, as evidenced by the polarized light data.[51] These changes probably take place first between the two anaphasic plates, where all

fibrous structures disappear and later between the poles and the centromeres, where they bring about a shortening of the fibers. The biochemical basis of this complex mechanism is unknown. The chemical constitution of the fibers themselves has not been determined, with the exception of some histochemical work indicating that their proteins are rich in sulfhydryl groups (cf. Subsection 17.4–2).

Any theory linking "narcosis" to spindle changes requires additional investigations with a wider use of specimens from both animals and plants. The Swedish author Östergren[71, 92] has presented evidence for the "narcosis theory" using *Allium* root tip cells as a major testing material. The relationship demonstrated to exist between lipo-solubility and the c-mitotic activity for many substances fits the hypothesis quite well, but there are unanswered questions that do not give us as much supporting evidence as everyone would desire. Therefore, the hypothesis put forward by Östergren at this time requires additional testing. Repeating from the preceding paragraph, it is to be stressed that the lack of specific biochemical evidence drastically limits our understanding, particularly when trying to formulate basic mechanisms for reactions such as the c-mitosis.

Colchicine is a spindle poison with a low thermodynamic activity and extremely high solubility in water. Therefore, this chemical is an exception to the general rule that applies to simpler aromatic derivatives.[91] These relationships are clearly illustrated in Figure 17.2, as drawn from experiments with cells of *Allium* and/or *Triticum*. The proposed theory of a narcosis, while interesting from the standpoint of the biochemistry of the spindle, cannot at the same time apply to colchicine, which appears to act on a *chemical* basis rather than *physically*. This conclusion was reached independently by the French authors.[43] Certain results will now be considered to show that ideas of a chemical relation between alkaloid and spindle appear promising for the ultimate explanation as to how a c-mitosis is accomplished.

### 17.4: Chemical Action

Two lines of research indicate that spindle poisoning may be related to definite chemical structures, and probably to chemical interference between poisons and spindle fibers. The first is the study of derivatives of colchicine and related molecules. This indicates that minor changes in this complex atomic structure may considerably affect the cytological activity. The second is the study of other mitotic poisons; while those which have been considered so far acted more physically than chemically, there is a small but important group of substances which inactivate the spindle and which possess specific

chemical reactivity. After studying these simple spindle poisons, some other substances acting like colchicine, or those with complex molecular structure will be examined briefly. The properties of colchicine will then be compared to those of other poisons.

*17.4–1: Colchicine derivatives.* These have been studied from three main points of view: their toxicity, their antimitotic activity,

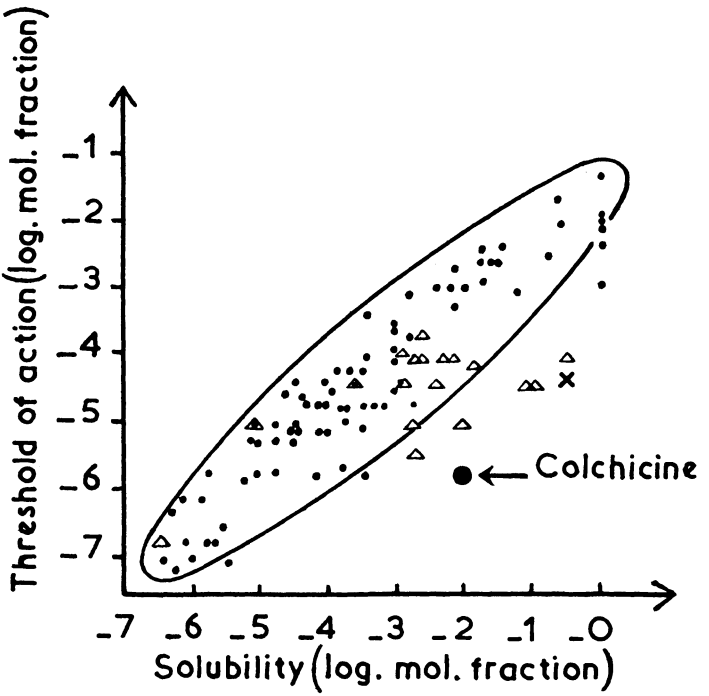
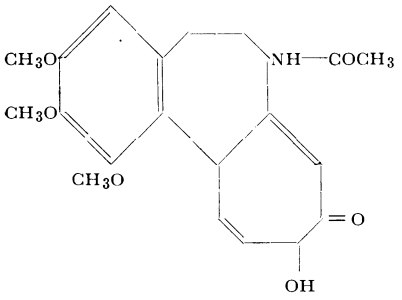



Fig. 17.2—Relation between c-mitotic activity in the **Allium** test and solubility in water. Each dot or triangle corresponds to a different substance. The singular behavior of colchicine is evident. (After Ostergren, 1951[92])

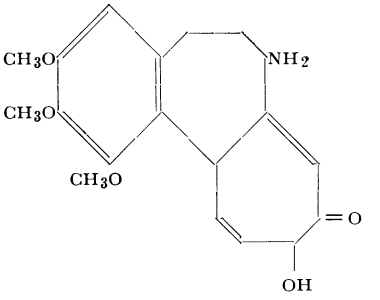
and their inhibition of tumor growth.[27] The spindle poisoning will mainly interest us here, and it should be made clear that this is not necessarily paralleled by other properties of these molecules. For instance, it has long been known that *colchiceine* (II) is less toxic, and also a weaker mitotic poison than colchicine. But desacetylcolchiceine, *trimethylcolchicinic acid* (III),[63] does not interfere at all with cell division in animals, while it may, like colchicine, kill frogs by central nervous paralysis. The opposite is also true; and results to be discussed further point to the possibility of synthesizing derivatives with lower toxicity and greater mitotic-poisoning effects than colchicine.

In the *Allium* test, *trimethylcolchicinic acid* (III) has been shown to induce c-mitosis, but it is thought that the mechanism is quite different from that of colchicine, and related to the amino group of ring B.[117] This derivative has a marked toxicity, while even 20 per cent solutions of colchicine are only slightly toxic for these plant cells.

Before considering in some detail artificial colchicine derivatives, it is important to remember that other closely related alkaloids exist in




**(II) Colchiceine**

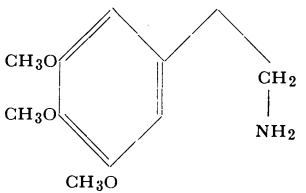



**(III) Trimethylcolchicinic Acid**

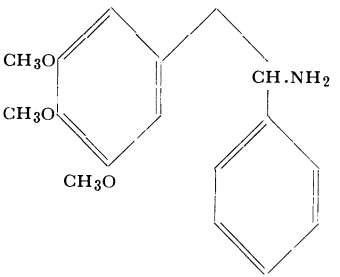
*Colchicum,* and also that colchicine is probably present in chemical combination with a glucoside. *Desmethylcolchicine* has been found in preparations of colchicine;[49] it differs from colchicine only by one methyl group missing in ring A. It has been proved that it poisons mitosis like colchicine, and demonstrates that two methyl groups are sufficient for this. It is probable that at least one is indispensable. Work by Lettré is interesting in this connection.[66] This author, searching for mitotic poisons with a simpler chemical structure, and basing his researches at the time on the old formula of Windaus in which rings B and C are 6-membered, showed that on fibroblasts in tissue culture, *mescaline* (IV) was without action, while *α-phenyl-β-(3, 4, 5-trimethoxyphenyl)-ethylamine* (V) is active. Further simplification demonstrated that spindle poisoning was retained in α-phenyl-β (*p*-metholxyphenyl)-ethylamine (VI), which was the simplest possible poison of this group.

The exact chemical structure of several other substances from *Colchicum* and closely related to colchicine is not known yet; they probably differ from the parent molecule by relatively minor changes,[100, 101, 65] and are all more or less active against mitosis.

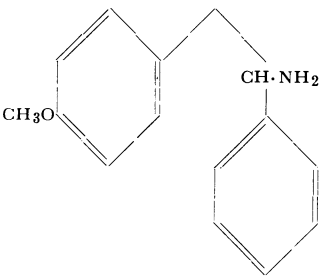
In *Colchicum,* a substance named *colchicoside,* resulting from a glucosidic linkage of colchicine, the exact chemical nature of which has not yet been established, has been isolated.[82] It is of interest to note that this poisons spindles, but is 40 times less active than colchicine towards plant mitoses. With diluted solutions, it is observed




( IV )



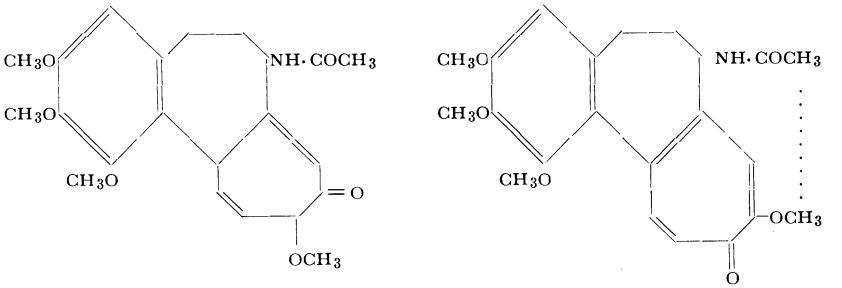

( V )




( VI )

that c-tumors (root-tip swellings) occur with solutions which are devoid of any mitotic action. The hypothesis has been put forward that colchicoside may be some kind of detoxication product of colchicine, a fact which may help to explain the resistance of *Colchicum* towards colchicine (cf. Subsection 17.2–2).

The principal changes affecting the action of colchicine are those affecting the *N*-substituted radicals in ring B and the esters of ring




**(VII) Colchicine**
**Isocolchicine**

C. Before considering some of these derivatives, it is important to study the results obtained with an isomer of colchicine, iso*colchicine,* (VII) in which the positions of the O and O-$CH_3$ radicals of ring C are reversed.[116, 117, 65]

The activity of *iso*colchicine has been studied on *Allium* root tips[117] and on fibroblast cultures.[66] Solubility and thermodynamic activity differ considerably from those of colchicine. While the latter is soluble in approximately all proportions in water, *iso*colchicine has a solubility of $50{,}000 \times 10^{-6} M/1$. The activity thresholds stand at 150 for colchicine and $14{,}000 \times 10^{-6}\, M/1$ for the *iso*-compound, the thermodynamic activity of which is 0.28, that is to say, about a thousand times higher than that of colchicine. As a conclusion of this work, it appears "that colchicine, with its low thermodynamic activity is a typical representative of the chemically acting substances, while *iso*colchicine with its 900 times higher thermodynamic activity belongs to the type of unspecifically acting substances." [117] *Iso*colchicine interferes thus with mitosis like the many substances mentioned in the previous paragraph of this chapter. In fibroblast cultures, the difference is not quite so great, for *iso*colchicine is only 50 times less active than colchicine. Two other similar molecules, *ethyl-colchiceine* and iso*ethylcolchiceine,* were compared on the same material: the second was about 200 times less active than the first. These substances have been isolated from *Colchicum.* Other *iso-* derivatives of

colchicine have also proved to be without action against neoplasms.[66]

It is premature to discuss the reasons for the weak activity of the *iso-* compounds. One reason which has been put forward is the formation of hydrogen bonds between the side-chains of ring C and ring B, because of the closeness of the methyl groups of these chains in the *iso-* forms. (VII) It has been suggested that the weak antimitotic activity of *colchiceine* may be the consequence of the *iso-* form of this molecule.[65] Other data prove that the activity of colchicine on mitosis is related to both these side-chains.

The substances to be studied now can all be considered as derivatives of *trimethylcolchicinic acid* (III). This compound was demonstrated in some of the first work on colchicine derivatives and mitotic cells in mammals, to be inactive. In cultures of fibroblasts and of neoplastic cells also, no activity could be detected (Table 17.1).[44]

Substitution on ring B *alone* does not yield effective mitotic poisons. On tissue cultures, N-*acetyl-colchicol* and its methyl ether (VIII) have only slight activity. Tables 17.1 and 17.2 give further evidence

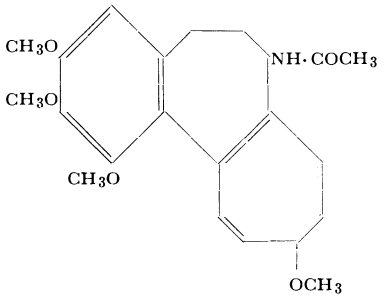
TABLE 17.1
LD 50's OF COLCHICINE DERIVATIVES IN MG/KG
(After Goldberg *et al.*[44])

| Substance | Mice | Rats | Cats |
|---|---|---|---|
| *N*-Benzoyl-TMCA* | >700 | ..... | ..... |
| TMCA | 200 | 200 | >10 |
| Colchiceine | 84 | 30 | >12.5 |
| *N*-Acetyl-colchicol | 56 | 200 | 10 |
| TMCA-methyl-ether | 46 | ..... | 5 |
| *N*-Benzoyl-TMCA-methyl-ether | 32 | ..... | <25 |
| *N*-Acetyl-TMCA-methyl-ether | 3.5 | 5.0 | 0.5 |

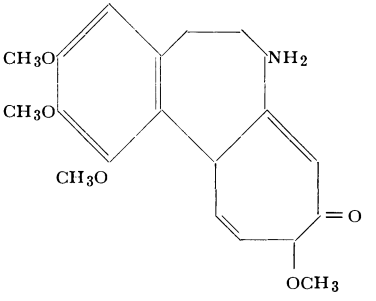
* TMCA = trimethylcolchicinic acid.

of this. The activity of this derivative is comparable to that of *colchiceine.*

However, when ring C remains as in colchicine, it is evident that *N*-substitution in ring B is not of great importance for activity. In tissue cultures, desacetylcolchicine, *trimethylcolchicinic acid methyl ether* (IX), is an effective spindle poison, while the parent substance, desacetylcolchiceine (=TMCA), is almost inactive. N-*benzoyl-tri-*




( VIII )




( IX )

*methylcolchicinic methyl ether* has been demonstrated to be one of the most effective derivatives in arresting mitoses in the stomach epithelium of mice.[11, 12, 36]

Substitutions in ring C are the most important, for they yield substances with a greater antimitotic activity than colchicine.[64, 78] These are derivatives of *colchicamide* (X). (This abbreviated spelling is to be preferred to *colchicineamide* or *colchiceinamide,* which are to be found in the literature.) Thirty-five derivatives of this type have been studied by Lettré,[66] who found *N*-methyl-, *N*-ethyl-, and *N*-dimethyl-colchicamide to be most effective in tissue-culture work, the activity decreasing when longer side-chains were added to the amino-group (Table 17.3).

Other derivatives with more extensive changes in ring C, for instance with a six-carbon aromatic ring C, *colchinol* series (XI), or

TABLE 17.2

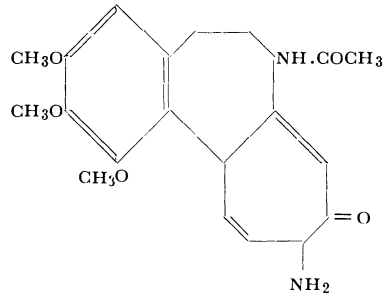
MINIMAL EFFECTIVE ANTIMITOTIC DOSE OF TMCA DERIVATIVES ON CORNEAL MITOSES OF MICE, SIX HOURS AFTER INJECTION, EXPRESSED AS THE FRACTION OF THE LD 50 IN CREASING THE MITOTIC INDEX ABOVE THAT OF CONTROLS AND MINIMAL EFFECTIVE ANTIMITOTIC DOSES IN VARIOUS TISSUES OF MICE

(After Goldberg *et al.*[44])

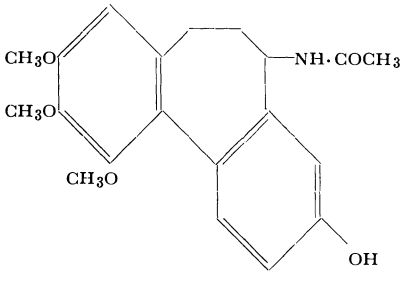
| Substance | Minimal Antimitotic Dose/ LD 50 | Minimal Effective Dose | | |
|---|---|---|---|---|
| | | Cornea (*mg/kg*) | Regenerating Liver (*mg/kg*) | Tissue Cultures (*μg/kg*) |
| Colchicine | 1/10 | 0.01 | 0.21 | 0.35 |
| *N*-acetylcolchicol | 1/2 | 1.0 | 9.01 | 28.0 |
| Colchiceine | 1 | 4.0 | 8.01 | 84.0 |
| TMCA (trimethyl-colchicinic acid) | >1 | inactive | inactive | inactive |

N-*benzoyl-colchicinic anhydride* (XII), have been tested on tumors.[63] None has shown an activity comparable to colchicine, and the reader should refer to the papers of the National Cancer Institute group for detailed data on this subject.[11, 12, 63, 64, 65]

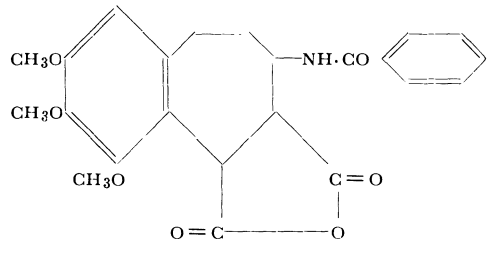
Although colchicine derivatives have been tested on few materials, the main purpose of the work having been a search for substances of




(X)




(XI) N-Acetylcolchinol




(XII)

interest in cancer chemotherapy, the following conclusions can be drawn for the papers published:

1. The *iso*colchicine derivatives, and *iso*colchicine itself, are considerably less active. It appears important that the esterified side-chains of rings B and C are at a proper distance one from another.
2. At least one methoxy group appears indispensable in ring A.
3. The amino group of ring B does not need to be esterified, though this increases the activity.
4. Ring C must be seven-membered, and the hydroxyl group esterified, or better, replaced by an amino group itself esterified (colchicamide derivatives).

These facts help to reveal which are the active groups of the colchicine molecule. However, they are yet of no help in explaining how these react with the spindle. Results obtained with spindle

poisons of very different chemical structure, and indicating relations between this structure and their action, throw further light on the subject of spindle inactivation.

*17.4–2: Sulfhydryl poisons.* With a few exceptions, most of the work in this field has been done on tissue cultures[50] or in intact warm-blooded animals.[44a] This method has an advantage in that, be-

TABLE 17.3
SMALLEST ANTIMITOTIC DOSES ($\mu$g/ml) EFFECTIVE IN ARRESTING MITOSES IN CULTURES OF CHICK FIBROBLASTS
(After Lettré [66])

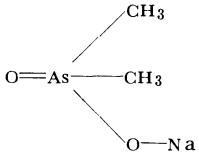
| Derivative | Dose |
|---|---|
| Colchiceine | 5.0 |
| Colchicine | 0.01 |
| Colchicamide | 0.01 |
| *N*-methylcolchicamide | 0.0025 |
| *N*-ethylcolchicamide | 0.003 |
| *N*-propylcolchicamide | 0.08 |
| *N*-butylcolchicamide | 0.9 |
| *N*-methyl-propyl-colchicamide | 0.5 |

cause of the necessity for avoiding toxic side-effects, only small doses may be used. Hence, substances acting as narcotics or producing a "physical" change of the spindle will not be found to have mitotic-poisoning properties.

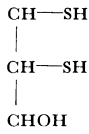
The most extensively studied in mammals,[96, 29, 73, 30] in invertebrates,[45] on tissue cultures,[74, 13, 55] and in plant cells[79, 22] are simple derivatives of arsenic. *Arsenious oxide* and *sodium arsenite* arrest metaphase by destroying the spindle, and these star metaphases are very similar to those described in Chapter 2. The most effective of the organic arsenicals appears to be sodium cacodylate, or dimethylarsinate (XIII).

In mice, it has been demonstrated that this action was reversible, that is to say, that arrested metaphases could be detoxicated and proceed to a normal telophase.[30] The inactivation of the spindle is thus the consequence of a labile combination of its proteins with arsenic. The detoxicating agent was *dimercaptopropanol* (BAL, British Anti-Lewisite) (XIV), a substance which combines rapidly and strongly with arsenic and other metals. This action of a chemical with two -SH functions suggested that arsenic may have combined with similar

SH groups in the spindle.[30] This hypothesis was in agreement with a theory of spindle activity in which reversible changes of SH to S-S functions were supposed to play a prominent part in the "contractile" properties of the spindle. The further discovery that -SH substances themselves were also spindle poisons, for instance, *dimercaptopropanol* and *sodium diethyldithiocarbamate,* was in agreement with this




( XIII )




( XIV )

hypothesis, if it was considered that a proper equilibrium between reduced and oxydized sulfhydryl functions was indispensable for spindle activity.[30]

This theory of chemical action on the spindle received further support from the discovery that many metals, known to combine with -SH groups, are mitotic poisons.[80] *Ethylmercurychloride* is an example of an organic poison of this type, active on plant cells,[56, 75] while *cadmium* salts are most effective in arresting mitosis in mammals.[122, 30, 2] The inhibition of metaphase by *beryllium* salts, which has been considered to be the result of nuclear phosphatase inhibition,[17] may possibly be explained by the combination of this metal with sulfhydryl groups.

It has been further demonstrated by work on tissue cultures and in injected mice, that the typical -SH poisons, *chloracetophenone, iodoacetic acid,* and *iodoacetamide,* arrested mitoses at metaphase.[44a, 50] However, these substances are very toxic, and have strong inhibitory actions on glycolysis, which may be important in explaining their action on cell division. Some of the complex molecules considered in the next Subsection may also act as -SH poisons.

This does not close the list of mitotic poisons which appear to act chemically on the cells. The most remarkable is *ethylcarbylamine* ($C_2H_5CN$), which has been demonstrated to modify the course of mitosis in tissue cultures exactly like colchicine.[120] Total inactivation of the spindle with exploded metaphase and, later, formation of numerous micronuclei were conspicuous. Ethylcarbylamine reacts chemically with metals; this chelating property is shared by *diethyldithiocarbamate,* another spindle poison.[30] These results point to some further complexities of the problem; the action of other organic spindle poisons will show how far we are from understanding the basic changes involved.

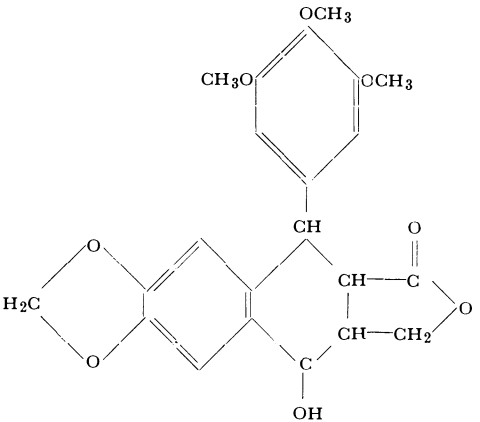
*17.4–3: Complex organic molecules.* The mechanism of action of most of the substances mentioned in this subsection is unknown; molecular structures are widely different. However, these drugs are all very active, and it is felt that they modify the spindle more by a chemical than by a physical change. The resin of *Podophyllum* sp. (mandrake) contains several toxic substances, the principal ones being *podophyllotoxin,* α- and β-*peltatins,* and *quercetin.* The crude resin was a popular remedy against warts in the United States, and this observation led to a scientific study of the active substances[54, 21] (XV). These proved to be efficient spindle poisons, and to act most similarly to colchicine, both in skin tumors of man, and in various animal materials.[118] From a chemical point of view, they are complex lactones.[69] Another instance of a lactone acting as a mitotic poison is the antibiotic *patulin* (Bacitracin, clavacin) (XVI). This inhibits remarkably the spindles of erythroblasts in the chick and in many tissues of mice.[1]

It is interesting to compare the formula of patulin with that of *coumarin* (XVII), which has been described as a weak metaphase poison in *Allium* and *Lilium.* Its action may be of the "physical" type, though combination with -SH groups is also possible.[121]

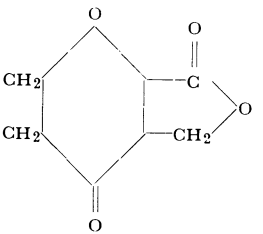
Other substances of plant origin have been found to inhibit mitosis, mainly in tissue cultures of fibroblasts. *Chelidonine*[69] is of interest because of its use in cancer chemotherapy (Chapter 10). In an extensive study of alkaloids, it has been shown that the only active ones were found in the group which is chemically related to *stilbylamine,* and thus to α-*phenyl*-β (*p-methoxyphenyl*)-*ethylamine* (cf. 17.4–1). These are *narcotin, gnoscopin, chelidonine, homochelidonine, methoxychelidonine,* and *protopin.*[69] Many other substances may yet be discovered when further systematic studies are conduced. This is already underway, and has demonstrated c-mitotic activity in extracts of *Chimaphila maculata* and *Sassafras albidum.*[7]

Other complex substances extracted from plants are *anethol*[62] and *apiol,*[41] which may induce polyploidy. This has also been observed in *Allium* root tips treated with *veratrine.*[128] *Sanguinarine* and *cryptopleurine* are also spindle poisons, and the second, extracted from *Cryptocaria pleurospora,* has been considered as effective as colchicine.[5] Positive effects on mitosis have also been found with extracts of the following plants: *Ervatamia angustifolia, Aristolochia elegans, Euphorbia peplus, Bulbina bulbosa,* and *Strychnos arborea. Protoanemomin* is an interesting poison,[33, 121] for its action on the spindle may be prevented by *dimercaptopropanol* (BAL); this is evidence of a chemical reaction.

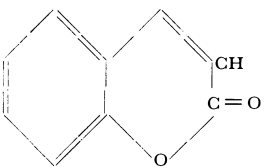
The list of c-mitotic active substances is much longer, and among chemicals of animal origin or related to the growth of animal cells, *adrenalin*[66, 69] has been found to arrest metaphases in fibroblast cul-


OCH3
CH3O
OCH3
CH
O
CH—C
O
O
H2C
O
CH—CH2
C
OH

( XV )


O
O
CH2
C
O
CH2
CH2
O

( XVI )


CH
C=O
O

( XVII )

tures at a concentration of 0.1 mg/ml, and the antifolic drug, *aminopterin (4-aminopteroylglutamic acid)* arrests mitoses in tissue culture.[52] This is a remarkable fact, for this antimetabolite when injected into mice, behaves as a strong and typical poison of the "radiomimetic" type, inducing chromosome breakages.[30]

*17.4–4: Colchicine compared with other spindle poisons.* The spindle structure, which can be destroyed by purely physical means, is evidently adversely influenced by a series of substances which appear to act through their chemical reactivity. Arsenic, the heavy metals (mercury and cadmium), and the sulfhydryl poisons of the iodoacetamide type indicate that -SH groups may play an important role in metaphase dynamics. Some more complex substances, such as the antibiotic patulin, and protoanemonin, may owe their antimitotic properties to the lactone structure, and perhaps also to interference with sulfhydryl. Podophyllotoxin may possibly belong to the same group, but the difficulties of understanding clearly the action of such complex molecules are formidable. There is no indication that colchicine may fit in this type of chemical theory, though the facts gathered by the protagonists of the "narcosis" hypothesis, as well as the study of colchicine derivatives, point towards a chemical combination of the alkaloid with some intracellular receptor.

The comparison of colchicine with other spindle poisons makes clear two facts: the great amount of work which is still necessary to understand the action of this drug, and the notable specificity of colchicine. For, if several chemicals have been quoted as acting similarly, few have been capable of inducing polyploidy, and still none has proved comparable in the practical work on polyploidy in plants. The extraordinary fact is the great efficiency and activity of colchicine, which will remain active when highly diluted, but concentrated solutions of which will not kill the cells. This points to some singular relation between the alkaloid and the spindle.

Further research about the biological activity of the tropolone compounds should help to understand better the chemical action of colchicine in the cell. Thus far, it has not been possible to "simplify" the molecule and obtain spindle poisoning. The few reports on tropolone derivatives indicate some action on mitosis, in *Tradescantia* staminal hair cells, far weaker than colchicine.[124] The necessity for such a complex molecule to achieve with the utmost efficiency what can be done by such simple agents as cold, arsenic, and ethylcarbylamine, is most puzzling. The solution of this problem should bring some important new insight on the submicroscopic and chemical mechanics of mitosis.

Often the mechanism of drug activity has been solved when a proper antagonist could be found, for instance *p*-aminobenzoic acid

and the sulfonamides. Some work in this direction has been carried along and will be summarized now.

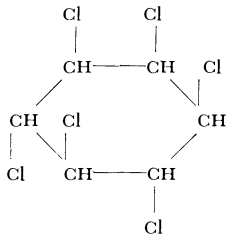
### 17.5: Synergists and Antagonists

A possible synergism between animal growth hormones and colchicine has been considered in Chapter 9. In plants, some changes visible after colchicine have been interpreted as evidence[8, 28, 46, 70, 79, 87] of hormonal action of the alkaloid. This has not been proved (cf. Chapter 4). In animal and plant cells, the antagonism of *meso*-inositol and colchicine is still a subject under discussion which merits to be reviewed here. Mention will also be made of a long series of experiments on fibroblasts in tissue cultures. These have led to a novel theory about c-mitosis which will be properly considered in the light of all the facts already gathered in this chapter.

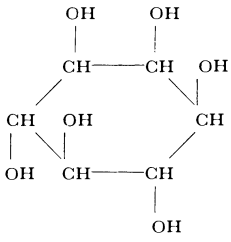
*17.5–1: Meso-inositol. γ-Hexachlorocyclohexane* ("Gammexane"), a widely used insecticide, has been reported by several authors to induce c-mitosis in *Allium* and other plant cells.[22, 56, 88] Both the γ and the δ isomers have been found to be active,[15] while the first only is of use as an insecticide. Polyploidy and chromosome fragmentation have also been recorded. Gammexane is probably an antagonist of a naturally occurring substance, meso-*inositol,* having the same stereoisomeric structure as this sugar, the biological significance of which appears from its presence in many types of cells.

It was thus not surprising that in 1948 it was announced that *meso*-inositol, (but neither *d*-inositol nor D-sorbitol) prevented, in proper concentrations, the c-mitotic activity of Gammexane in *Allium cepa.*[16] It was, however, more surprising and most interesting that *meso*-inositol was claimed to prevent also the spindle effect of colchicine. The results were given as percentages of the different stages of mitosis, and it is to be regretted that no counts of the total number of cell divisions were recorded. Inositol alone did not interfere with mitosis. The formation of c-tumors, both by Gammexane and colchicine, was also prevented.[16] These results were checked over a wider range of concentrations and times by another author, who found that *meso*-inositol merely delayed the c-mitotic effect of colchicine, which was visible, as in the controls, after 24 hours.[22] Similar delays were observed with other sugars, a solution of saccharose (0.95 mg/ml) suppressing all colchicine mitoses in root tips observed after four hours of treatment, while after 24 hours the c-mitotic effect was normal.[22] Modified cell permeability was thought to explain the results obtained with *meso*-inositol. A confirmation of these findings was found in the observation that colchicine and podophyllotoxine effects were antagonized in the egg of the sea urchin *Lytechinus variegatus* by glucose.[20] The antagonism was never total; it was suggested

that inositol may become changed into glucose in the cells. However, in *Allium,* it was demonstrated that the isomer of *hexacyclochlorohexane,* which could not act as an antagonist to *meso*-inositol, was also a spindle poison, and that no true protection was offered by *meso*-inositol against the effects of Gammexane.[15] The different tem-



(XVIII) γ-Hexachlorocyclohexane ("Gammexane")



(XIX) Meso-Inositol

peratures at which the experiments were conducted may explain the conflicting results.

Two papers published in 1951 renewed interest in this problem. In the first, the authors who discovered the action of *meso*-inositol first in plants, brought forward evidence that a similar antagonism existed in rat fibroblast cultures.[84] Here, for the first 12 hours, no difference was observed between colchicine alone and colchicine + inositol, but in the following hours, while the colchicine mitoses remained arrested, the cultures treated with inositol recovered almost completely. This period of 12 hours during which, quite contrary to the plant experiments, inositol does not prove to have any effect, except that of lowering the total numbers of mitoses, is considered to correspond to the duration of interkinesis. The authors suggest that *meso*-inositol may "allow the cell to prepare for a new mitosis," which is surprising, for this would lead one to think that there is no true detoxication of c-mitoses, similar to that of arsenite by BAL, and that these degenerate, and are no longer counted, while other cells enter mitosis. However difficult the interpretation of these results may seem to be, it is significant that neither sucrose, glucose, ribose, sorbitol, nor even *d*-inositol, *meso*-inosose or *epi*-inosose are capable of altering the action of colchicine.[84]

This result is also in contradiction with the facts observed in plant cells, and no conclusion can be drawn at this time. One interesting report, given only in a short note, is that some enzymes of bacterial origin capable of oxidizing inositol are inhibited by colchicine and the parent substances, tropolone and 4, 5-tetramethylene-tropolone.[37, 124] Further results on this aspect of the colchicine problem are eagerly

awaited; they may help to understand better the biochemistry of the spindle and the physiological functions of *meso*-inositol.[26] As for the action of γ-hexachlorocyclopropane, it may of course be of a "physical" type, similar to that of the numerous other c-mitotic and polyploidizing substances studied in plants.[103]

*17.5–2: Other antagonists and synergists.* In tissue cultures of rabbit heart fibroblasts, l-ascorbic acid was found to prevent, to a certain extent, the action of colchicine.[13] The numbers of arrested mitoses were smaller, and a careful study of the different types of mitotic abnormalities indicated that the vitamin decreased the amount of spindle inactivation. This was not the result of an action as a vitamin, for d-*araboascorbic acid,* whose properties as a vitamin are 20 times weaker, had the same effect. The two substances are equally reducing, and the interpretation of these results is difficult, for p-*quinone,* an oxydant, also depressed colchicine inhibition of mitoses.[13] An antagonism between colchicine and "soluble prontosil" (sulfanilamide) has been reported in plants,[6] but the effective concentrations of the sulfa drug were about a hundred times those of colchicine, and solubility effects were unavoidable. In animals, sulfanilamide has been claimed to influence colchicine-leukocytosis, but this was only remotely related to mitosis[127] (cf. Chapter 7).

An extract from hearts of embryonic warm-blooded animals has been reported to delay the cytotoxicity of colchicine in fibroblast and myoblast cultures. A colchicine concentration of $2 \times 10^{-5}$ *M* was without effect after 10 hours in cultures previously treated with the extract. If this was added after the alkaloid, no antagonism was visible.[123] Another more recent observation is that glycosidic substances endowed with cardiotonic activity decrease the action of colchicine in tissue cultures of chick heart fibroblasts.[59]

It appears evident from these data that no true antagonism has yet been found between any substance and colchicine, on a molar basis, and that the only effects observed depend on the presence of substances either of unknown chemical nature or in concentrated solutions.

On the contrary, the search for synergists of c-mitotic activity has yielded important results.[67, 25] Some synergists act mainly by increasing cellular permeability to the alkaloid, and the reader is referred to the paper of Deysson[25] for a detailed study of this type of false synergism. It has been observed only in plant cells. In fibroblast cultures, Lettré has conducted a very large series of experiments, and has discovered that many substances increased the action of colchicine, though having no c-mitotic activity of their own. These synergists belong to the most dissimilar groups of chemicals: alkaloids, steroid hormones, and carcinogenic agents (benzopyrene). The

amount of the synergist is always far greater, on a molar basis, than that of colchicine. For instance, while 5.5 mitoses per hundred were found after 0.01 mg/ml of colchicine, the addition of 5 mg/ml of bulbocapnin increased this figure to 23.8. Forty times this dose of bulbocapnin had no action on control cultures. With phlorizin the results are very striking also.

More than 8 times more mitoses are arrested when a solution of phlorizin, which has no antimitotic action, is added to a concentration of colchicine, which is only weakly antimitotic. This is truly a synergistic effect.[67] Its study may most probably increase our knowledge of the physiological action of colchicine, and further work along similar lines with different types of cells is to be expected.

Another interesting colchicine synergist has been reported by P. Rondini and A. Necco (Tumori, 39:161–63, 1953). *Italchine,* an acridine derivative, is itself a mitotic poison, affecting spindle and chromosomes. Small doses, which do not affect mitosis, increase markedly the action of colchicine on chick fibroblasts cultivated *in vitro.* The principal results are apparent from Table 17.4.

*17.5–3: The role of adenosine-triphosphoric acid (ATP).* That the spindle functions, partly at least, as a fibrous contractile structure has been affirmed repeatedly. The contraction which takes place has

TABLE 17.4

SYNERGIC ACTION OF ITALCHINE AND COLCHICINE ON TISSUE CULTURES OF CHICK FIBROBLASTS

(Mitoses counted after 48 hours' incubation with the drugs)

(After Rondoni and Necco)

| Substances and Concentrations | Pro-phases | Meta-phases | Ana-phases | Telo-phases | Total |
|---|---|---|---|---|---|
| Italchine (1/300,000) | 2.5 | 18.8 | 5.03 | 7.7 | 34.03 |
| Colchicine (0.0033 μg/ml) | 4.9 | 42.3 | 8.86 | 5.06 | 61.12 |
| Italchine + colchicine (same concentrations) | 0.5 | 79.4 | 2.15 | 1.07 | 83.12 |
| Controls | 4.9 | 13.8 | 8.00 | 10.5 | 37.2 |

also been compared to that of muscle. While biochemical data about the nature of the spindle proteins are lacking entirely, it could be imagined that colchicine acted on the contraction mechanism. Most cytological data (cf. Chapter 2) point to an action on the fibers themselves, which can be observed to "dissolve" into a "pseudospindle" or "hyaline globule" under the influence of the alkaloid. In muscular

contraction, the role of ATP is well known. Observations of colchicine synergists and theoretical considerations led Lettré to suppose that ATP may also be indispensable for spindle contraction and mitosis, and that colchicine acted on the cell by modifying this mechanism.[69]

Experiments *in vitro* demonstrated that strong concentrations of colchicine inhibited the viscosity fall of complexes of actomyosin and ATP.[3] It was further observed that ATP-ase was inhibited by colchicine at concentrations of $10^{-3}$ and $10^{-4}$ *M*. However, more dilute solutions ($10^{-8}$ *M*), which arrested mitosis, did not affect the enzyme.[61]

A direct antagonist action of ATP and colchicine was difficult to prove, because of the rapid destruction of ATP in fibroblast cultures. Only with very small doses of colchicine was such an antagonism visible. Cultures were grown for 24 hours, and then colchicine, at a concentration of 0.04 mg/ml was added.[68] This arrested, after 24 hours, 55 per cent of the cells in mitosis. When 1 mg/ml of ATP was added at the same time, mitotic inhibition did not start until four hours later. The results are given in Table 17.5. It is concluded that the higher the amount of ATP in a cell, the smaller the action of colchicine, and vice versa.[69]

ATP may play an important part in the conservation of cell form in cultured fibroblasts. The "resting" cells have been considered to be in a condition of permanent contraction, while cells intoxicated with various drugs, such as Victoria blue, have a lower content in ATP, and display a rounded form with rapidly moving surface blebs. If ATP is added to a fibroblast culture, the cells assume a spindle shape, even when dividing. In this condition, ATP would provide the energy necessary for this contraction, and would also protect the spindle against mitotic poisons.[69]

This hypothesis is only a tentative one, and it is not yet proven that colchicine acts by depressing ATP in the cells. Further experiments will be needed to explain the relation between cellular respiration and the formation of the spindle fibers, and also between ATP and the physiology of the spindle. It is apparent that more fundamental knowledge about the dynamics of mitosis is needed before the effect of colchicine and its various synergists may become clear. While these effects are still difficult to understand, there is no doubt that the discovery of the colchicine-mitosis has provided a considerable impetus to such fundamental studies.

### 17.6: Conclusion: the Singularity of Colchicine

From this chapter it has been made evident that destruction of the fibrillar properties of the spindle, and mitosis arrest at metaphase

or pro-metaphase, is by no means limited to colchicine or even to chemical agents. From some angles, it appears as an entirely non-specific reaction of metaphase to agents as different as cold, nitrogen, hydrostatic pressure, lipid-soluble hydrocarbons, or heavy metals. However, that it is in most cases more than a "narcotized" mitosis is evident from the data about sulfhydryl groups, colchicine deriva-

TABLE 17.5

PERCENTAGE OF MITOSES AFTER COLCHICINE AND ADENOSINE-TRIPHOSPHORIC ACID (ATP) IN CULTURES OF FIBROBLASTS

(After Lettré and Albrecht[68])

| Hours | Colchicine | *id.* + ATP |
|---|---|---|
| 1..... | 2.0 | 2.0 |
| 2..... | 7.7 | 3.0 |
| 3..... | 11.2 | 3.3 |
| 4..... | 13.0 | 5.0 |
| 5..... | 16.4 | 8.3 |
| 9..... | 27.4 | 9.4 |
| 14..... | 38.4 | 23.2 |

tives, and synergic activities. It is also evident at this point that further progress will only be possible when the biochemical and physiological properties of the spindle are better known. Mitotic poisons are useful tools for this purpose, and it may well be that the solution of this problem will lead rapidly to an understanding of the properties of colchicine. The difficulties of this task are great, and resemble in many aspects those of the study of muscle contraction. The spindle structure is however relatively simple, as far as can be known at this time, and its contractility and reversion to a nonfibrous "hyaline globule" are problems of which a solution appears possible in the not-too-distant future.

Colchicine, from all that has been said in this chapter, must be considered a singular substance. Not only does it possess remarkable side-effects, such as its action on gout, the colchicine-leukocytosis, its action on the nervous system and on muscular contraction, its induction of specific malformations in embryos; it is also the most efficient and active of all mitotic poisons known – with the exception of derivatives of the colchicamide series. It is also the mitotic poison to which the largest amount of work has been devoted. While some substances like podophyllotoxin have received great attention, others, such as the arsenical derivatives, have hardly been studied from the angle of mitosis. It is not because colchicine was one of the first-discovered spindle poisons that it received such attention. Chloral

hydrate, acenaphthene, and arsenic may have deserved more detailed studies. Colchicine was investigated from such diverse standpoints because it was not only a mitotic poison like others, but also an ideal tool for the study of growth, and, last but not least, the best polyploidogenic agent in plants. As the creation of new polyploid species was taken up with enthusiasm, chemists and morphologists studied more and more the structure and the properties of the alkaloid. It is probably more than mere chance that the unique structure of this tropolone derivative is associated with so many physiological activities. It is reasonable to prophesy that colchicine will long retain its prominent place in the vast chapter of mitotic poisons. Many observations point towards a high degree of specificity in the reactions between the alkaloid and the spindle; if these reactions could be properly understood, that fundamental process of all growth and evolution, mitosis, would appear in a new light.

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