NEW ASPECTS OF THE PHARMACOLOGY OF ANTIMITOTIC AGENTS

PIERRE DUSTIN, JR.

Laboratory of Pathological Anatomy, University of Brussels, Belgium

TABLE OF CONTENTS

I. Recent discoveries about normal mitosis 451
II. Definition and classification of antimitotic agents 453
III. Poisons of the achromatic apparatus (spindle poisons) 454
A. Sulfhydryl reactants 455
B. Colchicine and colchicine derivatives 456
C. Other spindle poisons of natural origin 460
1. Vinblastine 460
2. Podophyllin and other substances of plant origin 461
3. Griseofulvin and other antibiotics 463
D. Partial spindle inactivation: "polar chromosome" metaphases 464
E. Carbamates 466
F. Nonspecific spindle inhibition 466
IV. Chromosome poisons 467
A. Inhibitors of nucleic acid synthesis 467
B. Antibiotics 469
C. Other chromosome poisons 470
D. Alkylating agents 471
V. Other mitotic inhibitors 472
A. Corticoids (cortisone, hydrocortisone, prednisone, *etc.*) 472
B. Other substances inhibiting mitosis 473
VI. Conclusions 473

"Given complexity, we may yet avoid turning it into obscurity"
[D. Mazia, 1960 (142)].

Since the classical contributions of Politzer (180) and Gavaudan (85), the chemical inhibition of mitosis has been the subject of many review articles (13, 16, 37, 56, 70, 90, 105, 106, 122, 127, 135, 206). A monograph has been devoted to the most extensively studied and utilized mitotic poison, colchicine (76). New drugs are daily tested for their effects on mitosis, in normal or neoplastic tissues. Throughout the literature, innumerable but often incomplete data on "mitotic inhibition" are to be found, resulting from the search for specific inhibitors of neoplastic growth.

The present review does not aim to be complete. It will mention some of the interesting findings of the last ten years, and discuss them, as far as possible, in the light of the improved knowledge of normal mitosis. This has been described in a masterly way by Mazia in 1961 (143). Frequent references will be made to this important contribution. Progress in the study of cellular division has come from different directions. The synthesis of deoxyribonucleic acid (DNA) during the S-phase of intermitosis is better understood, thanks to Watson and Crick's (224) molecular model, and has benefited from the radioautographic studies with tritiated precursors of nucleic acids. Mazia and his group (139, 141, 142, 145, 226, 227, 233) have improved considerably our under-

standing of the structure and the chemical composition of the achromatic apparatus of mitosis (spindle and asters) by isolating this "mitotic apparatus" (MA) of invertebrate eggs. Electron microscopy has brought to light the intricate structure of the centriole, its relation to the spindle fibers, and the complex mechanism of its reproduction (10, 53, 82). It has also helped to understand the modifications of the cytoplasmic structure during the phases of cellular division.

Any review of the literature on antimitotic agents encounters difficulties arising from the different approaches used in this field. Various techniques and various types of cells have been studied. The direct microscopical approach has sometimes been neglected for indirect methods, such as the incorporation of isotopes or the microchemical determination of nuclear constituents. These may be of little value without proper morphological control. Radioautography with tritiated precursors provides a good instance of structural and biochemical integration.

Other difficulties arise from the fact that mitosis, although implying similar complex and coordinated series of changes leading from one to two daughter cells, may not be identical, at the biochemical level, in all types of cells. The cytoplasmic origin and the anchoring of the achromatic apparatus are different in plant cells, which have no centrioles, and in animal cells. These do appear to have a similar behavior during mitosis (181), although it is far from certain that they all derive the energy required for division from the same sources, and that the sequence of events leading to division is always the same. Furthermore, it is evident that in the same animal, each tissue has its proper mitotic regulation, and consequently, may react differently when confronted with some inhibitors of mitosis (30, 214).

These facts, which are not always seen in their proper perspective, indicate that cells grown in culture at a maximal rate cannot be compared to cells in whole tissues, where the mechanisms of mitotic regulation may interfere with the action of pharmacological agents. The studies of developing eggs have yielded most interesting data (201, 205), although their rapid succession of divisions with a very short intermitotic period is quite different from what is found in other cells. Neoplastic cells have been the subject of much work, complicated by the fact that they often spontaneously display mitotic irregularities similar to those induced by antimitotic drugs.

For these reasons, it does not appear necessary to analyze all the types of mitotic inhibition. It is better to limit this review to those discoveries which can be related to the fundamental changes of mitosis, and also to papers which give a detailed account of the morphology of the modified mitosis, without being content with vague terms such as "mitotic inhibition" or "mitotic arrest." Many findings have resulted from apparently simple experiments in which growing cells were directly in contact with inhibitors, especially plant cells, tissue cultures, and eggs. Some of these agents may cause toxic effects that restrict their study in whole animals—cyanide for instance. A systematic survey of hundreds of substances by these techniques may be useful, although it often indicates the lack of specificity of many antimitotic actions.

From personal experience, the author believes that the pharmacology of antimitotic substances acting in whole organisms is not without interest. The purpose of much recent work has been the search for specific poisons of cancer cells. These may be of some use only if they do not affect normal tissues. The fallacy of the chemotherapy of cancer with drugs which poison most normal mitotically growing tissues is apparent from the results of the last decade (64). Experiments on whole animals may, on the other hand, help to understand better the still obscure problem of the normal regulation of mitotic growth (39, 213, 214).

Emphasis will be laid on substances of which the mechanism of action, at the biochemical level, is the best understood. Naturally occurring substances—whether antibiotics, alkaloids, plant extracts, or hormones—deserve attention as indicators that mitotic inhibition is a problem for general biology, as suggested by Lettré in 1947 (121). Special attention will be given to the substances affecting the "mitotic apparatus" (143), which controls all the mitotic movements. The poisons directly affecting the chromosomes will be considered in their relations to the basic processes of cell division.

I. RECENT DISCOVERIES ABOUT NORMAL MITOSIS

The mitotic cycle, that is, the sequence of events which take place from one consecutive mitosis to another in the same tissue, implies the perfect partition between daughter cells of the material present in the mother cells, and especially of the genetic information carried by the chromosomes.

The mitotic cycle has a much longer duration than mitosis, and its principal events take place long before any morphological changes are seen. The timing of the main events is summarized in Table 1. Some of these take place very early—for instance, the formation of new centrioles, which may be completed at the end of the preceding division.

Electron microscopy has shown that the centrioles are complex cylindrical structures built of nine groups of tubules of about 20 mμ diameter, which is

TABLE 1
The mitotic cycle: main events

	Preparation	Prophase	Metaphase	Anaphase	Division
Nucleolus	RNA synthesis		Disappearance		Reappearance
Chromosomes	DNA synthesis and reproduction	Coiling and condensation	Alignment	Separation	Uncoiling
Nuclear membrane		Breakdown			Reappearance
Mitotic apparatus	Synthesis	Aster and spindle formation	Spindle elongation		Breakdown
Centrioles	Reduplication	Poleward separation			

about the diameter of the spindle fibers as seen with the electron microscope (10, 190, 191). These fibers may be spun out by the centrioles, in the same way as the fibers which constitute ciliae and flagellae originate from the basal granules, which have a closely related structure. In intermitotic cells new centrioles (procentrioles) are formed at right angles to the old ones (10, 82). The dividing cell is probably already provided with four centrioles, two which will orient the spindle and two procentrioles which will play their part only in the further divisions of the sister cells (144).

Complex mechanisms take place at the molecular level in the chromosomes. During the intermitotic period, the formation of new DNA, as demonstrated by radioautographic detection of the incorporation of precursors, takes place during the S or synthetic phase, preceded and followed by two "gap" intervals, G^1 and G^2 (216). It is generally accepted that in the formation of new threads of DNA, one new molecule is built along one old one, a process which implies the unwinding and rewinding of the Watson-Crick spiral structure of DNA (225). Whatever the exact process, it is not certain whether the chromosome represents a double or a quadruple thread of DNA, conveying the genetic information, or whether the chromosomal structure is not much more complex, and multistranded. Studies on the replication of DNA in viruses and bacteria favor the simple mechanism of unwinding and rewinding (32). Coiling plays a great part in mitosis, as evidenced by the structure of chromosomes.

All synthesis of DNA, for simple mechanical reasons, must be completed long before the chromatids shorten at prophase. The discovery of the role of folic acid derivatives in the biosynthesis of DNA and RNA explains some antimitotic actions. Tetrahydrofolic acid (THF) plays a manifold role in the transport of one-carbon atom groups. The step leading from deoxyuridylic acid to thymidylic acid, controlled by THF and taking place shortly before the polymerization of DNA, might provide the cell with "a very sensitive means of controlling DNA synthesis" (156). It may also explain a fact which has not been clearly understood, *i.e.*, why DNA synthesis is more sensitive to folic acid antagonists and other poisons than that of RNA (68).

Another problem closely linked to antimitotic action is that of the "energy requirements" for mitosis (87, 88, 89). Apparently, energy is stored in the cell before the beginning of the mitotic movements themselves. It has been suggested that some substances prevent mitosis by acting at this period (38). The exact nature of the energy required, the respective roles of glucose (28) and ATP (123), are still under discussion (87). Substances which simply prevent cells from entering mitosis, whether by a specific action or by their general toxicity, have been grouped under the heading of "pre-prophase poisons" (13, 37). The hypothesis that the energy would be stored in the form of the mitotic apparatus itself (spindle and asters) (213) is attractive. The MA is known to behave like a contractile protein, perhaps under the influence of adenosine triphosphate (ATP). Comparisons with muscle have actually been abandoned, the spindle contractility being more akin to that of the flagellae and ciliae. These, as mentioned above, originate from structures similar to the centrioles, and biochemical

data point to some resemblances between their proteins and those of the spindle (42). Identical antigens have been found in the spindle of the egg and the flagellae of the sea-urchin sperm (192).

In considering mitosis in complex tissues, the problem of the "trigger" (142, 143) cannot be neglected. At some moment during the intermitotic period, the cell is instructed to divide. It is clear that some special event is necessary, and that mitosis does not follow automatically the preparations of interphase-DNA reduplication, centriole formation, cytoplasmic growth. Cells ready to divide may be maintained in intermitosis (89). Most organs are controlled by homeostatic mechanisms which prevent the occurrence of mitosis except when needed (for instance, in liver, kidney, skin). These mechanisms may operate through the action of natural mitotic inhibitors (70). This problem will be discussed in relation to some actions of the adrenal cortical hormones. In some cases, the "trigger" action may be the lifting of an inhibition, in others a true stimulation (for instance by hormones). The intracellular mechanisms that are specifically linked with the onset of cell division remain unknown.

II. DEFINITION AND CLASSIFICATION OF ANTIMITOTIC AGENTS

Much has already been written about the classification of mitotic poisons. For Biesele (13), to this category belongs "any agent affecting mitosis." Such a definition is imprecise and neglects the most important fact that *only* the phenomena related to division are altered. As proposed earlier (70), antimitotic agents (or "mitotic poisons") will be defined as *those substances nearly specifically affecting dividing cells, while leaving unharmed the resting cells* (*i.e.*, postmitotic, differentiating cells or intermitotic cells not preparing for division).

From what has been said above, antimitotic agents may affect either the events taking place during intermitosis, such as the reduplication of DNA and centrioles and the synthesis of the future achromatic apparatus, or during mitosis itself. This definition, and the types of substances known, lends itself to a classification in two great groups: the spindle poisons and the chromosome poisons. This does not ignore other possibilities of injuring dividing cells. It takes into account the far greater sensitivity of the spindle and the chromosomes than that of other cellular components towards noxious agents.

There is still no specific poison of anaphase, and it seems that the late stages of mitosis are much less sensitive, either because they require less energy or because they do not imply such complex movements as the metaphase: "anaphase proceedings (are) events that are extraordinarily difficult to stop once they have started" (143).

Classifications always dangerously tend to over-simplify. Some substances may affect both spindle and chromosomes, according to the type of cell. For this reason, mitotic inhibitors will be grouped empirically, following their supposed molecular action, the similarity of their effects, or their origin in nature. Problems of classification have lost most of their importance, for pharmacology is tending towards another goal: defining antimitotic action in chemical terms.

One might be surprised, in the light of the modern knowledge of the ultra-

structure of the cytoplasm, that so few cytoplasmic changes will be mentioned. It is remarkable that little is known about the multiplication and division of the Golgi apparatus and the mitochondria, and about their partition between the daughter cells. This may be an evidence of our ignorance: the complex budding of the centrioles has been known only a few years (10). Toxic agents, specifically disturbing this centriolar mechanism, may be discovered one day. The same may be true for the mitochondria, their division, and its regulation.

III. POISONS OF THE ACHROMATIC APPARATUS (SPINDLE POISONS)

In order to assure the proper movements of the chromosomes, a complex fibrillar apparatus must be built before mitosis. It has been demonstrated that the main components of spindle and asters are already present in some cells before the formation of visible fibrils (139, 142). At the beginning of mitosis, these will be anchored to the centrioles, which have moved to the poles of the nucleus, and to a special region of each chromosome, the kinetochore (or centromere). The movements of the chromosomes towards the metaphase plate at prophase, towards the poles at anaphase after the division of the kinetochores, result from the activity of this fibrillar apparatus. It appears to pull the kinetochores towards the centrioles, while elongating at the same time (196). The fibrillar structure of the spindle and the asters disappears first between the two anaphase groups of chromosomes and fades progressively at the end of anaphase. The so-called spindle poisons of the colchicine type interfere with the fibrillar structure of the mitotic apparatus, without affecting the synthesis of its specific components. When this action is complete, in place of the spindle, a globular mass of material is found ["pseudo-spindle" (133)], which has lost the main physical property of the spindle, namely, its birefringence (108). The movements of the centrioles are also modified and they may come to lie in the middle of the cell (55), surrounded by the chromosomes. Eventually each chromosome may reform a micronucleus [caryomery (76)] or may divide in the absence of cytoplasmic division, resulting in the doubling of the number of chromosomes.

In plants, the cells without fibrillar spindles may undergo repeated chromosome reduplication, and become highly polyploid (133). In animals, on the contrary, it is apparent that many of the poisoned cells eventually degenerate; this is particularly true in mammals (176, 177).

Until recently the cause of this cellular degeneration occurring after a few hours of mitotic arrest was poorly understood. The discovery (182) that all (or most of the) cytoplasmic RNA is synthesized in the nucleus, and that this synthesis stops shortly before mitosis, and does not take place in colchicinized cells arrested at metaphase, may explain the irreversible changes which are observed.

Although colchicine and its derivatives have been the subject of an extensive literature (76), their mode of action is by no means understood in chemical terms. For this reason, sulfhydryl reactants will be considered first, for their action may be related to the chemical structure of the spindle and asters.

A. Sulfhydryl reactants

Relations between the sulfhydryl groups of glutathione and mitosis were suggested by Rapkine (183), the formation of S—S links explaining the contraction of the spindle. About the same time, following the observations of Wätjen (223) on the cellular destructions in lymphoid tissues of animals injected with arsenicals, it was discovered (179) that arsenous oxide and sodium cacodylate increase the number of mitoses in growing tissues of mice. This was the consequence of an arrest in metaphase resulting from the selective inactivation of the spindle, the other mechanisms of cell division (and, in particular, the changes associated with prophase and early metaphase) remaining unaffected. It was demonstrated that the inhibition of mitosis by sodium arsenite could be reversed by dimercaptopropanol (BAL) (61). Other work had indicated that arsenical derivatives are active in tissue cultures (26) and in plants (49). Other specific sulfhydryl reactants, such as chloracetophenone, monoiodoacetate (91), heavy metals such as mercury (202, 233) and cadmium (65), were found to have similar effects on the spindle. The importance of —SH groups was further suggested by the fact that SH-substances themselves, such as dimercaptopropanol (61), and sodium diethyldithiocarbamate (65) were also toxic for the spindle. This was supposed to indicate that a certain balance between SH and S—S is necessary for maintaining the integrity of the spindle fibers (65).

The isolation of the "mitotic apparatus" (MA) by Mazia (138, 142) brought further evidence to bear upon these relationships. The first results, with sea-urchin eggs, were obtained by dispersion of the cytoplasm after a short fixation in ethanol. Later, a better method was devised on the basis that "the chemical bonds of the mitotic apparatus having some properties of S—S bonds must be 'protected' and that they could be protected by an excess of an S—S compound" (143, 145). The substance chosen was dithiodiglycol. The mitotic apparatus thus isolated, without any fixation, was stable only in the presence of dithiodiglycol, a fact suggesting that this chemical may be "mimicking a comparable, though of course not identical, condition within the dividing cell" (143, p. 245). Histochemistry has repeatedly confirmed the presence of sulfhydryl groups in the spindle of the eggs of *Arbacia* (114): "The spindle and astral fibers are composed of proteins rich in —SH groups which arise from S—S protein in the region of the centrosphere."

Further work by Sakai and Dan (193) has suggested that a SH— polypeptide may be responsible for the properties of the MA. The hypothesis of Rapkine (183) that glutathione plays an important part in mitosis has been discarded (141).

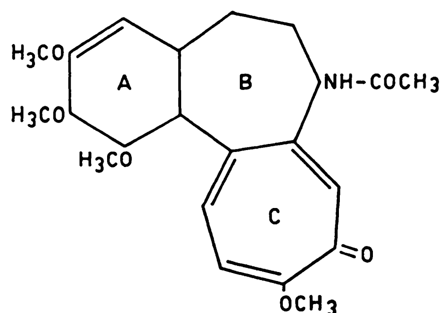
The relations between mitotic poisoning and this work on the isolated mitotic apparatus have been analyzed by Zimmerman (233). The MA was dissolved by mercury compounds, such as *p*-chloromercuribenzoate [a spindle poison (13)] and Salyrgan (mersalyl, a mercurial diuretic), and by thioglycolate. On the contrary, mercury acetate and mercaptoethanol [which has been shown to destroy reversibly the MA of eggs (140, 146)] did not affect the isolated MA. Its

destruction took place very rapidly: with Salyrgan at the concentration of 2×10^{-3} M, it was complete in 12 minutes; in a 10^{-2} M solution, it was immediate; this was true only for the recently isolated MA. For Zimmerman (233), "thiol groups play an essential role in the assembly and maintenance of the spindle and aster." What remains unclear, however, is the exact nature of the sulfhydryl bonds in the MA fibrils, and their participation in the polymerization leading to the fibrillar structures of mitosis.

These results provide a closer insight into the action of this group of spindle poisons, comprising sulfhydryl reactants and substances with reactive sulfhydryl groups. This may lead to a better understanding of the similar changes induced by more complicated molecules.

B. Colchicine and colchicine derivatives

The tropolone derivative (44, 229), colchicine, was the subject of a monograph in 1955 (76). Since then, the number of papers devoted either to aspects of its action on cells, or to its use as a tool for the study of growth and mitosis, has increased by several hundred. Modern cytogenetics owes much to the use of colchicine for increasing the number of visible mitoses displayed by cells in culture, and for facilitating the study of the chromosomes (220).



Colchicine

Colchicine was described in 1955 as a spindle poison of great potency (76): solutions of 10^{-9} g/ml (27) are still capable of inactivating the spindle in tissue cultures, and even larger doses, acting in isolated cells, seldom modify other events of mitosis.

The study of colchicine derivatives had so far yielded no molecule of simpler structure with a similar activity. The deacetyl-N-methylcolchicine derivative (colcemide) widely used now, is slightly less toxic for animals, but also less potent on the spindle. Among the active derivatives, colchicamide (57) and N-deacetylthiocolchicine (23) should be mentioned. The principal known requirements for activity were: 1) at least one oxymethyl group on ring A; 2) the esterification of the amino group of ring B; 3) a seven-membered ring C, and 4) a methoxy group on ring C or its replacement by an esterified amino or sulfhydryl group, preferably esterified (76). However, recent work on deacetylamino-

TABLE 2

Metaphase arrest in tissue cultures of a mast cell tumor after colchicine and deacetylamino-colchicine

	Metaphases*	Anaphase and Telophase*
Control	1.36	1.15
Colchicine (3×10^{-5} µmol/ml)	23.5	0.53
Deacetylamino-colchicine (3×10^{-6} µmol/ml)	30.6	0.36

* Percentage of total cells.

colchicine, which has no acetylated amino group on ring B, has shown, contrary to previous findings, that the mitotic inhibiting effect was not only preserved, but increased (195a). The cell growth of *in vitro* cultures of a transplantable murine mast cell tumor was strongly depressed, and deacetylamino-colchicine was approximately ten times more active than colchicine. The percentage of arrested mitoses was of the same degree as that produced by colchicine (Table 2). Another finding by the same author (195b) is that the carbonyl of ring C is indispensable: if C═O is replaced by C═N, all activity is lost.

Although no important discoveries about the molecular basis of the action of colchicine have been made recently, some data from studies of the isolated mitotic apparatus, the resistance of the hamster, and substances acting as antagonists, will be discussed.

The action of deacetyl-N-methylcolchicine (colcemide) has been studied on the mitotic apparatus of the sea urchin, *Strongylocentrotus purpuratus* (194). Cleavage is inhibited in 95 to 100% of all cells, with a concentration of 2.7×10^{-5} M. This is irreversible, even if the eggs are washed within 5 minutes after the treatment. The asters are involved before the spindle, and lose their fibrillar orientation. The MA isolated from eggs treated for 20 minutes does not show any fibrillar spindle. In its place is found a spherical body, similar to the "hyaline globules" reported earlier in grasshopper neuroblasts treated by colchicine (84). The MA, isolated by the alcohol–digitonin method, resists destruction by the direct action of colchicine. The antigenic composition of the MA treated by colchicine in the living egg does not show any abnormalities. It was also demonstrated, in eggs treated with colcemide, from which the MA had been isolated by the dithioglycol method, that the ATP activity linked to the spindle proteins is not affected by the changes in form brought about by the drug (194). Further developments in this most promising field should clarify the interrelation between colchicine and the isolated MA. The fact that colchicine effects are so similar in all cells tested so far, from unicellular forms to man, suggests some simple relation to the structure of the spindle.

There are some remarkable cases of cellular resistance to colchicine. Orsini and Pansky (159) described the resistance of the golden hamster, *Mesocricetus auratus*, to colchicine. Doses as great as 10 and 20 mg/kg did not affect mitoses; these are more than ten times higher than those effective in other rodents (mouse,

rabbit, rat). de Harven (50) was able to arrest mitoses in the bone marrow of the hamster by injecting still larger doses (30 and 50 mg/kg), which were well tolerated by the animal; thus, 50 mg of deacetyl-N-methylcolchicine per kg arrested 94% of all divisions at metaphase. An important finding was that another spindle poison, podophyllotoxin, was also inactive in the hamster in doses (10 mg/kg) that were effective in the mouse; mitotic arrest was observed only with doses of more than 30 mg/kg. On the contrary, sodium cacodylate was equally active in the mouse and the hamster, a fact pointing towards a special resistance of the latter species towards colchicine and podophyllotoxin, without any fundamental difference in spindle structure and biochemistry. The lethal dose of colchicine for the hamster was found to be as high as 700 mg/kg (221); however, the hamster is not resistant towards other toxic effects of colchicine, unrelated to mitoses (*vide infra*).

Wrba (230) studied the action of N-methylcolchicamide on the Walker tumor of the rat grafted to the golden hamster. Surprisingly, it was found that the cells of the heterograft became as resistant as those of the host. This could be explained by the existence of a detoxication mechanism preventing the drug from reaching the graft in effective quantities. However, the following results are more surprising (231). The Walker carcinoma of the rat, after passage in the hamster, was grafted back to normal rats. An acquired resistance towards N-methylcolchicamide was indicated by the fact that the injection of 100 to 200 mg, a dose which normally arrests all metaphases, had no action on its mitoses. This is not true for all tumors. In the case of the Ehrlich carcinoma of the mouse, only some cells became "resistant" when grafted to the hamster, and the tumor recovered its normal sensitivity when grafted back to the mouse. These results deserve further study. It would be interesting to find out whether some resistance to podophyllotoxin is also acquired, and what the mechanism of the resistance is at the cellular level.

The protection of mitosis against the action of colchicine has been subject to conflicting claims. Thus, mesoinositol (8) and ATP (124) have been considered to be colchicine antagonists. Some of the data concerning ATP indicate that this substance exerts a nonspecific inhibition of cell division, rather than a true antagonism. Any chemical that depresses the number of cell divisions will render the cytological action of colchicine less visible; a true antagonist, by itself, should have no action on mitosis. According to Lettré (123), ATP slows the action of colchicine on tissue cultures; thus, 9 hours after 0.04 g of colchicine per ml, 27.4 mitoses were observed in tissue cultures of chick embryo, while only 9.4 mitoses were seen with the same concentration of colchicine in the presence of 1 mg of ATP per ml. After 14 hours of exposure to these agents, the mitotic counts were 38.4 and 23.2, respectively, a finding which indicates a slight antagonism of colchicine by ATP. These results could not be confirmed by Benitez *et al.* (8). These authors also studied the antagonistic action of mesoinositol. This was tried because gammexane (hexachlorocyclohexane) was known to arrest mitosis at metaphase (35), and because of the structural analogies between the molecules of gammexane and inositol. In tissue cultures of rat fibroblasts,

treated with colchicine, mesoinositol has no effect during the first 12 hours, but later, the mitotic index decreases sharply. A similar effect has been observed with tropolone (8). Here also, a decline in arrested metaphases is observed between 16 and 22 hours after the beginning of the experiment. It is not clear whether or not cellular destruction could explain these results.

The antagonism between colchicine and cortisone is interesting in the light of similar observations with the spindle poison, vincaleukoblastine (vinblastine). This antagonism, discovered in a study of the mitotic activity of bone-marrow eosinophils (51, 54, 73), contradicts previous findings by Lettré *et al.* (127) in cultures of chick fibroblasts. Here a synergism between colchicine (0.01 mg/ml) and cortisone (added to cultures in crystal form) was noted (9 mitoses with colchicine alone, 31.3 with cortisone added).

In rats, colchicine and deacetyl-N-methylcolchicine had little effect on the mitoses of eosinophilic leukocytes, although all other mitoses were arrested. de Harven (51), supposing an intervention of the adrenals and cortisone, studied the mitotic index in the bone marrow of rats, 6 hours after deacetyl-N-methylcolchicine. In controls, and in adrenalectomized animals treated with cortisone, no mitotic arrest was evident, whereas in adrenalectomized animals, the percentage of cells in metaphase was increased and the number of eosinophil mitoses was up to nine times higher than that seen in the controls. For this type of cell, corticosteroids appeared to have a protective action, the spindle poisoning being visible only after adrenalectomy. Piscitelli *et al.* (178) studied further the action of deacetyl-N-methylcolchicine on the bone marrow of intact and adrenalectomized rats. It became clear that during the first 4 hours of action of the spindle poison, the mitotic arrest was about equal in the two groups. Later, in intact animals, the mitotic index dropped rapidly, and returned to normal after about 8 hours. In adrenalectomized animals, on the contrary, the mitotic index continued to rise until the eighth hour, with great individual variations. These results were not considered to express a simple pharmacological antagonism between cortisone and deacetyl-N-methylcolchicine, but rather to indicate a favorable action of cortisone on the recovery of the spindle. The mitoses in the hair-bulbs of the skin of rats were affected in the same way by cortisone: in adrenalectomized animals, the numbers of anaphases and telophases were higher 8 hours after deacetyl-N-methylcolchicine when cortisone was injected (52).

Colchicine acts similarly on most cells. Even unicellular organisms like *Chlamydomas*, which had been considered resistant (45), display mitotic abnormalities when treated with 0.2% solutions (24). Some differences in sensitivity may be linked differences of permeability; in the case of *Amoeba sphaeronucleus*, the typical spindle effect is observed only after intracellular injection of the drug (43). In mammals, cell sensitivities may vary from one tissue to another and are often related to the rate of cell division and the duration of mitosis. Immature eosinophils in the bone marrow of the rat, which had been thought resistant by de Harven (51, 54, 73), show arrested mitoses much later than other bone-marrow elements. This may be explained if these cells have a longer intermitotic time and a longer duration of mitosis. It has been estimated that the mitosis of

eosinophil granulocytes lasts about 2 hours, while the other bone-marrow cells divide in 45 minutes on the average. The intermitotic time for eosinophils is also probably two or three times longer. It is possible that still other cells may show similar differences in behavior, without being truly resistant to colchicine. This has been suggested for mastocytes (2), as an explanation of the fact that Padawer (161) was unable to find any mitoses in the mastocytes of colchicine-injected rats.

Colchicine may affect cell growth by indirect effects, quite unrelated to mitosis. It had been observed that colchicine modified the process of axon-sprouting in denervated muscles, apparently by reducing the viscosity of the cortical layers of the axon (104). Singer *et al.* (210) found that locally injected colchicine inhibited the regeneration of limbs in adult *Triturus*. This was the consequence of the degeneration of the axon, and lasted far longer than any mitotic effects. Although the nerves of the opposite limb showed some degeneration, it appears that the local concentration of colchicine was much higher than that required to affect cell division. By local infusions of colchicine (0.1 M) in the sciatic nerve of the mouse at the rate of 0.005 ml/hr, Angevine (1) found axonal degeneration after 24 hours, followed by myelin destruction, and Schwann cell proliferation. Smaller concentrations (0.01 to 0.04 M) produced more diffuse nervous alterations. These could be antagonized neither by mesoinositol nor by ATP, which appeared to increase the neuropathy. The intracerebral injection of colchicine (8.6 mg/kg) is rapidly fatal, as had been found by early authors (59). The resistance of the hamster towards the antimitotic effect was unrelated to this type of toxicity, for the intracerebral injection was rapidly fatal, with the same manifestations after a dose similar to that which killed the mouse. The changes in the nerves were also affected in the same way as in the mouse with similar concentrations (0.1 m and 0.2 M).

C. Other spindle poisons of natural origin

Colchicine is by no means the only spindle poison found in plants; moreover, antibiotics with similar properties have been described.

1. Vinblastine. In 1955, Beer (7) reported that certain extracts of the Jamaican periwinkle (*Vinca rosea*) injected into rats produced a severe leukopenia with infectious complications. Because of the frequent association of growth depression of the bone marrow and potential chemotherapeutic effects against tumors, these extracts have been in recent years the subject of many publications (34, 47, 48, 58, 103, 112). Cutts, Beer and Noble (47) found that not only the bone marrow but also the intestinal mucosa was damaged. The most active of the alkaloids isolated from *Vinca rosea*, originally named "vincaleukoblastine," and now called vinblastine, had an infrared spectrum nearly identical to that of an equimolecular mixture of two substances present in the plant, catharanthine ($C_{24}H_{24}O_2N_2$) and vindoline ($C_{25}H_{32}O_6N_2$). The exact chemical formula is not yet known, but these substances are pentacyclic derivatives of indole; they belong to a new family of mitotic poisons (153, 154). Vinblastine has the general formula $C_{46}H_{58}O_9N_4$ (112). After favorable results with leukemic mice, human cases of leukemia were treated with vinblastine. Arrested metaphases were ob-

served in the bone marrow of two such patients (103). Hepatic regeneration in the rat was not affected (47). In cultures of human cancer cells a linear increase of the mitotic index, up to 45%, followed the addition of vinblastine. This effect lasted as long as 53 hours (170). Anaphase bridges and pluricentric mitoses were also observed (112, 170). The inhibition of neoplastic growth *in vitro* could be partially antagonized by tryptophan or by preparations containing coenzyme A. A complete antagonism was found with glutamic acid, aspartic acid, α-ketoglutaric acid, ornithine, citrulline, or arginine (112).

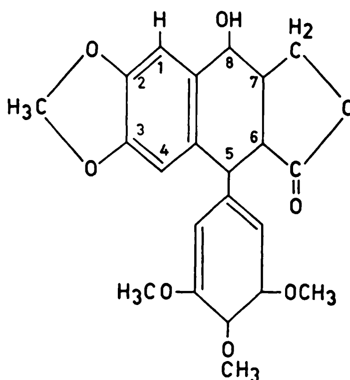
Cutts (48) compared the action of vinblastine with that of colchicine on the bone marrow of the rat and on ascites tumors of the mouse. The action of the new drug lasted longer than that of colchicine. Arrest in metaphase, secondary to an inhibition of the spindle activity, led to an accumulation of mitotic figures. The antagonism of this action by tryptophan and glutamic acid was studied in Swiss mice bearing the Ehrlich ascites tumor. The percentage of arrested mitoses was decreased from 9.2 with vinblastine alone to 1.84 with tryptophan, and to 1.39 with glutamic acid (controls: 0.93% mitoses). This was the consequence of an incomplete metaphase arrest, for up to 27.1 and 22.2 of postmetaphase stages per hundred cells could be seen, while none was present without antagonist, 12 hours after the injection. At 24 hours, however, smears of ascitic cells showed that the number of arrested mitoses was as great as in animals receiving vinblastine alone, indicating that the antagonism was only temporary.

Cardinali *et al.* (34) found similar effects on mitosis. In leukemic cells, vinblastine decreased the number of prophases during the first hours of its action, as demonstrated by the slow rise of the mitotic count. The mitotic activity in the Ehrlich ascites tumor was further studied in animals which received 0.025 mg of vinblastine per kg daily for four consecutive days, and cortisone, at doses of 25 and 50 mg/kg. The number of arrested mitoses was lower in the cortisone-injected animals. This may be evidence of a true antagonism or of some inhibition of mitotic growth before prophase. The mitotic index was followed for only 4 hours and it is not known whether poisoning is of shorter duration in animals receiving cortisone. A temporary antagonism between glutamic acid, glucose, or D-ribose, and vinblastine was also observed (58). These findings are interesting in relation to the above-mentioned work of de Harven (51) (section III B), which was apparently unknown to the Italian workers.

2. Podophyllin and other substances of plant origin. The spindle-poisoning activities of the active principles of the resin of *Podophyllum*, podophyllin and α- and β-peltatin, bring more evidence that substances of widely different chemical structure may suppress the fibrous structure of the spindle. The literature about podophyllin has been reviewed (115) and only some points will be mentioned here. The drug itself was discovered following the popular use of the resin in the treatment of benign papillomatous growths of the skin (77, 212). Colchicine has similar favorable effects on these tumors (18), as does another spindle poison, chelidonine (124, 126), extracted from *Chelidonium majus*, a plant used in popular medicine for the treatment of warts.

The cytological action of podophyllotoxin is quite similar to that of colchicine,

though in animal tissues more atypical cells with giant, highly polyploid nuclei have been described (115). Little is known about the biochemical aspects of the action of podophyllin (36).



Podophyllotoxin
[trans (8:7) trans (7:6) cis (6:5)]

Studies of the relation between chemical structure and antimitotic action of derivatives of podophyllotoxin have been carried on by Santavy *et al.* (137, 197). The relative toxicity is higher than that of colchicine, a fact which may be expressed by the index, LD100/smallest dose affecting the spindle. This is 1.5 to 2.7 for podophyllotoxin derivatives, 2.5 for colchicine, and 7 for deacetyl-N-methylcolchicine. Chemically, it appears that the spindle-poisoning activity requires 1) a free hydroxyl in position 1 or 8, 2) the integrity of the hydroxyl attached to carbon 8, and 3) a definite stereochemical configuration, the position trans (8:7) cis (7:6) trans (6:5) leading to an inactive compound [*cf.* the inactivity of *iso*colchicine as opposed to colchicine (76)].

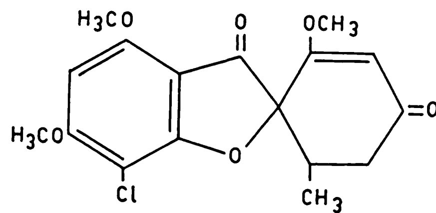
There is a slight suggestion of the possibility of a carcinogenic action of podophyllotoxin. The monstrous cells of the uterine cervical epithelium of the mouse after its administration resemble neoplastic cells found in cervical smears. Prolonged application of podophyllin to the mouse cervix was studied. Out of 50 animals, each painted twice weekly with podophyllin in liquid petrolatum, 30 were available for histologic study, and only one epidermoid carcinoma of the vagina and cervix was found; this animal also developed leukemia (113). However, podophyllotoxin had been previously found to have neither carcinogenic nor cocarcinogenic actions on the skin of mice (8).

Several other substances of plant origin have been tested by Lettré (122) in tissue cultures. Narcotin is a weak mitotic poison, less potent than colchicine (123) by a factor of 2000. Chelidonin is 80 times less active, and concentrations of 1 μg/ml of medium inhibit mitoses of chick fibroblasts. The complex structure of this substance bears some resemblance to that of podophyllotoxin (122). The cytological effects in human tumor cells of the HeLa strain are typical, with chromosomes scattered throughout the cell (124), a circumstance that leads to the formation of multiple small nuclei (caryomery) (126).

Extracts, yet unpurified, of other plants, have been shown to arrest mitosis at metaphase; *e.g.*, *Chimaphila maculata* (reported in the treatment of malignant disease in 1818) and *Sassafras albidum* (76).

3. Griseofulvin and other antibiotics. Colchicine can produce hypertrophy of hyphae and failure to form conidia in several species of fungi, and can profoundly modify the architecture of the alga, *Hydrocyction* (92), through a possible action on cell-wall formation.

The antifungal antibiotic griseofulvin was isolated in 1939 from *Penicillium griseofulvin* Dierckx (160) and shown to be like colchicine in inhibiting metaphase and modifying the shape of growing mycelium. It was also shown (22) to disturb the synthesis of the cell wall in plants. Its utilization in medicine results from its accumulation in keratin, leading to cures of infections caused by some dermatophytes resistant to all other drugs. In 1958, Paget and Walpole (162) found that large doses (100 to 200 mg/kg), given to the rat intravenously, arrested at metaphase the mitoses of bone marrow, intestinal mucosa, testis, and transplanted tumors. No mitotic action could be observed with oral doses (140 mg/lb) in the skin and the hair bulbs of man (37). The parenteral injection of 2 mg/kg in the rat for five consecutive days caused necrosis of the seminiferous tubules and intestinal lesions (163). The intravenous injection of 200 mg/kg leads, after 2 and 6 hours, to an accumulation of arrested mitoses in the Lieberkuhn glands of the intestine and in the bone marrow (118, 162). In plant cells (root tips of *Pisum*), the effect is similar to that of colchicine but less complete (163).

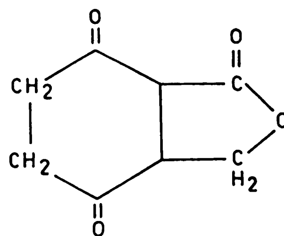


Griseofulvin

The mechanism of action of griseofulvin on dermatophytes has been extensively studied. The inhibition of growth is partially prevented by purines, pyrimidines, and their nucleotides (131). The structural analogies of griseofulvin and purine-ribosides have been emphasized. The antibiotic would interfere with the "polymerization state during the reduplication of chromosomes" and with the formation of the cell wall (131). This last aspect has been studied with the help of the electron microscope (17). The youngest and the most active cells of dermatophytes are the most affected, and the membrane "seems to lose its integrity, although it may become much thickened" (17).

A curious finding is that methylcholanthrene-induced papillomas grow faster and are more numerous in rats receiving griseofulvin at a level of 1% in the diet; at a level of 0.01%, given for 6 weeks before methylcholanthrene, the tumors appeared more rapidly and were larger and more numerous than in controls.

Griseofulvin alone had no similar effect. In the 1% group, some squamous-cell carcinomas did develop. Griseofulvin is not carcinogenic (5, 6, 118).



Patulin

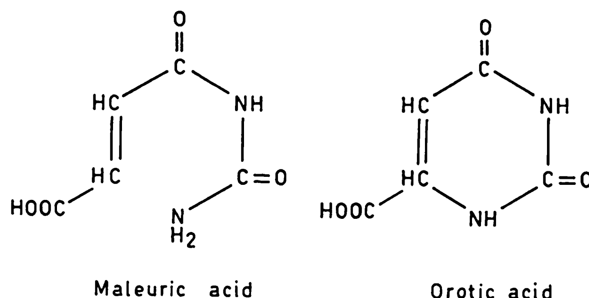
Patulin, a lactone with a much simpler chemical structure than other spindle poisons of natural origin, inhibits the mitoses of fibroblasts at concentrations of 5×10^{-6} g/ml (3). In the rat, doses of 0.01 mg/g had a typical action on bone marrow, lymph glands, and intestine, in which arrested metaphases could be found 24 hours later (169). In the liver of rat embryos, after an injection of 0.2 mg into the pregnant rat, the mitotic index rose within 12 hours from 39 to 103 per thousand cells, as a result of the metaphase arrest; this was already visible after 4 hours (169). Similar effects were reported on the developing eggs of *Triturus* and *Pleurodeles* (200), varying somewhat with the concentration. At 1:500 the spindle was destroyed, at 1:1000 multinucleated eggs were formed, and at 1:2000 multipolar spindles were observed. Chromosome ruptures and nuclear degeneration were visible at the lowest concentrations used, 1:4000 (206).

D. Partial spindle inactivation: "polar-chromosome" metaphases

Spindle poisons do not necessarily transform the whole spindle into a structureless mass. With small doses, the size of the spindle may simply be reduced, and chromosomes may be able to move normally to the equatorial plate. Often some chromosomes appear to be "lost" in this movement towards the equator, and remain undivided in the region of the centrioles; these are usually the smallest ones. Mitoses with lost chromosomes had long been known to occur spontaneously in malignant cells (74). A renewed interest in this type of abnormality stems from the demonstration in the mouse (166), that hydroquinone arrested the cells of the intestinal glands at metaphase, with an apparently intact and fibrillar spindle and small groups of chromosomes at each pole. This disposition of the chromosomes, which could be observed in tissue cultures treated with hydroquinone (165), appeared to be related to the mitoses with "lost" chromosomes described by von Moellendorff in his work on the action of steroid hormones on tissue cultures (149, 150). It was later (168) demonstrated that the metaphase plate could divide normally, the small chromosomes remaining at each pole (metaphase and anaphase with "polar chromosomes"). The interest in this problem was renewed with the finding that some early neoplastic changes in the cervical epithelium of the human uterus were frequently associated with this type of "polar chromosome mitosis" (74, 75, 98, 99, 167). Only some of the

phenols cause this effect (164), which is related to their spindle-inhibitory properties. Polar chromosomes were found after injection into the mouse of the following in order of decreasing activity: hydroquinone, toluhydroquinone, *o*- and *m*-cresol, and phenol. Related substances such as pyrogallol and aminophenol are inactive. The spindle disturbances are observed only during the first hours of action of hydroquinone, which later depresses all mitotic activity and leads to extensive nuclear degeneration similar to that observed with "chromosome poisons." The effect of hydroquinone varies from one species of animal to another (164). Phenol causes mitotic abnormalities also in dividing eggs (204).

Polar chromosome metaphases have been observed with several other substances that are toxic to the spindle: deacetyl-N-methylcolchicine (72), chelidonine (125); carcinogens (croton oil, benzpyrene) (195), adrenochrome (96) and maleuric acid (157, 158). This last substance bears a structural resemblance to orotic acid, and may interfere with nucleoprotein metabolism. It acts on a variety of cells, animal and vegetable. In mice bearing the Ehrlich carcinoma, intraperitoneal injection of maleuric acid produces within 3 to 6 hours typical polar-chromosome metaphases and arrested metaphases of the colchicine type. Orotic acid does not protect the cells, but the injection of reduced glutathione prevents the cytological effects, although it cannot reverse the arrest of mitosis. Maleuric acid may act as an alkylating agent (158).



Maleuric acid   Orotic acid

The action of hormones on tissue culture mitoses, studied by von Moellendorff (149, 150) and by Lettré (122, 127) has been extended by Siebs (208), who has given excellent illustrations of mitosis with polar chromosomes. Cultures of chick fibroblasts and of two strains of human malignant cells were treated with steroids, a small crystal of the hormone being placed in the medium, close to the culture. All types of metaphase arrest of the colchicine type and of partial arrest of the hydroquinone type could be observed 24 and 48 hours after the combined action of diethylstilbestrol and testosterone. The first arrested metaphases, while the second had no visible effect: the polar-chromosome metaphases were found in the zone influenced by the two steroids. Multipolar mitoses were also observed 24 hours later (209). Diethylstilbestrol alone may induce polar chromosome metaphases.

Several other substances of natural origin were found to have similar effects on tissue cultures, in particular adrenochrome in solution (80 to 120 μg/ml), alone

or in combination with testosterone, and adenosine (0.02 M). Of the other substances having similar action, dichlorbenzimidazole (in solid form), colchicine and colchicine derivatives, 6-puryltryptamine (125) and 6-(β-indolylethyl)-aminopurine may be mentioned (208, 209). The abnormal mitoses may lead to the formation of trinucleated cells, each of the three groups of chromosomes building a nucleus. A study of the abnormal metaphases in living cells by phase contrast confirmed that the migration of the chromosomes towards the equatorial plate at early metaphase was disturbed (208).

E. Carbamates

The mitotic arrest at metaphase produced by phenylurethane (phenylethylcarbamate) was studied by Warburg on sea-urchin eggs in 1910 (222). The rather extensive literature on carbamates, following the discovery of the therapeutic properties of ethylcarbamate in human leukemias (171), has been reviewed by Cornman (46). While methylcarbamate is devoid of any cellular action and has a very low toxicity in mammals, ethylcarbamate has a complex action on spindle and nuclei (62).

An extensive study of the antimitotic action of phenylurethane on the eggs of *Triturus* and *Pleurodeles* has been made by Sentein (207). Complex actions on the spindle and the asters have been described. These indicate an antagonism between the formation of fibers and the reduplication of the centrioles. When phenylurethane inhibits the achromatic apparatus, the centrioles undergo a rapid multiplication which results, after the drug has been removed, in the appearance of multicentric mitoses. The hypothesis of a nuclear control of the multiplication of the centrioles has been suggested. This is somewhat comparable to the atypical spermatogenesis of snails, where multiple centrioles are formed only in the cells in which the chromosomes become detached from the spindle, without contributing to the formation of the spermatozoal nucleus (82).

F. Nonspecific spindle inhibition

Only a few of the hundreds of drugs acting on the spindle have been mentioned. From the extensive work which has been carried out on plant cells, it appears that a great number of chemicals, quite unrelated to one another, may prevent normal spindle activity if their concentration is high enough. This nonspecific inhibition, however interesting it may be for cytologists, is outside the field of this review (56, 128).

Some results are confusing—for instance, spindle inhibition by folic acid antagonists, such as aminopterin. This drug, when injected into animals, acts mainly on nuclei and chromosomes. However, following the work of Hughes on the immediate action of various drugs on cells in culture (105), aminopterin has often been described as a spindle poison (110). Its action is quite different from that of a drug like colchicine. It has an immediate and transient effect on the spindle in tissue cultures, without any accumulation of arrested mitoses (8, 110). It would be premature, however, to discard any possibility of a role of folic acid in the activity of the spindle. Mazia (143), confirming other observations on the

basophilia of the spindle, has shown that there is a RNA fraction associated with the mitotic apparatus. Aminopterin may interfere with the formation of this RNA, the exact significance of which awaits further research.

The findings of Taylor (215) on the action of chloramphenicol indicate an altogether different type of spindle inhibition. This antibiotic inhibits the synthesis of proteins; in tissue cultures it has no action on the already formed spindle of cardiac cells of *Triturus viridescens*, while the cells treated before mitosis develop only very small spindles or none at all. The small spindles lead to a rosette arrangement of the chromosomes "around a small birefringent body": this may be similar to the "star-metaphases" after colchicine, where the centrioles occupy the center (55). This appears to result from the inhibition of the synthesis of the proteins of the mitotic apparatus.

Other examples of spindle inhibition will be mentioned in reviewing the cellular action of the so-called alkylating agents.

IV. CHROMOSOME POISONS

Under this heading will be considered a vast group of substances which modify the structure of the chromosomes or induce nuclear destruction in mitotically dividing tissues. For the microscopist, these effects have much resemblance to these of ionizing radiations; hence the expression "radiomimetic poisons," which has been widely used and criticized (67, 117, 119). As there are reasons for believing that on the molecular level the changes may be quite different—the chromosome poisons intervening mainly during the synthesis of new DNA or the assembly of chromosomes and the ionizing radiations being capable of altering already formed chromosomes—the expression "radiomimetic" will no longer be used.

The chromosome poisons belong to several groups, and their mode of action is certainly varied. They act mainly during the intermitotic period of DNA reduplication. The consequences are visible only during the succeeding cell division. The overall effect will often be one of severe mitotic depression, the mitotic index falling to zero in the most sensitive tissues. One difficulty is to distinguish such mitotic inhibition from a toxic or lethal effect unrelated to chromosome synthesis. For this reason, research on whole animals may be preferred to the study of isolated cells, for it will clearly indicate whether the cellular lesions are limited to zones of active growth, without damage to postmitotic cells.

A considerable difficulty arises from the fact that many substances of this group have been tested for their inhibitory growth only on neoplastic tissues, without any precise description of their action on normal cells.

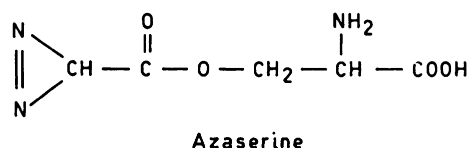
A. Inhibitors of nucleic acid synthesis

Recent work on the pathways of RNA and DNA synthesis explains how cell growth may remain unaffected, while the synthesis of DNA is halted (156). Folic acid antimetabolites of the aminopterin type prevent the onset of mitosis, while the cells continue to enlarge with a large nucleus and nucleolus and a normal cytoplasmic (RNA) basophilia (66, 70, 71, 93, 94).

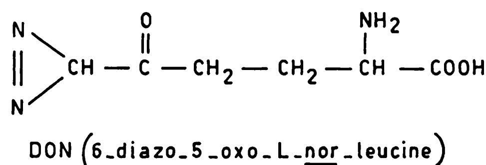
In mammals, this type of inhibition affects the most rapidly growing tissues (bone marrow and intestine). It results first in a sharp decrease of the mitotic index. In the subsequent hours, the cells of the germinative regions, those which are not differentiating and are not in a postmitotic condition, undergo a rapid destruction, with a sudden condensation of their chromatin into irregular masses; this is followed by necrosis. In mice, this is most striking about 8 hours after the injection of a chromosome poison: the cells appear unable to resist the increased duration of the intermitotic period (70). A similar effect is observed after irradiation with X-rays. It may not be fundamentally different from the death after metaphase of arrested mitoses without spindles.

The antagonists of folic acid (173) (aminopterin, Methotrexate, *etc.*) have the best understood action of all chromosome poisons. The inhibition of mitoses, leading to aplasia of tissues such as bone marrow, intestine, testis; the pycnonecrosis appearing after the cessation of mitotic activity (66); and the persistence of nuclear, nucleolar, and cellular growth without increase in the amount of DNA, have been thoroughly described (93, 94). The inhibition of spindle activity, shortly after the addition of antifolic substances to tissue cultures (110), which has been already mentioned, is not visible in whole animals (66). The metabolic pathways in which the active form of folic acid, tetrahydrofolic acid (THF), is involved in the synthesis of nucleic acids, have been reviewed recently (156). The synthesis of thymidylic acid is especially sensitive to inhibition by aminopterin. It is by no means the only reaction interfered with by this substance, which inhibits the transformation of folic acid to dihydrofolic acid (DHF) and affects particularly the conversion of DHF to THF, catalyzed by DHF-reductase. The binding of aminopterin to this enzyme "is undoubtedly the single most important action of the (antifolic) drugs" (156).

This observation indicates that the various antimetabolites which interfere with cell division inhibit synthesis, not usually as abnormal building-blocks, but more frequently as inhibitors of the formation of coenzymes (132). This statement applies also to the large number of antimetabolites of purine and pyrimidine bases which have been tested for their growth-inhibitory properties. These agents, which have been reviewed in detail by Biesele (13, 14), are mainly analogs of purines and pyrimidines, such as 2,6-diaminopurine, 2,4-diaminopyrimidines, 8-azaguanine, 6-azauracil (and its ribonucleoside, 6-azauridine), 4-aminopyrazolo-pyrimidine, 6-mercaptopurine, 6-thioguanine, 6-(β-indolylethyl)-aminopurine, fluorinated purines and pyrimidines (particularly 5-fluorouracil and its deoxyribonucleoside, 5-fluorodeoxyuridine), 5-fluoro-orotic acid, 6-selenopurine, 6-chloropurine, *etc.* The list is long, and the search is still going on for antimetabolites which would prevent neoplastic cells from dividing without harming normal tissues. Most of these substances have in common the property of inhibiting mitosis by interfering with DNA metabolism. The most evident morphological lesions affect the chromosomes at metaphase and anaphase (breakage, bridges, translocations, *etc.*). The spindle effects seem always to be negligible. Several reviews having been written on this subject, no detailed discussion will be given here (14, 100, 132, 136).



Azaserine



DON (6-diazo-5-oxo-L-nor-leucine)

To this same group belong two substances which are at the same time antimetabolites and antibiotics: *azaserine* and *diazonorleucine* (DON). These compounds are antagonists (or analogs) of glutamine, which is essential for the formation of a phosphorylated ribosylamine that reacts with glycine to form glycinamide ribonucleotide, a precursor of the first purine, inosinic acid (132). The role of glutamic acid in preventing the action of some spindle poisons should be recalled here (58). Azaserine decreases the number of mitoses of ascites tumor cells, although their volume continues to increase (80).

B. Antibiotics

The search for carcinostatic agents has led to the study of many substances extracted from molds that inhibit mitosis; griseofulvin, patulin, and azaserine have been mentioned. In this field, few morphological studies are available.

The actinomycins, a group of polypeptides (111), were shown in 1951 to increase for a short time duodenal mitoses in mice injected intravenously with 2.5 μg of the impure material; the percentage of metaphases was higher than normal. Later, all mitoses were depressed, including those of neoplastic tissues. The antimitotic effect leads to an atrophy of thymus, spleen and lymph glands (97). A recent study of the action of actinomycins on human cells in tissue cultures confirmed the mitotic inhibition. There was a tendency towards nucleolar fragmentation (11). Intestinal nuclear destruction was observed in mice injected with 750 μg of actinomycin D per kg (129).

Sarcomycin and numerous derivatives have been tested in animals and cancerous patients. Pycnonecrotic nuclear degeneration and mitotic arrest have been mentioned (33).

Mitomycin, which has been used in the chemotherapy of neoplastic diseases, inhibits the growth of several transplanted tumors in the mouse, the rat, and the hamster. In the rat (175), repeated injections induced intestinal and gastric lesions and thymic atrophy. In the dog, anemia, bone marrow aplasia, and "severe karyorrhexis in the basal third of the epithelial glands" of the intestine have been mentioned. This antibiotic is supposed to act selectively on the synthesis of purines and DNA (175).

In a recent review (134), more than a dozen antibiotics with possible antimitotic effects in neoplastic growth were listed. It may be regretted that in the search for a drug that might control at least some forms of cancer, so few precise morphological descriptions of the antimitotic actions in normal tissues have been published.

C. Other chromosome poisons

One of the earliest chromosome poisons discovered was acriflavine (trypaflavine), described by Hertwig (102) as a "Radiumersatz," the first mention of a "radiomimetic" action of a chemical. Basic dyes are known to combine readily with DNA and RNA, a fact which explains why their noxious effects are complex. The acriflavine group appears to be especially toxic for mitosis (25). Of a series of derivatives tested on chicken fibroblasts *in vitro*, 5-aminoacridine hydrochloride was most active (120). Divisions under way were not disturbed, but cells were prevented from starting new mitoses, and pycnotic nuclear degeneration was observed. These effects were similar to those of small doses of radiation (120). Meyer (147) described various mitotic and chromosomal anomalies in the cornea of *Triturus* treated with trypaflavine. This substance also affects the division of amphibian eggs (199) and fibroblasts in tissue culture (25).

Ethylcarbamate (urethane), already mentioned for its spindle-poisoning properties, produces alterations of the intestinal mucosa in mice similar to those of chromosome poisons, *i.e.*, mitotic arrest (intermitotic inhibition) followed by pycnonecrotic degeneration of cells of the germinative zone (62). In the Walker tumor of the rat, a decrease of the number of mitoses and of chromosomal fragmentation was observed (20). The injection of thymine (though not of uracil) decreased the number of abnormal mitoses, suggesting that ethylcarbamate may interfere with the synthesis of thymine (20). It may also inhibit the incorporation of uracil into DNA (78).

Ethylcarbamate has also a carcinogenic action [lung adenomas and other neoplasms in mice (115a, 152, 186)], and it may act as a cocarcinogen (11). For Cornman (46) "almost any radical added to the urethan molecule increases its effectiveness as a mitotic poison and decreases or removes its carcinogenic action." The mitotic abnormalities produced by carbamates are varied, and range from decrease of mitotic activity to spindle inhibition and chromosome fragmentation. In eggs, segmentation may also be inhibited (46).[1] In the bone marrow of mice, an increase in the numbers of metaphases, chromosome bridges, and telophasic aberrations was found after a single injection of urethane, as well as giant promyelocytes, after repeated doses (187). These compounds depressed granulopoiesis more than the formation of red blood cells, a fact confirmed by the observations of human leukemic patients treated with urethane (63).

[1] About the pycnonecrotic effects observed in the intestine (62), Cornman thinks that it is "reasonable to suggest caution . . . regarding conclusions drawn from effects on whole animals." Nevertheless, ethylcarbamate having been used in the treatment of human leukemia and myeloma, it appears on the contrary indispensable to study its effects on whole animals.

Another group of chromosome poisons which has not attracted much notice includes simple quinones and aromatic diamines (164). Apart from the spindle effects of some of these, mentioned above, very severe destructive effects on germinative regions, in particular in the intestinal mucosa of the mouse, have been described (164). Of the diphenols, only the *para-* and *ortho-*forms are active. Pyrogallol is the most potent phenol, while phloroglucinol is without any effect. The mono-aminophenols are only slightly active. The phenylenediamines are also strong chromosome poisons, *p*-phenylenediamine being the most potent. The possible pathways of biochemical action may be inactivation of SH-substances, disturbances of oxidation-reduction, or chelation of metal enzymes (164). Naphthoquinones, which have been shown to inhibit mitosis and rupture chromosomes in tumor cells (148), were found to be inactive in these experiments (164).

D. *Alkylating agents*

The so-called alkylating agents, the prototype of which is *bis*(2-chloroethyl)sulfide, or mustard gas, have complex cellular actions which resemble closely those of radiation, for they are chromosome poisons, mutagens, and carcinogens, as well as agents which inhibit some types of malignant growth (228). Several review articles have been devoted to this group of chemicals, which has found many applications in cancer chemotherapy (13, 15, 19, 79, 83, 107, 116, 174, 188, 189). The principal cytological effects are chromosomal rupture and a general inhibition of mitosis. The mechanism of action of these substances is poorly understood. It appears possible that alkylation may occur at the level of enzymes or cofactors (132, 228) rather than of DNA molecules, which had been supposed to explain chromosome breakage (117). The formation of antimetabolites by the alkylation of purines or pyrimidines has been suggested (219).

The substances belonging to this group fall into several categories from the chemical point of view, but their cytological action is similar, although their antineoplastic properties may be quite different. The principal groups are the chloroethylsulfides and amines ("mustards") and their more complex derivatives, the ethyleneimines, the sulfonoxides, typified by busulfan (Myleran, sulfone-oxy-butane). Epoxides and certain N-alkyl-N-nitroso compounds with alkylating properties also inhibit cell division (219).

These substances have complex cytological effects, and their action on chromosomes can be masked by important modifications of the achromatic apparatus, which have been thoroughly studied in eggs (203). Koller (116), in a study of 18 alkylating agents of the sulfonoxy group, considers three successive cellular actions: 1) mitotic arrest and suppression; 2) "radiomimetic" nuclear damage; and 3) nonspecific, toxic alterations.

The action of busulfan on tissue cultures has been extensively studied by Chèvremont *et al.* (41), who found various cytoplasmic changes and disturbances of DNA reduplication, as measured spectrophotometrically, with abnormally high DNA contents per nucleus. Observations with tritium-labeled busulfan indicate that this substance becomes selectively fixed on nuclei (151).

V. OTHER MITOTIC INHIBITORS

A few substances must be considered here, in particular cortisone and other corticosteroids of the adrenal gland.

A. *Corticoids (cortisone, hydrocortisone, prednisone, etc.)*

Mention has already been made of antimitotic effects of hormones (127, 149, 150, 208). The action of the steroids of the cortisone type deserves further study. The discovery that cortisone may influence dividing cells stems from the works of A. P. Dustin (60) on "caryoclastic shock," and of Selye (198) on "stress" and the "general adaptation syndrome." It was found that various drugs may have a nonspecific damaging action on mitotic growth. This is limited to certain tissues, in particular the thymus and the lymphoid tissues (spleen, lymph glands and lymphatic tissue of the intestine). Selye (198) was the first to demonstrate that this action is mediated through the hypophysis and the adrenal, and that the secretion of adrenocorticotrophic hormone (ACTH) and cortisone was responsible for the destruction of lymphoid cells. The effects of many poisonous drugs and nonspecific aggressions (such as cold, excessive heat, and forced immobility) on lymphoid tissue disappear after adrenalectomy.

These changes are similar to those produced by the chromosome poisons, and involve a selective action on the germinative regions of lymphoid tissue (thymic cortex, germinative centers in lymphatic follicles), the most striking effect being the pycnonecrotic destruction of the cells of these regions after a period of mitotic depression (4, 184, 211). The depression of mitoses has been observed by several authors (109, 217, 218). The mitotic activity of the regenerating liver of hepatectomized mice is also strongly decreased by the subcutaneous injection of cortisone (172). This is paralleled by a marked increase of glycogen content and a decrease of total proteins in the hepatic cells. The increase of RNA is not affected, while that of DNA is strongly depressed. The mitotic activity of some other tissues, *e.g.*, the intestinal mucosa, is not affected, indicating that "any effect of cortisone on mitosis apparently varies greatly from one tissue to another" (185).

The relations between mitosis and nuclear damage have been discussed previously (69); it is not cetrain whether or not postmitotic cells in lymphoid tissue can be destroyed by cortisone. No precise quantitative studies on this subject are available. In a recent analysis of "stress" in new-born rats, a nearly complete disappearance of mitoses in the thymus has been observed preceding the nuclear destruction (95).

Although these lesions may superficially resemble those observed after any chromosome poison, it should be pointed out that cortisone has never been mentioned as a chromosome-damaging agent. On the contrary, it promotes the growth of some tissues or cells, *e.g.*, the bone marrow erythroblasts (81, 232). Cortisone has also been identified as an antagonist of such spindle poisons as deacetyl-N-methylcolchicine (54) and vinblastine (34). The metabolic effects of cortisone (*e.g.*, the depression of protein synthesis) may explain the nucleotoxic effects, but the absence of any depression of mitotic activity in a tissue as sensi-

tive as the intestinal mucosa indicates a selective toxicity towards the lymphoid cells. Cortisone, in this respect, is the opposite of the antifolic substances, which may depress bone marrow and intestinal growth without affecting the thymus and lymph glands (71, 72).

The antimitotic action of cortisone and the corticoid hormones which are supposed to act as "endogenous mitotic poisons" (70) clearly deserves further study. It appears that various steroids may stimulate mitosis (estrogens, *etc.*), may act as spindle poisons (127, 149, 208, 209), and may be inhibitors of the "chromosome" type (69).

B. Other substances inhibiting mitosis

Cortisone is not the only endogenous mitotic poison: epinephrine (28), adrenochrome, and a closely related substance, trihydroxy-N-methylindole (39), in some circumstances may inhibit mitosis. For Bullough (29), epinephrine would be one of the principal endogenous mitotic inhibitors. The mitotic inhibition by trihydroxy-N-methylindole would result from alterations of the mitochondria, which become rounded (as observed in tissue cultures) (39). The inhibition of mitotic growth by the nucleases is of great interest (21, 40), but too little is known about the mechanisms involved to warrant further discussion. The already vast literature on mitotic inhibition by compounds such as chloral hydrate (86, 149) and various substances inducing "cell-narcosis" will not be discussed, for it does not apply to mitotic growth in animals.

VI. CONCLUSIONS

At this point, it may appear that, although only a small number of the mitotic inhibitors has been considered, the problem of antimitotic action remains rather confused. Clear-cut classifications are no longer possible; in the same group of substances (*e.g.*, the steroid hormones) actions are to be found varying between mitotic stimulation and selective inhibition of one of the steps of mitosis. Moreover, several substances may affect either spindle or chromosomes, depending on the experimental conditions. The biochemical basis of most antimitotic action is shrouded in obscurity, even though some discoveries about the chemistry of the mitotic apparatus, the role of folic coenzymes in nucleic acid metabolism, the structure of the spindle fibers, and the antagonists of mitotic poisons, open the path for further clarifications of the subject.

It was pointed out several years ago (64) that mitotic poisons appeared far more promising in the biochemical analysis of cellular division than in cancer chemotherapy. Since then, thousands of drugs have been tested, and so far, the only ones which have found some use in medicine remain dangerous, for the fundamental reason that they are mitotic poisons, and that some normal tissues grow faster than many neoplastic ones. If an antimitotic substance acting selectively on neoplastic growth is to be discovered, it should have no action on normal cells. That such a possibility may exist is indicated by the rare examples of mitotic antagonists with a specific action on some tissues: for instance, cortisone *vs.* lymphoid tissue, and griseofulvin *vs.* skin.

On the positive side, it must be emphasized that knowledge not only of cell division, but also of many fundamental biochemical changes related to cellular growth, has benefited from work with mitotic inhibitors. The study of DNA synthesis, the isolation of the mitotic apparatus, morphological studies of chromosomes, have considerably progressed because some of the mitotic poisons have proven to be useful tools.

Mitotic inhibitors are more than possible drugs for cancer chemotherapy; they are tools which enable the cytologist to dissect the interrelated phases of the mitotic cycle. This type of approach would certainly be facilitated if more nearly uniform methods were used. On the other hand, chance observations, like those which led to the discovery of griseofulvin and vinblastine (155), will always play a fundamental part in the development of science.

It should be evident from this review that many important aspects of mitosis have not been touched by the use of mitotic inhibitors. The reduplication of the centrioles (a remarkably complex phenomenon), the disappearance and synthesis of the nucleolus, the mechanisms of cleavage, the modifications of cell surface, the multiplication of mitochondria and other cell organelles, the control of mitosis itself in the pluricellular animal, remain poorly understood. Mitosis in plants and animals appears to be quite similar, and many mitotic poisons, among them the most active, act similarly on both. Cortisone, however, would never had been found to have an antimitotic action if only plant cells had been used. In pluricellular organisms, growth may be controlled by organ-specific substances that inhibit mitosis, but these remain to be isolated.

Apart from the pharmacological and toxicological aspects of these problems, it should be pointed out *in fine* that the antimitotic properties of so many natural substances—hormones, plant extracts, antibiotics—indicate that the various types of mitotic inhibition belong to general biology, and that their study helps to understand better the normal control of mitotic activity.

ACKNOWLEDGMENT

I wish to acknowledge the precious help of Mrs. L. Gistelinck in the preparation and typing of the manuscript.

REFERENCES

1. Angevine, J. B., Jr.: Nerve destruction by colchicine in mice and golden hamsters. J. exp. Zool. 136: 363–391, 1957.
2. Allen, A. M.: Mitosis and binucleation in mast cells of the rat. J. nat. Cancer Inst. 28: 1125–1152, 1962.
3. Astaldi, G.: Azione antimitotica di alcuni antibiotici. Atti. I. Symposio sugli antimitotici, pp. 271–287. San Remo, 1955.
4. Baker, B. L., Ingle, D. J. and Li, Choh Hao: The histology of the lymphoid organs of rats treated with adrenocorticotropin. Amer. J. Anat. 88: 313–350, 1951.
5. Barich, L. L., Nakai, T., Schwarz, J. and Barich, D. J.: Tumour-promoting effect of excessively large doses of oral griseofulvin on tumours induced in mice by methylcholanthrene. Nature, Lond. 187: 335–336, 1960.
6. Barich, L. L., Schwarz, J. and Barich, D.: Oral griseofulvin, a cocarcinogenic agent to methylcholanthrene-induced cutaneous tumors. Cancer Res. 22: 53–55, 1962.
7. Beer, C. T.: The leucopenic action of extracts of *Vinca rosea*. Brit. Emp. Cancer Campaign 33: 487–488, 1955.
8. Benitez, H. H., Murray, M. R. and Chargaff, E.: Studies on inhibition of the colchicine effect on mitosis. Ann. N. Y. Acad. Sci. 58: 1288–1302, 1954.
9. Berenblum, I.: The effect of podophyllotoxin on the skin of the mouse, with reference to carcinogenic, cocarcinogenic and anticarcinogenic action. J. nat. Cancer Inst. 11: 839–841, 1951.
10. Bernhard, W. and de Harven, E.: L'ultrastructure du centriole et d'autres éléments de l'appareil achromatique. Verh. Vierter Int. Kongress f. Elektronenmikroscopie, vol. 2, pp. 217–227. Springer Verlag, Berlin, 1960.

11. Bierling, R.: Die Wirkung von Actinomycinen auf menschliche Gewebe in vitro. Z. Krebsforsch. **63**: 519–522, 1960.
12. Biesele, J. J., Berger, R. E., Clarke, M. and Weiss, L.: Effects of purines and other chemotherapeutic agents on nuclear structure and function. Exp. Cell Res. **2**: 279–303, 1952.
13. Biesele, J. J.: Mitotic Poisons and the Cancer Problem. Elsevier Publ. Co., Amsterdam, 1958.
14. Biesele, J. J.: Action of certain antimetabolites as mitotic poisons. In: Fundamental Aspects of Normal and Malignant Growth, ed. by W. W. Nowinski, pp. 926–951. Elsevier Publ. Co., Amsterdam, 1960.
15. Biesele, J. J.: On the mechanisms of antimitotic action, as studied with cancer cells and antimetabolites. Path. Biol. **9**: 466–473, 1961.
16. Biesele, J. J.: Experimental and therapeutic modifications of mitosis. Cancer Res. **22**: 779–787, 1962.
17. Blank, H., Taplin, D. and Roth, F. J., Jr.: Electron microscopic observations of the effects of griseofulvin on dermatophytes. Arch. Derm. **81**: 667–680, 1960.
18. Bourg, R. and Dustin, P., Jr.: Le traitement des papillomes vulvaires par l'application locale de colchicine. Pr. méd. **53**: 578–579, 1945.
19. Boyland, E.: Mutagens. Pharmacol. Rev. **6**: 345–364, 1954.
20. Boyland, E. and Koller, P. C.: Effects of urethane on mitosis in the Walker rat carcinoma. Brit. J. Cancer **8**: 677–684, 1954.
21. Brachet, J. and Ledoux, L.: L'action de la ribonucléase sur la division des oeufs d'Amphibiens. Exp. Cell Res. suppl. **3**: 27–36, 1955.
22. Brian, P. W.: Studies on the biological activity of griseofulvin. Ann. Bot., Lond. **13**: 59–77, 1949.
23. Branceni, D., Peterfalvi, M. and Jequier, R.: La N-désacétylthiocolchicine. Etude expérimentale des propriétés antimitotiques. Arch. int. Pharmacodyn. **107**: 156–158, 1956.
24. Buffaloe, N. D.: Some effects of colchicine on cells of *Chlamydomonas eugametos* Moewus. Exp. Cell Res. **16**: 221–231, 1959.
25. Bucher, O.: Zur Kenntnis der Mitose. VI. Der Einfluss von Colchicin und Trypaflavin auf den Wachstumsrhythmus und auf die Zellteilung in Fibrocytenkultmen. Z. Zellforsch. **29**: 283–322, 1939.
26. Bucher, O.: Zur Kenntnis der Mitose. Die Wirkung von Arsenik auf Fibrocyten Kulturen. Z. Zellforsch. **30**: 438–462, 1940.
27. Bucher, O.: Über die Wirkung sehr kleinen Colchicindosen nach Untersuchungen an *in vitro* gezüchteten Bindgewebszellen. Schweiz. med. Wschr. **75**: 715–718, 1945.
28. Bullough, W. S.: Stress and epidermal mitotic activity. 1. The effects of the adrenal hormones. J. Endocrin. **8**: 265–274, 1952.
29. Bullough, W. S.: A study of the hormonal relations of epidermal mitotic activity in vitro. III. Adrenalin. Exp. Cell Res. **9**: 108–115, 1955.
30. Bullough, W. S.: The control of mitotic activity in adult mammalian tissues. Biol. Rev. **37**: 307–342, 1962.
31. Burgoon, C. F., Jr., Graham, J. H., Keiper, R. J., Urbach, F., Burgoon, J. S. and Helwig, E. B.: Histopathologic evaluation of griseofulvin in *Microsporum audouini* infections. Arch. Derm. **81**: 724–732, 1960.
32. Burton, K.: Deoxyribonucleic acid. Brit. med. Bull. **18**: 3–9, 1962.
33. Caputo, A., Brunori, M. and Giuliano, R.: Antitumoral action of new sarcomycin derivatives. I. Importance of ethyl radical and substituted methylene groups. Cancer Res. **21**: 1499–1509, 1961.
34. Cardinali, G., Cardinali, G. and Blair, J.: The stathmokinetic effect of vincaleukoblastine on normal bone marrow and leukemic cells. Cancer Res. **21**: 1542–1544, 1961.
35. Carpentier, S. and Fromageot, C.: Activité c-mitotique des isomères γ et δ de l'hexachlorocyclohexane, avec des observations sur l'influence du mésoinositol et du mésoinositophosphate de sodium. Biochim. biophys. Acta **5**: 290–296, 1950.
36. Cervigni, T. and Massarelli, A.: Podofillina: azione inibente sull'attivita catalasica. Boll. Oncol. **29**: 533–544, 1955.
37. Chèvremont, M.: Le mécanisme de l'action antimitotique. Path. Biol. **9**: 973–1004, 1961.
38. Chèvremont, M.: La préparation à la mitose. Quelques modalités de son inhibition par des substances antimitotiques. Chemotherapia **2**: 191–209, 1961.
39. Chèvremont, M. and Chèvremont-Comhaire, S.: Recherches sur le trihydroxy-N-méthylindole et l'adrénochrome en culture de tissus. I. Action sur la croissance et la mitose. II. Action sur le chondriome. Arch. Biol. **44**: 399–437, 1953.
40. Chèvremont, M., Chèvremont-Comhaire, S. and Firket, H.: Etude de l'action de la ribonucléase sur des cellules vivantes cultivées in vitro et en particulier de ses effets sur la mitose. Arch. Biol. **47**: 635–656, 1956.
41. Chèvremont, J. F. and Baeckeland, E.: Contribution à l'étude de l'action cellulaire du Myleran. I. Modifications cytologiques dans les fibroblastes cultivés in vitro et traités pendant des temps plus ou moins longs. II. Modifications quantitatives de leurs acides désoxyribonucléiques. Bull. Acad. Méd. Belg. 6ème série **25**: 141–177, 1959.
42. Child, F. M.: The characterization of the cilia of *Tetrahymena pyriformis*. Exp. Cell Res. **18**: 258–267, 1959.
43. Comando, J. and De Fonbrune, P.: Action de la colchicine sur *Amoeba sphaeronucleus*. C. R. Soc. Biol., Paris **136**: 410–411, 423, 460, 461, 746, 747, 748, 1942.
44. Cook, J. W. and Loudon, J. D.: Colchicine. In: The Alkaloids, Chemistry and Physiology, ed. by R. H. F. Manske and H. L. Holmes, vol. 2. Academic Press, New York, 1952.
45. Cornman, I.: Susceptibility of *Colchicum* and *Chlamydomonas* to colchicine. Bot. Gaz. **104**: 50–61, 1942.
46. Cornman, I.: The properties of urethan considered in relation to its action on mitosis. Int. Rev. Cytol. **3**: 113–130, 1954.
47. Cutts, J. H., Beer, C. T. and Noble, R. L.: Biological properties of Vincaleukoblastine, an alkaloid in *Vinca rosea* Linn with reference to its antitumor action. Cancer Res. **20**: 1023–1031, 1960.

48. Cutts, J. H.: The effect of Vincaleukoblastine on dividing cells in vivo. Cancer Res. 21: 168–172, 1961.
49. D'Amato, R.: Attivita citologica del dimercaptopropanolo (BAL) e del metilarsinato di sodio (arrhenal) e loro azione combinate. Caryologia 2: 13–22, 1949.
50. de Harven, E.: A propos de la résistance du hamster doré (*Mesocricetus auratus*) à certains poisons stathmocinétiques. Bull. Acad. Belg. Cl. Sci. 41: 1056–1060, 1955.
51. de Harven, E.: Recherches expérimentales sur l'éosinophilopoièse du rat. Action des hormones corticosurrénaliennes et des poisons stathmocinétiques. Rev. belge Path. 25: 93–154, 1956.
52. de Harven, E.: Action de la colchicine et de certaines hormones corticosurrénaliennes sur les mitoses des follicules pileux du rat. Rev. belge Path. 25: 277–285, 1956.
53. de Harven, E. and Bernhard, W.: Etude au microscope électronique de l'ultrastructure du centriole chez les Vertébrés. Z. Zellforsch. 45: 378–398, 1956.
54. de Harven, E. and Dustin, P., Jr.: La régulation hormonale de l'éosinophilie. II. Antagonisme entre certains poisons stathmocinétiques et la cortisone au niveau des myélocytes éosinophiles du rat. Rev. Hémat. 11: 131–140, 1956.
55. de Harven, E. and Dustin, P., Jr.: Etude au microscope électronique de la stathmocinèse chez le rat. In: Action antimitotique et caryoclasique de substances chimiques. Coll. int. Cent. nat. Rech. sci., Paris 88: 189–197, 1960.
56. Deysson, G.: Les facteurs de la mito-inhibition végétale. Exposés Annuels de Biologie Cellulaire, pp. 241–274. Masson et Cie., Paris, 1956.
57. Deysson, G.: Relations entre constitution chimique et activité mitoclasique dans la série de la colchicine. In: Action antimitotique et caryoclasique de substances chimiques, pp. 215–219. Cent. nat. Rech. sci., Paris, 1960.
58. Di Marco, A., Soldati, M. and Gaetani, M.: Ricerche sull'azione della "vincaleukoblastine sulphate" in associazione ad altre sostanze. Tumori 67: 279–288, 1961.
59. Dixon, W. and Malden, W.: Colchicine with special reference to its mode of action and effect on bone-marrow. J. Physiol. 37: 50–76, 1908.
60. Dustin, A. P.: La pycnose expérimentale—ou crise caryoclasique—réalisée par l'injection de dérivés de l'aniline. C. R. Soc. Biol., Paris 93: 465, 1925.
61. Dustin, P., Jr.: Some new aspects of mitotic poisoning. Nature, Lond. 159: 794–797, 1947.
62. Dustin, P., Jr.: The cytological action of ethylcarbamate (urethane) and other carbamic esters in normal and leukaemic mice, and in rabbits. Brit. J. Cancer 1: 48–59, 1947.
63. Dustin, P., Jr.: L'uréthane et son association à la radiothérapie dans les leucémies humaines. Rev. belge Path. 19: 115–174, 1948.
64. Dustin, P., Jr.: Mitotic poisons in the chemotherapy of malignant growth. Acta Un. int. Cancr. 6: 466–477, 1949.
65. Dustin, P.: Mitotic poisoning at metaphase and —SH proteins. Exp. Cell Res. suppl. 1: 153–155, 1949.
66. Dustin, P., Jr.: Lésions cellulaires provoquées par les acides 4-aminopteroyl-glutamiques chez la souris. Rev. Hémat. 5: 603–617, 1950.
67. Dustin, P., Jr.: Imitation chimique des radiolésions cellulaires par les agents "radiomimétiques." J. Radiol. Électrol. 32: 333–344, 1951.
68. Dustin, P., Jr.: Les poisons mitotiques "radiomimétiques" et le métabolisme des nucléoprotéines. Rev. belge Path. 22: 55–69, 1952.
69. Dustin, P., Jr.: Mitoses et caryoclasie. Contribution à l'étude des lésions radiomimetiques du tissu lymphoïde. Rev. Hémat. 8: 462–476, 1953.
70. Dustin, P., Jr.: Les facteurs de la mito-inhibition des cellules animales. Leur rôle dans la régulation de la croissance mitotique chez les Mammifères. Exposés Annuels de Biologie Cellulaire, pp. 189–240. Masson et Cie., Paris, 1956.
71. Dustin, P., Jr.: Die Zytostatischen Substanzen und ihre Wirkung auf die Hämopoese. In: Handbuch der gesamte Hämatologie, ed. by L. Heilmeyer and A. Hittmair, vol. 2, pp. 1–47. Urban u. Schwarzenberg, München, 1960.
72. Dustin, P., Jr.: Unpublished observations.
73. Dustin, P. Jr. and de Harven, E.: La régulation hormonale de l'éosinophilie sanguine et son mécanisme. Rev. Hémat. 9: 307–340, 1954.
74. Dustin, P., Jr. and Parmentier, R.: Données expérimentales sur la nature des mitoses anormales observées dans certains épithéliomas du col utérin. Gynéc. et Obstét. 52: 258–265, 1953.
75. Dustin, P., Jr. and Parmentier, R.: Données nouvelles sur les mitoses à chromosomes polaires. Atti I. Symposio sugli antimitotici, pp. 23–40. San Remo, 1955.
76. Eigisti, O. J. and Dustin, P., Jr.: Colchicine, in Agriculture, Medicine, Biology and Chemistry. Iowa State College Press, Ames, 1955.
77. Eisenmann: Über die locale Wirkung der Sabina. Virchows Arch. 18: 171–172, 1860.
78. Elion, G. B., Bieber, S., Nathan, H. and Hitchings, G. H.: Uracil antagonism and inhibition of mammary adenocarcinoma 755. Cancer Res. 18: 802–817, 1958.
79. Fell, H. B. and Allsopp, C. B.: The action of mustard gas (β,β-dichlorodiethylsulfide) on living cells *in vitro*. Cancer Res. 8: 145–161, 1948.
80. Fernandes, J. F., Le Page, G. A. and Lindner, A.: The influence of azaserine and 6-mercaptopurine in the *in vivo* metabolism of ascites tumor cells. Cancer Res. 16: 154–161, 1956.
81. Fruhman, G. J. and Gordon, A. S.: A quantitative study of adrenal influences upon the cellular elements of bone marrow. Endocrinology 57: 711–718, 1955.
82. Gall, J. G.: Centriole replication, a study of spermatogenesis in the snail *Viviparus*. J. biophys. biochem. Cytol. 10: 163–193, 1961.
83. Galton, D. A.: The use of Myleran and similar agents in chronic leukemias. Advanc. Cancer Res. 4: 73–112, 1956.

84. GAULDEN, M. AND CARLSON, J.: Cytological effects of colchicine on the grasshopper neuroblast *in vitro*, with special reference to the origin of the spindle. Exp. Cell Res. **2**: 416-433, 1951.
85. GAVAUDAN, P.: Pharmacodynamie de l'inhibition de la caryocinèse. Librairie Le François, Paris, 1947.
86. GAVAUDAN, P.: Les facteurs de la cytonarcose. Ds: Exposés Annuels de Biologie Cellulaire, pp. 275-361. Masson et Cie., Paris, 1956.
87. GELFANT, S.: The energy requirements for mitosis. Ann. N. Y. Acad. Sci. **90**: 536-549, 1960.
88. GELFANT, S.: A study of mitosis in mouse ear epidermis *in vitro*. Exp. Cell Res. **19**: 65-82, 1960; **21**: 603-615, 1960.
89. GELFANT, S.: Initiation of mitosis in relation to the cell division cycle. Exp. Cell Res. **26**: 395-403, 1962.
90. GELFANT, S.: Inhibition of cell division: a critical and experimental analysis. Int. Rev. Cytol. **14**: 1-39, 1963.
91. GOMPEL, C.: Sur l'inactivation du fuseau chez la souris par les substances thioloprives. Rev. belge Path. **22**: 85-92, 1952.
92. GORTER, C.: De invloed van colchicine op den groei van den celwand van wortelharen. Proc. Kon. Ned. Akad. Wetenschap. **48**: 3-12, 1945.
93. GRAMPA, G. AND DUSTIN, P., JR.: Analyse, par la colchicine, des effets radiomimétiques de l'acide 4-aminopteroylglutamique (aminoptérine). Rev. belge Path. **22**: 115-125, 1952.
94. GRAMPA, C. AND DUSTIN, P., JR.: Impiego associato di aminopterina e colchicina e anomalie nucleari: ricerce sperimentali sull intestino del topo. Tumori **39**: 63-71, 1953.
95. GRÉGOIRE, A.: Recherches histophysiologiques sur la surrénale du Rat au cours de la période périnatale. Arch. Biol. **72**: 413-460, 1961.
96. GROPP, A.: Über den Einfluss von Adrenalin auf das Wachstum eines organoiden Systems in vitro. Z. Krebsforsch. **60**: 52-65, 1954.
97. HACKMANN, CH.: Experimentelle Untersuchungen über die Wirkung von Actinomycin C (HBF 386) bei bösartigen Geschwülsten. Z. Krebsforsch. **58**: 607-613, 1951-1952.
98. HAMPERL, H.: Three group metaphases and carcinoma in situ of the cervix uteri. Acta Un. int. Cancr. **10**: 128-131, 1954.
99. HAMPERL, H., KAUFMAN, C. AND OBER, K. G.: Histologische Untersuchungen an der Cervix schwangerer Frauen. Die Erosion und das Carcinoma in situ. Arch. Gynäk. **184**: 181-280, 1954.
100. HANDSCHUMACHER, R. E. AND WELCH, A. D.: Agents which influence nucleic acid metabolism. In: The Nucleic Acids, ed. by E. Chargaff and J. N. Davidson, vol. 3. The Academic Press, New York, 1960.
101. HARAN, N. AND BERENBLUM, I.: The induction of the initiating phase of skin carcinogenesis in the mouse by oral administration of urethane (ethylcarbamate). Brit. J. Cancer **10**: 57-60, 1956.
102. HERTWIG, G.: Trypaflavin als Radiumersatz zur Gewinnung haploidkerniger Froschlarven. Anat. Anz. **58**: 223-227, 1924.
103. HODES, M. E., ROHN, R. J. AND BOND, W. H.: Vinkaleukoblastine. I. Preliminary clinical studies. Cancer Res. **20**: 1041-1049, 1960.
104. HOFFMAN, H.: Acceleration and retardation of the process of axon-sprouting in partially denervated muscles. Aust. J. exp. Biol. med. Sci. **30**: 541-566, 1952.
105. HUGHES, A. F. W.: The effect of inhibitory substances on cell division. Quart. J. micr. Sci. **91**: 251-277, 1950.
106. HUGHES, A.: The Mitotic Cycle. The Cytoplasm and Nucleus during Interphase and Mitosis. Butterworths Sci. Publ., London, 1952.
107. HUGHES, A. F. W. AND FELL, H. B.: Studies on abnormal mitosis induced in chick tissue cultures by mustard gas (β,β'-dichlordiethyl sulfide). Quart. J. micr. Sci. **90**: 37-55, 1949.
108. INOUE, S.: The effect of colchicine on the microscopic and submicroscopic structure of the mitotic spindle. Exp. Cell Res. suppl. **2**: 305-318, 1952.
109. ISOTALO, A. AND TEIR, H.: Influence of cortisone on mitosis. II. Effects of simultaneously applied cortisone and colchicine. Ann. Med. exp. Fenn. **31**: 301-304, 1953.
110. JACOBSON, W.: The mode of action of folic acid antagonists on cells. J. Physiol. **123**: 603-617, 1954.
111. JOHNSON, A. W.: The chemistry of Actinomycin D and related compounds. Ann. N. Y. Acad. Sci. **89**: 336-341, 1960.
112. JOHNSON, I. S., WRIGHT, H. F., SVOBODA, G. H. AND VLANTIS, J.: Antitumor principles derived from *Vinca rosea* Linn. I. Vincaleukoblastine and Leurosine. Cancer Res. **20**: 1016-1022, 1960.
113. KAMINETZKY, H. A. AND MCGREW, E. A.: Podophyllin and mouse cervix: effect of long-term application. Arch. Path. **73**: 481-485, 1962.
114. KAWAMURA, N.: Cytochemical and quantitative study of protein-bound sulfhydryl and disulfide groups in eggs of Arbacia during the first cleavage. Exp. Cell Res. **20**: 127-138, 1960.
115. KELLY, M. G. AND HARTWELL, J. L.: The biological effects and the chemical composition of podophyllin: a review. J. nat. Cancer Inst. **14**: 967-1010, 1953.
116. KOLLER, P. C.: Comparative effects of alkylating agents on cellular morphology. Ann. N. Y. Acad. Sci. **68**: 783-801, 1958.
117. KOLLER, P. C.: Comparison of the biological effects of X-rays and radiomimetic chemical agents. Radiobiology **2**: 281-286, 1961.
118. KOLLER, P. C.: The cytological effects of griseofulvin. Trans. St. John's derm. Soc. **45**: 38-41, 1961.
119. KOLLER, P. C. AND CASARINI, A.: Comparison of cytological effects induced by X-rays and nitrogen-mustard. Brit. J. Cancer **6**: 173-185, 1952.
120. LAZNITZLI, I. AND WILKINSON, J. H.: The effect of acridine derivatives on growth and mitosis of cells in vitro. Brit. J. Cancer **2**: 369-375, 1948.
121. LETTRÉ, H.: Mitosegifte und cancerogene Faktoren als Antibiotica. Z. Krebsforsch. **1**: 5-35, 1948.
122. LETTRÉ, H.: Über Mitosegifte. Ergebn. Physiol. **46**: 379-452, 1950.

123. LETTRÉ, H.: Synergists and antagonists of mitotic poisons. Ann. N. Y. Acad. Sci. **58**: 1264–1275, 1954.
124. LETTRÉ, H.: Dissoziabilität einzelner Mitoseschritte und ihre Bedeutung für das Tumorproblem. Verh. dtsch. Ges. Path. **40**: 355–359, 1957.
125. LETTRÉ, H.: L'action cellulaire des dérivés de l'adénine. Ds: Action antimitotique et caryoclasique de substances chimiques. Cent. nat. Rech. sci., pp. 242–254. Montpellier, 1959.
126. LETTRÉ, H.: Variations du cours normal des mitoses sur l'influence de facteurs chimiques. Chemotherapia **2**: 163–177, 1961.
127. LETTRÉ, H., LETTRÉ, C. AND PFLANZC, C.: Über Synergisten von Mitosegiften. VIII. Sexual Hormone und Colchicin. Naturwissenschaften **38**: 70–71, 1951.
128. LEVAN, A. AND OSTERGREN, G.: The mechanism of c-mitotic action. Observations on the naphthalene series. Hereditas (Lund) **29**: 381–443, 1943.
129. MADDOCK, C., D'ANGLIO, G. J., FARBER, S. AND HANDLER, A. H.: Biological studies of actinomycin D. Ann. N. Y. Acad. Sci. **89**: 386–398, 1960.
130. MCGREW, E. A. AND KAMINETZKY, H. A.: The genesis of experimental cervical epithelial dysplasia. Amer. J. clin. Path. **35**: 538–545, 1961.
131. MCNALL, E. G.: Biochemical studies on the metabolism of griseofulvin. Arch. Derm. **81**: 657–661, 1960.
132. MANDEL, H. G.: The physiological disposition of some anticancer agents. Pharmacol. Rev. **11**: 743–838, 1959.
133. MANGENOT, G.: Action de la colchicine sur les racines d'*Allium cepa*. Herman et Cie., Paris, 1942.
134. MARINONE, G.: Il problema degli antibiotici ad azione antimitotica. Att. I. Symposio suglo Antimitotici, pp. 315–345. San Remo, 1955.
135. MASCRE, M. AND DEYSSON, G.: Les poisons mitotiques. Biol. méd., Paris **40**: 323–376, 1951.
136. MATTHEWS, R. E. F.: Biosynthetic incorporation of metabolite analogues. Pharmacol. Rev. **10**: 359–406, 1958.
137. MATUROVA, M., MALINSKY, J. AND SANTAVY, F.: The biological effects of some podophyllin compounds and their dependence on chemical structure. XX. J. nat. Cancer Inst. **22**: 297–302, 1959.
138. MAZIA, D.: SH and growth. In: Glutathione, ed. by S. Colowick, A. Lazarow, E. Racker, D. R. Schwarz, E. Stadtman and H. Waelsch, pp. 209–228. Academic Press, New York, 1954.
139. MAZIA, D. AND ROSLANSKY, J. D.: The quantitative relations between total cell proteins and the proteins of the mitotic apparatus. Protoplasma **46**: 528–534, 1956.
140. MAZIA, D.: SH components in mitosis. I. The action of mercaptoethanol on the eggs of the sand dollar *Dendraster excentricus*. Exp. Cell Res. **14**: 486–494, 1958.
141. MAZIA, D.: The role of thiol groups in the structure and function of the mitotic apparatus. In: Sulfur in Proteins, ed. by R. Benesch, R. E. Benesch, P. D. Boyer, I. M. Klotz, W. R. Middlebrook, A. G. Szent-Györgyi and D. R. Schwartz, pp. 367–389. Academic Press, New York, 1959.
142. MAZIA, D.: The analysis of cell reproduction. Ann. N. Y. Acad. Sci. **90**: 455–469, 1960.
143. MAZIA, D.: Mitosis and the physiology of the cell division. In: The Cell, ed. by J. Brachet and A. E. Mirsky, vol. 3, pp. 77–412. Academic Press, New York, 1961.
144. MAZIA, D., HARRIS, P. J. AND BIBRING, T.: The multiplicity of the mitotic centers and the time-course of their duplication and separation. J. biophys. biochem. Cytol. **7**: 1–20, 1960.
145. MAZIA, D., MITCHINSON, J. M., MEDINA, H. AND HARRIS, P.: The direct isolation of the mitotic apparatus. J. biophys. biochem. Cytol. **10**: 467–474, 1961.
146. MAZIA, D. AND ZIMMERMAN, A.: SH compounds in mitosis. II. The effect of mercaptoethanol on the structure of the mitotic apparatus in sea urchin eggs. Exp. Cell Res. **15**: 138–153, 1958.
147. MEYER, M.: Trypaflavinwirkungen auf Amphibienmitosen. Z. Zellforsch. **47**: 731–759, 1958.
148. MICHELL, J. S. AND SIMON-REUSS, I.: Experiments on the mechanism of action of tetra-sodium 2-methyl-1,4-naphthohydroquinone diphosphate as a mitotic inhibitor and radiosensitizer, using the technique of tissue culture. Brit. J. Cancer **6**: 305–316, 317–338, 1952.
149. MOELLENDORFF, W. VON: Zur Kenntnis der Mitose. VIII. Zur Analyse der pathologischen Wachstum, hervorgerufen durch Chloralhydrat, Geschlechtshormone und cancerogene Kohlenwasserstoffe. Z. Zellforsch. **29**: 706–749, 1939.
150. MOELLENDORFF, W. VON: Der Einfluss von Wasserlöslichen Vitaminen auf die durch Steroide und cancerogene Kohlenwasserstoffe in Gewebekulturen bewirkte Teilungstörung. Z. Zellforsch. **32**: 445–469, 1943.
151. MOUTSCHEN-DAHMEN, J. AND M., VERLY, W. G. AND KOCH, G.: Autoradiogram with tritiated Myleran. Exp. Cell Res. **20**: 585–588, 1960.
152. NETTLESHIP, A. AND HENSHAW, P. S.: Induction of pulmonary tumors in mice with ethylcarbamate (urethane). J. nat. Cancer Inst. **4**: 309–319, 1943.
153. NEUSS, N., GORMAN, M., SVOBODA, G. H., MACIAK, G. AND BEER, C. T.: *Vinca* alkaloids. III. Characterization of leurosine and vincaleukoblastine. J. Amer. chem. Soc. **81**: 4754–4755, 1959.
154. NEUSS, N., GORMAN, M. AND SVOBODA, G. H.: *Vinca* alkaloids. IV. Structural features of leurosine and vincaleukoblastine, representative of a new type of indole-indoline alkaloids. J. Amer. chem. Soc. **81**: 4745–4746, 1959.
155. NOBLE, R. L., BEER, C. T. AND CUTTS, J. H.: Role of chance observations in chemotherapy: *Vinca rosea*. Ann. N. Y. Acad. Sci. **76**: 882–894, 1958.
156. O'BRIEN, J. S.: The role of the folate coenzymes in cellular division: a review. Cancer Res. **22**: 267–281, 1962.
157. OKADA, T. AND ROBERTS, E.: Antimitotic action of maleuric acid. Proc. Soc. exp. Biol., N. Y. **99**: 329–332, 1958.
158. OKADA, A. AND ROBERTS, E.: Cytological analysis of effects of maleuric acid on Ehrlich ascites tumor cells. Cancer Res. **20**: 1154–1159, 1960.
159. ORSINI, M. W. AND PANSKY, B.: The natural resistance of the golden hamster to colchicine. Science **115**: 88–89, 1952.
160. OXFORD, A. E., RAISTRICK, H. AND SIMONART, P.: Studies in the biochemistry of microorganisms. LX. Griseofulvin $C_{18}H_{17}O_6Cl$, a metabolic product of *Penicillium griseofulvin* Dierckx. Biochem. J. **33**: 240–248, 1939.

161. PADAWER, J.: Effect of colchicine and related substances on the morphology of peritoneal mast-cells. J. nat. Cancer Inst. 25: 731–747, 1960.

162. PAGET, G. E. AND WALPOLE, A. L.: Some cytological effects of griseofulvin. Nature, Lond. 182: 1320–1321, 1958.

163. PAGET, G. E. AND WALPOLE, A. L.: The experimental toxicology of griseofulvin. Arch. Derm. 81: 750–757, 1960.

164. PARMENTIER, R.: Etude des lésions cellulaires provoquées par divers phénols et amines aromatiques. Rev. belge Path. 22: 1–54, 1952.

165. PARMENTIER, R.: Reproduction sur cultures des lésions cellulaires provoquées par l'hydroquinone in vivo. Bull. Acad. Belg. Cl. Sci. 5ème série 39: 734–736, 1953.

166. PARMENTIER, R. AND DUSTIN, P., JR.: Early effects of hydroquinone on mitosis. Nature, Lond. 161: 527–528, 1948.

167. PARMENTIER, R. AND DUSTIN, P., JR.: Reproduction expérimentale d'une anomalie particulière de la métaphase des cellules malignes ("métaphase à trois groupes"). Caryologia 4: 98–109, 1951.

168. PARMENTIER, R. AND DUSTIN, P., JR.: On the mechanism of the mitotic abnormalities induced by hydroquinone in animal tissues. Rev. belge Path. 23: 20–30, 1953.

169. PARMENTIER, R., RONDANELLI, E. G. AND STROSSELLI, E.: Effecti citologica della patulina nell'animale vivente. Haematologica 41: 67–80, 1955.

170. PARMER, C. G., LIVENGOOD, D., WARREN, A. K., SIMPSON, R. J. AND JOHNSON, I. S.: The action of vincaleukoblastine on mitosis *in vitro*. Exp. Cell Res. 20: 198–201, 1960.

171. PATERSON, E., AP THOMAS, I., HADDOW, A. AND WATKINSON, J. M.: Leukemia treated with urethane compared with deep X-ray therapy. Lancet 1: 677–683, 1946.

172. PEREZ-TAMAYO, R., MURPHY, W. R. AND IGNEN, M.: Effect of cortisone and partial starvation on liver regeneration. Arch. Path. 56: 629–636, 1953.

173. PETERING, H. G.: Folic acid antagonists. Physiol. Rev. 32: 197–213, 1952.

174. PHILIPS, F. S.: Recent contributions to the pharmacology of bis(2-haloethyl) amines and sulfides. Pharmacol. Rev. 2: 281–323, 1950.

175. PHILIPS, F. S., SCHWARTZ, H. S. AND STERNBERG, S. S.: Pharmacology of Mitomycin C. I. Toxicity and pathologic effects. Cancer Res. 20: 1354–1361, 1960.

176. PISCITELLI, N.: Recherches sur l'action de la désacétyl-N-méthylcolchicine sur la moelle osseuse de rats normaux et surrénalectomisés. Rev. belge Path. 25: 219–231, 1956.

177. PISCITELLI, N.: Sull'azione statmocinetica della desacetil-N-metilcolchicina nel midollo osseo di ratti normali e di ratti surrenalectomizzati. Boll. Oncol. 30: 139–155, 1956.

178. PISCITELLI, N., DE HARVEN, E. AND DUSTIN, P., JR.: Etude quantitative de l'inhibition mitotique des cellules médullaires de rats normaux et surrénalectomisés par la méthode colchicinique. Bull. Acad. Belg. Cl. Sci. 42: 201–208, 1956.

179. PITON, R.: Recherches sur les actions caryoclasiques et caryocinétiques des composés arsénicaux. Arch. int. Méd. exp. 5: 355–411, 1929.

180. POLITZER, G.: Pathologie der Mitose. Protoplasma Monographien. Gebrüder Borntraeger, Berlin, 1934.

181. PORTER, K. R. AND MACHADO, R. S.: Studies on the endoplasmic reticulum. IV. Its form and distribution during mitosis in cells of onion root tip. J. biophys. biochem. Cytol. 7: 167–180, 1960.

182. PRESCOTT, D. M. AND BENDER, M. A.: Synthesis of RNA and protein during mitosis in mammalian tissue culture cells. Exp. Cell Res. 26: 260–268, 1962.

183. RAPKINE, L.: Sur les processus chimiques au cours de la division cellulaire. Ann. Physiol. Physicochim. biol. 7: 382, 1931.

184. ROBBINS, G. P., COOPER, J. A. D. AND ALT, H. L.: Effect of corticotropin on cellularity and mitosis in the rat bone marrow, spleen and thymus. Endocrinology 56: 161–166, 1955.

185. ROBERTS, K. B., FLOREY, H. W. AND JOKLIK, W. K.: The influence of cortisone on cell division. Quart. J. exp. Physiol. 37: 239–257, 1952.

186. ROGERS, S.: Studies of the mechanism of action of urethane in initiating pulmonary adenomas in mice. II. Its relation to nucleic acid synthesis. J. exp. Med. 105: 279–306, 1957.

187. ROSIN, A.: Effect of urethane (ethylcarbonate) on the mitotic activity in the bone-marrow of normal mice. Blood 7: 652–660, 1951.

188. ROSS, W. C. J.: The chemistry of cytotoxic alkylating agents. Advanc. Cancer Res. 1: 396–449, 1953.

189. ROSS, W. C. J.: Biological Alkylating Agents. Butterworths Sci. Publ., London, 1962.

190. ROTH, L. E., OBETZ, S. W. AND DANIELS, E. W.: Electron microscopic studies of mitosis in amebae. I. *Amoeba proteus*. J. biophys. biochem. Cytol. 8: 207–220, 1960.

191. ROTH, L. E. AND DANIELS, E. W.: Electron microscopic studies of mitosis in Amebae. II. The giant Ameba *Pelomyxa carolinensis*. J. Cell Biol. 12: 57–78, 1962.

192. RUBY, A.: Ph.D. Thesis, University of Berkeley, California, 1961 (quoted by Mazia, 138).

193. SAKAI, H. AND DAN, K.: Studies on sulfhydryl groups during cell division of sea urchin egg. I. Glutathione. Exp. Cell Res. 16: 24–41, 1959.

194. SAUAIA, H. AND MAZIA, D.: Action of colchicine on the mitotic apparatus. Path. Biol. 9: 473–476, 1961.

195. SCARPELLI, D. G. AND VON HAAM, E.: A study of mitosis in cervical epithelium during experimental inflammation and carcinogenesis. Cancer Res. 17: 880–884, 1957.

195a. SCHINDLER, R.: Desacetylamino-colchicine: a derivative of colchicine with increased cytotoxic activity in mammalian cell cultures. Nature, Lond. 196: 73–74, 1962.

195b. SCHINDLER, R.: Personal communication.

196. SCHRADER, F.: Mitosis. The Movement of Chromosomes in Cell Division, 2d ed. Columbia University Press, New York, 1953.

197. SEIDLOVA-MASINOVA, V., MALINSKY, J. AND SANTAVY, F.: The biological effects of some podophyllin compounds and their dependence on chemical structure. J. nat. Cancer Inst. 18: 359–372, 1957.

198. SELYE, H.: The physiology and pathology of exposure to stress. A treatise based on the concepts of the general-adaptation-syndrome and the diseases of adaptation. Acta Inc. Med. Publ., Montreal, Canada, 1950.
199. SENTEIN, P.: Analyse du mécanisme de la caryocinèse par l'action de substances antimitotiques sur l'oeuf en segmentation. J. Physiol. **41:** 269A-270A, 1949.
200. SENTEIN, P.: Altérations du fuseau mitotique et fragmentation des chromosomes par l'action de la patuline sur l'oeuf d'Urodèles en segmentation. C. R. Soc. Biol., Paris **149:** 1621-1622, 1955.
201. SENTEIN, P.: Action de la désacétyl-méthyl-colchicine sur la segmentation et les mitoses de l'oeuf d'Urodèle. Comparaison avec la colchicine et le colchicoside. Arch. Anat. micr. **45:** 99-138, 1956.
202. SENTEIN, P.: Action antimitotique de l'Ethylmercurithiosalicylate de Sodium. Acta anat. **30:** 787-801, 1957.
203. SENTEIN, P.: Action de la 2, 4, 6 triéthylèneimino-1, 3, 5 triazine (TEM) sur les mitoses de segmentation, les mitoses épithéliales et les mitoses épendymaires chez les Batraciens. Arch. Biol. **68:** 581-632, 1957.
204. SENTEIN, P.: Action du phénol sur les mitoses de segmentation des oeufs d'Amphibiens. Acta anat. **34:** 201-234, 1958.
205. SENTEIN, P.: Le test de l'oeuf d'Urodèle en segmentation. Renseignements qu'il donne sur le mode d'action de quelques antimitotiques et sur les caractères particuliers des premières mitoses embryonnaires. In: Action antimitotique et caryoclasique de substances chimiques, pp. 143-166. Cent. nat. Rech. sci., Paris, 1960.
206. SENTEIN, P.: L'action des antimitotiques pendant la segmentation de l'oeuf et le mécanisme de cette action. Path. Biol. **9:** 445-466, 1961.
207. SENTEIN, P.: Le déterminisme des mitoses pluripolaires et leur mécanisme d'après l'action interrompue du phényluréthane sur l'oeuf d'Urodèle. Chromosoma **13:** 67-105, 1962.
208. SIEBS, W.: Mitoseablaufstörungen. 1. Mitteilung: Polchromosomen- und Dreigruppen-metaphasen (Chromosomenspindelfaserstörung). Z. Zellforsch. **51:** 497-534, 1960.
209. SIEBS, W.: Mitoseablaufstörungen. II. Mitteilung. Sternkonfiguration arretierter Metaphasen (Zentralspindelstörung). Z. Zellforsch. **51:** 535-544, 1960.
210. SINGER, M., FLINKER, D. AND SIDMAN, R. L.: Nerve destruction by colchicine resulting in suppression of limb regeneration in adult *Triturus*. J. exp. Zool. **131:** 267-299, 1956.
211. STUDER, A.: Zur Frage der Angriffsorte von Compound E (Cortisone). Eine experimentelle Studie. Z. ges. exp. Med. **121:** 287-418, 1953.
212. SULLIVAN, M. AND KING, L. S.: Effects of resin of podophyllum on normal skin, condylomata acuminata and verrucae vulgaris. Arch. Derm. Syph. **56:** 30-45, 1947.
213. SWANN, M. M.: The control of cell division: a review. I. General mechanisms. Cancer Res. **17:** 727-757, 1957.
214. SWANN, M. M.: The control of cell division: a review. II. Special mechanisms. Cancer Res. **18:** 1118-1160, 1958.
215. TAYLOR, E. W.: Dynamics of spindle formation and its inhibition by chemicals. J. biophys. biochem. Cytol. **6:** 193-196, 1959.
216. TAYLOR, J. H.: Nucleic acid synthesis in relation to the cell division cycle. Ann. N. Y. Acad. Sci. **90:** 409-421, 1960.
217. TEIR, H. AND ISOTALO, A.: Influence of cortisone on mitosis. I. Effect of single dose and prolonged application. Ann. Med. exp. Fenn. **31:** 171-180, 1953.
218. TEIR, H. AND ISOTALO, A.: Influence of cortisone on mitosis. III. Effect of simultaneously applied cortisone and cell suspensions. Ann. Med. exp. Fenn. **32:** 1-4, 1954.
219. TIMMIS, G. M.: The action of antimetabolites and biological alkylating agents on the synthesis of deoxyribonucleic acid and a possible relation between the mechanisms of action. Biochem. Pharmacol. **4:** 49-56, 1960.
220. TJIO JOE HIN AND LEVAN, A.: The chromosome number of man. Hereditas **42:** 1-6, 1956.
221. TURBYFILL, CH. L. AND SODERWALL, A. L.: Sensitivity of hamster to colchicine. Science **126:** 749, 1957.
222. WARBURG, O.: Über die Oxydationen in lebenden Zellen nach Versuchen am Seeigelei. Hoppe-Seyl. Z. **66:** 305, 1910.
223. WÄTJEN, J.: Über experimentelle, toxische Schädigungen des lymphatischen Gewebes durch Arsen. Virchows Arch. **256:** 86-116, 1925.
224. WATSON, J. D. AND CRICK, F. H. C.: Molecular structure for deoxyribose nucleic acid. Nature, Lond. **171:** 737-738, 1953.
225. WATSON, J. D. AND CRICK, F. H. C.: Genetical implications of the structure of desoxyribonucleic acid. Nature, Lond. **171:** 964-966, 1953.
226. WENT, H. A.: Dynamic aspects of mitotic apparatus protein. Ann. N. Y. Acad. Sci. **90:** 422-429, 1960.
227. WENT, H. A. AND MAZIA, D.: Immunochemical study of the origin of the mitotic apparatus. Exp. Cell Res. **7:** 200-218, 1959.
228. WHEELER, G. P.: Studies related to the mechanisms of action of cytotoxic alkylating agents: a review. Cancer Res. **22:** 651-689, 1962.
229. WILDMAN, W. C.: Colchicine and related compounds. In: The Alkaloids (Chem. Physiol.), ed. by R. H. F. Manske, vol. 6 (suppl. to vol. 2, chap. 10). Academic Press, New York, 1960.
230. WRBA, H.: Zur Colchicinresistenz heterolog wachsender Tumoren des Goldhamsters. Z. Krebsforsch. **64:** 88-95, 1961.
231. WRBA, H. AND RABES, H.: Die Entstehung stabiler colchicinresistenter Stämme von Impfgeschwülsten durch heterologe Passage auf Goldhamstern. Z. Krebsforsch. **64:** 348-352, 1961.
232. YOFFEY, J. M., ANCIL, R. J., HOLT, J. A. G., OWEN-SMITH, B. AND HERDAN, G.: A quantitative study of the effects of compound E, compound F, and compound A, upon the bone marrow of the guinea-pig. J. Anat. **88:** 115-130, 1954.
233. ZIMMERMAN, A. M.: Physico-chemical analysis of the isolated mitotic apparatus. Exp. Cell Res. **20:** 529-547, 1960.