

Neoplastic Growths

– In Animals and Plants

10.1: Colchicine in Cancer Research

Mitotic changes induced by colchicine in a Crocker sarcoma of the mouse were described by Professor A. P. Dustin, Sr., in 1934²⁴ (Fig. 10.1). This now recognized classic research marked a new trend in the study of cancer. At that time, the toll of life from bacterial diseases was declining as a result of the use of the sulfa drugs, and the relative incidence of cancer was gaining the impressive figure it has reached today in civilized countries. It is not surprising that the discovery of a specific action upon mitosis, the metaphase arrest, attracted wide attention. This research made clear for the first time the possibility of arresting cell division with chemicals acting specifically. Such a relation had, it is true, been demonstrated several years earlier in the Brussels laboratory,^{24, 25} but colchicine, being such a unique chemical, helped greatly in convincing research men of the possibility of cancer chemotherapy. A. P. Dustin, Sr., grasped immediately the potentiality of this new approach.²⁴ His 1934 publication and the demonstration given by his school at the Second International Cancer Congress, held in Brussels in 1936, marked a turning point and led many people to work on neoplastic growth.

It is quite remarkable that colchicine, like other plant substances used in popular medicine, such as chelidonine,²⁰ may have been utilized in cancer treatment long before that date. At least two French textbooks of pharmacology^{46, 50} mention that Dominici, the great French hematologist and radiotherapist who died in 1919, had observed favorable effects of colchicine in cancerous patients who had received X-ray while under treatment for gout. We have been unable so far to discover the original text of Dominici's observation and his publication. The idea of some interrelation between gout and cancer was mentioned in 1920 in Belgium by A. P. Dustin, Sr.²⁴ Again,

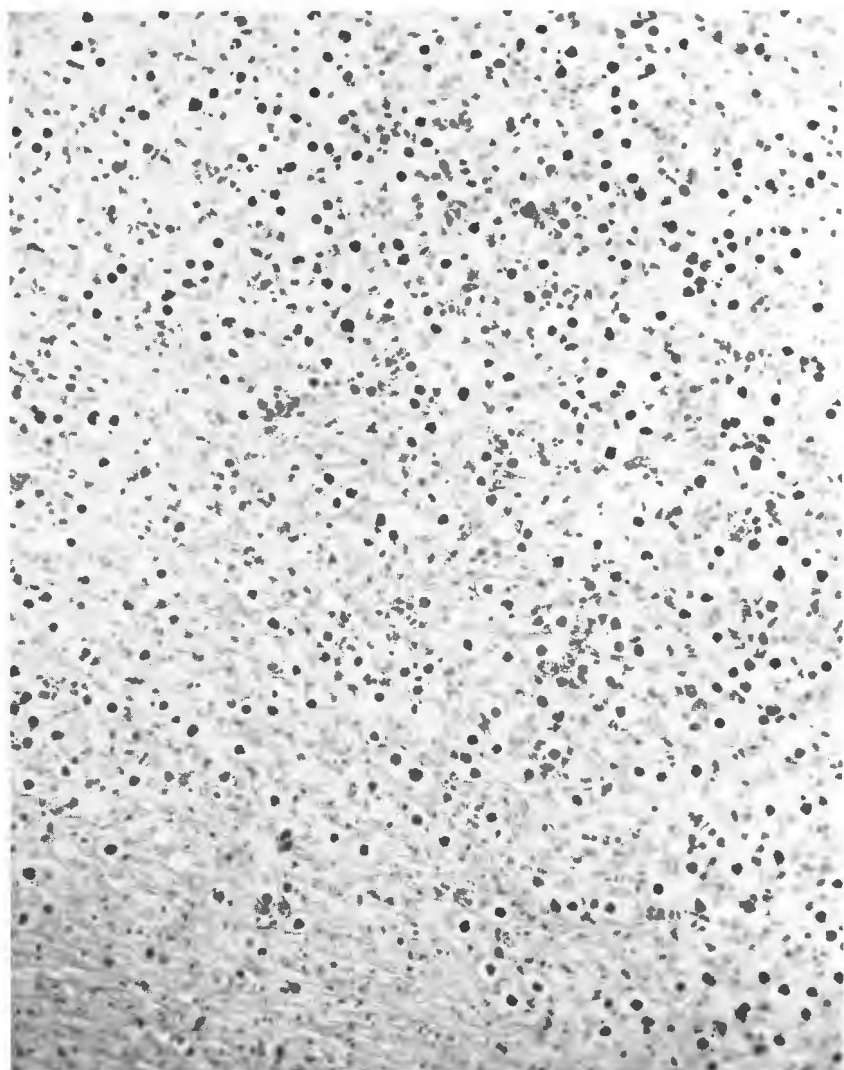


Fig. 10.1—Action of colchicine on the Crocker sarcoma in a mouse. All the nuclei which appear as black dots are in a condition of arrested metaphase of the "ball" type, with clumping and progressive fusion of chromosomes. There is no hemorrhagic effect in this area. Nuclear staining: iron-hematoxylin. (From an original preparation from the collections of the Department of Pathology, Brussels University. A. P. Dustin, 1934²⁴)

in the first report of favorable effects of colchicine on tumors in mice and in one epithelial cancer in a dog,¹ made in 1935, the author, E. C. Amoroso, did not mention any of the work done in Brussels, but writes:

Following on some earlier observations (unpublished, 1927) which I made with the late Prof. M. R. J. Hayes on the beneficial effects of deep X-ray therapy on neoplasms in patients suffering from acute attacks of gout, which were being treated with colchicum, a series of experiments was . . . planned. *

These results are only known in a preliminary form, and no detailed paper appeared later. They may have influenced one report on favorable results of the treatment by colchicine of a malignant growth in a mare.¹⁶ The first report in English on the action of colchicine on normal and malignant cells in tissue cultures, which was published in 1936,⁴⁷ acknowledges these references and claims not to have been influenced by the work done in Brussels.^{24, 44} It is, however, surprising that this paper also describes the effects of arsenical derivatives on the spindle, for this was discovered in Belgium in 1929 and had only received scant attention.^{60, 25}

Many experiments and also practical applications of colchicine in experimental and human tumors were made; this subject has been reviewed recently.⁴⁷ The conclusion was reached that colchicine is no cure for cancer. However, much work is now in progress^{10, 22} in the search for chemicals, more or less related to colchicine, with a lower general toxicity and a more specific action against malignant cells. The study of these will be described in the last chapter of this book.

The discovery of colchicine heralded a greater search for mitotic poisons, i.e., substances specifically harmful to dividing cells. This subject has become so extensive that is more and more difficult, even for specialized workers, to review it all.

It has been shown in previous chapters what a unique substance colchicine is as a tool for detecting cellular proliferation. It could be used as such for the study of carcinogenesis, on the one hand, and malignant growth on the other. A surprisingly limited amount of research has been conducted in this direction.^{54, 62, 68} However, interesting results have been obtained recently with the use of colchicine *in vitro*. This work demonstrates the quite unexpected fact that, apparently, cells from acute leukemia, a disease in which cellular proliferation was always believed to be extremely rapid, grow much more slowly than the normal constituents of the human bone marrow.³

A section related to the problem of plant overgrowths and tumors is included in this chapter because some careful work has been done

* E. C. Amoroso, "Colchicine and Tumour Growth," *Nature*, 135 (1935), p. 266.

in this field. The basic relationship between the action of colchicine and abnormally proliferating plant cells remains unsolved. An induced vascularization similar to that referred to in Chapter 4 may be related to this problem, and would provide a promising new approach.

The combined action of colchicine and X-irradiation on animal and plant materials has been studied in several laboratories. No decisive results appear to have been obtained. However, some recent research indicating the action of irradiation on metaphasic chromosomes, shows that this work is well worth reviewing.

All the studies on neoplastic cells point towards the same inescapable fact: Whereas colchicine, as a treatment for gout, may well have been observed prior to 1934 to have some favorable action against cancer, all the papers which connect both have been published since 1934. This clearly indicates the significance of the cytological work published at that time by A. P. Dustin²⁴ and demonstrated at the 1936 Cancer Congress.

10.2: Experimental Study of Neoplastic Cells

Malignant cells, especially in animal tumors, often display "spontaneous" mitotic abnormalities. These have been compared to those induced by colchicine, and it has been suggested that the cells were under the influence of some mitotic poison acting like colchicine.²⁹ It has been suggested that this may be lactic acid.⁷⁴ However, these spindle disturbances often appear to be the consequence of more deep-seated nuclear changes, closely related to the cause of malignancy itself, and leading to chromosome breakages and rearrangements. In early human carcinomas, however, it has been pointed out that the spindle changes appeared first.⁵⁵ The behavior of such cells when brought under the influence of colchicine is of great importance, for it would be of value to determine whether a specific destruction of malignant cells by a spindle poison is possible.

The effect of colchicine on cancerous growths has been studied either by injecting the animals with the drug, or by explanting the abnormal cells *in vitro* and using the methods of tissue culture. This last procedure has been followed with a mammary carcinoma⁷³ and a sarcoma¹⁸ of the mouse, and with Ehrlich mouse carcinoma growing as an "ascites tumor" in the abdominal cavity.^{40, 63} Concentrations of $100 \times 10^{-6} M$ to $1.25 \times 10^{-6} M$ inhibit outgrowth from the explants and arrest cell divisions. This effect is still evident on carcinoma cells at a concentration of $0.5 \times 10^{-6} M$. In culture containing explants of both tumor and embryonic kidney, the latter showed the greatest cellular destruction following the mitotic arrest. Differences of sensitivity between various strains of carcinomas were found, while the Crocker sarcoma showed fewer arrested metaphases.⁴⁷

The ascites tumor enables colchicine to be brought in direct contact with the malignant cells *in vivo*. The tumor cells float freely in the fluid which gradually fills the abdominal cavity. It is possible, simply by pipetting cells from the abdomen, to examine all the changes brought about by the injection of colchicine.^{11, 40, 63} Growth curves of the tumor indicate that on the average each cell divides every 2 to 2½ days. After an injection of colchicine, the percentage of mitotic cells rises in 9½ hours from 1.2 to 14.2. Thirteen hours after injection, it reaches 18.2, and falls to 2.0 after 48 hours. From these figures, the normal average duration of mitosis can be calculated as follows: $1.2 \times 9.5/14.2 \cong 1.2 \times 13/18.2 \cong 0.8$ hours, or 48 minutes.

Scattered groups of chromosomes and micronuclei are observed in the colchicine-treated tumor cells.^{11, 37} Resting (intermitotic) nuclei are also affected; their chromatin network becomes coarser.¹¹ In sarcoma-bearing mice, a series of experiments was carried out to determine whether administration of colchicine had any effect upon subsequent growth of the tumor cultivated *in vitro*.¹⁸ Colchicine (0.004 to 0.06 mg.) was administered by subcutaneous or intravenous injection, and fragments of sarcoma were removed for cultivation at various intervals after treatment. The growth of tumor tissue *in vitro*, obtained from an animal treated with colchicine, was inhibited to a large extent. Colchicine arrested mitoses, both normal and neoplastic.

In human malignant growth, colchicine has been found useful for the study of cellular multiplication. In 11 patients injected with 1.5 to 4 mg. subcutaneously or intramuscularly, modification of tumor mitoses were observed.⁵³ Four other patients did not show any response, a fact which is not surprising, the dose being kept relatively small by comparison with doses administered in animal work, because of the great toxicity of colchicine in man. In one case of adenocarcinoma of the bowel, the progressive increase of the mitotic index could be followed by repeated biopsies. The control specimens had an index of 2.6, which rose to 7.3 five hours after colchicine and reached 19.6 after 12 hours. This last biopsy demonstrated a considerable increase of arrested mitoses. It is regrettable that, owing partly to the too great danger of colchicine poisoning (cf. Chapter 7), no further research of this type has been conducted. Now that new and less toxic colchicine derivatives are available¹⁰ (Chapter 17), a more thorough study of the rate of growth of human neoplasms may be possible. This could then be compared with data on normal tissues obtained by the same method.

Colchicine may yet be used on explanted human tissues, and it is surprising that only two papers on that subject can be recorded up to now. In polycythemia vera, a disease in which the abnormal number of red blood cells has often been considered closely related

to malignant growth, and which may end in leukemia, the increase of metaphases of bone-marrow cells explanted *in vitro* in a solution of colchicine was found not to differ from normal.⁵ The striking results obtained with marrow of patients with acute leukemia have been mentioned in Section 10.1.³

10.3: Cancer Chemotherapy

It is evident that the data on the growth of neoplastic cells treated with colchicine are meagre. Workers were quickly attracted by the false idea of finding a cancer cure, and they injected colchicine into animals bearing various tumors. Botanists, also, painted plant tumors with colchicine. Neither were much interested in the fundamental changes taking place. As a result, the cytological data are often incomplete and only mention "cellular destruction," "nuclear fragmentation," or "tumor necrosis and hemorrhage." This emphasis on the gross changes in animal tumors has led to a neglect of the fundamental problem which is at the base of any cancer chemotherapy: Are malignant cells more severely damaged than normal ones? This is of great importance with a chemical like colchicine which affects all types of mitoses. The appearance of large zones of hemorrhage in tumors treated with colchicine has led some workers^{9, 47, 70} to the conclusion that this is the main action of the drug and the only possibility of obtaining a destruction of the neoplastic growth. This problem will be discussed first, though it is quite evident to all engaged in cancer chemotherapy that a drug the main action of which would be hemorrhagic destruction, is of no use in medicine.

10.3-1: The hemorrhagic effect and metabolic changes. Many reports on experimental tumors in mammals, whether induced by carcinogens or grafted, showed that colchicine was unable to prevent the neoplastic growth.^{62, 66, 18, 65} In the sarcoma 180 of the rat even the largest tolerated doses were unable to arrest all mitoses at metaphase.¹⁴ From the unaffected ana- and telophases the malignant growth resumed its activity once colchicine was discontinued.

On the other hand, the metabolic changes in tumors treated by colchicine were being investigated. In grafted tumors in rats the metabolism, measured *in vitro*, was found to decrease. At the same time, the ascorbic acid content of the tumors was considerably lowered, and large zones of hemorrhage were seen.⁹ This last change was believed to play a great part in the regression of the tumors. Similar changes could be observed after the injection of *Bacillus typhosus* extracts. It was not reported that these bacterial products induced any nuclear or mitotic change.⁹ Similar hemorrhages were also noticed in other grafted carcinomas, in spontaneous mammary tumors, and in methylcholanthrene-induced tumors of mice. They were most apparent 18 to 20 hours after colchicine. The spontaneous tumors ap-

peared the most resistant towards this new "colchicine-effect." A parallel decrease in ascorbic acid content, respiration, and glycolysis was observed.⁹

The significance of these hemorrhages, which appear only with sublethal doses,² is not clear. It has been suggested that mitotic poisoning of the endothelial cells of the tumor capillary bed (cf. Chapter 9) may play an important part.⁴⁷ *Escherichia coli* filtrates have similar hemorrhagic properties, and add their effect to those of colchicine, but the over-all toxicity is also increased. The polysaccharide extracted from *Serratia marcescens* is interesting, for it also produces hemorrhages in tumors and has been shown to interfere with cell division.⁷⁰

Tumors treated with colchicine become quite fragile. In the Flexner-Jobling carcinoma of rats the injection of distilled water in the tumor has a destructive action 15 hours after colchicine. These experiments, which were done on a great number of animals, have been reported only in a short note.³⁰

In a recent review,⁴⁷ the effects of colchicine on 17 different strains of tumors and 49 spontaneous mammary carcinomas in mice have been summarized. While the effects vary according to age, genetic constitution, rate of tumor growth, toxicity of colchicine, and histological structure, the hemorrhagic effect was considered to be the main factor in tumor regression. In highly cellular and soft tumors growing on RIII mice, complete cures were reported. Regression is obtained only by doses very close to the lethal one and far above those that simply arrest mitosis. Soft and rapidly growing tumors respond well, while slowly growing and fibrous tumors are resistant.

This conclusion applies only to the experience of one group of authors, and instances can be found of malignant growths which respond to colchicine without any hemorrhage. Such is the case of a benzopyrene-induced sarcoma (HL tumor) in albino rats.⁷ The regression appeared here to bear some relation to a decrease in the pyrophosphatase of the neoplasm, while liver and kidney pyrophosphatase were not affected.

Further examples will be given of favorable effects unrelated to hemorrhage, which is clearly related to very toxic doses and is of no practical interest in chemotherapy. The hemorrhagic effect is one more of the riddles of colchicine, but to insist too much on it as the main mode of action of the drug on tumors is to discourage any further work on nontoxic derivatives with mitosis-arresting properties.

10.3-2: Animal tumors. One of the most striking effects of colchicine noticed in the first experiments on animals⁴⁴ was the destruction of lymphoid and thymic cells following the metaphase arrest of their mitoses. This action is certainly related to the general toxicity of

colchicine and to a "stress" releasing cortisone and other lymphocyte-damaging hormones from the adrenals (Chapter 7). It led to the idea of treating lymphoid tumors in C3H strain mice with colchicine.⁴⁵ The malignant lymphocytes, like those of thymus and spleen, underwent a pycnotic destruction after injections of 0.025 mg. repeated every third day. The average duration of life of the animals after the tumors had been grafted was significantly prolonged. In controls it was 31.5 days; in those injected with colchicine, 50.5 days. Histological study showed that the reticulum cells and some of the neoplastic lymphocytes escaped destruction, and resumed growth when the injections were interrupted. In another series of experiments⁴ a permanent regression of the 6C3HED lymphosarcoma (in C3H mice) was obtained by daily injections of 0.5 to 0.75 mg/kg after the tumor had reached a diameter of about 1 cm. The animals cured from the grafted neoplasm became immune to further graftings of the same tumor. No similar effects were observed after cortisone. This appears to rule out the possibility of colchicine acting on tumor growth by the indirect pathway of the pituitary-adrenal system. In these lymphoid tumors, colchicine destroyed the cells and their mitoses, and no mention is made of hemorrhage playing any part in the chemotherapeutic action.^{45, 4}

In epithelial tumors the results vary considerably. For instance, the Brown-Pearce carcinoma of the rabbit showed some increase in the percentage of metaphases after 1 mg/kg of colchicine. The response was, however, so unpredictable as not to warrant further study.²³ Some authors have reported an important prolongation of life in mice bearing the Ehrlich carcinoma,⁴⁰ while in various other tumors of mice and rats no such prolongation could be claimed.^{52, 61, 66}

Studies on virus-induced malignant growths in fowl are of interest. In animals grafted with the Rous sarcoma, doses capable of arresting the testicular mitoses did not modify the tumor growth. Larger doses killed the birds.¹⁶ In avian erythroblastosis, a dose of 1 mg/kg injected over a period of five days did not alter the evolution of the malignant growth of blood cells.⁶² Some inhibition of the growth of the Rous virus has, however, been observed,²¹ especially when this is cultivated on the chorio-allantoic membrane of eggs.

It appears that considerable variations in sensitivity towards colchicine exist from one tumor to another,^{41, 48} and that the toxicity of the drug has often limited its use. Further work should clearly be aimed at many different tumors and at the use of the new colchicine derivatives, which are discussed in Chapter 17.

10.3-3: *The Shope papilloma in rabbits.* This is a virus-induced tumor, which is very widespread in this species. A closely related virus, myxomatosis, has even been advocated as a tool for the extermination of rabbits in Australia and other countries. This tumor

is benignant, but under the influence of carcinogens it may become malignant. A series of papers has been devoted to its possible cure by colchicine.^{57, 58, 59} This may be obtained after injections of colchicine in the animal.⁵⁷ While one is always limited by the toxicity reactions, it was found that the local application of a colchicine ointment to the skin tumors could definitely cure a great number of animals. A remarkable and rather perturbing fact was noticed.⁵⁹ If both ears of a rabbit are inoculated with the Shope virus, and a cure is obtained on one side with the colchicine ointment, the tumors of the other ear become more liable to undergo a malignant change into carcinomas. The conclusions of these papers are most important for they opened a new pathway for the use of colchicine in human pathology.⁵⁹ To quote: “. . . these experimental data suggest the possibility of using colchicine in human therapeutics . . . by local applications, to precancerous lesions or benignant skin tumors.” * The results obtained in tumor-bearing patients will now be discussed.

10.4: Chemotherapy of Human Neoplasms

The suggestion of a local application of colchicine, enabling a strong concentration to act upon abnormal cells without general toxicity symptoms, was taken up in 1941. Colchicine, either in a paste or an injection as an oily solution, was applied to metastatic nodules of epithelial cancers.⁶⁷ The volume of the treated metastases clearly decreased.

However, it appeared more logical to begin with benign growths of the skin. Some of these, such as the venereal papillomas or warts, may be very extensive, and their treatment by usual methods involves large surgical excisions. These are virus-induced growths, comparable to the papillomas of the rabbit. A colchicine-lanoline paste (0.05 per cent) was applied twice daily to six of such cases.¹¹ Remarkable regressions were observed after several weeks of treatment. The tumor became more and more resistant to colchicine, and in the last stages, had to be removed surgically. This was facilitated considerably by the regression of the size and extension of the tumor. Colchicine-mitoses can be found in great numbers in biopsies of treated papillomas.⁸ It is quite evident that the regression of the neoplastic growth is a simple consequence of the arrest of its cell divisions. No hemorrhage is to be seen. It appears also that the mitoses of normal skin are less modified by the treatment, for there is no skin ulceration, and after the tumor has disappeared, the skin has a normal aspect.^{11, 8}

* A. Peyron, G. Poumeau-Delille, and B. LaFay. La tumeur de Shope du lapin et sa sterilisation par la colchicine. *Bull. Assoc. Franc. Étude Cancer* 26:633. 1937.

Colchicine has now been replaced in the treatment of such warts and papillomas by another substance of plant origin, podophyllin, a resin extracted from *Podophyllum* sp.³⁶ This substance is a complex mixture of chemicals, the most active being podophyllotoxin and the peltatins. These are, quite like colchicine, mitotic poisons, and they interfere mainly with the spindle formation.³⁶ The use of the resin of podophyll was known in the United States as a popular medical remedy; it is remarkable that another plant, known in Europe to have good effect on warts, *Chelidonium majus*, contains an alkaloid, chelidonine, which has also been demonstrated to inhibit spindle formation in tissue cultures.⁴⁰ Chelidonine was advocated for the treatment of cancer at the end of the nineteenth century.²⁰

These empirical remedies, probably centuries old, are most interesting, and it may be recalled that Dioscorides recommended the use of *Ephemerum*, a species containing colchicine, in the treatment of some tumors. Colchicine-paste has also proved to be successful in the treatment of some skin cancers of the basal-cell type.^{11, 17} In ulcerating mammary tumors, interesting results have been obtained. A striking fact is that here again the growth of normal skin appears to be less altered than that of the neoplasm.¹¹

In human malignant tumors, the effect of colchicine has so far proved quite disappointing, and from the reports available, it is difficult to understand how it could have been observed to be of any benefit to cancerous patients.¹ It may arrest tumor mitoses in man,⁵³ but this effect is never powerful enough to stop the malignant growth. The toxicity of colchicine is redoubtable. Even in a series of four patients, where some favorable effects were noticed, one case of severe leukopenia was noted, and another patient lost almost all his hair.⁶⁵ In another series, two out of three patients died of agranulocytosis, which was probably the consequence of mitotic inhibition in the bone marrow.¹²

In severe neoplastic blood diseases, colchicine has also been tried by a few investigators. In lymphoid tumors the results were of no practical interest,³⁹ and intramedullary injections did not change the fatal course of acute leukemia.²⁸ In chronic myeloid leukemia, a disease which is known to respond favorably to many mitotic poisons, more promising results have been recorded. In one patient, who received 0.5 mg. of colchicine three times and later twice daily, the leukocyte count was found to fall from 110,000 to 2400. This improvement was only of short duration.^{38, 56}

These data, which are very sketchy, may seem to rule out colchicine for the treatment of cancer in man. However, recent developments are more promising, though still in an experimental stage. In Hodgkin's disease, a neoplastic condition affecting mainly the lymph-

oid tissue, excellent effects have been described. Colchicine administered intravenously produced a sharp fall in temperature, which in these patients is often very high.³⁵ Substances chemically close to colchicine but less toxic are being tested; "methyl-colchicine" has quite recently proved to be of value in the management of cases of chronic myeloid leukemia.⁵¹ It is quite evident that it is too early to draw a conclusion about the future of colchicine in cancer therapy, and that far more work remains to be done.

10.5: A Tool for the Study of Cancer Chemotherapy

The mitotic stasis resulting from spindle destruction can make visible small changes in the mitotic rate which would pass unnoticed in microscopic sections (cf. Chapter 9). Some promising work has been initiated in this field. Urethane, at a dose of 0.5 gm/day, has been demonstrated not to modify the number of mitoses, studied with the colchicine method, in the Walker rat carcinoma 256.²⁷ Azaguanine,^{69, 77} on the other hand, has been proved to be one of the most remarkable chemotherapeutic substances. This antagonist of guanine and adenine can be demonstrated not to affect normal mitoses, while strongly decreasing those of the Brown-Pearce carcinoma. This tumor was studied while grafted in the anterior chamber of the guinea pig's eye.⁶⁹ This type of mitotic depression is made more evident by the use of colchicine.

Another type of experiment was planned for the study of an antifolic drug, aminopterin. This substance is widely used in the treatment of acute leukemia. When large doses are injected into mice, the cell divisions in the intestine do not take place any more for about 48 hours. During this period of mitotic inhibition, cellular and nuclear growth are not impaired, and very large nuclei are formed. When these divide again, the mitoses are of exceptional size. Colchicine was used as a tool to arrest these mitoses and to provide a greater number for study, as a consequence of the mitotic stasis. Also, the shortening of the chromosomes made their counting easier, and ball metaphases provided excellent material for photometric measurements. These experiments indicated that the increase in nuclear size was neither the result of polyploidy nor of polypeny.²⁶

10.6: Plant Tumors

Whatever may be the exact relation between tumors in animals—and, in particular, cancerous growths—and the various types of gall formations induced in plants by *Bacillus tumefaciens*, insects, etc., it is interesting to compare the effects obtained with colchicine with those described for animal neoplasms. In a series of experiments on *Lycopersicum esculentum* inoculated with *B. tumefaciens*, a 1:10,000

solution of colchicine, locally applied, decreased the number and the volume of the induced tumors without disturbing the growth of the plant itself.³¹ An extensive series of experiments was started shortly after on seven species.¹⁹ By injecting colchicine in plants at the time of infection by *B. tumefaciens*, tumor growth was only prevented in 9 out of 61 plants. On the contrary, to arrest the growth of tumors and to destroy them later were possible in most cases by several techniques of application of the alkaloid.¹ In *Tagetes patula*, these tumors, after daily paintings with a 1 per cent colchicine solution, stop growing after 7 days and then progressively decrease and die. The principal microscopic effect is a great enlargement of the tumor cells, four or five of the colchicized ones occupying the area of 30 normal ones. This enlargement is the most visible with rather concentrated solutions of colchicine (up to 0.1 per cent). The smallest cells are 64-ploid (1536 chromosomes), the larger 1014-ploid (24,500 chromosomes). Some nuclei have irregular shapes and some cells are multinucleated. Cellular death is a direct consequence of the extreme degree of polyploidy which is reached, the giant cells becoming at some stage quite unable to divide any further. There is no effect on the bacterial growth.¹⁹ Similar results have been obtained in *Pelargonium* and *Ricinus*.⁷² It was supposed that the death of the tumor was the consequence of its isolation by a layer of cork.⁷²

Though animal cells, through failure of centromere division, cannot usually go through repeated colchicine mitoses, it is thought-provoking, however, to compare these effects with those of X-rays in animal tumors. Cellular proliferation after X-ray therapy is also stopped when cells become gigantic and highly polyploid through repeated abnormal mitoses.

10.7: Colchicine and X-rays Associated

When the first work on colchicine and tumors was done in 1934, ionizing radiations were supposed to have the most harmful effects on mitotic chromosomes, and it was expected that accumulating such a great number of divisions, as seen in sarcomas for instance, would increase the radiosensitivity of the tumors (Fig. 10.1). Most recent work, however, shows that the sensitive period of the mitotic cycle is before prophase, and thus, accumulating metaphases could not be expected to increase radiosensitivity since the rate of prophases is not disturbed.⁶⁴ This is confirmed by most work on colchicine and tumors, whether in animals or in plants.

10.7-1: *Animal tumors.* X-rays were observed to be considerably more efficient in killing *in vitro* tumor cells when these had been previously treated by colchicine (Flexner-Jobling grafted carcinoma of the rat).³⁰ Here the test used was the grafting of fragments of

tumor, the number of "takes" being decreased. Colchicine (1 mg/kg) administered 15 hours before irradiation (188 r. twice weekly) increased also the effects of X-rays as measured by the size of tumors in surviving animals. No similar increase in mice and rats, even with large doses of colchicine, was found.¹² In the Yale carcinoma of the mouse, 2 mg/kg produced extensive necrosis and hemorrhage, but a border of viable tissue was always seen to persist.¹⁴ The addition of 2500 r. produced only a slightly higher rate of curability "not significant to warrant further investigation."³² In the Ehrlich carcinoma, colchicine was injected every day (5 mg.) and 260 to 300 r. delivered.¹¹ Some results seemed to indicate an improvement of the colchicine action by X-rays, which alone are not effective. However, if the dose of irradiation was increased, the life span of the colchicized mice became shorter than the nontreated controls. From Table 9.2, it is clear that no significant improvement is obtained by combining the two treatments. It must, however, be pointed out that this is a radio-resistant tumor, not well suited for such studies.

One paper mentions that in a case of gastric carcinoma, two metastases were irradiated with the same dose of X-rays, while one was injected with colchicine; the post-mortem disclosed that the latter was severely necrotic, a fact which is not surprising in view of a large local injection of colchicine and which does not demonstrate a true synergism between the two agents.¹³

The action of colchicine on human tumors has been followed by multiple biopsies.⁴³ The patients were injected intramuscularly with 2 mg. of colchicine. An increase of the metaphase percentage was noted, as well as some hemorrhage and cells with highly polyploid nuclei. These data, which are supposed to open the way towards a treatment with colchicine and X-ray combined, were not examined critically, and the variations observed may be entirely fortuitous.

A series of clinical reports have been published^{33, 49, 43} about colchicine increasing the effectiveness of X-rays, but these results are not statistically valid and cannot be accepted without further research. Colchicine was used for some time as a routine in irradiated cancerous patients at the Cancer Hospital, Brussels, with no convincing results (unpublished).

10.7-2: *Plant overgrowths*. In plants, experimental work⁷⁵ brings some significant detailed cytological data on the action of irradiation on mitoses previously arrested by colchicine, which appear to be abnormally fragile. Root tips of *Pisum sativum* and *Allium cepa* were dipped into a 1:2000 solution of the alkaloid, and irradiated (3500 r. in one minute) at various intervals later. Prophases were observed to be quite resistant, but the c-metaphases were very rapidly modified, the chromosomes clumping together and later undergoing katachro-

matic changes into apparently normal restitution nuclei (6 hours after irradiation). The nuclear membrane may give some protection to the prophasic chromosomes.

The results of these changes on the growth of the root tips and of the leaves of bulbs of *Allium cepa* have been studied.⁴² Exposure to 0.01 per cent solutions of colchicine induces the well-known root tip swelling, the so-called c-tumors, and when the plants are replaced in water, growth is resumed. If the root tips are irradiated with 900 or 1500 r. after 48 hours of colchicine, growth is arrested and leaf development is strongly impaired. These effects are greater than those obtained by irradiation alone. The action of X-rays appears to be independent of the nuclear division stage. After 48 hours of colchicine, "some non-recognizable toxic effects in the cell . . . sensitize it to irradiation." * The same author has published detailed results of investigations on the combined action of colchicine and X-irradiation on onion root tips.⁴³ It appears evident that the two actions add their effects, but the mechanism is not clear, and does not seem to be related to an increase of mitotic cells at the time of irradiation. For instance, the 48-hour colchicine bulbs are more vulnerable to X-treatment, "even though the time of exposure occurred when the number of dividing cells had passed the peak of metaphase arrest." † Irradiation by 900 r., which has only a temporary retarding effect on growth, inhibits completely cellular multiplication and growth without any immediate death of the tissues when the roots have been previously treated for 48 hours with a 0.01 per cent solution of colchicine. A long exposure to the alkaloid seems necessary, for, "while colchicine causes analogous cytological changes at 6, 12, 18, 24 and 48 hours, the larger exposures induce some microscopically unrecognizable alterations. This . . . arrests growth permanently and completely [with 1500 r.]" ‡ The optimum growth-inhibition effects were observed after 1500 r. and a more than 36 hours' exposure to colchicine.

On the other hand, onion bulbs treated for 45 minutes in a 0.05 per cent solution of colchicine, then irradiated with 300 r. and replaced in the solution, showed less chromosome rearrangements than controls, while the number of breakages was not appreciably altered. It is supposed that the short colchicine treatment could not have increased the metaphases, but impairment of the spindle function may slow the movements of chromosomes. This would leave less opportunity for the broken ends to reunite into abnormal structures.¹⁵

* M. Levine, "The Action of Colchicine on Cell Division in Human Cancer, Animal and Plant Tissues." *Ann. N. Y. Acad. Sci.*, 51 (1951), p. 1400.

† *Ibid.*, p. 1397.

‡ *Ibid.*, p. 1399.

It is evident that work in this field is particularly difficult, because the interpretation of the results depends on the action of two agents, each having a complex nature. It has recently been shown that metaphase chromosomes could be singled out and destroyed in a beam of neutrons.⁷⁸ Modern cytological and radiobiological methods should enable similar experiments to be performed with arrested metaphases. The exploded type would be an excellent test object for a study of the action of irradiation on isolated chromosomes.

10.8: The Study of Carcinogenesis

Chapter 9 has shown how useful colchicine could be in the analysis of growth. It is regrettable that more studies have not been done on the first stages of malignant change under the effect of various carcinogens. For instance, the action of azo-dyes on the liver, and the various factors which are known to influence the origin of liver carcinomas have never been subjected to the colchicine method. From the few instances which will be quoted here, there is little doubt that the early changes in mitotic activity in the liver would be fascinating to study with the colchicine tool.

In one of the first modern papers on colchicine, this was described as a tool for the detection of the increased mitotic rate in the skin of animals painted with the methylcholanthrene.²⁴ Shortly after, in the 39th Annual Report of the Imperial Cancer Research Fund, similar findings were described in mice painted with benzo-pyrene. This British work does not appear to have ever been published *in extenso*. These early results, demonstrating for the first time that mitotic activity is increased shortly after the application of carcinogens, is in agreement with later findings.⁶ These confirm the idea that some subtle cellular change takes place soon after the first painting with a carcinogen even when no malignant growth will develop for several weeks. Colchicine could evidently be used for studying all the intermediate stages between benignancy and cancerous growth.

Another observation published in 1934 is remarkable.²⁴ In methylcholanthrene-treated mice a great increase in the numbers of mitoses, as detected by colchicine, was found in the thyroid, in the salivary glands, and in histiocytes. The meaning of this remains unknown.

A single paper gives a detailed cytological study of the hair follicles of mice,⁵⁴ in normal skin, in embryos, and in skin painted with methylcholanthrene. Ultracentrifugation studies were carried out to study the cellular viscosity. This was not found to be modified, even in arrested mitoses.

There is also a possibility that colchicine may act as an anti-carcinogen. In mice implanted with methylcholanthrene and in-

jected with colchicine, no skin tumors appeared.⁶² This result is contradicted by experiments demonstrating that methylcholanthrene tumors appeared in 30 days in mice injected with colchicine.⁶⁸ The time for the controls was 100 days. There is no evidence from the data of the literature that colchicine may be itself a carcinogen.

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