CHAPTER 7

Pharmacology

7.1: Colchicine in Medical Therapeutics and Forensic Practice

The nineteenth century medical literature contains many references to *Colchicum* preparations.[18] These were widely used in the treatment of gout, a disease in which severe pain is associated with the deposition of uric acid crystals near the joints. It was logical to attempt to cure other painful joint ailments with the same drug, and references may be found dealing with the treatment of various types of "rheumatism." The medical interest in the drug had two very different consequences. Scientists took up precise pharmacodynamic experiments in order to reach a better understanding of the therapeutic effects of colchicine. Various animals and organs were treated with the drug, and important new facts were proclaimed in learned papers. A typical paper of this type is that of Jacobj, which summarizes all that was known of the drug in the 1890's.[35] Frequent reference will be made to it, and to a chapter contributed by Führer[27] in Heffter's textbook of pharmacology. Most of the contributions of the last century are now only of historical interest and will not be reviewed in this chapter. Today interest in colchicine pharmacology has been revived,[23] and it is apparent that many conclusions will have to be changed in the light of modern work. In 1952, it was stated that the mechanism of action of colchicine, from a pharmacological point of view, was "largely unknown."[23]

Another and more redoubtable consequence of the use of the drug against gout in the nineteenth century was the increasing number of cases of fatal human poisoning.[74, 44] While one author is claimed to have taken as much as 20 mg. of colchicine in an experiment to study the toxic reactions,[67] there are reports of severe physiological disturbances and even death in patients that had absorbed only a few milligrams of the drug.[15] It is quite difficult to compare all these findings, for the preparations of *Colchicum* may have been different. Even after the crystallization of the alkaloid by Houdé, preparations

were not standardized. Recent work reviewed in other chapters indicates the complexity of the alkaloidal content of *Colchicum* and the great differences in toxicity of substances chemically very close to colchicine.

Forensic medicine quite naturally was often interested in the problem of human poisoning, accidental or criminal. A vast amount of literature on this subject exists, but it has not been found necessary to include it in this book. However, one most important fact made clear in this field is the long persistence of the alkaloid in the body after death.[27] The problems of the metabolism of colchicine will be taken up further in this chapter.

All work on colchicine before 1934, excepting only that on blood-forming tissues and blood cells, which will be discussed later, was confined to pharmacological methods and chemical testing. No study of the morphological changes was made, and these remained unsuspected for a long time. The aim of this chapter is not to give a detailed study of the pharmacology of colchicine, but to place it in a new perspective, that of spindle-poisoning. The significance of this in a field apparently so distant from cytology can be illustrated by modern descriptions of death from colchicine poisoning. These will show some of the complexities of the pharmacology of that very ancient drug, *Colchicum*.

7.2: Colchicine Poisoning in Man

The junior author happened to make the first detailed post-mortem study after the discovery of the action of colchicine on cell division.[22] In 1941, a woman of 42, attempting suicide, swallowed 60 1-mg. pills of colchicine "Houdé." She lived eight days after this very high dose; delayed lethality is nearly always found in colchicine poisoning. Vomiting and diarrhea were prominent, the blood urea increased to 1.5 gm. per thousand, and there were nervous troubles which were considered to be evidence of polyneuritis. An important decrease in the number of white blood cells and of platelets was noticeable. A bone-marrow study was performed only two hours before death, that is to say, eight days after colchicine had started to act. The abnormal percentage of metaphases, mainly of the star type, illustrated that spindle activity had not yet entirely recovered (Fig. 7.1).

Microscopic evidence of this was found at the post-mortem examination.[22] Arrested metaphases could be seen in lymph glands, in the spleen, and in the Lieberkühn glands of the intestine. The histological changes in the liver were remarkable. Here, 4 per cent of all liver cells were in a condition of arrested metaphase. About 15 per cent of these mitoses were ball metaphases, while the others showed scattered chromosomes. Other findings interesting from the point of view of the

general action of the alkaloid were hypertrophy of the adrenal cortex, where no mitoses were to be seen, hypertrophy of the Langerhans' islets, and hyperbasophilia of the anterior lobe of the pituitary. These were considered to bring evidence of an "alarm-reaction," that is to say, a nonspecific pituitary-adrenal stimulation. The kidneys did not show any particular changes, with the exception of a very small

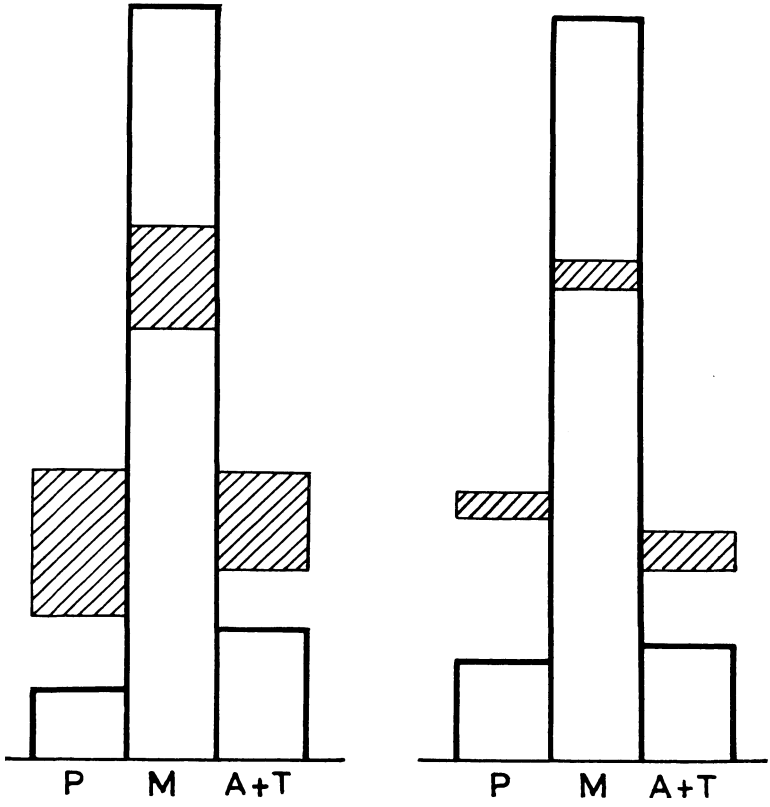


Fig. 7.1—Colchicine poisoning in man. Metaphasic arrest in the bone marrow. **Left,** granulocytes; **Right,** erythroblasts. The shaded areas indicate the normal repartition and variation in the percentage of each stage. (After P. Dustin[23])

number of mitoses. Mitoses arrested by colchicine could be found both in exocrine and endocrine tissues of the pancreatic gland.

The principal findings were (1) the persistence of mitotic changes long after the ingestion of colchicine, indicating that this substance is only slowly metabolized, (2) evidence of a general toxic reaction, and (3) considerable changes in the liver, where the proliferation of hepatic cells was made evident by the mitotic "stasis" produced by

spindle destruction. These changes were considered at the time as evidence of mitotic stimulation by colchicine (cf. Chapter 9); they are probably only an indirect effect, the alkaloid having destroyed hepatic cells and later arrested the mitoses needed for regeneration.

One other similar pathological description has recently been published.[38] This was a case of acute poisoning. A five-year-old girl swallowed an unknown number of seeds. These were later identified as belonging to the genus *Colchicum*. Repeated vomiting and abdominal pain were the first signs of toxicity. The central temperature rose and the pulse became fast. Death followed in 38 hours. Cerebral edema was conspicuous. Small hemorrhagic dots were seen on the pericardium and the peritoneal serosa. The duodenal mucosa was swollen and dotted with many hemorrhagic zones.

Evidence of mitotic poisoning was visible in the liver, where some cells were in a condition of arrested metaphase. Others showed evidence of degenerative alterations. Arrested metaphases were conspicuous in the bone marrow; a small number could be found in the duodenal mucosa. Pycnotic destruction of lymphocytes in lymph glands, Peyer's patches, and the thymic cortex was probably the result of the combined action of the mitotic poison and of the general alarm-reaction.[69]

Colchicine was detected by a biological method, while chemical reactions remained negative. Large quantities were found in several organs, in particular the liver, the kidney, and the brain. Extracts from these tissues displayed a typical spindle-poisoning effect when brought into contact with chick fibroblast cultures.

In the complex changes which take place when a large dose of colchicine is absorbed in man, it is evident that some are related to the poisoning of cell division, for instance bone-marrow inhibition,[9, 68] while others, such as the destruction and regeneration of liver cells, and the evidence of stress, are of a more complex nature. Vomiting, which may appear shortly after the drug is taken, is one major sign of a series of disturbances which clearly have nothing to do with the cytological effects which have been studied so far. These will now be described from data on various mammals and vertebrates, before analyzing the changes possibly related to spindle inhibition. The important problem of the metabolism of colchicine in the body will be discussed in a later paragraph.

7.3: Disturbances Unrelated to Mitotic Poisoning

Vomiting, diarrhea, bloody stools, and a progressive paralysis of the central nervous system are the most evident signs of toxicity. Death occurs within several hours in warm-blooded animals, or several days in cold-blooded vertebrates, after injections of the largest doses. In 1906,

colchicine was called "this most remarkable slow poison."[20] Progressive nervous paralysis leading to respiration arrest, appears to be the main cause of death, whatever the animal tested. Recent research has brought new emphasis on this nervous action of colchicine.[23]

7.3–1: Nervous system, central and peripheral. An experiment performed nearly 50 years ago gives a remarkable demonstration of the sensitivity of the nervous system towards colchicine. While the injection of even the largest doses killed a cat only after several hours, the intracerebral injection of the drug had a spectacular and rapid action. Very soon the blood pressure was found to increase, and the respiration became rapid and deeper. After 35 minutes, a sharp fall in the blood pressure indicated vasomotor paralysis. One hour after the injection, the animal died of respiratory paralysis.[20]

An important series of findings in rats and cats points to the nervous system as one of the principal causes of the various effects of colchicine poisoning. This work can only be summarized here.[23] Some of the most significant observations are listed. Vomiting cannot be, as was sometimes thought, the consequence of pathological modifications of the gastrointestinal tract brought about by mitotic arrest. The same is true for diarrhea, a frequent symptom, which would appear to be a consequence of intestinal congestion and ulcerations.[26] No diarrhea and almost no vomiting is found in animals injected with barbiturates, even when the dose of colchicine is lethal.

The central temperature falls sharply after colchicine. This may be partly a result of stress and nonspecific toxicity[14, 69] (Fig. 7.2), but the curves indicate that the decrease taking place in the first ten hours has another cause. This is now believed to be a central nervous effect.[23]

Another fact points in the same direction: Animals treated with colchicine display an increased sensitivity. While unanesthetized cats die only after eight to ten hours, the same dose of colchicine brought death in less than two hours when the animals had received barbiturates previously.[23] Barbiturate or ether anesthesia also proved to be abnormally dangerous in animals which had received the alkaloid first.

Arterial constriction leading to high blood pressure has been mentioned. Experiments of brain transsection in the cat demonstrated that this also was a consequence of a central nervous stimulation.[23]

However, other territories of the nervous system are affected by colchicine. The neuromuscular apparatus appears to be the most sensitive, though only after repeated administration of the alkaloid can the modifications be detected. An atrophy of the hind quarters of cats injected daily with 0.05 mg. per kg. of body weight was observed after two weeks. The leg muscles were converted into thin strands. There was no evidence of muscular damage. Abnormal responses to

acetylcholine were observed. There was no true neuromuscular block.

Anesthetic properties have also been described; these are probably of central origin. Death often follows a period resembling narcosis. In the dog, this appears before the muscle paralysis. In cold-blooded animals, the nervous changes may be very slow to appear. In frogs

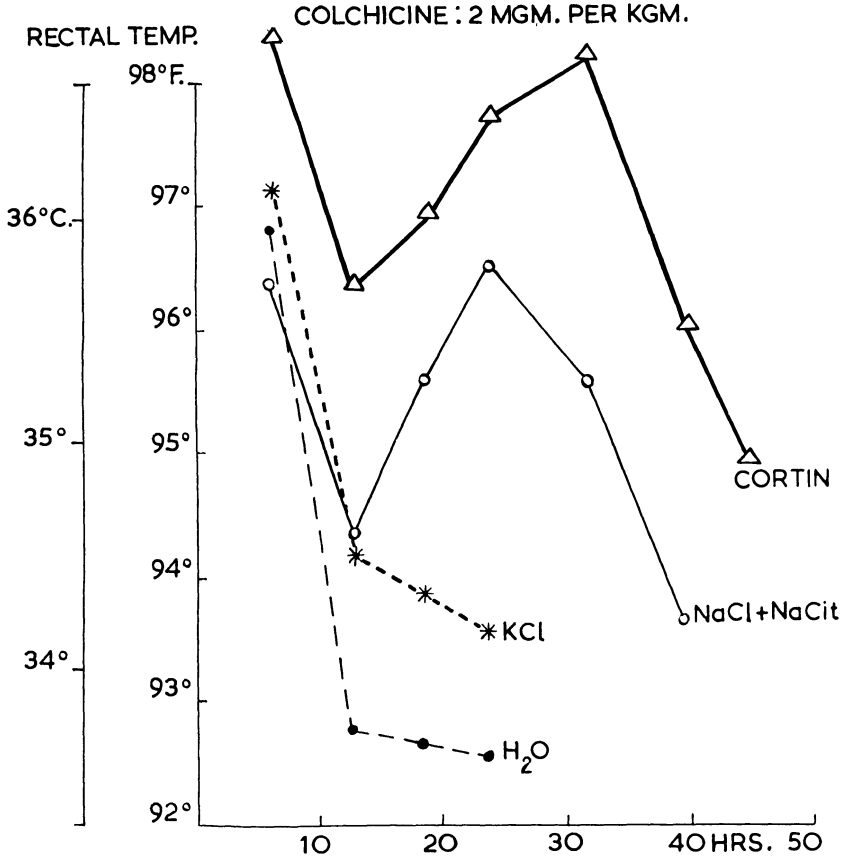


Fig. 7.2—Action of cortin and sodium on the temperature fall of rats after colchicine intoxication. (After Clark and Barnes[14])

kept at low temperature, reflexes disappear progressively, the corneal being the last, and this not until several weeks after an injection of colchicine.[27]

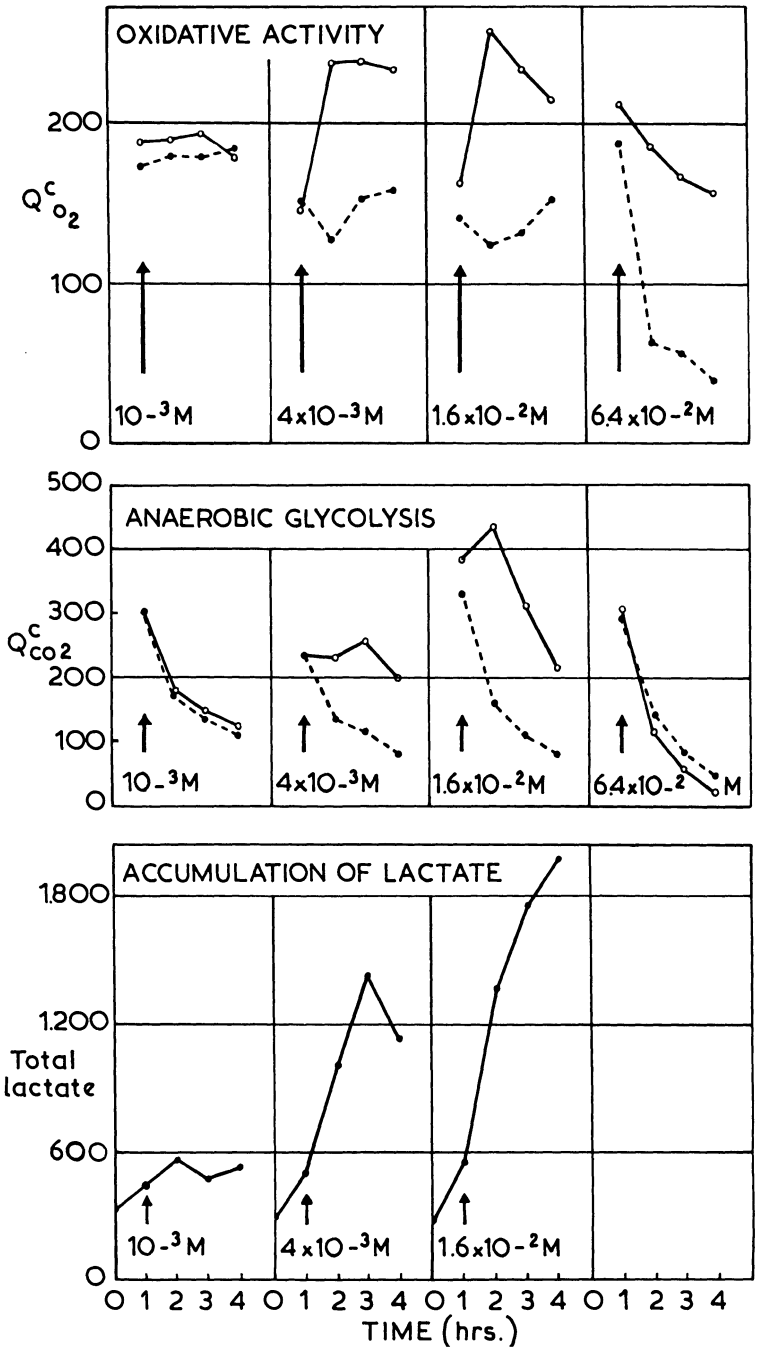
7.3–2: Striated muscle. Recent studies of the frog's sartorius muscle have brought new evidence of a muscular action of colchicine. In 1875, irreversible changes in striated muscles of frogs injected with a large dose were first reported.[27] Later "oxycolchicine" was shown to be

extremely toxic in frogs.[35] If the injected animals leapt within a few minutes after the drug took effect, their legs remained stretched and exhibited fibrillary twitchings. The rectus abdominis muscle of the frog was also modified by colchicine, and contracture appeared after repeated stimulation.[43] This was considered to be a "Lundsgaard effect," identical with that induced by many substances interfering with glycolysis.

A detailed analysis of the sartorius muscle of frog treated with especially purified preparations of colchicine has brought to light many facts, which will be summarized here and which are illustrated by Figure 7.3. The curarized muscle preparation was subjected to supramaximum electrical stimulation. Colchicine concentrations above 10^{-3} *M* produced a sustained increase in contractile force, which reached more than 60 per cent with 1.6×10^{-2} *M*. Larger doses resulted in contracture and failure to respond to stimulation. The increased contractility was paralleled by an increased demand for oxygen, which may be the double of the controls after two hours. Caffeine appeared to act synergically on this increase in oxidative processes, while metabolic inhibitors such as azide, fluoroacetate, and malonate prevented this action of colchicine. The rate of glycolysis was increased two to three times with colchicine concentrations of 6.4×10^{-2} *M*, as evidenced by the amount of lactate produced. Hydrolyzable, but not inorganic, phosphorus was also increased. These facts do not appear to point towards a change in ATP utilization. They resemble closely those of caffeine. The action of colchicine in increasing the available energy is called "relative rarity," and thus one more curious effect of the alkaloid appears to have been discovered.[23]

7.3–3: Smooth muscle and intestine. Conflicting reports have been published on this subject. The discovery that diarrhea is of central origin may be the explanation. A strong increase in the intestinal movements has been described in animals under ether anesthesia.[35] A similar effect has been found in frogs.[20] It was abolished by atropin. Increased tonus and automatic movements have also been described in spleen, uterus, and bronchioli. In the dog, the action on smooth muscle has been said to be immediate, resembling that of pilocarpin, and to be antagonized by atropin.[21] Quite different results have been reached by other workers on isolated intestine.[27, 59] The immediate effect was one of depression. The reactions towards adrenalin and atropin were not altered.

The local action on the intestine is paralytic, and was found to be related to the changes taking place in the mucosa, especially hemorrhage.[26] In a cat, injections of colchicine (1 mg. in saline) were made in ligated segments of the small intestine. A strong congestion and hemorrhages are to be seen locally within 24 hours. With larger doses,


OXIDATIVE ACTIVITY
200
100
0
$Q^c_{O_2}$
10-3M
4x10-3M
1.6x10-2M
6.4x10-2M
500
400
300
200
100
0
ANAEROBIC GLYCOLYSIS
$Q^c_{CO_2}$
10-3M
4x10-3M
1.6x10-2M
6.4x10-2M
1.800
1.200
600
0
ACCUMULATION OF LACTATE
Total lactate
10-3M
4x10-3M
1.6x10-2M
0 1 2 3 4 0 1 2 3 4 0 1 2 3 4 0 1 2 3 4
TIME (hrs.)

up to 5 mg. colchicine, the hemorrhages are apparent after 8 hours. This does not appear to be in any way related to a release of histamine,[3] which is one of the toxic actions of colchicine locally applied on the skin.[30]

Recent work[23] indicates that colchicine has no direct action on the smooth muscle of the intestine.

7.3–4: Heart and circulation. The heart is apparently insensitive to colchicine, either in frogs or in mammals. The isolated heart of the frog may beat in a 4 per cent solution of colchicine.[26] In mammals, the heart may go on contracting regularly for as long as two hours after death by colchicine poisoning.[61] As a consequence, blood pressure is only depressed immediately before death.

There is no general agreement about action on vasomotor nerves. While having no action on the heart's sympathetic fibers,[36] colchicine has been found to increase the hypertensive action of epinephrine in the rabbit under urethane anesthesia.[12] In a dog under chloralose anesthesia, a similar potentiating effect could be measured by changes in blood pressure and intestinal contraction.[58] This latter observation has not been confirmed, and only the excitatory actions of epinephrine on the vascular bed appear to be well proved.[23]

7.4: Disturbances Possibly Related to Mitotic Poisoning

Several remarkable effects of the alkaloid will be gathered under this heading. Our purpose is, when possible, to relate pharmacological effects to the histological changes resulting from spindle destruction. However, this is obviously far from being simple, and this paragraph should only be considered as a tentative grouping of cellular reactions. It will be noticed that the leukocytosis-promoting effect of colchicine, which nearly led to the discovery of its action on mitosis,[20, 21] is probably only remotely linked to mitotic arrest. Its origin may be the action of the drug on the central nervous system. However, it is associated with some of the first descriptions of tissues altered by colchicine, and has often been quoted as the origin of modern cytological work in this field. For this reason, the problem will receive more attention here.

7.4–1: Action on the blood. A substance that arrests for some hours the mitoses taking place in the bone marrow and destroys many of them, would be expected to depress blood formation. Extensive cellular destruction has been found in the bone marrow of mice.[47] Considerable congestion and a decrease in the number of nucleated cells are the consequence of this destruction. In some experiments, 20

Fig. 7.3—Action of colchicine on the isolated Sartorius muscle of the frog. Broken lines: controls. The oxidative activity and anaerobic glycolysis are measured on caffeinated muscle (1.9×10^{-3}M). The lactate concentration is expressed in microgm/gm of muscle. (After Ferguson,[24] slightly modified)

per cent of all the nucleated cells of the marrow were arrested at metaphase.[77] That this actually decreases the output of young red blood cells was made clear by reticulocyte counts in the blood of rabbits. Normal animals and rabbits with phenylhydrazine-induced hemolytic anemia were utilized (Fig. 7.4 and 7.5). A sharp but transient fall in the percentage of reticulocytes is a convincing demonstration of the inhibition of blood formation.[22]

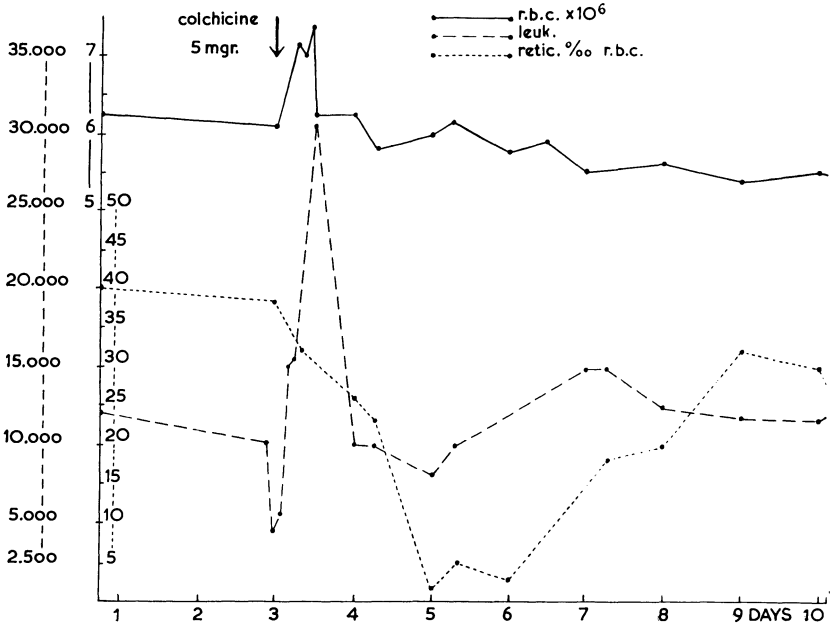


Fig. 7.4—Blood changes in the adult rabbit. Colchicine-leukocytosis and sharp fall of the numbers of reticulocytes (immature red-blood cells). The importance of the mitotic disturbances of the erythroblasts is evidenced by the slow return of the reticulocyte number to normal, and by a slight anemia. (Unpublished, after P. Dustin[23])

On the other hand, Dixon and Malden[21] discovered that in rabbits and dogs an injection of colchicine was followed by a considerable increase in the number of circulating white blood cells (Figs. 7.6 and 7.7). These authors, while reporting this curious effect, mentioned that 12 hours after the injection, the bone marrow of rabbits appears empty of most of its nucleated cells. This is in agreement with observations of bone-marrow aplasia, sometimes fatal, which have since been recorded in the medical literature (cf. Chapter 10).

The British authors[21] expressed their conclusions in a rather misleading way, to quote: "evidence is conclusive that colchicine is a powerful stimulant to the bone-marrow, since it turns out into the circulation all the elements including the erythroblasts, and leaves the

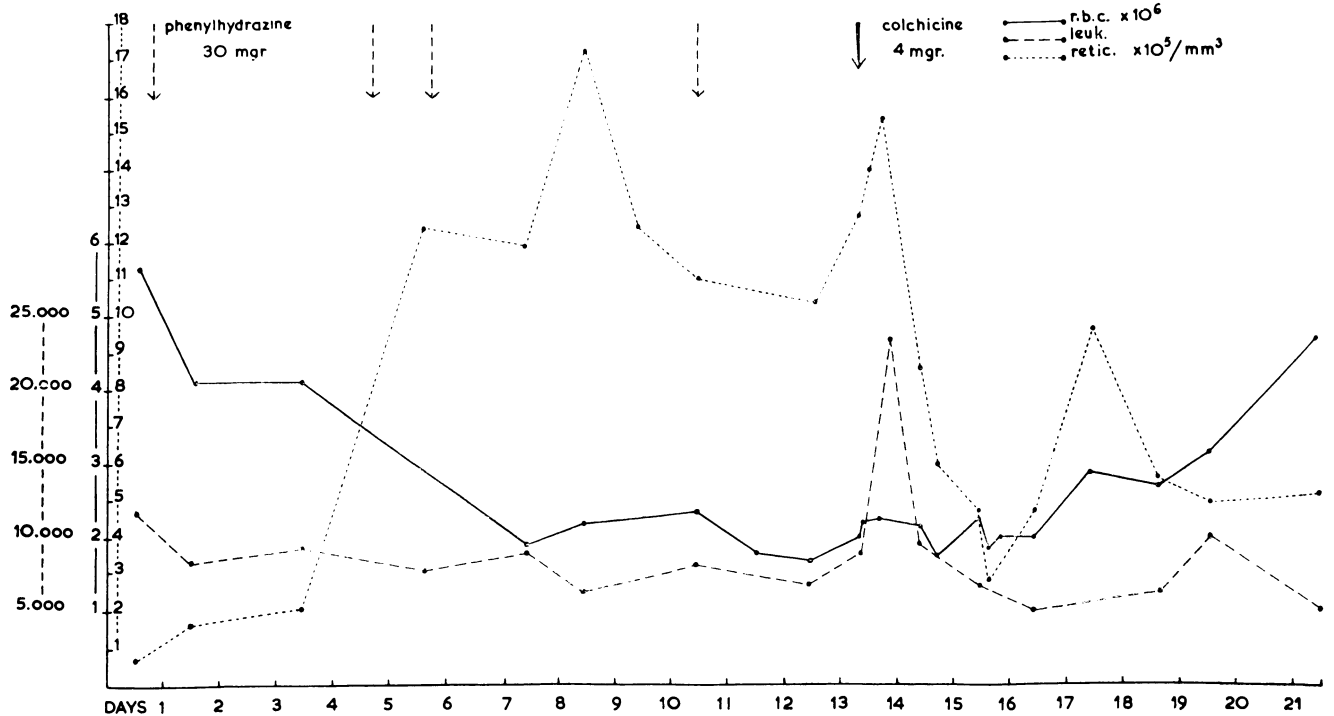


Fig. 7.5—Blood changes in an adult rabbit previously intoxicated by the hemolytic drug, phenylhydrazine. This has considerably increased the number of reticulocytes. The curve of this graph, contrary to Fig. 7.4, gives the number of reticulocytes per cmm. The injection of colchicine is followed by the usual leukocytosis and by a sharp but transient drop in the numbers of reticulocytes. This decreases later because the effect of phenylhydrazine fades off and the anemia disappears. (Unpublished, after P. Dustin[23])

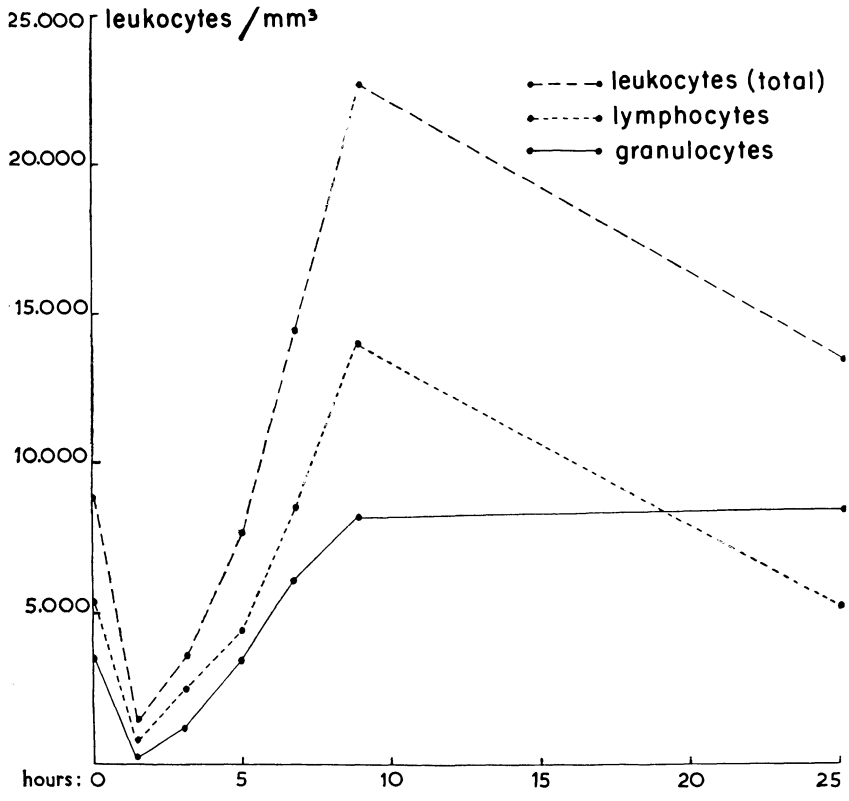


Fig. 7.6—Modifications of the leukocyte count in the blood of a rabbit injected 7.8 mg/kg colchicine, after 5.2 mg/kg atropine sulfate. (After Dixon and Malden[21])

marrow relatively denuded of corpuscles."* This is no true stimulation, and the authors are more precise when in the same paper they mention that the cells "are swept out . . . of the bone-marrow . . . into the circulation" (see Table 7.1).†

It appears evident, however, that these authors did observe some of the facts of mitotic arrest. But not being histologists, they failed to appreciate the exact significance of the facts. In 1906, Dixon[20] wrote:

A further effect of colchicine is to excite karyokinesis. This action on the marrow cannot be adequately determined at present, but it should not be regarded as specific to the leukocytes, but rather a type of the action which goes on to a greater or less degree in other tissues of the body, but is necessarily more easily investigated in the wandering cells of the blood.‡

*W. Dixon and W. Malden, "Colchicine, With Special Reference to Its Mode of Action and Effect on Bone-Marrow," *Jour. Physiol.*, 37 (1908), p. 73.

† *Ibid.*, p. 62.

‡ W. Dixon, *A Manual of Pharmacology* (London: Arnold, 1906), p. 96.

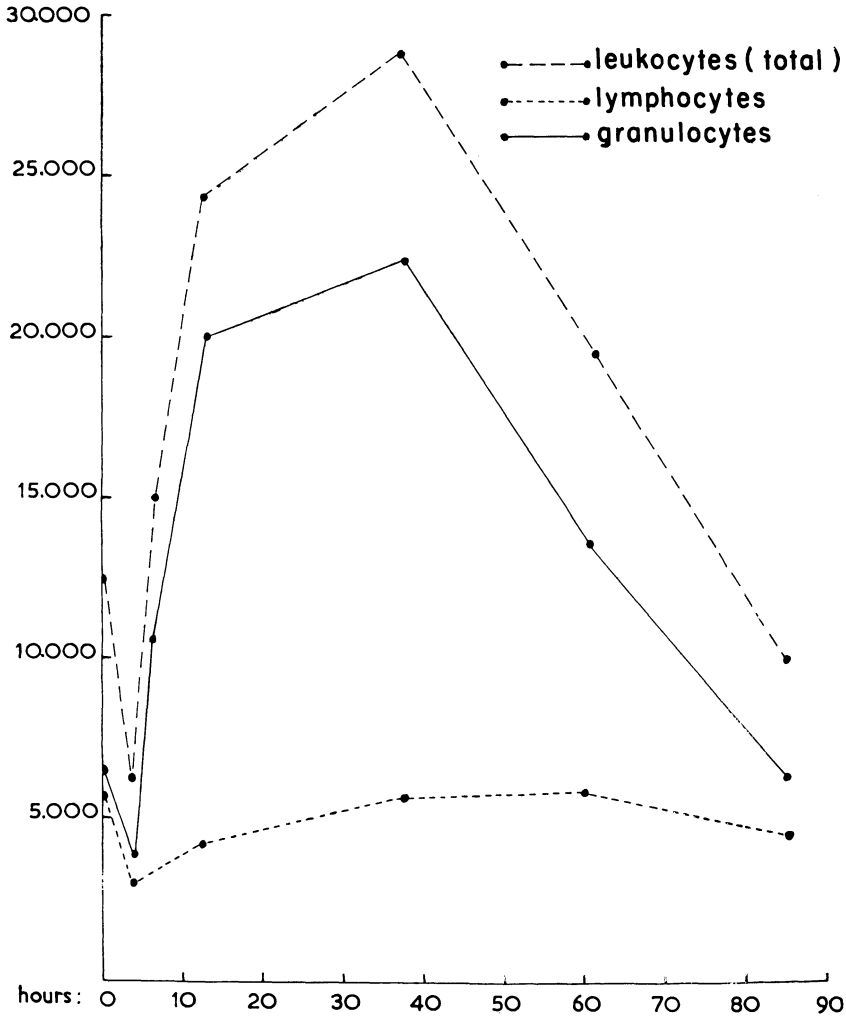


Fig. 7.7—Modifications of the leukocyte count of a dog injected 0.34 mg/kg colchicine. (After Dixon and Malden[21])

In a later paper,[21] it is mentioned that after repeated injections of colchicine in rabbits, "sections of smears of the bone-marrow . . . exhibit proliferation . . . : *plentiful mitotic forms can occasionally be observed*" [our italics].*

There can be no doubt today that the significance of these histological changes was not grasped. These publications on colchicine pharmacology were widely quoted, and for 26 years text books

* *Jour. Physiol.*, 37 (1908), p. 76.

mentioned that colchicine increased the numbers of leukocytes. Nobody appears to have been interested enough to study more precisely the bone-marrow changes, and it is only in 1934 that this was done.[47] Colchicine-mitosis was then discovered at once, for in the laboratory of A.P. Dustin, Sr., problems of mitosis and mitotic stimulation had been studied for many years, and the proper techniques had been developed.

TABLE 7.1
EFFECT OF COLCHICINE ON BLOOD COUNT IN RABBIT*
(Injection with 0.02 gm. colchicine made at 1:05 P.M.)
(After Dixon and Malden)

	Time of Blood Count				
Cellular Types	1 P.M.	1:30	3:00	5:00	9:15
Total leukocytes per cmm.	8850	4600	6700	9650	20,000
Granulocytes (%) (pseudoeosinophils)	37	16	50	36	16
Eosinophils	1	1	1	0.5	1
Mast cells	7	4	7	6.5	1
Myelocytes			3	7.5	7
Monocytes	10	4	7	4.5	4
Lymphocytes	45	75	32	45	71
Erythroblasts (per cent leukocytes)		5	4	8	11

* Weight of rabbit, 1800 gm.

While the changes occurring in the blood-forming tissues were then described, first in mammals,[47] then in amphibia,[17] the Dixon and Malden experiments were repeated in rabbits by another author, unaware of the problems of mitotic regulation and poisoning.[16] The effect of repeated small (from 1 to 5 mg.) daily injections was studied. Immature white and red blood cells were found in the blood stream. The percentage of hemoglobin and the number of red blood cells progressively decreased. The marrow was very cellular, with leukoblastic areas far in excess of the erythroblastic ones. The following conclusion was reached, to quote: "Colchicine, undoubtedly, stimulates the formation of new cells in the marrow, and induces immature cells . . . to appear in the peripheral blood, but . . .its destructive powers outweigh its stimulant effect."* Here again, the action on the mitotic spindle was missed.[16]

*C. R. Das Gupta, "The Action of Leucopoietic Drugs," *Indian Jour. Med. Res.*, 26 (1939) p. 997.

At present, no clear relation can be discovered between the inhibition of mitotic growth and the colchicine-leukocytosis, and clearly new work is badly needed in this field. Some facts are of interest however.

It has been discovered that in leukemic patients and in normal men a single dose of colchicine (2 mg.) may increase considerably the number of platelets. The bone-marrow megakaryocytes do not change in number, but there is evidence of a greater platelet-building activity by their cytoplasm.[37, 41] In essential thrombopenia, where megakaryocytes are present but appear to be unable to produce platelets, this effect of colchicine was not found. It is evidently not related to mitosis, but may be similar to some other membrane changes induced by the alkaloid (Chapter 4).

Some recent work attempts to relate the bone-marrow changes and leukocytosis. This is often preceded by a transient period of leukopenia, which appears to have no causal influence on the leukocytosis.[77] Bone-marrow studies in mice and rabbits all confirm the increase of arrested metaphases, which is about 15-fold in the rabbit after 15 hours. The erythroblastic cells become progressively more numerous than the granuloblastic; the increase is from 10–15 per cent to more than 60 per cent in mice. The immature cells increase in proportion, because the adult cells leave the marrow. There is no visible relation between this phenomenon and the mitotic changes.[77] However, repeated daily injections of 12 μg. of colchicine increase considerably the number of leukocytes in the blood of mice (more than 250,000 per cmm.). It has been suggested[77] that these changes may be the consequence of a central nervous stimulation of the bone marrow. This is in line with more recent pharmacological data (see above) and merits close attention.

The following changes of blood cells after colchicine may be mentioned here, though an explanation is not evident. Young rats, aged 1 and 3 days, develop anemia, and a single injection decreases the red blood cell diameter.[71] These two facts may bear some relation to the decrease in the numbers of reticulocytes, which have a larger diameter than average red blood cells. An increase of "monocytoid" leukocytes in a case of fatal human poisoning[39] parallels the observation of abnormally great numbers of histiocytes in guinea-pig tissues after repeated injections.[56] Several important data on blood cells studied by culture *in vitro* with the help of colchicine will be reported in Chapter 9.

7.4–2: Skin, hair, and feathers. Colchicine arrests the mitoses in the hair follicles in mammals. Inhibition of hair growth can be seen in rats in the vicinity of colchicine injections, and loss of hair has been found in human intoxication.[44] In birds, similar changes may be ex-

pected to exist, but the following results are not necessarily the consequence of mitotic poisoning.

In hens, 1.5 mg/kg of colchicine causes death in 36 to 48 hours. The symptoms are those already described: diarrhea, vasomotor disturbances, and nervous paralysis. Injections of 1.2 mg/kg are not fatal. They cause a shedding of the feather buds in places where the feathers were removed 15 days previously.[2] The feathers which grow next have a white extremity. Two similar injections, 7 and 14 days later, give to these feathers a deep black barring. The other feathers of the animals darken. An analysis of the rate of growth of the feathers demonstrates that colchicine acts immediately and that it modifies the feather growth for 48 hours. It was demonstrated later[13] that the section of the spinal nerves could bring about similar changes of color. The authors are led to the conclusion that colchicine may act by affecting the nervous system, a conclusion remarkably in line with later research.[23]

7.5: Nonspecific Toxic Changes

In considering the modifications of an organism which has been injected or which has received by any route a substance as toxic as colchicine, nonspecific changes must be taken into account.[69] These may be difficult to separate from effects of the drug itself, and only future work will enable this aspect of the subject to become clearer. For instance, while the influence of the pituitary-adrenal system is known to be great in all types of "stress," there are only two papers on the action of colchicine in adrenalectomized animals.[24, 42] It was demonstrated that an important number of the nuclear pycnoses of thymus and lymphoid tissue are only indirectly the consequence of mitotic poisoning. Pycnosis is much less apparent in adrenalectomized animals.[42] No work has been reported on the general effects of the alkaloid after hypophysectomy. This should be important, considering the possibility of the pituitary gland taking part in some central nervous stimulation of leukocytosis.

The facts assembled here may only have a distant relation to stress and the alarm-reaction. It is known, however, from experimental work[38] and from human pathology[23] that this reaction can appear after colchicine. Also, several of the changes reported have also been observed after other mitotic poisons, chemically unrelated to colchicine.[73] It is logical to believe that they belong to the vast group of nonspecific tissue changes.[69]

7.5–1: The "hormone-mimetic" actions of colchicine. The idea of colchicine having some direct hormonal action was put forward by botanical work.[32] It led to some curious experiments which are im-

portant to consider when one knows how often the alkaloid has been used for the detection of hormone-stimulated growth (Chapter 9).

During the breeding season, the fish *Rhodeus amarus* displays brilliant red "nuptial colors," which are related to the expansion of chromatophores and to local hyperemia. These colors appear in animals treated with male hormones. Colchicine alone has the same effects.[32, 33] Nuptial colors are displayed by fish subjected for 10 minutes to a 1.5/1000 solution, or for 35 minutes to a concentration of 0.75/1000. Colchicine and hormones add their effects, and the full skin changes could be produced in 2 instead of 20 hours with hormone alone. The oxygen consumption of the animals was also increased.[50] However, the "endocrine" mechanisms of this action of colchicine may be questioned. In females of the same species, no increase in the size of the ovipositor was noted.[8] The changes of the male fishes, where vasomotor mechanisms play a great part, may have been either the consequence of a nervous action, or of the general toxicity of colchicine.

The possibility of stimulating the action of pituitary hormones by the alkaloid was strongly suggested by experiments on the ovulation of isolated ovaries of *Rana pipiens*. This was considerably accelerated, both in whole animals and on isolated ovaries (Fig. 7.8). The eggs were fertilizable, but none ever divided. Colchicine was believed to bring a "true potentiation" of the pituitary hormones controlling ovulation.[52] In the rabbit, however, no potentiation of the action of pregnant mare's serum, containing gonadotropic hormones, on the rate of ovulation could be detected.[52] Colchicine had no action on the weight of ovaries of mice similarly injected, or on the seminal vesicles of rats injected with testosterone.[52] Neither do results of experiments on silk-worms[33] justify the conclusion that colchicine is "hormone-mimetic." The only possibility is that through nonspecific action, this toxic drug could stimulate the secretion of hormones by endocrine glands, in particular the pituitary.

7.5–2: Liver and kidney damage. The mechanism of these changes is not clearly understood, but it certainly plays an important part in the general toxicity of the drug. Though bile secretion has been supposed to be increased, severe degenerative changes and necrosis have been described in the livers of mice,[56] especially after repeated injections.[40] In mice, the LD_{50} dose induces liver cell steatosis in one hour.[62] Steatosis of heart muscle cells and kidney tubules was also noted. Female mice appear to be more resistant to this damage than males.

Mitoses of liver cells have been described in human poisoning by colchicine. There are often arrested metaphases, even long after the drug has been administered, a fact which is explained by its slow excretion.[11] Three days after injection of colchicine in mice, normal mitoses also

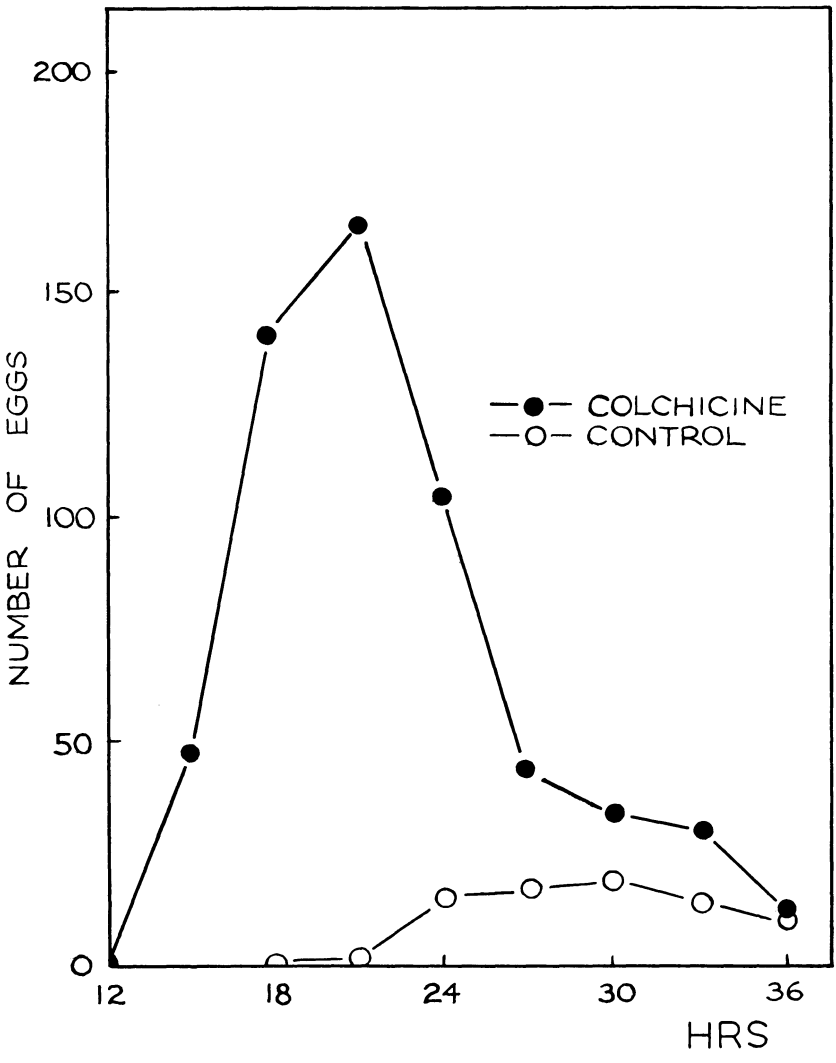


Fig. 7.8—Action of colchicine on the release of eggs from the ovary of the frog, treated in vitro with pituitary powder. (After McPhail and Wilbur[52])

have been observed in liver cells. These will be discussed in the next paragraph. After several injections of colchicine, many arrested mitoses are to be seen. The stages of recovery lead often to bizarre nuclei which may resemble those of megakaryocytes. Cellular damage may not be evident at all, and the cause of these divisions is not clear. A hormonal stimulation related to stress and the adaptation syndrome is possible.[53]

In chronic intoxication of mice, after daily injections of 12 to 15 μg. for 20 to 30 days a great number of liver nuclei are irregularly shaped. More than 40 per cent of these contain spherical bodies resembling huge nucleoli. These are diffusely stained by acid dyes. They persist 13 days after the end of the injections. No mitoses were seen, a rather surprising fact.[40] It may be suggested that these intranuclear bodies result from arrested mitoses, and represent spindle material, similar to the hyaline globules and pseudospindles (Chapter 3).

Kidney damage has been mentioned repeatedly,[19, 62] but has never been described in detail. It should be borne in mind while considering in Chapter 9 the use of colchicine in studies on the mitotic growth of kidney tubules.

7.5–3: The "late" mitoses. In many experiments on mitotic poisons, and in particular after the injection of trypaflavine (acriflavine), normal mitoses could be found in unusual locations several days after the mitotic poisoning itself.[73] Colchicine is also effective, and this is one of the observations that led to the belief that a true mitotic stimulation existed. Actually, things are probably far more complicated.

In adult mice,[47] divisions could be observed in many locations: liver cells and Kupffer cells, endothelial and epithelial cells of the pancreas, salivary cells, histiocytes, and renal epithelial cells. Some of these may be abnormal, but normal mitoses are usually found in liver, pancreas, kidney, and adrenals, from one to two days after an injection. While some of the divisions may be of a regenerative character, for instance in liver and kidney, the important fact is that this is not a phenomenon observed with colchicine alone. It obviously needs further investigation, because very few authors appear to have taken notice of it. In the light of all recent work on stress, the hypothesis that pituitary-adrenal stimulation of cellular division has taken place as a consequence of the general toxicity of colchicine, deserves notice.

7.5–4: Chemical changes of the blood. The idea of the alkaloid producing a stress effect may help to explain some unrelated facts mentioned in the pharmacological literature. The hyperglycemia following the intravenous injection of 1 gm/kg of glucose in the dog is increased 10 to 12 hours after colchicine.[49] The lethal dose of the drug in this species is 1 mg/kg. It decreases the blood sugar and also the body temperature.[64] The action on the glycemia does not appear to be related to pancreatic islet activity. The LD_{50} dose has the same effect. In pancreatectomized dogs, on the contrary, the glycemia again reaches its normal level within 6 to 14 hours.[65] The influence of the adrenal cortical hormones has not been studied in these experiments. Evidence has been presented that the adrenal plays an important part in controlling the temperature fall observed after colchicine poisoning (Fig. 7.2).

Considerable changes of blood-clotting time have also been reported in rabbits injected with large doses of colchicine. This may be five times too long.[48] It will be mentioned elsewhere that hemorrhage has been considered an important factor in the action of the drug on neoplastic growth.[7] One author has found that the direct action of colchicine, added *in vitro* to oxalated blood plasma containing thrombin, was to decrease the clotting time from 20 to 15 seconds.

Much remains to be learned about what happens when a complex organism is under the influence of such a poisonous chemical. It is evident that much of the reviewed work is incomplete, that even the exact chemical structure of the "colchicine" that is injected is not always known, and that we are confronted with a puzzle in which specific effects of colchicine are intermingled with general toxic reactions involving hormonal stimulation and metabolic changes. The importance of all these apparently unrelated facts emerges when one considers colchicine's action in gout, which will be discussed later. It is first necessary to have some idea of the metabolic changes, if any, of colchicine within the body. The study of this problem has recently received some new light.

7.6: Metabolism of Colchicine

Forensic medicine demonstrated long ago that colchicine could be detected, apparently unchanged, in the bodies of patients who had died of an overdose.[27] Experiments on cold-blooded animals, which can withstand considerable amounts of the alkaloid (Table 7.2), demonstrated that this remained unchanged. They also brought attention to the considerable variations in toxicity depending on body temperature.[27, 63, 75] For instance, a frog is able to withstand an injection of 50 mg. of colchicine. For several days the chemical may be detected unchanged in the urine. If such an animal, two to three weeks after the injection, is warmed to 32°C., a temperature in itself harmless, death supervenes in a few days. Progressive nervous paralysis is evident, a typical manifestation of colchicine poisoning. Similar facts are to be found in hibernating bats, which do not appear to be affected by colchicine.[31] Once the animals are warmed and awake, the characteristic nervous poisoning becomes visible.[31]

After injection in dogs and cats, colchicine is chemically detected in the feces and urine. Similarly in man, it is excreted unchanged in the urine. However, only a fraction of the initial dose can be recovered.[27] This suggested to early workers that the alkaloid was modified and metabolized in the animal and human body. The striking effect of temperature suggested that some of these changes may only be possible in warm-blooded animals, or in artificially warmed amphibians. Table 7.2 shows that the toxicity of colchicine is

about the same in mammals and frogs when the latter are kept at 30–32°C.

It was also known that solutions of colchicine that had been left standing and have become brownish, probably as a result of oxidation, become far more toxic to frogs, even at low temperatures.[35] In 1890, an attempt was made to separate the toxic fraction of these oxidized

TABLE 7.2
RELATIVE TOXICITY OF COLCHICINE
(After Fuehner[28])

Species	Lethal Doses, After Subcutaneous Injection (*gm/kg of body weight*)
Rana esculenta, 15–20°C.	1.200–2.000
Rana esculenta, 30–32°C.	0.002–0.004
White mouse	0.003–0.010
Rabbit	0.003–0.005
Dog	0.001
Cat	0.0005–0.001

preparations, and a substance tentatively named "oxydicolchicine" was isolated. This was believed to be made of two molecules of colchicine linked by an oxygen atom.[35] Artificial oxidation of colchicine with ozone yielded a similar substance. A further experiment attempted to prove that the kidney was the organ in which colchicine was oxidized to a more toxic product. About 330 mg. of amorphous colchicine were added to defibrinized hog's blood, and this was slowly perfused through the hog's kidney. From this organ 42 mg. of a brown substance were recovered. This, like "oxydicolchicine," displayed a rapid toxic action in the frog, where the symptoms were visible about one hour after the injection of 30 mg.

These experiments do not appear to have been checked by modern methods. This would be interesting now that the chemistry of the alkaloid has made such great progress (cf. Chapter 6). No substance of the structure assigned to "oxydicolchicine" has been described. On the other hand, experiments with mitotic poisoning are conflicting. In mice, solutions of colchicine lose about 20 per cent of their cytological activity after five weeks of standing.[47]

The fate of colchicine in the animal body has been studied by modern methods, chemical, biological, and physical. A colorimetric

method of titration was checked by measuring the mitosis-arresting properties of solutions either by injecting them in mice or by studying their action on tissue cultures.[11] After a single injection the blood level in the adult rat decreased rapidly, and remained stable after a few minutes. The tissues contained less alkaloid than the blood. Elimination was by the bile and intestine, and within a few hours, 10 to 25 per cent of the dose injected was to be found in the intestine and its contents. Elimination by the urine only lasted a short time, while the blood concentration was at its highest. Within 16 hours, 50 per cent appeared to have been eliminated. There was neither evidence of a change into a more toxic substance, nor of any selective tissular fixation. The cumulative toxicity of repeated injections is a simple consequence of the slow excretion.

By growing *Colchicum* in an atmosphere containing radioactive carbon, C^{14}, in the form of CO_2, a biological synthesis of radioactive colchicine has been made possible.[76] The fate of this in the body of mice has been tested. One fact of importance is that four hours after the injection, no more colchicine could be detected in the central nervous system, muscle, heart, or blood. Most of the radioactive alkaloid was detected in the kidney, the spleen, and the intestine. Neoplastic tissue (sarcoma 180) did not contain more colchicine than the liver. An unexplained fact is that while the spleens of control animals were a site of active fixation, no more colchicine could be found in this location in tumor-bearing mice.[4] These observations appear to demonstrate that the alkaloid brings about quite rapidly some change in the brain without becoming fixed in this tissue.[4] Evidence will be presented elsewhere (Chapter 9) that colchicine may be retained for some time in tissues of cold-blooded animals (*Xenopus* tadpoles).

Further research is also necessary in this field, for there appears to be some contradiction between the stability of colchicine as evidenced from old and modern work, and the biological activity and specificity of this molecule. These problems will be discussed in the last chapter of this book.

7.7: The Treatment of Gout

Logically, colchicine pharmacology should be an introduction to its use in medicine and should enable us to understand why this plant alkaloid is effective in treating a disease of uric-acid metabolism. However, as will be noticed, actual data on pharmacology are of small help in understanding the curative properties of *Colchicum*. Many complicated side-effects have been described, many strange properties investigated, but modern medicine is apparently not much closer than the Ebers Papyrus in explaining the medical use of this plant.

Gout, which was still called a forgotten disease in 1946,[51] has regained much medical attention. New methods of treatment and new methods of study have brought this change. Also, the frequency of cases of gout may have increased in some countries. The principal and painful lesion that affects the joints of gouty patients results from deposits of uric acid. This chemical was believed to be mainly related to nucleoprotein metabolism. Studies with radioactive uric acid, marked with N^{15}, have helped to understand the origin of the so-called "miscible pool" of uric acid, which is considerably increased in some cases of gout. This has been demonstrated to originate from many pathways of metabolism. All proteins, carbon dioxide, ammonia, glycine, serine, and carbohydrates may be used as building blocks for uric acid. Methods for studying the changes of the "miscible pool" of uric acid have been developed.[28, 29, 70]

This has been mainly the consequence of the discovery that steroid hormones like cortisone,[6] and the adrenotropic hormone of the pituitary (ACTH) may play an important part in gout and may possibly be used for its treatment.[25, 28, 29] Now, the nonspecific toxic reactions of colchicine poisonings have been described. These would result in an increased secretion of ACTH and cortisone.[69, 57] Could colchicine possibly act in a nonspecific way in this disease?

The considerable amount of work, mainly clinical, which has been published these last years on this subject can only be rapidly reviewed here.[34, 45, 46, 60, 78, 79, 80] Current practice of handling gouty patients with colchicine has recently been summarized.[29]

The doses which elicit in animals the alarm-reaction and ACTH secretion are far larger than those effective in human therapeutics. The Thorn test of adrenal stimulation demostrates effectively that in patients with diseases other than gout, therapeutic doses of colchicine do not stimulate the pituitary and the adrenal. The urinary elimination of 17-cetosteroids is not modified either.[45, 46] A positive Thorn test is demonstrated by a rapid fall in the numbers of eosinophil leukocytes in the blood. In one case only was this positive, the eosinophils falling to 53/cmm. and later rising to the normal number of 269. This, however, was in a man who had taken 24 mg. of colchicine in 24 hours, that is to say more than six times the usual dose.

On the other hand, while ACTH and cortisone may be effective in the treatment of gout, they have by no means taken the place of colchicine. This is now used either at the same time or after the injections of hormones, and it is recognized that its action is unrelated to the alarm-reaction, and to put it shortly, "entirely unknown." [25]

Some workers believe that the acute crisis of gout, the origin of which is by no means clear, is related to allergy. Colchicine has been found to decrease the intensity of the anaphylactic shock in guinea pigs injected with ovalbumine.[1] In patients suffering from diverse types

of allergy, such as serum sickness, Quinke's edema, or urticaria, colchicine has been used with results comparable to those of the antihistamine drugs.[54, 66] Colchicine, however, does not antagonize histamine, and this new use in therapeutics now presents further unsolved problems.

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