CHAPTER 1

The Parent Plant

1.1: The Knowledge of *Colchicum* in Ancient Civilizations

The history of *Colchicum,* the drug of ancient and modern materia medica, is rooted in the myths and the written records of ancient Egypt, India, and Greece, and runs its course through the ages into the world of today. Not only do modern formularies admit *Colchicum,* the producer of the pure substance *colchicine,* but this plant is probably one of those mentioned in the Ebers Papyrus. This Egyptian document was prepared about 1550 B.C., and is our oldest medical text. *Colchicum* could be one of the saffron plants of the Papyrus. From this early age through thirty-five centuries of medical history to the compilation of the modern pharmacopeias, very few drug plants have survived. In fact, only eighteen, among seven hundred plants[44] originally listed as material for ancient Egyptian practitioners, achieved such historical fame.

The Egyptian civilization developed a code for practicing medicine in which plant products played an important role, and the Ebers Papyrus summarized this accumulation of knowledge. Egyptian doctors were advised in the Papyrus to give various seeds to their patients for relief from aches and pains. The seeds were administered on bread.[5] While *pure* colchicine was not given in these doses, we can assume that the drug was used in treating rheumatism and gout, ailments which then and even yet afflict the human race. It is probable also that, if seeds were used, a large quantity would have been administered to the patient.

A danger associated with using colchicine in the crude form is the poisonous property of the drug. Enough active substance can be given to cause death in warm-blooded animals. Dry seeds may have as much as four parts of the drug per thousand of dry raw material. Perhaps some patients died from the colchicine prescription, for severe punishments were said to be meted out to ancient doctors when a patient succumbed. In some instances the physician even paid with

his life.[29] Since gout and rheumatism were common ailments among the noble and the wealthy, the attending physicians, who were often servants of the court, must have held a rather precarious position. Yet, in spite of its poisonous nature, *Colchicum* in correct dosage was capable of relieving pain if administered as seed, powdered corm, or even dried flowers. It is probable that substitutes for *Colchicum,* as well as similar plants containing very small amounts of colchicine, were employed.

Plants were frequently used in ancient days without sound basis, and more magic than medicine was practiced; in fact, magic and the medicine man have been associated through the ages. Our modern word *pharmacy* originates[24] from an Egyptian term *pharmaki* and the Greek *pharmakon.* These terms are in turn related to another Egyptian word *pharmagia,* which means the art of making magic.

Another civilization, the Hindu, developed a medical system independent of the Egyptian and the Babylonian. This period is known as the Vedic,[29] and extends from 2000 B.C. to 800 B.C. Much information about treating diseases with plants is transmitted in the Vedic text.[29] Although in this book specific plants are mentioned and certain diseases noted, and while *Colchicum luteum,* a producer of pure colchicine, is common in the Indus River area of the Himalayas, the present Indian *Colchicum* cannot be deciphered from this book.

At some time during the Vedic period a traffic in drugs was established between the Orient and Arabia. Good evidence is at hand to show that Hindu medicine had an influence upon Arabian medical knowledge. There was a serious decline in Hindu medicine, but the traffic in drugs continued. This exchange reached such proportions that Pliny the Elder complained about his money being drained to the Orient for drugs. Two species, known as the Kashmir hermodactyls,[7] could have been among these drugs. They are identified as *Colchicum luteum* and *Merendera persica.* Although both contain colchicine, the respective quantities differ markedly, as will be described later.

Botanical historians[21] tell of an ancient class in Greece known as the Rhizotomi, or root gatherers. They were pharmacobotanists practicing their art in the pre-Hippocratic era; their powers resembled those of magicians, associating all manner of ritual with the collection, preparation, and dispensing of roots. Such details as the wind direction, time, season, as well as astronomical signs were observed.

Since foods were primarily grain and leaves, the roots must have served other purposes such as medicine. Driving away evil spirits that caused disease may have been helped by using underground plant parts, and the trade in roots by the Rhizotomi flourished.[21]

More than fifty species containing colchicine are native to the region where the Rhizotomi practiced.[41] The most notable species is

Colchicum autumnale,[41] that produces flowers in autumn followed by leaves, fruits, and seeds the next spring. Such an unusual habit must have attracted these pharmacobotanists.[21]

Perhaps the best link between ancient and modern medicine is seen in the two drugs found in Oriental bazaars: the Surinjan-i-talkh and the Surinjan-i-chirrin.[7] These corms are distinguished as bitter and sweet surinjan and are obtained from the Kashmir hermodactyls growing in the northwest Himalayan foothills.[7] Botanically the drugs are identified as (1) *Colchicum luteum,* the bitter, and (2) *Merendera persica,* the sweet; both contain colchicine, 0.2 per cent and 0.02 per cent, respectively.[30] Pharmacists advise their use for rheumatism as well as for aching joints.

If these same hermodactyls entered the drug trade from the Orient to Arabia, then early Arabian physicians may have borrowed their ideas for treating gout from this source. It is difficult to determine how many centuries have passed since the Hindu specialists began collecting the hermodactyls and other plants useful in medical practice. But their knowledge of herbs has been handed down for countless generations to their successors of the present day.

The ancient usage of *Colchicum,* along with an antiquity in medicine, can be established through several sources: the Ebers Papyrus, a drug traffic from the Orient, and the evidence about a pharmacobotanical trade practiced by the Rhizotomi. Present-day surinjan may link the past to modern medicine.

Our discussion of the knowledge of *Colchicum* in the ancient world turns for a moment to Greek history and mythology, and it is in Greece that the period we are examining will close with the organization of medical knowledge around the system of Hippocrates.

Colchicum is named for the land of Colchis at the eastern tip of the Black Sea.[47, 22] In this area the plants are most abundant. When Colchis was mentioned to the Greek, visions of sorcery immediately arose. This was the land where Jason secured the Golden Fleece. Here he met the sorceress Medea, famous for her powerful life-giving brews. She was said to have rejuvenated Jason's aging father by substituting a special potent mixture for his blood. Many of her directions for poisonous mixtures required underground roots. Magic powers were associated with these ingredients that figured in Medea's sorcery.[6]

Among the instructions for making a certain mixture were specific details for collecting the poisonous plants.[6] In one instance, only during a hoarfrost could roots be dug. While boiling the juices in a pot, it was said olive branches touching the brew would immediately bring forth flowers and fruits.

The ancient Colchian kings had gardens containing poisonous species. Undoubtedly the knowledge of the toxic properties of plants

was at their disposal. Such plants might have served their intrigues and provided means for the elimination of competitors or persons convicted of crime.

1.2: Botanical Studies of *Colchicum* From Dioscorides to Twentieth-Century Investigators

In the land of Colchis, along the Black Sea, an autumn-flowering crocus-like plant occurs in abundance (Fig. 1.1). Dioscorides, first century botanist-physician, knew about this particular species from either personal observations in the area or through reports by travelers to this region. This fall-blooming meadow saffron was named the

Fig. 1.1—Flowers of **Colchicum autumnale** showing only the floral parts above ground. (Photograph, courtesy of General Biological Supply House, Chicago, Ill.)

Colchicon,[22] a name which has been continued in its Latinized form to the present time.

Dioscorides made very careful descriptions dealing with such phases as growth, development, and morphology of the plant. His drawings involving two plants (Fig. 1.2), one with fruits, seeds, and leaves, the other with flowers only, clearly show that he associated

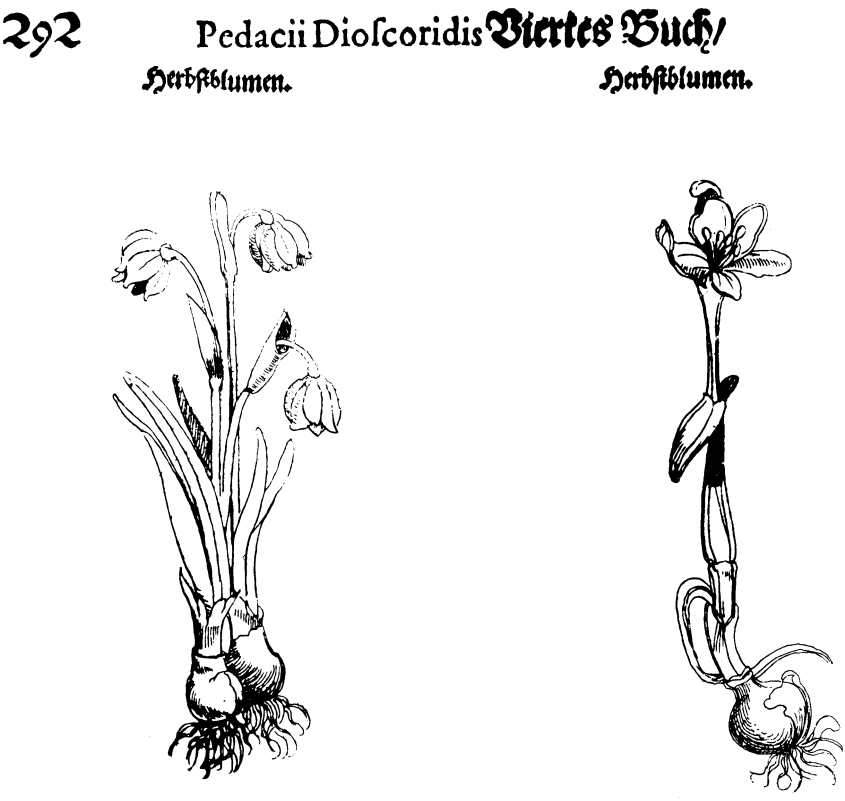


Fig. 1.2—Diagrams showing the seed-producing portion of **Colchicum autumnale**, and the flower stalk appearing in autumn. **A**, fruiting; **B**, flowering. (After drawings by Dioscorides)

autumnal flowering with spring fruiting, both having the same underground portion. This was a careful scientific observation for his day. Such great detail was given to the corm, bud, leaf, flower, and seed that writers copied his observations and drawings for the next fifteen centuries.

Since the botanical and medical professions were closely allied in the times of Dioscorides, it was natural that the objective of his study

should extend beyond strictly botanical descriptions and that his primary interest should be in the medical application of plants. He warned that *Colchicon* was a dangerous poison and compared it with the mushroom that causes death (Fig. 1.3). He was concerned that this plant might be used by practitioners unaware of its poisonous nature, and the effect of his careful descriptions and stern warnings was so profound that many followers avoided the use of *Colchicon*.

Herbſtblumen/ Spinnblumen/ Colchicon, Bulbus Agreſtis. Cap. lxxx.

Beſchreibung.

Spinnblumen / Nachtblumen / Herbſtblumen / Griechiſch Colchicon, zu Latein Bulbus Agreſtis, ſindt weißlechte Blumen / den Saffran Blättern ehnlich / vnnd wachſen im außgang deß Herbſts / nach den Blumen gewinnen ſie Blätter wie die Blätter der Wurtzeln / die man Griechiſch vnd zu Latein eygentlich Bulbos nennt / außgenommen daß ſie feyſter ſindt: Sie haben Stengel einer Spannen hoch / mit rohtem Samen / rohtlechte Wurtzeln / die bekleidet ſindt mit braunroht / etwas ſchwartzfärbigen Rinden / wenn man die Rinde abthut / ſo ſindt die Wurtzeln weiß / zart / ſüß / voller Safft / jhre Wurtzel hat in der mitte an einer Seitten von vnden auff ein Kerff oder Ritz / dardurch die Blume wächſt vnd außbricht. Der Herbſtblumen wachſen viel in Meſſenia vnd Colchis.

Krafft vnd Vermögen.

Die Wurtzeln geſſen / tödten wie die gifftige Schwämm / mit würgen vnd erſtecken. Dieſes Kraut haben wir auch allein darumb beſchrieben / damit niemandt daſſelbige / oder ſeine Wurtzeln vnwiſſentlich an ſtatt der Bulbenwurtzeln eſſe / denn etliche durch jhre ſüſſigkeit darzu werden gereitzt. Wider dieſes Gifft braucht man bequemlich die Artzney / die droben wider die gifftige Schwämm beſchrieben worden ſindt / Kühmilch iſt auch gut darwider getruncken / alſo daß man keiner andern Artzney bedarff / wo Kühmilch vorhanden iſt.

Bb ij Wey-

Fig. 1.3—Dioscorides' description of **Colchicum** taken from the **Krauterbuch** of Pedanius Dioscorides, printed by J. Bringern, Frankfurt, 1610. Reproductions obtained through courtesy of John Crerar Library, Chicago, Ill.

In spite of such warnings, Dioscorides believed plants were very useful in the medical practice. Accordingly, other less poisonous species were recommended. In one case he suggested the *Ephemeron* instead of the *Colchicon,* particularly for those tumors that had not yet spread into the body. The *Ephemeron* is now identified as *Colchicum lingulatum*,[21] which contains less colchicine than *C. autumnale*, the autumn-flowering plant, his *Colchicon*.[21] There can be no doubt that his careful attention to species difference distinguished him as a great botanist.

The Greek physicians at the beginning of the Christian era developed a distrust for Oriental medicine, notably the plants that were used in drug traffic.[22] This suspicion had been aroused as early as the time of Hippocrates. Perhaps there was some basis for their doubt. If our assumption was correct that Kashmir hermodactyls were introduced into this drug traffic from the Orient to the West,

then two very similar drugs would have appeared. These are *Colchicum luteum* and *Merendera persica,* which were described in the last section. While the alkaloid contents of these two plants differ considerably, it is probable that then as now they were sold under the name *surinjan.* A careful worker like Dioscorides would not have been misled by these substitutions, but not all Greek physicians were skilled in distinguishing botanical specimens, and they undoubtedly appreciated the excellent services rendered by Dioscorides through his botanical investigations.

In the following fifteen centuries, down through the period of the Herbalists, nothing different was added to the description of *Colchicon.* In fact, the Herbalists merely copied and repeated what Dioscorides and several other botanists of his period had written.[47] The great contributions made during the fifteenth to seventeenth centuries, of course, were the translation, copying, and printing which made book production easier than at any previous period in history.

The Herbalists[22] collected interesting names that became associated with *Colchicon.*[47] These usually refer to the poisonous features or to some unusual habit such as fall flowering and spring fruiting. The plants were called *"mort au chien,"* or "death to dogs.[47] The name *"bulbus agrestis,"* or "wild bulb," was commonly used.[47] Since the flowers appeared in clusters out of the ground without leaves associated, a descriptive name "naked ladies" was given. Probably the most involved name was the Latin *"Filius ante patrem,"* translated "son before the father," meaning a deviation from established biological laws.[47] This is understandable, for when they associated the spring seeds and fruiting with the flowers that came up the same year in autumn, several months later, it was an instance of the offspring preceding the parents. However, Dioscorides had made the correct interpretation because his diagrams (Fig. 1.2) clearly associated buds, flowers, leaves, and fruits at the correct season and he realized that the flowering plants of autumn put forth fruits the next spring. Some Herbalists devoted much discussion to the growth habits involving flowering and fruiting. Finally, the common name *Hermodactyl* caused confusion for a long time until it was clearly shown that the *Colchicon* and *Hermodactyl* were the same plant.[39]

Linnaeus kept the original name given by Dioscorides, changing it from the Greek *Colchicon* to Latin *Colchicum,* when he devised his extensive system of nomenclature. A binomial affixed to the autumn crocus was published in *Species Plantarum,* 1753: *Colchicum autumnale* L. The species describes the fall-flowering character, and the genus retains the original reference to the land of Colchis. Very few changes were made in descriptions as originally given by the Greek botanist. Linnaeus made an important contribution in showing re-

lationships between the *Colchicum* group and other families of plants.[41]

The genus *Colchicum* L. belongs to the tribe Colchiceae, which also includes *Merendera* Ram., *Bulbocodium* L., and *Synsiphon* Regel. This tribe is a part of the subfamily Melanthoideae. The family Liliaceae shows many relationships to the species *Colchicum*; hence their correct position is within the lily family. At one time the family Colchicaceae was on the same level of importance that was given the Liliaceae, but this became changed to the system listed above.

An excellent monograph[41] dealing with *Colchicum* was published by Stefanoff in 1926. Considerable revision has been made and ten new species have been added. The text is in Bulgarian, but the descriptions and keys are printed in Latin, thus making this information available to specialists of any nationality. Useful distribution maps are attached to the monograph.[41]

The genus is divided into two subgenera:[41] (1) *Archicolchicum* including seven sections, and (2) *Eucolchicum* with a single section. An Indian species, *C. luteum* Baker, official in the *Indian Pharmacopoeia* belongs to the first subgenus, whereas the most notable drug species, *C. autumnale* L. is placed in the subgenus *Eucolchicum*. All species belonging to the latter subgenus flower in the autumn, while the members of the first subgenus have many members that bloom in the spring.

A total of 64 species are described and extensively reviewed for their geographical distribution. All belong to the Northern Hemisphere and are primarily indigenous to the Mediterranean region, although many species range over Europe and North Africa and extend eastward into India along the northwestern Himalayan ranges.

Thirty-six species flower in the months of September to November. Except for several unknown, the remaining twenty-five species bloom during the spring, early in January, or late in June. These characteristics are noted in the list of species given in Table 1.1.

Cytological investigations include eleven species for which exact chromosomal determinations have been made.[26, 30] There is no evidence that speciation has proceeded along a polyploidy series with or without hybridization. In fact, the number for these at hand is entirely heteroploid. No correlation exists between taxonomic position and chromosome number. Certainly the diploid numbers ranging from 36 to 54 are not exceptionally high. In light of the polyploidizing effect of colchicine on many plant cells, the suggestion has been made that perhaps within this group high numbers may be found. Chapters 4 and 17 deal with this problem and show by resistance to the drug how polyploidy could not be developed. Furthermore, there is no indication that other species of plants found in the

TABLE 1.1

THE GENUS COLCHICUM LINNAEUS

(After Stefanoff)

Family: Liliaceae
Subfamily: Melanthoideae
Tribe: Colchiceae

Species Name	Authority	Flowering Date	Chromosome Number
Subgenus 1. *Archicolchicum:*			
	Section 1. Luteae		
C. luteum	Baker	Feb.–May	$2n = 38$
C. regelii	Stef.	Feb.–March	
C. hissaricum	Stef.	July	
C. robustum	Stef.	Feb.–May	
	Section 2. Bulbocodiae		
C. szovitsii	F. M.	Jan.–April	
C. crocifolium	Boiss.	Feb.–March	
C. fasciculare	Boiss.	Jan.–Feb.	
C. libanoticum	Ehrenb.	June	
C. ritchii	R. Br.	Nov.–Jan.	
C. schimperi	Janka	Dec.	
C. tauri	Siehe	Feb	
C. serpentinum	Woronow ap. Mischenko	not given	
C. hydrophilum	Siehe	May–June	
C. hirsutum	Stef.	April–May	
C. nivale	Boiss. et Huet	April–June	
C. biebersteinii	Rouy	Feb.–March	
C. davidovi	Stef.	Feb.–April	
C. catacuzenium	Heldr	March–May	
C. hungaricum	Janka	Dec.–April	
C. doerfleri	Hal	Feb.–April	
C. macedonicum	Kosanin	June	
C. triphyllum	Kze	March	
C. kurdicum	Stef.	June	
C. caucasicum	Spreng.	March–May	
C. soboliferum	Stef.	Feb.–April	
C. atticum	Sprun.	Nov.–March	
C. jordanicolum	Stef.	not given	
C. sieheanum	Hausskn.	Sept.	
C. procurrens	Baker	Oct.	
	Section 3. Vernae		
C. vernum	Ker-Gawl.	March–May	
	Section 4. Montanae		
C. montanum	L.	Sept.–Oct.	$2n = 54$
	Section 5. Cupaniae		
C. cupani	Guss.	Sept.–Dec.	
C. psaridis	Heldr.	Sept.–Dec.	
C. boissieri	Orph.	Sept.–Dec.	

(*continued on next page*)

Table 1.1 (*continued*)

Species Name	Authority	Flowering Date	Chromosome Number
	Section 5. Cupaniae (*continued*)		
C. pusillum	Sieb.	Oct.–Nov.	
C. hiemale	Freyn	Dec.–Jan.	
C. troodi	Kotschy	Oct.	
C. steveni	Kunth.	Sept.–Jan.	
C. parlatoris	Orph.	Aug.–Nov.	
	Section 6. Filifoliae		
C. filifolium	Stef.	Oct.–Nov.	
	Section 7. Arenariae		
C. arenarium	W. K.	Sept.–Oct.	
C. alpinum	Lam. et DC.	Aug.–Sept.	
Subgenus 2. *Eucolchicum*:			
	Section 8. Autumnales		
C. corsicum	Baker	Sept.	
C. micranthum	Boiss.	Sept.	
C. borisii	Stef.	Aug.	
C. umbrosum	Stev.	Aug.–Sept.	
C. laetum	Stev.	Sept.	
C. kotschyi	Boiss.	Aug.–Nov.	
C. decaisnei	Boiss.	Oct.	
C. neapolitanum	Ten.	Aug.–Sept.	$2n = 38$
C. longifolium	Cast.	Aug.–Oct.	
C. kochii	Parl.	Aug.–Sept.	
C. lingulatum	Boiss. et Sprun	Sept.–Oct.	
C. haynaldii	Heuff.	Sept.–Oct.	
C. autumnale	L.	Aug.–Oct.	$2n = 38$
C. lusitanum	Brot.	Sept.–Nov.	
C. tenorii (*C. byzantium* Ten.)	Parl.	Sept.	$2n = 40$
C. levieri	Janka	Sept.	
C. visianii	Parl.	Sept.	
C. turicum	Jka	Aug.–Oct.	
C. variegatum	L.	Sept.–Oct.	$2n = 44$
C. latifolium	S. S.	Aug.–Oct.	$2n = 54$
C. speciosum	Stev.	Aug.–Oct.	$2n = 38$
C. bivonae	Guss.	Sept.–Oct.	$2n = 36$

regions where *Colchicum* is abundant are unusually high in chromosome numbers. This question was raised after the cytological work revealed an action on mitotic processes in plants.

Additional references and details concerning the botanical features of the official drug-producing species are given in Chapter 5.

1.3: Medical Applications of Colchicine

Hippocrates founded modern medicine; he swept away many mystical concepts, introduced new explanations for disease, and left a profound influence upon the medical profession. About three or four hundred drugs were kept in his materia medica, some of them introduced from the East where he was a visitor. The ritual of magic and charm was eliminated as much as possible, but his direct contacts with Hindu medicine did leave impressions. He made no reference to a specific treatment for gout, although he was familiar with the ailment called *podagra*[19] in various aspects. It is possible that the bitter hermodactyls were a part of his materia medica.

A *History of Plants* prepared by Theophrastus (372?–285 B.C.) described five hundred plants[19] for medicinal use. This study marks a new age, which continued the advancement of medicine started by Hippocrates. Gout was a familiar disease in Theophrastus' day, but he does not record specifically the form of drug for treating the difficulty. However, Theophrastus gave stern warning that the bitter hermodactyls were powerful poisons. There can be no doubt that the practice of medicine was enlarged by the work of Theophrastus.

The first materia medica with accurate descriptions was firmly established by Dioscorides in the first century A.D. He showed an acquaintance with the studies of Theophrastus and gave many new details from his private observations that became useful to practicing doctors. *Colchicon* was very poisonous and in its place the *Ephemeron* was recommended for those "tumors" that had not yet "spread into the body." This same plant, the *Ephemeron,* was advocated by Galen in the second century A.D. The *Colchicum* treatment for gout may have been advocated by Galen because the bitter hermodactyls were listed in his materia medica and he was well acquainted with gout. The hermodactyls and *Ephemeron* are both members of the *Colchicum* genus.

Aretaeus, the Cappadocian, contemporary with Galen, clearly recognized podagra and noticed that many remedies were advocated. He observed innumerable remedies were suggested for gout; in fact, this calamity usually made the patient "an expert druggist."[19] Many plants were dispensed from the pharmacist. In light of the widespread distribution of colchicine-producing species, a large selection might have been in the hands of the druggists.

About this same time, the "Doctrine of Signatures" was promoted by Pliny,[46] who also made his mark upon medical thought. Plants were chosen for a specific disease by means of suggestive associations. For instance, saxifrages grew among rocks; therefore kidney stones

could be dissolved by juices from this plant. Solomon's seal in cross section of the root looked like the King's seal; hence the plant should be used to seal wounds. Perhaps gout, frequently attacking the fingers, was treated by the hermodactyls since these flowers came up like the fingers of a hand. Recalling that a translation of *hermodactyl* means "fingers of Hermes," the doctrine would have provided good basis for treating these ills and aches.

Emperors, rulers, and the wealthy were most frequently afflicted with gout and arthritic rheumatism. One medical councilor, J. Psychriste, who was attached to the court of the Byzantine ruler Leon the Great (457–475 A.D.), used one single dose of bitter hermodactyl to cure gout.[19] Doctors attached to ruling classes found gout a prevalent disease among these personages, though specific directions for curing gout have not been recognized in most historical records. *Colchicum,* or the bitter hermodactyls are usually mentioned as first used in the sixth century.

Alexander of Tralles (*ca.* 560 A.D.) has been credited as the first to advocate bitter hermodactyl[19] to alleviate the pains from gout. He used a drastic purgative combining scammony, colocynth, aloes, hermodactyls with anise, myrrh, peppers, cinnamon, and ginger. His twelve books on medicine include many references to drug plants.

The seventh century physician,[39] Paul of Aeginata, recommended the hermodactyls when treating gout or other arthritic complaints. His record is likewise well established by the medical historians. Following him, two Arabian doctors, Rhazes and Avicenna, specifically proposed hermodactyls in cases of gout. The latter wrote from traditional belief and personal experience about the "Souradjan" from Arabia. Undoubtedly this is the same as the surinjan, or bitter hermodactyl, *Colchicum luteum* of the Indus River area. The combined periods of Paul of Aeginata, Rhazes, and Avicenna extend from the seventh century to 1037 A.D. The translations made by these physicians included many documents dealing with science and medicine,[39] and they exerted a profound influence upon medicine generally as well as upon the specific knowledge passed on about gout.

An extensive treatise on gout dedicated to the Emperor Michael Paleologus was prepared by a famous thirteenth century Greek physician, Demetrius Pepagomeus.[39] In this account, specific directions were stated for making a pill of hermodactyl, aloes, and cinnamon, to be used in treating podagra.

From the thirteenth to the sixteenth century, records about gout and drugs are scarce. Confusion embroiled the Greek doctors because of the widespread distrust for Arabian medicine and advice from the East. Others suggest that the stern warnings noted about the toxic property of *Colchicon,* beginning with Theophrastus and

Dioscorides, discouraged its uses. While relief was obtained quickly, the dangers associated with treatment were always present. As some writers believe, the chance of death was so great the gamble wasn't "worth the candle."

A German writer, Wirtzung (1500–1571), revived interest in bitter hermodactyl by his discussions on treating gout, and about this time joined in the call for return to *Colchicum* as a treatment for gout.[39] Later John Quincy published a *Complete English Dispensatory* and called attention to hermodactyls, identifying these drugs with *Colchicum*. Accordingly, the British formularies carried both *Hermodactyl* and *Colchicum* in the 1618 edition.[39] This practice was continued in subsequent editions of the *London Pharmacopoeia*: 1627, 1632, 1639; but both plants were dropped in 1650. The omissions continued for 149 years — until 1788, when *Colchicum* was admitted as official. *Hermodactyl* was dropped, never to be heard from again in materia medica.[39] This revival, after such a long period without recognition, requires some explanation.

Without doubt the renewal in the eighteenth century was largely due to the thorough studies by Baron Anton von Storck[39] (1731–1803), who experimented with *Colchicum* in a Vienna hospital. His own body was used for testing sensations as well as bodily changes induced by *Colchicum*. Students joined him in experiments that involved rubbing the tongue with some of the drug to experience the numbness, then recording the time necessary to render the tongue "void of sensation."

Dr. von Storck determined lethal doses for dogs, observing that "two drams killed the animal in 13 hours." Post-mortem studies established the changes induced by the drug, particularly among the internal organs. These tests aided in formulating correct dosages such as the oxymel colchici, used by many practitioners throughout Britain, France, and Germany. Undoubtedly the place gained for *Colchicum* in materia medica by the middle eighteenth century was a direct result of von Storck's effort.

While debates were going on as to the efficacy of *Colchicum*, Husson,[39] a military officer in the pay of the French king, gave out a vinous preparation called "Eau Médicinale," especially useful for gout. The identity of the effective ingredient was kept secret, known only to Husson. There arose quack preparations, i.e., Wilsons Tincture, Reynolds Specific, and others. Their true nature was always kept secret, but an English pharmacist discovered in 1814 that the active ingredient in Husson's preparation was *Colchicum*.

The combined research by Dr. von Storck and the popular success achieved by the "Eau Médicinale" preparations established *Colchicum* in modern materia medica as a specific for gout.

During the latter eighteenth and beginning nineteenth centuries, many English and French physicians wrote extensively about gout, recommending *Colchicum* for relief. The great nineteenth century doctor, Thomas Sydenham, who styled himself as the English Hippocrates,[19] was a martyr to gout. He offered theories for its nature and cause, and advocated treatment with *Colchicum*. Another successful student and physician was Alfred Baring Garrod, whose books[19] and papers contained valuable data about the changes induced by gout. In the nineteenth century almost every prominent doctor with a knowledge of gout had a particular theory as to its origin and nature. The forty-seven cases studied by Garrod are classic examples of sound scientific investigation. Like others, he stood behind the *Colchicum* treatment even though the poisonous nature of this crude drug was well known.

An application of colchicine reported in modern medical practice is the treatment of Hodgkin's disease in which instance remissions were obtained.[3]

1.4: Chemical Studies of the Pure Substance Colchicine

Accuracy in treating gout and in performing critical experiments demanded pure substances. Until the chemists' analysis and extraction of crystalline compounds from corm and seed, only the crude material was available to provide the active principles in the drug. A toxic principle involving pure colchicine was detected in substance from *Colchicum* seed in 1820,[38] but the compound was confused with veratrine. Later the name *colchicine*[16] was proposed for a crystalline material extracted by chemical procedures developed for this purpose. Thus, the first steps were taken toward solving the problems in the chemistry of colchicine. Chapter 6, devoted to the chemistry of this substance, illustrates the exceedingly complicated analytical work necessary to understand colchicine chemistry, much less to contribute to its development. But the rewards in a broad field of biology appear promising for experimenters who can obtain derivatives of known chemical organization and apply the same to critical biological test organisms.

Thorough descriptions characterizing crystalline colchicine were prepared by Zeisel in 1883, and by Houdé in 1884.[8] The formula $C_{22}H_{26}O_6N$ was proposed.[38] These analytical developments formed the groundwork for later work. Pharmacological studies using colchicine and its derivatives could then proceed on a sounder basis, as shown by the work done during the next several decades from the laboratories of Jacobj and Fuhner.[8]

One of the first derivatives studied was colchiceine, obviously demonstrating different biological[42] activity from that of colchicine.

This information has been linked with modern concepts of specific biological activity associated with certain chemical structures.[1] The Symposium on the Chemistry of Colchicine at the 1951–52 meeting of the American Association for the Advancement of Science at Philadelphia, Pennsylvania, dealt with this problem.

Advancement was made in colchicine chemistry when Adolph Windaus, after a long series of investigations, set forth the concept of a three-ring structure.[38] Upon analysis of oxidation products, his case was developed for three rings, A, B, and C, each constructed of 6 carbons, respectively. The first ring A is aromatic, 6 carbon with three associated methoxyl groups. This much of the Windaus formula has been confirmed and remains as earlier constructed.[9] Other parts required modification as will be shown below and in more detail in Chapter 6.

Unusually high water solubility characterizes colchicine in spite of a deficiency of the groups generally associated with this capacity.[9] To account for this feature and others, Dewar speculated that the structural concept should include a "tropolone" system and proposed that ring C was a 7-membered structure.[11]

Earlier than this proposal, doubts were raised by Cohen, Cook, and Roe in 1940[8] that led to changes in the central part of the structure, ring B. Changing ring B, as well as C, from a 6- to 7-membered ring appeared necessary. This first evidence for the need to modify Windaus' structure, which came from the Glasgow Laboratories,[9] has since led to extensive studies dealing with the structure of colchicine. Dr. James Loudon, a member of this team, has generously contributed the chapter on chemistry. Degradative work provided thorough evidence that ring B is 7-membered instead of 6 as originally proposed. Further confirmation came through synthesis work[34] upon dl colchinol methyl ether, also establishing the position of the amino group on ring B.

A compound described as octahydrodemethoxydesoxydesacetamidocolchicine,[33] has been obtained by degradation. Such a product derived from colchicine that is more or less a carbon skeleton for rings B and C presents opportunities for making some definitive proof of the structure of colchicine through synthesis.

Tropolone, as originally suggested by Dewar has been synthesized;[11] therefore, ring C of colchicine is essentially as predicted in earlier speculations. Much might be expected here for biological experimental procedures. Interesting tests with tropolonoid compounds have been tried. The "radiomimetic" action of a tropolonoid compound is of considerable interest.[43]

Polarographic evidence supports the work with colchicine and derivatives in several aspects.[36] Santavy and associates beginning in

1942 have been contributors.[35] Other similar results comparing in particular the infrared spectra of colchicine and its derivatives with the tropolone structure, also offer supporting evidence for the correctness of the structure of colchicine.[36]

Tools for deeper insight to biological problems arise from the many derivatives obtained with chemical studies.[25] There are also natural compounds accompanying the crude product from *Colchicum* which can be of value for experimental work. Numerous areas where such may be introduced shall be considered in chapters throughout this work.

When *iso*colchicine was prepared, additional c-mitotic* analysis could be made. Significant changes in the biological activity accompanied changes in chemical structure. The new compound has a c-mitotic activity 100 times lower than colchicine.[42] In this instance, ring C appears to be decisive through the interchanges of keto and methoxyl groups. Another well-known derivative, colchiceine, demonstrates little or no c-mitotic action in any concentrations tested.[42] These and other cases call for cooperative work between two highly complex laboratory operations, chemistry on one hand and experimental biology on the other. These areas are exceedingly difficult; the lack of control in biology often becomes frustrating to the physical scientist. Control or direction over life processes such as mitosis by designing chemical structures are intriguing fields for investigation.

1.5: New Biological Uses for Colchicine

Colchicine causes a "veritable explosion"[27] of mitoses when in contact with mitotically active tissues. The sudden increase in published reports dealing with colchicine was also described as a "veritable explosion" of publications,[16] particularly from 1938 to 1942. For this reason, Wellensiek proclaimed a new "fad" in biological research,[45] the "colchicine fad." An immense bibliography[16] has accumulated, chiefly since 1934.

Accurate historical records have established the way in which colchicine research began in new fields[45] and chronologies[23] have been written; no attempt shall be made to review this aspect.[10] Such sudden increase in research with a drug known to man for thirty-five centuries does arouse interesting speculations as to the causes for an immediate switch to this particular line of work. After research in several fields had shown unusual results, much work was soon under way. Here we touch upon the initiation of research with colchicine; extensive details are found in subsequent chapters.

* The adjective *c-mitotic* is derived from *c-mitosis,* which designates a mitosis occurring under the influence of colchicine.

An early experimenter with plants and colchicine was Charles Darwin who applied the drug to "insectivorous" and "sensitive" plants. The reactions in leaf movements were tested, but no conclusive results were obtained for colchicine, nicotine, or morphine. This work was done about 1875 and is of historical interest only. No modern colchicine papers cite Darwin's study.

Another report, untouched for sixty years, was obviously closer to the central theme: Pernice in 1889 clearly described the action of colchicine on mitosis.[17] His figures (Fig. 1.4) showing arrested metaphase are remarkable even though their significance was not entirely realized. Pernice conducted research far ahead of the knowledge at hand in his day.

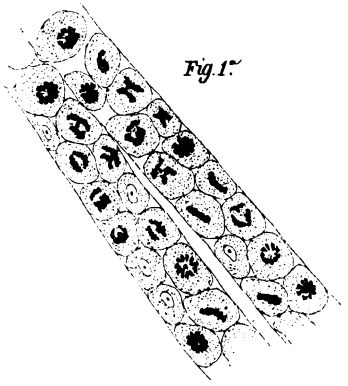
Many references credit Malden with the first observation on mitotic effects of colchicine because he said the drug appeared to "excite karyokinesis" [9] in white blood cells. The full significance was not realized at this date, but Dixon and Malden[28] prepared an excellent report on the effects of colchicine on the blood picture.

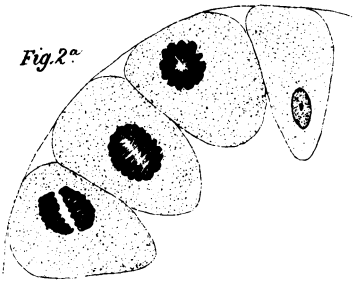
This relationship between colchicine and leukocytosis was reexamined by Lits,[27] a student in the Pathology Laboratory, University of Brussels, Belgium, under the direction of the late Professor A. P. Dustin, Sr., in 1934. Since the mitotic effects induced by colchicine were so similar to those previously reported by Dustin and Grégoire[13] with sodium cacodylate, more than passing attention was paid to the results by Lits. The situation was ideal for striking at the basic biological issues since Professor Dustin had already devoted much time to the study of the action of chemicals upon mitosis.[12] Colchicine was effective in much less concentration and the volume of arrested metaphases in a given treated tissue was an impressive sight.

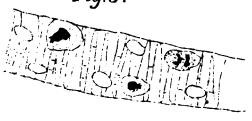
The Dustin school immediately established that colchicine acts upon mitosis whether using animal or plant tissues.[14] Their contribution was important and significant. With regard to polyploidy in *Allium* root tips they did not grasp its significance even though the preserved slides today show restitution nuclei that have multiples of chromosome sets.[14]

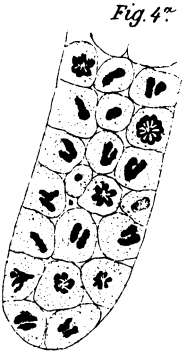
Independently, a penetrating analysis of colchicine acting upon mitosis was made by Ludford[28, 28a] with tissue culture methods using normal and malignant cells *in vivo* and *in vitro*. His results showed that metaphases were arrested. Amoroso urged using colchicine.

Attention was called to the possibilities of colchicine as a tool for cancer chemotherapy.[12] Two other projects specifically mention the use of colchicine as a means of attacking problems of cancer. One was done by Amoroso in 1935 when colchicine was given to mice bearing specific tumors.[9] The other reported regression of a spindle-









La Sicilia Medica. A.I. Fas. 4.° Lit. E. Sanzo. C.

Fig. 1.4—Pernice's first description of colchicine-mitoses (in dog). 1. Gastric gland. 2. Arrested metaphases at the tip of a villosity of gastric mucosa. 3. Endothelial mitoses in the vessels of the mucosa. 4. Lieberkühn's gland crowded with abnormal mitoses. Note absence of anaphases and telophases. (After O. Eigsti, P. Dustin, et al.)

celled sarcoma of a mare that received colchicine by intramuscular injections.[9]

Reference to Dominici,[27] a pioneering investigator with irradiations and treatment of cancer, is frequently made, but his original studies have not been found except for a sentence carried in a textbook. Dominici died in 1919, so the relation between his work and modern studies is not as direct as many have been led to believe.

While the late Professor G. M. Smith of Yale attended the Second International Cancer Congress in Brussels in September, 1936, the work by the Dustin school came to his attention. Here an elaborate demonstration of research with colchicine was made. Before leaving Europe, Professor Smith purchased colchicine with the hope that specific research could be done in his laboratory in the United States.[18] Along with Professor D. U. Gardner and the late Professor E. Allen, he developed assay methods to test estrogenic hormones. Their preliminary paper was published in 1936.

In another laboratory Dr. A. M. Brues[4] and associates reported important observations on the effect of colchicine upon mitosis in regenerating liver. These studies struck at the basic mitotic problem.

At Cold Spring Harbor, Long Island, New York, Mr. E. L. Lahr initiated research similar to that reported by the Yale group. An Atlantic City A.A.A.S. sectional meeting, 1936–37, presented the work by Allen, Gardner, and Smith, which paper was heard by Carnegie staff scientists. Mr. Lahr performed two valuable services: first, he informed the geneticists at the Carnegie Institution about research with colchicine at the regular seminar attended by all the *Datura* workers; and secondly, his excellent slides showed metaphasic stages in tremendous numbers when colchicine was present. These results were freely demonstrated and thoroughly discussed with all who visited Mr. Lahr's laboratory.[15]

One day in February, 1937, the slides were shown to the senior author. The demonstration was so impressive that he obtained colchicine for *Allium* root tip tests before leaving the laboratory. Appropriate concentrations were determined for the experiment with plant materials. Within 72 hours, large bulbous tips appeared on onion roots immersed in colchicine; the cells showed polyploid restitution nuclei by acetocarmine methods. Since the senior author had been privileged to attend seminars in cytophysiology by Professor C. F. Hottes, University of Illinois, the polyploid cells found in treated root tips at the Carnegie Laboratories received more than average passing attention.[15]

The *Allium* root tip tests at the Carnegie Institution Laboratories were followed by seedling treatments. Each test pointed toward a

potential use for inducing polyploidy. These preliminary results aroused discussion at Cold Spring Harbor which continued up to April 30, 1937.[15]

On this date, the senior author severed connections with the Carnegie Laboratories. Working conditions for continuing colchicine research with plant materials were obtained for him May 1, 1937, through the generosity of Dr. Geo. H. Conant in his Triarch Laboratories, Ripon, Wisconsin. Here the *Allium* test was repeated. *Datura stramonium* seedlings were treated with colchicine, and the drug was applied to the generative cell in pollen tube cultures. Remarkable results at Wisconsin confirmed the previous opinion that colchicine was an unusually effective substance. From these experiments the senior author developed a deep interest in colchicine research, and he has maintained a continued contact with various phases of it through the years.

Following the departure of the senior author from the Carnegie Laboratories, research workers investigating cytogenetic problems of *Datura* began treatments of seeds of this species with recommended dosages of colchicine.[40] Announcement of these results was made in a publication[2] by the French Academy of Science in September, 1937. By December, 1937,[2a] the evidence from *Datura* and other species clearly established the fact that colchicine was a new and effective tool for making polyploids experimentally. Since there are sufficient historical notes[45] and colchicine chronologies,[23, 40] an elaborate discussion does not seem needed here, except to recommend an article from the Botanical Review,[10] published in 1940, for important details of historical significance concerning the pioneering work with colchicine pursued at Cold Spring Harbor from January to December, 1937.

Independently, Doctors B. R. Nebel and M. L. Ruttle began research in April, 1937, and concluded important experiments that year, clearly demonstrating that colchicine acted upon mitosis.[32] Furthermore, this drug was an important tool for inducing polyploidy in plants.[32] Dr. D. F. Jones of Connecticut is credited with calling their attention to colchicine; however, they also acknowledged a bibliography in their early publications, mentioning the work by Dustin,[12] Ludford,[28] and Brues.[4]

In France, Dr. P. Gavaudan and associates published the first account[20] that called attention to polyploidy induced by colchicine. This paper was presented in June, 1937, but little notice was given to the contribution. The text clearly described doubling of the chromosomes along with specific figures. While Havas claims an earlier date in publication,[23] his paper completely disregarded polyploidy as a consequence of the colchicine treatment. In this regard

Gavaudan was more closely associated with cytogenetic aspects than Havas.

During the summer of 1937, a Swedish geneticist, Dr. A. Levan, visited genetics laboratories in eastern United States and was shown by Dr. Nebel data obtained from his colchicine studies. When Dr. Levan returned to Sweden, he began experiments with colchicine and made basic contributions to the concepts of polyploidy and colchicine mitosis.[26]

The Cold Spring Harbor studies exerted an influence that spread around the world. These activities plus the other biological work created an intense and wide interest that led to the "colchicine fad."[45] Many scientists went to work establishing facts about colchicine.[16] Generally, the cooperation was genuine, ideas were exchanged freely, mutual problems were discussed, and knowledge advanced rapidly. Significant contributions were made within a short time.

By 1938 colchicine was applied to many kinds of living cells, plant and animal, with outstanding specific reactions obtained by the treatment. Cancer control continued to be injected into the discussions. Geneticists discovered a very useful tool at their disposal for theoretical and practical work. These data were linked to publicity that developed a common language for layman and scientist.

In spite of volumes published, there remain unexplored problems which appear to have promise for more discoveries. Excellent research has been accomplished; future progress in agriculture, medicine, pharmacy, biology, and chemistry will be facilitated by the possession of such a tool as colchicine.[31]

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