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Colchicine—

in Agriculture, Medicine, Biology, and Chemistry

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To the memory of Albert Pierre Dustin, 1884–1942, whose concepts concerning the regulation of mitotic activity prepared a foundation for the broad scope of biological research that followed the rediscovery in 1934 of the effects of colchicine upon mitosis.

Preface

When an American botanist and a Belgian pathologist collaborate in writing a book, the obstacles to be encountered are necessarily numerous, and this is true of the present work even though the subject is limited to the single substance, *colchicine*. Our collaboration has required intercontinental travel, hours spent together in discussing factual materials from plant and animal sciences, countless days assembling a vast bibliography.

Finally, our cooperative project made it necessary to overcome barriers inherent in our widely different research fields, to resolve problems arising from the use of different languages, and to recognize the dissimilar perspectives of the American and European educational systems. But a common ground of interest was maintained, irrespective of personal interests, through a constant realization of the remarkable and singular properties of colchicine as a mitotic poison and as a tool for experimental work. Moreover, research programs in mitotic problems which each of us had developed prior to the work with colchicine provided a basis of mutual interest.

This work actually had two beginnings when in 1942, almost simultaneously, two scientists commenced manuscripts, each without knowledge of the other. One of them was A. P. Dustin, Sr., of Brussels, whose untimely death occurred in the year his review was started. The task of completing this study fortunately passed to Dr. Dustin's son, and in 1947 the botanical writing done in America by the senior author and the medical studies under way in Europe were brought together into one joint project. It was decided to integrate the many lines of research with colchicine into one study. This book is the result of that cooperative effort.

A survey of the chapters comprising this study will indicate the many lines of research that have been included. The modern literature on colchicine is vast. The references to *gout* alone would require

pages. Rather than catalog titles, we have brought together significant contributions and have attempted to correlate the various lines of research. Whenever possible, we summarize the basic contribution, point out differences of opinion, and, most important, call attention to work that needs to be accomplished. Finally, in retrospect over the modern period of studies of colchicine, one of our purposes has been to point out the progress made, rather than to predict what is to come.

For the shortcomings, the errors of interpretation, statements of viewpoints not pleasing to all specialists, which may be found in any portion of this book, the authors assume full responsibility. We who have assembled as many as possible of the important facts about colchicine welcome corrections and comments concerning the conclusions which we have reached.

The modern period of research with colchicine began in 1889, when Pernice described metaphasic arrest produced by this drug. Until Pernice's report was rediscovered, Dixon and Malden were cited as the pioneers. Thus, our search for *all* references to colchicine was rewarded. Special recognition is due to Nancy Gay-Winn, whose diligent quest led to this classic work by Pernice.

Colchicine in its present role as a mitotic poison and as a tool for biological research was discovered in 1934 at Brussels, Belgium, in the laboratory of Professor A. P. Dustin, Sr., who for a long time had been investigating means of altering mitosis. When colchicine was suggested by a Brussels medical student, F. Lits, the characteristics of colchicine were quickly measured. Our review covers the period from 1934 to the middle 1950's.

In 1937 botanical research began in several countries, generally following descriptions or reports of unusual observations from animal cells. In this same year, the scientists at Brussels included *Allium* root tips for their tests. Other botanists chose *Allium* root tips or plant materials to illustrate the action of colchicine. In this year the role of colchicine as an agent for the induction of polyploidy was conclusively demonstrated.

The horizons of colchicine research widened quickly when botanists learned how effectively the drug could be used in their work. Laymen became interested in the drug as references to cancer entered the discussions and as the creation of new varieties of plants stimulated new programs in agriculture. A broad scope of research was opened up by this single substance.

Organic chemists realized that Windaus' concept of the structural formula for colchicine needed revision. In 1940 definite evidence was at hand. There followed an unusually large volume of research on

the chemistry of colchicine. In 1947 we realized the need for specialized help. Fortunately, Dr. James D. Loudon of Glasgow University, Scotland, who worked with the group that began the revision of colchicine structure, generously contributed to this aspect of the study. We express our gratitude to him for the writing of Chapter 6.

Colchicum, which is a drug plant of antiquity, has a long history in the annals of pharmacy. Professor F. Santavy of the Medical Institute of Olomouc, Czechoslovakia, provided special materials for Chapter 5. Many facts about the pharmacognosy of *Colchicum* were compiled by Mr. Ikram Hassan of the University of Panjab, Lahore, Pakistan. We appreciate their special aid in the preparation of Chapter 5.

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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

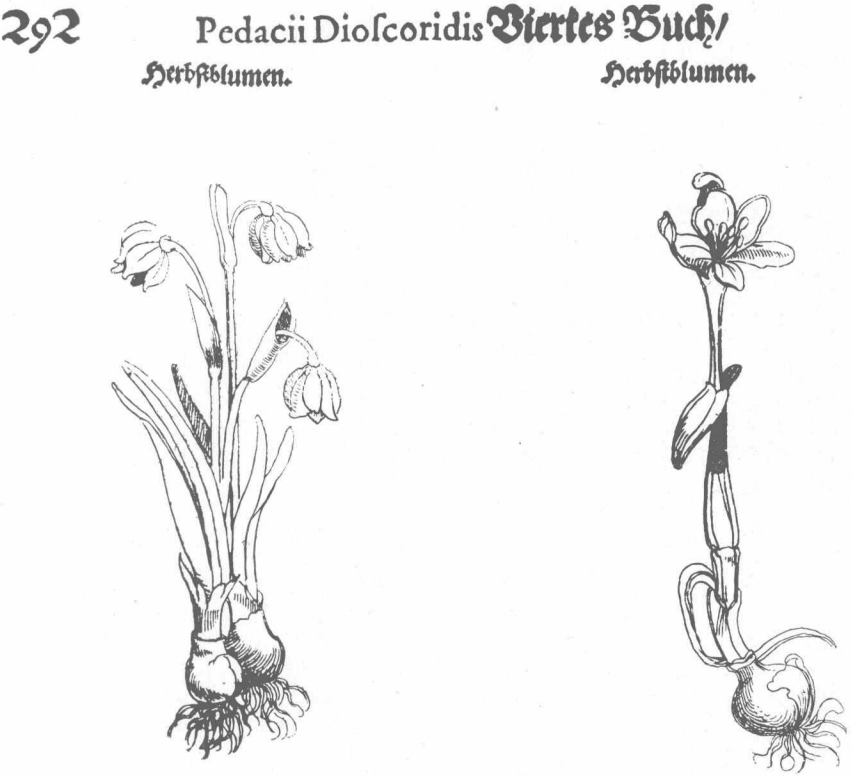

Fig. 1.2—Diagrams showing the seed-producing portion of **Colchicum autumnale**, and the flower stalk appearing in autumn. **A**, fruiting; **B**, flowering. (After drawings by Dioscorides)

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. Die Wurtzeln geſſen / tödten wie die gifftige Schwämm / mit würgen vnd erſtecken. Dieſes Krafft vnd Kraut haben wir auch allein darumb beſchrieben / damit niemandt daſſelbige / oder ſeine Vermögen. 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 Mey-

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,* [41] which contains less colchicine than *C. autumnale,* the autumn-flowering plant, his *Colchicon.*[47] 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-