exception of *S. Ballsii* and *S. sucrense*.[2] These two species were tested over a three-year period in soil heavily infested with the golden nematode, and very few nematodes developed on their roots. In 1947, resistance was measured by a determination of the number of immature females per gram of root by the washed-root technique of Chitwood and Feldmesser (*3*); in 1948, by counts of the cysts on roots at harvest time; and in 1949, by counts of the number of immature females on the roots exposed when the intact soil ball was removed from the clay pot (*4*). Low and consistent readings were obtained with *S. Ballsii* and *S. sucrense* over the three-year period regardless of the different procedures employed (Table 1).

TABLE 1

RESISTANCE OF *Solanum sucrense* AND *Solanum Ballsii* TO ATTACK BY THE GOLDEN NEMATODE

Variety or species	1947		1948		1949	
	No. plants	Immature females/g of root	No. plants	No. cysts found by inspection	No. plants	No. immature females/soil ball
Green Mountain	6	129	10	Many	16	294
Solanum sucrense	2	0.0	3	4	13	4
Solanum Ballsii	2	3	10	2	4	0.25

All available stocks of *S. Ballsii* originate from the Commonwealth Potato Collection at Cambridge, England. Plants from this collection are completely infected with a virus. Virus-diseased plants of *S. Ballsii* grow slowly and flower poorly. All attempts to cross this species with commercial potato varieties or to produce seed by selfing have resulted in failure.

On the other hand, there are indications that *S. sucrense* will cross rather easily with standard potato varieties. Unfortunately, the *S. sucrense* material eventually was lost because of virus infection. Additional tubers of *S. sucrense,* to replace those lost with virus infection, were obtained from the Commonwealth Potato Collection. Plants produced from this second importation of *S. sucrense,* although resistant as compared to the variety Green Mountain, were more susceptible than plants produced from the original tuber importation.

To test the possibility of the existence of strains varying in golden nematode susceptibity within *S. sucrense,* a plant was inbred. The seedlings produced were tested for resistance in soil heavily infested with the golden nematode. As shown by counts of immature females on their roots, considerable variation in nematode infection existed among these inbred seedlings (Table 2). From the 80 inbred seedlings

TABLE 2

RESISTANCE OF INBRED SEEDLINGS OF *Solanum sucrense* TO ATTACK BY THE GOLDEN NEMATODE

	No. plants tested	No. immature females/soil ball (2-yr av)
Solanum tuberosum L. var. Green Mountain	40	729
S. sucrense from Commonwealth Potato Collection	107	69
S. sucrense seedlings		
#31	4	50*
#25	5	38*
#59	4	33*
#30	28	8
#16	13	7
#68	14	4
#62	8	3
#51	10	3
#43	29	1
#78	4	1

* 1950 results only.

tested for resistance to the nematode, 7 with low infection readings were selected for further testing. When these 7 seedlings were tested the following year, results consistent with those of the first test were obtained. These results indicate that variation in susceptibility to the golden nematode exists within the species *S. sucrense.* Attempts to further purify the 7 selected strains of *S. sucrense* with respect to their resistance to the golden nematode will be continued, and use will be made of them in a potato breeding program.

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[2] Tubers of the wild species of Solanum were kindly supplied by J. G. Hawkes and P. S. Hudson from the Commonwealth Potato Collection, Cambridge, Eng., and by Donald Reddick, of the Department of Plant Pathology, Cornell University, Ithaca, N. Y.

Biosynthesis and Isolation of Radioactive Colchicine[1]

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The availability of carbon 14 for biological purposes, and the extreme sensitivity of the radioactive isotope tracer technique, make possible studies on the metabolism of drugs and poisons in the animal body at levels well below the lethal dose. In this paper are given (*a*) the procedures used for the growth of the

[1] This work was done under a contract between the Atomic Energy Commission and the University of Chicago.

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Colchicum autumnale plants in the laboratory under conditions permitting the incorporation of carbon 14 into all the constituents of the plants; (*b*) the extraction methods used for the isolation of the crude alkaloids from the radioactive plants; (*c*) the chromatographic methods used for the separation and purification of colchicine from the other alkaloids; and (*d*) the identification methods used to establish the degree of purity of the final product.

was then allowed to filter through a 2 × 15 cm column of alumina (activated alumina, Grade F-20, manufactured by Aluminum Ore Company). After washing the column with fresh benzene, the colchicine was eluted with chloroform (cp; 0.7% ethyl alcohol). The residue was dissolved in 1 ml chloroform and 30 ml of petroleum ether was added to precipitate the colchicine. The preparation was then centrifuged, the petroleum ether decanted, and the process repeated.

TABLE 1

		Colchicine	Radioactive colchicine
Melting point	Crystallized from ethyl acetate–ethyl ether	155°–157° (Uncorrected) 154°–156° (*3*)	155°–157° (Uncorrected)
Optical rotation	In chloroform	$[\alpha]_D^{26} = -122°$ $c = 0.6708$ $l = 1$dm $[\alpha]_D^{28} = -118.6°$ (*3*)	$[\alpha]_D^{25} = -121.6°$ $c = 0.5371$ $l = 1$dm
	In water	$[\alpha]_D^{25} = -411.7°$ $c = 0.5670$ $l = 1$dm $[\alpha]_D = -409°$ (*3*)	$[\alpha]_D^{25} = -410.6°$ $c = 0.5426$ $l = 1$dm
Ultraviolet absorption spectrum in ethyl alcohol		λ max 350 mμ ($\log \varepsilon = 4.20$) and 247 mμ ($\log \varepsilon = 4.45$) (*3*, *4*)	λ max 350 mμ ($\log \varepsilon = 4.23$) and 245 mμ ($\log \varepsilon = 4.48$)
Polarographic reduction		$E_{1/2} = -1.38$ v *vs* SCE $c = 1 \times 10^{-3}$ M	$E_{1/2} = -1.36$ v *vs* SCE $c = 0.75 \times 10^{-3}$ M
Paper chromatograms R_f values	Carbon tetrachloride, ethyl alcohol, and water 1: 2: 1	0.76 ± 0.01	0.77 ± 0.01
	Carbon tetrachloride, methyl alcohol, and water 1: 1: 1	0.32 ± 0.17	0.34 ± 0.01
Color reactions	H_2SO_4	Yellow	Yellow
	Phosphotungstic acid	Yellow ppt	Yellow ppt
	$FeCl_3$	Negative	Negative
	$FeCl_3$, after warming with 1% HCl	Green	Green

C. autumnale bulbs that had been wintered were allowed to develop about 5–8 in. of leaf growth. At that point they were transferred to a sealed growing chamber into which radioactive carbon 14 was introduced in the form of carbon dioxide (*1*). They were given 100 ml carbon dioxide which contained 0.5 μc carbon 14/ml gas, approximately every third day, the total amount given to each plant being 300–500 μc of carbon 14.

The plants were allowed to grow under these conditions for 20–30 days, after which the corms were harvested, sliced, dried, and powdered. Extraction of 10–20-g portions of this material was carried out for 6 hr in a Soxhlet extractor with methyl alcohol. The extract was evaporated to a small volume, diluted with 400 ml of distilled water, and extracted with two 250-ml portions of petroleum ether in a separatory funnel. The aqueous phase was made slightly alkaline with sodium hydroxide and extracted five times with 100-ml portions of chloroform.

Purification of the crude colchicine was accomplished by resolution on an alumina column. The chloroform solution of the crude drug was evaporated to dryness, and the residue taken up in benzene, which

The almost colorless product was then collected and stored over P_2O_5 in a desiccator protected from light. Yields were 0.1–0.2%.

Colchicine was also isolated from the leaves of radioactive *C. autumnale* plants. The extraction method used for the corms was not feasible because of the pigments and other constituents of the leaf; 2–8 g of the ground leaves were extracted with methyl alcohol in a Soxhlet extractor. The methyl alcohol solution was evaporated to dryness, suspended in 100 ml of distilled water, and filtered. The clear filtrate was extracted twice with 100-ml portions of petroleum ether and once with 100 ml of ethyl ether. The aqueous fraction was then extracted with five 50-ml portions of chloroform. The chloroform extracts were combined and evaporated to dryness. The residue was dissolved in benzene and filtered through a 1.8 × 14 cm column of Florisil (60–100 mesh), and the column washed with 30 ml of benzene. A mixture of benzene and acetone (9:1) was then used to develop the column. A green pigment was eluted with 60–100 ml of this mixture, and the colchicine remained behind. The colchicine was then eluted by 300 ml of acetone; an orange pigment was left on the column. The acetone

solution was evaporated to dryness, and the residue taken up in benzene and allowed to filter over an alumina column, as described above for the crude colchicine fraction from the corms. Elution of colchicine was accomplished with chloroform. Yields were from 0.04% to 0.08%.

Various methods of identification were used for the characterization of our radioactive colchicine. Table 1 gives the comparative results obtained with our radioactive colchicine and with a pure sample of colchicine which was obtained by purifying a U.S.P. sample using the method suggested by Ashley and Harris (*2*). Wherever possible, comparisons were made with the constants for pure colchicine which were determined by Santavy (*3*) and Santavy and Reichstein (*4*).

Melting points were obtained with a Fischer-Johns melting point block. There was no depression of the melting point when a sample of the radioactive colchicine was mixed intimately with an equivalent amount of the pure nonradioactive alkaloid. The optical rotations were in close agreement and were determined with the use of a J. and J. Fric research model polariscope. A model DU Beckman quartz spectrophotometer was used to obtain the ultraviolet absorption spectrum.

The polarographic reductions were carried out using a Heyrovsky polarograph, Model XII, manufactured by E. H. Sargent. All solutions polarographed were 0.1 *M* in respect to lithium chloride, which was used as the supporting electrolyte. When equal concentrations of the normal and radioactive colchicine were polarographed, the diffusion currents were equal in magnitude.

Paper chromatograms were obtained using strips of Whatman #1 filter paper. The values reported are averages of several determinations, and comparative samples were always run simultaneously (*5*). The solvent systems found to be best suited for our purposes were the bottom phases of well-shaken mixtures of carbon tetrachloride, ethyl alcohol, and water 1 : 2 : 1, and carbon tetrachloride, methyl alcohol, and water 1 : 1 : 1. In order to visualize the chromatogram we used a solution of phosphotungstic acid (Scheibler's reagent), which was found to be the best of a number tested by us. This gave a bright-yellow color against a colorless background and also gave a color with all the other related alkaloids which occur with colchicine in *C. autumnale*. Another method of visualizing the colchicine and colchicinelike compounds was found to be ultraviolet light, which gave a yellow color to the spots where the substances were to be found, whereas the rest of the paper strip remained blue.

The carbon tetrachloride–methyl alcohol–water system was found to be the best for separating colchicine from the many similar compounds with which it occurs. Using this solvent system, we were able to find at least 4 components other than colchicine in commercial U.S.P. colchicine preparations, and hence it was for this reason that the comparisons were done on the colchicine prepared by the method of Ashley and Harris (*2*), which gave only one band on the paper chromatogram.

A small amount of radioactive colchicine was mixed with carrier and chromatographed, using the carbon tetrachloride–methyl alcohol–water system. It was found that all the disintegrations that were due to the radioactive colchicine were found at the spot identified as the pure nonradioactive compound.

The ferric chloride reaction which is given by colchiceine was negative for our radioactive compound. However, upon warming it with 1% HCl, the $FeCl_3$ reaction was positive, indicating the conversion of the radioactive colchicine to colchiceine. Upon chromatographing the hydrolysis product in the carbon tetrachloride–methyl alcohol–water system, we found that it had an R_f of essentially zero. A sample of colchiceine prepared from colchicine by hydrolysis (*6*) gave an identical R_f.

The radioactive colchicine was also subjected to microanalysis.[3] The alkaloid was crystallized from ethyl acetate-ethyl ether, and dried for 2 hr under high vacuum over P_2O_5 at 100° C, $C_{22}H_{25}O_6N$ (399.43):

Calcd: C, 66.15%; H, 6.31%.
Found: C, 65.78%; H, 6.64%.

The specific activity of the colchicine obtained was 27 μc/g as determined with the internal Geiger counter in use in our laboratories (*7*). This is equivalent to 40,000 cpm/mg colchicine under optimal solid sample counting conditions, thus permitting the detection of as little as 0.1 μg of colchicine. Since the extraction of colchicine from biological samples is relatively simple, the level of radioactivity is adequate for *in vitro* and *in vivo* experiments. Preliminary investigations covering the metabolism of radioactive colchicine in normal and tumor-bearing mice have already been reported (*8*).

In addition to the colchicine, we have isolated in a radioactive condition five other related alkaloids from *C. autumnale*. At the present time we are growing colchicum plants in a large chamber using daylight illumination. Preliminary results as evidenced by the uptake of carbon 14 by the plants indicate that we shall have a five- to eightfold increase in the specific activity of colchicine.

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