that the cellular component with which colchicine and Gammexane interfere is not *meso*-inositol itself, but a

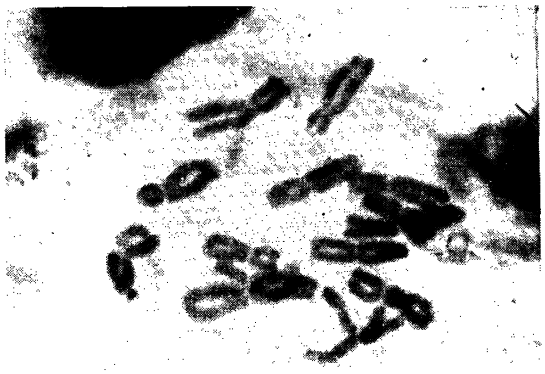

FIG. 1. Metaphase in *Allium Cepa* treated with 0.00035 M Gammexane and 0.033 M *d*-inositol solution, showing failure of the *d*-inositol to inhibit c-mitosis. (Magnification, approximately 1,000 ×.)

substance to which it gives rise and the formation of which is prevented by the mitotic poisons in question.

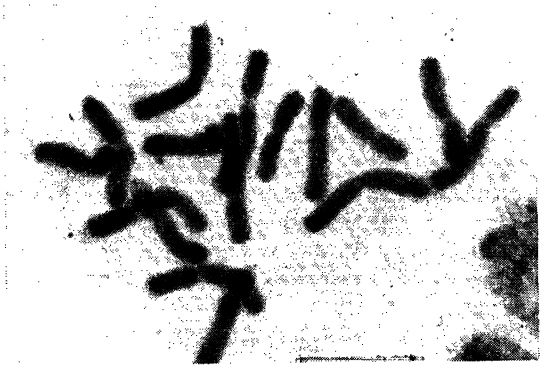

FIG. 2. *Allium Cepa* treated with 0.000035 M Gammexane and 0.0033 M *meso*-inositol solution, showing inhibition of c-mitosis. (Magnification, approximately 1,000 ×.)

Experiments on these and other aspects will form the subject of a detailed communication which will appear at a later date.

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Biosynthesis of Radioactive Drugs Using Carbon 14[1]

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The therapeutic dose of many important drugs is so small that neither the compound itself nor its possible breakdown products can be detected in the body by ordinary chemical or biological methods. Incorporation of radioactive isotopes into such drugs makes possible studies of their distribution and fate in the animal organism because of the great sensitivity of the isotope tracer technique. Obtaining the organic compound containing the isotope constitutes the major practical difficulty in the application of this technique to biological problems. At the present time many of the needed compounds can be prepared only by biological synthesis.

This report deals with our experiences in obtaining radioactive digitoxin and nicotine from the medicinal plants *Digitalis purpurea* and *Nicotiana rustica*.

Plants were grown from seed and transplanted into suitable containers containing soil, sand, or crushed mica. Growth was supported by an inorganic nutrient solution containing calcium nitrate (0.1%), magnesium sulfate (0.06%), potassium nitrate (0.05%), potassium acid phosphate (0.04%), ammonium sulfate (0.01%), and smaller amounts of other salts needed in trace amounts (*2*). As soon as the young transplanted plants were sufficiently well established, they were sealed in a closed system, usually consisting of two battery jars placed with their open ends in apposition. Suitable holes were drilled in the glass jars for the introduction of nutrient solution and of radioactive carbon dioxide, for the attach-

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Because work of this kind involves many techniques unusual for the pharmacologist and because certain precautions must be taken when radioactive substances are used, we consulted numerous colleagues in our own University and elsewhere. Among the many who aided us, we should acknowledge especially the help of Dr. Austin Brues, chief of the Biological Division, Argonne National Laboratory, and Drs. Wright Langham and Lloyd Roth, Los Alamos Scientific Laboratory, who instructed us in the procedures used in the assay of radioactive carbon and in the necessary precautions. Dr. Crooks, of the U. S. Department of Agriculture, offered helpful hints concerning the culture of these plants and also furnished us with seeds. Dr. Burris, of the University of Wisconsin, kindly gave us the benefit of his experience with technics in plant culture and in the study of plant respiration. Prof. Ezra Kraus, chairman of the Department of Botany, University of Chicago, gave us many suggestions and also placed at our disposal the facilities of the University's greenhouses, where the chief gardener, Mr. Michael Costello, was indispensable to us in the seeding, transplanting, and care of the plants in the greenhouse. We are also grateful to the Lilly Research Laboratories and to the Sandoz Chemical Company for generous supplies of the pure glycosides.

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ment of a water manometer to determine adequacy of the sealing, and for drainage. The enclosed volume was approximately 22 liters. Three 40-watt fluorescent lights were placed 24″ above the plants. Preliminary experiments showed that digitoxin and nicotine could be synthesized by the plants under these conditions.

CO_2 was generated from a solution of sodium carbonate containing 1, 10, or 100 μc of C^{14} and 1 mM of carbonate/cc by placing 1 cc into a 50-cc syringe having a two-way valve and adding an excess of sulfuric acid. The CO_2 was injected into the closed system containing the plant. Gas samples were analyzed for C^{14} from time to time, thus giving a measure of the CO_2 uptake by the plant. After 2–6 weeks the plants were harvested, air dried, and pulverized.

Radioactive digitoxin. A 30-gm sample of the dried and powdered *Digitalis purpurea* plant was mixed with 500 cc of 50% alcohol and the mixture allowed to stand overnight. It was filtered through a sintered glass filter and the residue washed twice with 250-cc portions of 50% alcohol. The combined filtrates were evaporated to a thick paste at or below room temperature under a current of air, the volume restored to approximately 10 cc with water, 100 mg of sodium bicarbonate added, and the suspension extracted three times with 30-cc portions of chloroform. The chloroform extracts were evaporated to dryness, 10 cc of chloroform added, and the solution filtered through a 5 × 50-mm adsorption column of water-alcohol-chloroform washed 60–80 mesh Decalso. The chromatogram was developed with 30 cc of chloroform, then with 30 cc of 1% ethyl alcohol (95%) in chloroform (U.S.P. XIII), and the digitoxin fraction eluted with 3% alcohol-in-chloroform. After evaporation of the solvent, the residue was dissolved in chloroform and adsorbed and eluted as before. The residue from the 3% alcohol-in-chloroform eluate was dissolved in 0.2 cc of chloroform, 2 cc of carbon tetrachloride and 5 cc of petroleum ether added. After removal of the precipitate by centrifuging, it was dried and weighed. Seven mg were obtained.

A solution of the isolated material, when injected into the ventral lymph sac of frogs, resulted in systolic arrest of the heart with doses of 4 μg/gm of frog. Similar results were obtained with known digitoxin. Colorimetric assays (*1*) of extracted and known digitoxin solutions were also in agreement. The majority of the radioactivity contained in 0.1 mg of the isolated material was recovered in the precipitate when 10 mg of known digitoxin was added as a carrier and the material recrystallized from dilute alcohol. The level of radioactivity was such that 2,000 cpm/mg were obtained from a 10-cm^2 surface with an end-window Geiger counter when plants exposed to 100 μc of C^{14} were used. Subsequent experiments indicate that higher amounts of the isotope (approximately 400–700 μc/plant) may be used.

Radioactive nicotine. The stems, leaves, and roots of the radioactive *Nicotiana rustica* plant were ground with 70 cc of 0.1 N sodium hydroxide in a Waring blendor. The homogenate was steam distilled, and 450 cc of distillate were collected in 20 cc of 0.5 N hydrochloric acid. This solution was analyzed for nicotine qualitatively by the characteristic absorption curve between 220 and 290 mμ and quantitatively at the absorption peak at 260 mμ, measured with the Beckman ultraviolet spectrophotometer. The solution was then concentrated to 50 cc on a steam bath, the pH adjusted to 2.3, and 5 cc of 12% silicotungstic acid added for each milligram of nicotine in the solution. The mixture was warmed on the steam bath for 30 min and allowed to cool overnight. The precipitate was filtered, washed with 10 cc of .005 N hydrochloric acid, and dissolved with 10 cc of 0.5 N sodium hydroxide. This solution was extracted by shaking with 50 cc of ethyl ether in a 125-cc separatory funnel and the aqueous phase re-extracted with another 50-cc portion of ether in a second separatory funnel. The ether phases were successively washed with 10 cc of 0.1 N sodium hydroxide and extracted with successive 3-cc portions of 0.1 N hydrochloric acid. This purified acid extract was made up to 10 cc and again analyzed spectrophotometrically for nicotine. The radioactivity was measured by spreading an aliquot of the solution on a 10-cm^2 surface, drying, and counting with an end-window Geiger counter. Ten thousand six hundred cpm/mg of nicotine were obtained when 100 μc of C^{14} were used for the plant.

Evidence that the material isolated from *Digitalis purpurea* is digitoxin lies in the specificity of the extraction procedure, the agreement between chemical and biological assay procedures, and experiments which show the radioactivity to persist in the digitoxin recrystallized from a 1: 100 mixture of radioactive and nonradioactive digitoxin. As yet we have no direct information as to which carbon atoms are labeled or, in fact, whether the sugar or the genin moiety of the digitoxin is so tagged. However, since the only source of new carbon for the synthetic processes of the plant was the CO_2 of the atmosphere, random labeling of all carbon compounds must occur. This may be an advantage in the use of such materials in biological problems, since it permits the tracing of all carbon-containing metabolic fragments of the drug rather than only the single atom usually labeled in synthetic drugs.

Evidence for the purity of the nicotine prepared from *Nicotiana rustica* is found in its ultraviolet absorption curve, the reaction with silicotungstic acid, the ether-water solubility characteristics of the material, and by the melting point of the picrate (218°).

Biological experiments with these drugs are in progress and will be reported elsewhere. Other plants, including *Digitalis lanata, Papaver somniferum,* and *Atropa Belladonna,* are also being investigated.

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