material does not thaw after the initial freezing, and that the microradiogram be taken before appreciable evaporation can occur. Specimens that do not require sectioning (for example, single nerve or muscle fibers) can be employed in a similar way. After the microradiogram has been taken, the sample is dried while it is still frozen to minimize morphological change (*6*), preferably with modern equipment (*7*), and the desiccated sample is used to obtain the second microradiogram.

2) If a microradiogram of only the dried sample is taken with 8 A or softer x-rays, the photographic positive can be taken as a true representation of the dry matter, since the microradiographic method of weighing cell structures gives the weight per unit area. The negative, however, will approximate a representation of the water: The "effective thickness" of the dry matter (t_S) in a given structure equals m_S/ρ_S, so that the "thickness" of the water present in the fresh material is

$$t_W = t_T - m_S/\rho_S, \qquad (6)$$

where t_T is the measured total thickness of the fresh sample. The mass of water (density equals 1) per unit area then is

$$m_W = t_T - m_S/\rho_S. \qquad (7)$$

It can be shown that the total x-ray extinction of a fresh tissue section of unit thickness changes relatively little with the varying proportions of water and dry substances common to soft tissues. Therefore, as was pointed out before, a photographic negative of a microradiogram showing the distribution of dry mass will show the distribution of water.

The application of procedure 2 is illustrated by the following. If earlier data on the dry weight of cells in dog gastric mucosa, obtained by microradiography of frozen-dried tissue sections (*8*), are substituted in Eq. 7, assuming $\rho_S = 1.3$, the approximate water content of the chief cells, epithelial cytoplasm, and parietal cells is 70, 76, and 85 percent, respectively. Similarly, if earlier data on the dry weight of ventral horn cells in the cat spinal cord (*9*) are used, calculations of the water content give 30, 65, and 75 percent for nucleolus, cytoplasm, and nucleus, respectively.

Procedure 1 is the more unequivocal, but it has the disadvantage of requiring a microradiogram of the fresh-frozen sample in addition to one of the dried sample. Although procedure 2 requires only the latter, the thickness of the fresh sample must be accurately known, since this will usually be 2 to 5 times the "effective" thickness of the organic material —that is, (m_S/ρ_S). A newer method permits the measurement of the thickness of a 5-μ section to within a few percent (*10*), while the older method of measurement by focusing on the upper and lower surfaces with a microscope usually involves an error of about 10 percent (*11*). Only a small error will result from the common deviations in the assumed value of 1.3 for ρ_S.

In both procedures it is necessary to consider the morphological effects of the drying process. Although freezing-drying minimizes these, the use of imbedding media may involve loss of substance on removal of the media. Whether or not such a loss is significant will depend on the nature of the specimen and of the medium. For frozen-dried gastric mucosa that is imbedded in paraffin, the deparaffinizing by xylol has no appreciable effect, owing to the low fat content of this tissue. Chemical fixatives are to be avoided, because they produce structural distortions and also affect the quantity of substance remaining.

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Biosynthesis of Pelargonidin-3-glucoside-C^{14}

In connection with current research conducted in this department on the stabilization of color in strawberry preserves (1, 2), it was desirable to obtain a quantity of C^{14}-labeled strawberry pigment to facilitate studies of its degradation (*3*). In view of the complex structure of the major strawberry pigment, pelargonidin-3-glucoside (3,5,7,4′-tetrahydroxyflavylium chloride 3-beta-glucoside), biosynthesis of the compound seemed more practical than its synthesis by conventional chemical means (*4*).

Several means of introducing C^{14} into the growing strawberry plants were considered. They included (i) exposure of the plants to $C^{14}O_2$ in a closed system, (ii) submersion of the roots in synthetic nutrient media incorporating C^{14} in an absorbable form, (iii) direct injection of glucose-C^{14} solution into stems of fruit during the final stages of berry maturation, and (iv) diffusion of glucose-C^{14} by direct immersion of the berries in a solution of radiosugar.

In view of the low activity of radiocarbon and the limitations on the amounts of tracer compounds used (microcurie levels were used in all preliminary experiments), only those methods appearing to channel the label most directly into the fruit were first tried. Injection of glucose-C^{14} solutions into either stems or fruits was unsuccessful owing to the absence of cavities in these that are capable of storing even small amounts of solutions. A slow, drip-feeding of glucose-C^{14} solution into the stems was equally unsuccessful. The diffusion of glucose-C^{14} into the green berries by direct immersion was not attempted because a preliminary trial, using an inactive glucose solution, resulted in a deleterious effect on the fruit.

However, two of the methods tried did result in the assimilation of the radiocarbon with no apparent injury to the plants. These methods were the exposure of strawberry plants to $C^{14}O_2$ and the application of crystalline, uniformly labeled glucose-C^{14} to freshly cut sections of fruit stems. In both instances, it was possible to extract, using suitable solvents, the pigments that were present and, upon chromatographic purification, to isolate the crystalline pelargonidin-3-glucoside-C^{14}. To eliminate the possibility that the pigment was merely contaminated with some of the radioglucose when the C^{14} was introduced in this form, tagged glucose was added to a portion of inactive juice from which the pigment was then also extracted. The absence of activity in pigment recovered from this sample is indicative of the effectiveness of the purification procedure used. Through acid hydrolysis of the active pelargonidin-3-glucoside to the pelargonidin, it was also established that the activity was uniformly distributed throughout the pigment molecule and not merely attributable to the sugar moiety.

The labeled sugar was introduced into the plant by placing the dry crystals in small, longitudinal, V-shaped grooves that were cut in the stems and allowing the plant juices to dissolve them. Forty grams of ripe strawberries was harvested from plants to which about 2μc (about 80 mg) of glucose-C^{14} was administered.

The berries were frozen and thawed, and the major pigment was isolated from the hand-expressed juice by the paper chromatographic method described in a subsequent paragraph. About 8 mg of crystalline pelargonidin chloride-3-monoglucoside-C^{14} was obtained.

Individual strawberry plants were covered with glass bell jars into which $C^{14}O_2$, generated by the action of HCl on $Na_2C^{14}O_3$ or on $BaC^{14}O_3$, was introduced to create an atmosphere of about 0.8 percent CO_2. In a preliminary greenhouse experiment, 2 μc of $C^{14}O_2$ was administered to a single plant in two doses within 3 weeks. During the time between the two doses, the plant was left uncovered for 4 days, because a few necrotic spots had appeared on its leaves. Fourteen grams of ripe berries was obtained from this plant, and about 3 mg of tagged pigment was crystallized from them.

In the subsequent field experiment, 1 mc of $C^{14}O_2$ was similarly applied over a 4-day period to four strawberry plants located in an outdoor bed. From the berries that were harvested from these plants 59.5 mg of pelargonidin-3-glucoside-C^{14} was recrystallized.

Juice from the strawberries was acidified to contain 1 percent HCl, and the resulting solution was extracted with 1-butanol. On treating the alcohol extract with petroleum ether, the water was separated from the butanol in which it had been dissolved. This aqueous phase contained all of the colored matter present and could be concentrated under vacuum at temperatures below 50°C to almost one-third of the original volume. The concentrate was streaked on strips of Whatman No. 1 or 4 paper, the resulting bands being dried immediately after application. After ascending development for 12 to 16 hours, using a solution of 1-butanol, acetic acid, and water (40/10/50, respectively, by volume) as the solvent, the anthocyanin bands were fully separated (*5*). Bands corresponding to pelargonidin-3-glucoside were cut out and eluted by immersing one end of each strip in a beaker of methanol that contained a trace of HCl and allowing this solvent to descend by capillarity into a lower beaker. The methanolic eluate was evaporated under vacuum, leaving a noncrystalline residue, which was redissolved in 1-percent HCl and chromatographed by the same method as that employed for the aqueous concentrate. Methanolic eluates, obtained from pelargonidin-3-glucoside bands from the second chromatograph, were acidified with HCl and allowed to evaporate slowly at room temperature. Red, needlelike crystals of pelargonidin-3-glucoside-C^{14} were obtained.

Measurements of activity were carried out using a Tracerlab Autoscaler equipped with a thin (1.4 mg/cm²) end-window Geiger-Müller tube, except for the determination of activity on the pigment resulting from the 1-mc $C^{14}O_2$ experiment. For this, a Tracerlab Superscaler equipped with a windowless, gas-flow counter was employed. Specific activities for the pigment samples recovered in the microcurie experiments were low, but counts were statistically significant at the 1-percent level. Pigment from strawberries harvested from the plants treated with glucose-C^{14} showed a specific activity of 379 ± 7 disintegrations per minute, per milligram of pigment. Pigment from the plant exposed to 2 μc of $C^{14}O_2$ showed a specific activity of 951 ± 19 disintegrations per minute, per milligram. These correspond to yields, based on activity, of 0.068 percent for the glucose-C^{14} method and 0.064 percent for the $C^{14}O_2$ method .

In the preparatory run, utilizing 1 mc of $C^{14}O_2$, pelargonidin-3-glucoside-C^{14} with a specific activity of 1512 disintegrations per minute, per milligram was obtained. The percentage yield, based on activity for this experiment was 0.004.

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Optical Isomerism and Pharmacological Action, a Generalization

The study of the biological activity of drugs with optical isomerism has been unduly influenced by the known optical specificity of the amino acids, wherein, for example, *d*-serine is reported not only to be inactive but to produce kidney damage (*1*). *d*-Glutamic acid is reported to be cyclicized into a pyrrole compound and excreted in the urine (*2*). Such complete failure of the body to utilize the unnatural isomer might be expected with chemicals such as amino acids, which are the building blocks for proteins. Drugs, and even the naturally occurring humoral agents, probably act by modifying cellular responses and are thus not as geometrically specific as are the amino acids. Yet, one finds frequently in the pharmacological literature the statement that the active form of the drug is the *l* or *d* isomer without adequate trial of higher doses of the more inactive isomer.

Consideration of the specificity of the optical isomers of thyroxin is omitted from this report, since the asymmetric carbon atom occurs at the portion of the molecule that may be involved in synthesis of the thyroxin molecule into cellular proteins where the optical configuration may be as crucial as that of the amino acids. Several individual factors that may dominate in the drug action of optical isomers also have been omitted from consideration in this generalization. These factors are (i) rate of absorption or destruction of the isomers, (ii) competitive inhibition between isomers, (iii) molecular size such as *d-l* complex formation by hydrogen bonding between isomers, (iv) affinity for the receptors of the isomers, (v) differential penetration to the site of drug action, and (vi) the possible racemization by the body of the less active isomer.

Several years ago we were disappointed when the anticonvulsant *d*-atrolactamide was found to have a ratio of potency only 1.68 times greater than that of *l*-atrolactamide. This difference is easily measurable in terms of the LD_{50} in the mouse and is a statistically significant difference, as is shown by the following oral LD_{50}'s and their standard deviations: *d,l*-atrolactamide, 1.86 ± .07 g/kg; *d*-atrolactamide, 1.39 ± .04 g/kg; and *l*-atrolactamide, 2.33 ± .06 g/kg (*3*). However, atrolactamide as an anticonvulsant is relatively nonpotent in that it must be given in a dose of 1.5 g four times daily to control the seizures of epileptic patients. In retrospect, it becomes clear that potency per se, as expressed by the LD_{50}, is probably a measure of the degree of reactivity of the drug with cells or enzymes. Therefore, with a nonpotent drug, such as atrolactamide, the degree of geometric conformation to the locus of action must be relatively poor and, hence, would not be greatly influenced by the optical isomerism of the compound.

Since effective dosage of a drug is a measurement of potency, the dose can be used as an approximation of this geometric conformation, and the postulate can be made that the lower the effective dose of a drug, the greater the difference in the pharmacological effect of the optical isomers. In order to document this thesis, one would wish to have LD_{50}'s in the same species of animal for all of the commonly used drugs that can exist as *d* and *l* isomers. However, a survey of the literature does not provide such data