because of the numerous, partially hidden orifices to these lobes. The peribronchial connective tissues have become fibrosed within 3 to 4 weeks and they contract, compressing and buckling the bronchial cartilages, to produce a partial collapse of the lumen of the bronchus. Proliferation of fibrous and granulation tissue from the submucosa further compresses the mucosa and occludes the lumen of the bronchus.

The procedure described is quite safe and surprisingly efficient. It is so benign that the bronchi of an entire lung may be safely treated at one bronchoscopy. Grossly, at bronchoscopy, there can be seen no ulcerations on the mucosa. There have occurred no pulmonary hemorrhages, and sections show little or no damage to the blood vessels. The dogs following bronchoscopy, even after the bronchi of an entire lung have been treated, appear in good condition, and it is rare that they run one to 2 degrees of temperature. They eat well and, except for a hacking cough, appear entirely normal. In our first 12 animals, when the technical application had not been entirely determined, 4 deaths occurred, 2 from pushing the applicator through the bronchus, and 2 from overcauterization. In the last 25 animals 100% were given satisfactory and complete stenoses.

It would appear from such observations on these dogs, that a simple and efficient method for the production of bronchial stenosis has been obtained.

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Ascorbic Acid from Iris and Other Plants by a Simplified Method.*

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We have had great difficulty in obtaining more than a few milligrams of the antiscorbutic vitamin, ascorbic acid, from either plant sources or suprarenal glands by the published methods of Szent-Györgyi[1] or of Waugh and King[2] and Svirbely and King.[3] Large

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1 Szent-Györgyi, A., *Biochem. J.*, 1928, **22**, 1387.

2 Waugh, W. A., and King, C. G., *J. Biol. Chem.*, 1932, **97**, 325.

3 Svirbely, J. L., and King, C. G., *J. Biol. Chem.*, 1931, **94**, 483.

and uncontrolled losses occur at many places in both processes as published. Svirbely and Szent-Györgyi[4] have just reported a somewhat modified procedure, using the Hungarian sweet pepper from which paprika is made, which contains considerably more vitamin C than we have obtained from the American sweet pepper—another variety of *Capsicum annuum.* We have studied the causes of these losses with a view to developing a more certain method of preparation. A survey of common plants was also made to find richer sources of the vitamin. Some of these results have been published by Marine, Baumann and Webster.[5] Leaves of German iris *(Iris germanica)* are by far the most satisfactory material from which to extract vitamin C, both because the vitamin is present in high concentration and because there are fewer substances in this plant that interfere with its separation. Most plants have a relatively high concentration of ascorbic acid in the young actively growing portions but on maturation, the vitamin rapidly disappears. In German iris leaves, however, the concentration of the vitamin, which is 600 mg. per 100 gm. of fresh leaf in early spring, remains as high as 250 or 300 mg. per 100 gm. in late summer, and this plant offers a very rich source during the entire growing season. Skunk cabbage *(Symplocarpus foetidus)* gathered during April and May and sweet green peppers as purchased from January to May both contained between 100 and 200 mg. per 100 gm.

We have developed a comparatively simple method for preparing 5 to 20 gm. of ascorbic acid at a time from 5 to 10 kg. of these plants. Several of the steps developed by King and his associates in extracting crystalline vitamin C from lemons have been utilized. We are also indebted to Nelson,[6] who showed that in the presence of traces of copper, ascorbic acid is rapidly oxidized, and to Marine,[7] who finds ferric iron acts similarly though it is only about half as active as copper. The ascorbic acid is isolated as the monoacetone derivative described by Vargha.[8]

From iris leaves, about 70-75% of the reducing substances originally present was obtained as the crystalline acetone derivative of ascorbic acid. From skunk cabbage leaves and from sweet green peppers, the yields were 35% and 60% respectively. From the

[4] Svirbely, J. L., and Szent-Györgyi, A., *Biochem. J.*, 1933, **27**, 279.

[5] Marine, D., Baumann, E. J., and Webster, B., *J. Biol. Chem.*, 1930, **89**, 213.

[6] Nelson, E. K., *Science*, 1932, **76**, 345.

[7] Marine, D., unpublished data.

[8] Vargha, L. v., *Nature*, 1932, **130**, 847.

mother liquors, which can be combined, considerable additional amounts have been readily obtained on further purification.

The specific instructions which follow are simpler, more readily followed and better adapted to the apparatus ordinarily found in most laboratories than those published hitherto.

Place 5 kg. of iris leaves in cheese cloth or scrim press cloth and steam for 30 minutes in an instrument sterilizer. Hash and press out juice in a wine press. The press cake may be reextracted with acidulated water or methyl alcohol over night and an additional 10-15% may be obtained in this extract, though ordinarily this is not worth the extra effort. Concentrate the press juice *in vacuo* at a water bath temperature of 40-50°C., with a capillary through which CO_2 is passed, to 1/5 its volume. Add 3-4 volumes methanol gradually. Filter on a large Buchner, wash the gelatinous precipitate once on the filter and then again by rubbing up in a mortar with a large volume of 75% methanol. Concentrate the filtrate and washings *in vacuo* to 400-500 cc. Extract 3-5 times with 100-125 cc. butanol in a separatory funnel. If separation does not take place reasonably quickly, the fluid may be centrifuged in 250 cc. cups and the aqueous layer aspirated off. Extract the butanol solution of fats and pigment once or twice with 50 cc. water, combine the aqueous extracts and chill to 0-5°C. Sufficient hot saturated solution of neutral lead acetate is added to make the concentration 25-30%. Keep chilled and gradually add 15% ammonium hydroxide to a pH of 7.6-7.8. Centrifuge off the precipitate and wash once in the cups with a large volume of water. Rub the precipitate up to a thick paste with cold water, transfer to a liter beaker and add cold 15% HCl to a pH of 2 (pink with thymol blue). Filter on Buchner, wash the PbCl in a mortar with cold water acidulated with HCl. Concentrate filtrate and washings *in vacuo* with a capillary through which CO_2 passes, to a thick syrup. The fluid darkens but apparently no loss of the vitamin takes place. Add 200 cc. of methanol, evacuate flask and heat under hot water (50-70°C.) until the gum is dissolved. Cool and gradually add 3-5 volumes of acetone, until precipitation is nearly complete. Centrifuge off gummy precipitate or oil (most remains in flask). Dissolve the gum in the centrifuge bottles in 50 cc. methanol and add to the main part of the gum in the flask which is then also dissolved as before. Then add 5 volumes of acetone gradually. Centrifuge again and treat the gum a third time with methanol and acetone. Concentrate the combined alcohol-acetone extracts *in vacuo* to a thick syrup.

Dissolve the syrup in an evacuated flask in 50 cc. of dry methanol, cool and add slowly with shaking about 10 volumes dry acetone and finally 500 cc. of pentane. Centrifuge off. Dissolve the oily residue in 50 cc. dry methanol, add 10 volumes of dry acetone and 10 volumes of pentane and centrifuge again. The oily residue is treated in this way 4-6 times. Concentrate the combined acetone pentane extracts to a thick syrup *in vacuo*. Add 30-40 cc. dry acetone. Evacuate the flask and warm in hot water until the syrup dissolves. The evacuated flask is kept chilled to 0-5°C. for about 2 days. A crop of crystals is then filtered off and washed with cold acetone until nearly white. 9.3 gm. of the acetone derivative were obtained at this point.

Dilute the mother liquor to 200-300 cc. with dry acetone and add 1½-2 volumes of pentane. Centrifuge off the oily residue, dissolve in 5 cc. methanol and treat again with acetone and pentane until nearly all of the vitamin has been extracted. Concentrate extracts *in vacuo* to a syrup, add 25 cc. of dry acetone and store in an ice box in an evacuated flask over night when another crop of crystals separates. 6.6 gm. were obtained, making a total of 15.9 gm. of the acetone derivative from 5 kg. of iris leaves. More can be separated from this mother liquor.

The amount of vitamin present at each stage can be conveniently followed by titrating small aliquots (0.5-2.0 cc.) with 0.01 N iodine solution in acid solution, with starch as an indicator.

Press juices from skunk cabbage leaves and green peppers have been treated similarly but an additional treatment with methyl alcohol and acetone is necessary in the first part of the process, before pentane can be used, and an additional fractional separation with acetone and pentane may also be needed.

Purification of Reagents. Synthetic methanol is distilled from alkali. Acetone is refluxed for half an hour with $KMnO_4$ and then distilled through a fractionating column. Acetone and methanol are dried sufficiently with anhydrous Na_2SO_4 for use in the latter part of this process and then distilled from alkali in the case of methanol or refluxed with $KMnO_4$ and distilled in the case of acetone. Normal butanol is used as purchased. Commercial pentane (B.P. 30-35°C.) is shaken with concentrated H_2SO_4 in a separatory funnel and distilled. Lead acetate (analytical grade) is recrystallized twice from water to remove traces of copper.

The crude acetone ascorbic acid from iris melted at 219.5°C. with effervescence. On recrystallization from acetone it melted at 219.5-221°C. Vargha gives the melting point at 220-222°C. The acetone

derivative was converted to the free vitamin by dissolving in water and warming for 10 minutes in an evacuated flask. It was dried in a vacuum desiccator over barium oxide. Without further purification this slightly yellow product melted at 189.5°. The melting point of the pure substance is 192°C. The acidity and the amount of iodine reduced by these preparations were within 3% of the theoretical.

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Purgative Effect of Some Aliphatic Alcohols.

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In connection with a pharmacological study of the first 18 primary alcohols of the aliphatic series made in this laboratory in collaboration with Professor E. Emmet Reid of the Laboratory for Organic Chemistry, Johns Hopkins University, an investigation was also made of the higher members of the series, most of which are solids, to ascertain whether any of these exert a laxative or purgative action. The reason for such an inquiry was the finding by Macht and Barba-Gose, while studying the pharmacology of the oil of the *Ruvettus pretiosus* fish, that the active principle responsible for its laxative effect was the acetate of a hexadecyl or cetyl alcohol.[1] Because of the small amount of material available and the insolubility of these higher alcohols in water and physiological saline, the writers employed a new method.[2] Briefly, it consists of the introduction through a "stomach tube" of an emulsion of finely divided animal charcoal into the stomachs of full-grown white rats, previously fed on a standard dry diet composed of wheat (25 gm.), ground maize (25 gm.), rolled oats (28½ gm.), flaxseed meal (10 gm.) casein or whole milk, dried (10 gm.), sodium chloride (1 gm.), and calcium carbonate (0.5 gm.). An hour after the introduction of such an emulsion, the animal is quickly killed and the entire gastrointestinal tract is excised and stretched out on the operating table. The distance traversed by the black emulsion in a

[1] Macht and Barba-Gose, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 772.

[2] Macht and Barba-Gose, *J. Am. Pharm. Assn.*, 1931, **20**, 558.