Pigments I, II and III had adsorption maxima similar to beta-carotene in petroleum ether. Biological assay by the U.S.P. XI procedure indicated that pigment II had vitamin A activity equivalent to that of beta-carotene, while pigment I had half the potency. Pigment III was completely devoid of vitamin A value.

These findings may be summarized as follows: (1) A new adsorption technic was developed for the separation of petroleum soluble carotenoids from mineral oil; (2) four carotenoids were separated as products of bacterial metabolism with mineral oil as the sole source of carbon; (3) two of these pigments possess vitamin A potency; (4) no xanthophylls were present; (5) one pigment was definitely shown to be astacin, a carotenoid found primarily in crustacea, and not hitherto associated with bacterial metabolism.

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SYNTHESIS OF ASCORBIC ACID IN EXCISED TOMATO ROOTS

ROOTS which have been carried through many passages in nutrient solution with no ascorbic acid supplied must either be able to grow without it or they must be able to synthesize it from the materials contained in the culture solution. Previous tests[1] conducted with excised roots of the white moonflower, grown in darkness for several weeks, had shown no more ascorbic acid than was present in the original explants. The cultures kept in light developed chloroplasts and contained 4 to 10 times the quantity of vitamin C present in the original explants. The results thus suggested that only the roots which contained chlorophyll had the capacity to synthesize ascorbic acid. In the light of more recent studies, however, it seems probable that synthesis occurred in the non-chlorophyllous roots, but that utilization of the product occurred also and at about the same rate as synthesis, resulting in the maintenance of a constant content per root.

A continuation of this type of study with non-chlorophyllous roots of another type was considered desirable. For this purpose cultures of tomato roots were prepared, using a modified Pfeffer's solution containing 1 per cent. cane sugar with 10 mμ moles of vitamin B_1 and 50 mμ moles of vitamin B_6 per flask. As inoculum, root segments were used which had grown for 60 passages during a period of 5 years in Pfeffer's solution + cane sugar + vitamin B_1. This procedure of long-continued culturing and numerous passages guarantees that the roots used as inoculum contained nothing other than that derived from the basal solution or synthesized by the roots in culture. The roots were grown in the culture solution 4 to 5 weeks, half of the cultures being kept in diffuse light and half in darkness. Ascorbic acid determinations were made by the indophenol method. Table 1 presents the results of these experiments.

TABLE 1

ASCORBIC ACID CONTENTS OF EXCISED TOMATO ROOTS

No. of expt.	No. of roots	cc of indicator required	Green weight per root (g)	Ascorbic acid	
				mg/root	mg/gram
			Light		
1	35	1.36	0.076	.0033	.043
2	30	1.41	0.192	.0055	.029
			Darkness		
1	35	0.48	0.069	.0010	.014
2	30	1.50	0.155	.0058	.037

A definite indophenol-reducing action of the root extracts was observed and with approximately the same speed of reaction as occurs with vitamin C. Moreover, the reaction was of such magnitude as to eliminate the possibility of transference of the total quantity of the reducing substance from the original explants. The reducing activity per root of cultures kept in the light in the second test was considerably higher than that of similar cultures in the first test but lower on a per gram basis. The weight per root was more than twice that of the roots in the first test. The reason for the higher reducing action per root of the cultures kept in darkness in the second test as compared to that in the first is not clear but it may be in part the effect of the slightly higher temperature at which the second set of cultures were kept. The weight of the roots increased from 70 to 200 fold during the culture period. Presumably the ascorbic acid increased in approximately the same proportion.

These results indicate fairly definitely that sterile cultures of excised tomato roots kept either in darkness or in light have the capacity to utilize sucrose in the synthesis of ascorbic acid, but a final conclusion on the effect of light on the synthesis of vitamin C by excised roots is not possible from these experiments. It is probable that intact plants have the ability to synthesize vitamin C at night by utilizing some of the stored carbohydrates. However,[2, 3, 4, 5] not only has no gain in absolute amount of ascorbic

[1] M. E. Reid and R. L. Weintraub, SCIENCE, 89: 587–8, 1939.

[2] E. F. Kohman and D. R. Porter, SCIENCE, 92: 561, 1940.

[3] H. G. Moldtmann, *Planta*, 30: 297–342, 1939.

[4] A. M. Smith and J. Gillies, *Biochem. Jour.*, 34: 1312–1320, 1940.

[5] M. E. Reid, *Am. Jour. Bot.*, 27: 18.S., 1940.

acid per plant been found to occur at night, but instead, losses of 15 to 20 per cent. have been observed. It is thus evident that neither the total gain nor loss at night can be estimated by the methods which have been employed and that the total losses are probably considerably greater than have been estimated. The results thus suggest that vitamin C may play a much more important part in the economy of the plant than has been previously attributed to it.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN APPARATUS FOR VACUUM DRYING IN THE FROZEN STATE[1]

IN the fractionation of thermo-labile biological products it often becomes necessary to recover small amounts of solids from comparatively large volumes of solution. Vacuum drying in the frozen state, as recommended by Flosdorf and Mudd[2] and Link, Eggers and Moulton,[3] is often preferred, particularly if the material is protein in nature.

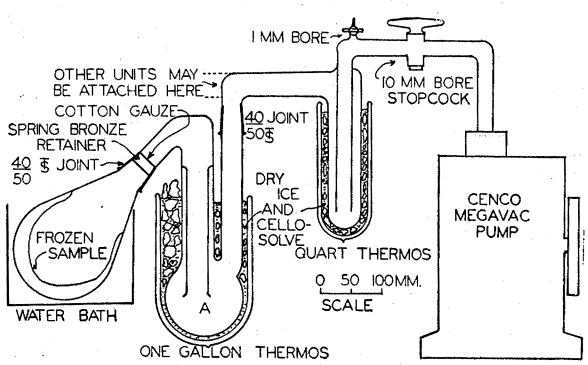

FIG. 1

The apparatus shown in the diagram is designed so that each receiver (A) is capable of receiving 500 to 600 cc of the distillate within a period of twelve hours. A Cenco Megavac pump serves to create the vacuum. A mercury vapor diffusion pump is not necessary. The water bath surrounding the flask containing the sample is left at room temperature and serves to prevent ice formation on this flask during evaporation. An acetone bath chilled by solid carbon dioxide is used to freeze the sample to be evaporated, while methyl cellosolve is used in place of acetone in the vacuum bottles in order to minimize loss through evaporation. A short spring bronze cylinder is used to hold a piece of cotton gauze in the neck of the sample flask to prevent loss of dry particles due to rapid vapor currents.

The apparatus is constructed of pyrex glass, is simple, compact and sturdy and has become nearly indispensable in our laboratory. It has been used to recover biologically active materials from as much as one liter of solution in a period of twenty-four hours with a single change of the receiver and recharging with solid carbon dioxide after twelve hours. Solutions containing the gonadotropic or thyrotropic fractions from the pituitary, choline esterase, bacterial toxins, whole blood, minced tissues and various extracts have been shown to retain their solubility and biological activity under such treatment. Solutions treated with toluene according to the method of Railton, Cunningham and Kirk[3] yield sterile preparations.

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ELIMINATION OF DEHYDRATION IN HISTOLOGICAL TECHNIQUE

THE classical methods for preparing tissues for microscopic study have a number of disadvantages. One of the more important is the extensive dehydration of the specimen by passage through increasing concentrations of alcohol. Another, consequent upon this, is the solution of cellular fat in the alcohol; this requires, for fat stains, the setting aside of special blocks of tissue and the use of the frozen section technique.

In the search for an improved method which would avoid dehydration, we thought of the recently synthesized group of resins, the polyvinyl alcohols, more familiar in the trade as PVA. These substances are readily soluble or dispersible in water, forming sols which penetrate easily into tissues. They can be made to form gels of a consistence comparable with that of celloidin. Sections may be cut as thin as four to five micra without difficulty. These stain excellently with hematoxylin-eosin, Masson's trichrome, Weigert-van Gieson; staining for fat is done with Sudan III on sections cut from the same block. The polyvinyl alcohols in solution are considered stable to the action of bacteria and most fungi; in our tests, the gels as well

[1] This work was supported in part by a grant from Armour and Co.

[2] E. W. Flosdorf and S. Mudd, *Jour. Immunol.*, 29: 389, 1935.

[3] G. K. K. Link, V. Eggers and J. E. Moulton, *Bot. Gaz.*, 102: 590, 1941.

[3] I. R. Railton, B. Cunningham and P. L. Kirk, SCIENCE, 94: 469, 1941.