connected with the Hall of Marine Mammals. Habitat groups include one showing the fishes of the Bahama coral reefs, another showing the rocky coast of Maine, and one of the sandy ocean floor of the Texas coast. In addition, there is an extensive systematic collection of fishes in kindred forms running from the giant whale-shark down to the tiny frog-fish from the Sargasso Sea.

Throughout this report there are cited many instances of new exhibits which have been opened to the public. It is only natural that any reader would attribute full credit to the department sponsoring each exhibit. Little thought or appreciation is given to the Division of Maintenance or the Division of Engineering through whose efforts the painstaking details of case-planning, lighting, construction and even to a large extent the actual installation are carried out. I am pleased to call especial attention to the effectiveness, thoroughness and spirit of cooperation with which these divisions carry on their work.

There are many persons whose names are not found in the press reports or on the labels of the museum exhibits, who contribute valuable service without which the museum could not continue. I acknowledge a debt of gratitude to the many men and women who perform routine jobs with skill and extreme care, and who thus contribute to the maintenance of the good name of this institution.

SPECIAL ARTICLES

THE SEPARATION AND CHARACTERIZATION OF CAROTENOID PIGMENTS PRODUCED FROM MINERAL OIL BY BACTERIA[1]

DURING the course of research studies[2] on the utilization of petroleum products by microorganisms a culture of *Mycobacterium* was isolated which produced "oil-soluble" yellow and orange pigments when grown on a substrate composed of mineral salts and mineral oil.[3]

The identification of these pigments, which were subsequently found to be carotenoids, was complicated by the fact that they could not be removed from the oil by the conventional methods used in the analysis of plant tissues. Extraction methods employing various solvents were not successful because the pigments were either not removed or, if removed, were accompanied by some of the oil.

In the preliminary trials, the chromatographic adsorption technique used by Strain[4] in the separation of leaf xanthophylls was used with a mixture of 50 per cent. MgO (Micron Brand No. 2641), and 50 per cent. siliceous earth as the adsorbent; however, when saponified pigment-bearing oil was passed through the column, incomplete separation resulted. When an attempt was made to wash the column free from mineral oil by the use of petroleum ether (B. P. 30°–60°), the pigments migrated with the ether and oil mixture. However, a column of the MgO alone was found to retain the pigments very tenaciously in the upper one-fourth inch of the column. Repeated washings with petroleum ether failed to remove the pigments, but the oil was removed by this means.

Since the pigment fraction in all separations was confined to the extreme upper portion of the column and because the passage of the oil through the column was too slow, the orthodox type of adsorption tube used in chromatographic analysis was discarded for the preliminary separation of the pigments and oil. Instead, a Jena glass filtering crucible (capacity, 30 cc; height above disc, 45 mm; diameter of disc, 30 mm) of the Gooch type was used. This device, when packed with adsorbent, permitted a rapid separation of the pigment from the oil because of the increased surface area of the adsorbent. Complete separation of the pigments was effected from as much as 100 ml of oil by this technic.

The pigmented portion of this column was then removed mechanically, and the pigments eluted by petroleum ether containing a small amount of ethyl alcohol. Extraction of the petroleum phase with 90 per cent. methanol at this point showed xanthophylls to be absent. The pigment solution was then evaporated to dryness under vacuum. The semi-crystalline residue was redissolved in a minimum amount of petroleum ether (1.0 to 2.0 ml) and chromatographed on a column containing 50 per cent. MgO and 50 per cent. silicious earth. Three distinct pigmented bands were obtained in this manner.

The color of the successive bands from the lowest to highest were: (I) yellow, (II) orange and (III) pink. In addition to the above-described pigments, a red pigment (IV) appeared in the 90 per cent. alcoholic-KOH solution used in the saponification of the oil. This pigment exhibited all the chemical properties of astacin[5] and possessed a single absorption maximum at 5,000 Å in carbon disulfide.

[1] Contribution No. 213, Department of Bacteriology, and No. 273, Department of Chemistry.

[2] H. F. Haas, M. F. Yantzi and L. D. Bushnell, *Kansas Acad. Sci. Trans.*, 44: 39–45, 1941.

[3] Refined light mineral oil having a specific gravity of 0.84 at 25° C. and a viscosity of about 105 (saybolt) at 100° F.

[4] "Leaf Xanthophylls," H. H. Strain, Carnegie Institution of Washington, Pub. No. 490, 1938.

[5] G. Wald and H. Zussman, *Jour. Biol. Chem.*, 122: 449–460, 1937.

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.

H. F. HAAS
L. D. BUSHNELL
W. J. PETERSON

KANSAS AGRICULTURAL EXPERIMENT STATION

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.