CXLV. THE LIPOCHROMES OF ETIOLATED WHEAT SEEDLINGS.

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THE association of vitamin A with some lipochrome (generally carotene) in all recorded cases of its occurrence in plant tissues [Steenbock and Gross, 1919; Steenbock, Sell and Boutwell, 1921; Steenbock and Sell, 1922; Coward, 1923] and the rapidity with which wheat seedlings grown in the dark form vitamin A on exposure to light, made it seem desirable to make a careful examination of the pigments occurring in these etiolated shoots.

The formation of a yellow pigment in small grains in the cells of plant structures grown in the dark has long been recognised. The pigment was known to be soluble in alcohol and was called "etioline" by Pringsheim [1874] because it occurred in etiolated structures. Willstätter and Stoll [1913] identify "etioline" with carotene of carrots, etc., on the strength of the similar solubilities and crystal form of the two substances. Other workers report various findings and employ various terms to designate the pigments. This has given rise to considerable confusion in the literature, a summary of which is given by Palmer [1922]. The term "etioline" is now obsolete although shoots grown in the absence of light are still spoken of as "etiolated." It is proposed to give here the results of an investigation of the lipochromes of etiolated wheat seedlings in the light of the later work on lipochromes of Willstätter and his co-workers and of Tswett [1906, 1911].

The seeds used throughout the experiments were Carter's "Red Stand-up" variety. No detectable traces of lipochromes were found in them, even on saponifying and extracting 100 g. They were germinated in ordinary garden mould mixed with about a quarter of its weight of sand, in boxes in a large airy dark room at a fairly uniform temperature of 6–10°. They were allowed to grow for 17 days and the shoots used in the investigation were cut immediately below the first outstanding leaf.

The pigments of the etiolated shoots were roughly identified as lipochromes by grinding up the tissue with anhydrous sodium sulphate, extracting with ether and applying the usual tests. Carotene and xanthophyll were both identified, the latter being present largely in excess of the former.

For reasons explained in the preceding paper, the quantitative estimation of the lipochromes was made, after saponification of the tissue with about

twice its weight of 20 % aqueous potash for 3 days, by addition of alcohol which seems to liberate the pigments, and extraction with light petroleum (B.P. below 40°). The extract was washed six times with water but not dried with anhydrous sodium sulphate. Apparently light petroleum takes up only a very small quantity of water, and this would not interfere materially with the estimation. The extract was evaporated down in nitrogen, an aliquot part taken, the carotene and xanthophyll separated by the addition of an equal volume of 90 % alcohol as in the Kraus phase test, the resultant volume of each phase noted, and the concentration of each estimated by the Hellige colorimeter. In this way 1.97 mg. carotene and 10.3 mg. xanthophyll were obtained from 400 g. fresh shoots.

CHROMATOGRAPH OF LIPOCHROMES OF ETIOLATED WHEAT SEEDLINGS.

It was attempted to make a chromatograph of the lipochrome solution obtained by the saponification of 400 g. fresh shoots as detailed above. This was found to be impossible, a certain amount of pigment being retained in the chalk, but most of it coming through in the filtrate.

Other attempts were equally unsuccessful and hence it must be concluded that the very long saponification, although carried out in nitrogen, had influenced the lipochromes somehow. A quick saponification of a smaller quantity (about 20 g. for 6 hours) gave a quite good chromatograph whose different layers gave the following spectra in ethereal solution.

	Band I	Band II	Band III	Substance presumably
Filtrate	489.5-472	455-442	428 to end	Carotene
Layer 1 (at base)	485 -472	453-440	426 "	Xanthophyll α
" 2	481 -468	452-438	424 "	" "
" 3	478 -467	450-438	424 "	" "
" 4 (at top)	476 -465	448-436	422 "	" "
Size of slit of spectroscope	0.08 0.08	0.08 0.095	0.12 mm.	

Apparently the four xanthophylls identified by Tswett [1906, 1911] in green leaves are also present in etiolated ones.

A fairly complete extraction can also be made by grinding up the fresh material with sand and alcohol, but as alcoholic solutions pass straight through the chalk column, the solution has to be filtered, evaporated to dryness in nitrogen under reduced pressure and then taken up in light petroleum. With the greatest care this cannot be really quantitative as there must be partial loss of one or more constituents by incomplete extraction and during the evaporation to dryness. A solution of the lipochromes of etiolated wheat seedlings made in this way gave four distinct layers in a chromatograph and a coloured filtrate.

The solutions of the layers of lipochromes from many chromatographs have been examined spectroscopically. The results given above are typical but slight variations have been noticed. When, however, it is realised that it is almost impossible to get solutions of the same strength for purposes of

comparison and that the edges of the bands are very ill-defined, the discrepancies may be pardoned. Tswett [1911] suggests that the slightly different spectra of carotene recorded by different observers may indicate the existence of "different homologous carotenes" but the differences appear to be only such as might result from the difficulties mentioned above.

The solutions of the four xanthophylls from etiolated wheat seedlings described above and identified spectroscopically were combined and concentrated. The spectrum of the solution was then 484-465, 452-434, 426 to end, which appears to be a simple combination of the four individual xanthophylls.

It was originally intended to make an estimation of the relative proportions of the four xanthophylls in etiolated wheat seedlings. Tswett mentions the desirability of investigating his chromatographic analysis quantitatively but does not appear to have done so.

With this purpose in view, the four layers of a chromatograph of lipochromes from etiolated wheat seedlings were separated. Each part was dissolved out with ether, filtered and the residue washed as completely as possible; the volume of the filtrate was noted and the concentration estimated by the Hellige colorimeter. The whole solution was evaporated just to dryness in a test-tube in a current of nitrogen, dissolved in light petroleum and the solution made up to its original volume. Its concentration was again determined to estimate loss by heating and drying, it was passed through a fresh chalk column, dissolved out again with ether as completely as possible and the solution again made up to its original volume. The concentration of this was estimated to see whether the extraction from the chalk had been complete. Even with the greatest care, there was loss of lipochrome in each process as may be seen from the following table.

		Vol. cc.	First extract from chalk		Light pet. solution		After second filtration		Bulk of chalk in second filtration
			H.R.	Xanth. mg.	H.R.	Xanth. mg.	H.R.	Xanth. mg.	
Xanth.	α	5.3	76.5	1.99	84.5	1.06	96.5*	0.1325	Greatest, about 1 inch depth
"	α'	6	82.3	1.35	90.0	0.6	92.6	0.36	Least, " 0.15 " "
"	α''	3	88.5	0.375	91.5	0.225	95	0.12	" 0.2 " "
"	β	4	82	1.0	86.3	0.6	91	0.3	" 0.2 " "

* Too dilute a solution for an accurate comparison.

Apparently the greater the bulk of chalk over which any one xanthophyll solution spreads itself, the greater the loss of lipochrome in the process. No strict comparison is possible in these figures as neither the total xanthophyll nor the concentration of the different solutions used was equal. In any case, as these factors cannot be controlled in the making of a chromatograph, it seems to be impossible to make a quantitative comparison of these lipochromes by this method.

SUMMARY.

1. The pigments of etiolated wheat seedlings have been identified with the carotene and four xanthophylls isolated by Tswett from green leaves.
2. It is not possible to make a quantitative estimation of the four xanthophylls separated from each other by Tswett's chromatographic method.

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