XV. THE NATURE OF THE UNSAPONIFIABLE FRACTION OF THE LIPOID MATTER EXTRACTED FROM GREEN LEAVES.

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In an investigation now being carried out by E. M. Hume at the Lister Institute, the unsaponifiable matter of the lipoid substances extracted from spinach leaves was being prepared by her to supply the vitamin A in a certain diet. During the course of this preparation we noticed that a white crystalline substance readily separated when this unsaponifiable matter was being taken up with hot alcohol, and we therefore decided to investigate its nature.

Method of preparation. The spinach leaves were dipped for a few seconds into boiling water before drying at 37° under a fan. The preliminary dipping expels air from the tissue and leaves so treated are crisp and green when dry. They were then powdered and extracted with light petroleum (B.P. 40–60°) at the laboratory temperature, being shaken for part of the time of each extraction. The petroleum was removed by evaporation and the residue dried to constant weight, dissolved in ether and a 5 % alcoholic solution of sodium ethoxide added. After standing overnight part of the alcohol was removed by evaporation, water added and the aqueous solution extracted with ether; an orange brown substance was obtained from this ethereal extract. Working in this manner, 2 kg. of spinach gave 230 g. of dried leaf tissue and, after this had been eleven times extracted, each time with 2 litres of light petroleum, 18·4 g. of lipoid substance were separated containing 4·6 g. of unsaponifiable matter. The latter is an orange brown solid giving with concentrated sulphuric acid the blue colour characteristic of carotene. The sterol content was determined by precipitation with digitonin, and was found to be less than 5 %. The sterol was removed from the rest of the unsaponifiable matter and the residue taken up with hot alcohol. On cooling yellowish white plates separated from the orange-coloured solution: after two or three crystallisations they were obtained as glistening white plates, M.P. 68–68·5°. The substance was a hydrocarbon containing 85·1 % C and 14·6 % H (micro-analysis). From these data the substance was identified as the hentriacontane, $C_{31}H_{64}$, originally prepared by Krafft [1882].

If green cabbage leaves are worked up similarly, the crystals separating from the hot alcoholic solution of the unsaponifiable matter melt at 72 to 75° and analysis shows that they contain about 5 % of oxygen. From these

a small amount of crystals melting at 68° was isolated, the melting point of which was unchanged when the crystals were mixed with the hentriacontane obtained from spinach. In cabbage leaves therefore the hentriacontane is accompanied by an oxygen-containing substance which is not present in spinach and which is now the subject of further investigation. The occurrence of these saturated normal hydrocarbons in green leaves is of some interest. A survey of the literature shows that three of them, containing 27, 31 and 35 carbon atoms, have been definitely identified in green leaves. These were originally prepared by Krafft [1882] by heating the barium salts of the fatty acids and reducing the resulting ketones with hydrogen iodide and phosphorus.

$$C_nH_{2n+1}.COO.Ba.OOC.C_nH_{2n+1} \rightarrow \begin{matrix} C_nH_{2n+1}.CO.C_nH_{2n+1} + BaCO_3 \\ \rightarrow C_nH_{2n+1}.CH_2.C_nH_{2n+1}. \end{matrix}$$

From myristic, palmitic and oleic (or stearic) acids, the acids most commonly occurring in plant fats, the hydrocarbons which would be produced by the above reactions are those containing respectively 27, 31 and 35 carbon atoms.

The following table shows their occurrence in plants.

$C_{27}H_{56}$ Heptacosane M.P. 59·5° C=85·2 % H=14·8 %	$C_{31}H_{64}$ Hentriacontane M.P. 68·5° C=85·2 % H=14·8 %	$C_{35}H_{72}$ Pentatriacontane M.P. 75° C=85·3 % H=14·7 %
	Occurrence	
	Beeswax*	
Leaves of tobacco†‡	Leaves of tobacco†‡	
	Seeds of Ko-Sam§	
	Leaves of *Gymnema sylvestre*‖	
	,, *Grindelia robusta*¶	
	,, *Morinda longiflora***	
	,, *Erodyction*††	Leaves of *Erodyction*††
	,, olive‡‡	Leaves of olive‡‡
	Bark of olive§§	
	Leaves of spinach	
	,, cabbage	

* Schwalb [1886]. † Thorpe and Holmes [1901]. ‡ Mabery [1905]. § Power and Lees [1903]. ‖ Power and Tutin [1904]. ¶ Power and Tutin [1905]. ** Barrowcliff and Tutin [1907]. †† Power and Tutin [1906]. ‡‡ Power and Tutin [1908, 1]. §§ Power and Tutin [1908, 2].

Two of the richest sources of vitamin A are found in the unsaponifiable matter obtained respectively from green leaves and from fish-liver oils and it is interesting that there is a certain parallelism between the constituents occurring in them.

Both contain (1) a highly unsaturated hydrocarbon, (2) products which may be regarded as obtained from the higher fatty acids by processes of condensation and reduction, and (3) sterols. The first of these is represented in the leaf material by the hydrocarbon carotene, containing 11 ethylenic linkages [Kuhn and Winterstein, 1928]: in the liver oil by squalene with six unsaturated linkings (or by similar unsaturated hydrocarbons). Both these hydrocarbons are built up from a number of isoprene units, and show considerable resemblance [Chapman, 1923; Heilbron, Kamm and Owens, 1926]. The second constituent is represented in the leaf by the high normal saturated

hydrocarbons enumerated above, in the liver oil by the batyl, selachyl and chimyl alcohols which are now known to be condensation products of glycerol with the higher fatty alcohols corresponding to stearic, oleic and palmitic acids [Heilbron and Owens, 1928]. Although the available evidence is against the identification of any of these substances as the active agent [Drummond, Channon and Coward, 1925], the existence of a certain similarity of composition in these two materials obtained from such widely different sources, both rich in vitamin A, is not without interest.

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