proline increases continuously during development. Nevertheless, even on the 5th day of development, 58 per cent of the hydroxyproline is in insoluble form. It is of interest that in young embryos not only soluble collagen but also free hydroxyproline constitute a noteworthy part of the total hydroxyproline. Similar values for free hydroxyproline have also been reported recently during the early development of carrageenin granuloma[4,8] and fibroblast cultures[10]. It has been generally accepted that administered hydroxyproline is not utilized for collagen synthesis and that the collagen hydroxyproline is derived from proline that is hydroxylated in bound form[17]. Therefore the part played by free and peptide-bound hydroxyproline in the metabolism of collagen is of interest. Recent work suggests that the ultra-filtrable hydroxyproline present in tissues during the early development of collagen represents a precursor of collagen[4,8,10]. It is evident, too, that part of the urinary hydroxyproline is derived from collagenic precursors[3,5,7]. The present work, likewise, suggests that in young chick embryos free hydroxyproline may be derived from precursors of insoluble collagen.

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Oxidation of β-Carotene : Formation of Vitamin A Acid

Although Moore[1] had demonstrated that β-carotene was transformed into vitamin A in the animal body, the actual process of this transformation has not as yet been fully understood. There are two views: one postulating oxidation of β-carotene at the central double bond, with the formation of, at first, two molecules of retinene[2,3] (vitamin A aldehyde), which are immediately reduced to give two molecules of vitamin A alcohol; the other claiming that if an excentric bond be attacked it would give only one molecule of vitamin A, resulting from the further degradation of the larger fragment. Moore[4] has given an outline of the evidence for and against these views.

The oxidation products of β-carotene therefore are of interest as they can help in giving an insight into the problem of provitamin–vitamin A transformation. A number of investigations in the oxidation of β-carotene, has been reported. When β-carotene was oxidized with chromium trioxide, semi-β-carotenone and β-carotenone were produced[5]. When, however, alkaline permanganate was used β-apocarotenals were formed[6]. Hunter and Williams[7] oxidized β-carotene with hydrogen peroxide in chloroform–acetic acid medium and found retinene as one of the oxidation products which gave vitamin A alcohol on

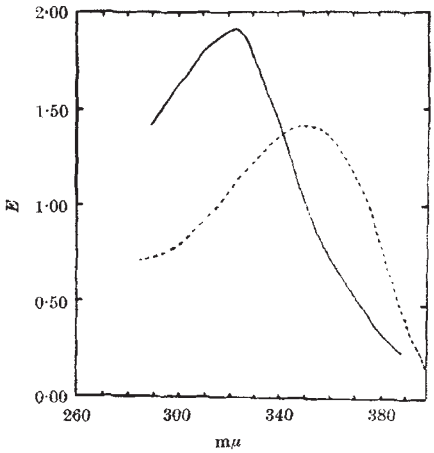

Fig. 1. Broken line, absorption spectrum of vitamin A acid obtained by oxidation of β-carotene and subsequent chromatography; solid line, absorption spectrum of vitamin A alcohol obtained after reduction of vitamin A acid followed by chromatography

reduction, the yield being only 0·4–0·5 per cent. With osmium tetroxide as catalyst a moderate yield of 30 per cent was reported[8,9]. Meunier[10] reported formation of vitamin A aldehyde, retinene hydrate, and vitamin A alcohol from carotenoids, especially from β-carotene by oxidation with various metallic oxides, namely, TiO_2, V_2O_5, Fe_2O_3. His reported yield of about 95 per cent was, however, disputed by Sebrell and Harris[11], who were unable to reproduce Meunier's results.

In none of the foregoing oxidation investigations, however, was the formation of vitamin A acid reported from β-carotene. In an investigation carried on in this laboratory, it has been possible to obtain vitamin A acid as one of the products of oxidation of β-carotene. When β-carotene in petroleum ether or ether solution was kept over a mixture of V_2O_5/MnO_2 (5 : 2) for about 80–90 h the solution changes to a greenish yellow colour. This was then chromatographed on a column of deactivated alumina[12] (8 per cent water), when three zones separated at the top. The first, second and the third zones gave on extrusion and elution with ethanol, spectra with $\lambda\lambda_{max}$ at 335 mμ, 285–287 mμ, and 350–351 mμ, respectively (solvent–ethanol). The third zone has spectral characteristics corresponding to vitamin A acid (λ_{max} at 347 mμ in petroleum ether, 350–351 mμ in ethanol). With $SbCl_3$, it gave a purple colour having λ_{max} at about 570 mμ. The acid was then selectively reduced and chromatographed on deactivated alumina, when it gave a product spectroscopically identical with vitamin A alcohol (λ_{max} at 325 mμ in ethanol, and with $SbCl_3$ gave a blue colour with λ_{max} at 618 mμ). Fig. 1 shows the absorption spectra in ethanol of vitamin A acid (shown with broken line) and that of vitamin A alcohol obtained after reduction and chromatography (shown with unbroken line).

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