## Separation of vitamins $A_1$, $A_2$ and allied substances by reverse phase paper chromatography

The characterization of mixtures of vitamin $A_1$ and its derivatives by reverse phase paper chromatography has the drawback of complicated procedures and imperfect resolutions[1–6]. Furthermore, separation of vitamin $A_2$ and allied compounds has not been hitherto effected.

Irrigation (4–5 h), of circular paper chromatograms[7] (Whatman No. 1) im-

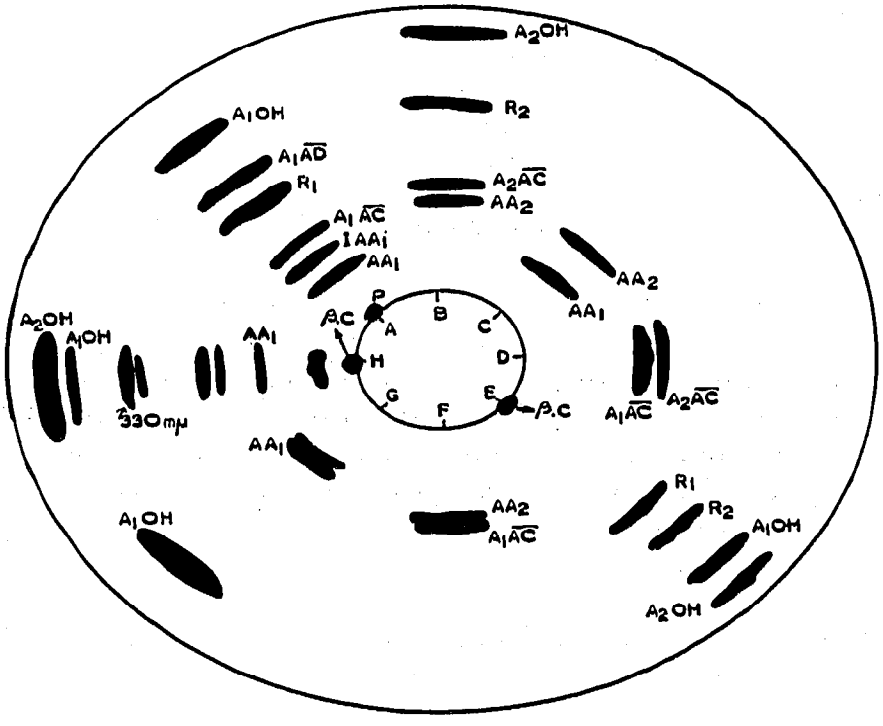


Fig. 1. Schematic representation of the separation of vitamins $A_1$, $A_2$ and their derivatives on 3 % vaseline coated paper using 90 % methanol. Unsaponifiable fraction of liver oil from: (G) *Zygena* species, (H) *Wallago attu*. P = Vitamin $A_1$ palmitate; $AA_1$ = Anhydrovitamin $A_1$; $IAA_1$ = Isoanhydrovitamin $A_1$; $A_1\overline{AC}$ = Vitamin $A_1$ acetate; $R_1$ = Vitamin $A_1$ aldehyde; $A_1\overline{AD}$ = Vitamin $A_1$ acid; $A_1OH$ = Vitamin $A_1$ alcohol; $AA_2$ = Anhydrovitamin $A_2$; $A_2\overline{AC}$ = Vitamin $A_2$ acetate; $R_2$ = Vitamin $A_2$ aldehyde; $A_2OH$ = Vitamin $A_2$ alcohol; $\beta$-C = $\beta$-Carotene; 330 m$\mu$ = Component X.

pregnated with 3 % vaseline (snow-white petroleum, Stanvac, India), by 90 % methanol effects their clear-cut separation (see Table I and Fig. 1). The absorption spectra of the substances eluted with ether–petrol ether (1:1) from bands located under ultraviolet light have been recorded.

Following this procedure, the constituents of marine and freshwater fish liver oils have been characterized. The resolution of the constituents of two representative fish liver oils (*Zygena* species and *Wallago attu*) after saponification is illustrated in Fig. 1. While vitamin $A_1$ alcohol together with anhydrovitamin $A_1$ and an uncharacterized component ($R_F$ 0.15) seem to be characteristic of the marine sample, the *Wallago attu* oil, in agreement with the results of column chromatography[8],

TABLE I

REVERSE PHASE CIRCULAR PAPER CHROMATOGRAPHIC RESOLUTION OF VITAMINS $A_1$, $A_2$ AND THEIR DERIVATIVES

| *Substance* | *Band as observed in U.V. light* | *$R_F$ values** | *$\lambda_{max}$ in petrol ether (mμ)* |
|---|---|---|---|
| Vitamin $A_1$ palmitate | Blue fluorescence | 0.00 | 325 |
| Anhydrovitamin $A_1$ | Bright yellow fluorescence | 0.20 ± 0.01 | 350, 370, 390 |
| Isoanhydrovitamin $A_1$ | Blue fluorescence | 0.29 ± 0.03 | 330, 350, 370 |
| Vitamin $A_1$ acetate | Blue fluorescence | 0.35 ± 0.03 | 325 |
| Vitamin $A_1$ aldehyde | Dull red absorbance | 0.55 ± 0.01 | 370 |
| Vitamin $A_1$ acid | Dull red absorbance | 0.65 ± 0.02 | 350 |
| Vitamin $A_1$ alcohol | Blue fluorescence | 0.82 ± 0.03 | 325 |
| Anhydrovitamin $A_2$ | Bright yellow fluorescence | 0.33 ± 0.01 | 350, 370, 390 |
| Vitamin $A_2$ acetate | Green fluorescence | 0.39 ± 0.02 | 350 |
| Vitamin $A_2$ aldehyde | Dull red absorbance | 0.67 ± 0.03 | 385 |
| Vitamin $A_2$ alcohol | Green fluorescence | 0.93 ± 0.01 | 350 |
| β-Carotene | Orange coloured band | 0.00 | — |

* Mean values of ten observations.

gives nine prominent bands corresponding to β-carotene, vitamin $A_1$ alcohol, anhydrovitamin $A_1$, vitamin $A_2$ alcohol, together with traces of a compound X with $\lambda_{max}$ 330 mμ[8] and four other uncharacterized components. The use of this procedure for the identification of vitamin $A_1$ derivatives occurring in rat tissues during metabolism of vitamin $A_1$ epoxide, will be described elsewhere.

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