range of acids, in the production of cow-milk fat than in that of human-milk fat.

So far as may be concluded from the small specimen of late lactation fat of lower unsaturation than the rest, the amount of octadecadienoic acid, as well as that of oleic acid, has been reduced by about one-fifth; when increase of saturation is observed in cow-milk fats, diminution in unsaturated acids has been as a rule confined exclusively to oleic acid. Moreover, in cow-milk fats diminution in oleic acid is paralleled by increases in the contents of the lower saturated acids, whereas in the present instance the myristic acid content alone seems to be materially increased. No definite conclusion should be drawn here, however, until larger specimens covering a wide range of mean unsaturation have been studied.

Two points of some interest appear to be established by the analyses recorded in the course of this work. First, since cow's milk may be substituted for human milk in infant nutrition, it follows that the variation in the component acids of the two milk fats has little if any effect on the nutritive value of the milk. Secondly, any inference that butter from the cow is superior for human consumption to other forms of edible fat by reason of its characteristic milk fat composition is discounted, since the data presented demonstrate that human-milk fat, qualitatively and to some extent quantitatively, resembles modern types of margarine more closely than it does butter. The presence in human-milk fat of vegetable-seed fat linoleic acid, of

definite proportions of lauric and myristic acids (characteristic of coconut and other 'nut oils') and of somewhat more unsaturated C_{20-22} acids than usual, coupled with the complete absence of butyric and other saturated acids of low molecular weight, cause it to be much more analogous to a margarine fat than to butter fat.

SUMMARY

1. The chief component acids of human-milk fat are oleic (30–37 %) and palmitic (22–24 %). Of unsaturated acids, diethenoid C_{18} acids (c. 7 %) include a considerable proportion of ordinary linoleic acid, unsaturated C_{20-22} acids amount to 3–4 %, and there are minor quantities of hexa- and tetradecenoic acids. The other saturated acids include stearic and myristic (c. 8–9 % each), lauric (5–7 %) and decanoic (2–3 %); no acids of lower molecular weight than decanoic are present.

2. The acids of human-milk fat differ from those of cow-milk fat in that they do not contain butyric or other acids below decanoic acid, and in their higher content of diethenoid C_{18} acids (including linoleic acid) and unsaturated C_{20-22} acids. Human-milk fat, in regard to its component acids, has more resemblance to a typical margarine fat-blend than to butter fat.

We offer cordial thanks to Dr S. K. Kon for placing the experimental material for this paper at our disposal, and to Mr H. Weatherall, F.I.C., of Messrs J. Bibby and Sons, Ltd., for assistance in the determination of some of the analytical characteristics of the milk fats.

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The 'Cyclization' of Vitamin A and Allied Compounds

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(Received 11 November 1943)

During the last two years a physico-chemical method for the estimation of vitamin A based on 'cyclization' and chromatographic separation of the 'cyclized' product has been devised in this laboratory. We hope to furnish a complete account of this method when further data have been accumulated with regard to comparison with biological assays. A recent paper by Shantz, Cawley & Embree

(1943) anticipates to some extent our own conclusions with regard to the nature of 'cyclized' vitamin A and it appears desirable to place on record the results so far obtained. The best concentrate of 'cyclized' vitamin A which we obtained by chromatography on alumina had $E_{1\text{cm}}^{1\%} = 2630$ at $370\text{m}\mu$ in cyclo-hexane, which agrees with the value given for material obtained in this way by

the American investigators; since the publication of their paper we have obtained from a similar concentrate a specimen of the crystalline compound, m.p. 77–78°, $E_{1\text{cm}}^{1\%} = 3760$ at 372m μ .

Our failure to 'cyclize' compounds such as β -apo-2-carotenal (Ia), axerophthylideneacetone (Ib), and the C₂₀ aldehyde of Haworth, Heilbron, Jones, Morrison & Polya (1939) by treatment with alcoholic HCl suggested to us that a terminal hydroxyl group is essential to the reaction. Furthermore, the alcohol obtained by Ponderff reduction of axerophthylideneacetone (Ib with CHOH in place of CO) furnished a substance with the characteristic fine-structure ultra-violet absorption of 'cyclized' vitamin A but with bands displaced some 20m μ towards the region of longer wave-length. Finally, the absence of the hydroxyl group, required by the formula (II) originally suggested by Heilbron, Morton & Webster (1932) for 'cyclized' vitamin A was confirmed by Zerewitinoff determination on a specimen of the chromatographed material.

A terminal hydroxyl group is not the only necessary condition for the reaction, since β -apo-2-carotenol (Ia with CH₂OH in place of CHO) did not undergo 'cyclization' under the usual conditions. This may be explained on the allene formula of Shantz *et al.* (1943) for 'cyclized' vitamin A (III). On the other hand, the alcohol obtained by reduction of the C₂₀ aldehyde also failed to undergo 'cyclization', and, if it has the constitution (IV), this does not appear to be explicable on the basis of any of their formulae.

(Ia) $R = \text{CH}:\text{CH}:\text{CH}:\text{CMe}:\text{CH}:\text{CH}:\text{CH}:\text{CMeCHO}$

(Ib) $R = \text{CH}:\text{CH}:\text{COMe}$

(Ic) $R = \text{CH}:\text{CH}:\text{CH}_2\text{OH}$

IV

These results are of interest in connexion with the structure of vitamin A₂. None of the suggested formulae appears to take account of the fact that vitamin A₂ undergoes 'cyclization' to give a substance having the wave-lengths of the ultra-violet absorption maxima identical with those of ordinary 'cyclized' vitamin A (Embree & Shantz, 1940), but distinguishable from the latter by the absorption

band at 693m μ in the antimony trichloride reaction. The original formula of Gillam, Heilbron, Jones & Lederer (1938) for vitamin A₂ (Ic) differs from axerophthylideneisopropyl alcohol only by a methyl group and would lead one to expect 'cyclization' products of practically identical ultra-violet absorption spectra. As already mentioned, the latter gives a substance with absorption maxima displaced some 20m μ from those of 'cyclized' vitamin A. The formula (IV) also seems to be ruled out by the failure of the alcohol obtained by reduction of the C₂₀ aldehyde to undergo 'cyclization'. The formula for vitamin A₂ suggested by Karrer, Geiger & Bretscher (1941) (V) bears the same relationship to vitamin A as lycopene to β -carotene and would, if the 'cyclization' reaction is of the same type in both cases, lead one to expect a difference in wave-length for the absorption maxima of the 'cyclized' products.

With regard to the 'cyclized' subvitamin A of Embree & Shantz (1943), it may be mentioned that we have observed the formation of this substance both in the product of 'cyclization' of the unsaponifiable matter of acetylated shark-liver oil and of a similar liver oil which had been deliberately oxidized in stages by aeration. In the latter experiment, subvitamin A was still present in substantial amount when some 80% or more of the original vitamin A alcohol had been destroyed, suggesting that it is probably a primary oxidation product of vitamin A. If the 'cyclization' reaction involves the production of an allene structure as

II

III

V

suggested by Shantz *et al.* (1943), it is probable that subvitamin A is formed by attack of the double bond of the β -ionone ring in vitamin A by oxygen. It is therefore interesting to note that the shift of ultra-violet absorption maxima towards the region of shorter wave-length in the 'cyclized' derivatives is similar to that associated with the conversion of β -carotene into β -carotene oxide, in which oxygen

is presumed to attack the double bond of one of the terminal β -ionone rings (Euler, Karrer & Walker, 1932).

Substance	Position of highest absorption peak ($m\mu$) (chloroform solution)
β -Carotene	463
β -Carotene oxide	437
Difference	26
	(cyclohexane solution)
'Cyclized' vitamin A	372
'Cyclized' subvitamin A	350
Difference	22

EXPERIMENTAL

β -Apo-2-carotenol was prepared by oxidation of β -carotene with alkaline $KMnO_4$ (Karrer & Solmassen, 1937) and the apo-2-carotenol was obtained from this by reduction with aluminium isopropoxide (Euler, Karrer & Solmassen, 1938). Attempts to 'cyclize' these substances by treatment with $N/25$ alcoholic HCl at 50° for 30 min. failed, the products showing the original absorption bands without any evidence of the development of fine structure.

Axerophthylideneacetone. Since fish-liver oils contain appreciable amounts of vitamin A_2 , a distilled concentrate of vitamin-A alcohol, with $E_{1\text{cm}}^{1\%} = 1240$ at $325m\mu$ in cyclohexane, obtained from whale-liver oil unsaponifiable matter, was used for the preparation of axerophthylideneacetone.

This was prepared by heating the vitamin-A alcohol concentrate, purified acetone, and aluminium isopropoxide in benzene for 22 hr. in a stream of nitrogen, and purified by chromatography on alumina under slight nitrogen pressure (cf. Hunter & Scott, 1941), and showed an absorption band at $395m\mu$ in the ultra-violet region ($E_{1\text{cm}}^{1\%} = 1460$) and a maximum at $735m\mu$ in the antimony trichloride reaction. Batty, Burawoy, Harper, Heilbron & Jones (1938) recorded the maxima as 401 and $646m\mu$ respectively. The positions of the maxima were unaltered by treatment of the substance with alcoholic HCl at 50° for 30 min.

Axerophthylideneisopropyl alcohol, obtained by reduction of axerophthylideneacetone with aluminium isopropoxide, showed an absorption band at $351m\mu$ and a maximum at $713m\mu$ in the antimony trichloride reaction. Heilbron, Johnson & Jones (1939) recorded 354.5 and $712m\mu$ respectively.

A solution of the substance in $N/25$ alcoholic HCl was heated at 50° for 30 min. The product showed the typical ultra-violet absorption spectrum for 'cyclized' material with bands at 415, 393 and $370m\mu$ and an inflexion at $330m\mu$. The position of the maximum in the antimony trichloride reaction remained unaltered. On chromatography on alumina, it gave a weakly adsorbed yellow zone, similar in appearance to 'cyclized' vitamin A, which furnished material showing maxima at 415, 393, 374 and $290m\mu$. A small crystalline specimen obtained by cooling a solution in light petroleum to about -70° had $E_{1\text{cm}}^{1\%}$ values of 2970, 3280 and 1900 at 420, 395 and $372m\mu$ respectively.

The C_{30} aldehyde. A mixture of the vitamin-A alcohol concentrate from whale-liver oil (see above) (7.05 g.), aluminium isopropoxide (7.1 g.), diethyl ketone (25 g.),

and benzene (250 ml.) was heated in a stream of nitrogen for 45 hr. and the product chromatographed on alumina. The chromatogram formed a series of six zones (1, yellow; 2, red-brown; 3, deep red-brown; 4, dark red-brown; 5, almost black; and 6, light brown: in order of increasing adsorption). Zones 3, 4 and 5 furnished 0.22, 1.086 and 1.63 g. of products which showed bands at 385, 390 and $390m\mu$ ($E_{1\text{cm}}^{1\%}$ values of 900, 1380 and 740) in the ultra-violet and at 735, 725 and $725m\mu$ in the antimony trichloride reaction respectively. The material from zone 5 on being again chromatographed gave 0.686 g. of a syrup which showed a band at $395m\mu$ ($E_{1\text{cm}}^{1\%} = 1310$) and a maximum at $730m\mu$ in the antimony trichloride reaction. Haworth *et al.* (1939) record the maxima as 401 and $740m\mu$ respectively.

The oxime, prepared by heating a solution of the aldehyde (1 g. of combined fractions 3, 4 and twice chromatographed 5, having $E_{1\text{cm}}^{1\%} = 1075$) in glacial acetic acid with hydroxylamine hydrochloride (2 g.) and sodium acetate (2 g.) in nitrogen at 50° for 4 hr., separated from light petroleum in orange-yellow crystals, m.p. $175-177^\circ$. (Found: C, 80.1, 80.2; H, 9.55, 9.75; N, 4.5, 4.8. Calc. for $C_{30}H_{47}ON$: C, 80.8; H, 9.2; N, 4.7%.) It showed an absorption band at $380m\mu$ ($E_{1\text{cm}}^{1\%} = 1490$) and gave a claret colour in the antimony trichloride reaction.

The aldehyde showed no evidence of 'cyclization' on treatment with alcoholic HCl under the usual conditions.

The alcohol, obtained by reduction of the aldehyde with aluminium isopropoxide, showed a fairly broad absorption band at $350-370m\mu$ ($E_{1\text{cm}}^{1\%} = 830$), and gave a blue-green colour with a maximum at $722m\mu$ in the antimony trichloride reaction. After chromatography on magnesium sulphate it showed an absorption band at $360-370m\mu$ and a maximum at $722m\mu$ in the antimony trichloride reaction. These spectroscopic characteristics were unaltered by treatment with alcoholic HCl under the usual conditions.

'Cyclization' of vitamin A_2 . A concentrate of vitamin- A_2 alcohol obtained by chromatography of the unsaponifiable matter from minced salmon livers on 'weakened' alumina was 'cyclized' and the product chromatographed on alumina. The lower yellow zone furnished material which showed the usual maxima of 'cyclized' vitamin A at 390, 370 and $350m\mu$ but a band at $697m\mu$ as well as one at $620m\mu$ in the antimony trichloride reaction. This product was again chromatographed and the chromatogram developed with light petroleum when two yellow zones, separated by a white space, were obtained. The lower of these showed a single band at $620m\mu$ in the antimony trichloride reaction. The upper zone furnished material exhibiting a prominent band at $697m\mu$ with a very faint additional band at $620m\mu$ and evidently consisted of cyclized vitamin A_2 contaminated with a small amount of cyclized vitamin A. It showed ultra-violet absorption maxima at 390, 370 and $350m\mu$. A long cell was used for this determination, but no evidence was obtained of the presence of a band of higher wave-length.

Subvitamin A. The unsaponifiable matter from shark-liver oil (100 g.) was treated with a stream of air at 50° for 2 hr. and a portion was removed, dissolved in ethanol, 'cyclized', and chromatographed on alumina. The chromatogram formed four zones (1, yellow; 2, yellow; 3, yellow; 4, pink: in order of increasing adsorption) which furnished

products showing the following absorption bands (wavelengths in $m\mu$):

	Ultra-violet	SbCl ₃
1	390, 370, 350	620
2	368, 350, 330	620
3	368, 350, 330	614
4	304	614 and faint band at 575

The spectroscopic characteristics of the material from zone 3 are those of 'cyclized' subvitamin A, and zone 2 appears to be the 'cyclized' isovitamin A of Embree & Shantz (1943).

Oxidation was continued with oxygen at 70° for 110 hr. and samples were removed at intervals, 'cyclized', and chromatographed. In each case, except towards the end of the oxidation, a zone was obtained which showed ultra-violet absorption maxima at 370, 350 and 330 $m\mu$ (the band at 350 $m\mu$ being the highest maximum) and a band at 614 $m\mu$ in the antimony trichloride reaction.

SUMMARY

1. β -Apo-2-carotenol, β -apo-2-carotenol, axerophthylideneacetone, the C₂₀ aldehyde obtained by oxidation of vitamin-A alcohol, and the alcohol obtained by Ponderff reduction of the latter, all fail to undergo 'cyclization' on treatment with alcoholic HCl, whereas axerophthylideneisopropyl alcohol gives a compound which shows the characteristic fine structure of a 'cyclized' product in the ultra-violet region.

2. The bearing of these and other observations on the structure of 'cyclized' vitamin A, vitamin A₂, and subvitamin A is discussed.

We wish to thank the directors of Lever Brothers and Unilever Ltd. for permission to publish these results.

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The Influence of Sodium Nitrate Concentration on the Metabolism of *Penicillium sclerotiorum* van Beyma

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(Received 29 November 1943)

Penicillium sclerotiorum produces two metabolic products: sclerotiorine, a chlorinated pigment (Curtin & Reilly, 1940), and sclerotiose, a poly-glucose (Albericci, Curtin & Reilly, 1943). The present paper records the effect of different concentrations of nitrate in the culture medium on the utilization of chloride and on the production of sclerotiorine and sclerotiose. The results support the earlier conclusion that sclerotiorine production is not directly connected with chloride utilization, and further show that sclerotiose production is affected by the nitrate concentration of the medium.

METHODS

Acid Czapek-Dox medium, in which the NaNO₃ content ranged from 0 to 0.4% by increments of 0.05%, was used: glucose, 50 g.; KCl, 0.5 g.; MgSO₄, 7H₂O, 0.5 g.; KH₂PO₄, 1.0 g.; FeSO₄, 7H₂O, 0.01 g.; NaNO₃, 0.4-0 g.; distilled water to 1000 ml. Nine 8 l. batches were distributed in 100 ml. amounts in 20 oz. medical flat bottles and sterilized by steaming for 1 hr. on each of three consecutive days. The batches were then inoculated with a spore suspension of *P. sclerotiorum* van Beyma (2 ml./bottle) taken from a 14-day-old culture, remotely transferred from that obtained in March 1940, from the Centraalbureau voor Schimmelcultures, Baarn, Holland.