CCXXVII. ADSORPTION EXPERIMENTS WITH VITAMIN A CONCENTRATES.

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THE unsaponifiable matter of halibut-liver oil, after removal of the major part of the sterols, is a viscid orange-brown oil containing 60–70 % vitamin A, which on fractional distillation yields a pale yellow oil [Heilbron *et al.*, 1932]. If the vitamin concentrate, whether purified by distillation or not, be dissolved in light petroleum and passed through a column packed with adsorbent (Al_2O_3 or $\text{Ca}(\text{OH})_2$) a red zone appears. Recovery of the adsorbed material by elution of the adsorbent with methyl alcohol shows that the colour is much more intense than that of the original concentrate. In view of the important biological relation between the red pigment carotene and vitamin A, this phenomenon has been studied in detail. At the same time, it was convenient to examine the reported separation of vitamin A into α - and β -forms [Karrer, Walker *et al.*, 1933; Karrer and Morf, 1933; Euler *et al.*, 1934]. As the main distinctions between these two forms and the other fractions obtained depend upon the spectroscopic examination of the colours they produce with antimony trichloride, a brief résumé of the earlier spectroscopic data on this reaction is necessary.

The blue colour characteristic of vitamin A [Carr and Price, 1926] has been shown to exhibit two absorption maxima at 620 and 583 $m\mu$ in concentrates, and 606 and 572 $m\mu$ in oils [Drummond and Morton, 1929; Morton *et al.*, 1931]. Measurements of the intensities of the two absorption bands in the antimony trichloride blue solution for numerous liver oils and concentrates [Gillam and Morton, 1931] showed that the two bands varied so much in relative intensity as to make it seem probable that they were derived from two separate, but related, chromogenic substances. This was the more probable when it was found [Heilbron *et al.*, 1931] that in oils which showed the 572 $m\mu$ band more strongly than the 606 $m\mu$ band, the intensity at 606 $m\mu$ could be increased by oxidation with a little ozone or hydrogen peroxide. Further, the 606 $m\mu$ band could be completely suppressed by adding a few drops of antimony trichloride before the main bulk of the reagent.

Certain indole-like substances likely to be present in liver extracts were found to inhibit the formation of the blue colour [Emmerie *et al.*, 1931]. Thus, a liver oil giving a blue colour with antimony trichloride showing a strong 606 $m\mu$ band and a weak satellite at 572 $m\mu$, when treated with a trace of 7-methyl-indole before addition of the antimony trichloride, gave a violet colour showing only the 572 $m\mu$ band, the 606 $m\mu$ band having been completely suppressed. These workers [Eekelen *et al.*, 1931; 1932; 1933] regarded this evidence as supporting the theory of two separate chromogens responsible for the two

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absorption bands. The inhibitor substances act in the same way with rich concentrates, suppressing the 617 $m\mu$ band very easily whilst scarcely affecting the 583 $m\mu$ maximum [Morton, 1932].

When richer concentrates of vitamin A ($E_{1\text{cm.}}^{1\%}$, 328 $m\mu$ = 1350) were obtained [Karrer *et al.*, 1931, 1, 2; Heilbron *et al.*, 1932] it was still found that with antimony trichloride the blue colour showed a 617–620 $m\mu$ band and a masked maximum at 583 $m\mu$, the ratio of their intensities being approximately 2 : 1. Later, by a refinement of the distillation method, Carr and Jewell [1933] prepared a still purer product ($E_{1\text{cm.}}^{1\%}$, 328 $m\mu$ = 1600) which with antimony trichloride gave a blue colour showing a clear band at 620 $m\mu$ with only a masked maximum at 583 $m\mu$ (ratio of intensities 5.0 : 2.6). The constant association of these two maxima, even in the purest vitamin preparations, made it seem highly probable that what were originally thought to be two separate chromogens were actually two chromophoric groupings in the same molecule. Karrer and his collaborators [1933; 1934; Euler *et al.*, 1934] have however claimed that, by adsorption on calcium hydroxide, vitamin A concentrates yield two substances, which they suggest correspond to the two chromogens already mentioned. The theory is attractive as it brings vitamin A into line with carotene, where the existence of α - and β -forms is fully established.

EXPERIMENTAL.

After numerous preliminary adsorption experiments we have found that the following experimental conditions yield the best separation of products for a quantitative study.

A distilled concentrate ($E_{1\text{cm.}}^{1\%}$, 328 $m\mu$ = 1300, 500 mg.) was dissolved in light petroleum (75 ml., B.P. 60–80°) and run into an adsorption tube filled with calcium hydroxide¹ (column of adsorbent 51 × 4 cm.). The chromatogram was developed by washing with several litres of petroleum (B.P. 60–80°). A faint yellow-brown zone formed almost at once, and at the end of the experiment it had moved nearly half-way down the tube and consisted of two main bands of dark chocolate-brown colour, and, in addition, a complicated series of narrow bands. The column was dried in a stream of nitrogen and pushed out of the tube, transverse sections being cut off and eluted with light petroleum containing methyl alcohol. The residue from each fraction was dried *in vacuo* and weighed before being examined spectroscopically. Table I summarises the results obtained.

This typical adsorption shows that a separation into five main fractions has been accomplished:

- (a) Faintly coloured thick oil, adsorbed strongly at the top of the tower and relatively weak in vitamin A.
- (b) Less strongly adsorbed material very rich in the vitamin.
- (c) Deeply coloured oil from the region including the bands. This, whilst still containing vitamin A, also contains a red substance.
- (d) The material immediately below the colour, with which it overlaps.
- (e) The least strongly adsorbed material, small in amount and definitely yellow in colour. This is markedly different from all the other fractions.

¹ The preparation of a satisfactory column of calcium hydroxide is much more difficult than that of one of alumina, and since the success of this experiment depends entirely on the use of an evenly packed column the following directions are given. The calcium hydroxide is vigorously shaken with several volumes of petroleum and the mixture allowed to stand for half an hour with occasional gentle agitation to remove air bubbles. The tube is filled with this mixture in several small portions, each portion being carefully stirred and allowed to settle before adding the next. The preparation of a satisfactory column takes several hours.

Table I.

Adsorption of vitamin A on $\text{Ca}(\text{OH})_2$ (T_{12}). Starting material, 500 mg. of concentrate $E_{1\text{ cm}}^{1\%}$ 617 $m\mu$ = 3680, 583 $m\mu$ = 1800, 328 $m\mu$ = 1300.

Frac- tion	Length of zone (cm.)	Colour of zone	Colour of extract	Wt. of extract (mg.)	Reaction with SbCl_3				Ratio of in- tensities 617:583 $m\mu$	Ultra-violet absorption
					Absorption bands ($m\mu$)	$E_{1\text{ cm}}^{1\%}$	+ 7-methyl- indole (max. in $m\mu$)	617/583 $m\mu$		
1	3	White	Pale yellow	64	622 (weak 697)	617 $m\mu$ 583 $m\mu$ 660 $m\mu$	580	1.7:1	Broad summation 280 + 328 $m\mu$ bands	$E_{1\text{ cm}}^{1\%}$ 328 $m\mu$ 280 $m\mu$ 250 —
2	6	White	Yellow	89	620 (weak 700)	3100 1420 —	580	2.2:1	328 $m\mu$ band	1080 (approx.) —
3	7	Dirty yellow	Red	142	620	3420 1740 —	583	2:1	328 $m\mu$ band	1280 —
4	5	Two chocolate- brown bands	Red	62	620 and satel- lite 583	1450 850 —	580 and 655	1.6:1	Single 328 band. Inflexion 430 $m\mu$	550 —
5	10	Red-pink	Red	18	Two clearly 645, 583	360 370 340	580 and 650	1:1	328 band. Inflexion 430 $m\mu$ Absorption 280 $m\mu$	375 280
6	12	Dirty yellow	Red	7.3	One band only, 582 (642 on fading)	270 410 —	582 and 642 on fading	0.7:1	328 band. Inflexion 420 $m\mu$ Absorption 280 $m\mu$	317 190
7	8	Pale yellow	Bright yellow	0.8	617 and 580-585	(1400) (1080) —	580	1.3:1	328 band. Inflexion 370 $m\mu$ (660)	—
8	Filtrate	Pale yellow	Yellow	24	620 (579)	606 342 —	579 and 625	1.8:1	Triplet of bands at 349, 369 and 390 $m\mu$ + 390 $m\mu$ and 278 $m\mu$ summation	150 —

Table II. The coloured zones.

Exp. No.	Adsorbent	Colour	Maxima ($m\mu$)	Antimony trichloride test				Ratio of 617/583 $m\mu$ intensities	Bands 7-Methylindole + SbCl_3 ($m\mu$)
				$E_{1\text{ cm}}^{1\%}$	650 $m\mu$	617 $m\mu$	583 $m\mu$		
T_{13}	Al_2O_3	Blue	620 and 583	1070	—	890	1.2:1	580	—
T_{14}	$\text{Ca}(\text{OH})_2$	Blue	660, 617 and 578	Three bands equal in intensity	340	360	1:1	660 and 583	—
$T_{15}^{(6)}$	$\text{Ca}(\text{OH})_2$	Slate-blue	645 and 583	240	240	250	1:1	650 and 583	—
C_{13}	Al_2O_3	Blue	645 and 583	84	122	146	0.8:1	645 and 585	216
C_{14}	Al_2O_3	Slate-blue	655, 620 and 585	76	218	200	1.1:1	656 and 585	220
C_{15}	Al_2O_3	Blue	617 and 585	—	—	—	—	660 and 582	—

It is important to note that although 500 mg. of concentrate were originally used in this experiment, only 417 mg. were recovered, and further that, taking the usual criteria for vitamin A, the total amount of this substance recovered was as follows:

By the 328 $m\mu$ band the vitamin recovered	= 52 %	} mean 52 %.
„ 617 $m\mu$ „ „	SbCl ₃ test = 50.4 %	
„ 583 $m\mu$ „ „	„ = 53.8 %	

Thus in this experiment some 18 % of the material was lost mechanically, being adsorbed so strongly that it could not be wholly eluted even by boiling methyl alcohol, whilst a further 30 % was converted into material giving no reactions characteristic of the vitamin.

The coloured zones.

To isolate the coloured fraction either aluminium oxide¹ or calcium hydroxide² can be used. With the former the solute is very strongly adsorbed and the colour is produced as a compact dark brown band. As obtained from this adsorbent, however, the coloured fraction contains much vitamin A even after several re-adsorptions. With calcium hydroxide the adsorption is much weaker, the colour in the tube being much more pale and diffused, but the separation of the vitamin from the colour is decidedly more effective even in one adsorption. The relative adsorbing powers of the two solids will be seen from the following data. A tube of dimensions 40 × 5 cm. when filled with alumina will adsorb the whole of 20 g. of vitamin concentrate, whereas the same tube filled with calcium hydroxide will only hold about 0.5 g. of the same concentrate after washing with light petroleum.

When the coloured band is eluted with methyl alcohol-petroleum and the mixture washed with water to remove alcohol, it is found that the solution so obtained has a much more intense red colour than that originally used. Thus, in one case the original solution was matched by a standard solution of azobenzene. After adsorption the recovered material had to be diluted 12 times to match the standard colour. It is therefore clear that colour is produced during the adsorption process, probably by the catalytic action of the adsorbent on some constituent of the concentrate. This production of colour cannot be reproduced by merely shaking a solution of the material with adsorbent; apparently it is only to be effected by the slow washing of the vitamin concentrate over the adsorbent, such as occurs in a column. During the actual adsorption, colour is produced continuously and washed down into the coloured zone. On passing a solution of the material extracted from that part of the column immediately above the coloured zone (that is the region richest in vitamin A) through another column and developing the chromatogram as usual, a broad red band is again produced, and the process may be repeated several times with the continuous production of colour. It thus seems probable that vitamin A itself is the precursor of the colour, which possibly accounts for the 30 % destruction of the vitamin during adsorption.

The colour is obviously complex in nature, appearing in a well-developed chromatogram as a number of narrow red bands (at least 5) superimposed one on the other (see Plate XI). It is usually obtained as a dark red oil which always contains some vitamin A and from which no solid derivative has yet been obtained. It slowly fades in the dark with the production of a white decomposition product insoluble in petroleum, and is not acted upon by boiling caustic alkali or by carbonyl reagents.

¹ Brockmann's Al₂O₃ for adsorption analysis. E. Merck, Darmstadt.

² Calcium hydricum pulvis alcoholisatus, from A. G. vorm B. Siegfried, Zofingen, Switzerland.

The combustion results agree approximately with $C_{20}H_{30}O$ required for vitamin A, but the mol. wt. is high, suggesting the presence of polymerised material. (Found: C, 82.5; H, 10.7 %. Mol. wt. 444, 434. $C_{20}H_{30}O$ requires C, 83.9; H, 10.5 %. Mol. wt. 286.)

The *absorption spectra* of the colour concentrates always show the presence of the $328\text{ m}\mu$ band associated with vitamin A. In addition there is absorption in the visible region in the form of a broad inflexion on the vitamin A curve near $400\text{--}430\text{ m}\mu$ (Fig. 1). No evidence of fine structure in the absorption band has been obtained such as one observes in the case of carotene and many carotenoids.

Fig. 1. Absorption spectra of (1) the extract from the coloured zone, 0.0036 % in alcohol, 1 cm. cell; (2) the extract from the bright yellow zone least easily adsorbed, 0.004 % in alcohol, 1 cm. cell [$T_{86}^{(a)}$].

With antimony trichloride the coloured fractions give a blue solution showing the absorption spectrum characteristic of vitamin A but, in addition, increased absorption near $583\text{ m}\mu$ and a new band at $642\text{--}660\text{ m}\mu$, the exact location of which is probably dependent on the other absorption present. This band is almost certainly due to the red substance and, like the $583\text{ m}\mu$ band in the vitamin A blue colour, is not suppressed by 7-methylindole. Thus in some fractions (e.g. C_{14} and T_{21} , Table II) the blue antimony trichloride solution shows three bands near 660 , 617 and $580\text{ m}\mu$. When 7-methylindole is added before the antimony trichloride, the $617\text{ m}\mu$ band is no longer visible, only the 660 and $580\text{ m}\mu$ bands remaining.

The conclusions to be drawn about the nature of the colour are that it is due to a substance difficult to characterise, almost certainly derived from vitamin A, and very probably, as obtained by us, a complex mixture of polymerised material containing some unchanged vitamin.

The fractions immediately above the colour.

The zone immediately above the colour was found to be richest in vitamin A. The fractions were syrupy and usually of an orange-yellow colour. In alcoholic solution they exhibit a clear absorption band of high persistence at $328\text{ m}\mu$ and correspond to the β -vitamin A of Karrer, Walker *et al.* [1933]. These workers claim that this material differs from ordinary vitamin A in that it shows only one absorption band at $622\text{ m}\mu$ in the blue antimony trichloride solution. In all the fractions examined by us the only band visible in the spectroscope lies at $617\text{--}622\text{ m}\mu$, but if intensity measurements are made at 617 and $583\text{ m}\mu$ the

ratios are found to be approximately 2 : 1, as has always been observed with the purest vitamin A preparations. Moreover, if a small quantity of 7-methylindole is added to the preparation before the antimony trichloride, only a clear band at 583 $m\mu$ is seen, at nearly half the intensity of that originally present at 617 $m\mu$. By adding still less 7-methylindole the 617 $m\mu$ band is only partially suppressed and can be observed side by side with the 583 $m\mu$ band, thus proving that the inhibitor does not merely displace the 617 $m\mu$ band but, by reducing its intensity, reveals the presence of the previously masked 583 $m\mu$ band.

Table III shows some collected data on a number of these fractions, and it is clear that in all cases the ratio of the intensities of absorption at 617 and 583 $m\mu$ is approximately 2 : 1.

Table III. *Fractions immediately above the colour (rich in vitamin A).*

Exp. No.	Adsorbent	Antimony trichloride $E_{1\text{ cm.}}^{1\%}$		Ratio of intensities 617 : 583 $m\mu$
		617 $m\mu$	583 $m\mu$	
T ₃₆	Al ₂ O ₃	3930	1900	2.07
C ₂₅	Fuller's earth	3430	1730	1.98
	+ Ca(OH) ₂			
T ₃₄	Ca(OH) ₂	3100	1560	1.98
T ₃₃	Ca(OH) ₂	3970	1850	2.14
T ₃₂	Ca(OH) ₂	3860	1760	2.19
T ₃₁	Ca(OH) ₂	3540	1700	2.08
T ₂₉	Ca(OH) ₂	3420	1740	1.97
T ₂₈	Ca(OH) ₂	3310	1700	1.94
T ₂₇	Ca(OH) ₂	3940	1970	2.0
T ₁₉	Al ₂ O ₃	3630	1850	1.96
T ₁₄	Ca(OH) ₂	4720	2350	2.0
T ₉	Ca(OH) ₂	3460	1905	1.82
T ₅	Al ₂ O ₃	3510	1740	2.01
Average				2.01

Thus, Karrer's β -vitamin A is qualitatively identical with what has been previously prepared by adsorption and by distillation. With regard to the above author's claim to have prepared a concentrate having $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ = 1700, we have also obtained values approaching this by adsorption, but products of this high potency are rare, lower values being more usual. In this respect the adsorption method is very little superior to the distillation method where values of $E_{1\text{ cm.}}^{1\%}$ 328 $m\mu$ = 1600 have been obtained.

Vitamin A thus gives with antimony trichloride a blue colour exhibiting two absorption bands, one only of which is readily suppressed by the addition of certain reagents (*e.g.* 7-methylindole). The two bands must be derived from two different chromophoric centres in the same molecule, only one of which is affected by 7-methylindole. That this state of affairs is possible is clearly shown by the following facts. We have observed that pure lutein and pure zeaxanthin give a blue colour with antimony trichloride, exhibiting an absorption band at 617 $m\mu$ and a weaker satellite band near 585 $m\mu$ (unpublished work). These bands are very similar to those due to vitamin A, except that their intensities are only about one-tenth of those characteristic of the vitamin. If a trace of 7-methylindole be added to these pure substances before the antimony trichloride reagent, the resulting colour shows only one band at 585 $m\mu$, the band at 617 $m\mu$ having been entirely suppressed.

The top zones of the adsorption tubes.

The results of the examination of the material from the top zones of the adsorption columns are given in Table IV. Absorption spectrum measurements indicate the presence of some vitamin A, together with other material absorbing selectively at 270–280 $m\mu$ and giving a violet colour with antimony trichloride possessing an absorption band near 580 $m\mu$. This material thus agrees in its properties with the strongly adsorbed “580 chromogen” of Eekelen *et al.* [1931; 1932] who have reported the separation of “two chromogens” from vitamin A by means of fuller’s earth. The adsorbed fraction on recovery was found to give a violet colour with antimony trichloride, showing only one band near 580 $m\mu$, whilst the unadsorbed fraction gave a colour showing only one obvious maximum at 620 $m\mu$. The fact that the “580 chromogen” is so strongly adsorbed suggests that it consists of partially oxidised vitamin A. We have submitted this hypothesis to direct test by carefully oxidising vitamin A with very dilute chromic acid, using the method of Kuhn and Brockmann [1933], and have actually obtained a product showing selective absorption in the ultraviolet at 275–280 $m\mu$ and giving a violet colour with antimony trichloride with an absorption maximum at 572–580 $m\mu$ (*cf.* Table IV). Products of this nature have also been frequently obtained by other oxidative treatments of vitamin A [Morton and Heilbron, 1928; Morton *et al.*, 1931; Edisbury *et al.*, 1932; Brockmann and Tecklenburg, 1933].

The fractions immediately below the colour.

It has been shown that the red substance is actually formed in the tube during adsorption and is therefore diffused somewhat into the fractions immediately above and below it. This lower fraction appears to correspond to the “ α -vitamin A” (hepaxanthin) of Karrer, Walker *et al.* [1933] and Karrer [1934]. These authors claim to have obtained by adsorption of rich vitamin A concentrates on calcium hydroxide, in addition to the main fraction (β -vitamin A) adsorbed in the upper part of the tube, a small amount of an isomeric substance (α -vitamin A) in an impure state from the lower zones. α -Vitamin A is characterised by an absorption band at 270–280 $m\mu$ and by a violet antimony trichloride colour showing only one band at 580 $m\mu$, which, however, soon disappears giving place to one at 620 $m\mu$. It is further suggested that the latter develops at the expense of the former, presumably owing to the passage of the labile “ α -vitamin A” into the “ β -form.”

Our detailed examination of the material from this zone shows that, in alcoholic solutions, it exhibits an absorption band at 275–280 $m\mu$ accompanied in most cases by the usual 328 $m\mu$ band (*cf.* Fig. 1). With antimony trichloride it gives a violet or blue colour showing a band near 580 $m\mu$ (*cf.* Table V). These observations are in accord with those of Karrer and his collaborators and further, we confirm the observation that the violet antimony trichloride solution rapidly changes on standing, and that a band near 620 $m\mu$ (or 640 $m\mu$, if colour was present in the fraction) appears to increase in intensity at the expense of the 580 $m\mu$ band. Careful and repeated spectrophotometric measurements of the intensities of the two bands during this fading process, however, reveal that the 620–640 $m\mu$ band is actually present all the time but is initially masked by the strong 580 $m\mu$ band. This latter fades so rapidly, however, that the band at 620–640 $m\mu$ becomes more and more visible in the spectroscope, although in no case have we observed it to increase in intensity as the intensity of the other band decreases.

Table IV. *Fractions from the tops of the adsorption columns.*

Exp. No.	Adsorbent	Wt. of fraction mg.	Antimony trichloride test					7-Methyl-indole SbCl ₅		Ratio of intensities 617 : 583 mμ	Ultraviolet absorption			
			Colour	Maxima in mμ	E ₁ % _{1 cm.}		Maximum in mμ	Maxima in mμ	E ₁ % _{1 cm.}					
					617 mμ	583 mμ			573 mμ		328 mμ	280 mμ		
C ₇	Al ₂ O ₃ + "Fasertonerde"	88	Purple-blue	617 (583)	1380	1160	—	—	1.2 : 1	578	—	—	—	—
T ₁₇	Ca(OH) ₂	53	Blue	617	945	580	—	—	1.6 : 1	—	—	—	—	—
T ₂₉	Ca(OH) ₂	64	Blue	622 (597)	802	470	—	—	1.7 : 1	580	—	—	250	—
C ₂₅	Fuller's earth	30	Violet-blue	620, 575	283	—	224	—	1.3 : 1	577	—	—	—	—
C ₂₅	Fuller's earth	79	Violet-blue	617, 582	1030	1030	—	—	1 : 1	582	270 inflexion at 328	—	670	950
C ₂₇	Fuller's earth	7.5	Violet-blue	617, 583	475	485	—	—	1 : 1	584	328 and 280 summation	—	—	342
C ₂₉	Fuller's earth	13	Violet-blue	588	870	1440	—	—	0.6 : 1	588	330 and 280 summation	—	1000	900
Oxidation products of vitamin A														
T ₂₃	—	42	Violet-blue	618, 578	860	610	—	—	1.4 : 1	—	328 mμ band and inflexion near 280	—	334	—
T ₂₆	—	60	Strong violet	617, 573	833	—	1040	—	1 : 1	573	328 mμ band and absorption 260-300	260	260	230

Table V. *The zones immediately below the colour.*

Exp. No.	Wt. of fraction mg.	Adsorbent	Antimony trichloride test					Ultraviolet absorption				
			Colour	Maxima in $m\mu$	$E_{1\text{ cm.}}^{1\%}$		Ratio of 617 : 583 $m\mu$ intensities	7-Methyl-indole + SbCl_3 bands	Maxima in $m\mu$	$E_{1\text{ cm.}}^{1\%}$		
					617 $m\mu$	583 $m\mu$				328 $m\mu$	280 $m\mu$	
T_{34} (4)	12.6	$\text{Ca}(\text{OH})_2$	Violet-blue	628 and 582	178	178	1 : 1	642 and 583	—	—	—	
T_{22}	17.6	$\text{Ca}(\text{OH})_2$	Violet	622 and 577	90	105	0.85 : 1	623 and 577	330 inflexion band near 280	54	70	
T_{22} (6)	7.3	$\text{Ca}(\text{OH})_2$	Violet	582 only	270	410	0.7 : 1	640 and 582	330 band and inflexion 275 $m\mu$	320	210	
C_{25}	5	$\text{Ca}(\text{OH})_2$ with fuller's earth above	Violet	623 and 580	270	350	0.8 : 1	583	328 inflexion, 275 band	250	465	
C_{28}	15	$\text{Ca}(\text{OH})_2$ with fuller's earth above	Violet	585	54	72	0.8 : 1	—	275	—	90	

Table VI. *Material from the yellow zone (least easily adsorbed).*

Exp. No.	Adsorbent	Wt. of fraction mg.	Location in column	Bands with SbCl ₃ mμ		E ₁ %		7-Methyl-indole + SbCl ₃ bands		Ultraviolet absorption	
T ₂₁	Ca(OH) ₂	30.8	Below colour	622, 578	—	—	—	622, 577	—	Maxima in mμ	370 mμ
T ₂₈ (6)	Ca(OH) ₂	24	Below colour	622, 578	414	292	—	625, 579	—	Triplet of narrow bands max. 366	—
T ₂₈ (4)	Ca(OH) ₂	108	Well below colour	620, 579	605	342	—	625, 579	—	Triplet max. 370	195
T ₂₈ (6)	Al ₂ O ₃	97.3	Just below colour	622	1300	580	—	624, 580	—	Triplet max. 349, 369, 390	670
T ₂₈ (6)	Al ₂ O ₃	10.5	Just below colour	623, 583	1230	554	—	623, 582	—	Triplet max. at 348, 370, 387	560
T ₂₈ (6)*	Ca(OH) ₂	10.5	Below colour	628, 582	208	180	—	640, 583	—	328. Inflexion 390	107

* Contains some of the red colour also.

We do not consider that the optical properties provide evidence that this substance is an isomeric form of the vitamin. Its properties agree with those of the oxidation products of vitamin A found in the top zones and with those of the "580 chromogen" of Eekelen *et al.* It is, however, much less strongly adsorbed, being still obtained below the colour when a solution from which the "580 chromogen" has been removed by preliminary adsorption on fuller's earth is passed through a column of calcium hydroxide (*cf.* Table V). It is thus probable that it is in a lower state of oxidation than the material in the top zones. Euler *et al.* [1934] suggest that the growth-promoting activity of this fraction may be simply explained by the occurrence in it of 10 % of vitamin A itself.

The yellow zone least easily adsorbed.

Using alumina as adsorbent and employing 5–10 g. of vitamin A concentrate a distinct bright yellow band develops well below the other fractions in the adsorption-tube. With calcium hydroxide as adsorbent the yellow band is much more diffuse. On elution a small quantity (2–5 % of original concentrate) of a thick yellow oil is obtained (*cf.* Table VI) differing altogether in properties from the other fractions. Its absorption spectrum (in alcohol) shows sharply defined maxima at 348, 369 and 389 $m\mu$ (Fig. 1) and thus resembles the spectra of the typical carotenoids with the maxima displaced from the visible into the near ultraviolet. The antimony trichloride solution has a bluish colour showing absorption maxima at 624 and 579 $m\mu$. On partition of the pigment between 90 % methyl alcohol and light petroleum it goes largely, but not completely, into the alcohol. Attempts to characterise it further have so far been unsuccessful, as on standing in solution the intensity of absorption and chromogenic power rapidly decrease, but we are actively engaged in further work with a view to its more precise characterisation.

SUMMARY.

Complete chromatographic analysis of rich vitamin A concentrates controlled by spectroscopic examination of the alcoholic solutions and of the antimony trichloride blue colours shows that five main fractions may be obtained. In order of adsorption on alumina or calcium hydroxide they are:

(a) Strongly adsorbed material which with antimony trichloride gives a violet or violet-blue colour. This corresponds to the similar material obtained by Eekelen *et al.* [1931; 1932] and is considered to consist largely of oxidation products of the vitamin.

(b) The main fraction consisting of highly concentrated vitamin A characterised by an absorption band at 328 $m\mu$. This corresponds with Karrer's so-called β -vitamin A, but it is found that, in addition to the band at 620 $m\mu$ in the antimony trichloride blue colour, the usual band at 583 $m\mu$ is also present as a masked maximum. The intensities of absorption at these two wavelengths are approximately 2 : 1, as has always been observed with the purest vitamin A concentrates. It is therefore concluded that the " β -vitamin A" of Karrer is essentially the same substance as the best vitamin A preparations previously obtained.

(c) A small amount of strongly coloured red material which appears to be produced from the vitamin during the adsorption process. Its absorption spectrum shows, together with the usual 328 $m\mu$ band of vitamin A, a smooth inflexion near 430 $m\mu$ which is the complement of the red colour. With antimony trichloride it gives the blue colour usually characteristic of vitamin A but

Tube of $\text{Ca}(\text{OH})_2$ showing coloured zones produced when a vitamin A concentrate is adsorbed from light petroleum solution.

showing an additional absorption band between 640 and 660 $m\mu$. Molecular weight determinations suggest the presence of polymerised material in this fraction.

(d) The so-called α -vitamin A (hepaxanthin) of Karrer *et al.* This is only small in amount and shows an absorption maximum at 270–280 $m\mu$ and a violet colour with antimony trichloride exhibiting a 580 $m\mu$ absorption band. In general, this material closely resembles fraction (a) in its properties and is therefore probably a decomposition product of the vitamin, but in a lower state of oxidation than the more strongly adsorbed fraction. There is no evidence that this fraction contains an isomeric form of the vitamin.

(e) A hitherto unrecorded yellow substance characterised by three distinct absorption bands at 348, 369 and 389 $m\mu$. It gives a blue colour with antimony trichloride showing absorption maxima at 624 and 579 $m\mu$.

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