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Spectrophotometric Studies on Human Sebum

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The absorption spectra of the samples investigated were examined between 230 and 500 $m\mu$, with special reference to the ultraviolet region of the spectrum; in particular it was sought to establish evidence for vitamin E.

α -, β -, γ - and δ -tocopherols show an absorption band near 295 $m\mu$, $E_{1\text{ cm}}^{1\%}$ about 80. α -Tocopherol acetate shows a maximum at 285 $m\mu$, $E_{1\text{ cm}}^{1\%}$ about 40. MacKenna, Wheatley & Wormall (1950, 1951, 1952) have found sebum to contain appreciable amounts of FeCl_3 -reducing substances; part of this material is stated to be vitamin E, the amount not exceeding 0.2 mg. tocopherol per gram of sebum.

The investigations to be described were carried out using sebum samples of about 1 g. A simple calculation shows that under such circumstances detection of tocopherol must be difficult: if the whole amount (0.2 mg.) of tocopherol can be concentrated in a volume of 4 ml. (in a 1 cm. cell) then the extinction value at 295 $m\mu$ will only be 0.4 (by way of comparison as small a quantity as 0.009 mg. vitamin A will give a similar extinction value at its absorption maximum). The chromatographic separation must be such as to concentrate the tocopherol in one fraction. Furthermore, other absorbing materials will have to be eliminated for the maximum at 295 $m\mu$ to be evident.

A preliminary report of this work has already been given (Festenstien & Morton, 1951). Two samples of sebum and sebum unsaponifiable material were investigated. In addition, a further sample of unsaponifiable material, which had been assayed for FeCl_3 -reducing substances by MacKenna *et al.* was examined; also a 'sterol-like' fraction provided by the same workers.

EXPERIMENTAL

Chromatography. Separations were carried out using Spence's grade 0 alumina (weakened by controlled addition of water in some cases); chromatographic tubes of 9 and 14 mm.

internal diameter were used, depending on the amount of material.

The sebum or sebum fraction was usually dissolved in the minimum amount of light petroleum (A.R. quality) and the column developed at first with this solvent alone; any residue was discarded. Mixtures of ether and light petroleum, ether, methanol and ethanol were used as eluants. Arbitrary fractions were collected, but where distinct bands appeared to be eluting the fractionation was extended. All fractions were taken to dryness and weighed before spectroscopic examination; in the later work particularly, evaporation of solvent was carried out in an atmosphere of CO_2 or N_2 , to minimize oxidation of any tocopherol.

The ether used was peroxide-free (usually distilled over reduced Fe before use).

Spectroscopy. Absorption spectra were determined quantitatively using a Beckman photoelectric spectrophotometer. Ethanol and cyclohexane were used as solvents. Readings were taken every 5 $m\mu$ in the range 230–310 $m\mu$, and every 10 $m\mu$ in the range 310–500 $m\mu$.

RESULTS

Sebum. The sebum itself showed mainly end absorption, though there was a suggestion of an inflexion between 270 and 300 $m\mu$ (Fig. 1).

Chromatography resulted in a good fractionation (cf. Table 1), but no selective absorption obviously characteristic of vitamins E appeared in any fraction (Figs. 2 and 3). Since, however, fraction 2 (sample 1, Table 1) showed a faint inflexion near 290 $m\mu$, it was rechromatographed (Table 2), and absorption again examined (Fig. 4). No real evidence is found for the presence of tocopherol in any of these fractions. At least a marked inflexion at 290 $m\mu$ should be shown to be indicative of tocopherol.

In view of the low recoveries from the chromatography of the sebum (57% even after elution with ethanol) it was decided to remove the free fatty acids prior to chromatography, lest these should prevent the elution of any tocopherol.

Table 1. *Chromatography of sebum*

Fraction no.	Eluant	% of original wt.	$E_{1\text{ cm.}}^{1\%}$ 295 m μ .	Character of ultraviolet absorption spectrum
Sample 1. $E_{1\text{ cm.}}^{1\%}$ 295 m μ . = 3.0. 2 g., on 20 g. alumina column 10 $\times$ 1.4 cm.				
1	Light petroleum	27.9	2.4	Inflexion 250–270 m μ .
2	10% ether/p*	8.7	2.3	Inflexions 250–270, 245, 290 m μ . (?)
3	20% ether/p	2.9	4.5	Weak inflexion 260–280 m μ .
4	50% ether/p	7.4	4.5	Mainly end absorption
5	Ether	6.3	4.5	
6	Methanol	3.9	7.3	
57.1 % recovery				
Sample 2. $E_{1\text{ cm.}}^{1\%}$ 295 m μ . = 2.8. 1.2 g., on 20 g. alumina column 10 $\times$ 1.4 cm.				
1	Light petroleum	30.5	2.6	Inflexion 250–270 m μ .
	+ 5 % ether/p			
2	10% ether/p	4.4	2.3	Maximum 258 m μ .
3	50% ether/p	7.5	4.7	Mainly end absorption
4	Ether	13.1	1.8	
5	Ethanol	0.6	4.1	
6	Ethanol	1.3	5.7	
57.4 % recovery				

* p = light petroleum.

Table 2. *Rechromatography of a sebum fraction*(0.173 g., on alumina column 4.5 $\times$ 1.4 cm.)

Fraction no.	Eluant	% of original wt.	$E_{1\text{ cm.}}^{1\%}$ 295 m μ .	Character of ultraviolet absorption spectrum
1	2% ether/p*	2.9	5.4	Inflexions 235, 275, 305, 250–270 m μ .
2	5% ether/p	6.5	8.0	Weak maxima 233, 255 m μ .
3	10% ether/p	8.1	12	Inflexions 245, 280, 290, 205 m μ .
4	Ether	45.7	1.3	Maximum, 257 m μ .
5	Ethanol	9.8	11	Inflexions 290, 305 m μ .
73% recovery				

* p = light petroleum.

Table 3. *Chromatography of sebum from which free fatty acids and other materials had been removed*(0.914 g., on 20 g. alumina column 10 $\times$ 1.4 cm.)

Fraction no.	Eluant	% of original wt.	$E_{1\text{ cm.}}^{1\%}$ 295 m μ .	Character of ultraviolet absorption spectrum
1	Light petroleum	39.2	1.5	Weak inflexion 250–270 m μ .
2	10% ether/p*	16.7	1.2	Very weak inflexion 250–270 m μ .
3	50% ether/p	15.4	1.2	Very weak inflexion 260–280 m μ .
4	Ether	13.7	0.93	Mainly end absorption
5	Ethanol	3.0	2.8	
		88%		
		recovery		

* p = light petroleum.

A solution of sebum in light petroleum was shaken with 5% (w/v) Na_2CO_3 solution until no more colour was extracted. The light petroleum fraction was washed free from alkali, dried over anhydrous Na_2SO_4 and taken to dryness.

As a further means of concentration the residue obtained was partitioned between light petroleum and 90% ethanol (after Evans, Emerson & Emerson (1936) who washed a light petroleum solution of wheat-germ oil non-saponifiable

material with small volumes of 92% methanol to remove 'xanthophylls and other inert material'). After removal of solvent the light petroleum fraction was chromatographed (cf. Table 3).

The inflexions are not so marked as for the untreated sebum fractions; also the $E_{1\text{ cm.}}^{1\%}$ 295 m μ . values can be seen to be generally lower.

Fig. 1. Absorption spectra of sebum (in *cyclohexane*).
-----, Sample 1, 0.08 %; —, sample 2, 0.06 %.

Fig. 2. Chromatographed sebum fractions (sample 1) (in *cyclohexane*). —, Fraction 1, 0.042 %; -----, fraction 2, 0.087 %; ·····, fraction 3, 0.04 %.

Fig. 3. Chromatographed sebum fractions (sample 2) (in *cyclohexane*). —, Fraction 1, 0.037 %; -----, fraction 2, 0.132 %; ·····, fraction 3, 0.054 %.

Fig. 4. Rechromatographed sebum fraction 2 (sample 1) (in *cyclohexane*). —, Fraction 1, 0.025 %; -----, fraction 2, 0.033 %; ·····, fraction 3, 0.034 %.

Table 4. *Chromatography of sebum unsaponifiable matter*

Fraction no.	Eluant	% of original wt.	$E_{1\text{ cm}}^{1\%}$ 295 m μ .	Character of ultraviolet absorption spectrum
Sample 1. 0.5 g., on 20 g. alumina column 10 $\times$ 1.4 cm.				
1	Light petroleum	40.2	—	Weak maximum 270 m μ ., $E_{1\text{ cm}}^{1\%}$ 3.1
2	10% ether/p*	1.6	—	Maxima 235 m μ . $E_{1\text{ cm}}^{1\%}$ 360 255 m μ . 335
3	20% ether/p	0.4	—	Faint inflexions 290, 305 m μ . Maximum 258 m μ ., $E_{1\text{ cm}}^{1\%}$ 325 Inflexions 270, 290 m μ .
4	50% ether/p	15.0	4.7	Chiefly end absorption
5	Ether	21.6	5	
6	Methanol	5.4	4	
7	Methanol	0.4	15.8	
		84.6%		
		recovery		
Sample 2. 1 g., on 'weakened' alumina column 12 $\times$ 1.4 cm.				
1	Light petroleum	47.3	3.5	Inflexions 250–260, 290 m μ . (?)
2	10% ether/p	7.5	6.2	Inflexion 250–270 m μ .
3	10% ether/p	15.8	3	Mainly end absorption
4	10% ether/p	8.0	3	
5	20% ether/p	1.4	5.3	
6	50% ether/p	0.5	18	
7	Ether	5.0	6	
8	Ethanol	0.5		
		86%		
		recovery		

* p = light petroleum.

Unsaponifiable fraction of sebum. This material (as obtained by MacKenna *et al.*) was examined similarly. The first sample (batch *M*) showed an inflexion near 290 m μ . ($E_{1\text{ cm}}^{1\%}$ 7.5) consistent with the presence of some vitamin E (Fig. 5). Correction for 'irrelevant absorption' by assuming the latter to be represented by a continuous curve (see uppermost curve, Fig. 5) gives the $E_{1\text{ cm}}^{1\%}$ 290 m μ . of the selectively absorbing material as about 0.25. It follows that the tocopherol content of the fraction could be of the order 0.3%. The material was chromatographed (Table 4).

Since fractions 2 and 3 showed inflexions at 290 m μ . (Fig. 6), they were rechromatographed, as shown in Table 5.

Fraction 2" (Fig. 7) exhibits a genuine inflexion at 280–300 m μ . which could be due to a trace of tocopherol, although this is not certainly the absorbing entity.

A second sample of sebum unsaponifiable matter was obtained here by saponification according to the method of Emmerie (1940), who saponified wheat-germ oil under these conditions to obtain 98% recovery of tocopherol. MacKenna *et al.* (1952), using this method on sebum, obtained 40% recovery of added tocopherol; they recommend saponification in the presence of pyrogallol according to the method of Tošić & Moore (1945), when 64% of the tocopherol added to sebum is recovered.

Table 5. *Rechromatography of fractions 2 and 3*

Fraction 2 (0.008 g.) on alumina column 4 $\times$ 0.9 cm.	
Fraction no.	Character of ultraviolet absorption spectrum
1'	Maximum 232 m μ .; feeble inflexion 258 m μ .
2'	Maximum 235 m μ .; inflexions 245–260, 285 m μ .
3'	Maxima 240, 260 m μ . (weak)
4'	Maximum 258 m μ .; feeble inflexions 280, 290 m μ .
5'	Maxima 255, 261 m μ .
Fraction 3 (0.002 g.) (Table 4) plus 4' from the previous chromatography were rechromatographed on an alumina column 3 $\times$ 0.9 cm.	
1''	Maximum 258 m μ .; inflexions 280, 290 m μ .
2''	Maximum 265 m μ .; inflexion 290 m μ .
3''	Maxima 255, 261 m μ .; inflexion 275 m μ .

Fig. 5. Sebum unsaponifiable material (in *cyclohexane*). —, Sample 1, lower curve 0.04%; upper curve is lower curve $\times 2.5$. ····, Sample 2, 0.05%.

Fig. 6. Chromatographed sebum unsaponifiable material (sample 1) (in *cyclohexane*). —, Fraction 2, 0.002%; ----, fraction 3, lower curve 0.001%, upper curve is lower curve $\times 4$.

Fig. 7. Twice rechromatographed sebum unsaponifiable material (sample 1). —, Fraction 1''; ----, fraction 2''; ····, fraction 3''.

Fig. 8. Chromatographed sebum unsaponifiable material (sample 2) (in *cyclohexane*). —, Fraction 1, 0.095%; ----, fraction 2, 0.023%; ····, fraction 3, 0.063%.

The unsaponifiable sample (2) showed $E_{1\text{ cm}}^{1\%}$ 295 m μ . 4.5; if an inflexion is present at 290 m μ , it is very faint indeed (Fig. 5). Chromatography (Table 4) was carried out on 'weakened' alumina to minimize oxidation—in case the alumina was responsible for oxidation of any tocopherol.

Only fractions 1 and 2 showed selective absorption, and this was not very pronounced (Fig. 8). It is interesting to compare the curves with those obtained from chromatography of the first sample (see Fig. 6): the maxima at 235 m μ . and in the vicinity of 260 m μ . are not so evident, neither is there an inflexion at 290 m μ . except possibly for fraction 1.

the highest absolute (and relative) amount of FeCl_3 -reducing substances was fraction 6. Assuming the reducing material to be exclusively tocopherol, the absorption contributed by the latter would be of the order of 5% of the total. The absorption curve only shows a very faint inflexion at about 280 m μ . (see Fig. 9); correction of this curve (assuming tocopherol to be the reducing entity) eliminates some of the absorption but the inflexion is not entirely eliminated.

Rechromatography of this fraction was carried out as previously, except that the material was initially dissolved in a solution of 1% ethanol in light petroleum (Table 7).

Table 6. *Chromatography of sebum unsaponifiable fraction*

(0.37 g., on 'weakened' alumina column 12 $\times$ 1.4 cm.)

Fraction no.	Eluant	% of original wt.	$E_{1\text{ cm}}^{1\%}$ 295 m μ .	Character of ultraviolet absorption spectrum
1	10% ether/p*	29.6	3.1	Inflexion 260–290 m μ .
2	10% ether/p	21.2	2.0	Weak inflexion, 260–290 m μ .
3	50% ether/p	29.6	2.9	Chiefly end absorption
4	50% ether/p	7.3	4.3	
5	Ether	4.3	7.7	
6	Ethanol	4.8	5.0	

Correlation of reducing properties and spectrophotometric data

Fraction no.	FeCl_3 -reducing substance as ' α -tocopherol' ($\mu\text{g.}$)	$E_{1\text{ cm}}^{1\%}$ 295 m μ . due to ' α -tocopherol'	$E_{1\text{ cm}}^{1\%}$ 295 m μ . obs.	Contribution to absorption by ' α -tocopherol' (%)
1	13	0.009	3.1	0.3
2	28	0.027	2.0	1.4
3	50	0.035	2.9	1.2
4	16	0.046	4.3	1.1
5	18	0.087	7.7	1.1
6	53	0.23	5.0	4.6

* p = light petroleum.

Correlation of absorption spectra and FeCl_3 -reducing properties of sebum unsaponifiable fractions

The sample was provided by Dr V. R. Wheatley and Prof. A. Wormall. It had been saponified in the presence of pyrogallol according to the method of Tošić & Moore (1945); hydrocarbons had been removed and the remainder assayed for FeCl_3 -reducing substances. The value 0.86 mg. ' α -tocopherol'/g. of sample was obtained. The absorption spectrum showed a weak inflexion between 260 and 290 m μ ., $E_{1\text{ cm}}^{1\%}$ 295 m μ . = 3.2. After chromatographic separation (Table 5), the various fractions were separately tested for the presence of FeCl_3 -reducing substances by a modification of the Emmerie-Engel colorimetric test used by Tošić & Moore (1945).

It should be noted that the FeCl_3 -reducing properties are spread over all the fractions; there is destruction of reducing power on chromatography, but less than 50%. The fraction which contained

The absorption of fraction 2 is shown in Fig. 10; it is the chief fraction showing selective absorption near 290 m μ . The lower curves show correction

Table 7. *Rechromatography of richest FeCl_3 -reducing fraction*

(0.016 g., on 'weakened' alumina column 8 $\times$ 0.9 cm.)

Fraction no.	Eluant	Character of ultraviolet absorption spectrum
1	1% ethanol/p*	Weak inflexion 250–260 m μ .
2	5% ethanol/p	Inflexion 265–290 m μ .
3	10% ethanol/p	Weak inflexion 270–290 m μ .
4	10% ethanol/p	Mainly end absorption
5	10% ethanol/p	
6	20% ethanol/p	
7	Ethanol	

* p = light petroleum.

assuming 20 and 10% of the absorption at 295 m μ . to be contributed by α -tocopherol. It is evident from consideration of the 'corrected' curves that there may be small amounts of tocopherol, but the main absorbing entity is not tocopherol.

Table 8. *Chromatography of 'sterol-like' fraction from sebum unsaponifiable matter*

(0.100 g., on 'weakened' alumina column 9 × 0.9 cm.)

Fraction no.	Eluant	% of original wt.	Character of ultraviolet absorption spectrum
1	5% ethanol/p*	9	Maximum 233 m μ .: $E_{1\text{ cm.}}^{1\%} = 132$; weak maximum 278 m μ .: $E_{1\text{ cm.}}^{1\%} = 43$
2	7.5% ethanol/p	50	Weak maximum 235 m μ .: $E_{1\text{ cm.}}^{1\%} = 83$
3	10% ethanol/p	9	Chiefly end absorption
4	20% ethanol/p	2	

* p = light petroleum.

'Sterol-like' fraction. This fraction was obtained from Dr V. R. Wheatley and Prof. A. Wormald; for the method of obtaining it see MacKenna *et al.* (1952). The spectrum of the fraction showed a weak inflexion at 265–320 m μ ., $E_{1\text{ cm.}}^{1\%}$ 280 m μ . = 27.5. It was chromatographed as shown in Table 8. The absorption of fraction 2 is shown in Fig. 11.

instrument; in the Rosenheim test fraction 1 also gave a faint 600 m μ . band.

As pointed out by MacKenna *et al.* (1952) it is possible that the compound responsible for the coloration is 7 α -hydroxycholesterol. 7 α -Hydroxycholesterol dibenzoate (m.p. 175°) was therefore examined: a band at 660 m μ . accompanied by a

Fig. 9. Spectrum of most potent FeCl₃-reducing fraction and the effect of correcting for α -tocopherol as absorbing material. (In ethanol.)

Both fractions 1 and 2 gave a blue colour with 90% trichloroacetic acid (after Rosenheim, 1929) and with antimony trichloride in chloroform (after Carr & Price, 1926). Both these colorations showed principal absorption maxima in the vicinity of 660 m μ . when examined on the Hilger-Nutting

Fig. 10. Fraction from rechromatography of most potent FeCl₃-reducing concentrate and correction for α -tocopherol assuming 10 and 20% of the absorption at 295 m μ . to be contributed by the tocopherol.

weaker one at 575 m μ . was observed in the Carr-Price test.

At no stage in the work was spectrophotometric evidence obtained for the presence of provitamins D, vitamins A and K or carotene.

Chemical investigations of the various fractions obtained from chromatography of sebum unsaponifiable material, sample 2, were carried out as follows:

The hydrocarbon fraction (1) was three times recrystallized from boiling ethanol when it was possible to separate a white wax-like material, m.p. 56° (C, 85.12; H, 14.42%; mol.wt. (Rast), 560). The remainder of the fraction was taken up in acetone and treated with dry hydrogen chloride gas. After taking to dryness some crystalline material separated (m.p. 106–109°; Cl, 32.5%). This was probably a mixture of the various isomers of squalene hydrochloride (Heilbron, Kamm & Owens, 1926). The calculated Cl content of squalene hydrochloride is 33.86%.

Fig. 11. Chromatography of 'sterol-like' fraction (in ethanol). —, Fraction 1, 0.0072%; ---, fraction 2, 0.0075%.

The main sterol fraction (4) was twice recrystallized from methanol and shown to be cholesterol (m.p. 145°).

The strongly adsorbed fraction (7) was recrystallized from methanol when some light yellow-brown crystalline material (m.p. 79–81°) was obtained.

In these investigations the findings of MacKenna *et al.* (1950) have been confirmed.

DISCUSSION

The primary question is whether there is any vitamin E in sebum. The first sample of sebum unsaponifiable material has a spectrum with an inflexion at 290 mμ. (see Fig. 5). Correction for 'irrelevant absorption' (see p. 171) results in an

estimate of 0.3% tocopherol for the particular fraction examined. This fraction after chromatographic separation yielded two fractions with inflexions at 290 mμ.: one with maxima at 235 and 255 mμ. as well, and the other with a maximum at 258 mμ. Again using the method of extrapolation for eliminating 'irrelevant absorption', the tocopherol in the original fraction is 0.1% calculated from fraction 2 and 0.06% calculated from fraction 3.

Fig. 12. Spectrum of sebum (sample 1) plus addition of 5, 10 and 20% of absorption at 295 mμ. as α-tocopherol.

These estimates are at best very approximate, yet there is an indication that there is loss of '290 mμ. material' on chromatography. It is doubtful whether the absorption at 290 mμ. is due to tocopherol or is merely part of the absorption spectrum of the 235 mμ. and/or the 255 mμ. absorbing material.

Some of the fractions obtained from the sebum itself (see fraction 2, Fig. 2, and fraction 2, Fig. 4) show faint inflexions at 290 mμ. There, too, there is other and more pronounced selective absorption—at 235 and 255 mμ. It seems certain, however, that the 290 mμ. material was not produced during the saponification process.

Assessment of tocopherol content where there is no 295 mμ. maximum is not easy. An approximate

figure for the percentage tocopherol required to produce an inflexion in a sample showing mainly end absorption has been calculated as follows: a curve showing chiefly end absorption—in this case of sebum sample 1—was plotted; then assuming 5 % of the absorption at 295 m μ . to be due to tocopherol, the effect of superimposing the amount on the original curve is shown. The procedure is also carried out for the effect of 10 and 20 % of the absorption at 296 m μ . estimated as tocopherol (Fig. 12).

In the case of 20 % the effect on the curve is quite marked, and 10 % produces a distinct inflexion. It is therefore suggested that it is a reasonable approximation to assume that if no inflexion is evident then the absorption due to tocopherol cannot exceed 5 % of the total absorption observed at 295 m μ . (The values for the tocopherol absorption spectrum have been interpolated from the graphical data provided by Robeson & Baxter (1943) for α -tocopherol, $E_{1\text{ cm}}^{1\%}$ 292 m μ . = 73.) On this basis the maximum amount of tocopherol in any of the non-saponifiable fractions is 0.03 % for the first sample and 0.02 % for the second sample (expressed as percentage of sebum). The amount assessed by MacKenna *et al.* (1952) is of the order of 0.02 %.

Cuthbertson, Ridgeway & Drummond (1940) have carried out the spectroscopic examination of various rat tissues; they claim that small amounts of tocopherols may easily be missed when they are insufficient (i.e. <0.1 %) to produce an inflexion in the ultraviolet absorption curve of the fat itself. They also found that amounts of tocopherols sufficient to give rise to distinct inflexions in the absorption curves of the crude extracts from tissues do not always reveal themselves by maxima at 290–294 m μ . Low concentrations tend to influence the curve in the vicinity of 270–280 m μ .; increasing concentration tends to shift the position of the maximum towards the region characteristic of the tocopherols. According to Cuthbertson *et al.* direct spectroscopic estimation of tocopherols in crude tissue extracts is unreliable if the proportion is much below 1 %, but examination of the unsaponifiable fraction will enable quantities down to 0.1 % to be determined satisfactorily. These conclusions have been drawn from experiments where known amounts of tocopherol were added to rat kidney fat and rat body fat. (These rats were fed on a vitamin E-deficient diet and the spectra of the original extracts show end absorption only.)

Regarding the detection of 0.1 % in unsaponifiable material, this would correspond to 0.03 % as percentage of sebum (MacKenna *et al.* (1950) quote the unsaponifiable content of sebum as 32 %).

The inflexions between 260 and 300 m μ . may on this basis be indicative of small amounts of toco-

pherols; this assumption is best treated with caution. The fraction obtained from rechromatography of the richest FeCl₃-reducing material (see Fig. 10) shows a distinct inflexion between 260 and 290 m μ . Correcting this curve for tocopherol eliminates some of the selectively absorbing material, but not all of it, suggesting the presence of something other than tocopherol.

It is of interest in this connexion to recall the findings of Moore & Rajagopal (1940), who investigated the spectroscopic properties of various rat tissues, with special reference to vitamin E content. Unsaponifiable fractions of tissues were examined from: (1) vitamin E-deficient rats; (2) rats fed on a diet reinforced with synthetic α -tocopherol; (3) rats fed on a diet containing wheat-germ oil. In all the rats absorption due to unidentified substances was shown, usually by an inflexion at 280 m μ .

It should be borne in mind that the selectively absorbing material may be oxidation products of squalene. MacKenna *et al.* (1952) have detected oxidation products of squalene in three of their five principal fractions obtained from sebum unsaponifiable material.

With regard to the 'sterol-like' material, if it were 7-hydroxycholesterol, then it should be precipitated by digitonin, and according to MacKenna *et al.* (1952) it is not. The only evidence MacKenna *et al.* have in favour of the substance being a sterol is a positive Liebermann-Burchard and Rosenheim test. It has been suggested by Dr T. Moore that the compound may arise by heating cholesterol.

Kaishio & Gardner (1930) found that specimens of cholesterol heated in open test tubes at various temperatures from 160 to 200° developed a blue colour with Rosenheim's reagent, with a band near 660 m μ . and a faint subsidiary band near 610 m μ . This appears to be identical with the fractions tested. It is known that the 7 position of the sterol nucleus is the most susceptible to oxidation, and it is quite likely that Kaishio & Gardner produced 7-hydroxycholesterol by heating cholesterol. 7-Hydroxycholesterol has no absorption band at 235 or 278 m μ ., but 7-ketocholesterol would show a maximum in the vicinity of 235 m μ . and it may well accompany 7-hydroxycholesterol as the next oxidation product.

SUMMARY

1. Spectrophotometric studies have been carried out on human sebum and sebum unsaponifiable fractions. The principal selectively absorbing substance shows $\lambda_{\text{max.}} = 258 \text{ m}\mu$. This has not been identified.

2. No vitamin A, β -carotene, vitamin K or provitamin D has been detected.

3. Vitamin E may be present in small amounts. The evidence is doubtful; if tocopherol is present it is outside the range of detection of normal spectroscopic measurement. It is estimated that the amount of tocopherol cannot exceed 0.03 % of the sebum.

4. Two fractions from a 'sterol-like' substance provided by MacKenna *et al.* (1952) have been shown to both give a blue colour with trichloroacetic acid (λ_{max} 660 m μ .) and with antimony trichloride in chloroform (λ_{max} 664 m μ .). One shows λ_{max} 235 m μ . and the other λ_{max} 278 m μ . (weak) as well.

5. Cholesterol, squalene, a hydrocarbon, m.p. 56°, and an unidentified substance, m.p. 79–81°, have been isolated.

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The Reactivity of Radiomimetic Compounds

1. CROSS-LINKING OF PROTEINS

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Within the last five years a number of different compounds has been shown to produce biological effects similar to those of X-rays. Radiomimetic activity has been defined by Loveless (1951) as a highly specific effect upon the resting cell producing an alteration of the genetic material which is revealed by chromosome breakage and rearrangement in subsequent divisions. Radiomimetic compounds are powerful growth inhibitors and have found limited use in the chemotherapy of malignant disease. Several have been demonstrated to be both carcinogenic and mutagenic (Haddow, 1951). Chemically, all the compounds in this class can be considered as alkylating agents (Loveless, 1951); they include sulphur mustard and nitrogen mustards, epoxides, ethyleneimines and *O*-methanesulphonyl derivatives of hydroxy compounds. Haddow, Kon & Ross (1948) demonstrated that, in the bischloroethylamine (nitrogen mustards) series, the struc-

tural condition requisite for biological activity is the presence of two halogenoalkyl groups. It has since been found that this requirement holds throughout the whole series and that, with the exception of a few isolated cases, only compounds with two alkylating groups in the molecule are active in the intact animal (Hendry, Homer, Rose & Walpole, 1951; Haddow, private communication). In tests *in vitro*, unifunctional compounds are capable of bringing about radiomimetic effects, but only at concentration 50 to 100 times those required for corresponding multifunctional compounds (see Bieseke, Philips, Thiersch, Burchenal, Buckley & Stock, 1950; Loveless & Ross, 1950).

These observations led Goldacre, Loveless & Ross (1949) to propose the hypothesis that the radiomimetic compounds exert their biological action by cross-linking genetic material such as chromatid threads. This hypothesis could not be tested directly,