CLXIX. THE ABSORPTION SPECTRA OF OILS AND OIL CONSTITUENTS WITH SPECIAL REFERENCE TO PRO-VITAMIN D.

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ERGOSTEROL, which shows three absorption bands at $293.5\mu\mu$, $281.5\mu\mu$ and $270\mu\mu$ respectively undergoes the reaction

[Heilbron, Kamm and Morton, 1927; Pohl, 1927; Windaus and Hess, 1927; Rosenheim and Webster, 1927.] Various investigators have shown that vegetable oils also acquire antirachitic potency on irradiation. We have therefore sought to determine whether ergosterol can be detected in vegetable oils by means of the spectroscope.

The possibility of other absorbing constituents in the oils, masking the presence of ergosterol, must first be considered. Aliphatic esters rarely, if ever, exhibit marked selective absorption. The type of curve found corresponds in most cases with feeble general absorption in the near and middle ultra-violet regions, and more powerful end absorption in the extreme ultra-violet. There is no evidence that the glycerides of fatty acids behave differently from the simple esters. Free fatty acids do not exhibit selective absorption to any marked degree. It has been shown [Heilbron, Kamm and Morton, 1927] that pure cholesterol shows no selective absorption, and we have recently found that sitosterol, which is the chief constituent of the unsaponifiable matter of vegetable oils, shares this property.

Hence, the detection of ergosterol in oils by means of the spectroscope will depend only on (a) the chance occurrence of substances peculiar to a particular oil, or particular process of extraction, and (b) the capacity of small amounts of the selectively absorbing ergosterol to show themselves in the presence of the general absorption of the other constituents already mentioned. This was put to direct test.

A very transparent sample of kapokseed oil, showing no selective absorption in the ultra-violet, was used as a solvent for ergosterol. Thin films of oil of a thickness of the order $1/10$ mm. were transparent down to $240\mu\mu$.

With the addition of 0.2 % of ergosterol, the three bands were detectable with certainty, while lesser amounts gave positive results, although these results were not always unequivocal. The sensitivity of the spectrographic method will depend primarily on the transparency of the oil under investigation, and in many cases of clear samples of high purity, percentages as low as 0.01–0.02 have been detected, *e.g.* cottonseed oil.

In what follows a "negative" test does not, therefore, *exclude* a concentration of ergosterol less than 0.2 %.

The reaction, ergosterol $\xrightarrow{\text{light}}$ vitamin D is accompanied by the appearance of a new band at 247μ which is, in all probability, characteristic of the vitamin [Morton, Heilbron and Kamm, 1927]. Unfortunately a very considerable concentration of pure vitamin (of the order of 0.01 g. per 100 cc. alcohol) is necessary to secure the detection of this absorption band. In view of the fact that such a concentration is equivalent to some thousands of doses of cod-liver oil (in respect of vitamin D), it is not surprising that the test will not serve as a criterion of the absence or presence of the antirachitic vitamin. As a further indication of the limits of the spectroscopic method, it may be pointed out that cod-liver oil, the source of our original "active" cholesterol, does not exhibit the pro-vitamin bands, although it does, of course, contain the precursor substance. A pronounced inflection, only, is shown in the same region as the ergosterol bands. It will thus be seen that the method only enables us to discriminate between oils containing relatively little ergosterol, and those containing quite reasonable amounts.

A large number of oils have been subjected to spectrographic examination, and the results are summarised in Table I. (See also Figs. 1–5.)

Certain of these oils will now be discussed in more detail.

Cottonseed oil. The unsaponifiable matter obtained by the saponification of a large quantity of oil, gave a high yield of crystalline sterol, which was fractionally crystallised from ethyl acetate, the various fractions then being examined spectroscopically. The least soluble fraction was found to contain 2.5 %, the most soluble 0.75 % of ergosterol. Cottonseed oil would thus appear to be a very rich source of this compound.

Sesame, illipi nut, shea nut and pumpkinseed oils. These all show well-defined selective absorption in the middle ultra-violet. Since, however, these bands do not exhibit the fine structure characteristic of ergosterol, there is no valid reason for connecting them with the pro-vitamin. Moreover, a preliminary examination of the unsaponifiable matter of shea nut oil has so far given no indication of the presence of ergosterol.

Yeast-fat. As would be expected from the work of Smedley MacLean and Thomas [1920], who found it to contain as much as 40 % of ergosterol, this fat gave by far the most pronounced absorption of all the oils examined.

Ground nut oil. This was probably the most interesting oil examined, showing as it did, big variations from sample to sample. In some cases there was no sign of selective absorption, in others very well-defined ergosterol

bands were obtained. Moreover, in all cases where the ergosterol bands were plainly present, there was also a more or less well-defined band in the neighbourhood of $320\mu\mu$. This latter band has only been found hitherto in oils of animal origin such as wool-fat, whale oil, cod-liver oil, etc. [cf. Schlutz and Morse, 1925; Heilbron, Kamm and Morton, 1927], and there is no definite evidence of its connection with vitamin chemistry. Experiments are, however, now in progress, which have been designed to elucidate the nature of the compound exhibiting this band.

Table I.

Oil	Ergosterol bands	Band at or near $320\mu\mu$	Remarks
Olive	—	—	Band at λ max. = $407\mu\mu$
Nux vomica	—	—	
Tiger nut	—	—	
Alfalfaseed	Very feeble	—	
Coconut	—	—	
Babassu	—	—	
Yeast-fat	+ + + + +	—	
Sesame	?	—	Well defined band at λ max. = $287\mu\mu$
Kapokseed	—	—	
Rape	—	—	
Soya bean	—	—	
Mowraseed	Inflection	—	
	$265-280\mu\mu$	—	
Illipi nut	?	—	Selective absorption λ max. = $270\mu\mu$
Shea nut	?	—	„ λ max. = $278\mu\mu$
Cottonseed	+ +	—	
Linseed	+ +	—	
Maize... ..	+ +	—	
Ground nut sample A	Inflection	—	
„ „ B	—	—	
„ „ C	—	—	
„ „ D	+	—	
„ „ E	+ +	+	
„ „ F	+	—	
„ „ G	+ +	+ +	
„ „ H	+ +	+ +	
Brazil nut	—	—	
Palm oil	—	—	
Palm kernel	—	—	
Rice meal	—	—	
Pumpkinseed	?	—	Well defined selective absorption λ max. = $276.5\mu\mu$. No fine structure
Sunflower	—	—	
Whale	—	?	
Wool-fat	Inflection	+ +	
Cod-liver oil... ..	?	+	
Ditto. Unsap.	?	+	
Extracted C.L.O.	?	+ +	
“Ostelin”	?	+	

Olive oil. Lewkowitsch [1927] examined the absorption of olive and tea oils. A band at $275\mu\mu$ was observed in the former oil. In view of the frequent contamination of olive oil with cottonseed oil, and also since a sample known to be free from adulterants, examined by us, did not show any band, the observation of Lewkowitsch should be accepted with reserve.

The activation of oils by irradiation bears a close relation to the stimulation of certain vital processes in phototherapeutics. It is well known that skin is

rich in cholesterol and that there is reason to suppose that a part at least of the mechanism of light therapy is the formation of vitamin D in the epidermis. This has not yet been proved to be true.

Thin sections of skin have been obtained by us (by the kindness of Prof. Blair Bell) and examined spectroscopically. The results were in approximate agreement with the data of Glitscher and Hagenbach [1926]. Sections of 0.1 mm. were not transparent below 297μ , and with thinner sections it was difficult to retain continuity when the sections were placed between quartz plates. Several experiments with very thin sections were carried out, and although inflections were shown in the middle ultra-violet regions of the curves, more definite evidence of selective absorption could not be obtained. Such a result is not surprising as the concentration of ergosterol in the epidermis must in any case be exceedingly small.

SUMMARY.

1. A method of detecting and estimating ergosterol in oils by direct spectrographic examination has been studied and its limits have been defined.
2. A large number of oils and oil extracts have been examined, and their absorption curves have been obtained.

In conclusion we desire to express our thanks to Messrs J. Bibby and Sons, Ltd., to Messrs Joseph Nathan & Co., Ltd. (proprietors of Glaxo), and to Messrs R. Silcock and Sons, Ltd., for samples of oils, and to the Food Investigation Board of the Department of Scientific and Industrial Research for a grant which has enabled the work to be carried out.

Fig. 1

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

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