CXXIII. THE ABSORPTION SPECTRUM OF VITAMIN A.

BY RICHARD ALAN MORTON AND ISIDOR MORRIS HEILBRON.

From the Department of Organic Chemistry, Liverpool University.

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THE absorption spectrum of cod-liver oil possesses a quite special interest because of the fact that the oil contains three known important substances which are sensitive towards light, namely, vitamin A, ergosterol or provitamin D and vitamin D itself.

A number of workers have studied the absorption spectrum of the medicinal oil, and Schlutz and his colleagues [1925, 1926] and Heilbron, Kamm and Morton [1927, 1] agree that selective absorption occurs in the regions 320–328 $\mu\mu$ and 270–290 $\mu\mu$. Woodrow [1928], using a photoelectric spectrophotometer, has been able to find not only a band at about 320 $\mu\mu$, but has also recorded the three absorption bands of ergosterol in thin films of the oil. The latter observation is of special interest as demonstrating the extreme sensitiveness of the spectroscopic method under favourable conditions. Even the cholesterol fraction from cod-liver oil contains only about 1 part in 4000 of ergosterol, and the proportion of cholesterol in the cod-liver oil is only some 0.5 %. Moreover, Woodrow's work definitely shows that the absorption band near 270–290 $\mu\mu$ is sometimes, if not necessarily, associated with provitamin D.

Nearly two years ago we adopted the following working hypotheses:

(a) that provitamin D should show selective absorption in the near ultra-violet;

(b) that vitamin A should show selective absorption in the nearer ultra-violet, since it has been demonstrated by many workers, *e.g.* Peacock [1926], that vitamin A may be destroyed by sunlight or the light from an incandescent lamp, and especially by the radiation from a quartz mercury lamp.

The first hypothesis was confirmed by a spectrographic study of cholesterol from brain or from cod-liver oil. Three absorption bands were shown to be a criterion of provitamin D and full use of these observations has been made in the later work by various authors on the photochemical conversion of ergosterol into vitamin D. In order to test the second hypothesis, it has been necessary to collect samples of materials varying widely in respect of the proportion of vitamin A present.

Since cod-liver oil remains the best-known source of the growth-promoting factor, it seems reasonable to test the assumption that the selective absorption at 320–328 $\mu\mu$ is connected with the vitamin. In most medicinal oils it is only

possible to record a marked inflexion at this point. If, however, samples of unrefined oil (obtained from fresh livers) are examined, it is possible to observe a real, well-defined absorption band. We have been fortunate in obtaining from Professor T. P. Hilditch samples of untreated oil from livers 1 day after their removal from the fish, and on examination we have found that very distinct selective absorption is shown in the near ultra-violet, the band exhibiting a clear maximum at $328.5\mu\mu$. It is interesting and significant that the oil gives an unusually good test with the antimony trichloride reagent which is generally regarded as being almost specific for vitamin A.

By the kindness of Professor J. C. Drummond we have been able to examine three other specimens of cod-liver oil differing widely in respect of vitamin A potency. An examination of these has shown that the intensity of the $328\mu\mu$ absorption band varies directly with the depth of the blue colour obtained with the antimony trichloride test.

Up to this point the parallelism between the colour test and the absorption band seems unmistakable, but it might be argued that the agreement is fortuitous, arising from the fact that all the observations were carried out on cod-liver oil. Difficult as such an objection might be to sustain, the possibility of a coincidence could not be overlooked. The examination of a large number of liver oils from various marine animals appears, however, definitely to have eliminated any such objection. In some cases oils were used which showed by the colour test no signs of the presence of vitamin A, and in such it proved impossible to record a definite inflexion in the absorption curve in the region of $320\text{--}328\mu\mu$. Other oils were rich in the chromogen reacting with antimony trichloride to give the blue colour, and with these in every case the $328\mu\mu$ absorption band was evident. Further, as is clearly indicated in the accompanying table, a strict correspondence is manifest between the intensity of the blue colour and that of the absorption band.

At this stage two apparent difficulties may be explained. In a dogfish-liver oil known to be rich in vitamin A, both by the colour test and by feeding experiments, the definition of the $328\mu\mu$ absorption band was apparently poor. It will be noted that in the region of $280\mu\mu$ (see Fig. 1) the oil shows relatively high selective absorption, so that actually two absorption bands are superimposed in the ultra-violet, and the $328\mu\mu$ band is partially masked. When, however, the oil is "blown" with oxygen for 1 hour at 100° a marked change takes place. Instead of the characteristic blue colour with antimony trichloride, a red colour is at once obtained. The absorption spectrum shows a marked increase in transmission over the whole region $270\text{--}350\mu\mu$. If now the curve for the "blown" oil is subtracted from that for the crude oil, the result is a well-defined absorption band with a maximum near $320\mu\mu$. The investigation of this oil demonstrates clearly that the absorption band can only be used as a measure of the vitamin A content if due care is observed in allowing for the part played by any substances absorbing strongly in a neighbouring region of the spectrum. In this connection it should be pointed out

that column (5) in Table I (p. 993) may not always represent strictly quantitative data, and that in this communication we are seeking merely to establish the generalisation that the absorption band is a true characteristic of the vitamin.

The other difficulty is that we have observed [1927, 2] a band at 318–319 $\mu\mu$ in certain samples of ground-nut (arachis) oils. Vegetable oils are often considered to be devoid of vitamin A, but Bacharach [1928] has proved that soya bean oil may be a good source. Whilst it is quite possible that arachis oils may contain vitamin A, the quantity present cannot exceed 5 % of that present in cod-liver oil. The difference between 319 and 328 $\mu\mu$ may be due to solvent shift, since alcoholic solutions are used in all cases other than arachis oil, which is used without solvent.

Various processes have been proposed for the extraction of vitamins from cod-liver oil. The well-known proprietary article "ostelin" is definitely rich in vitamin D, but is not claimed to be rich in vitamin A. Judged by the presence of an inflexion in the absorption curves of certain samples it is not always entirely free from vitamin A and this is in agreement with the method of manufacture which is such as to make it quite likely that traces of the latter will occasionally be present. Another commercial preparation, namely "essogen," gives a marked reaction with antimony trichloride, and also shows very clearly the absorption band at 328 $\mu\mu$. In addition to the cod-liver oils already mentioned, Professor J. C. Drummond has also sent us samples of vitamin A concentrates prepared in his laboratories. The materials were "unsaponifiable" extracts from cod-liver oil and sheep-liver fat, and were free from sterols precipitable by digitonin. The intensity of the 328 $\mu\mu$ band indicated that the cod-liver oil extract was 200–300 times, and the sheep-liver fat extract 800–1000 times, as potent as a typical cod-liver oil. Professor Drummond's independent estimates were 500 and 775, the figures being based on the colour test.

The correlation between vitamin A and an absorption band at 328 $\mu\mu$ would therefore seem to be proved. The connection can, however, be subjected to even stricter tests. Vitamin A is known to be easily destroyed by oxidation, and if our contention is justified this process should simultaneously destroy also the chromogen responsible for the antimony trichloride blue and effect the disappearance of the 328 $\mu\mu$ absorption band. Professor Hilditch has supplied us with samples of cod-liver oil "blown" under different conditions. Aeration at 40° for 4 hours resulted in no perceptible decrease either in the intensity of the blue colour, or in the persistence of the absorption band. On the other hand, aeration for 4 hours at 80° resulted in a very noticeable change, reflected to the same extent by the colour test and by the spectroscopic criterion. We have also found that after treatment of "essogen" or shark-liver oil with oxygen at 100° for 2 hours, the absorption band had entirely disappeared and the blue colour was so feeble as to be negligible.

The instability of vitamin A towards light makes it necessary to demonstrate not only that the vitamin can be destroyed by irradiating an oil, but

Fig. 1. Absorption spectra of dogfish-liver oils.

- I. Untreated oil 1 % solution 3 mm. cell.
- II. Same oil "blown" with oxygen for 1 hour at 100°, 1 % solution 3 mm.
- III. Same oil exposed to sunlight in a quartz vessel.
- IV. 320 $\mu\mu$ band obtained by subtracting II from I.
- V. Absorption of materials destroyed by irradiation, probably vitamin A and ergosterol: curve obtained by subtracting III from I.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Table I.

Material	Band or inflexion near 230 $\mu\mu$	Band or inflexion near 270-290 $\mu\mu$	Band or inflexion 320-328 $\mu\mu$	Units of absorption*	Units of "blue"†
Marine animal oils of known origin:					
(1) <i>Hexanchus</i>	—	Faint inflexion	Absent	0	0
(2) <i>Spinax niger</i>	—	Definite	Absent	0	0
(3) <i>Scyllium canicula</i>	—	Faint	Absent	0	0
(4) <i>Seymouria ringieris</i>	—	Faint	Absent	0	0
(5) <i>Raja clavata</i>	Inflexion	Definite	Doubtful	0	0
(6) <i>Raja batia</i>	Inflexion	Definite	Absent	0	0
(7) <i>Symmerichthys lithia</i>	Inflexion at 240 $\mu\mu$	Marked at 275 $\mu\mu$	Absent	0	1.0 circa
Cod-liver oils from Prof. Hilditch:					
(1) Oil from fresh livers	—	Ergosterol bands	Good band	10.8	9.9
(2) Chilled and filtered oil	—	Masked if present	Good band	11.0	9.9 approx.
(3) Non-freezing oil	No inflexion	Masked if present	Good band	12.0	10.5
(4) Stearin-rich oil	—	Masked if present	Good band	10.0	10.5 approx.
Blown oils from fresh livers:					
4 hours at 43°	—	No change	No change	12	Not measured
4 hours at 80°	—	Definite inflexion	Less intense	6	Not measured
4 hours at 80°	—	Definite	Less intense	<2.5	Not measured
Irradiated oil:					
Fresh liver oil irradiated for 4 days	—	Definite	Absent	0	0
Cod-liver oils of high medicinal quality from Prof. Drummond:					
"G" Scotch oil from Aberdeen	—	Ergosterol	Good band	11.5	10.0
"K" Lofoten oil	—	Inflexion 275	Smaller band	5.8	6.6
"L4" Lofoten oil	—	Inflexion 275	Clear inflexion	3.2	3.3
Essogen (Lever Bros.)	—	Ergosterol definite	Clear band	<112	Not measured
Essogen blown with oxygen for 1 hour at 100°	—	Clear inflexion	Absent	0	0
Ostelin	Inflexion	Clear inflexion	Doubtful	?	?

* The arbitrary unit of absorption here used may be defined as follows: when $\log I_0/I$ (intensities of incident and emergent light respectively) represents the extinction E , 1 unit of absorption for a 1 % solution and a 1 cm. cell corresponds with a persistence of $E=0.04$ for the 328 $\mu\mu$ band. † 1 unit is given by $\log I_0/I=0.275$ at 608 $\mu\mu$ using 2 cm. cells, 0.3 cc. of 10 % cod-liver oil in chloroform plus 3 cc. of 30 % antimony trichloride in the same solvent. This unit stands in close relationship with Lovibond units.

— indicates that definite results were not obtained.

Table I (continued)

Material	Band or inflexion near 230 $\mu\mu$	Band or inflexion near 270-290 $\mu\mu$	Band or inflexion 320-328 $\mu\mu$	Units of absorption	Units of "blue"
Shark-liver oils:					
Sample (1)	Inflexion	Ergosterol (?)	Inflexion	?	?
Sample (2)	—	Inflexion	Inflexion	<12.5	12
Sample (3)	—	Band at 280 $\mu\mu$	Very weak	<12.5	14.5
Sample (4)	Good inflexion	Masked if present	Good band	72	80
Sample (5)	Good inflexion	Masked if present	Good band	94	90
Irradiated shark oil (4) (silvered quartz test-tube)	—	Inflexion	Much weakened	Not measured	
Blown shark oil (5):	—	Inflexion	Weaker	Doubtful	13} doubtful
(1) oxygen for 1 hour at 100°	—	Marked inflexion at 275 $\mu\mu$	Much weaker	Doubtful	11} values
(2) oxygen for 2 hours at 100°	—	Small inflexion	Very doubtful	0	0
Porbeagle shark-liver oil	—				
Skate-liver oils:					
Sample (1)	—	Clear inflexion at 275 $\mu\mu$	Poor	?	<3.5
Sample (2)	—	Marked inflexion	Good band	Order 25	19
Dogfish-liver oils:					
Sample (1)	—	Band at 280 $\mu\mu$	Inflexion (see Fig. 1)	38	28
Sample (1) (blown with oxygen 1 hour at 100°)	—	Band at 280 $\mu\mu$	No inflexion	0	?
Sample (1) (exposed to sunlight in a quartz vessel)	—	Faint inflexion	Weak if at all	?	8.5
Whale oil	—	Inflexion	Faint	?	?
Wool fat	—	Inflexion	Faint band	?	Positive
Spent cod-liver oil from the Zucker process	—	Inflexion	Small band	Order 2	Not measured
"Un-sap." from cod-liver oil after removal of all sterols precipitable by digitonin (from Prof. Drummond)	No inflexion	Masked if present	Good band	600-1000	1650
"Un-sap." from sheep liver similarly prepared (from Prof. Drummond)	No inflexion	Masked if present	Good band	2500-3000	2580

that the chromogen and the absorption band can also be made to disappear under the same circumstances. It will be seen from the figures and the accompanying table that irradiation of cod-liver oil has the expected effects.

In order to put the photochemical mechanism to a final test the following procedure was adopted. A 4-inch quartz test-tube of $\frac{3}{8}$ inch external diameter was covered with a bright film of silver on the outside. The transmission spectrum of silvered quartz almost coincides with the $328\mu\mu$ absorption band, so that if an oil rich in vitamin A is placed in the tube and subjected to the action of the light from a quartz mercury lamp, the only rays capable of reaching the oil are those within the absorption band. After 24 hours' irradiation, the blue colour with antimony trichloride was noticeably weaker, and after 48 hours' irradiation the very rich shark oil used gave a test little stronger than that given by an ordinary cod-liver oil. The intensity of the $328\mu\mu$ absorption band had by this time decreased almost to zero, at the concentration used initially.

It will be noticed that in the table and figures many oils show an inflexion or band in the neighbourhood of $275\text{--}285\mu\mu$. In some of these oils, the vitamin had been destroyed during the experiments, and in others the colour test indicated the absence of the vitamin at the outset. We have recorded a similar band in fractions obtained from cod-liver oil concentrates, and it seems not at all unlikely that a decomposition product of vitamin A is characterised by an absorption band at $275\text{--}285\mu\mu$. In this connection it is interesting to recall the fact that bands in exactly the same region have been observed in un-saponifiable extracts from cotton-seed oil, and in some cases in vegetable oils themselves. Whether this is fortuitous or not is a matter which we have now under investigation.

We have also recorded in many oils an inflexion in the absorption curves at about $230\mu\mu$, but it has not so far proved possible to attach any significance to this band.

SUMMARY.

Hitherto there have been two criteria of vitamin A, the feeding test and the antimony (or arsenic) trichloride colour reaction. It is now claimed as a third property that vitamin A is characterised by an absorption band with a maximum at $328\mu\mu$.

It is probable that one of the decomposition products of vitamin A has an absorption band near $275\text{--}285\mu\mu$.

In conclusion we would like to express our thanks to Professors Drummond and Hilditch, to Mr A. L. Bacharach and Miss V. Hazley of the Glaxo Research Laboratory, and also to Messrs Creed, Gillam and Sexton for assistance in various directions. We are also indebted to the Food Investigation Board of the Department of Scientific and Industrial Research for supporting the investigation.

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