CCLXIII.—*The Absorption Spectrum of Ergosterol in Relation to the Photosynthetic Formation of Vitamin* D.

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It has been shown (Heilbron, Kamm, and Morton, *Biochem. J.*, 1927, **21**, 78) that the antirachitic potency developed on irradiation of ordinary cholesterol, either from cod-liver oil or from brain, may be traced to the presence in it of a minute quantity of some foreign substance which exhibits well-defined absorption bands near 293·5 μμ, 281·5 μμ, and 270 μμ. These bands disappear on irradiation, concomitantly with the development of antirachitic potency in the product. A similar conclusion was almost simultaneously arrived at by Pohl (*Nach. Ges. Wiss. Göttingen*, 1926), using an entirely different spectroscopic technique (monochromatic light and photo-electric cells). These results are also substantiated by the fact that cholesterol regenerated from its dibromide could not be activated by irradiation with ultra-violet light. Subsequent work developed by using the spectroscopic method rapidly led to the conclusion that ergosterol—or a similar sterol—is the actual pro-vitamin, and this has been fully substantiated by animal tests (Rosenheim and Webster, *Biochem. J.*, 1927, **21**, 127; Windaus and Hess, *Nach. Ges. Wiss. Göttingen*, 1926).

In extension of our earlier work, it became necessary to examine in detail the absorption spectrum of ergosterol, in order to establish an accurate standard of comparison by means of which we could determine the relative richness of various materials in respect of the pro-vitamin.

In agreement with the work of Pohl as briefly reported by Windaus and Hess (*loc. cit.*), our results correspond in a very remarkable degree with the absorption curve found for ordinary cholesterol :

	λ max.	λ max.	λ max.
Cholesterol	293 μμ	280 μμ	269 μμ
Ergosterol	293·5 μμ	281·5 μμ	270 μμ

Further, the results indicate that ergosterol is 2—4 × 10^3 times as active as our specially prepared cholesterol (Heilbron, Kamm, and Morton, *loc. cit.*).

We next studied the change in the absorption spectrum brought about by irradiation of ergosterol in alcoholic solution. A solution of 0·05 g. of ergosterol in 250 c.c. of optically pure alcohol was prepared and portions were exposed to the light of a practically *new* (Hewittic) quartz mercury-vapour lamp of the vacuum type. Samples were withdrawn every 15 minutes and the absorption spectrum was determined. For this purpose two Hilger E_3 quartz spectrographs were used alternately, each being fitted with a rotating disc sector photometer. Two series of absorption curves were obtained showing the selective absorption at half-hour intervals. The two sets of curves were reasonably concordant and only one will therefore be described.

1. Ergosterol. Fresh solution not irradiated. Bands at 270 μμ, 281·5 μμ, and 293·5 μμ. Minimum 230 μμ. "ε" max. 10,200. "ε" min. 2,400.

2. Irradiated for 30 minutes. Broad maximum near 275 μμ. "ε" max. 8,500. Minimum near 230 μμ. "ε" min. 3,750. The absorption band is wider in the region 230—265 μμ than the corresponding band for the fresh solution.

3. Irradiated for 60 minutes. Broad maximum near 275 μμ. "ε" max. 7,150. Minimum near 230 μμ. "ε" min. 4,850. The curve shows a marked step-out in the region 240—260 μμ, indicating the incipient appearance of a band in that region of the spectrum.

4. Irradiated for 90 minutes. The curve shows a new maximum near 247 μμ. "ε" max. 6,300 and a minimum near 230 μμ. "ε" min. 5,800. The persistence of the new band is therefore low. At the same time the absorption curve shows marked absorption over the region 260—300 μμ, indicating that the ergosterol has not entirely disappeared.

5. Irradiated for 120 minutes. The band at 247 μμ has increased in persistence, whilst the absorption over the range 260—300 μμ shows a collateral decrease.

6. Irradiated 150 minutes. The band at 247 μμ is well developed. "ε" max. 5,250, a minimum is shown at 225 μμ, and the persistence is considerable. From 247 μμ—269 μμ the absorption curve is fairly straight, but at 270 μμ a sharp inflexion commences and the curve falls slowly to the limiting position near 310 μμ.

7. Irradiated for 6 hours. The solution is now almost diactinic and all trace of selective absorption has disappeared.

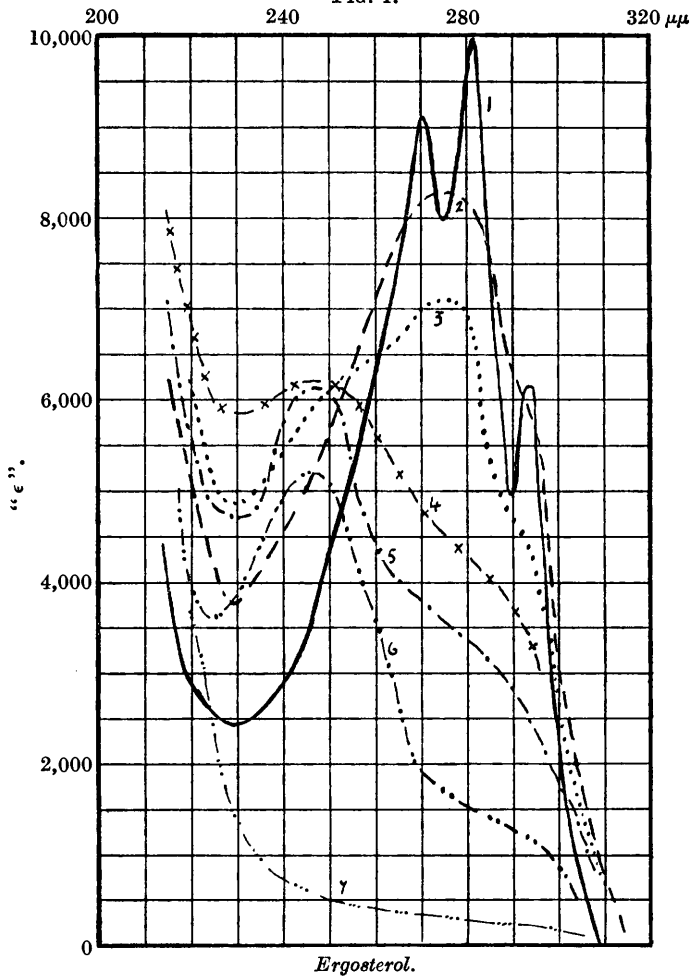
The curves (Fig. 1) show that the reaction

$$\text{ergosterol} \xrightarrow{\text{u.v. light}} \text{vitamin } D \quad . \quad . \quad . \quad . \quad (1)$$

is accompanied by the disappearance of selective absorption in the region 260—300 μμ and by the appearance of a new selective absorp-

tion in the region 230—260 μμ with a maximum at 247 μμ. This new band itself disappears with further irradiation, a fact which is

FIG. 1.

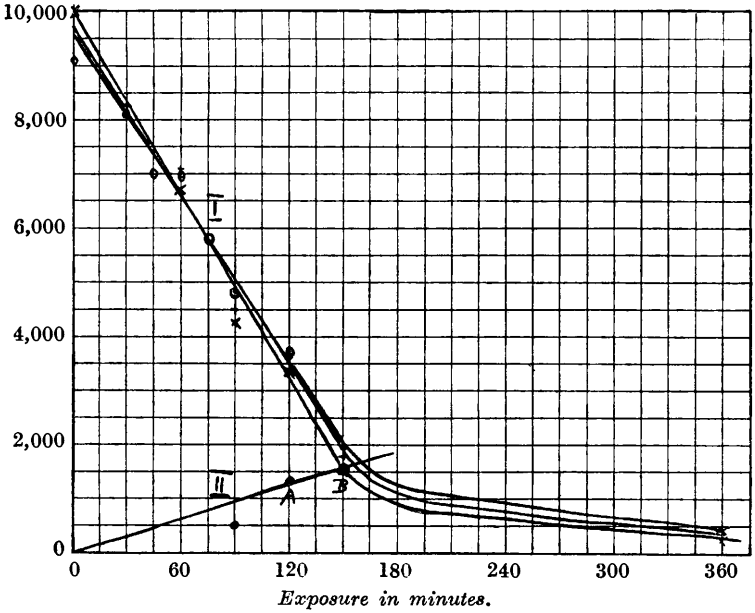


not inconsistent with the view that it is due to vitamin *D*, since it is known that "excessive" irradiation of cod-liver oil induces a loss of vitamin potency.*

* The results of biological experiments have now come to hand and show that the product giving the 247 μμ band at maximum intensity possesses very

When the extinctions at 270—275 μμ and 280 μμ are plotted against time, the result is, within experimental error, a straight line, indicating that the rate of disappearance of ergosterol depends solely on the energy input. By plotting the persistence of the new band against time, a roughly linear relation is likewise obtained (Fig. 2). These results can only mean that the *whole* of the incident energy is absorbed and is photochemically effective, within the absorption band and within the limits of the straight line relationship.

FIG. 2.



I, *extinction coefficients at* 281·5 μμ, 275 μμ, 270 μμ, *ergosterol curves.*
II, *persistence of new band* (*line* AB *produced cuts axes at origin*).

In our experience, the irradiation of thin layers of solid ergosterol is unsatisfactory. Complete disappearance of the original bands is secured only after long exposure and under conditions in which consecutive reactions are not precluded.

The absorption curves indicate a fact of considerable significance, namely, that the irradiation of ergosterol in solution or in the solid state is quite unlikely to give pure vitamin *D* unless special precautions are taken. The existence of a well-marked absorption

high antirachitic potency, whereas the product which has undergone irradiation until this band has disappeared is not effective at the same dosage. The tests were kindly carried out by Messrs. H. Jephcott and A. L. Bacharach, using the method described in their paper (*Biochem. J.*, 1926, **20**, 1351).

band between 225 μμ and 270 μμ characteristic of a photochemically unstable substance, *viz.*, vitamin *D*, means that if photodecomposition of this compound is to be avoided, *these radiations must be screened off*. The most suitable material for transmitting only light of the middle ultra-violet region is the proprietary " Vitaglass." Since, however, the rays between 270 and 293 μμ are absorbed to a considerable extent by vitaglass, very prolonged exposures will be necessary. Rosenheim and Webster (*Biochem. J.*, 1927, **21**, 392) state that " the action of ultra-violet light on ergosterol leads to an obvious physical change and the production of a yellowish resin." The nature of the intramolecular change which gives rise to the vitamin formation is at present unknown. The first step towards solving this problem is the preparation of pure vitamin *D*, and at present the line of attack most clearly indicated is the prevention of its photochemical decomposition by suitable screening.

The present results have a very definite bearing on the problem of the industrial production of vitamin *D*. Anything approaching a satisfactory yield in the reaction (1) requires prolonged irradiation —an expensive process. Screening a quartz mercury-vapour lamp with vitaglass is obviously wasteful, since the large energy emission of the 265 μμ and 253·6 μμ (resonance) lines is detrimental to the stability of vitamin *D*, and must not have access to the solution undergoing irradiation. It would seem, therefore, that the mercury vapour lamp is unsuitable for this particular photochemical reaction.

The alternative light sources are carbon or metal arcs. It is quite feasible to obtain suitably treated carbon arcs with little or no emission on the ultra-violet side of 270 μμ. On the other hand, there can be little doubt that the working cost of mercury vapour lamps is lower than that of carbon or tungsten arcs producing the same intensity of ultra-violet light of all wave-lengths. These considerations show that the photochemical formation of vitamin *D* in good yield presents a formidable problem both to research and to costing. There seems, however, every reason to expect a solution if the experiments are accurately controlled by spectroscopic means.

Some comment is necessary on a statement by Rosenheim and Webster (*Biochem. J.*, 1927, **21**, 395) to the effect that the absorption of ergosterol " extends well into the ultra-violet region of sunlight." The facts appear to be as follows. Dorno found that the limiting detectable ultra-violet wave-lengths of sunlight were : December—February, 3120 Å.; September—November, 3080 Å.; March—May, 3010 Å.; June—August, 2962 Å.; the relative intensities being Jan. 100, April 400, July 1000, October 600.

Only a very small area of the ergosterol absorption is covered by wave-lengths on the long-wave side of 2960 Å., and appreciable

photochemical reaction only appears possible in summer sunlight. This is, of course, in harmony with the seasonal incidence of rickets.

A brief remark on the paper by Hume, Lucas, and Smith (*Biochem. J.*, 1927, **21**, 362) is also necessary. These authors suggest that the mechanism of synthesis of vitamin *D in vivo* is the photochemical transformation of traces of ergosterol or a substance with similar absorption bands. Unfortunately, the transmission of epidermis falls to zero near 296 $\mu\mu$, so that a direct experimental test is excluded. We propose to investigate the matter further in two ways :

(1) To ascertain spectroscopically whether ergosterol, in solution in a quartz vessel, can be transformed into vitamin *D* by exposure to sunlight.

(2) To ascertain whether an extract containing ergosterol can be prepared from skin by means of solvents.

During the course of this work preliminary attempts were made to ascertain whether the formation of vitamin *D* is due to polymerisation. Determinations of molecular weight by the Rast micro-method failed to establish any such change, but we were not completely satisfied with the conditions under which the determinations were carried out and are repeating and extending the experiments.*

We desire to express our thanks to Mr. W. Doran and Mr. M. N. Leathwood for help in the experimental work, to Mr. F. H. Carr, C.B.E., of British Drug Houses, Ltd., for the ergosterol, and to the Food Investigation Board for a grant which has enabled this research to be carried out.

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