CXLIII. THE VITAMIN D PROBLEM¹.

I. THE PHOTOCHEMICAL REACTIONS OF ERGOSTEROL.

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IN the course of the last year several publications have appeared, *e.g.* by Kon, Daniels and Steenbock [1928], Webster and Bourdillon [1928], in which the authors came to the conclusion that the photochemical reaction of ergosterol, caused by irradiation with ultra-violet light, is independent of the wave-length used in the range from $300\mu\mu$ onward. Our own experiments on this subject, which have since been repeated and refined, prove the incorrectness of this conclusion, for we found that irradiation with light of wave-length $> 275\mu\mu$ gives rise to a series of reaction products which differs from that obtained by irradiation with light of wave-length $254\mu\mu$. This follows from the fact that the changes in the absorption spectrum are different in the two cases.

EXPERIMENTAL.

(1) *Absorption spectra.* In order to obtain the absorption-indices, we have chosen the photographic method, as worked out under the direction of Prof. L. S. Ornstein in the Physical Laboratory of Utrecht, for measuring intensities [cf. Dorgelo, 1925]. The photographs were taken with a source of light which emitted the continuous spectrum of hydrogen. It was constructed substantially according to the prescriptions given by Bay and Steiner [1927]. The use of this very suitable source of light enabled us to evaluate the absorption spectra at arbitrary wave-lengths, and thus to locate exactly all singularities of the absorption curves. The quartz spectrograph was one by Hilger, type E2; the photographs were measured with an especially constructed rapid-recording photoelectric photometer.

(2) *Irradiation.* In our earlier experiments we irradiated the ergosterol, dissolved in hexane, in a glass tray with a cover-plate of quartz. In order to exclude oxygen, argon was continually blown over the solution. As we learned however, from further experiments, that the smallest traces of oxygen might influence the results, we afterwards used only all-quartz *cuvettes*. These

¹ The present paper is the first of a series of three, which present the results of a joint research carried out at the Pharmaco-Therapeutical Institute of the University, Leyden, by Prof. W. Storm van Leeuwen, J. W. R. Everse and J. van Niekerk, and at the Physical Laboratory of the N. V. Philips' Gloeilampenfabrieken, Eindhoven, by ourselves.

were filled with the solution, cooled in solid CO_2 , evacuated with a high-vacuum pump, and then sealed off. In these latter experiments the absorption spectra were taken immediately after the irradiation, without opening the *cuvette*, in order to prevent oxidation after irradiation.

As the production of pure monochromatic radiation of sufficient intensity is very difficult, we have provisionally limited our research to experiments with two different ranges of wave-lengths, which may be obtained separately with appropriate light-sources and filters. The following light-sources were used.

(a) A quartz-mercury arc (Heraeus).

(b) A condensed magnesium spark.

(c) A mercury column in argon, which has been worked out in this laboratory: under definite conditions this column emits very strongly the resonance-line $253.7\mu\mu$, whilst the other lines are weak.

The above light-sources were used in combination with the following filters.

I. A quartz-tray of thickness 0.5 cm., filled with a 10 % solution of benzene. This filter cuts off all radiation of wave-length $< 275\mu\mu$, whereas the other radiation is scarcely weakened.

II. A quartz-tray, of thickness 2 cm., filled with saturated chlorine vapour of 15° (pressure ca. 6 atm.). This filter, whilst transmitting the line $254\mu\mu$ for about 90 %, absorbs the longer wave-lengths very strongly, so that between $\lambda = 275\mu\mu$ and $\lambda = 390\mu\mu$ less than 0.1 % is transmitted.

III. A quartz-tray, of thickness 0.5 cm., filled with a 0.5 % solution of KNO_3 , in distilled water. This filter absorbs all radiations shorter than $241\mu\mu$, whereas the other radiation is scarcely weakened.

Two separate ranges of wave-lengths were obtained.

(1) $\lambda > 275\mu\mu$, by combining the mercury arc (a) or magnesium spark (b) with the benzene filter (I).

(2) $\lambda = 254\mu\mu$, by combining the mercury column (c) with the chlorine filter (II) and the KNO_3 filter (III).

The ergosterol was prepared from yeast and purified *via* the acetate. Its melting-point was 163° in air and 165° *in vacuo*. As already mentioned, hexane was used as a solvent because of its small absorption (the absorption beginning only beyond $200\mu\mu$); moreover it may be considered as a highly indifferent solvent, as has been proved in many cases by V. Henri and his collaborators. During the irradiation, the solution was vigorously stirred by an iron stirrer, which was operated by means of an electromagnet.

Experimental results.

The changes in the absorption spectrum of a solution irradiated with light of $\lambda > 275\mu\mu$ ("long-wave" irradiation) are quite different from those caused by irradiation with light of $\lambda = 253.7\mu\mu$ ("short-wave" irradiation). This difference becomes more and more marked as the duration of the irradiation

becomes longer, but is already clearly visible after a short exposure to ultra-violet light. In order to interpret the photochemical changes by means of the resulting absorption spectra, it would be desirable to know the absorption-curves of all the substances formed. This constitutes the main difficulty, as it appears that the reaction does not stop after the formation of a first product, but that this product itself is subsequently changed, and it is not possible to separate all these products.

In order to find the absorption curve of the first-formed substance, we have chosen the following way. The absorption-curve was measured of a solution irradiated for such a short time that most of the ergosterol was left unchanged. Afterwards the amount of unchanged ergosterol was evaluated by precipitation with digitonin. On the assumption that the irradiation has not lasted long enough to cause a noticeable conversion of the first reaction product, the absorption curve of this substance can be determined as the difference between the absorption curves of the irradiated solution and of the unchanged fraction of ergosterol. As long as the curves deduced in this way from solutions irradiated during increasing lengths of time prove to be identical (of course after reduction to the same concentration), it follows that the product has not changed.

This method, of course, does not answer the question whether the first reaction product is a single substance or a mixture of substances, formed simultaneously. This would be the case, *e.g.*, if the ergosterol molecule, after absorbing a quantum of radiant energy, had different possibilities of rearrangement. This method moreover does not give insight into the further photochemical changes, only an indication of their existence.

No objection can be made against the above procedure, as the absorption spectra are taken in the absence of the damaging influence of oxygen, and as the estimation of the unchanged ergosterol may be considered to be unimpaired by oxidation of the reaction product.

The most obvious way to determine the absorption spectrum of the reaction product, *i.e.* measuring the absorption of the solution after precipitation of the unchanged ergosterol, is a very risky one. Indeed we found it only practicable, if every precaution had been taken to exclude even traces of oxygen during the whole process. The best proof that the precautions have been successful lies in the fact that the absorption spectrum established in this way tallies with that determined in the previously described way.

(a) *Long-wave irradiation.* Fig. 1 gives the absorption curve¹ of an un-irradiated ergosterol solution (concentration 80 mg./l.), Figs. 2–10 those of solutions irradiated during 1, 3, 7, 15, 45 minutes, 1½, 13, 25 and 40 hours respectively. These figures show clearly that during the first 15 minutes the

¹The absorption coefficient a is given by: $a = -1/d \log \text{nat } I/I_0$, where I_0 and I represent the intensities of incident and transmitted light (after correction for losses by reflection on the walls of the *cuvette* and for the absorption of the solvent); d measures the thickness of the layer in mm.

irradiation substantially causes an increase in absorption over the whole range; while later on the absorption diminishes in the long-wave range, whereas it remains about the same at shorter wave-lengths. One very interesting fact however is, that the absorption in the maximum at $282\mu\mu$ diminishes from the beginning, while in that at $271\cdot5\mu\mu$ the first change is an increase in absorption. This proves conclusively that for the first reaction product the absorption index at $282\mu\mu$ is smaller than that for ergosterol, whereas at $271\cdot5\mu\mu$ it is larger. Fig. 11 gives the absorption curve of this substance, which we will provisionally call *L*, calculated as described above, for the concentration of 80 mg./l. As a consequence of a possible accumulation of errors associated with this calculation the curve obtained may be incorrect in minor details, but it certainly is substantially correct. The dots and the crosses are calculated from different irradiated solutions, and agree very well. Further, it can be shown in the following way that as long as the degree of conversion of the solution does not exceed a certain amount the first reaction product is not destroyed.

Fig. 1.

Since for an arbitrary wave-length the absorption indices are now known for ergosterol and for the substance *L*, say α_1 and α_2 , the absorption index of an irradiated solution, say α_i , must satisfy the condition: $\alpha_i = x\alpha_1 + y\alpha_2$ in which x and y represent the relative amounts of unchanged and changed ergosterol respectively. This is true as long as no secondary reaction takes place. As analogous equations hold for all wave-lengths (with other values of α but with the same x and y), we can write down a system of equations that must be satisfied simultaneously by the same values for x and y . By applying this procedure to the absorption curves Figs. 2, 3, 4 and 5, and solving for x and y by the method of the least squares, we found the figures given in Table I.

Table I. *Calculated composition of ergosterol exposed to long-wave irradiation.*

Time of irradiation mins.	Ergosterol %	Substance <i>L</i> (vitamin D) %	Sum %
1	90.0	10.4	100.4
3	77.1	22.8	99.9
7	64.8	34.7	99.5
15	48.8	50.5	99.3

Figs. 2-10.

With the aid of these values for x and y , the absorption curves of the irradiated solutions can be calculated. Table II shows the very close agreement between the calculated and experimental absorption indices.

Table II. *Comparison of calculated and experimental absorption coefficients (α), of ergosterol exposed to long-wave irradiation.*

Wave-length (μ)	Irradiation 1 min.			Irradiation 3 mins.		
	α exp.	α calc.	Difference in %	α exp.	α calc.	Difference in %
234.8	0.098	0.100	-2	0.135	0.132	+2
238.8	0.110	0.111	-1	0.146	0.146	0
246.1	0.141	0.143	-1.5	0.184	0.182	+1
250.7	0.187	0.187	0	0.224	0.224	0
253.7	0.215	0.211	+3	0.247	0.248	-0.5
255.2	0.231	0.224	+2	0.265	0.260	+2
262.3	0.319	0.322	-1	0.340	0.345	-1.5
264.3	0.320	0.326	-2	0.341	0.350	-2.5
271.5	0.449	0.437	+3	0.447	0.444	+1
276.3	0.365	0.367	-1	0.371	0.378	-2
282.0	0.446	0.452	-1.5	0.439	0.442	-1
289.3	0.241	0.242	-0.5	0.260	0.250	+4
293.5	0.267	0.265	+1	0.265	0.261	+1.5

Wave-length (μ)	Irradiation 7 mins.			Irradiation 15 mins.		
	α exp.	α calc.	Difference in %	α exp.	α calc.	Difference in %
234.8	0.165	0.163	+1.5	0.209	0.204	+2.5
238.8	0.177	0.179	-1	0.221	0.224	-1.5
246.1	0.221	0.220	+0.5	0.266	0.270	-1.5
250.7	0.257	0.261	-1.5	0.305	0.309	-1
253.7	0.280	0.282	-1	0.329	0.331	-1
255.2	0.295	0.294	+0.5	0.348	0.340	+2
262.3	0.359	0.369	-3	0.395	0.401	-1.5
264.3	0.360	0.372	-3.5	0.406	0.405	0
271.5	0.435	0.448	-3	0.438	0.459	-5
276.3	0.392	0.387	+1.5	0.408	0.403	+1
282.0	0.418	0.430	-3	0.412	0.420	-2
289.3	0.281	0.258	+8	0.291	0.269	+8
293.5	0.271	0.258	+4.5	0.273	0.254	+7

The fact that in Table I the sum of x and y is very nearly equal to 1, is a further proof for the correctness of our assumptions. If this method of calculation is applied to the curves of Figs. 6-10, the results are no longer satisfactory. This means that the conditions on which the calculations are based do not hold in this case, *i.e.* part of the first reaction product itself has been destroyed. In agreement herewith, the figures show that prolongation of the irradiation after at least 50 % of the ergosterol has been converted causes a gradual diminution of the absorption in the range about 270μ . This change, however, is much slower than the one first mentioned, and indicates a relative stability of the substance L toward this long-wave radiation. After 25 hours of irradiation only small traces of bands at about 290μ and 280μ are visible. These disappear completely after 40 hours, whilst the resulting absorption curve then shows an ill-defined band between 250μ and 240μ . In experiments in which the oxygen has not been carefully excluded, the absorption in this range proves to be much more marked.

After this stage has been reached, the photochemical reaction slows down still further, subsequent changes, if present, being hardly noticeable.

In the case of long-wave irradiation we succeeded in preparing the first reaction product, free from ergosterol, in such a way that its absorption spectrum was identical with that calculated above (see Fig. 11, where the unbroken line represents the measured absorption curve of this substance).

This was done by performing all operations in a metal box in which the air was replaced by a mixture of nitrogen and hydrogen, carefully freed from oxygen by leading it over platinised asbestos at 250°.

This product will be shown later to be vitamin D. It can be obtained in a crystalline state, but the melting point of the crystals is well below 0°. At room temperature the substance has the appearance of a colourless vitreous mass.

Fig. 11.

As the low melting-point makes further investigation of the crystals rather difficult, we restrict ourselves at the moment to the statement that the crystals have the appearance of lozenge-shaped plates.

(b) *Short-wave irradiation.* That short-wave irradiation gives rise to a series of reactions different from those caused by long-wave irradiation, follows from a comparison of Figs. 2-10 with Figs. 12-17, in which the absorption-spectra of a solution of 80 mg./l., irradiated during 2½, 7, 14, 60, 135 and 330 minutes respectively, with short-wave light are shown. (The exposures in the two series are not directly comparable, as the intensities of the light-sources are different.)

Now, after 2½ minutes' irradiation, the absorption has increased over the whole range of wave-lengths, 280 μμ included. Prolongation of the irradiation causes this increase to be much more marked, until after 14 minutes the absorption at longer wave-lengths is much higher than in any case of long-wave irradiation. The most striking features of the change are the gradual disappearance of the band at 262 μμ, and the large increase of the bands at 282 μμ and 293 μμ with respect to that at 271.5 μμ.

Here, too, prolonged exposure to radiation causes a further photochemical change, in this case betraying itself by a gradual decrease of the absorption in the whole range of wave-lengths which was explored. After 330 minutes no appreciable absorption is left.

In the case of long-wave irradiation we obtained an insight into the reaction mechanism by initially calculating the absorption spectrum of the first reaction product in the way outlined on p. 1296. This method appears to be impracticable here, as the calculated absorption spectrum is not independent of the degree of conversion of the solution. This shows that secondary reactions take place even when only little of the ergosterol has been changed.

However, there appears to be another way to attack the problem. This is shown by a quantitative comparison of the curves of Figs. 14, 15 and 16. Table III shows that in the wave-length range $> 262\mu\mu$, the ratios of the absorption indices of these three curves are independent of the wave-length. From this we conclude that, when the reaction has come to the stage characterised by Fig. 14, the absorption in this range of wave-length is due to one substance only, which upon prolonged irradiation is gradually transformed into a substance without appreciable absorption in this range. This is in agreement with Fig. 17, which shows the absorption spectrum of nearly the final stage.

Table III. *Ratio of the absorption coefficients (α) after 60 and 135 minutes of short-wave irradiation, to those after 14 minutes of irradiation.*

Wave-length ($\mu\mu$)	α 60/ α 14	α 135/ α 14
262.2	0.79	0.50
265.7	0.78	0.47
270.0	0.77	0.45
273.6	0.75	0.43
280.3	0.75	0.43
288.0	0.77	0.43
291.1	0.78	0.43
300.7	0.81	0.48
311.1	0.75	0.50

The curve of Fig. 14 may thus be considered as the absorption spectrum of a single product, formed in the course of the reaction. For the sake of convenience we denote this product by the letter *S*. We do not know the precise concentration of the solution for which Fig. 14 holds. However, as it follows from the foregoing, that this substance *S* is destroyed by the radiation, evidently this concentration is less than 80 mg./l. (*i.e.* the concentration of the original ergosterol solution).

Now we know already that in the earlier stages of the reaction there must be at least three absorbing substances present. Ergosterol and the substance *S* are very likely to be two of them. As we have no knowledge of the relative amounts in which they are present, we cannot, in order to evaluate the spectrum of a third substance, use a method similar to that described before. Yet we did not think it unreasonable to try whether the absorption curves

Figs. 12-17.

could be calculated on the assumption that, besides ergosterol and *S*, the substance *L* (see p. 1297) was also present.

On this hypothesis we calculated the relative amounts of these three substances, by the method of least squares. Table IV shows the observed absorption indices and those calculated on this basis for the spectra of Figs. 12 and 13.

Table IV. *Comparison of calculated and experimental absorption coefficients (α) of ergosterol exposed to short-wave irradiation.*

Irradiation 2½ mins.				Irradiation 7 mins.			
Wave-length ($\mu\mu$)	α exp.	α calc. (65.8 % erg. 13.3 % <i>S</i> 15.6 % <i>L</i>)	Difference in %	Wave-length	α exp.	α calc. (37.6 % erg. 61.4 % <i>S</i> 4.9 % <i>L</i>)	Difference in %
240.5	0.139	0.144	-3.5	239.4	0.160	0.159	+0.5
245.1	0.166	0.162	+2.5	243.7	0.186	0.185	+0.5
249.6	0.203	0.204	-0.5	248.6	0.236	0.231	+2
253.7	0.238	0.239	-0.5	253.7	0.298	0.294	+1.5
257.5	0.288	0.292	-1.5	258.7	0.384	0.386	-0.5
261.0	0.340	0.338	+0.5	263.6	0.450	0.448	+0.5
264.5	0.348	0.347	+0.5	266.6	0.511	0.512	-0
271.0	0.445	0.447	-0.5	270.3	0.579	0.585	-1
276.0	0.400	0.398	+0.5	274.7	0.573	0.580	-1.5
281.5	0.465	0.467	-0.5	280.4	0.661	0.662	-0
289.1	0.284	0.284	0	289.0	0.494	0.493	+0
292.9	0.296	0.295	+0.5	292.0	0.501	0.497	+1

The very close agreement between calculated and observed curves supports our assumption. In order to elucidate the first stage of the reaction, we calculated in the same way spectra obtained after very short periods of irradiation. In Table V a comparison is given between calculated and observed values for a time of irradiation of 45 seconds only. Here too the agreement is very good.

Table V. *Comparison of calculated and experimental absorption coefficients (α) of ergosterol, irradiated during 45 seconds with short-wave light.*

Wave-length ($\mu\mu$)	α exp.	α calc. (90.4 % erg. 0.9 % <i>S</i> 6.1 % <i>L</i>)	Difference in %
238.0	0.089	0.096	-7
242.1	0.110	0.110	0
246.2	0.134	0.129	+4
250.0	0.170	0.168	+1
253.7	0.200	0.198	+1
257.5	0.248	0.257	-3.5
261.1	0.307	0.306	+0.5
264.3	0.314	0.307	+2
271.4	0.424	0.423	+0
276.7	0.355	0.356	-0
282.1	0.445	0.447	-0.5
289.4	0.234	0.233	+0.5
293.7	0.259	0.262	-1

From the amounts of the different substances determined, it follows that in the first period of irradiation mainly the substance *L* is formed, and only relatively little of *S*; further, that the rate of destruction of *L* is also much

greater than that of *S*, so that after 7 minutes of irradiation the amount of *L* has already passed its maximum and fallen to a value less than that after 45 seconds of irradiation. The concentration of *L* never reaches a high percentage in the course of the irradiation.

Without for the moment going into details, we may state that the substance *S* is readily changed by the action of long-wave light, just as is *L* by short-wave light. This we learned from special experiments; in a subsequent paper we will return to this subject more in detail.

Antirachitic activity.

The antirachitic activity of different samples of ergosterol exposed to long- or short-wave irradiation has been tested on rats in the Institute of Prof. Storm van Leeuwen, by his collaborators J. W. R. Everse and J. van Niekerk, who will publish the outcome of this research in detail. In the meantime, he has kindly allowed us to communicate here the preliminary results.

For the samples exposed to long-wave irradiation, it was found that in the first period of irradiation the activity increased rapidly, fairly parallel with the amount of the substance *L* formed, as calculated from the absorption-spectra. After continued exposure, when the substance *L* is being gradually destroyed, the activity also decreases.

This proves that the substance *L* is identical with vitamin D.

The minimal daily dose able to cure a rat of rickets in 14 days, was 0.00001 mg. for the most potent sample that was tested. Of this last preparation about 50 % consisted of vitamin D¹.

All samples of ergosterol exposed to short-wave irradiation have an activity of 0.1 or less of the best sample of ergosterol exposed to long-wave irradiation. This activity is just of the order of magnitude we should expect on the basis of the content of substance *L* (vitamin D) found after short-wave irradiation.

A preparation of ergosterol exposed to long-wave irradiation, containing about 30 % of vitamin D, has been used by Prof. E. Gorter and Dr. J. J. Soer in the Children's Hospital of Leiden (Director Prof. E. Gorter), to cure rachitic children. They kindly permit us to mention here that complete healing was attained in all cases within 14 days by a daily dose of 0.04 mg. This dose is a hundred times smaller than that used in general.

¹ In judging the value of the figures given, account should be taken of the fact that our collaborators Everse and Van Niekerk in their physiological standardisation of our preparations consider as a "curative" dose a dose of vitamin D which is able to cure *completely* in 2 weeks rickets induced in young rats by a deficient diet given during the preceding 4 weeks. This detail is of interest since many investigators apply a less severe test and are consequently apt to find relatively lower curative doses.

DISCUSSION.

The original conception, based upon the work of Pohl [1926], that vitamin D is characterised by an absorption band at $247\mu\mu$, which was the starting point for the considerations of Morton, Heilbron and Kamm [1927, 1, 2], has of late been abandoned.

The results obtained by Rosenheim and Webster [1927], Webster and Bourdillon [1928], Bills, Honeywell and Cox [1928], Smakula [1928], Windaus, Westphal, Werder and Rygh [1929], Delaplace and Rebière [1929], Lahousse and Gonnard [1929], Fabre and Simonnet [1929] give a high degree of probability to the hypothesis that the irradiation of ergosterol produces several substances in succession, and that the antirachitic activity is connected with the first stage of the reaction. It was especially emphasised by Webster and Bourdillon [1929], that the consecutive changes in the absorption spectrum of ergosterol after increasing periods of irradiation are in agreement herewith. These authors found that an increase in antirachitic activity goes parallel with an increase in the absorption in the range of longer wave-lengths. As has been shown above, the first stage of the photochemical reaction is always accompanied by an increase in the absorption in this range, but while this increase is only comparatively small in the case of long-wave irradiation, it is very marked when short-wave ultra-violet light is used. Now we believe that in the experiments of Webster and Bourdillon [1928] the increase in absorption was mainly due to the action of the short-wave light of the mercury-arc (which light they never absolutely excluded in their experiments on absorption spectra), while the antirachitic activity will have been chiefly caused by the long-wave radiation. Thus we are convinced that the parallelism found by them is mainly accidental. A special experiment showed us that indeed in the earlier stages of the reaction irradiation with the unfiltered light of the mercury arc causes a change in the absorption-spectrum mainly due to the specific action of the short-wave light, *i.e.* the formation of substance *S*.

It is not surprising that the curve which Webster and Bourdillon give for the absorption of the reaction-product, and which they think to be of the type of that of vitamin D, has not much resemblance to our vitamin curve.

The absorption curves given by Windaus *et al.* [1929] have more similarity with our vitamin curve. They all have their maximum at greater wave-lengths however; from this and from their shape it is to be concluded that the products studied by Windaus contained an appreciable percentage of the substance *S*. So, even in the cases where the magnesium spark was used for the irradiation, the short-wave light has been of influence. As a matter of fact, Windaus *et al.* used the unfiltered light of the spark, in which, though the lines at $280\text{--}290\mu\mu$ are by far the strongest, those of shorter wave-lengths are not to be neglected.

Kon, Daniels and Steenbock [1928] found that the efficiency of the radiation for the formation of vitamin D from ergosterol was independent of the

wave-length in the range from 256 to 293 $\mu\mu$. Though at first sight there seems to be a discrepancy with our results, a closer inspection of their experimental method shows us, that, on the contrary, their results may be used to support our assumption concerning the influence of the short-wave irradiation. As a matter of fact they irradiated relatively large amounts of ergosterol with monochromatic light for such short durations that only very little of the substance was converted. We conclude for example from their data that in no case more than about 1 % had been changed. On the basis of our calculations it is probable that at such a low degree of conversion practically only vitamin D is formed, as well by short-wave as by long-wave irradiation.

SUMMARY.

1. It is shown that irradiation of ergosterol with light of wave-length 254 $\mu\mu$ ("short-wave" irradiation) gives rise to a series of reaction-products, different from that caused by irradiation with light of wave-length $> 275 \mu\mu$ ("long-wave" irradiation).

2. In the case of long-wave irradiation, the reaction proves to be comparatively simple, in so far as only one product is directly formed from the ergosterol. This product itself, which appears to be vitamin D, is after longer irradiation gradually transformed into a new and much more stable product. As the rate of destruction of the first product is rather small, it is possible to convert about 60 % of the ergosterol into vitamin D before the secondary reaction becomes important.

3. The short-wave irradiation causes changes of a more complex character. In this case there are two reaction-products present during the first period of irradiation.

It is made probable that one of these products, which is very quickly destroyed on prolonged irradiation, and thus does not accumulate to an appreciable amount, is identical with vitamin D. The other product, into which the main portion of the ergosterol is transformed, is much more stable in respect to this irradiation.

4. The absorption spectra of both vitamin D and the "short-wave" product have been evaluated.

5. By means of the absorption spectrum it is possible to estimate with much precision the amount of vitamin D present in correctly irradiated solutions.

6. Feeding-tests on children and rats have shown the first reaction-product, produced by long-wave irradiation, characterised by a distinct absorption spectrum and obtained in crystalline state, to have an antirachitic activity of a magnitude not hitherto reached.

In conclusion we wish to express our thanks to Mr J. Houtgast and Mr H. A. Kruuk for their valuable assistance in the performance of the experiments.

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