Physics. — *On the photochemical reactions of ergosterol.* By E. H. REERINK and A. VAN WIJK. (Communicated by Prof. G. HOLST.)

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In the course of the last years, the photochemical conversion of ergosterol has aroused a great deal of interest. This is mainly due to one of the substances that are formed during the mentioned reaction, the vitamin-D, which is of high importance in medicine and biology.

According to our view insufficient attention has been paid to the influence of the wavelength used for the irradiation. Researches concerning this question were made in collaboration with Prof. Dr. W. STORM VAN LEEUWEN and his co-workers Messrs. J. W. R. EVERSE and J. VAN NIEKERK, who charged themselves with the biological part. It appeared already from preliminary experiments, in which light-sources of different energydistribution over the wavelengths were used, that the course of the reaction depends on this distribution. By a systematical research we arrived at the conception that there exist two wavelength-ranges which have a typically different action on the ergosterol, see Fig. 1,

1. the wavelength-range $300\ \mu\mu > \lambda > 270\ \mu\mu$ ("longwave" range)
2. the wavelength-range at about $250\ \mu\mu$ ("shortwave" range)

By means of suitably selected light-sources and filters it was possible to realise irradiations, with each of these wavelength-ranges without light of the other range. For the purpose of irradiation the ergosterol was solved in hexane and brought into an all-quartz cuvette, which was evacuated and then sealed-off. The progress of the reaction was studied by means of the determination of the absorption-spectrum of the liquid in the cuvette. For this determination we choose the method of photographical measurement of light-intensities, as worked out in the Physical Laboratory of Utrecht, under the direction of Prof. Dr. L. S. ORNSTEIN. A tube emitting the continuous hydrogen-spectrum was used as a lightsource.

It appeared, that during the first period of the reaction (in which less than about 60 % of the ergosterol had been converted) caused by longwave irradiation, the absorption-spectra can be interpreted by the assumption, that besides the ergosterol only one single substance is present. The absorption-spectrum of this reaction-product has been established in two different manners, which gave well agreeing results :

1°. By means of digitonine the amount of unchanged ergosterol present in an irradiated solution, was determined. The absorption due to this ergosterol was substracted from that of the solution, determined before; the remaining absorption was then calculated for a solution of the reaction-

product of a fixed concentration. In case the conversion of the solution used for the determination did not exceed about 60 % the spectrum calculated in the above manner was independent on the degree of conversion.

2°. The absorption-spectrum of an irradiated solution, after precipitation of the unchanged ergosterol by means of digitonine was directly measured. This method, though being the most obvious way to determine the absorption of the reaction-product, is only mentioned in the second place, as it appears to be very difficult to perform all necessary operations under such circumstances as are required by the great sensitiveness of the product for oxygen.

Only by the fact that the product separated by us, shows an absorption-spectrum which totally agrees with that calculated in the first-mentioned manner we can be certain that we have succeeded in preparing the unchanged reaction-product.

The absorption-spectrum shows a broad band with a maximum at about 265 $\mu\mu$ and a height that is of the same order of magnitude as the maximum-height of the egosterol-curve, see Fig. 2.

From the experiments of Messrs. EVERSE and VAN NIEKERK, which are for the moment still in progress with a great number of animals, it follows that the antirachitic activity of longwave-irradiated ergosterol goes parallel with the presence of the above mentioned first reaction-product and that the activity of this product is the highest that has been found till now. *This proves the identity of this substance with the vitamine-D.*

We were able to obtain the vitamin-D in a crystallised state : the crystals form lozengeshaped transparant plates which melt below 0° C. to a colourless vitreous mass.

The absorption-curves of solutions irradiated for a longer time can no more be calculated from those of ergosterol and vitamin-D. In this following stage of the reaction the photochemical conversion of the first reaction-pro-

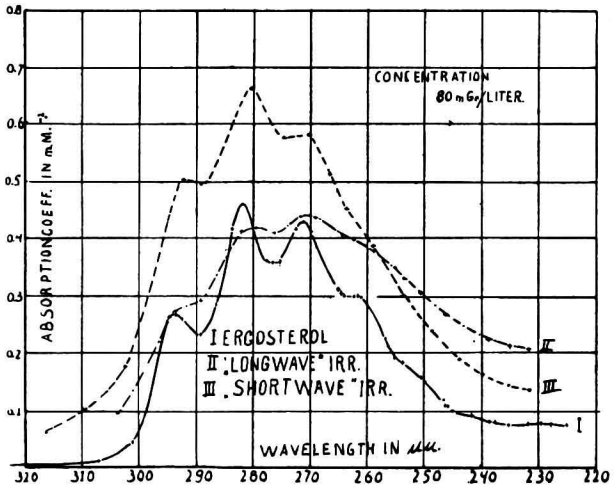

Fig. 1.

duct itself becomes appreciable, which is shown by a progressive decrease of the absorption in the range from 300—260 $\mu\mu$ (Fig. 3 shows the spectrum of the final substance formed by longwave-irradiation (curve I) in comparison with that of the final state formed by shortwave-irradiation, c.f. furtheron.)

From the absorption-curves of the irradiated solutions, it is obvious that upon shortwave irradiation the course of the reaction is totally different from that upon longwave-irradiation (See fig. 1 in which the curve I

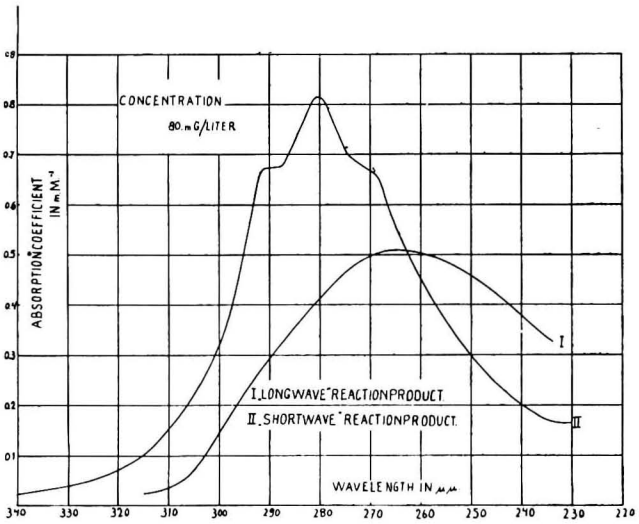

Fig. 2.

represents the absorption-spectrum of unirradiated, curve II that of longwave-irradiated, and curve III that of shortwave-irradiated ergosterol). While upon longwave-irradiation the total absorption does not change very much during a great part of the process, upon shortwave-irradiation it increases very quickly in the beginning, attains a maximum of about twice the original value and then decreases slowly till almost no absorption is left. In this case it appears impossible to describe the first stage of the reaction in such a simple manner as before. As the absorption-curves calculated from those of the irradiated solutions after substraction of the absorption of the unchanged ergosterol (determined with digitonine) depend much on the degree of conversion of the solutions, it is certain that now more than one new substance has been formed. In the second stage however, after the absorption has passed its maximum, we found that the absorption-curves of solutions irradiated during increasing times, show decreasing but proportional heights, over a great range of wavelengths. This proves that in this stage of the reaction one single absorbing substance is gradually converted into a substance with low specific absorption. These results suffice to determine the form, but not the absolute magnitude of the absorption-curve of the reaction-product present in the beginning of the

second stage. (See fig. 2 which gives this absorption-spectrum, curve II, in comparison to the curve of the vitamin-D, curve I.)

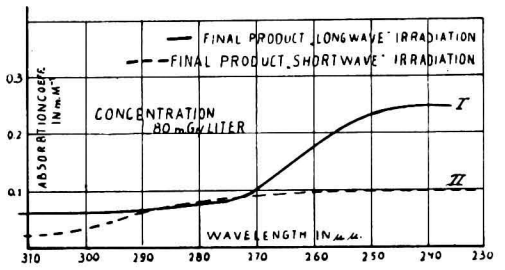

Fig. 3.

Now it appears that the absorption-curves in the first stage can be calculated in the assumption, that, besides the ergosterol, this shortwave reaction-product and vitamin-D are present. One finds that after a very short time the formed amount of vitamin-D reaches a maximum of at most 15 % of the original quantity of ergosterol and then decreases quickly, whereas the amount of the other product increases gradually till the reaction comes into the second stage. The biological experiments which can check these hypotheses, as far as the vitamin-D contents is concerned, are not in contradiction herewith, but are still in progress in order to permit us to draw definitive conclusions.

It follows from our research, that for the formation of vitamin-D the wavelength of the used light is of great importance. While by the use of light of wavelength between 300 and 270 $\mu\mu$ up to about 60 % of the ergosterol can be converted into vitamin-D *without the formation of by-products,* it is impossible to obtain more than a yield of about 15 % by the use of light of wavelength 254 $\mu\mu$, in which case *the formation of by-products cannot be prevented.* It will be necessary to pay attention to this point in case a standard for the irradiation will be chosen.

It has further become possible to determine the vitamin-D contents of irradiated ergosterol-solutions in a physical way, as the absorption-spectrum of the vitamin and the other occurring substances are established. The biological experiments show that the activity of this vitamin for the curation of a rachitic rat lies at a daily dose of 5/1000.000 mgr. during 10 days.

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