[CONTRIBUTION NO. 135 FROM THE DEPARTMENT OF BIOLOGY AND PUBLIC HEALTH, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Quantitative Measurement of the Ultraviolet Activation of Sterols. I. Ergosterol

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Ten years ago Kon, Daniels and Steenbock[1] reported that the ergs of radiant energy necessary to form from ergosterol an amount of vitamin D sufficient to demonstrate a deposition of calcium in the bones of rachitic rats was constant (700–1000 ergs) for mercury lines 2560, 2652, 2804 and 2937 Å.

Another paper, that by Marshall and Knudson,[2] reported that the rate of production of vitamin D is directly proportional to the number of light quanta absorbed by ergosterol and is independent of the wave length. They also stated that one quantum produced an average of 0.3 molecule of vitamin D.

A more recent publication[3] has reported that one International Unit of vitamin D was synthesized from ergosterol by 900 ergs of ultraviolet energy and that lines 249, 254, 265, 275, 280, 297 and 303 mμ were equally effective on a quantum basis. These results have created confusion, since they indicate that the photochemical conversion of ergosterol into vitamin D by various frequencies is dependent upon (1) the ergs of radiant energy irrespective of wave length (in the active region), (2) the quanta of energy irrespective of wave length and (3) equal ergs and equal quanta for the various wave lengths. Since ergs and quanta do not have a constant relation as wave lengths are varied, there is some obvious discrepancy.

The study which is being reported at this time was undertaken to determine more accurately the antirachitic effectiveness of the various lines in the mercury arc spectrum, and to determine the efficiency of the photochemical reaction in which ergosterol is converted into its antirachitically active isomer vitamin D (vitamin D_2 or calciferol). In this study 600 rachitic rats were used with a double quartz monochromator in a study of the active ultraviolet lines.

Procedure.—In this study we used the five active lines[4] of the mercury arc spectrum. The quantum–erg relationship of these lines is as follows.

Å.	Ergs/quantum
3025	6.50×10^{-12}
2967	6.62×10^{-12}
2804	7.01×10^{-12}
2652	7.41×10^{-12}
2537	7.74×10^{-12}

Irradiation Procedure.—In all experiments a cylindrical quartz vessel (27 mm. diameter, 152 mm. long) with flat ends was filled to capacity (approximately 80 cc.) with a 0.1% solution of ergosterol (du Pont) in C. P. anhydrous ethyl ether. This cell was mounted against the exit slit of a Bausch and Lomb quartz monochromator[4] (type no. 33-86-05) with prisms 8 × 8 cm. in such a way that the incident energy entered the front face of the vessel and passed into the ergosterol solution along the axis of the vessel.

By the use of photographic paper and malachite green leucocyanide reagent,[5] it was shown with all wave lengths studied that the energy was all absorbed in passing through the ergosterol solution.

These experiments were designed to compare the relative ability, on a quantum basis, of the different lines to activate ergosterol. Exposure times were calculated so that in a given experiment the ergosterol solution was exposed to the same number of quanta. Because of variations in the energy emission of the mercury arc the exposure times were different for each line. The emission from this lamp was measured from time to time during the course of the investigation.

To ensure the greatest accuracy the time was varied until an exposure would produce an amount of vitamin which, when fed to eight standard rachitic rats (Steenbock diet 2965), would produce a Steenbock unit of healing. Concurrent assays were run with USP Reference Oil so that in all cases a conversion into USP or International Units was possible.

The amount of energy entering the quartz cell was determined with the thermopile–galvanometer (type HS, Leeds and Northrup), using a Bureau of Standards K-4 lamp as a reference. The vertical six-inch (15 cm.) Uviarc was housed in a white transite box with an open window. The lamp was placed in the principal optical axis of the monochromator, at a distance of 13 cm. from its front face. The lamp was operated at 170–174 v. and 4.0–4.5 amp. The voltage was controlled by a photoelectrically operated voltage regulator (Stockbarger, to be published).

Following exposure the ergosterol solution was run into 20.0 g. of pure olive oil, three rinsings with anhydrous ether were added to the oil. The ether was removed from the oil under carbon dioxide by vacuum and the oil solution of irradiated ergosterol then thoroughly incorporated into 360 g. of Steenbock diet 2965. The mixture was divided into eight portions of 45 g. each and fed to eight

(1) Kon, Daniels and Steenbock, THIS JOURNAL, **50**, 2573 (1928).

(2) Marshall and Knudson, *ibid.*, **52**, 2304 (1930).

(3) Haman and Steenbock, *Ind. Eng. Chem., Anal. Ed.*, **8**, 291 (1936).

(4) Bunker and Harris, *N. E. J. Med.*, **216**, 165 (1937).

(5) L. Harris and J. Kaminsky, THIS JOURNAL, **57**, 1154 (1935).

rachitic rats. Additional 2965 diet was placed in the cages of the animals when this special mixture had been consumed. The animals were killed at the end of ten days, their leg bones X-rayed and line tested so that the degree of healing could be calibrated. In all cases, Wistar strain albino rats were placed in individual cages and given the rachitogenic diet when twenty-eight days old. They were fed the irradiated ergosterol supplement after twenty to twenty-four days on the diet.

Results

Early in the research it was found that the efficiency of our irradiation procedure was better than that reported by Marshall and Knudson,[2] for 11.6×10^{13} quanta in our hands produced more than one USP unit of vitamin D. In six successive stages the irradiation time was decreased until it was established that 7.5×10^{13} quanta produced a unit of rachitic healing.

Space does not permit a presentation of the data in full. A total of sixteen complete experiments has been run with each active mercury line, the last ten of which are summarized in Table I.

TABLE I

QUANTUM EFFICIENCY OF ACTIVATION OF ERGOSTEROL IN ETHER

Number of Runs	Animals	Lambda in Å.	Energy received in Ergs/sec.	Quanta	Average healing
10	77	2537	81.1	7.5×10^{13}	2.26
10	77	2652	86.2	7.5×10^{13}	2.39
10	78	2804	48.5	7.5×10^{13}	2.58
10	78	2967	60.3	7.5×10^{13}	2.20
10	78	3025	120.9	7.5×10^{13}	2.46
10	74	USP Reference Oil			2.33

Discussion

The arithmetical values of healing displayed in Table I show a variation between 2.20 and 2.58 for the averages of recorded response to administration of ergosterol activated by monochromatic ultraviolet of various wave lengths. The interpretation of these mathematical differences is complicated by the fact that there is no evidence that the scale of healing (1+ to 4+) represents corresponding linear variations in physiological response. The intent of the experimental work was to produce a degree of healing as nearly identical as possible with that induced by measured dosages of USP Reference Oil in the neighborhood of 2.0. Individual animal variations in response are compensated to some degree by employing groups of at least eight animals for each feeding level.

The mathematical symbols used to represent observational data in the bioassay for vitamin D are in general recognized to be approximations rather than precise values. Differences in such values, to be significant, must be greater than those which can be computed mathematically as significant upon a purely statistical basis. This defect in the assay procedure is universal and is not peculiar to the experiments reported herein. We believe that the results herein reported comprise a minimum of probable error for such work.

Considering the results for the several wave lengths on the basis of the averages for eighty animals and comparing them with the value 2.33 which is the average for control animals fed USP Reference Oil concurrently, we are of the opinion that no significant differences have been demonstrated in the quantum efficiency of the several active wave lengths of ultraviolet in the photochemical activation of ergosterol.

Upon examining the original data from another point of view, namely, by groups of eight animals, and having regard for the slight changes in severity of experimental rickets, one is impressed with a tendency for more effective activation by the mercury line 2804, and whether or not it be coincidence that this line is one that shows maximum absorption by ergosterol or whether it be an indication of correlation between absorption and activatability only further work, with probably greater refinements than are now known to us, can definitely demonstrate.

Conclusion

1. The photochemical activation of ergosterol in ether by monochromatic ultraviolet light of 2537, 2652, 2804, 2967 or 3025 Å. is substantially uniform per quantum of energy applied.

CAMBRIDGE, MASS. RECEIVED JULY 25, 1938