Summary

The true heat capacity of silicon from 60 to 300°K. has been determined and its entropy at 298°K. calculated as 4.52 entropy units.

BERKELEY, CALIFORNIA

[CONTRIBUTION FROM THE RESEARCH LABORATORY, GENERAL ELECTRIC COMPANY AND THE DEPARTMENT OF BIOLOGICAL CHEMISTRY, ALBANY MEDICAL COLLEGE]

THE FORMATION OF VITAMIN D BY MONOCHROMATIC LIGHT[1]

BY ABRAHAM LINCOLN MARSHALL AND ARTHUR KNUDSON

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It has now been demonstrated fairly conclusively by the work of Rosenheim and Webster[1a] and of Hess and Windaus[2] that ergosterol is the parent substance and apparently the only sterol from which it is possible to make a preparation by irradiation with light which is effective in curing rickets. Previous to this work it was thought that cholesterol was the provitamin, but this activity has been shown to be due to the presence of about 0.05% of ergosterol in the cholesterol, which can be destroyed by brominating the cholesterol.[1,2,3]

Several investigators have experimented with the effect of monochromatic light on cholesterol. Hess and Anderson,[4] using a quartz monochromator, concluded that the upper limit of wave length which gave any effect was 3130 Å. Light of wave length 2800 and 3020 Å. produced material of marked potency. The energy density of radiation was 3.9 ergs per second per sq. mm. but the total dose is not given. Sonne and Rekling[5] have rayed rats directly with lights of various frequencies obtained from a monochromator. The wave lengths used were 366, 313, 302–297, 280, 265, 254, 248 and 227–220$\mu\mu$ obtained from a mercury arc. The area rayed was 8.1 sq. cm. and the energy density varied from 5–0.3 $\times$ 10^{-4} g. calories per sq. cm. per minute. All the rats were rayed for thirty minutes daily. In the range 302–254$\mu\mu$ the rats were exposed to approximately 1.3 $\times$ 10^6 ergs daily and were protected from rickets by the light when fed McCollum's ricket producing diet; 248$\mu\mu$ and 240$\mu\mu$ were found to have less effect and 227$\mu\mu$ no effect. However, in the last three cases the energy used was considerably less than in those cases giving complete protection; 313$\mu\mu$ had only a doubtful action and

[1] A report of this work was presented at the Swampscott Meeting of the American Chemical Society, September, 1928.

[1a] Rosenheim and Webster, *Biochem. J.*, **21**, 389 (1927).

[2] Hess and Windaus, *Proc. Soc. Exptl. Biol. Med.*, **24**, 461 (1927).

[3] Windaus and Hess, *Nachr. Ges. Wiss. Göttingen, Math.-phys. Klasse*, 175 (1926).

[4] Hess and Anderson, *J. Am. Med. Assocn.*, **89**, 1222 (1927).

[5] Sonne and Rekling, *Strahlentherapie*, **25**, 552 (1927).

366 no effect whatever. Fosbinder, Daniels and Steenbock[6] have rayed solid cholesterol with light of varying wave lengths obtained from a monochromator. The cholesterol was fed to rats having severe rickets over a ten-day period. The animals were killed and healing was detected by means of the line test. The minimum amounts of energy required to produce a measurable effect were as follows: 234 ergs at 2650 Å., > 350 ergs at 2537 Å., 1170 ergs at 2800 Å. and 2730 ergs at 3020 Å.

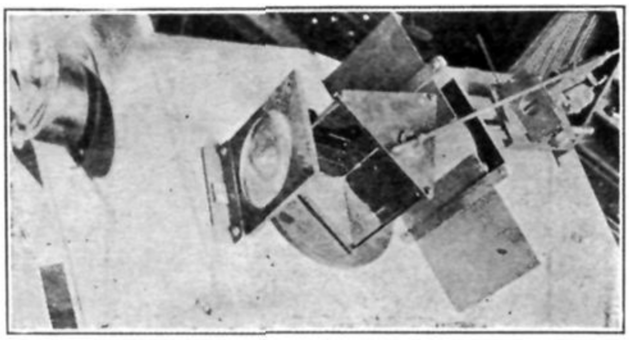


Fig. 1.—The monochromator.

In a subsequent paper[7] it was found that the quantity of radiant energy necessary to form an amount of vitamin D from ergosterol sufficient to cause demonstrable deposition of calcium in the bones of a rachitic rat was constant over the range 256–293$\mu\mu$, 700–1000 ergs being necessary.

Apparatus

A monochromator similar in design to that described by Harrison and Forbes[8] was used in this investigation.

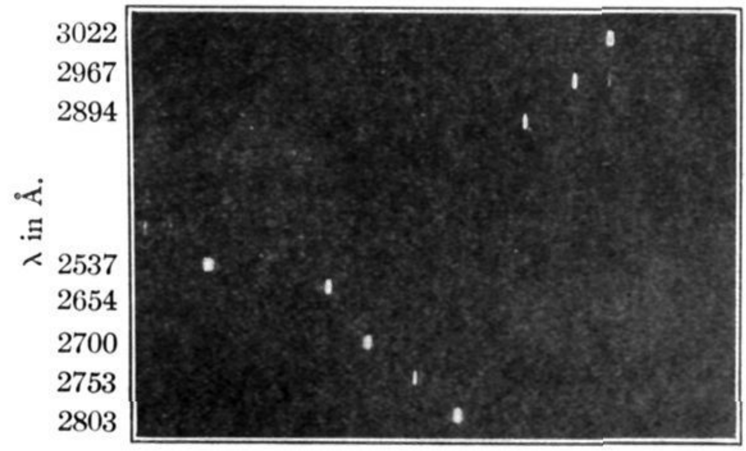


Fig. 2.—Photograph to show purity of monochromatic light obtained.

The 60° prism was made from fused quartz 135 × 135 × 135 mm. The fused quartz lenses had a ratio of focal length to diameter of 2 at 2800 Å. There was a considerable amount of spherical aberration with both lenses and this was corrected in part by the diaphragm shown in Fig. 1. This spherical aberration is largely responsible for

[6] Fosbinder, Daniels and Steenbock, This Journal, **50**, 923 (1928).

[7] Kon, Daniels and Steenbock, *ibid.*, **50**, 2573 (1928).

[8] Harrison and Forbes, *J. Opt. Soc. Am.*, **10**, 1 (1925); see also Villars, This Journal, **49**, 326 (1927).

the considerable amount of continuous background shown in Figs. 3, 4 and 5 and for most of the impurity present in the individual lines in Fig. 2. The collimator slit was made from invar and curved slightly in order to neutralize distortion in the optical system and to obtain a vertical image at the telescope slit. Both slits were adjustable as to width. A linear Moll thermopile was mounted behind the rear slit and connected to a Moll galvanometer mounted on a Julius suspension. The lamp and scale were placed two meters from the galvanometer and the latter was found to have a current sensitivity of 10^{-9} amp. for 1 mm. deflection with a current of 1 amp. in the magnet coil. A photograph of the monochromator is given in Fig. 1. Figure 2 gives a series of spectrographs taken with a large Hilger quartz spectrograph of the light incident on the telescope slit of the monochromator at various settings using a mercury arc as light source. The times of exposure at the different settings were chosen to give roughly the same exposure on the plate for each setting, which thus gives a good idea of the degree of purity of the light obtained. The dispersion of the instrument was found to be given by the equation

$$\lambda = 1100 + \frac{11{,}800}{d}$$

where d is a scale in cm. showing position of telescope slit.

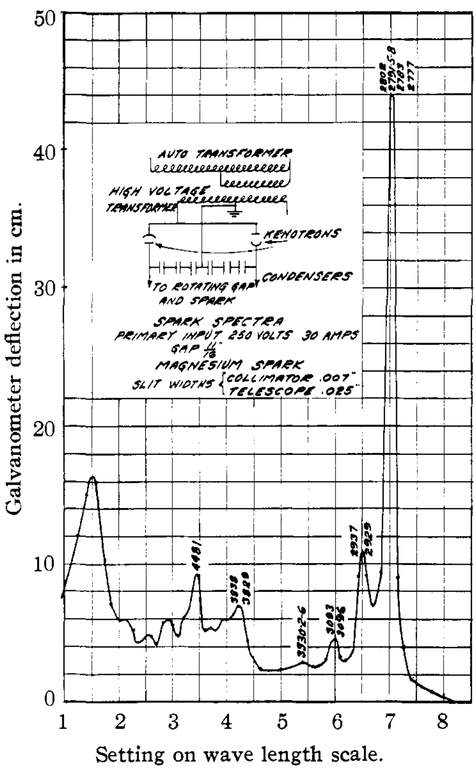


Fig. 3.—Energy distribution of magnesium spark.

Energy Calibration.—The thermopile–galvanometer system was calibrated in absolute units by means of a standard carbon lamp No. C63 supplied by the Bureau of Standards. The thermopile mounted behind the telescope slit was set at 2 meters from the lamp. The slit width was 1.22 mm. Table I gives the results of the calibration.

TABLE I

CALIBRATION OF THERMOPILE

Length of telescope slit, 27 mm. Area of slit, 33 sq. mm. 1 cm. scale deflection = 83 × $(10)^{-7}$ watt. Incident on slit = 83 ergs per second.

Lamp current, amp.	Energy incident on slit per sq. mm.	Scale reading	Incident energy/sq. mm. per cm. deflection
0.300	47.0 $(10)^{-8}$ watt	1.85	25.4 $(10)^{-8}$
0.400	85.8 $(10)^{-8}$ watt	3.53	24.3 $(10)^{-8}$
		Average	24.8 $(10)^{-8}$

Light Sources.—In order to obtain monochromatic light of high intensity at the various wave lengths, a number of light sources were used and a brief summary of the energy distribution curves of these sources will not be out of place at this point. A magnesium spark operated by means of a rotating gap from a high voltage direct current outfit illustrated in Fig. 3 was used as a source of 2900 Å. as was also the high pressure mercury arc. The spark was operated with an energy input of 7.5 kw. Variation of the number of breaks per minute between 3600 and 24,000 made no difference in the light intensity. The capacity used was 0.30 mf.; when this was reduced to 0.002 mf. the

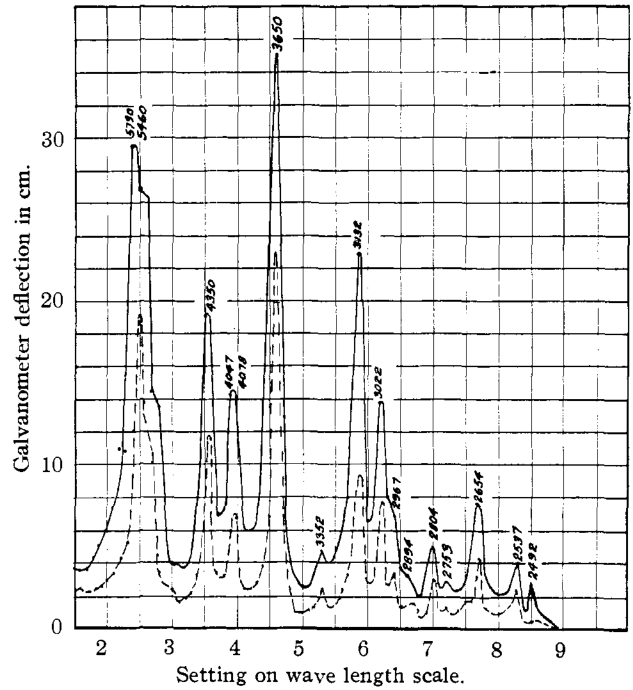


Fig. 4.—Energy distribution in mercury arc. Rating: 83 volts, 6.0 amps. Lower curve: front slit, 0.007"; rear slit, 0.010". Upper curve: front slit, 0.015"; rear slit, 0.010". Arc length, 8.5 cm.

spark would only take an input of 2.25 kw. at the original setting of the electrodes and this gave about 16% of the original light intensity. The electrodes were cylinders of magnesium $^5/_8$" in diameter tapered at the end to an area $^1/_8$" × $^1/_4$" and operated without any artificial cooling over long periods of time with very small wastage of magnesium. The energy distribution for this source is remarkable in having such a very large portion of the energy concentrated in the group of lines about 2790 Å. A number of experiments were made raying the material directly with the light from the spark. Since the absorption of ergosterol is very small for wave lengths longer than 3100 Å., this means that all the energy absorbed lies in the region 3095, 2930 and 2790 Å.; 3095 contributes 4%, 2930, 18% and 2790, 77%. This amounts to approximately monochromatic light of wave length 2800 Å. as far as ergosterol absorption is concerned.

Figure 4 illustrates the manner in which the intensity varies as the slit widths of the monochromator are changed and also give the energy distribution for the high-pressure mercury arc. From this source bands at 3022 and 2804 Å. were used. Figure 5 illustrates the energy distribution of a low-pressure mercury arc and shows that the 2536 Å. line is very strong under these conditions. It was used as a source of this wave length. A subsequent paper will discuss in detail the characteristics of this source.

A quartz cadmium arc was constructed as a source of 2300 Å. The anode consisted of an all-tungsten cylinder and a hot tungsten cathode was used as a source of electrons. The main arc tube was about 6″ long and 0.25″ inside diameter. Great care was taken in baking out the lamp and distilling in cadmium to avoid any contamination by mercury. About 2 mm. pressure of neon was admitted to the arc before it was sealed off. The bulbs containing the anode and cathode were heated so that the coldest portion was at 365°. This arc was operated continuously at 4 amps. and 64 volts for almost a week and showed no change in intensity at 2300 Å. as measured by the monochromator.

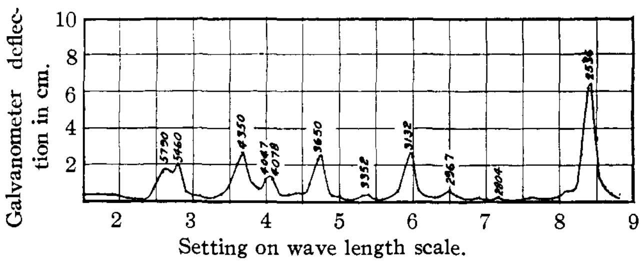


Fig. 5.—Energy distribution for low-pressure mercury arc. Mercury pressure, 0.0036 mm.; rating, 32 volts, 28.8 amps.; arc length, 30 cm.; front slit, 0.007″; rear slit, 0.025″.

Experimental Technique.—A solution of ergosterol in absolute alcohol containing 20 mg. per 100 cc. of solution was used for all the work to be reported. A quartz cell 0.5 cm. thick by 1.0 cm. wide by 3.0 cm. high was used with two sides made from ground and polished plates. The cell was placed in contact with the rear slit of the monochromator and the light path through the solution was 0.5 cm. long. Of the light incident on the slit, 5% was reflected at the polished quartz surface of the cell and the remainder passed into the solution. From the absorption curve[9] for this solution given in Fig. 6, it is possible to calculate the percentage of the light incident on the slit which was absorbed by the solution; 40% was absorbed at 3022 Å., 93% at 2800 Å., 76% at 2536 Å. and 73% at 2300 Å.

The test for antirachitic potency of the different preparations was carried out with rats, using the technique which was reported in a previous paper from these Laboratories.[10]

In brief, rickets was produced in rats by feeding them the Steenbock rachitic diet 2965 for a period of three weeks; after this time had elapsed they had usually developed a marked and uniform degree of rickets. X-

[9] These absorption curves were made by the courtesy of the Eastman Kodak Research Laboratory.

[10] Knudson and Moore, *J. Biol. Chem.*, **81**, 49 (1929).

ray pictures were taken of the rats before starting the experiment. The experimental period lasted for twenty-one days and x-ray pictures were again taken at the end of the period and in many cases after seven days. Comparisons were made with the radiograph, taken at the beginning of the experimental period, to judge the degree of healing. A "beginning healing" is indicated when there is a scattering of calcium salts which cast a hair-line shadow in the epiphyseal region; "moderate healing" when a narrow band shows in the epiphyseal area; advanced "healing" when about two-thirds of the epiphyseal area is filled up with deposit; and "complete healing" when the epiphyseal region is almost closed up, showing only a narrow cartilaginous area and the bone approaches its normal appearance. The radiographic technique as developed in our Laboratory is in most cases as sensitive as the line test; however, there are a few instances in which we have observed that the line test showed perceptible healing which was not visible by the radiograph.

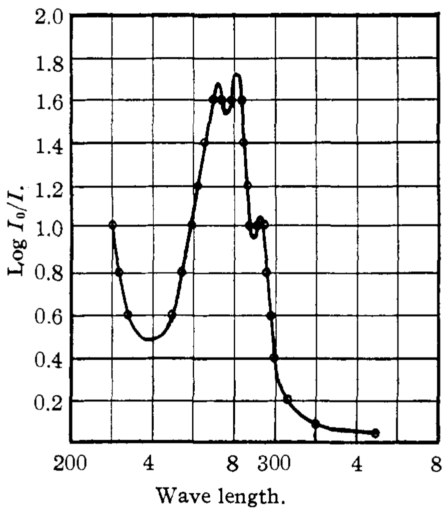


Fig. 6.—Absorption of ergosterol: 1 mg. in 10 cc. of absolute alcohol, 1-cm. layer.

Before irradiation with monochromatic light the ergosterol was dissolved in absolute alcohol and the alcoholic solution after irradiation was then added to a measured amount of olive oil. The alcohol was distilled off from the olive oil under reduced pressure, below a temperature of 60°. The test preparation dissolved in olive oil was then diluted with appropriate amounts of oil in order to have a concentration suitable for administration to the rat. In all cases we have diluted the preparations so that the dose required for administration was dissolved in 0.05 cc. of oil, and this was given directly into the mouth by means of a capillary pipet.

In arriving at the maximum potency of the preparation we would start with the dilution which we considered would give a beginning or moderate healing. We then diluted the preparations in the order of five-fold and tested them until we found a preparation that would give a beginning healing, and one that was negative. In certain instances we were unable to carry this procedure to the extinction point due to a lack of material, because of the fact that some of these preparations were radiated as long as seven days continuously, and the amount that we could irradiate at one time was 0.3 mg.

Results

Experiments were first made on the effect of varying light intensity on the minimum daily dose of radiated ergosterol necessary to show the first incidence of healing. In all exposures in a given experiment the product of intensity and time was kept constant. If the production of the vitamin varies directly with the light intensity, the minimum dose obtained from all these preparations should be constant. The magnesium spark was used as a light source and the light intensity varied by a hundredfold by moving the specimen progressively further from the source. Table II gives the result of these experiments.

TABLE II

EFFECT OF LIGHT INTENSITY ON PRODUCTION OF VITAMIN D

Time of radiation, seconds	Distance from spark, cm.	Approx. energy[11] absorbed, ergs	Daily minimum dose, mg.
2	30	2000	1/2500
20	100	2000	1/2500
200	300	10,000	1/500

In ensuing experiments the wave length was varied from 3022 Å. to 2300 Å. and the time of exposure varied 30,000-fold in some cases The data are given in Table III. Column 1 gives the light incident per second on the rear slit of the monochromator; Col. 4 the total energy absorbed by 0.3 mg. of ergosterol present in the cell during the period of radiation; Col. 6 the minimum amount of the radiated ergosterol which had to be fed daily to show perceptible healing by a radiographic method after three weeks and Col. 5 gives the amount of energy received by the ergosterol which produced this healing effect; this energy is thus the minimum amount necessary to produce a perceptible effect. Part of the experiments with 2800 Å. were performed using the magnesium spark as a source without recourse to monochromator; in this case the energies are estimated by comparison with other experiments using the monochromator.

Figure 7 shows a plot on logarithmic paper of the results of Table III. The minimum daily dose is shown as a function of the total energy absorbed by the constant amount of ergosterol which was irradiated for each experiment. The log–log plot is used to condense the wide range covered into a single plot. In the initial stages of irradiation the minimum dose varies inversely with the amount of energy absorbed; this shows that the amount of vitamin D formed is directly proportional to the light absorbed. With prolonged irradiation this linear relationship no longer holds, the net amount of vitamin D obtained being smaller for each succeeding period and finally a stage is reached where there is a loss of the

[11] This is the total energy absorbed by the ergosterol which was fed to the rat over a period of 21 days and which produced a beginning healing.

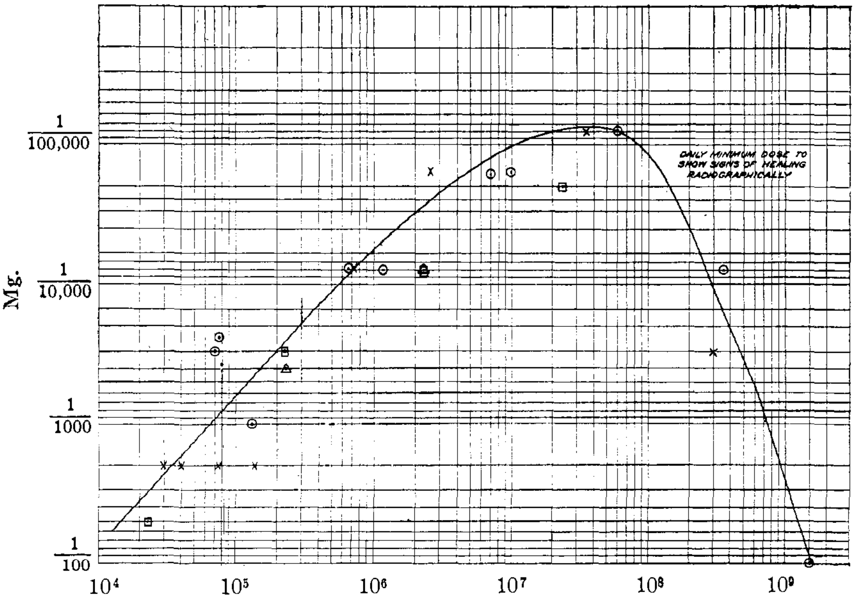
TABLE III

EXPERIMENTAL DATA

Light intensity, ergs/sec.	Time of exposure, sec.	λ in Å.	Total energy absorbed in cell, ergs	Minimum energy received by rat in 3 weeks	Daily minimum dose, mg.
2500	240	3022	$2.4(10)^5$	16,000	1/1000
2500	2400	..	$2.4(10)^6$	13,000	1/12,500
465	19,800	2800	$9.2(10)^6$	9,300	1/62,500
3850	20		$7.7(10)^4$	1,700	1/3000
1070	120		$1.2(10)^5$	8,000	1/1000
1070	1200		$1.2(10)^6$	6,400	1/12,500
	2	Mg	$7(10)^4$	2,000	1/2500
	20	spark	$7(10)^5$	4,000	1/12,500
	200	direct	$7(10)^6$	8,000	1/62,500
	2000		$7(10)^7$	40,000	1/125,000
	14,400		$3.5(10)^8$	$1.9(10)^6$	1/12,500
	57,600		$1.4(10)^9$	$9.3(10)^8$	1/100
880	60	2536	$4(10)^4$	5,300	1/500
3300	20		$2.9(10)^4$	3,800	1/500
3300	95		$1.4(10)^5$	3,800	1/2,500
3300	1,800		$2.6(10)^6$	2,600	1/62,500
3400	10,800		$3.7(10)^7$	20,000	1/125,000
3400	86,400		$3.0(10)^8$	$8(10)^6$	1/2,500
3400	590,000		$2.6(10)^9$	....	<1/100
2600	30		$7.8(10)^4$	5,000	1/1,000
2600	300		$7.6(10)^5$	4,000	1/12,500
400	60	2300	$2.3(10)^4$	6,100	1/250
400	600		$2.3(10)^5$	6,100	1/2,500
400	6000		$2.5(10)^6$	13,300	1/12,500
400	60,000		$2.4(10)^7$	24,000	1/62,500

vitamin with further irradiation. It is thus evident that we are dealing with two simultaneous reactions of light: first, a synthesis of vitamin D from ergosterol and second, a destruction of the vitamin. As the reaction proceeds the ergosterol concentration is decreasing and the vitamin D increasing, so that in the later stages the destructive role of the light predominates. We have only carried out prolonged irradiations at 2800 Å. and 2536 Å., since these experiments require intense sources of light to enable one to carry them out in a reasonable time; at 2536 Å. the longest period of irradiation was seven days and to obtain a similar amount of energy at 2300 Å. would have taken five weeks. In the two cases investigated the results were identical and there is no reason to assume that the results with other frequencies would have been different. Vitamin D has to absorb light in the same wave length region as ergosterol in order to be destroyed by light of the same frequency as that which forms it. About 10^8 ergs of light energy were necessary to produce the maximum concentration of vitamin D in 1 mg. of ergosterol dissolved in 5 cc. of absolute alcohol. From these results it is now possible to state that a concentration of vitamin D corresponding to a minimum daily dose of 1/125,000 mg. is the maximum concentration which can be produced by direct irradiation of ergosterol. The results for all the wave lengths are identical within the accuracy of the biological method used for testing.

Tanret[12] gives the formula $C_{27}H_{42}O\cdot H_2O$ for ergosterol, which gives it a molecular weight of 400. One milligram of ergosterol contains 1.5×10^{18} molecules. A quantum of 2800 Å. contains 7×10^{-12} ergs. Two thousand ergs of 2800 Å. absorbed by ergosterol and fed to a rat with rickets over a



Total energy in ergs for 0.3 mg. of material.

△, 3022 A°; ×, 2536 A°; ⊙, 2800 A°; ⊡, 2300 A°.

Fig. 7.

period of three weeks produced a perceptible healing. This amount of energy contains 3×10^{14} quanta. The rat received 1/150 mg. of the irradiated material over a period of three weeks or about 10^{16} molecules. The purest preparation of vitamin D which has been prepared by Bourdillon and Webster[13] produced a perceptible healing with 1/20,000 mg. fed over a three-week period, so that if we assume this preparation to have been pure vitamin D, our material in this case contained about 0.7% of the vitamin or approximately 10^{14} molecules. This means that 1 quantum produces on the average 0.3 molecule of vitamin D. In another experiment where a concentration of 16% vitamin D was reached, 3.7×10^{17}

[12] Tanret, *Compt. rend.*, **147,** 75 (1908).

[13] Bourdillon and Webster, *Proc. Roy. Soc.* (London), **104B,** 561 (1929).

quanta produced 7.2×10^{16} molecules of the vitamin, giving a quantum efficiency of 0.20. Fourteen-fold increase in the amount of radiation received by the sample increased the vitamin D concentration to 35%; in other words, 5.3×10^{18} quanta produced 16×10^{16} molecules of vitamin D instead of 1×10^{18} molecules as calculated from the quantum efficiency in the previous experiment. There were only 4.5×10^{17} molecules of ergosterol present in the cell; this means that the quantum efficiency for the transformation of vitamin D to some inert substance is greater than for its production from ergosterol. It is evident from these experiments that vitamin D must itself possess a strong absorption band in the same region as ergosterol, contrary to the work of Heilbron, Morton and Kamm.[14] Since this work was completed two papers[15,16] have been published giving experimental proof that the absorption spectrum of vitamin D occurs in the same spectral region as that of ergosterol. Smakula has shown that a solution of ergosterol from which air is not excluded completely loses its characteristic absorption spectrum after standing for a few months. He has measured the absorption spectrum of a solution of ergosterol during the course of radiation and found at first a slight decrease in absorption and then an increase followed later by a decrease and disappearance of the characteristic absorption. On the basis of a rather questionable rate of disappearance of ergosterol he calculates the absorption spectrum for vitamin D with two maxima at 293 and 260$\mu\mu$. Bourdillon and Webster have examined the absorption spectra of irradiated ergosterol solutions both before and after the removal of ergosterol and have found maxima at 270 and 280$\mu\mu$ which they believe to be due to vitamin D. The material with an absorption maxima at 240$\mu\mu$ is formed by radiation from vitamin D and has no antirachitic action; it in turn is changed by further irradiation to a substance showing no specific absorption. The extinction coefficient for a substance which they think is mainly vitamin D is 40% greater than that of ergosterol and the absorption curve is practically the same shape. They have prepared a material whose biological action can be detected radiographically in doses of 1/400,-000 mg. daily and we have assumed that this material is pure vitamin D in calculating quantum efficiencies in this paper.

Summary

1. Several intense sources are described for obtaining monochromatic light of various wave lengths.

2. The rate of production of vitamin D from ergosterol is proportional to the first power of the light intensity.

[14] Morton, Heilbron and Kamm, *J. Chem. Soc.*, 2000 (1927).

[15] Smakula, *Nach. Ges. Wiss. Göttingen, Math.-phys. Klasse*, 49 (1928).

[16] Bourdillon, Fischmann, Jenkins and Webster, *Proc. Roy. Soc.* (London), **104B**, 561 (1929).

3. The rate of production of vitamin D is directly proportional to the number of light quanta *absorbed* by ergosterol and independent of the wave length of the light used.

4. Vitamin D absorbs in the same wave length region as ergosterol and is destroyed by light of the same wave length as that which forms it.

5. The highest concentration of vitamin D which can be produced by direct irradiation of ergosterol is 35%. This is an absolute maximum and the probable value is lower.

6. The quantum efficiency is 0.3 molecules of vitamin D per quantum of light absorbed.

SCHENECTADY, NEW YORK

[CONTRIBUTION NO. 31 FROM THE EXPERIMENTAL STATION, E. I. DU PONT DE NEMOURS AND COMPANY]

REDUCTION OF METAL OXIDES BY HYDROGEN

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A common method for the preparation of metal catalysts consists in first preparing a finely divided oxide and then reducing the oxide with hydrogen. Usually the lower the temperature of reduction the greater the catalytic activity. Low temperature means a slow process of reduction. The present article is concerned with a new method for studying the rate of reduction. Nearly all prior work in this field has been done by passing hydrogen over the heated oxide and collecting and weighing the water formed at definite time intervals. The present method involves following the rate of reduction by measuring the volume of hydrogen consumed.

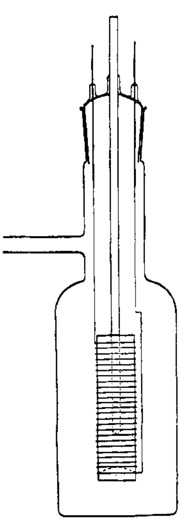

Fig. 1.

Method.—The essential feature of the apparatus is shown in Fig. 1. Platinum wire was wound on a mica cylinder to make a small electric heater, 2 by 6 cm. This heater was suspended on stout nickel wires in a pyrex vessel. The nickel wires were brazed to tungsten wires for sealing through the ground stopper of the vessel. These stout wires served both as supports and electrical leads. A small glass tube sealed to the stopper extended to the middle of the heater and served as a well for a fine-wire chromel–alumel thermocouple. The bottom of the mica cylinder was closed with a piece of platinum gauze wedged on the inside.

The rest of the apparatus consisted of a compensated gas buret, electrolytic hydrogen generator, and Hyvac pump. Electrolytic hydrogen was generated from sodium hydroxide with nickel electrodes, passed over a glowing platinum wire to free it from traces of oxygen, and dried by Dehydrite. Suitable stopcocks were provided so that the buret could be refilled rapidly and repeatedly during the course of a run.

The heater was charged by putting a layer of porcelain chips in the bottom, then