Summary

Benzalaniline is reduced by a mixture of magnesium and magnesium iodide to the iodomagnesium derivative of dianilino-dibenzyl.

Benzophenone-anil is reduced by the binary system to the iodomagnesium derivative of N-benzohydrylaniline.

Benzil-anil takes up two MgI groups to give a stilbene derivative which is very reactive to iodine, oxygen and carbon dioxide. Hydrolysis of the reduction product yields anilbenzoin.

The results of this investigation substantiate the hypothesis that the active reducing agent is MgI.[16]

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF PURDUE UNIVERSITY AND THE ELI LILLY LABORATORIES]

THE ACTIVATION OF ERGOSTEROL WITH RADIUM EMANATION

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Introduction

The existence of an antirachitic vitamin (D) has been recognized for some time. Recently, however, it has become possible to prepare an antirachitic substance by "irradiative" or "activating" ergosterol with ultraviolet radiation. Such a product is called viosterol and is considered by some to be vitamin D.

To Huldschinsky[2] perhaps should go the credit for first using ultraviolet light on children afflicted with rickets. Two years later in 1924, Steenbock reported on his work[3] with rats cured by the use of activated cholesterol. He took out patents that same year. The high-speed electrons from a Coolidge tube have been used by Knudsen.[4] He and C. N. Moore activated cholesterol in a few minutes with 200,000 volt electrons, such that 0.0050 g. per day was the minimum dosage required to cure rachitic rats, whereas 0.00002 was the minimum dosage using ultraviolet activated cholesterol.

The activation of cholesterol or ergosterol with radium has not been

[16] In a recent article [*Z. physik. Chem.*, **153**, 83 (1931)], Sven Bodforss reports results obtained from a study of magnesium potentials in aqueous salt solutions, which are best explained by considering that magnesium metal can send into solution univalent ions, Mg^{+}.

[1] Deceased Jan. 20, 1931.

[2] Hess, "Rickets, Osteomalacia and Tetany," p. 107.

[3] Steenbock, *J. Biol. Chem.*, **61**, 405 (1924); **64**, 263 (1925).

[4] Knudsen, *Proc. Soc. Exptl. Biol. Med.*, **24**, 366 (1927); *J. Biol. Chem.*, **81**, 49 (1929).

reported. Chick and Tazellar[5] used radium emanation in a study of vitamin A deficiency in rats. Their results were negative. Kofman and Chizet[6] demonstrated that radium emanation through x-rays made sterols photoactive even as ultraviolet had done.

In this investigation ergosterol was activated with radium emanation and the results as given below show that a reasonably active viosterol can so be produced.

Experimental Methods and Results

Reasonably pure ergosterol was activated with radium emanation on four different occasions under different experimental conditions and with two varieties of ergosterol. The one sample, prepared at the Eli Lilly Laboratories, had a melting point of 148°, and a specific rotation of −100° in chloroform solution. The other sample, sent from Germany by C. H. Boehringer Sohn, had a more crystalline appearance, a melting point of 157° and a specific rotation of −121° in chloroform.

The activated samples were tested on white rachitic rats by C. R. Eckler of the Eli Lilly Laboratories under the direction of Mr. H. W. Rhodehamel. A modification of the line test[7] was employed for the determination of the antirachitic potency. The extent of healing is designated in the tables by the following notation:

− − − − no healing	+ healing (calcification line continuous)
+ − − − very slight healing	++ moderate healing
+ − − slight healing	+++ marked healing
+ − healing (calcification line not continuous)	++++ complete healing

There is also included in the tabulation of results a quantity called "millicurie hours per cubic centimeter" (mch/V) which is a measure of the concentration of radium emanation multiplied by the time of its action on the ergosterol and is therefore a measure of the amount of radiation to which different samples were exposed and so can be used in the comparison of samples. The quantity millicurie hours is given by the definite integral

$$mch = \int_{t_1}^{t_2} Q \, dt \tag{1}$$

where

$$Q = Q_0 e^{-kt} \tag{2}$$

which is the radioactivity decay formula. If the units of time are in hours or days, the relations become

$$\log Q = \log Q_0 - 0.00326\, t \text{ (hours)} \tag{3}$$

$$\log Q = \log Q_0 - 0.0782\, t \text{ (days)} \tag{4}$$

$$mch = 133.2\,(Q - Q_0) \tag{5}$$

[5] Chick and Tazellar, *Biochem. J.*, **18**, 1346 (1924).

[6] Kofman and Chizet, *Compt. rend.*, **189**, 45 (1929).

[7] Bills, *J. Biol. Chem.*, **51**, 41 (1922).

In each of the four series, the radium emanation was mixed with air in a Ramsay style gas buret over mercury and admitted to the evacuated tube containing a known amount of ergosterol. In Series I, approximately one gram of the Eli Lilly sample was placed in each of three tubes, with stopcocks attached. Each tube had a volume of about 15 cc. About 0.2, 0.3 and 0.5 of 60 millicuries of radium emanation was admitted to each tube, and left in contact for three and one-half, eighteen and seventy-two hours and labeled as R-1, R-2 and R-3, respectively. At the end of seventy-two hours there was no visible sign of decomposition except a pale yellow color. The results given in Table I show that for a 0.0001 g. dosage, 20 millicurie hours per cc. is sufficient to activate ergosterol. For a 0.00001 g. dosage it is necessary to submit it to about 100 *mch* per cc.

Sample R-3 was retested after three months and also after five months, at which time the results were practically identical. This would indicate that the activated samples were permanent.

TABLE I

AMOUNT OF IRRADIATION AND EXTENT OF HEALING FOR SAMPLES OF SERIES I

Q_0 = 12, 18 or 30 millicuries, respectively. Vol. of containers, 15 cc.

Sample no.	Withdrawn after	*mch/V*	Dosage in grams per day: 0.0002	0.0001	0.00001	0.000001	0.0000001
R-1	3.5 hours	2.7	(2) ++ (3) +− (1) −	lost weight	died	−	
R-2	18 hours	20.2	(6) ++	++	−	−	
R-3	72 hours	110.1	(8) +++ (2) ++	++	++	+−−	
Ultraviolet treated					(6) +++	(2) +++ (1) ++	+−−

The numbers in parentheses indicate number of rats giving the indicated results. No figure means only one rat used.

Series II.—The second set of samples was prepared with the purpose of determining whether prolonged radiation would modify the antirachitic properties of the ergosterol. For this experiment, 114 millicuries of emanation was available and after mixing with air in the usual way, it was placed with 7 g. of ergosterol in a 30-cc. tube, fitted at one end with a rubber tube (one end closed) and a pinch clamp, so that samples could be withdrawn without loss of emanation or admitting any undue amount of air. Five samples were withdrawn, the first after seventeen hours, the last after having been in contact with the emanation for two hundred eleven hours. The results (see Table II) substantiated those of the first series. The lowest radiation this time was 50 *mch* per cc., and was more than sufficient to activate for dosages of 0.0001 g. For dosages of 0.00001 g. per day, more than 100 units of radiation were required. The results also indicated that there was no noticeable deleterious effect due to the pro-

longed irradiation; in fact, such samples were more efficient, as shown by the results when dosages of 0.000001 g. were used.

The result for X-5 with the 0.000001 g. dose was perhaps not as conclusive as might be desired. The rat which gave this negative result seemed to be a poorer specimen than usual.

TABLE II

AMOUNT OF IRRADIATION AND EXTENT OF HEALING FOR SAMPLES OF SERIES II

Q_0 = 114 millicuries. Vol. of container, 30 cc.

Sample no.	Withdrawn after	*mch/V*	Dosage in grams per day			
			0.0001	0.00001	0.000001	0.0000001
X-1	17 hours	47	(2) +++	(2) −	(2) −	−
X-2	41 hours	134	(2) +++	(2) +−−−	(2) −	−
X-3	93 hours	254	(2) ++++	(2) +−	(2) −	−
					−	
X-4	162 hours	356	(2) ++++	(2) ++	+−−−	−
X-5	211 hours	400	(2) ++++	+−−−	−	−
				+		−

Series III.—The third series of samples was prepared using the ergosterol prepared by Boehringer Sohn in Germany. It was also our belief that the active rays from the emanation did not penetrate very deeply into the ergosterol, so that by crushing the material, fresh surfaces would be exposed to the rays and so increase the potency of the product. For the latter a specially designed Pyrex tube, filled half full with glass rods, was constantly rotated at the rate of twelve revolutions per minute. The effective volume of this apparatus was 56 cc. About 4 g. of ergosterol was put in with 31 millicuries of emanation. As a comparison experiment with Series I and II, about 2 g. of the German ergosterol was placed in the 30-cc. tube used for Series II with 16 millicuries of emanation. (The emanation was first mixed with air and this mixture subdivided itself between the two containers in the ratio of their volumes.)

It should be mentioned that Sample R-19 was the ergosterol which had caked on the inside of the rod mill and was dissolved out with alcohol. Upon evaporation of the solvent, there was evidence that organic decomposition products were present. There was only a small amount of yellow product at the crystallizing edge, however.

In Table 3A are listed the results for the control samples and in 3B the results for the stirred samples.

Series IV.—The fourth series of samples was prepared to obtain a check on the previous results and to compare the efficiency of stirring with Eli Lilly product in place of the German material.

The samples were activated in substantially the same way as in Series III. A smaller rod mill was made, with 23-cc. capacity. The A-1 to A-7 samples were prepared in it. The B-1 to B-7 samples were activated in the 30-cc. tube used previously. At the start 120 millicuries of emanation

TABLE III

AMOUNT OF IRRADIATION AND EXTENT OF HEALING FOR SAMPLES OF SERIES III

A—German ergosterol, not stirred

Q_0 = 16 millicuries. Vol. of container, 30 cc.

Sample no.	Withdrawn after	*mch/V*	Dosage in grams per day 0.0001	0.00001	0.000001
S-1	2 hours	1.0	(4) −	(2) −	(2) −
S-2	3 hours	1.6	(2) −		
S-3	7 hours	3.6	(2) −		
S-4	17 hours	8.1	(2) −	(2) −	
S-5	41 hours	18.8	(2) −	(2) −	
S-6	93 hours	35.7	(2) −	(2) −	
S-7	165 hours	50.3	(4) −	(2) −	(2) −
			(2) +−		
S-8	310 hours	64.0	Broken in mail		

B—German ergosterol, stirred

Q_0 = 31 millicuries. Vol. of container, 56 cc.

Sample no.	Withdrawn after	*mch/V*	Dosage in grams per day 0.0001	0.00001	0.000001
R-11	1 hour	0.5	(4) −	(2) −	
R-12	3 hours	1.6	(2) −		
R-13	7 hours	3.7	(2) +		
R-14	17 hours	8.7	(2) ++	(3) −	
R-15	41 hours	19.5	(2) ++	(2) +−	
				(1) −	
R-16	93 hours	37.0	(2) +++	(1) +	
				(4) +−−	
R-17	165 hours	52.3	(2) +++	(4) +−−−	(2) −
				(3) −	
R-18	310 hours	66.8	(2) ++	(4) +−	(2) −
				(4) −	
R-19	310 hours	66.8	(2) +++	(4) +−	(2) −
				(4) −	

was available, and admitted into the evacuated apparatuses connected in series. The concentration of emanation was therefore 2.8 m.c. per cc., since the ergosterol occupied about 10 cc. of the total volume.

TABLE IV

AMOUNT OF IRRADIATION AND EXTENT OF HEALING FOR SAMPLES OF SERIES IV

A—German ergosterol, not stirred

Q_0 = 70 millicuries. Vol. of container, 30 cc.

Sample no.	Withdrawn after	*mch/V*	Dosage in grams per day 0.0001	0.00001
B-1	20 minutes	1.0	(2) −	
B-2	65 minutes	3.0	(1) −	
B-3	160 minutes	7.5	(2) −	
B-4	330 minutes	15	(2) +−	(2) +−−−
B-5				
B-6	39.6 hours	96	(2) ++ to +++	(2) + to ++

TABLE IV (*Concluded*)

B—Lilly ergosterol, stirred

Q_0 = 50 millicuries. Vol. of apparatus, 23 cc.

Sample no.	Withdrawn after	*mch/V*	Dosage in grams per day: 0.0001	0.00001	0.000001	0.0000001
A-1	23 minutes	1.0	(2) −			
A-2	65 minutes	3.0	(2) −			
A-3	160 minutes	7.5	(1) −			
A-4	330 minutes	15	(2) +−		(1) −	
A-5	15.7 hours	42	(2) ++		(2) −	
A-6	39.5 hours	96		(2) ++	(2) −	
A-7	184.0 hours	280		(2) ++	(2) +−−−	(2) −

The results of previous series were well confirmed (see Tables IVA and IVB) although the results with 0.0001 g. doses seem to indicate that the German product activates slightly faster, being capable of producing moderate healing after 10 *mch* per cc. of radiation, while the Eli Lilly ergosterol required 40 units of radiation. At 70, both gave results of "marked healing." The 0.00001 dosage also indicated that the German product had only a slightly higher degree of potency.

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Summary

1. Ergosterol can be activated with radium emanation to a degree of potency perhaps 0.01 of that of a good grade of ultraviolet irradiated ergosterol.

2. Stirring the sample while under the influence of the emanation increases the speed of activation but does not increase the potency appreciably.

3. There is no appreciable loss in potency if the activation is carried on to even as much as a twenty-fold excess. There are some decomposition products formed in all cases.

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