THE ULTRAVIOLET IRRADIATION OF CALCIFEROL IN VARIOUS SOLVENTS

By

Robert Yates

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

1952

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. D. T. Ewing for his encouragement and valuable guidance throughout this investigation.

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THESIS ABSTRACT

The irradiation of calciferol has been studied to some extent previously but the reactions involved and their relationships have not been completely elucidated. This investigation was undertaken to study these reactions and their relationships and to determine the effects of wavelength of irradiating energy and solvent on the reactions.

The irradiations were conducted in ethanol, n-hexane and a mixture of n-hexane and ethyl ether using several lines of the mercury spectrum. The absorption spectra of the irradiated solutions were measured at various time intervals and the compositions of the mixtures were estimated on the basis of absorption spectra data.

Evidence was obtained for the presence of a substance which has a spectrum similar to protachysterol which has not previously been reported in the irradiated calciferol solutions. This substance appeared to be formed simultaneously with toxisterol and the suprasterols and to react photochemically after its formation.

The nature of the solvent appeared to have no effect on these reactions. The wavelength of irradiating light appeared to have no effect on the course of the reactions, although the rate of conversion of calciferol varied to some extent.

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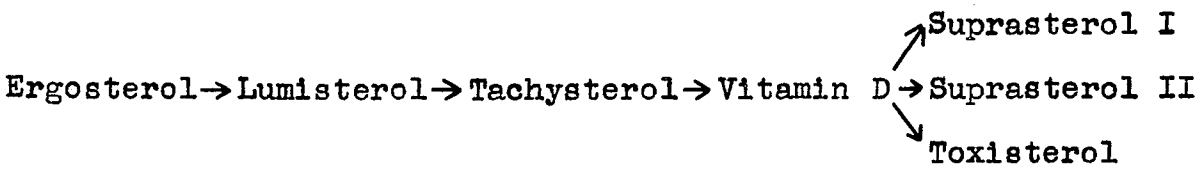
INTRODUCTION

The conversion of ergosterol into an antirachitically active substance by means of ultraviolet light has been studied rather extensively for a number of years. Our present knowledge of the process is largely the result of the investigations of Windaus. In 1930, Windaus, Gaede, Köser, and Stein (1) announced the isolation of two crystalline irradiation products which had no antirachitic activity and which showed no ultraviolet absorption at wave lengths greater than 250 mu. These compounds were named suprasterols I and II because they were formed by over-irradiation of ergosterol. Some time later, Askew and co-workers (2) and Windaus and co-workers (3) announced the isolation of active crystalline products by quite different methods. These products later proved to be different, although both were found to contain the true vitamin D. The product isolated by Windaus and co-workers (4, 5, 6), called vitamin D_1, was shown to be an addition compound of the vitamin, now called vitamin D_2, and an isomeric alcohol, called lumisterol. In 1932, Windaus, von Werder and Lüttringhaus (7) obtained tachysterol from the irradiation mixture. Its high reactivity with oxygen led Windaus to believe tachysterol preceded calciferol in the sequence of reactions, since the presence

of oxygen in the process reduces the yield of vitamin. Dimroth (8) found that on short irradiation both ergosterol and lumisterol yield a substance resembling tachysterol in its absorption spectrum. In 1932, Windaus, Lüttringhaus and Busse (9) isolated toxisterol as an oil and concluded that it was an irradiation product of the vitamin itself because vitamin preparations which were over-irradiated had a higher toxic factor than those which were not. Windaus and Auhagen (10) obtained evidence for the existence of another substance, which they called "protachysterol," in irradiated ergosterol solutions. This substance appeared to be transformed both thermally and photochemically into a compound resembling tachysterol in its absorption spectrum. Windaus and co-workers (7) concluded that the following sequence of reactions was involved in the irradiation of ergosterol.



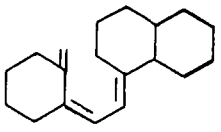
The importance of the vitamin and its formation from ergosterol caused the primary interest in the problem to center around the early stages of the process, with the consequence that only minor stress was placed on the decomposition of the vitamin itself. The futility of the experiments conducted

by Windaus and Auhagen (11) and by Windaus, Busse, and Weidlich (17) also deferred interest in this phase of the process.

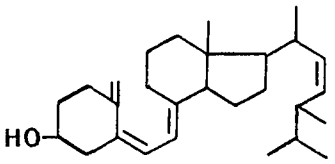
In 1943, Dimroth and Stockstrom (13) attempted to study more carefully the photo-conversion of the vitamin into toxisterol and the suprasterols. They irradiated an ethanol solution of a synthetic model (I) which contained the same nuclear arrangement of unsaturation as the vitamin (II). They concluded that the decomposition of calciferol followed the sequence

Calciferol ⟶ Toxisterol ⟶ Suprasterols

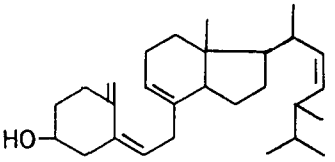
and proposed structure III as that representing toxisterol.



I



II



III

Recently Green (14) studied the irradiation of calciferol in ether and benzene using the direct antimony trichloride method for determining concentrations of calciferol. Throughout these experiments he observed the absorption spectra of the mixtures, but found no evidence for the presence of toxisterol. However, he obtained from irradiated solutions another material which he called suprasterol III.

The factors governing its formation were unknown as indicated by the varying amounts formed under similar conditions.

Green also observed in the irradiation of ergosterol in ether and benzene that maximum potency of the product was obtained after about the same length of time in both ether and benzene, this maximum potency being about one and one-half times as great in ether as in benzene. Bills, Honeywell and Cox (15) had previously observed a solvent effect in the irradiation of ergosterol in ether, cyclohexane and ethanol. The maximum potency was reached in ethanol more rapidly than in cyclohexane, and most slowly in ether. The maximum potency was greater in ether than in cyclohexane or ethanol.

Green also observed a solvent effect in the irradiation of calciferol in benzene and ether, the rate of destruction being slower in benzene than in ether, which may be due to the ultraviolet absorption properties of benzene.

The reactions which occur when ergosterol is irradiated have been shown to be very complex. They include the formation and subsequent decomposition of calciferol. The latter has been studied to some extent but the reactions which occur when calciferol is irradiated have not been completely elaborated. The purpose of this investigation was to study the relationship between the reactions which occur when calciferol is irradiated with ultraviolet light and to determine what effects, if any, solvent and activating wave length have on these reactions.

EXPERIMENTAL

Chemicals and Equipment

1. The calciferol used in this investigation was obtained as "Deltaxin" from Sterwin Chemicals, Inc., New York City, New York. This product was purified by recrystallization from ethanol and stored at about -20° C. under carbon dioxide.

The value of E (1%, 1 cm.) at 265 mu of the purified calciferol (Figure 1) was found to be 480 in ethanol and 474 in hexane. No change in the absorption spectrum was observed over a period of several months when the calciferol was stored as described.

2. n-Hexane, obtained as "Skellysolve B" from the Skelly Oil Company, 3711 California Avenue, Chicago, Illinois, was purified by the method of Mair and White (16), using a chromatographic column (4 cm. in diameter by 75 cm. in length) of silica gel which had been activated by heating at 250° for 24 hours.

3. Anhydrous ethyl ether was obtained in purified state by distilling the commercial anhydrous ethyl ether (C. P.) from a mixture of anhydrous sodium sulfite (C. P.) and sodium hydroxide immediately prior to use.

4. Commercial absolute ethanol was purified by first removing the aldehydes with silver nitrate and sodium

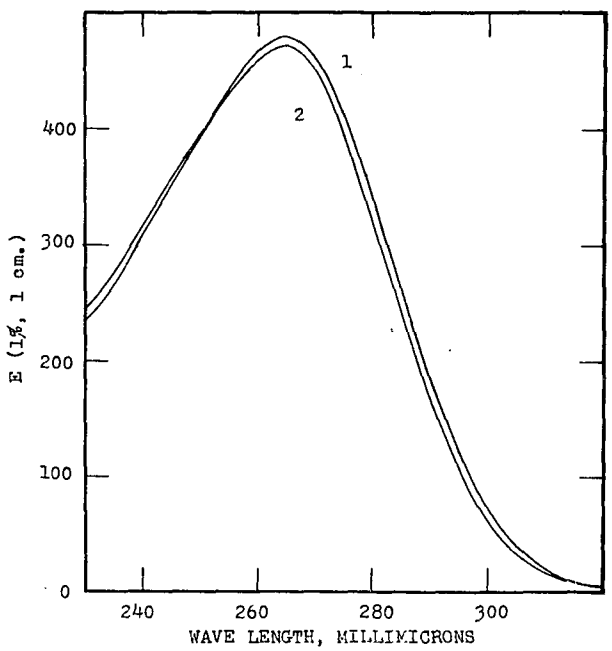


FIGURE 1

ABSORPTION SPECTRA OF CALCIFEROL IN
(1) ETHANOL AND (2) HEXANE

hydroxide (17) and then dehydrating with amalgamated aluminum foil (18).

5. The absorption spectra were obtained using a Beckman Quartz Spectrophotometer, Model DU, with a hydrogen discharge lamp source. Figure 2 is a diagram of one of the absorption cells used. These were composed of the elements of cells designed for use with the Bausch and Lomb Sector Photometer. Each cell consisted of two optically flat quartz discs separated by a 0.5 cm. glass spacing ring and held in place by a brass holder. The cells were mounted in a special brass cell carriage for use with the Beckman spectrophotometer.

6. The Bausch and Lomb Grating Monochrometer, catalogue number 33-86-40, equipped with a Hanovia Quartz Alpine Sun Burner, type S-100, was used as a source of monochromatic radiation. The monochrometer has an equivalent aperture of f/4.4, a focal length of 250 mm. and linear dispersion of 66 A. per mm. The grating has a ruled surface of 50 x 50 mm., containing 600 grooves per mm., blazed for first order in the range 200-400 mu.

The Validity of Beer's Law as Applied to Calciferol

Five solutions of calciferol in n-hexane containing 2.09, 1.67, 1.26, 0.84 and 0.42 mg./100 ml., and five solutions of calciferol in ethanol containing 2.00, 1.60, 1.20, 0.80 and 0.40 mg./100 ml. were prepared and their absorption spectra were measured over the wave length range 230-300 mu.

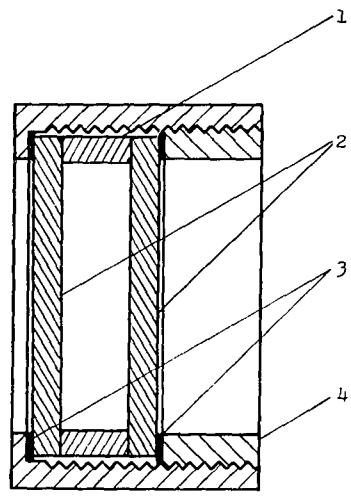


FIGURE 2

DIAGRAM OF ABSORPTION CELL

1. 0.50 cm. GLASS SPACING RING
2. QUARTZ DISKS
3. TEFLON PRESSURE RINGS
4. BRASS HOLDER

⟵1 cm.⟶

It was found that the measured extinctions of the solutions varied directly with concentration at wave lengths 230 mu, 250 mu, 265 mu and 280 mu as shown in Figure 3 and Figure 4.

Irradiation Procedure

A standard irradiation procedure was devised after some preliminary experimentation to determine the most practical arrangement. This general procedure consisted of placing the absorption cell containing the solution to be irradiated at the point of focus of the monochromator beam. After the desired time interval, the absorption spectrum of the solution was measured and the cell was replaced in the monochromator beam for the next time interval.

It was found most convenient to conduct all irradiations with entrance and exit slit widths of 2.5 mm., the exit beam thus having an effective band width of 16.5 mu. The approximate irradiation times at which the absorption spectra were measured were 0, 10, 20, 35, 60, 95, 145 and 210 minutes.

The irradiations were carried out in ethanol, n-hexane and a mixture of ethyl ether and n-hexane (one part by volume ether to 19 parts hexane). Monochromatic light of wave lengths 2483, 2537, 2654, 2753, 2967 and 3132 A. were used for each solvent system. All initial concentrations of calciferol were of the order of 2 mg./100 ml.

Results

The absorption spectra of the irradiated solutions of calciferol in ethanol are shown in Figures 5-10, those for

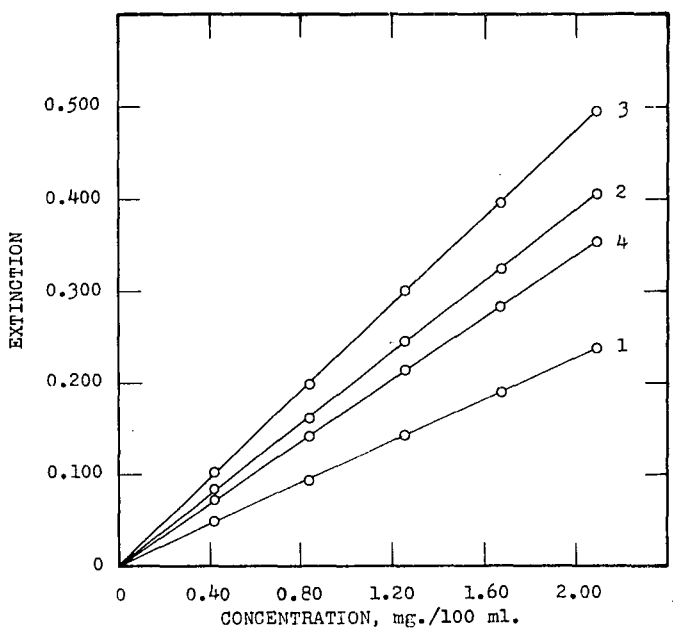


FIGURE 3

RELATIONSHIP BETWEEN EXTINCTION AND CONCENTRATION FOR CALCIFEROL IN HEXANE AT (1) 230 mu, (2) 250 mu, (3) 265 mu, (4) 280 mu.

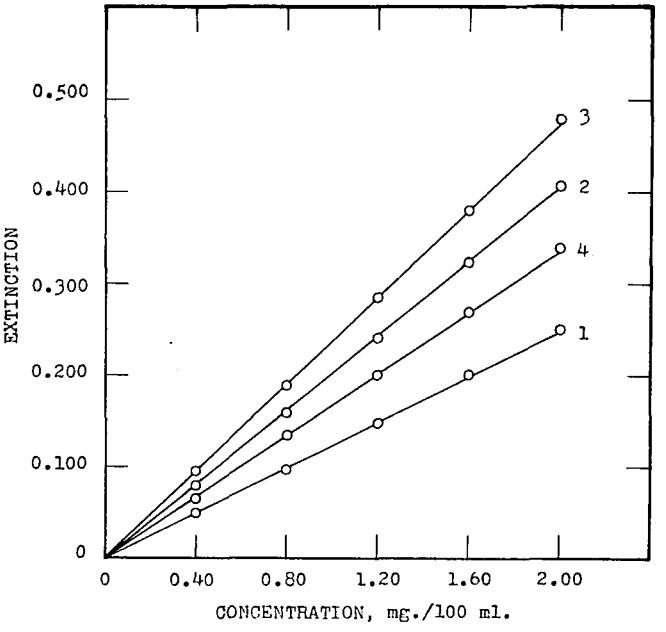


FIGURE 4

RELATIONSHIP BETWEEN EXTINCTION AND CONCENTRATION FOR CALCIFEROL IN ETHANOL AT (1) 230 mu, (2) 250 mu, (3) 265 mu, (4) 280 mu.

the irradiations in hexane in Figures 11-16, and those for the irradiations in hexane-ether mixture in Figures 17-22.

A number of these experiments were repeated and the results were found to agree generally although variations in the rates of decrease of calciferol concentrations were observed. These variations were attributed to variations in the intensity of the irradiating light.

The calculated percentages of toxisterol, calciferol, protachysterol and combined suprasterols for the various irradiations conducted are recorded in Tables I-XVIII along with the E (1%, 1 cm.) values upon which these calculations are based.

The method of calculation of the composition of the irradiation mixtures will be discussed later.

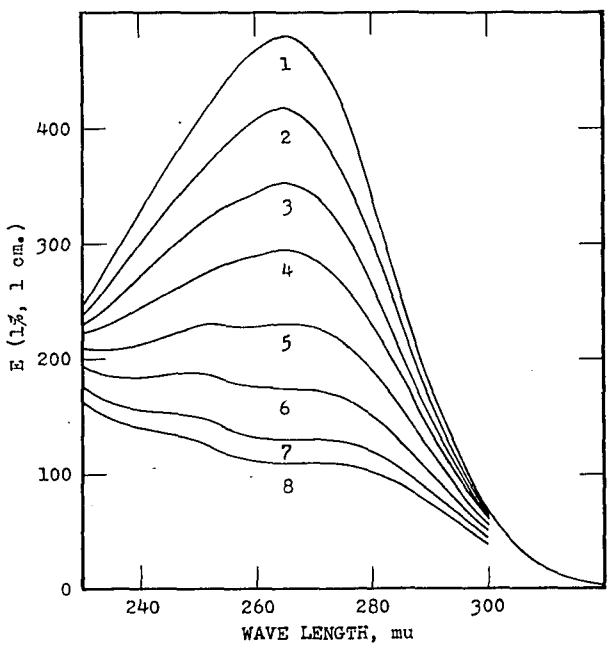


FIGURE 5

IRRADIATION IN ETHANOL WITH Hg 2483 A. LINE

(1) 0 MINUTES	(5) 60 MINUTES
(2) 10 MINUTES	(6) 95 MINUTES
(3) 20 MINUTES	(7) 145 MINUTES
(4) 35 MINUTES	(8) 210 MINUTES

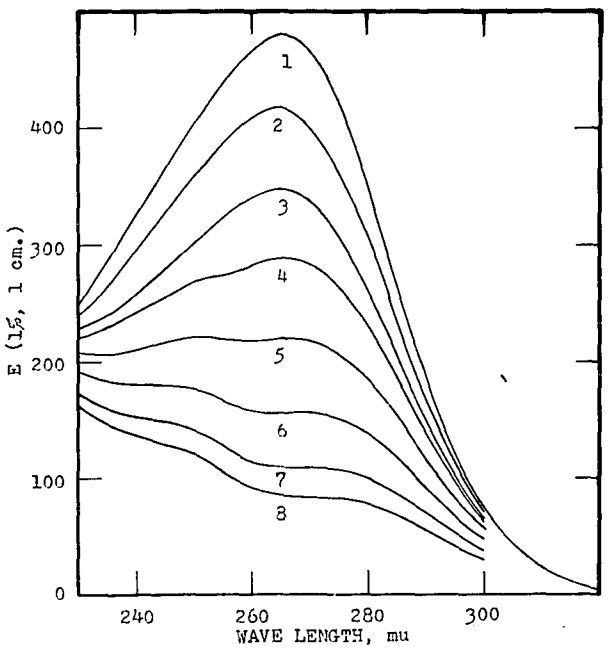


FIGURE 6

IRRADIATION IN ETHANOL WITH Hg 2537 A. LINE

(1) 0 MINUTES	(5) 60 MINUTES
(2) 10 MINUTES	(6) 95 MINUTES
(3) 22 MINUTES	(7) 145 MINUTES
(4) 37 MINUTES	(8) 205 MINUTES

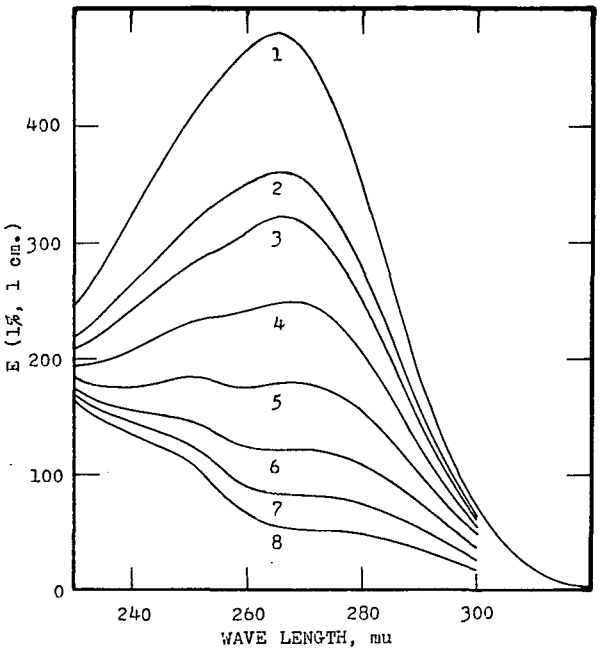


FIGURE 7

IRRADIATION IN ETHANOL WITH Hg 2654 A. LINE

(1) 0 MINUTES	(5) 60 MINUTES
(2) 13 MINUTES	(6) 97 MINUTES
(3) 20 MINUTES	(7) 145 MINUTES
(4) 35 MINUTES	(8) 210 MINUTES

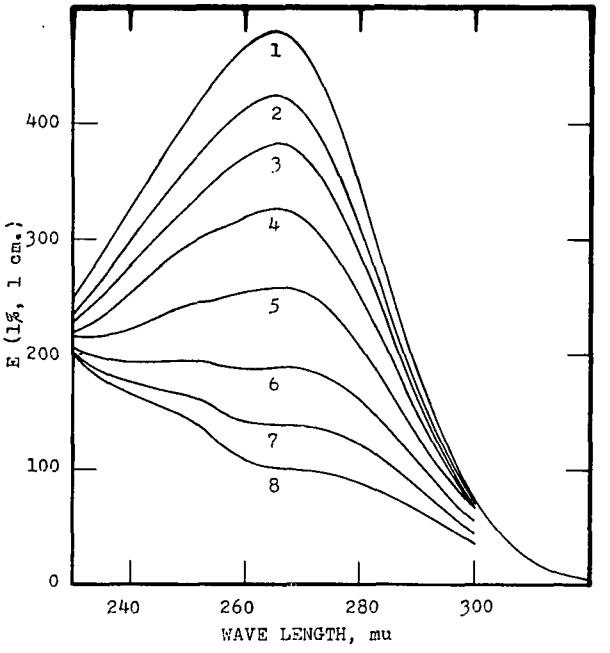


FIGURE 8

IRRADIATION IN ETHANOL WITH Hg 2753 A. LINE

(1) 0 MINUTES
(2) 11 MINUTES
(3) 20 MINUTES
(4) 35 MINUTES
(5) 60 MINUTES
(6) 95 MINUTES
(7) 145 MINUTES
(8) 210 MINUTES

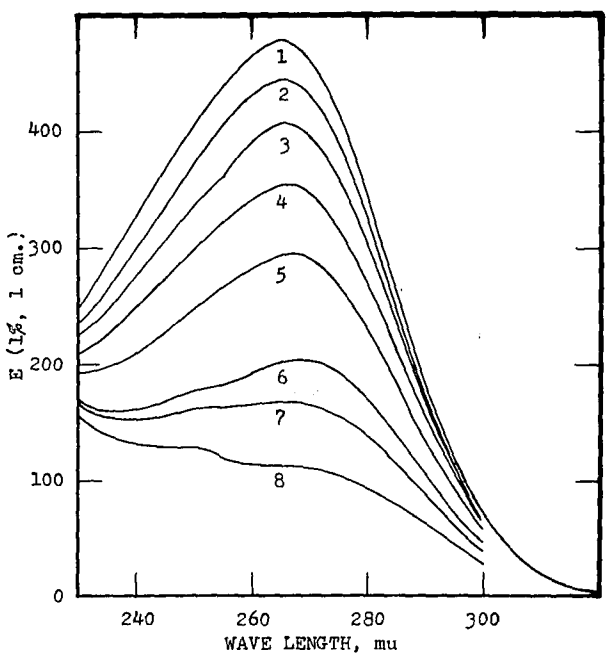


FIGURE 9

IRRADIATION IN ETHANOL WITH Hg 2967 A. LINE

(1) 0 MINUTES	(5) 60 MINUTES
(2) 10 MINUTES	(6) 113 MINUTES
(3) 20 MINUTES	(7) 147 MINUTES
(4) 35 MINUTES	(8) 210 MINUTES

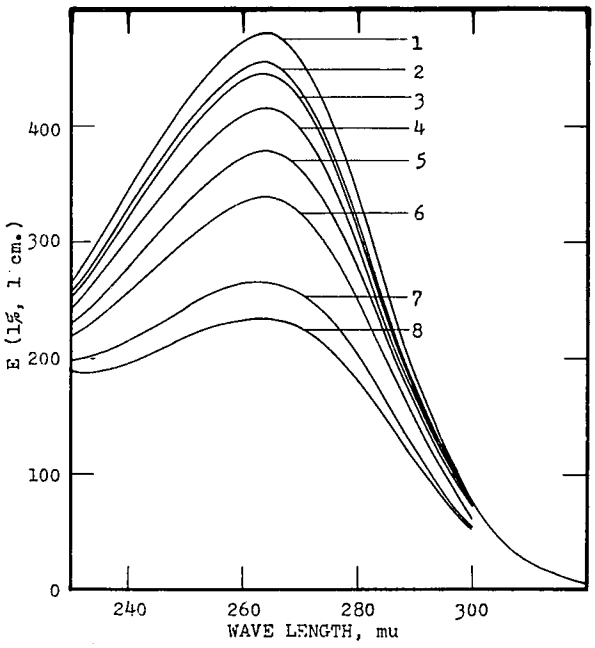


FIGURE 10

IRRADIATION IN ETHANOL WITH Hg 3132 A. LINE

(1) 0 MINUTES	(5) 60 MINUTES
(2) 10 MINUTES	(6) 95 MINUTES
(3) 20 MINUTES	(7) 165 MINUTES
(4) 35 MINUTES	(8) 216 MINUTES

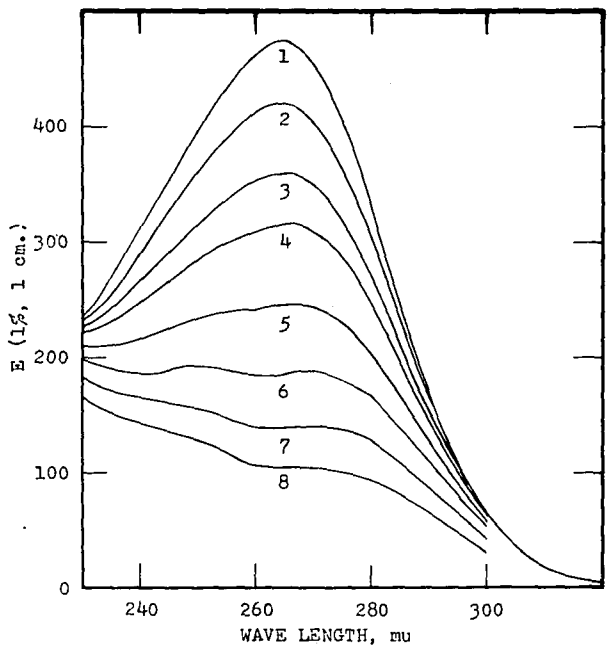


FIGURE 11

IRRADIATION IN HEXANE WITH Hg 2483 A. LINE

(1) 0 MINUTES	(5) 60 MINUTES
(2) 10 MINUTES	(6) 95 MINUTES
(3) 23 MINUTES	(7) 145 MINUTES
(4) 35 MINUTES	(8) 210 MINUTES

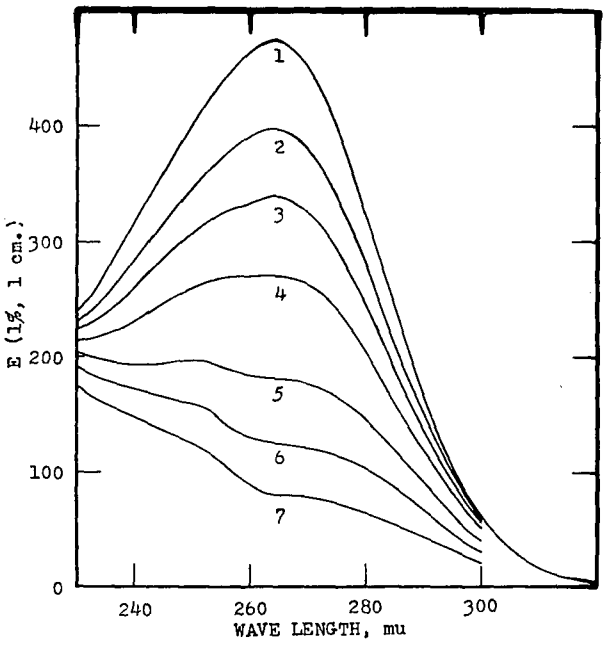


FIGURE 12

IRRADIATION IN HEXANE WITH Hg 2537 A. LINE

(1) 0 MINUTES
(2) 10 MINUTES
(3) 20 MINUTES
(4) 35 MINUTES
(5) 65 MINUTES
(6) 105 MINUTES
(7) 160 MINUTES

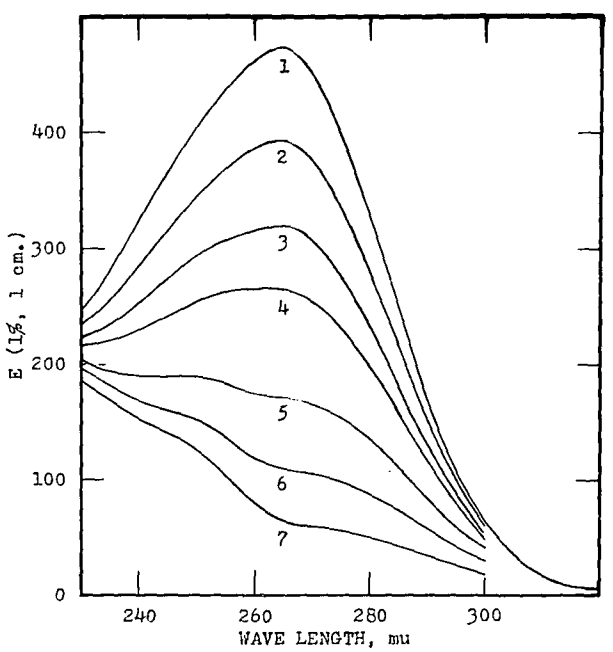


FIGURE 13

IRRADIATION IN HEXANE WITH Hg 2654 A. LINE

(1) 0 MINUTES
(2) 10 MINUTES
(3) 20 MINUTES
(4) 35 MINUTES
(5) 60 MINUTES
(6) 95 MINUTES
(7) 145 MINUTES

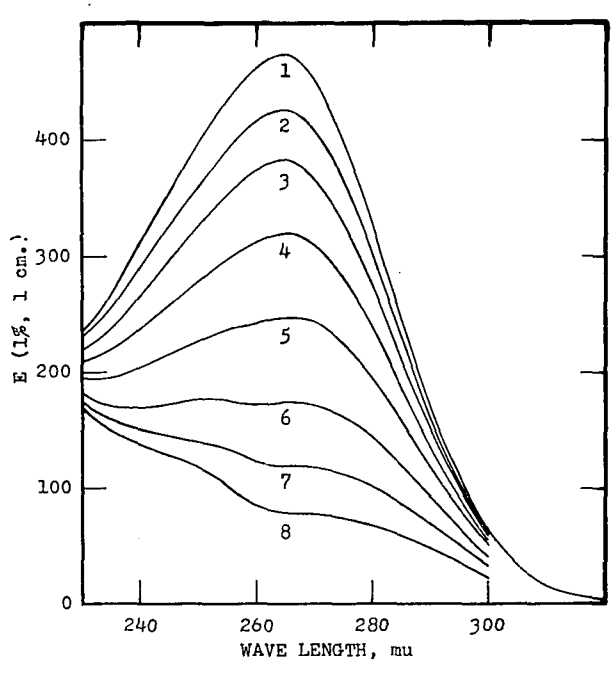


FIGURE 14

IRRADIATION IN HEXANE WITH Hg 2753 A. LINE

(1) 0 MINUTES
(2) 10 MINUTES
(3) 20 MINUTES
(4) 35 MINUTES
(5) 60 MINUTES
(6) 97 MINUTES
(7) 145 MINUTES
(8) 213 MINUTES

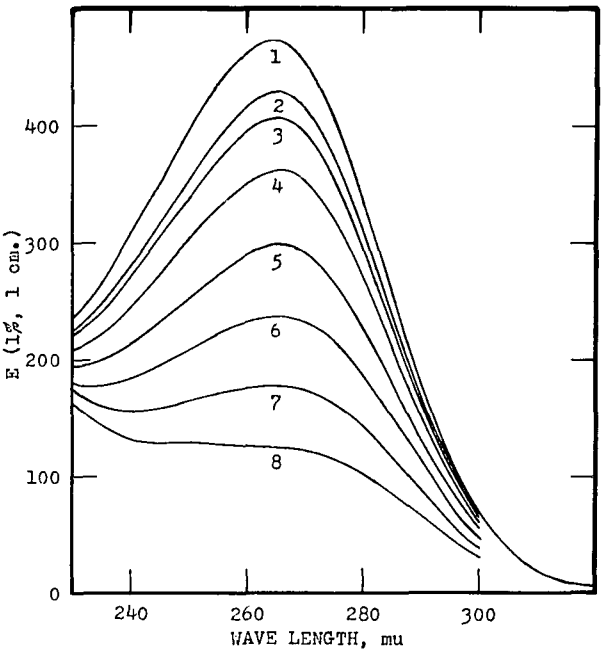


FIGURE 15

IRRADIATION IN HEXANE WITH Hg 2967 A. LINE

(1) 0 MINUTES	(5) 62 MINUTES
(2) 13 MINUTES	(6) 95 MINUTES
(3) 20 MINUTES	(7) 145 MINUTES
(4) 35 MINUTES	(8) 210 MINUTES

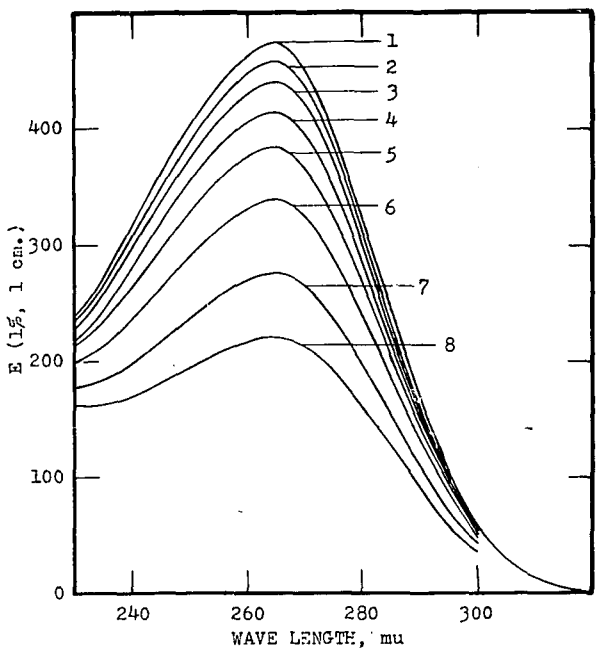


FIGURE 16

IRRADIATION IN HEXANE WITH Hg 3132 A. LINE

(1) 0 MINUTES	(5) 60 MINUTES
(2) 10 MINUTES	(6) 96 MINUTES
(3) 20 MINUTES	(7) 145 MINUTES
(4) 40 MINUTES	(8) 210 MINUTES

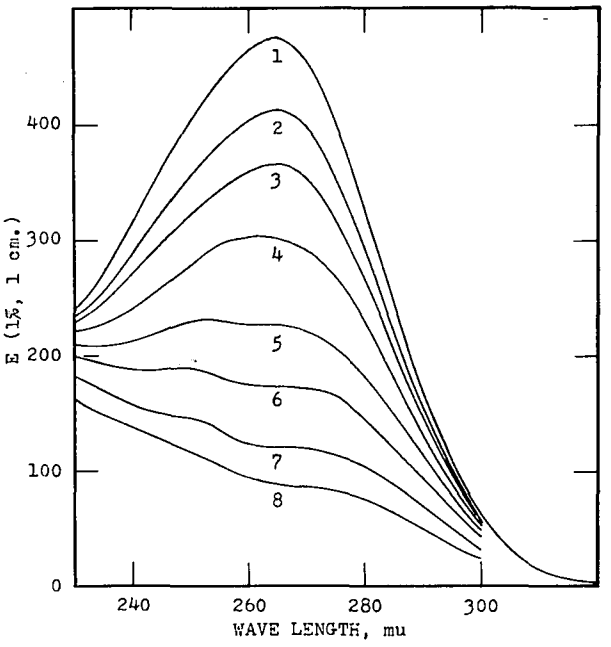


FIGURE 17

IRRADIATION IN HEXANE-ETHER WITH Hg 2483 A. LINE

(1) 0 MINUTES	(5) 60 MINUTES
(2) 11 MINUTES	(6) 90 MINUTES
(3) 20 MINUTES	(7) 145 MINUTES
(4) 35 MINUTES	(8) 210 MINUTES

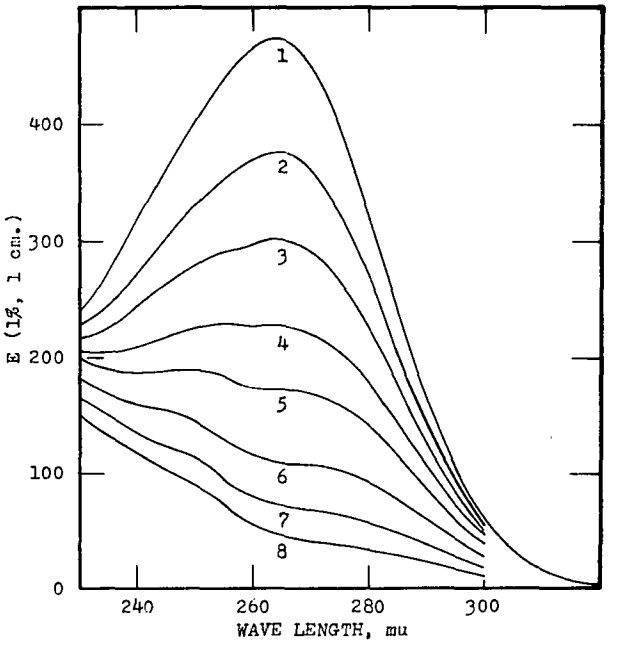


FIGURE 18

IRRADIATION IN HEXANE-ETHER WITH Hg 2537 A. LINE

(1) 0 MINUTES	(5) 55 MINUTES
(2) 10 MINUTES	(6) 96 MINUTES
(3) 21 MINUTES	(7) 150 MINUTES
(4) 36 MINUTES	(8) 218 MINUTES

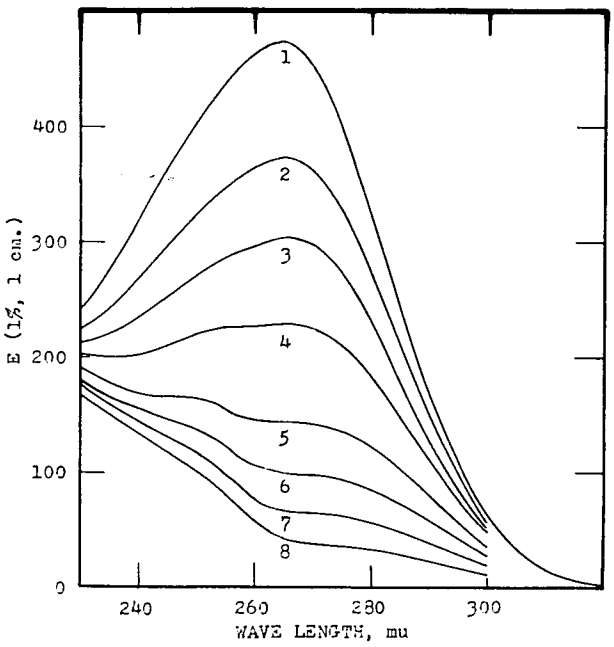


FIGURE 19

IRRADIATION IN HEXANE-ETHER WITH Hg 2654 A. LINE

(1) 0 MINUTES	(5) 66 MINUTES
(2) 10 MINUTES	(6) 98 MINUTES
(3) 20 MINUTES	(7) 144 MINUTES
(4) 35 MINUTES	(8) 210 MINUTES

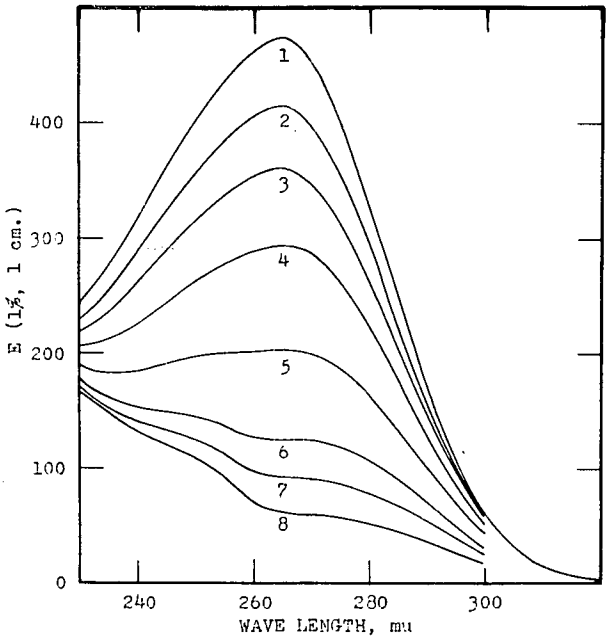


FIGURE 20

IRRADIATION IN HEXANE-ETHER WITH Hg 2753 A. LINE

(1) 0 MINUTES	(5) 65 MINUTES
(2) 10 MINUTES	(6) 112 MINUTES
(3) 20 MINUTES	(7) 150 MINUTES
(4) 35 MINUTES	(8) 210 MINUTES

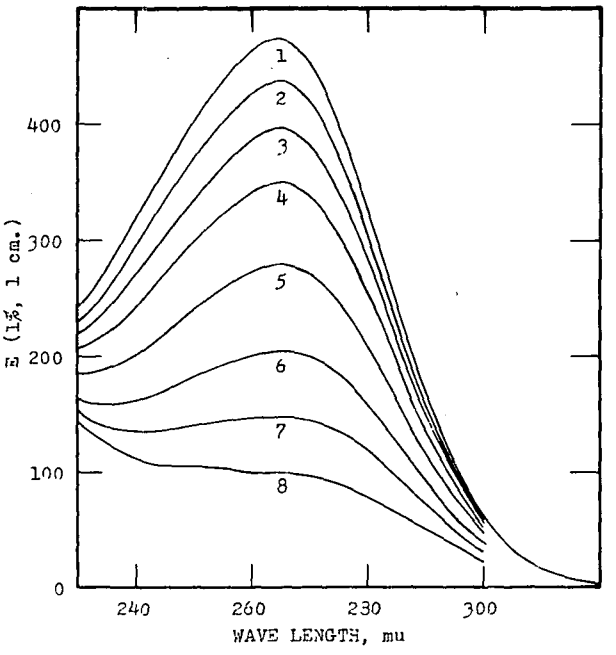


FIGURE 21

IRRADIATION IN HEXANE-ETHER WITH Hg 2967 A. LINE

(1) 0 MINUTES	(5) 62 MINUTES
(2) 10 MINUTES	(6) 100 MINUTES
(3) 21 MINUTES	(7) 145 MINUTES
(4) 35 MINUTES	(8) 205 MINUTES

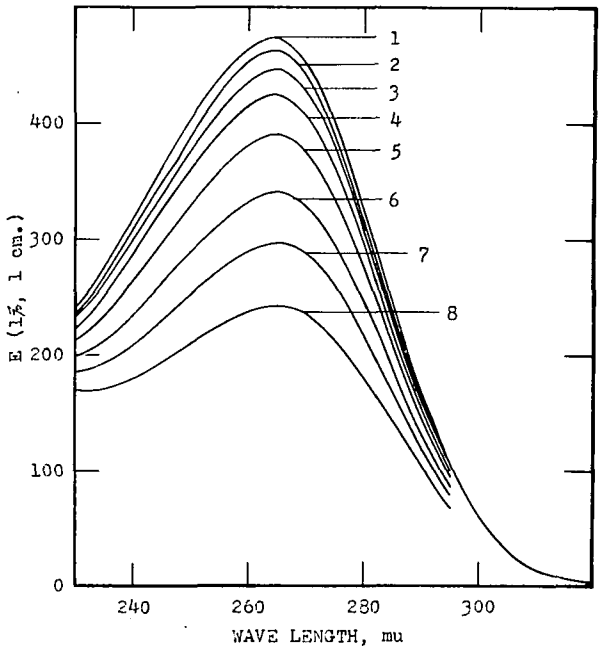


FIGURE 22

IRRADIATION IN HEXANE-ETHER WITH Hg 3132 A. LINE

(1) 0 MINUTES	(5) 60 MINUTES
(2) 10 MINUTES	(6) 100 MINUTES
(3) 20 MINUTES	(7) 145 MINUTES
(4) 35 MINUTES	(8) 210 MINUTES

TABLE I

IRRADIATION IN ETHANOL WITH Hg 2483 A. LINE

Initial concentration: 2.07 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	406	480	341	248	3	97	0	9
10	361	417	304	240	7	79	6	32
20	316	353	266	231	11	60	13	55
35	274	295	231	223	14	44	19	78
60	229	231	193	209	17	26	25	94
95	187	174	151	194	19	15	24	108
145	149	130	121	176	18	5	25	114
210	130	109	102	163	17	4	22	112

t = Irradiation time

E_1 = E (1%, 1 cm.) at 250 mu

E_2 = E (1%, 1 cm.) at 265 mu

E_3 = E (1%, 1 cm.) at 280 mu

E_4 = E (1%, 1 cm.) at 230 mu

T = % concentration of toxisterol

D = % concentration of calciferol

P = % concentration of protachysterol

S = % concentration of suprasterols

TABLE II
IRRADIATION IN ETHANOL WITH Hg 2537 A. LINE
Initial concentration: 2.08 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	404	480	348	250	4	93	6	10
10	359	417	304	240	6	79	6	33
22	304	348	260	229	8	62	11	59
37	270	289	230	220	14	41	22	76
60	222	220	184	208	18	24	24	99
95	177	157	139	192	20	11	24	115
145	142	111	100	176	20	5	19	124
205	121	86	78	162	19	3	16	124

TABLE III
IRRADIATION IN ETHANOL WITH Hg 2654 A. LINE
Initial concentration: 2.08 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	409	480	349	248	6	92	6	7
13	316	362	277	220	9	60	17	38
20	282	323	252	210	9	50	19	48
35	232	249	205	196	13	31	24	71
60	185	179	156	186	17	15	25	99
97	148	122	111	175	19	6	22	116
145	127	84	77	170	22	2	16	131
210	112	57	50	164	23	1	10	139

TABLE IV
IRRADIATION IN ETHANOL WITH Hg 2753 A. LINE
Initial concentration: 2.08 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	407	480	346	251	5	94	4	10
11	362	425	311	236	5	80	8	24
20	329	383	287	228	7	68	12	38
35	296	327	253	219	12	52	18	54
60	244	258	208	218	14	35	22	92
95	197	189	162	207	18	18	24	115
145	166	139	123	203	20	9	22	138
210	145	101	89	202	23	5	16	159

TABLE V
IRRADIATION IN ETHANOL WITH Hg 2967 A. LINE
Initial concentration: 2.08 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	408	480	341	248	4	97	0	9
10	372	446	322	236	2	88	4	15
20	338	408	302	225	3	76	10	22
35	300	356	272	210	5	60	16	32
60	248	295	230	193	5	47	17	48
113	178	203	170	170	8	24	22	72
147	163	168	139	166	11	20	17	85
210	129	114	96	156	14	11	14	103

TABLE VI
IRRADIATION IN ETHANOL WITH Hg 3132 A. LINE
Initial concentration: 2.08 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	422	480	337	266	8	99	-3	26
10	400	454	320	257	8	93	-2	31
20	392	444	314	253	8	90	-1	32
35	368	415	298	243	9	81	3	37
60	334	378	276	231	9	71	7	44
95	303	339	252	220	10	60	10	53
165	244	266	202	199	10	44	11	69
216	219	234	181	190	11	36	13	76

TABLE VII
IRRADIATION IN ETHANOL WITH Hg 2483 A. LINE
Initial concentration: 2.18 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	397	474	327	236	2	100	3	-6
10	360	421	303	232	6	80	13	16
23	316	360	272	227	10	60	21	41
35	285	316	248	222	12	47	26	59
60	234	246	207	212	16	28	31	84
95	192	186	167	197	18	14	32	100
145	156	139	129	184	18	7	28	103
210	129	104	96	166	18	5	20	103

TABLE VIII
IRRADIATION IN HEXANE WITH Hg 2537 A. LINE
Initial concentration: 2.18 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	400	474	325	239	2	100	2	0
10	346	396	282	231	8	77	10	30
20	307	339	247	224	11	62	13	52
35	259	271	206	215	14	44	17	76
65	198	181	146	203	19	23	19	112
105	160	124	103	190	21	12	16	128
160	125	80	65	174	21	8	9	138

TABLE IX
IRRADIATION IN HEXANE WITH Hg 2654 A. LINE
Initial concentration: 2.18 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	403	474	328	246	4	98	4	6
10	345	392	279	234	8	76	10	34
20	293	319	234	224	12	57	14	63
35	254	266	199	217	14	45	14	83
60	190	172	137	204	18	22	16	117
95	153	110	89	196	22	12	12	141
145	126	66	51	186	24	6	6	157

TABLE X
IRRADIATION IN HEXANE WITH Hg 2753 A. LINE
Initial concentration: 2.18 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	397	474	328	235	2	99	4	-6
10	359	426	302	230	4	84	9	14
20	326	384	277	220	5	73	12	24
35	280	320	241	210	8	54	18	47
60	227	248	204	196	12	31	28	68
97	177	174	146	182	15	19	22	94
145	141	119	106	177	18	8	20	120
213	119	79	71	171	20	4	14	133

TABLE XI
IRRADIATION IN HEXANE WITH Hg 2967 A. LINE
Initial concentration: 2.18 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	396	474	333	236	2	96	8	-6
13	353	430	308	226	1	84	12	8
20	338	408	296	221	3	77	14	14
35	303	363	270	210	4	64	18	25
62	253	299	228	196	5	50	19	45
95	208	238	187	181	7	36	20	62
145	165	177	143	174	9	24	18	87
210	129	125	103	164	11	15	14	106

TABLE XII
IRRADIATION IN HEXANE WITH Hg 3132 A. LINE
Initial concentration: 2.18 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	403	474	321	240	3	103	-2	-1
10	389	458	311	234	3	99	-1	1
20	376	441	303	229	4	93	2	6
40	352	414	286	218	3	86	3	8
60	327	385	268	214	3	79	4	20
96	290	340	240	199	4	68	7	29
145	238	276	198	177	4	53	8	39
210	195	221	161	163	5	41	8	54

TABLE XIII
IRRADIATION IN HEXANE-ETHER WITH Hg 2483 A. LINE
Initial concentration: 2.07 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	402	474	328	240	4	99	4	-1
11	356	414	293	235	6	82	9	25
20	323	367	265	231	8	69	12	46
35	279	302	230	223	13	49	20	69
60	231	228	183	210	18	30	23	86
90	190	174	145	200	19	19	22	113
145	147	121	105	182	19	10	18	122
210	118	89	76	165	17	7	13	124

TABLE XIV
IRRADIATION IN HEXANE-ETHER WITH Hg 2537 A. LINE
Initial concentration: 2.07 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	401	474	325	240	3	100	2	0
10	332	378	270	229	8	73	10	36
21	280	302	226	218	13	51	16	64
36	226	228	180	207	16	32	20	91
55	190	173	142	199	19	20	20	112
96	145	110	93	183	20	10	15	129
150	114	72	58	166	19	7	8	135
218	90	47	35	151	17	5	3	135

TABLE XV
IRRADIATION IN HEXANE-ETHER WITH Hg 2654 A. LINE
Initial concentration: 2.07 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	402	474	325	242	3	100	2	2
10	326	374	273	226	8	69	14	36
20	271	305	230	214	9	51	18	60
35	220	229	184	202	14	31	22	85
66	165	144	123	190	19	13	20	117
98	138	99	87	183	21	6	16	133
144	118	67	58	177	22	3	11	147
210	102	42	34	167	22	2	6	150

TABLE XVI
IRRADIATION IN HEXANE-ETHER WITH Hg 2753 A. LINE
Initial concentration: 2.07 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	400	474	325	243	2	101	2	2
10	356	415	292	230	5	83	7	18
20	316	362	262	219	7	68	12	36
35	264	296	223	207	9	50	17	56
65	198	204	164	190	13	27	20	86
112	146	125	108	178	17	11	19	114
150	126	93	82	171	19	6	16	127
210	110	63	55	168	20	3	11	139

TABLE XVII
IRRADIATION IN HEXANE-ETHER WITH Hg 2967 A. LINE
Initial concentration: 2.07 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	400	474	326	241	3	100	2	0
10	370	438	306	223	3	89	6	0
21	336	398	283	218	4	78	9	16
35	300	352	254	208	5	67	11	30
62	241	280	208	187	6	50	14	47
100	183	204	159	164	8	31	16	62
145	140	147	118	154	9	20	14	84
205	105	99	81	144	10	12	11	99

TABLE XVIII

IRRADIATION IN HEXANE-ETHER WITH Hg 3132 A. LINE

Initial concentration: 2.07 mg./100 ml.

t	E_1	E_2	E_3	E_4	T	D	P	S
0	402	474	324	242	3	101	1	1
10	388	463	315	237	1	100	0	5
20	376	447	309	232	2	93	4	6
35	361	425	297	225	4	87	6	9
60	329	391	276	213	3	78	8	15
100	289	342	246	200	4	66	10	29
145	254	297	216	187	4	55	11	39
210	210	243	181	172	5	43	12	51

DISCUSSION

The absorption spectra of the products of the irradiation of ergosterol, with the exception of that for protachysterol, are quite characteristic. The measurement of the absorption spectra of irradiated calciferol solutions is a convenient method for determining the composition of the mixtures obtained. The validity of the results of this method are dependent upon the applicability of Beer's Law to the mixtures obtained. The relationship of concentration to extinction for solutions of pure calciferol in hexane and ethanol has been determined and the results indicated that Beer's Law could be applied over the concentration range 0.4 - 2.0 mg./100 ml. and over the wave length range 230-300 mu. On the basis of these results and the observations of others in the cases of other sterols, it was assumed that mixtures obtained by the irradiation of calciferol would also follow Beer's Law.

Careful observation of the absorption spectra of irradiated calciferol solutions indicated that as the irradiation proceeded the rate of decrease of the extinction at 280 mu was not proportional to, but was somewhat less than proportional to, the rate of decrease of extinction at 265 mu. Because of the nature of the absorption spectra of toxisterol

and the suprasterols it would be expected that the rate of decrease of the extinction at 280 mu would be slightly greater than the rate of decrease of extinction at 265 mu. Therefore, a logical conclusion was that a substance other than toxisterol and the suprasterols was being formed and that the spectrum of this substance had a higher extinction at 280 mu than at 265 mu.

It was obvious that a spectrophotometric analysis of the mixtures must include a determination of the concentration of this component. In order to accomplish this the absorption spectrum of the substance must be known. The sterols most likely to be associated with calciferol in the irradiation mixtures were those arising from the irradiation of ergosterol. Of these sterols, those which satisfied the condition that the extinction at 280 mu be greater than that at 265 mu were ergosterol, lumisterol, tachysterol and protachysterol, and perhaps others which have not yet been isolated. In addition, there was the condition that the absorption spectrum calculated on the basis of the relative concentrations of the various substances must agree with the experimentally determined absorption spectrum. The closest agreement between observed and calculated absorption spectra in the range 250-300 mu was obtained using in the calculations the absorption spectrum of protachysterol as that representing the substance being formed.

Additional evidence that the compound being formed was protachysterol was the observation that in the final stages of the irradiation there appeared a slight inflection in the absorption spectrum at 280 mu, which may have been caused by the formation of a trace of tachysterol.

Theoretically, the analysis of the mixtures can be accomplished by solving a series of simultaneous equations. Inasmuch as the absorption spectra of suprasterol I and suprasterol II are identical, a spectrophotometric analysis cannot differentiate between them. The analysis would be expected to yield the concentrations of (1) the combined suprasterols (I and II), (2) suprasterol III, (3) toxisterol, (4) calciferol and (5) protachysterol. This would require the solution of five simultaneous equations. It was found, however, that no more than three equations could be utilized. The reason for this was that the measurements upon which these calculations were based contained only three significant figures. Upon completing the necessary arithmetical manipulations of fourth or fifth order determinants the number of significant figures in the calculated concentrations was reduced to one or zero.

It is evident from the absorption spectra shown in Figure 23 that small variations in concentrations in toxisterol, calciferol and protachysterol have a marked effect on the absorption spectra of the mixtures in the range

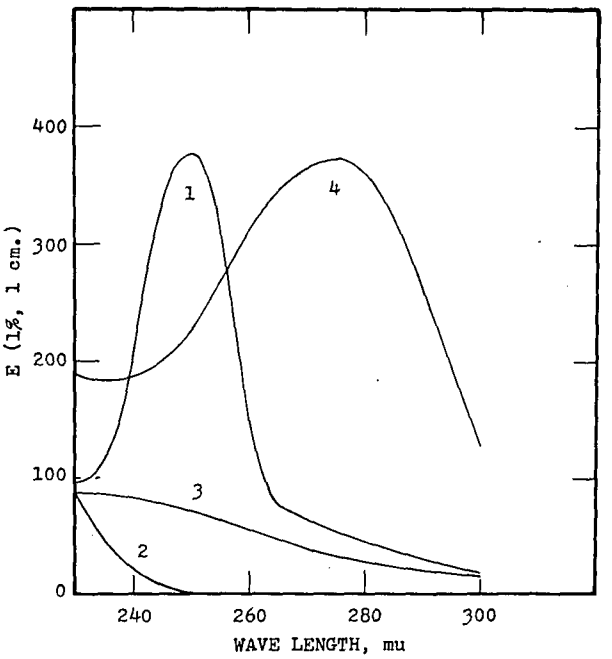


FIGURE 23

ABSORPTION SPECTRA OF (1) TOXISTEROL, (2) SUPRASTEROLS I & II, (3) SUPRASTEROL III AND (4) PROTACHYSTEROL.

250-280 mu, while changes in the concentrations of suprasterol I and suprasterol II have practically no effect at wave lengths of 250 mu and greater. Changes in suprasterol III concentrations affect, to a limited extent, the absorption in the range 250-280 mu. Therefore, the three equations which gave the best approximation to the concentrations of toxisterol, calciferol and protachysterol were those involving the extinctions at 250, 265 and 280 mu. These three equations were:

$$a_1T + b_1D + c_1P = E_1$$

$$a_2T + b_2D + C_2P = E_2$$

$$a_3T + b_3D + c_3P = E_3$$

where T is the fraction of calciferol which has been converted to toxisterol;

D is the fraction of calciferol which has not reacted;

P is the fraction of calciferol which has been converted to protachysterol;

a_1, a_2 and a_3 are E (1%, 1 cm.) values of toxisterol at 250, 265 and 280 mu, respectively;

b_1, b_2 and b_3 are the E (1%, 1 cm.) values of calciferol at 250, 265 and 280 mu, respectively;

c_1, c_2 and c_3 are the E (1%, 1 cm.) values of protachysterol at 250, 265 and 280 mu, respectively;

E_1, E_2 and E_3 are the E (1%, 1 cm.) values of the irradiation mixture at 250, 265 and 280 mu, respectively.

These three equations can be solved by the method of determinants to give the three expressions for T, D and P, which are, upon substitution of the values for the a's, b's and c's:

for ethanol solutions:

$$T = 0.00323\ E_1 - 0.00381\ E_2 + 0.00162\ E_3$$

$$D = -0.000733\ E_1 + 0.00721\ E_2 - 0.00642\ E_3$$

$$P = 0.000284\ E_1 - 0.00630\ E_2 + 0.00852\ E_3$$

for hexane solutions:

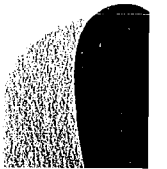
$$T = 0.00324\ E_1 - 0.00376\ E_2 + 0.00157\ E_3$$

$$D = -0.000582\ E_1 + 0.00671\ E_2 - 0.00598\ E_3$$

$$P = 0.000196\ E_1 - 0.00550\ E_2 + 0.00783\ E_3$$

The extinction at 230 mu of the irradiated solutions would be the sum of the extinctions of the constituents present. Since the concentrations of toxisterol, calciferol and protachysterol had been calculated, the contribution of each of these constituents to the extinction coefficient at 230 mu could be calculated.

Subtracting these contributions from the measured extinction and dividing the result by the E (1%, 1 cm.) value of the suprasterols yielded the approximate concentration of the combined suprasterols. This was expressed in the form



of an equation as follows:

$$S = \frac{E_4 - (k_1T + k_2D + k_3P)}{k_4}$$

where S is the fraction of calciferol which has been converted to suprasterols;

E_4 is the E (1%, 1 cm.) of the mixture at 230 mu;

T, D and P are concentrations of toxisterol, calciferol and protachysterol, respectively;

k_1, k_2, k_3 and k_4 are the E (1%, 1 cm.) values of toxisterol, calciferol, protachysterol and suprasterols (I, II and III), respectively, at 230 mu.

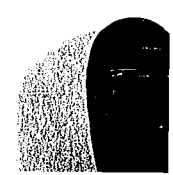
Upon substitution of the values of k_1, k_2, k_3 and k_4 this equation became:

$$S = 0.0115\ E_4 - (1.12\ T + 2.85\ D + 2.15\ P).$$

Using these equations the approximate percentages of calciferol, toxisterol, protachysterol and the combined suprasterols have been calculated for the irradiations conducted in ethanol, hexane and hexane-ether mixture, and are recorded in Tables I-XVIII.

Figures 24 to 41 represent the variation of concentrations of the constituents with time under the various conditions of the irradiation.

One of the most outstanding characteristics observed in these figures is the apparent variation of concentration of combined suprasterols with time. The values of these



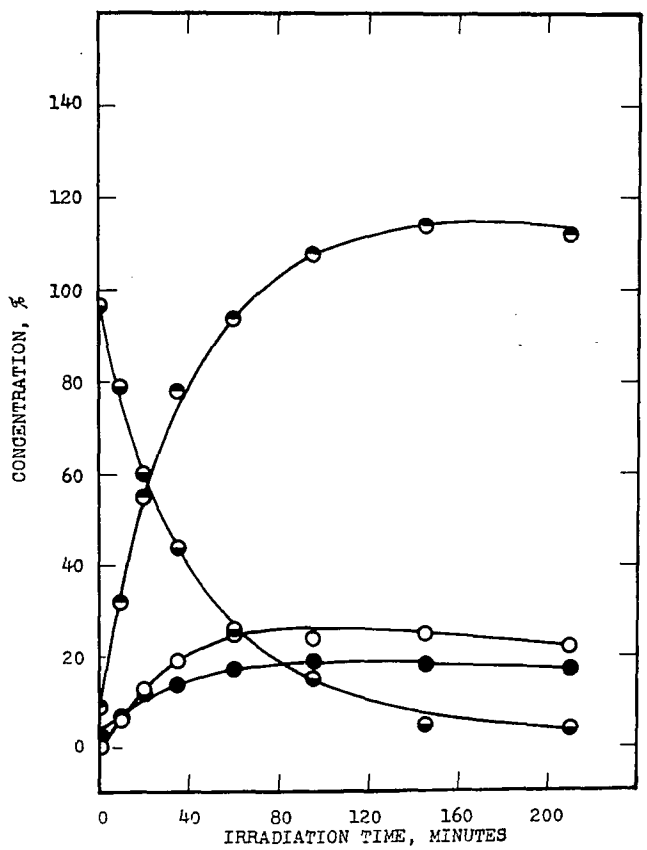


FIGURE 24

IRRADIATION IN ETHANOL WITH Hg 2483 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

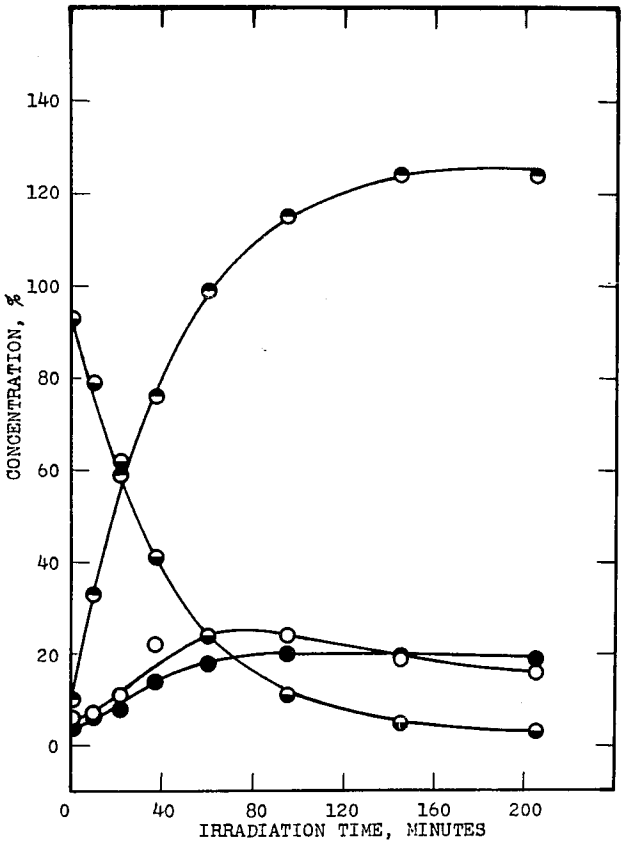


FIGURE 25

IRRADIATION IN ETHANOL WITH Hg 2537 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

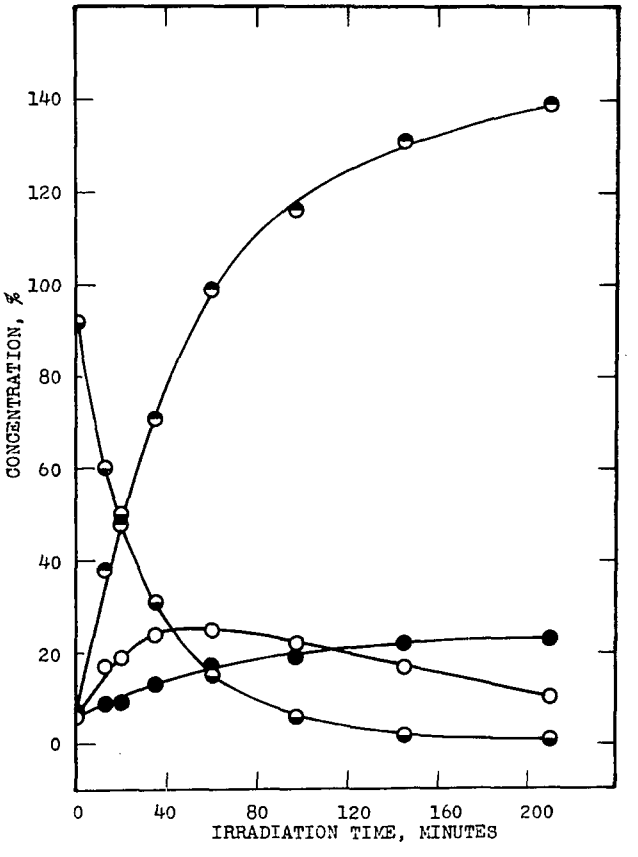


FIGURE 26

IRRADIATION IN ETHANOL WITH Hg 2654 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

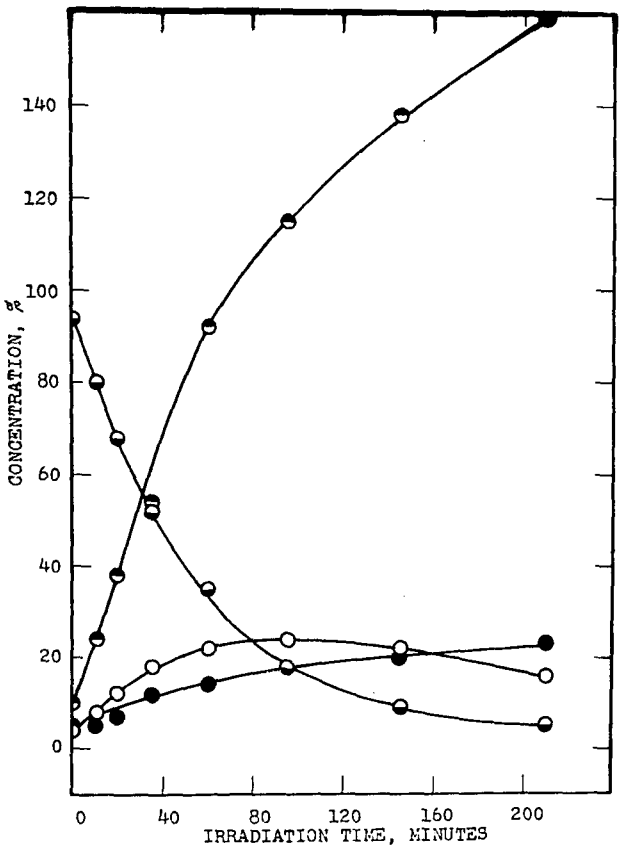


FIGURE 27

IRRADIATION IN ETHANOL WITH Hg 2753 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

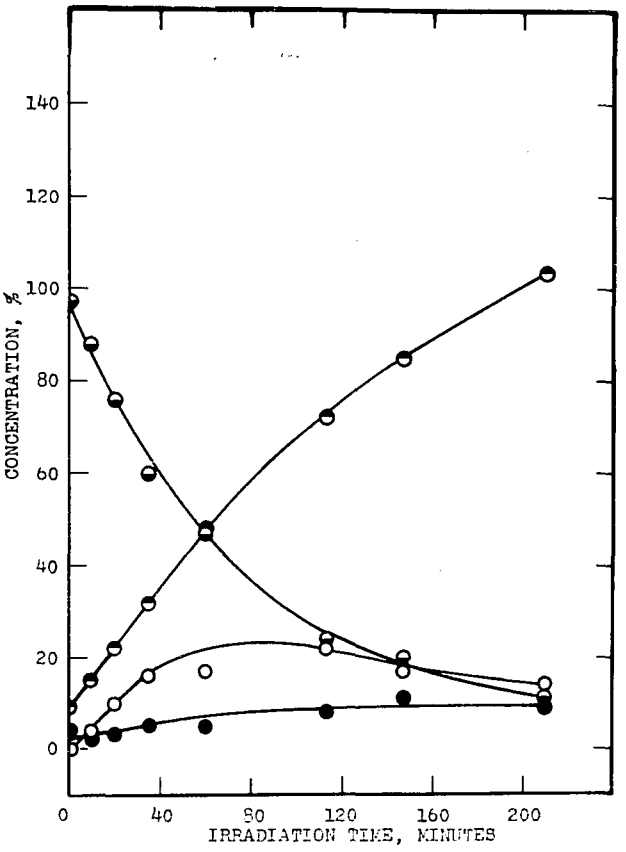


FIGURE 28

IRRADIATION IN ETHANOL WITH Hg 2967 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

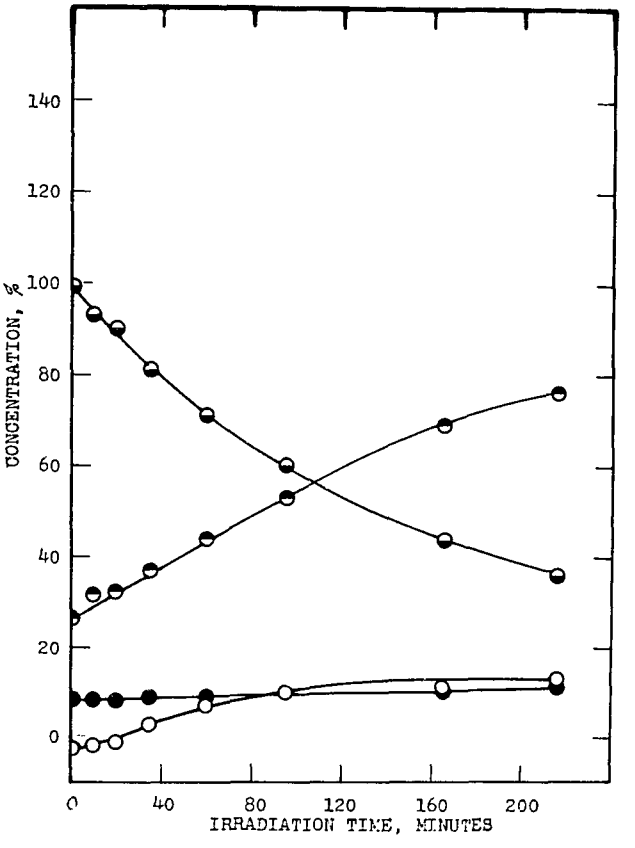


FIGURE 29

IRRADIATION IN ETHANOL WITH Hg 3132 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

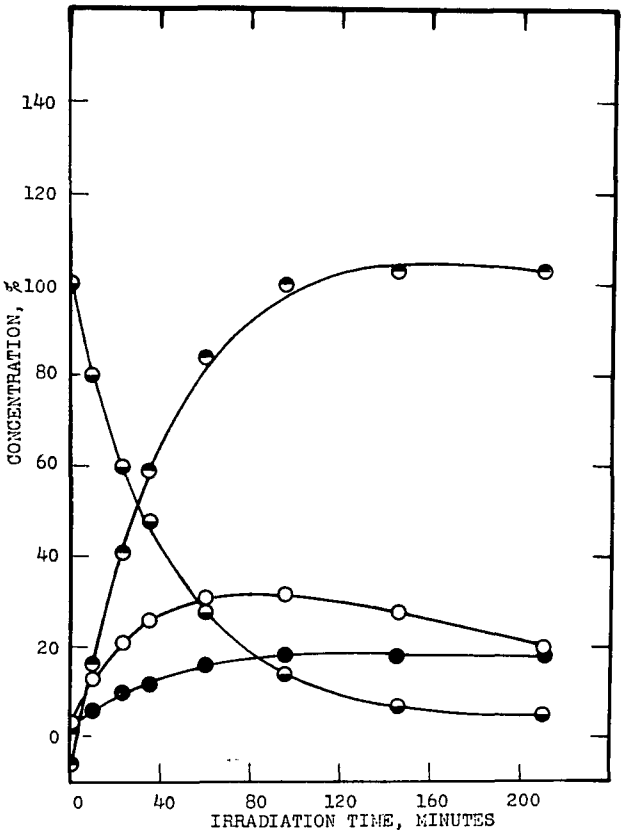


FIGURE 30

IRRADIATION IN HEXANE WITH Hg 2483 A. LINE

◒ = D ○ = P ● = T ◓ = S

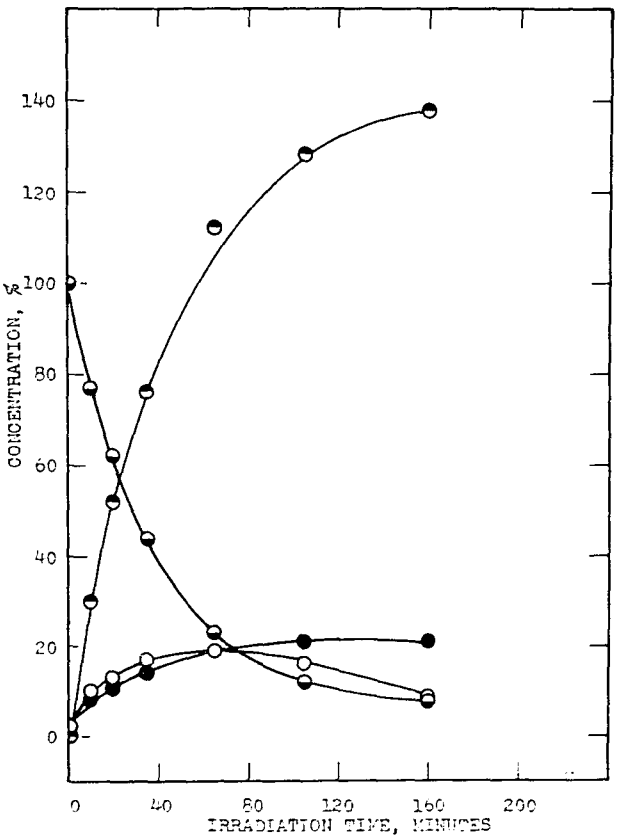


FIGURE 31

IRRADIATION IN HEXANE WITH Hg 2537 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

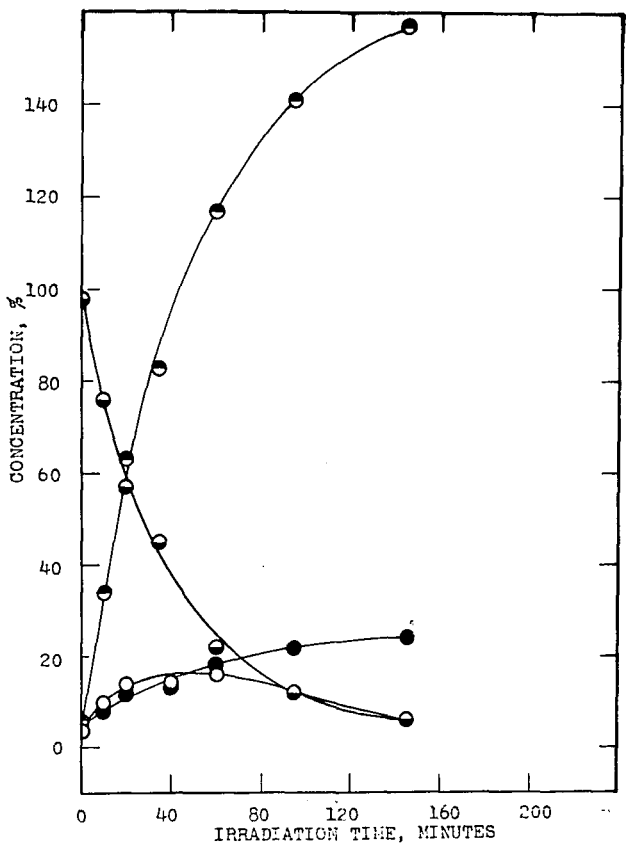


FIGURE 32

IRRADIATION IN HEXANE WITH Hg 2654 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

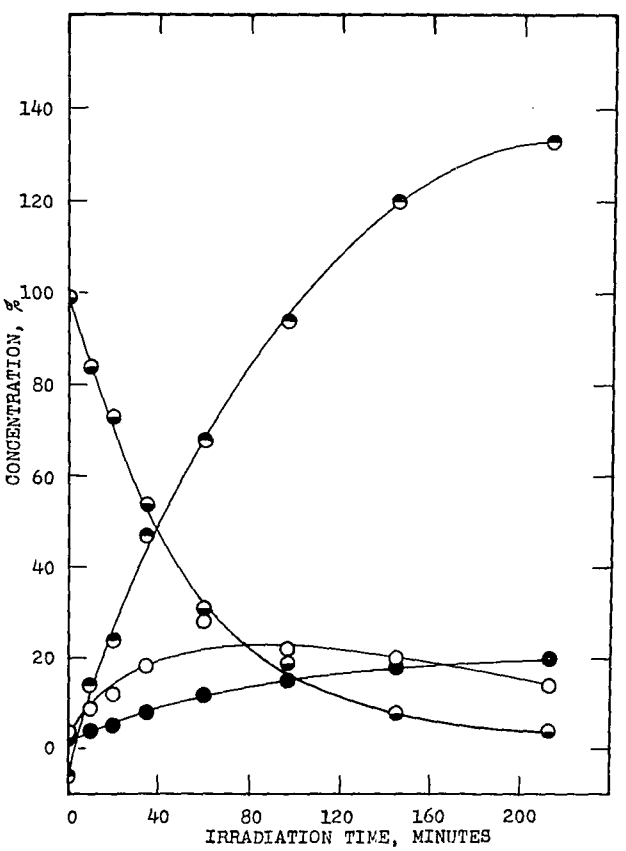


FIGURE 33

IRRADIATION IN HEXANE WITH Hg 2753 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

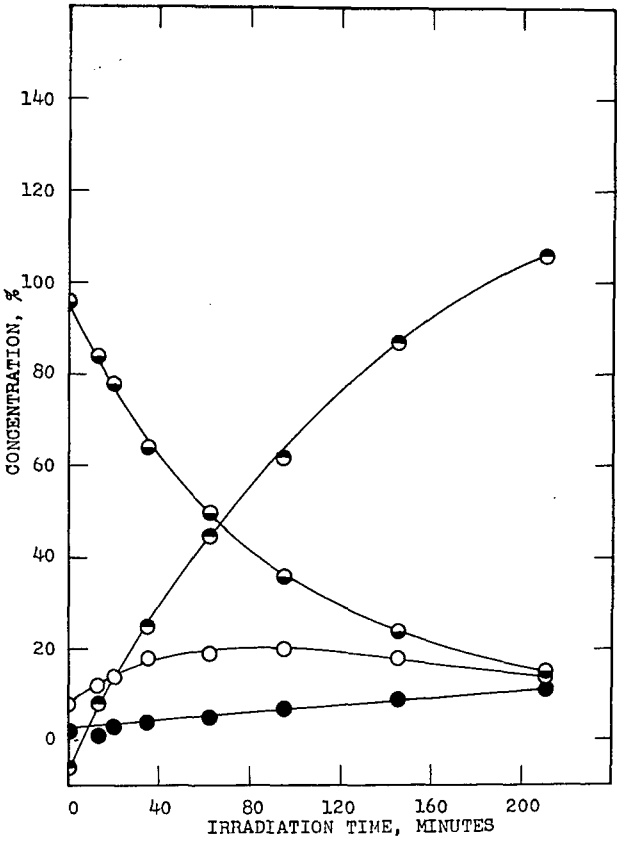


FIGURE 34

IRRADIATION IN HEXANE WITH Hg 2967 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

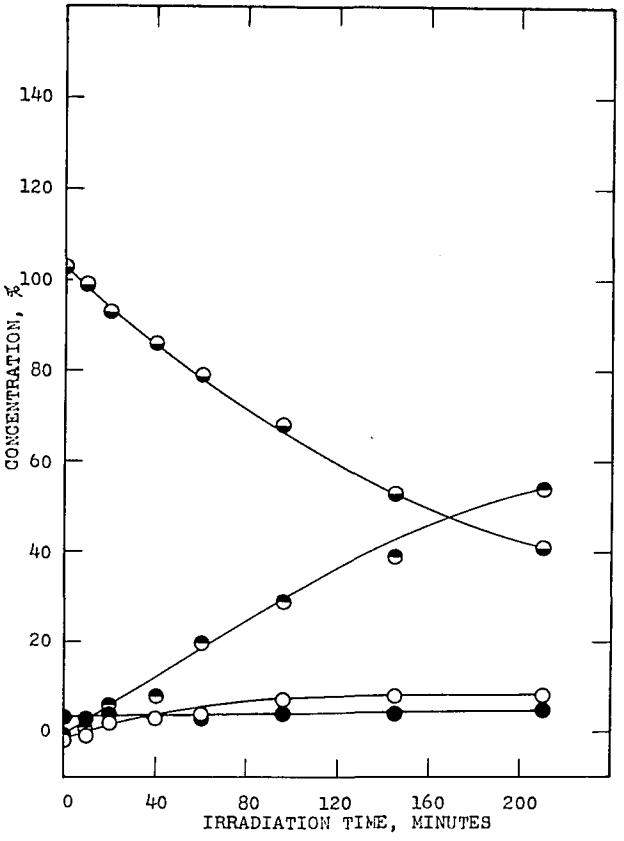


FIGURE 35

IRRADIATION IN HEXANE WITH Hg 3132 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

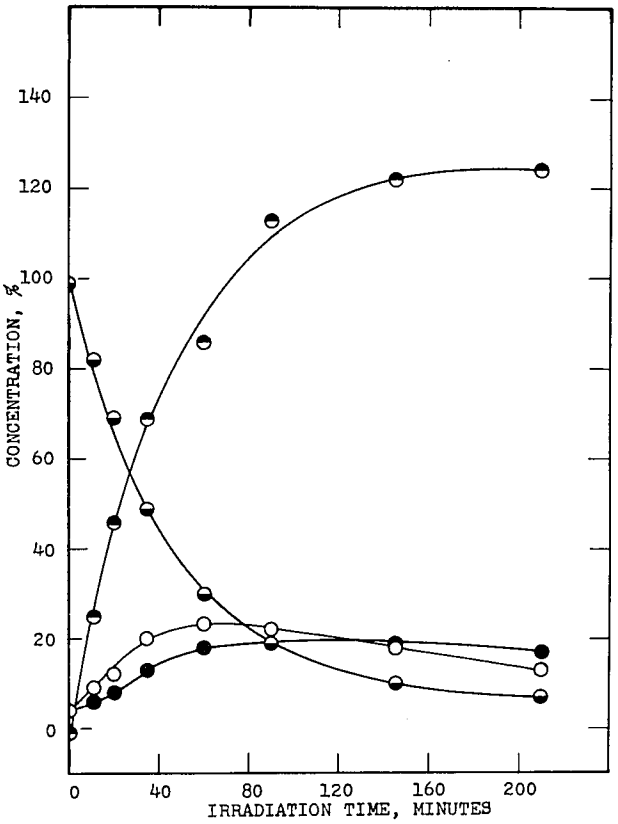


FIGURE 36

IRRADIATION IN HEXANE-ETHER WITH Hg 2483 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

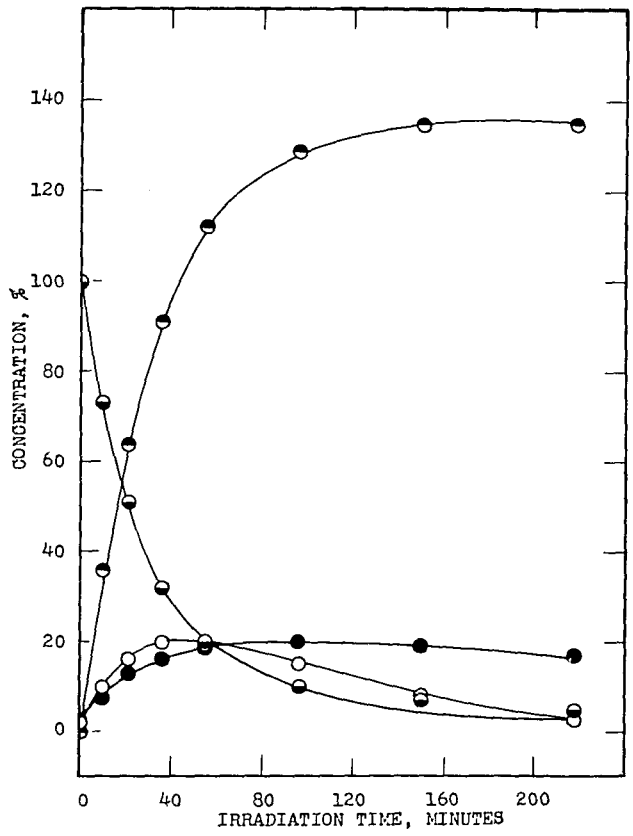


FIGURE 37

IRRADIATION IN HEXANE-ETHER WITH Hg 2537 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

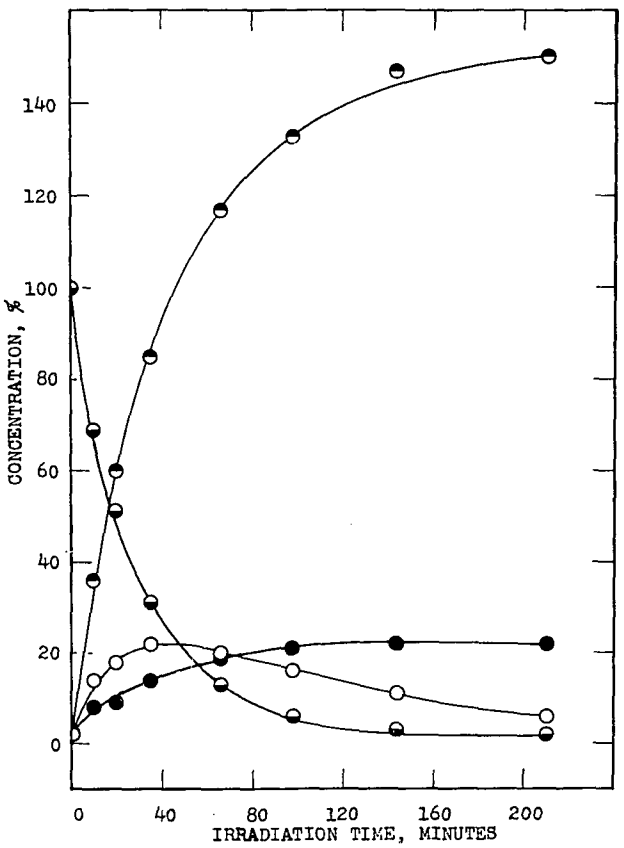


FIGURE 38

IRRADIATION IN HEXANE-ETHER WITH Hg 2654 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

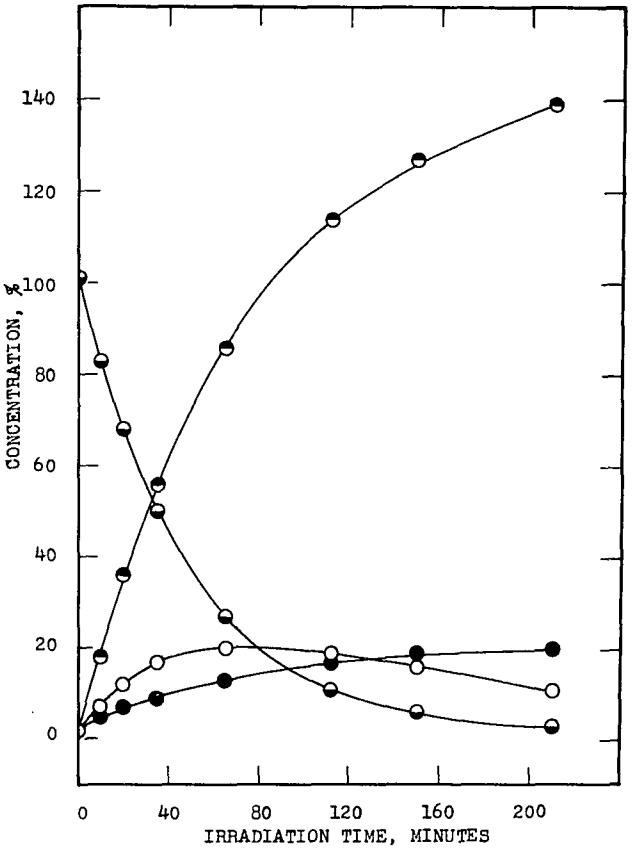


FIGURE 39

IRRADIATION IN HEXANE-ETHER WITH Hg 2753 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

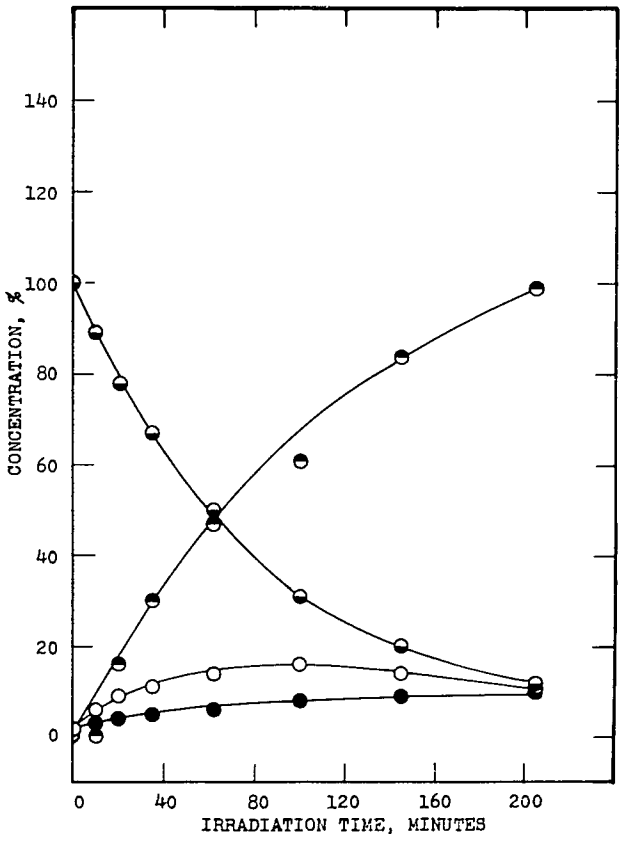


FIGURE 40

IRRADIATION IN HEXANE-ETHER WITH Hg 2967 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

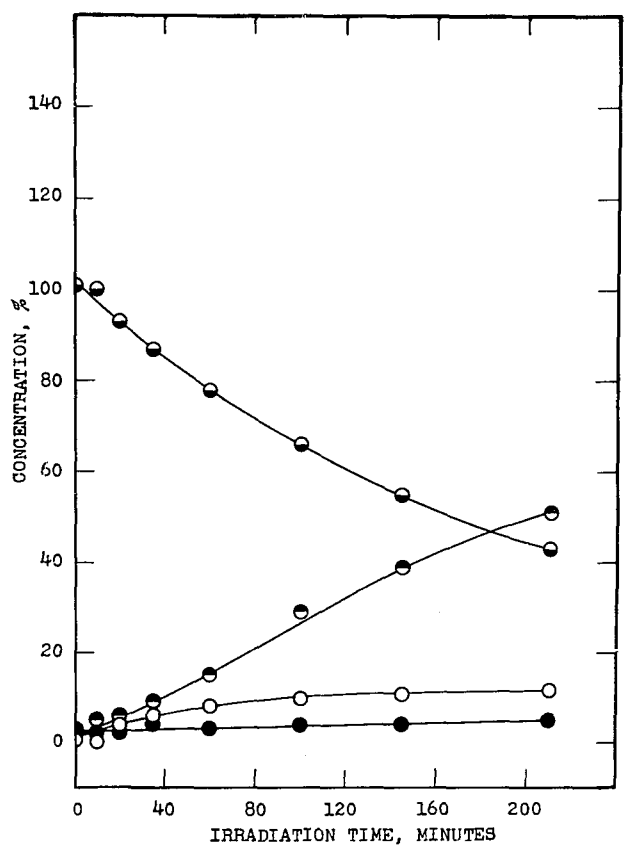


FIGURE 41

IRRADIATION IN HEXANE-ETHER WITH Hg 3132 A. LINE

◒ = D; ○ = P; ● = T; ◓ = S

concentrations were much greater than was possible. For example, in Figure 27 the calculated concentration of suprasterols after an irradiation time of 210 minutes was 159%, which was an absurd value. The method of calculation of the suprasterol concentrations was inadequate to obtain accurate values.

It was noted that the changes in suprasterol concentrations were similar to those of toxisterol concentration. For example, in Figure 24 the rate of change of toxisterol concentration was nearly constant after 95 minutes and the rate of change of suprasterol concentration was low, while in Figure 27 the toxisterol concentration was changing slowly at all times and the suprasterol concentration was changing rapidly. This effect was observed in every case, suggesting that the large errors in calculated suprasterol concentrations were caused by the inadequacy of the equations used, particularly with regard to toxisterol. This was probably partly due to the presence of suprasterol III in the irradiation mixture, which would contribute to some extent to the absorption at 250 mu, a contribution which has been neglected in the calculations of composition.

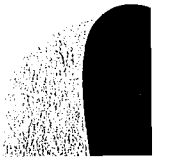
Because of the very low E (1%, 1 cm.) values of the suprasterols at 230 mu, the calculated concentrations which were based upon the measured extinction at 230 mu were very sensitive to small errors in absorption measurements in that

region. Unfortunately, the reproducibility of extinction values in the region 220-240 mu was much less than would be desirable.

As a result of the inaccuracies involved in the measurements and the inadequacies of the treatment, only qualitative conclusions regarding the suprasterols and toxisterol could be expressed.

The formation of suprasterols was the principal reaction which occured on irradiation of calciferol with ultraviolet light of wave lengths between 2483 and 3132 A. The formation of toxisterol accompanied this reaction but to a much lesser extent. There was no evidence to support a conclusion that the formation of toxisterol preceded the formation of suprasterols.

In Figures 24-41 the concentration of protachysterol increased rapidly early in the irradiation, reached a maximum value and finally decreased when the concentration of calciferol had reached rather low values. This indicated that the formation of protachysterol from calciferol was a process which occured simultaneously with the formation of toxisterol and suprasterols, with subsequent decomposition, probably to the suprasterols. There was no evidence that the formation of protachysterol preceded the formation of toxisterol or the suprasterols, but the possibility that it did was not excluded.



The rate of change of calciferol concentration, as shown in Figures 24-41, appeared to be exponential. In an effort to discover the order of the reaction the proposition was made that the reaction would follow the general rate expression:

$$- \frac{d(D)}{dt} = k\ (D)^n,$$

where (D) is the concentration of calciferol;

k is the rate constant for the reaction under the conditions of the irradiation;

n is the order of the reaction.

Taking logarithms of both sides of the equation there resulted:

$$\ln \frac{d(D)}{dt} = \ln k + n \ln (D).$$

As a good approximation the rate, $- \frac{d(D)}{dt}$, was replaced by $- \Delta(D)/\Delta t$ and the concentration, (D), was replaced by the average concentration, $\overline{(D)}$, in the time interval, Δt. A plot of $\ln - \Delta(D)/\Delta t$ as a function of $\ln \overline{(D)}$ would be linear, if n was constant. Figure 42 shows this plot using data from several different irradiation experiments. In no case was this plot linear, indicating the variance of n throughout the reaction. This result was in agreement with the conclusion of Green (14) that the reaction was of changing order.

On the basis of the evidence obtained in this investigation the photoreaction of calciferol has been considered a combination of three simultaneous reactions, one of which

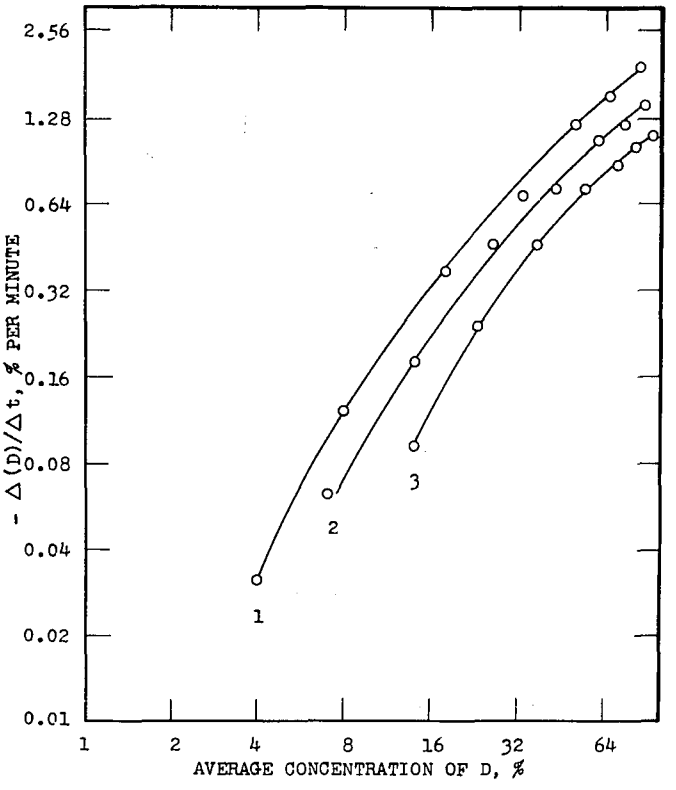
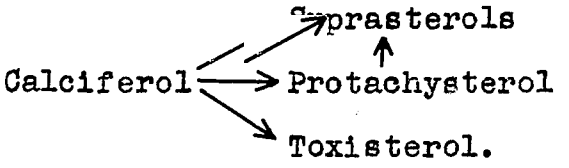


FIGURE 42

VARIATION OF REACTION RATE WITH CONCENTRATION OF CALCIFEROL IRRADIATED IN ETHANOL WITH (1) Hg 2537 A. (2) Hg 2753 A. (3) Hg 2967 A.

led to the formation of a substance resembling protachysterol which underwent further reaction, probably to form one or more of the suprasterols. This has been represented as follows:



The reaction resulted from the irradiation of calciferol with light of wave lengths between 2483 and 3132 A., although the reaction produced by light of wave lengths greater than 2967 A. was much slower than that produced by light of shorter wave lengths. The very slow rate of reaction produced by the Hg 3132 A. line may have been the reason why Bowden and Snow (19) did not observe a change in the absorption spectrum after irradiation of calciferol with light of that wave length.

There was no apparent variation of the reaction as a result of the influence of solvent when the irradiation was performed in ethanol, hexane or a mixture of hexane and ether.

SUMMARY

Solutions of calciferol in ethanol, n-hexane and a mixture of n-hexane and ethyl ether were irradiated with monochromatic light of wave lengths 2483, 2537, 2654, 2753, 2967 and 3132 A. The absorption spectra of the solutions were measured at various times during the irradiations.

Absorption spectra measurements indicated the presence of a substance resembling protachysterol in its absorption spectrum.

The composition of the irradiation mixtures with respect to (1) calciferol, (2) toxisterol, (3) protachysterol and (4) combined suprasterols was estimated on the basis of absorption spectra measurements.

Upon irradiation, calciferol was converted into toxisterol, protachysterol and suprasterols I, II and III; the protachysterol thus formed was converted photochemically to one or more of the suprasterols.

The photoreaction of calciferol was of changing order with respect to time.

The photoreaction of calciferol was brought about by light of wave lengths in the range 2483-3132 A., although the effectiveness of light of the longer wave lengths was somewhat less than that of shorter wave lengths. The reaction was apparently the same in each of the three solvents used.

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