STUDIES ON THE MODE OF ACTION OF IRRADIATED ERGOSTEROL

I. Its effect on the Calcium, Phosphorus and Nitrogen Metabolism of Normal Individuals [1, 2]

By WALTER BAUER, ALEXANDER MARBLE [3] and DOROTHY CLAFLIN

(From the Medical Clinic of the Massachusetts General Hospital, Boston)

(Received for publication June 6, 1931)

INTRODUCTION

The literature dealing with the development of irradiated ergosterol has been adequately reviewed in recent articles (1–13). Steenbock (14, 15) and Hess (16) in 1924 observed that certain foods, notably oils, milk, and cereals, can be made to possess antirachitic properties by exposure to ultraviolet light. It soon became evident that this acquired antirachitic property was lodged in the sterol portion of these foods, and at first it was thought that this sterol was cholesterol (17, 18, 19). By 1926 the investigations of Rosenheim and Webster (20) and of Hess and Windaus (21) had demonstrated clearly that the antirachitic properties were taken on not by cholesterol itself, but by an accompanying sterol, ergosterol. This sterol had been isolated years before by Tanret (22), who differentiated it from cholesterol and extracted it from ergot, yeast, mushrooms, and other fungi.

In the last five years irradiated ergosterol has been given extensive laboratory and clinical trial. Neglecting the data which have been acquired as to its chemistry, physical properties, preparation and activation, one may outline the present state of knowledge concerning it as follows:

(1) Irradiated ergosterol is definitely prophylactic and curative in rickets and rachitic tetany (1, 3, 8, 23). Coincident with clinical improvement, there occurs a rise in the values (if low) of serum calcium and phosphorus, and a normal deposition of lime salts in the bones. The efficacy of irradiated ergosterol in osteomalacia is practically as well established, but improvement is reported to take place more slowly than in rickets (24, 25). Its reported effectiveness in hastening the union of fractured bones (26, 27), in preventing dental caries (28), in increasing bodily resist-

[1] This is publication No. 4 of the Robert W. Lovett Memorial for the study of crippling disease, Harvard Medical School, Boston, Massachusetts.

[2] Part of the expense orthis study was paid by the William W. Wellington Memorial Research Fund.

[3] Medical Resident, Massachusetts General Hospital.

ance against infections (2, 29), in preventing rickets in infants by administration to pregnant and nursing mothers (30), and in other situations of less definite indication, remains to be settled by wider use and further study.

(2) Irradiated ergosterol is a very potent agent; the often quoted statement that 1 mgm. has the antirachitic potency of about 200,000 times that amount of standard cod liver oil is sufficiently accurate to serve as emphasis of this strength.

(3) Its administration in excessively large doses to animals (31–35) has resulted in anorexia, loss of weight and strength, often diarrhea, emaciation, and finally death. At necropsy calcium deposits have been found in various tissues, particularly the stomach wall, heart muscle, blood vessels, kidneys, bladder, ureters, and lungs (35, 36, 37). Some workers have ascribed the toxic effects of irradiated ergosterol to contained impurities (38, 39, 40).

Because no adequate explanation concerning the mode of action of irradiated ergosterol could be found in the literature previously mentioned, studies were undertaken to determine the effect of irradiated ergosterol on the calcium and phosphorus metabolism of normal individuals and individuals with various disorders of calcium metabolism. The data from these experiments and some observations on animals are here presented because they are of value in explaining the modus operandi of this vitamin D substance. A preliminary report of these studies has been published (58).

We are indebted to the Winthrop Chemical Company of New York for supplying us with large quantities of "Vigantol" (Irradiated Ergosterol-Winthrop) for use in these experiments. The late F. C. Waldecker of the Winthrop Chemical Company informed us that this was the same specially prepared product furnished to Shohl et al. (37) who found that "0.0001 to 0.00025 mgm. is sufficient, when fed to rats on the Steenbock diet, to protect against rickets."

METHODS OF STUDY

Patients were studied for varying lengths of time in the Metabolism Ward. Each patient was kept on a constant fluid intake and on a diet which was accurately weighed, adequate in caloric and vitamin content and the ash of which was neutral in reaction. The mineral content of each diet was carefully estimated and kept constant throughout each experiment. The urine and stools were carefully collected in three-day periods and prepared for analysis. The collecting of excreta was not begun until the patient had been on the special cliet for at least ten days. Thus the control periods represented the true excretions for the diet employed. The routine of the ward and the laboratory has been outlined in a previous paper (41). Calcium was determined by the method of Fiske (42), phosphorus, by the method of Fiske and Subbarow (43), carbon dioxide content of blood, by that of Van Slyke (44), cholesterol, by that of Bloor, Pelkan and Allen (45), and nitrogen, by the Kjeldahl method (46).

EXPERIMENTS

I. Normal individuals receiving small doses of irradiated ergosterol

Experiment I

Mr. R. L., an apparently normal 19 year old Italian school boy, who volunteered for this investigation, was studied for 51 days while receiving a *low* calcium diet. During the last 33 days of this period he was given *5 mgm.* of irradiated ergosterol per day. In Charts 1*A*, 1*B* and Table I are presented the data.

The fecal calcium averaged 0.25 gram during the medication period compared to an average of 0.20 gram during the control periods (see Table I). The average urinary calcium values for the same periods of

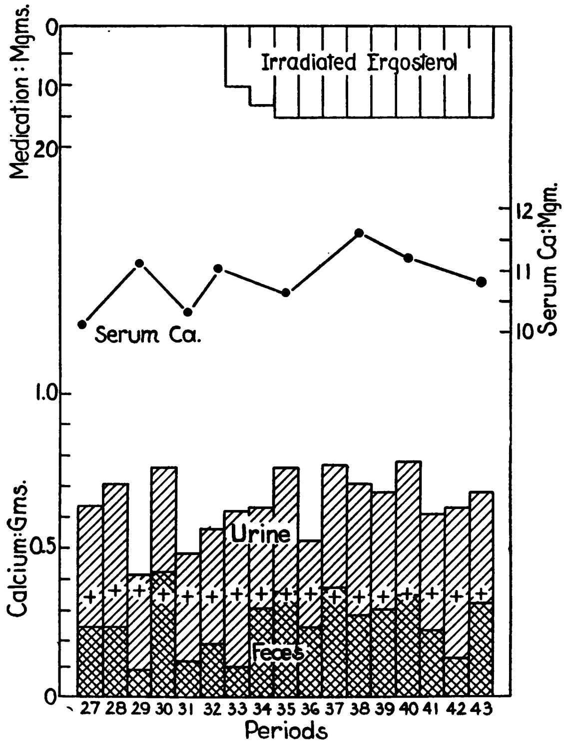

CHART 1*A*. GRAPHIC REPRESENTATION OF CALCIUM METABOLISM IN EXPERIMENT I

In this chart as well as in all subsequent charts the intake is designated by +, the urinary excretion by single hatching and the fecal excretion by double cross hatching.

TABLE I

The effect of irradiated ergosterol on the calcium, phosphorus and nitrogen metabolism of normal individuals. Average values in grams per three-day period

Experiment	Periods	Average weight	Phosphorus				Calcium				Nitrogen				Irradiated ergosterol per period	Remarks
			Intake	Output		Balance	Intake	Output		Balance	Intake	Output		Balance		
				Urine	Feces			Urine	Feces			Urine	Feces			
Small doses of irradiated ergosterol																
		kgm.	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*mgm.*	
I. Mr. R. L......	27 to 32	77.1	2.40	2.23	0.38	−0.21	0.34	0.38	0.20	−0.24	35.0	34.5	3.5	− 3.0	0	Low calcium diet
	33 to 43	73.8	2.40	1.72	0.47	+0.21	0.34	0.41	0.25	−0.32	35.0	33.7	3.5	− 2.2	15	Average dietary formula per three-day period = C_{650} P_{219} F_{215}
II. Mrs. M. A. R..	15 to 18	70.2	5.14	3.02	1.25	+0.87	4.28	0.75	2.67	+0.86	47.7	31.1	4.8	+11.7	0	High calcium diet
	19 to 23	70.1	5.16	3.13	1.07	+0.96	4.28	0.77	2.83	+0.68	47.4	35.6	4.7	+ 7.1	30	Average dietary formula per three-day period = C_{539} P_{300} F_{275}
	24 to 27	70.3	5.16	3.08	1.16	+0.92	4.28	0.61	3.01	+0.66	44.8	36.9	4.5	+ 3.4	60	
Large doses of irradiated ergosterol																
III. Mrs. M. A. R.	1 to 6	73.6	2.10	1.38	0.49	+0.23	0.28	0.25	0.28	−0.25	29.8	23.5	3.0	+ 3.3	0	Low calcium diet
	7 to 14	72.6	2.09	1.57	0.42	+0.10	0.27	0.39	0.17	−0.29	29.0	21.5	2.9	+ 4.6	90	Average dietary formula per three-day period = C_{785} P_{185} F_{250}
	12 to 14	72.3	2.07	1.51	0.31	+0.25	0.27	0.46	0.11	−0.30	29.0	23.1	2.9	+ 3.0	90	
IV. Mr. R. L.....	4 to 5	75.1	5.78	3.68	0.99	+1.11	4.46	0.88	1.91	+1.67	58.6	46.6	5.9	+ 6.1	0	High calcium diet
	6 to 14	75.7	5.78	3.67	0.93	+1.18	4.46	1.19	1.54	+1.73	58.6	42.5	5.9	+10.2	90	Average dietary formula per three-day period = C_{898} P_{366} F_{396}
	15 to 23	76.5	5.78	3.64	0.84	+1.30	4.46	1.19	1.61	+1.66	58.6	46.3	5.9	+ 6.4	30	
	24 to 26	76.9	5.78	3.60	1.25	+0.93	4.46	1.00	2.29	+1.17	58.6	53.0	5.9	− 0.3	0	

study were 0.41 gram and 0.38 gram respectively. One is hardly justified in calling such slight changes an effect of the administration of irradiated ergosterol. The serum calcium rose slightly.

The effect on the fecal phosphorus was little greater than that on the fecal calcium. The average for the control periods was 0.38 gram as compared to 0.47 gram during the medication period. The effect on the urinary phosphorus, however, was more marked, for it fell from an average of 2.23 grams in the control periods to 1.72 gram in the ergosterol periods. These changes resulted in a shift from a negative phosphorus balance of 0.21 gram to a positive balance of 0.21 gram. There occurred no variations in the serum phosphorus which could be attributed to irradiated ergosterol.

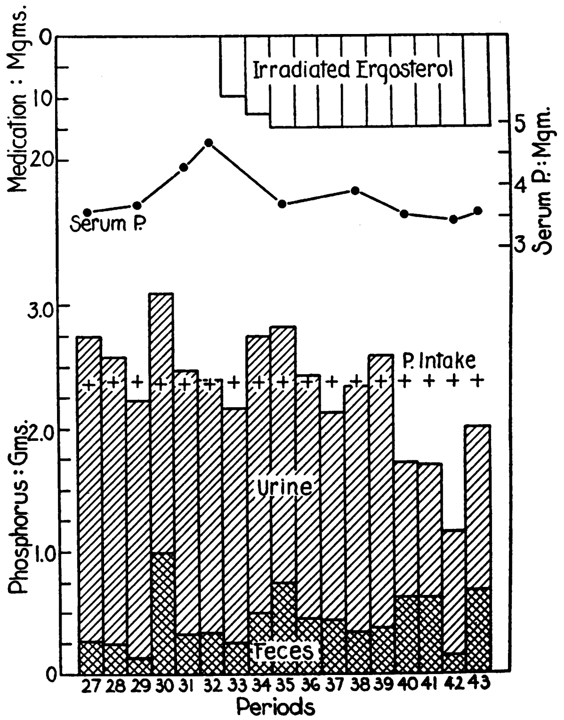

CHART 1*B*. PHOSPHORUS METABOLISM IN EXPERIMENT I

Experiment II

Mrs. M. A. R., an apparently normal 27 year old widow, who volunteered for this purpose, was studied for 39 days, while receiving a *high* calcium diet. During the last 27 days of this period she was given *small* doses of irradiated ergosterol. During the first 15 days she received *10 mgm.* a day; for the remaining 12 days, *20 mgm.* The data from this case are shown in Charts 2*A*, 2*B* and Table I.

One notes that doses of *10 and 20 mgm.* of irradiated ergosterol produced very little more effect on the calcium metabolism than did 5 mgm. in experiment I. True, the fecal calcium rose from an average of 2.67 grams during the control periods to 2.83 grams while receiving 10 mgm. a day and finally reached 3.01 grams during the periods in which 20 mgm.

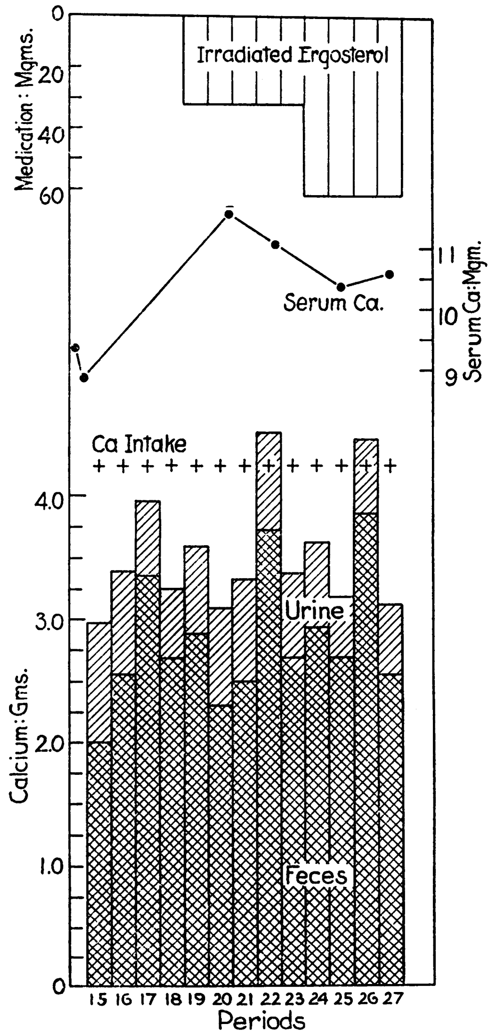

CHART 2*A*. CALCIUM METABOLISM IN EXPERIMENT II

a day were given. Instead of rising as it had done in experiment I, the urinary calcium fell from an average of 0.75 gram to 0.61 gram per period (see Table I). The serum calcium increased, reaching its high point 5 days after the institution of irradiated ergosterol therapy. It remained above the control values during the remainder of the study period.

The changes in the phosphorus metabolism were less marked than the calcium metabolism changes. The fecal phosphorus fell from an average of 1.25 gram in the control periods to 1.16 gram in the last three medication periods. The urinary phosphorus remained unchanged; the control value was 3.02 grams compared to an average value of 3.08 grams for the last three medication periods. These changes are very slight, yet the exact opposite of those noted in the phosphorus metabolism of experiment I. The serum phosphorus was unaffected.

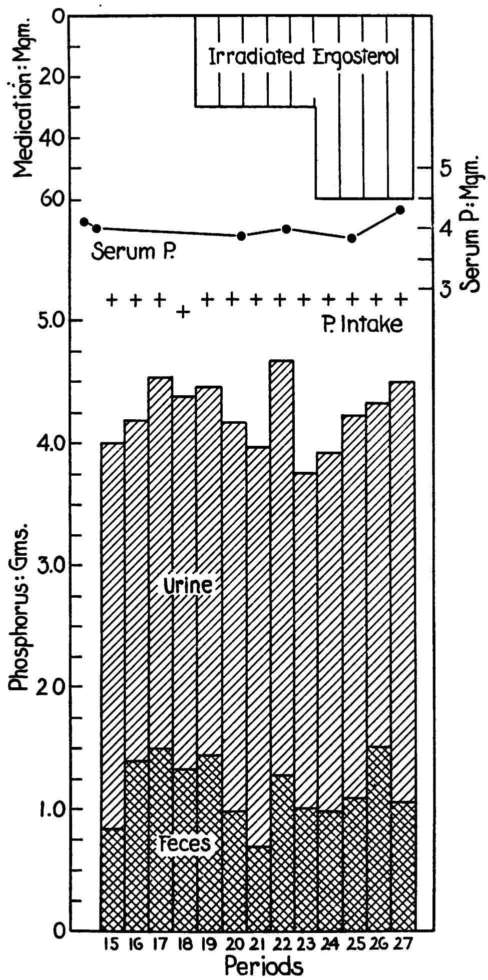

CHART 2*B*. PHOSPHORUS METABOLISM IN EXPERIMENT II

II. Normal individuals receiving large doses of irradiated ergosterol

Experiment III

Mrs. M. A. R. was also studied while receiving a low calcium diet and *30 mgm.* of irradiated ergosterol per day. An interval of 26 days was permitted to elapse between this period of study and the one made while the patient was on a high calcium intake. From Charts 3*A*, 3*B* and Table I, one observes that during the first period of irradiated ergosterol administration there was a marked rise in the fecal calcium and a fall in the urinary calcium. In the subsequent periods, there was a gradual fall in the fecal calcium. Coincident with this fall in fecal calcium there was a gradual rise in the urinary calcium of approximately the same magnitude. The average for the fecal calcium during the control period was 0.28 gram compared to an average of 0.11 gram during the last three periods of therapy. The urinary calcium averages for these same periods of study were 0.25 gram and 0.46 gram. There resulted a very slight increase in the already existing negative calcium balance (see Table I). The slight rise in serum calcium seen in Chart 3*A* probably falls within the limits of physiological variation, although it may conceivably represent an effect of irradiated ergosterol.

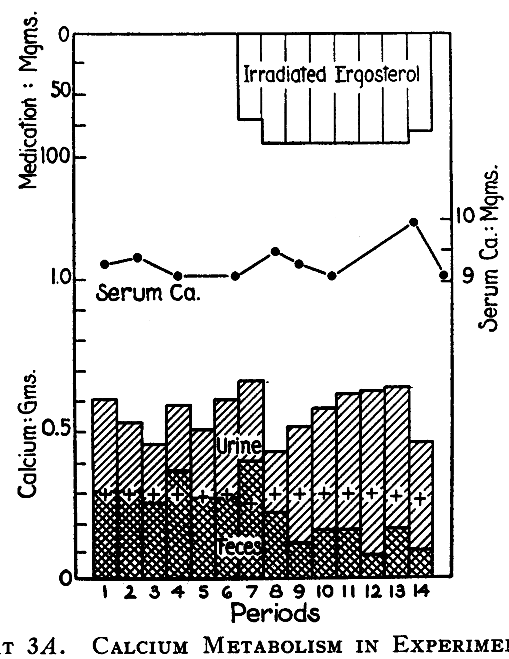

CHART 3*A*. CALCIUM METABOLISM IN EXPERIMENT III

The effect on the phosphorus metabolism was the same as that on the calcium metabolism, although the changes were less marked. At first there was a rise in the fecal phosphorus and a fall in the urinary phosphorus. Following this there resulted a gradual fall in the fecal phosphorus. The average fecal phosphorus excretion for the control periods was 0.49 gram, falling to an average of 0.31 gram during the last three periods of therapy. The effect on the urinary phosphorus was the opposite; it rose from 1.38 gram per three-day period before irradiated ergosterol was administered to 1.51 gram during the last three periods of ergosterol medication. The average phosphorus balance was + 0.23 gram during the control period (1 to 6) compared to + 0.10 gram in the medication period (7 to 14), yet the average for the last three periods (12 to 14) was + 0.25 gram. The changes in the serum phosphorus were not constant enough to be ascribed to the effect of ergosterol.

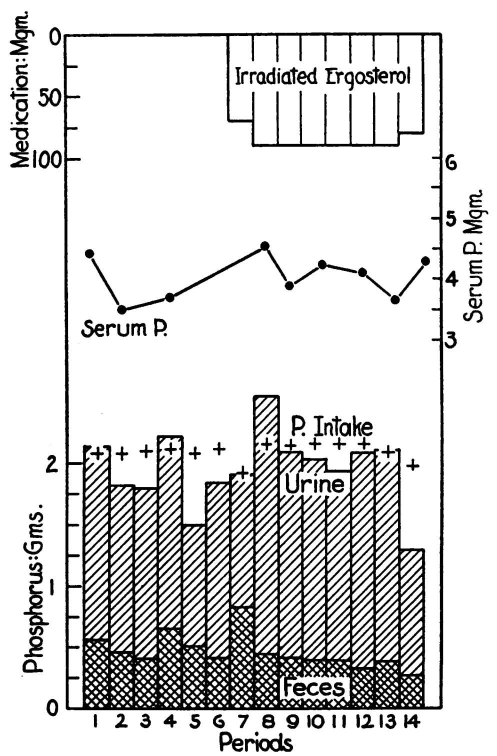

CHART 3*B*. PHOSPHORUS METABOLISM IN EXPERIMENT III

Experiment IV

In Charts 4*A*, 4*B* and Table I are presented the calcium, phosphorus and nitrogen metabolism data from Mr. R. L. for a period of 78 days in which he was given a high calcium diet. During periods 6 to 14 the dose of irradiated ergosterol was *30 mgm.* a day. During periods 15 to 23 it was reduced to *10 mgm.* a day.

The calcium data confirm the observations made in experiment III (see Table I). During the 9 periods (27 days) in which 30 mgm. a day were given, the fecal calcium per three-day period was 1.54 gram in contrast to the value of 1.91 gram for the control period. During this time the urinary calcium rose from a control value of 0.88 gram per three-day period to 1.19 gram. The positive calcium balance increased from + 1.67 gram to + 1.73 gram. With a reduction of the irradiated ergosterol dosage to 10 mgm. a day, the fecal calcium rose very slightly to 1.61 gram, while the urinary calcium remained unchanged, resulting in a

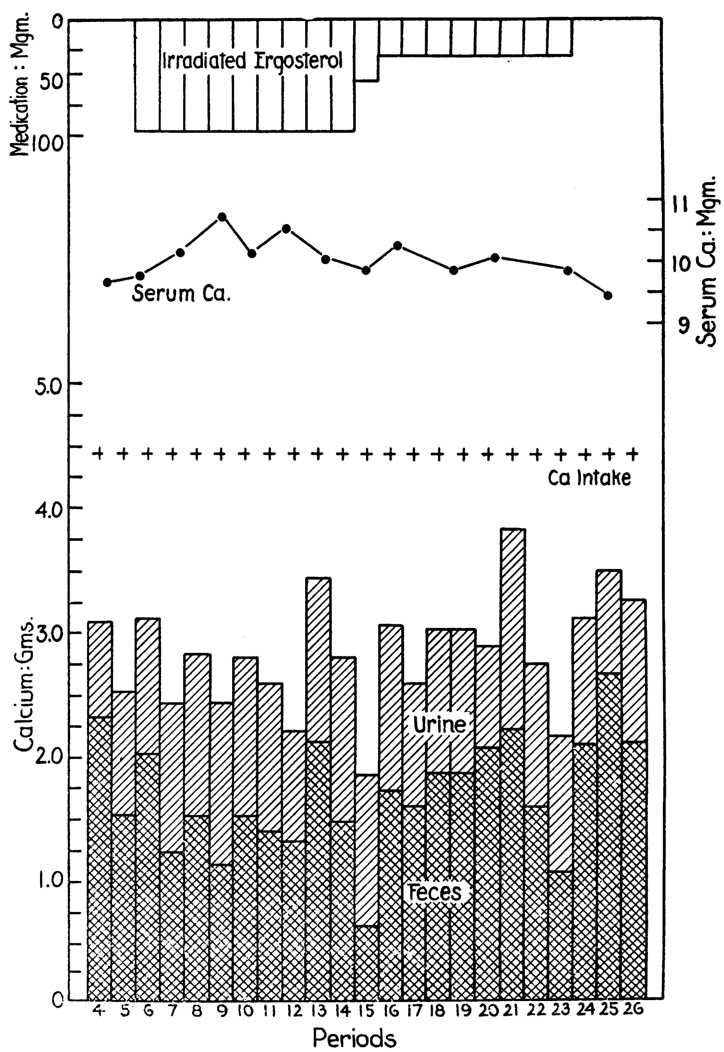

CHART 4A. CALCIUM METABOLISM IN EXPERIMENT IV

slight lowering of the total calcium balance to + 1.66 gram. Following the cessation of irradiated ergosterol therapy (periods 24 to 26), there was an abrupt rise in the fecal calcium to 2.29 grams, a level even higher than that observed in the control periods. The urinary calcium fell to 1.00 gram during these same two periods. The serum calcium showed changes which are slight, but probably significant, as they parallel closely the changes in the calcium balance. The serum calcium reached a high point of 10.8 mgm. per 100 cc. during the period of large ergosterol dosage. On reduction to 10 mgm. a day, the serum calcium fluctuated

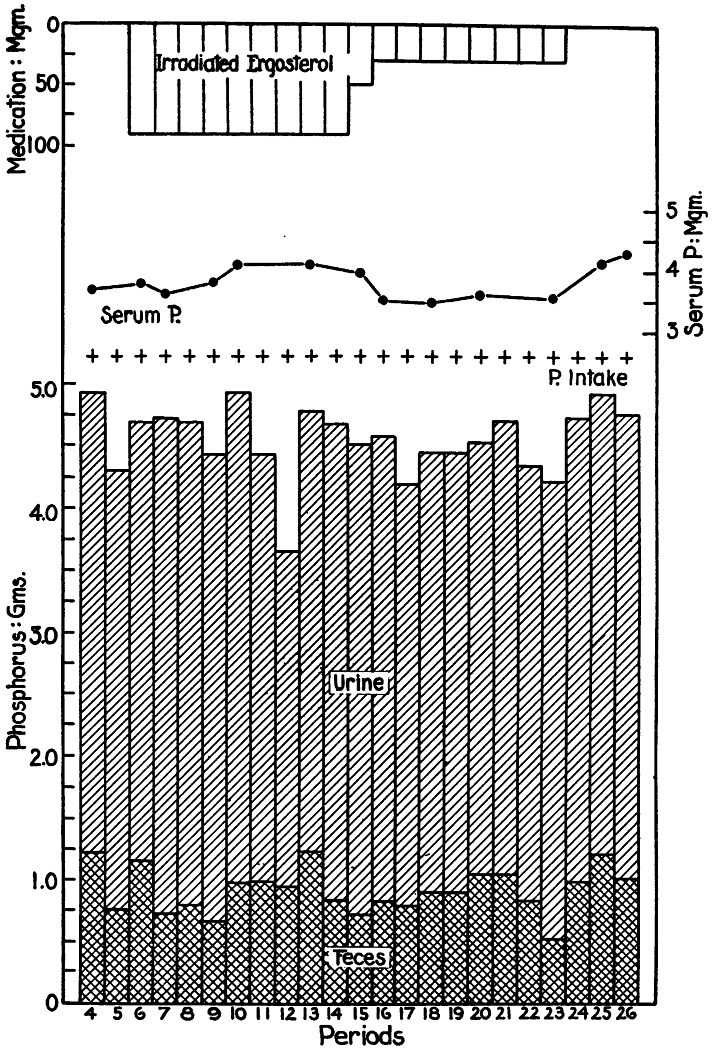

CHART 4*B*. PHOSPHORUS METABOLISM IN EXPERIMENT IV

between 9.9 and 10.3 mgm. per 100 cc. On discontinuance of the drug, it fell to 9.5 mgm. per 100 cc., the value obtained during the control period.

From Chart 4*B* and Table I one notes that the effect on the phosphorus metabolism was not so marked. With the administration of 30 mgm. of irradiated ergosterol per day, the fecal phosphorus fell to 0.93 gram as contrasted with a value of 0.99 gram during the control period. This fall continued during the subsequent periods, 15 to 23, to 0.84 gram despite the fact that the dose of irradiated ergosterol was reduced. When, however, the drug was discontinued, an abrupt and striking rise occurred. The average fecal phosphorus during these periods, 24 to 26, was 1.25 gram. The urinary phosphorus remained fairly constant during the control and medication periods, with perhaps a slight fall on discontinuance of the drug. These changes were associated with an increase in the positive phosphorus balance. The changes in the serum phosphorus were very slight, although the variations seemed to parallel those of the serum calcium.

During period 12 this patient had four or five stools a day for two days instead of his usual one or two. At the time it was thought that this might possibly be a symptom of overdosage of irradiated ergosterol. Although the same dose was continued for two more periods the bowel disturbance ceased.

III. The effect of irradiated ergosterol administration on the absorption of large doses of calcium lactate from the gastro-intestinal tract

In order to obtain further information concerning the effect of irradiated ergosterol administration on the absorption of calcium from the gastro-intestinal tract of normal individuals, the following experiments were performed on two normal individuals, Miss D. C. and Mr. A. M. After fasting twelve hours, each subject was given 10 grams of calcium lactate in 250 cc. of water, a control serum calcium having been obtained prior to the ingestion of the calcium lactate. Serum calcium determinations were made every hour for the first three hours and at the end of the sixth, ninth, twelfth and twenty-fourth hours. From Table II it will be noted that in each individual the serum calcium rose to a maximum height at the end of the third hour. In the case of Miss D. C. this elevation was 2.2 mgm. and in Mr. A. M., 2.5 mgm. above the fasting serum calcium value.

Irradiated ergosterol was then administered to each subject in doses of 30 mgm. a day for twelve days. On the twelfth day the same experiment was repeated. Again the serum calcium reached a maximum at the end of the third hour. However, in each subject this rise was approximately 1 mgm. higher than it had been in the control experiments.

These experiments would seem to serve as evidence that the administration of irradiated ergosterol increases the absorption of calcium from the gastro-intestinal tract. That the extra calcium absorbed is not necessarily retained is strongly suggested by the experiments performed on Mr. A. M. During each experiment the urinary calcium excretion was determined for the first twelve hours following the ingestion of the cal-

TABLE II

Showing the effect of the ingestion of 10 grams of calcium lactate on the serum calcium before and during the administration of irradiated ergosterol

Time	Miss D. C.		Mr. A. M.	
	Serum calcium		Serum calcium	
	Before therapy	During * therapy	Before therapy	During * therapy
	mgm.	*mgm.*	*mgm.*	*mgm.*
Fasting	11.0	11.6	9.8	10.6
	10 grams calcium lactate administered			
1 hour after	11.8	13.6	11.1	12.3
2 hours after	12.2	14.2	12.0	13.0
3 hours after	13.2	14.6	12.3	14.0
6 hours after	11.3	13.7	11.2	13.8
9 hours after	10.4	12.0	11.2	12.2
12 hours after	10.6	12.6	10.7	11.5
24 hours after	10.4	10.4	9.6	10.9

* Each subject had received 30 mgm. of irradiated ergosterol per day for twelve days. The second experiment was performed on the twelfth day of such therapy.

cium lactate. In the control experiment the amount of calcium excreted in the urine was 110 mgm.; in the experiment performed during the administration of irradiated ergosterol, 195 mgm.

The experiments reported by Warkany (47*b*) are sufficient proof that the administration of irradiated ergosterol increases the absorption of phosphorus as well as calcium. He administered 0.5 gram of Na_2HPO_4 per kilogram of body weight and noted the subsequent rise in the serum phosphorus. This same experiment was later repeated, irradiated ergosterol having been administered daily in the interim. In these latter instances, the increase in the serum phosphorus was frequently twice as great as that which he had observed in the control experiments.

DISCUSSION

These observations on the calcium and phosphorus metabolism of normal individuals show quite clearly the metabolic effects which result from the daily administration of irradiated ergosterol to normal individuals.

From the first two experiments it is seen that the effects of small doses of irradiated ergosterol (*5 to 20 mgm.*) when given to normal adults on either a high or low calcium diet were very slight and not constant. The fact that the changes were very slight, inconstant and opposite in nature in these two cases probably means that the variations noted were not significant.

From experiments III and IV one notes that the effect of *30 mgm.* of irradiated ergosterol on normal individuals is the same whether the calcium intake is high or low. This dose exerts the same effect on both the calcium and phosphorus metabolism. *The resulting changes are a fall in the fecal calcium and phosphorus accompanied by a rise in the urinary calcium and phosphorus.* The calcium and phosphorus balances were little affected, a finding which is in accord with that of other workers (9) (48) (49). The decreased fecal calcium excretion might be interpreted as signifying either increased absorption from the gastro-intestinal tract or failure to re-excrete calcium into the bowel because of increased retention resulting from the administration of irradiated ergosterol.[4] The latter theory seems unjustified because of the findings in experiment III. In this instance during the control period the calcium intake and the fecal calcium were identical, 0.28 gram. The fecal calcium fell to 0.11 gram during the last three periods of irradiated ergosterol medication, although the intake remained unchanged. If this decreased fecal excretion had been due to increased retention, one would not have expected the urinary excretion to increase as much as it did, from 0.25 gram to 0.39 gram. That the increased calcium absorbed was without benefit to the body calcium stores was apparent because the negative calcium balance increased during the period of medication.

Thus it would seem that the administration of irradiated ergosterol to individuals with adequate body stores of calcium and phosphorus does not necessarily result in the retention of these elements in the body. The increased amount of calcium and phosphorus absorbed is evidently re-excreted in the urine. The fact that the urinary calcium and phosphorus rise as the fecal calcium and phosphorus fall is best interpreted as signifying that increased retention does not occur in normal individuals as the result of irradiated ergosterol administration.

The difference in the effect of small and large doses of irradiated ergosterol in normal individuals cannot be ascribed to individual variation because the same patients were used in both types of experiments.

From our experiments it would appear that the action of irradiated ergosterol is an immediate one. Beumer's (50) and Hottinger's (51) data are in accord with such a view. György (52) stated that it took ten to fourteen days to produce a demonstrable ergosterol effect. In our experiments, the first effect noted (during the first period of its administra-

[4] There is considerable evidence in the literature which proves quite conclusively that calcium which is absorbed from one portion of the bowel may be re-excreted into another portion of the gastro-intestinal tract (46, 47*a*, 48). Proof of this has been previously published by one of us (W. B.) (57). In a group of normal subjects (46 three-day periods in all) studied on an inadequate intake (0.33 gram per three-day period), the average fecal calcium excretion per three-day period was found to be 0.60 gram. In other words, the fecal calcium excretion was 0.27 gram in excess of the intake.

tion) was a marked rise in the fecal calcium and a fall in the urinary calcium. The fecal phosphorus and urinary phosphorus were similarly affected. Following this immediate effect there then occurred a gradual fall in the fecal calcium and phosphorus and a gradual rise in the urinary calcium and phosphorus. Hottinger (51) noted similar changes when irradiated ergosterol was administered and referred to it as the diphasic ergosterol effect. Both Hottinger (51) and Kroetz (53) thought that a state of acidosis occurred during the first period of ergosterol administration, because they observed during the first twenty-four hours of such therapy an increased excretion of acid and a more acid urine. As the body returns to its normal state, the second phase sets in. We are unable to offer any explanation for this diphasic ergosterol effect observed in normal individuals.

In the normal individuals receiving small doses of irradiated ergosterol, the serum calcium was very slightly raised, although this may have been due in part in experiment II to the high calcium intake following a previous period of study on a low calcium diet. The serum phosphorus was unaffected in these two cases. When doses of 30 mgm. per day were administered, the changes in the serum calcium and phosphorus were slight, but probably represented an ergosterol effect. Other workers (54) (55) have demonstrated slight rises of both the serum calcium and phosphorus in normal individuals during irradiated ergosterol therapy, whereas Havard and Hoyle (56) failed to obtain such rises. The serum calcium can be elevated to higher levels by the ingestion of 10 grams of calcium lactate during the administration of irradiated ergosterol than under normal conditions. In any condition where calcium medication is required, the daily administration of irradiated ergosterol in conjunction with large doses of a calcium salt might be a better therapeutic procedure than the administration of a calcium salt alone.

No significant changes occurred in the nitrogen metabolism in any of the four experiments (see Chart 1). The plasma cholesterol was determined repeatedly in all these experiments, but because in no instance was there any noteworthy change as a result of irradiated ergosterol administration, these cholesterol values are not included in the tables. No untoward symptoms were observed in any of the four individuals.

SUMMARY

1. The administration of irradiated ergosterol in *small doses* to normal individuals produced no constant changes in either the calcium or phosphorus metabolism.

2. The administration of irradiated ergosterol in doses of *30 mgm.* per day to normal individuals resulted in an immediate increase of the fecal calcium and phosphorus excretion. The urinary calcium and urinary phosphorus were decreased. Following this there occurred a de-

creased fecal calcium and phosphorus excretion and an increase in the urinary calcium and phosphorus excretion. The calcium and phosphorus balances were only slightly affected. Following the cessation of irradiated ergosterol administration, the fecal calcium and phosphorus promptly rose and the urinary calcium and phosphorus fell slightly.

3. The serum calcium and phosphorus of normal individuals was only slightly affected by irradiated ergosterol therapy.

4. The nitrogen excretion was unaffected by irradiated ergosterol administration.

5. No constant changes in the blood plasma cholesterol were noted during the administration of irradiated ergosterol.

6. No untoward symptoms resulted from the administration of as much as *30 mgm.* of irradiated ergosterol per day.

BIBLIOGRAPHY

1. Hess, A. F., and Lewis, J. M., J. Am. Med. Assoc., 1928, xci, 783. Clinical Experience with Irradiated Ergosterol.
2. Pfannenstiel, W., Lancet, 1928, ii, 845. A Summary of Recent Work on Vigantol (Irradiated Ergosterol).
3. Karelitz, S., Am. J. Dis. Child., 1928, xxxvi, 1108. Activated Ergosterol in the Treatment of Rickets.
4. György, P., Klin. Wchnschr., 1927, vi, 580. Therapeutische Versuche mit bestrahltem Ergosterin.
5. György, P., Klin. Wchnschr., 1929, viii, 684. Sind die bisher üblichen Gaben von bestrahltem Ergosterin als überflüssig hoch zu bezeichnen?
6. Wieland, E., Ztschr. f. Kinderh., 1929, xlvii, 643. Vier Jahre indirekte Lichttherapie (mit bestrahlter Frisch- und Trockenmilch und verschiedenen Ergosterinpräparaten) bei Rachitis an der Basler Kinderklinik.
7. Blunt, K., and Cowan, R., J. Am. Med. Assoc., 1929, xciii, 1301. Irradiated Foods and Irradiated Ergosterol.
8. Hess, A. F., Rickets Including Osteomalacia and Tetany. Lea and Febiger, Philadelphia, 1929.
9. Brown, H. B., and Shohl, A. T., J. Biol. Chem., 1930, lxxxvi, 245. Rickets in Rats. XI. The Alteration of Calcium and Phosphorus Metabolism of Normal and Ricketic Rats Produced by Irradiated Ergosterol.
10. Mouzon, J., Presse Méd., 1927, xxxv, 1459. L'Ingestion de Substances Irradiées dans le Traitement et dans la Prévention du Rachitisme l'Ergostérine Irradiée.
11. Wilkes, E. T., Follett, D. W., and Marples, E., Am. J. Dis. Child., 1929, xxxvii, 483. The Treatment of Rickets with Irradiated Ergosterol.
12. Wiedow, München. med. Wchnschr., 1928, lxxv, 1501. Zur Ergosterinfrage.
13. Wurzinger, S., Klin. Wchnschr., 1928, vii, 1859. Erfahrungen mit Vigantol bei der Behandlung der Rachitis.
14. Steenbock, H., and Black, A., J. Biol. Chem., 1924, lxi, 405. Fat-Soluble Vitamins. XVII. The Induction of Growth-Promoting and Calcifying Properties in a Ration by Exposure to Ultra-Violet Light.
15. Steenbock, H., and Nelson, M. T., J. Biol. Chem., 1924, lxii, 209. Fat-Soluble Vitamins. XIX. The Induction of Calcifying Properties in a Rickets-Producing Ration by Radiant Energy.

16. Hess, A. F., Am. J. Dis. Child., 1924, xxviii, 517. Experiments on the Action of Light in Relation to Rickets.
17. Rosenheim, O., and Webster, T. A., Lancet, 1925, i, 1025. Rickets and Cholesterol.
18. Hess, A. F., Weinstock, M., and Helman, F. D., J. Biol. Chem., 1925, lxiii, 305. The Antirachitic Value of Irradiated Phytosterol and Cholesterol. I.
19. Steenbock, H., and Black, A., J. Biol. Chem., 1925, lxiv, 263. Fat-Soluble Vitamins. XXIII. The Induction of Growth-Promoting and Calcifying Properties in Fats and Their Unsaponifiable Constituents by Exposure to Light.
20. Rosenheim, O., and Webster, T. A., Biochem. J., 1927, xxi, 389. The Parent Substance of Vitamin D.
21. Hess, A. F., and Windaus, A., Proc. Soc. Exp. Biol. and Med., 1927, xxiv, 461. The Development of Marked Activity in Ergosterol Following Ultra-Violet Irradiation.
22. Tanret, C., Ann. de Chim. et Phys., 1890, 6s., xx, 289. Sur un Nouveau Principe Immédiat de l'Ergot de Seigle: l'Ergostérine. Ann. Chim. Phys., 1908, 8s., xv, 313. Sur l'Ergostérine et la Fongistérine.
23. Bakwin, H., Bakwin, R. M., and Gottschall, G., Am. J. Dis. Child., 1929, xxxvii, 311. Tetany in Infants; a Comparison of Various Agents Used in Treatment.
24. Starlinger, W., Deutsche med. Wchnschr., 1927, liii, 1553. Ueber die Beeinflüssung des Verlaufes einer schweren Osteomalazie durch Bestrahltes Ergosterin.
25. Hottinger, A., Deutsche med. Wchnschr., 1927, liii, 1549. Untersuchungen über bestrahltes Ergosterin: Beiträge zur direkten und indirekten Lichttherapie der Rachitis beim Tier, beim Kind und am Erwachsenen.
26. Bors, E., Fortschr. a. d. Geb. d. Röntgenstrahlen, 1928, xxxvii, 104. Vigantol und Frakturheilung.
27. Knoflach, I. G., Wien. klin. Wchnschr., 1928, xli, 739. Behandlung der Knochenbrüche mit bestrahltem Ergosterin.
28. Mellanby, M., and Pattison, C. L., Brit. Med. J., 1928, ii, 1079. The Action of Vitamin D in Preventing the Spread and Promoting the Arrest of Caries in Children.
29. Maurer, E., and Hofmann, P., Arch. f. Hyg., 1928, c, 367. Untersuchungen über die antibakterielle Resistenz experimentell-rachitischer Ratten.
30. Grant, A. R., and Goettsch, M., Am. J. Hyg., 1926, vi, 211. The Nutritional Requirements of Nursing Mothers; Effect of a Deficiency of the Antirachitic Vitamin only in the Diet of the Mothers upon the Development of Rickets in the Young.
31. Kreitmair, H., and Moll, T., München. med. Wchnschr., 1928, lxxv, 637. Hypervitaminose durch grosse Dosen Vitamin D.
32. Harris, L. J., and Stewart, C. P., Biochem. J., 1929, xxiii, 206. The Effect of Excessive Doses of Irradiated Ergosterol on the Calcium and Phosphorus Content of the Blood.
33. Klein, I. J., J. Am. Med. Assoc., 1929, xcii, 621. Effects of Massive Doses of Irradiated Ergosterol.
34. Pfannenstiel, W., München. med. Wchnschr., 1928, lxxv, 1113. Weitere Beobachtungen über Wirkungen bestrahlten Ergosterins im Tierversuch.
35. Hückel, R., and Wenzel, H., Arch. f. exper. Path. u. Pharmakol., 1929, cxli, 292. Über sklerotische Organveränderungen, insonderheit der Arterien. IV. Mitteilung: Über die Wirkung des bestrahlten Ergosterins auf die Nierengefässe von Kaninchen.

36. Smith, M. I., and Elvove, E., Pub. Health Rep., 1929, xliv, 1245. The Action of Irradiated Ergosterol in the Rabbit.
37. Shohl, A. T., Goldblatt, H., and Brown, H. B., J. Clin. Invest., 1930, viii, 505. The Pathological Effects upon Rats of Excess Irradiated Ergosterol.
38. Bamberger, P., Deutsche med. Wchnschr., 1929, lv, 399. Zur Frage der Vigantolschäden.
39. Von Wendt, G., Klin. Wchnschr., 1930, ix, 166. Ist das D-Vitamin eine einheitliche Substanz?
40. Windaus, A., Forschungen und Fortschritte, 1930, vi, 209. Ergosterin and Vitamin D.
41. Bauer, W., and Aub, J. C., J. Am. Dietet. Assoc., 1927, iii, 106. Studies of Inorganic Salt Metabolism. I. The Ward Routine and Methods.
42. Fiske, C. H., Unpublished Method. For outline see: Blackfan, K. D., and Hamilton, B., Bull. Johns Hopkins Hosp., 1927, xli, 322. A Study of the Inorganic Constituents of the Serum in Children with Acute Nephritis.
43. Fiske, C. H., and Subbarow, Y., J. Biol. Chem., 1925, lxvi, 375. The Colorimetric Determination of Phosphorus.
44. Van Slyke, D. D., and Neill, J. M., J. Biol. Chem., 1924, lxi, 523. The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement. I.
45. Bloor, W. R., Pelkan, K. F., and Allen, D. M., J. Biol. Chem., 1922, lii, 191. Determination of Fatty Acids (and Cholesterol) in Small Amounts of Blood Plasma.
46. Hawk, P. B., and Bergeim, O., Practical Physiological Chemistry. Philadelphia, P. Blakiston's Son and Co., 1926, 9th ed., p. 711.
47*a*. Warkany, J., Klin. Wchnschr., 1930, ix, 63. Über die Wirkungsweise des bestrahlten Ergosterins.
b. Warkany, J., Ztschr. f. Kinderh., 1928, xlvi, 1. Die phosphatämische Kurve des normalen und des rachitischen Organismus. Ibid., 1928, xlvi, 916.
48. Light, R. F., Miller, G., and Frey, C. N., J. Biol. Chem., 1929, lxxxiv, 487. Studies on the Effects of Overdosage of Vitamin D.
49. Heubner, W., and Holtz, F., Am. J. Physiol., 1929, xc, 387. Untersuchungen über bestrahltes Ergosterin.
50. Beumer, H., Monatschr. f. Kinderh., 1928, xxxviii, 44. Das antirachitische Prinzip.
51. Hottinger, A., Ztschr. f. Kinderh., 1929, xlvii, 341. Untersuchungen über bestrahltes Ergosterin. V. Stoffwechselversuche zur Ermittlung der biologischen Wirkungsweise.
52. György, P., Klin. Wchnschr., 1930, ix, 102. Die besondere Stellung der Subepiphysären Knochenschicht im Kalkstoffwechsel als eines leicht mobilisierbaren Kalkspeichers.
53. Kroetz, C., Klin. Wchnschr., 1927, vi, 1171. Über Wirkungen des bestrahlten Ergosterins auf den gesunden Erwachsenen.
54. Lasch, F., Klin. Wchnschr., 1928, vii, 2148. Über den Einfluss von Bestrahltem Ergosterin (Vigantol) auf den Cholesterin- und Calciumgehalt des Blutserums beim Erwachsenen.
55. Kaminsky, J., and Davidson, D. L., Am. Rev. Tuberc., 1930, xxii, 48. A Note on the Effect of the Oral Administration of Irradiated Ergosterol on the Calcium Concentration of the Blood Serum in Pulmonary Tuberculosis.

56. Havard, R. E., and Hoyle, J. C., Biochem. J., 1928, xxii, 713. Vitamin D in Adults; Its Effect on Calcium and Inorganic Phosphate of Blood.
57. Bauer, W., Albright, F., and Aub, J. C., J. Clin. Invest., 1929, vii, 75. Studies of Calcium and Phosphorus Metabolism. II. The Calcium Excretion of Normal Individuals on a Low Calcium Diet, also Data on a Case of Pregnancy.
58. Bauer, W., and Marble, A., New England J. Med., 1929, cci, 809. Preliminary Note on the Mode of Action of Irradiated Ergosterol.