SOME OBSERVATIONS ON VIOSTEROL AND COD LIVER OIL.*

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INTRODUCTION.

In 1922 McCollum, Simmonds, Becker and Shipley (27) found two vitamin factors in cod liver oil, one of which was destroyed by aeration. Up to that time it was thought that there was but one fat-soluble vitamin, and that deficiency of that vitamin was responsible for both rickets and xerophthalmia. These investigators showed that the factor which is destroyed by aeration is the one which prevents xerophthalmia, and that the process does not modify the influence of cod liver oil on the calcification of bone. They showed further that olive, cottonseed, maize and sesame oils possessed neither of these vitamin factors. Steenbock and Nelson (47) reported during the following year work which confirmed the presence of two factors in cod liver oil, and the results of their work were accepted as proof that cod liver oil contains two vitamins.

Hume's (24) study of the effects of radiation with the mercury-vapor quartz lamp convinced him that light could be substituted for vitamins. Steenbock and Nelson (47) drew the same conclusions. Steenbock, Nelson and Black (48), in 1924, demonstrated that a diet complete except for vitamins A and D caused cessation of growth before the appearance of xerophthalmia, and stated that irradiation of the animals results in stimulation of growth. During the same year the irradiation of animals, foods and oils with the mercury-vapor quartz lamp attracted world-wide attention, and stimulated much investigation and research. Steenbock and Black (42) demonstrated that irradiated lard and olive oil develop an antirachitic factor.

In 1925 Green and Mellanby (13) showed that fat-soluble vitamin A acts as a protective against infection, that many common infections occur in animals when there is a deficiency of this substance in the diet, and that no other vitamin can be substituted for fat-soluble vitamin A, which is essential to normal growth and which is apparently a factor in resistance to infection.

Steenbock, Hart, Hoppert and Black (46) demonstrated that irradiated milk was more effective and rapid as an antirachitic agent than direct irradiation of the animal, that irradiation destroyed fat-soluble A in butter, but does not destroy water-soluble B in yeast or C in lemon juice. Zilva (53) had made practically the same observations six years earlier (1919). During the same year cottonseed and linseed oils, cholesterol (35, 38, 43), phytosterol (18), yeast, commercial casein, oxbile, lanolin, grains, starch, flour and breakfast foods were added to the list of substances which could be made antirachitic by irradiation. It was demonstrated also that this antirachitic property was not produced in pure protein, carbohydrate or fat, or in such substances as salts, water, ether, phloroglucin or paraffin hydrocarbons (44).

Rosenheim and Webster (35), in 1926, proved that cholesterol failed to become antirachitic if it was first subjected to further purification. During the preceding

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* Scientific Section, A. PH. A., Miami meeting, 1931.

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year it had been suggested, on the basis of spectroscopic examinations (41, 44), that the acquired antirachitic property of cholesterol was dependent upon the presence of an impurity. Rosenheim and Webster found that irradiated ergosterol was protective in doses of 1 mg., and that cholesterol, under identical conditions, required 5 mg. to produce equivalent effects. Thus, irradiated ergosterol became the most potent antirachitic substance known. The same workers found that over-irradiation destroyed the provitamin, and that irradiated ergosterol, kept for one and one-half years in an inactive oil, was still potent when tested in daily doses of $^1/_{10,000}$ mg.

Hess and co-workers (16, 19–22) expressed the opinion that ergosterol is the impurity in cholesterol which is activated by irradiation, and that it is the only sterol which thus far has been made antirachitic by irradiation. Rosenheim and Webster (37), in 1927, demonstrated that the lowest protective dose of irradiated ergosterol is from $^1/_{10,000}$ to $^1/_{20,000}$ mg. per day.

The disagreeable odor and taste of cod liver oil have stimulated efforts to find a substance equally efficacious but more palatable. As a result there are in the market to-day, available to both the medical profession and the laity, many brands of flavored and "tasteless" cod liver oils and sugar-coated tablets of cod liver oil, and more recently, irradiated ergosterol, known by the trade-name "Viosterol." Because of the vagueness, or the indirectness, or the lack of clarity of some manufacturers' statements concerning irradiated ergosterol or "Viosterol," there has been a growing belief among pediatricians, physicians in general, pharmacists, nurses and the laity that this substance is a concentrated, acceptable and complete substitute for cod liver oil, and it is enjoying, consequently, an increasing popularity as a "substitute for cod liver oil." Although aware of the facts that cod liver oil contains two vitamins, and irradiated ergosterol but one, the average physician is better acquainted with the antirachitic vitamin, and is likely to disregard the importance of fat-soluble vitamin A and its less obvious influence on the child's ability to resist infections. Because of this increasingly wide-spread idea and the resulting popularity of irradiated ergosterol as a "substitute for cod liver oil," and the fact that there is no available comparative literature on the subject, this investigation was carried out in an effort to obtain some practical, comparative data illustrating the effects of such substitution.

The authors express their sincere appreciation to E. Clay Mitchell, M.D., to Ralph Bowen, M.D., and to J. A. McIntosh, M.D., all of Memphis, for their cordial coöperation, suggestions, criticisms and advice.

THE PROCEDURE.

Pure bred albino rats, obtained from the Sprague-Dawley Corporation, Madison, Wisconsin, were used as the experimental animals. The rats were provided with fresh tap water daily, and with as much diet as they would consume. Young rats were weaned when three weeks old, and were not placed on a purified diet until after their weight exceeded 30 Gm. The experimental animals were weighed on alternate days and were carefully watched for signs on any abnormalities. They were housed in individual cages designed and made by E. O. Prather, Jr. Each cage was kept over a pan of pine shavings at a sufficient height to prevent access to the shavings. The cages were cleaned every day, and the shavings renewed on

alternate days. No bedding of any kind was provided in order to avoid the possibility of the rats obtaining vitamins from such sources. The animals were kept in a well-lighted and ventilated room at a temperature range of from 60° to 75° F.

The *stock diet* used was a modification of that employed by Bethke, Steenbock and Nelson (1). It consisted of:

Powdered whole milk	40.0 parts
Wheat, finely ground	37.5 parts
Yellow corn, finely ground	37.5 parts
Soy beans, finely ground	15.0 parts
Crude casein	5.0 parts
Dried spinach	5.0 parts
Yeast*	5.0 parts
Iodized table salt	0.5 part
Calcium carbonate	0.5 part

* If this is reduced one-half, the diarrhea often observed in female rats after the birth of first litters is corrected.

The *purified diet* employed was a modification of that used by Yudkin and Lambert (52). Crisco was substituted for lard since the latter at one time had been reported to contain small amounts of fat-soluble vitamins. The purified diet consisted of:

Casein, extracted	18%
Osborne and Mendel salt mixture	4%
Crisco	20%
Cornstarch	58%

To prevent waste, water was added to make a paste. This diet is complete in proteins, calories and mineral salts, but deficient in all vitamins.

The crude casein was freed from fat-soluble vitamins by extraction (Soxhlet extractors) first for 24 hours with 95% alcohol, then for 24 hours with a mixture of equal parts of 95% alcohol and anesthetic ether, and finally for 48 hours with anesthetic ether. It was found that this extraction procedure is superior to that of Daniels and Loughlin (6) in that no lumping occurred. Crisco is freed from all fat-soluble vitamins by the process of hydrogenation (9). Commercial cornstarch is also free from fat-soluble vitamins (9).

The salt mixture of Osborne and Mendel (31) consists of:

Calcium carbonate	134.8000 Gm.
Magnesium carbonate	24.2000 Gm.
Sodium carbonate	34.2000 Gm.
Potassium carbonate	141.3000 Gm.
Phosphoric acid	103.2000 Gm.
Hydrochloric acid	53.4000 Gm.
Sulphuric acid	9.2000 Gm.
Citric acid plus HOH	111.1000 Gm.
Iron citrate plus $1^1/_2$ HOH	6.340 Gm.
Potassium iodide	0.0200 Gm.
Magnesium sulphate	0.0790 Gm.
Sodium fluoride	0.2480 Gm.
Potassium and aluminum sulphate	0.0245 Gm.

The acids were mixed, the salts then added using sufficient distilled water to facilitate mixing, and the mixture was finally evaporated to dryness at 90° C. and reduced to a fine powder.

The cod liver oil and the irradiated ergosterol used were secured from the same manufacturing house. Since the irradiated ergosterol used was reported to be one hundred times the antirachitic vitamin D potency of good cod liver oil (34), it was diluted one hundred times with olive oil, which does not contain any fat-soluble vitamins (27). Vitamin B was supplied in the form of powdered yeast.

Osborne and Mendel (31) reported that animals receiving an insufficiency of vitamins or a complete deficiency of fat-soluble vitamins soon lost their appetites and refused to eat. In these cases the vitamin-containing substances had been mixed with the food in definite proportion, and it was assumed that the animals would consume a definite quantity of food daily. Simmonds, McCollum and Becker (40), Jones (26) and Hopkins (23) found that if large quantities of food are mixed at one time for future use, a part of the vitamin content is destroyed through oxidation due to the iron content of these mixtures. To overcome these possible influences all of the vitamin-containing fluids were placed directly in the mouths of the animals by means of a graduated pipette; a definite quantity of yeast was weighed, diluted to a known volume with water and a measured quantity of this fluid was placed into the mouth of each animal daily; and the diets were mixed in sufficient quantities to last a period of one week.

When the young rats had reached a weight of 30 Gm. or more, they were divided into groups, each group, whenever possible, composed of an equal number of males and females.

The first group of rats was placed on *Diet B*, consisting of the purified ration and 80 mg. of yeast daily (32, 33). After these animals had begun to develop xerophthalmia, the diets of some of them were modified. Some were given $^1/_5$ drop of cod liver oil daily, in addition to Diet B, and $^1/_2$ drop of irradiated ergosterol, this being designated as *Diet Bb*. This quantity of cod liver oil cures xerophthalmia, but has little effect on bone. Others were given $^1/_2$ drop of cod liver oil only, in addition to Diet B; this constituted *Diet Bc*. The remainder of the rats were left on the original experimental diet, *Diet B*, which consisted of the purified ration, 80 mg. of yeast, and $^1/_2$ drop of irradiated ergosterol daily. After xerophthalmia developed, one-half of the animals were given $^1/_2$ drop of cod liver oil daily, in addition to the irradiated ergosterol (*Diet Da*). The rest of this group were kept on their original diet (*Diet D*) during the remainder of the observations.

TABLE I.—FIRST SERIES, DIETS B, D, C.

Diet.*	B.	D.	C.
Number of rats on the diet	18	15	15
Average days on the diet	62.1	55.6	54.4
Average wt. in Gm. at beginning of observations	47.7	47.0	46.3
Average wt. in Gm. at end of observations	55.1	58.2	80.6
Average gain per rat in Gm.	7.4	11.2	34.2
Average days to xerophthalmia	41.3	55.6	

* *Diet B*: Purified food plus 80 mg. of yeast daily. *Diet D:* Purified food plus 80 mg. of yeast and one-half drop of irradiated ergosterol daily. *Diet C:* Purified food plus 80 mg. of yeast and one-half drop of cod liver oil daily.

The third group of rats was placed on *Diet C*, composed of the purified ration, 80 mg. of yeast, and $^1/_2$ drop of cod liver oil daily.

The rats were killed and autopsied at the close of the periods of observation. The intestines, spleen, liver, kidneys, heart and lungs were examined for gross abnormalities. The left front and the right rear legs of each animal were severed, and roentgenograms were made. The heads were severed and dissected transversely and smears were made of the nasal sinuses.

None of the first series of animals had grown as had been expected, and some had lost hair and become bald in spots. Harris and Moore (15) believe this condition is caused by a deficiency of the vitamin B complex. Accordingly, a second series of studies was made, and the yeast increased to 1 Gm. per animal per day. The observations in the cases of the first series with the cod liver oil were satisfactory, so it was deemed unnecessary to repeat these observations. As a further test of irradiated ergosterol half of the animals receiving no fat-soluble vitamins (*Diet B*) were given $^1/_2$ drop of irradiated ergosterol after they had developed xerophthalmia (*Diet Ba*).

All of the rats used in the second series of observations had been raised on the same stock diet as the first series, the young were weaned at the same age and the animals were treated throughout in the same manner with the noted exceptions.

OBSERVATIONS AND DISCUSSION.

For a time the increase in weight of the three groups of rats (*Diets B, D* and *C*) were parallel, then the weights of the animals which received no fat-soluble vitamins (*Diet B*), and of those which received irradiated ergosterol (*Diet D*), became practically stationary. The weights of the rats which received cod liver oil (*Diet C*) continued at a regular rate of increase throughout the period of observation. The irradiated ergosterol animals (*Diet D*) gained very little more than those which received no fat-soluble vitamins (*Diet B*). These results seem contradictory to those of Steenbock and Nelson (47) who reported that direct irradiation of the animals or the feeding of irradiated goods stimulate the growth. However, these results are in accord with the observations of Hume (24), and of Goldblatt and Soames (12). The rats which received cod liver oil (*Diet C*) gained three times as much as those which received irradiated ergosterol (*Diet D*) during the 60-day period of observation.

All of the animals which received no cod liver oil (*Diets B* and *D*) developed xerophthalmia within an average period of about 40 days (Table I). The condition of the rats which received irradiated ergosterol (*Diet D*) was more severe than in the case of the group which received no fat-soluble vitamins (*Diet B*). Hume (24) reported similar results.

All of the external symptoms presented by the animals which had been kept on *Diets B* and *D* were absent in the rats which had been fed *Diet C* (cod liver oil). It is obvious, therefore, that these groups (*B* and *D*) were suffering from a deficiency of fat-soluble vitamin A, for, in addition to the xerophthalmia, it was noted that the weights of these animals became stationary for a few days or declined rapidly following the development of xerophthalmia, unless some treatment, *i. e.*, fat-soluble vitamin, was administered. Similar observations, in the absence of fat-soluble vitamin A or in low vitamin A diets, have been reported by Cramer, Drew

and Mottram (4), Steenbock, Sell and Nelson (49), Steenbock and Nelson (47), Wohlbach and Howe (50, 51), Davis and Outhouse (7), Coward (3), Hume and Smith (25), Sherman and Cammock (38), Steenbock, Nelson and Black (48), Daniels, Armstrong and Hutton (5), Bloch (2), Sherman and Munsell (39), and Halliday (14).

To test further the effects of irradiated ergosterol and of cod liver oil on the growth and the eye condition, rats which had developed xerophthalmia on Diet B (no fat-soluble vitamin) were given 1/5 drop of cod liver oil and 1/2 drop of irradiated ergosterol (*Diet Bb*) daily. These animals showed no appreciable gain in weight, although the xerophthalmia was cured in about 6 days. When 1/2 drop of cod liver oil was administered daily (*Diet Bc*), the xerophthalmia was cured in about the same length of time as with *Diet Bb*, but there was a noticeable increase in the rate of growth (Table II). Some of the animals which had been receiving only irradiated ergosterol (*Diet D*) were given 1/2 drop of cod liver oil daily in addition to the irradiated ergosterol (*Diet Da*). The xerophthalmia was cured in about six days, as with the other animals, and there was some increase in the weights of these animals (Table III).

TABLE II.—FIRST SERIES, DIETS BB, BC, B.

Prather, Nelson, Bliss.

Diet.*	Bb.	Bc.	B.
Number of rats on the diet	5	4	5
Average gain in wt. (Gm.) 10 days before treatment	0.5	0.7	0.6
Average gain in wt. (Gm.) on treatment	4.5	14.9	4.4
Average days to cure xerophthalmia	5.6	5.4	...
Average days' treatment	9.5	24.5	...

* *Diet Bb:* Purified food plus 80 mg. of yeast daily. After xerophthalmia developed, 1/5 drop of cod liver oil and 1/2 drop of irradiated ergosterol daily. *Diet Bc:* Purified food plus 80 mg. of yeast daily. After xerophthalmia developed, 1/2 drop of cod liver oil daily. *Diet B:* Purified food plus 80 mg. of yeast daily.

TABLE III.—FIRST SERIES, DIETS D, DA.

Prather, Nelson, Bliss.

Diet.*	D.	Da.
Number of rats on the diet	4	4
Average gain in wt. (Gm.) 10 days before treatment	0.2	1.2
Average gain in wt. (Gm.) on treatment	...	4.2
Average days to cure xerophthalmia	...	5.2
Average days treated	...	13.4

* *Diet D:* Purified food plus 80 mg. of yeast daily and 1/2 drop of irradiated ergosterol daily. *Diet Da:* Purified food plus 80 mg. of yeast and 1/2 drop of irradiated ergosterol daily; after xerophthalmia developed, 1/2 drop of cod liver oil daily.

Autopsy disclosed some marked differences in the conditions of the several groups of experimental animals. The rats which had been fed no fat-soluble vitamins (*Diet B*) showed a marked inflammatory condition of the lungs, the spleen and the liver were discolored, and in the majority of cases, were shrunken and infiltrated with fat, and the intestines were thin-walled and filled with gas. In some

cases the intestines displayed a dull brown color, but in others the inflammation was so intense that the color was almost red. In most instances abcesses had appeared at the base of the tongue, and these often extended over the entire posterior portion of the tongue.

The animals which had been on *Diet B* (no fat-soluble vitamin) but had had their diets modified *after the development of xerophthalmia* (*Diets Bb* and *Bc*) showed a few differences on autopsy from those which had remained on *Diet B* for the entire period of observation. The rats which had been fed *Diet Bb* ($^1/_5$ drop of cod liver oil and $^1/_2$ drop of ergosterol) presented practically the same appearance as in the cases of the *Diet B* rats. Those rats which had been fed *Diet Bc* ($^1/_2$ drop of cod liver oil) showed a less marked intestinal inflammation. It appeared that, once an appreciable decline in weight had started, the administration of relatively small quantities of fat-soluble vitamin A was of little benefit.

The rats which had been on *Diet D* ($^1/_2$ drop of irradiated ergosterol) disclosed, on autopsy, all of the changes exhibited by the animals which had received *Diet B* (no fat-soluble vitamins). In some of the animals the conditions were even more severe than in those which had received no fat-soluble vitamins. This was particularly true of the intestinal conditions. The animals which had received $^1/_2$ drop each of cod liver oil and of irradiated ergosterol (*Diet Da*), *after they had developed xerophthalmia*, showed some slight improvement over those which had received irradiated ergosterol only (*Diet D*). Here, too, it was evident that, after the weight had become stationary or had begun to decline, the addition of small quantities of fat-soluble vitamin A to the diet accomplished little toward increasing resistance to infection.

TABLE IV.—SECOND SERIES, DIETS B, D, C.

Prather, Nelson, Bliss.

Diet.*	B.	D.	C.
Number of rats on the diet	8	14	13
Average days on diet	57.2	58.1	48.6
Average wt. in Gm. at beginning of observations	41.8	38.3	37.5
Average wt. in Gm. at end of observations	73.6	88.5	169.5
Average gain in wt. (Gm.) per rat	31.8	50.2	132.0
Average days to xerophthalmia	43.8	44.9	...

* *Diet B:* Purified food plus 1 Gm. of yeast daily. *Diet D:* Purified food plus 1 Gm. of yeast and $^1/_2$ drop of irradiated ergosterol daily. *Diet C:* Purified food plus one Gm. of yeast and $^1/_2$ drop of cod liver oil daily.

All of the rats which had received *Diet C* (purified food plus 80 mg. of yeast and $^1/_2$ drop of cod liver oil daily) were normal. In every case there were large deposits of intraperitoneal fat visible, the intestines contained no gas, and there were no signs of intestinal inflammation. There were no indications of shrinkage of the liver or of the spleen, nor was there any fatty infiltration. None of these animals had developed xerophthalmia. Not a single abscess was found on the tongues of these animals. These observations are in agreement with the findings of Mori (29, 30), McKay (28), McCollum, Simmonds, Becker and Shipley (27), Steenbock and Coward (45), Steenbock, Sell and Nelson (49), Drummond (8), Bloch (2), Daniels, Armstrong and Hutton (5), Steenbock and Nelson (47), Coward (3),

Sherman and Cammock (38), Steenbock, Nelson and Black (48), Sherman and Munsell (39), Wohlbach and Howe (50, 51), Davis and Outhouse (7), and Halliday (14).

None of these observations verify the findings of Emmet and Allen (10), and Cramer, Drew and Mottram (4), who reported that no pathological changes were found in rats when fed diets which were deficient in fat-soluble vitamin or in which this vitamin was entirely absent.

In 1923 Daniels, Armstrong and Hutton (5), and Halliday (14) reported accumulations of pus in the nasal sinuses of rats on diets deficient in fat-soluble vitamin A. In the same paper Daniels and her co-workers reported snuffles in their animals. The observations herewith reported do not verify the latter findings of Daniels. With but two exceptions, one in the first series of animals and another in the second series, there were no audible or visible signs of difficult breathing. However, this may be explained, possibly, by the marked difference in the ages of the animals, and the differences in the degree of purity of the rations used in this work as compared with those employed by Daniels and co-workers. Unfortunately the smears from the first series of animals reported herewith were unsatisfactory and had to be discarded.

As already stated, the rats in the first series of these observations did not grow as had been expected, and some had lost hair and had become bald in spots. Harris and Moore (15) believe this condition is due to a deficiency in the vitamin B complex. In the second series of observations the yeast was increased to 1 Gm. per day, and this hair condition did not occur. The animals were very fond of the yeast, and eagerly consumed the paste composed of yeast and water which was placed in the food containers.

TABLE V.—SECOND SERIES, DIETS B, BA.

Prather, Nelson, Bliss.

Diet.*	B.	Ba.
Number of rats on the diet	4	4
Average gain in wt. (Gm.) 10 days before treatment	9.3	9.0
Average gain in wt. (Gm.) on treatment	10.9	6.1
Average days treated	..	9

* *Diet B:* Purified food plus 1 Gm. of yeast daily. *Diet Ba:* Purified food plus 1 Gm. of yeast daily. After xerophthalmia developed, $^1/_2$ drop of irradiated ergosterol daily.

TABLE VI.—SECOND SERIES, DIETS B, D, C.

Prather, Nelson, Bliss.

Diet.*	B.	D.	C.
Number of rats on the diet	8	14	13
Number of rats showing pus in the nasal sinuses	5	9	0
Number of rats showing abscesses on the tongue	6	8	0

* *Diet B:* Purified food and 1 Gm. of yeast daily. *Diet D:* Purified food, 1 Gm. of yeast, and $^1/_2$ drop of irradiated ergosterol daily. *Diet C:* Purified food, 1 Gm. of yeast, and $^1/_2$ drop of cod liver oil daily.

With the exception of a higher growth rate the external appearances of all of the animals of the second series (Table IV) were in accord with those of the first

series. The growth of the animals which had received no fat-soluble vitamins (*Diet B*) was stunted, and the hair was rough and coarse. Evans and Burr (11) reported that on highly purified diets the growth of their animals was stunted, but the animals remained normal physiologically, had glossy coats and appeared to be in the best of health. Their observations are not confirmed by this investigation, although the diets used were purified to the same degree as those employed by Evans and Burr. The appearance of the animals which had received *Diet D* (irradiated ergosterol) differed very little from those which had received no fat-soluble vitamins (*Diet B*). The animals on *Diet C* (cod liver oil) presented a normal appearance. Their fur was smooth and glossy, their posture was better than either of the other groups and they appeared very active and in perfect health. With the exception of the degree of xerophthalmia, it was difficult to distinguish between the rats which received *Diet B* (no fat-soluble vitamins), and those which received *Diet D* (irradiated ergosterol).

The gain in the weights of the animals of the second series was about five times greater than in the animals of the first series. The appearance of xerophthalmia occurred also in about 40 days (Table IV). Since it was demonstrated very satisfactorily and conclusively in the first series that cod liver oil will stimulate the growth and cure the xerophthalmia, it was deemed unnecessary to repeat that part of the experimentation. To test further the effects of irradiated ergosterol on the growth rate and on the xerophthalmia, half of the animals which had been fed *Diet B* (no fat-soluble vitamins) were given $^1/_2$ drop of irradiated ergosterol daily, *after they had developed xerophthalmia*. Again no improvement was observed in xerophthalmia; if anything, the condition appeared to have become somewhat aggravated and more severe. The growth was not stimulated, and, as a matter of fact, the rats which had been on *Diet B* (no fat-soluble vitamins) gained more than those which had been fed *Diet Ba* (irradiated ergosterol) (Table V).

In the cases of the rats which had received *Diet D* (irradiated ergosterol) there was no prolongation of the average time to the appearance of xerophthalmia (Table IV). However, the condition was much more severe and the decline in weight much more rapid than in the animals which had been fed *Diet B* (no fat-soluble vitamins).

The rats which had been fed *Diet B* (no fat-soluble vitamins) and those which had received *Diet D* (irradiated ergosterol) for a period of 40 days began to lose the complete use of their hind legs. This was followed by the loss of the use of the front legs, in some cases. The condition presented the appearance of rickets, for the animals which could walk at all showed a peculiar "flat footed" gait and were irritable. The rats which had received *Diet C* (cod liver oil) did not develop this condition. It is obvious, therefore, since the only differences in the three diets were in the cod liver oil and the irradiated ergosterol contents, that cod liver oil contains some essential factors which are essential to growth, the prevention of infections and the cure and prevention of xerophthalmia, and that these factors are lacking in irradiated ergosterol.

On autopsy all of those animals in the second series which had received *Diet B* (no fat-soluble vitamins) and all of those which had been fed *Diet D* (irradiated ergosterol) showed all of those conditions which had been found in the animals of the first series. In most cases these conditions were more severe. Six of the eight

rats which had been fed *Diet B*, and eight of the fourteen animals which had received *Diet D* exhibited abscesses at the bases of the tongues (Table IV). In some cases the abscesses were spread over the entire posterior half of the tongues.

The smears of the nasal sinuses were examined by J. A. McIntosh, M.D., Pathologist to St. Joseph's Hospital, Memphis. He reported (Table VI) that five of the eight rats which had received no fat-soluble vitamins (*Diet B*), and nine of the fourteen animals which had been fed irradiated ergosterol (*Diet D*) showed accumulations of pus in the nasal sinuses. In no case was pus found present in the nasal sinuses of the rats which had received $^1/_2$ drop of cod liver oil daily (*Diet C*). With the two exceptions noted above, no snuffles developed.

The roentgenograms showed that the degree of calcification in all of the rats which had received *Diet D* (irradiated ergosterol) was greater than that exhibited by the animals which had been fed *Diet B* (no fat-soluble vitamins), but the rats which had been on *Diet C* (cod liver oil) showed the greatest degree of calcification. Since irradiated ergosterol has been reported, by manufacturers, to possess 100 times the antirachitic potency of cod liver oil, and the irradiated ergosterol which was used in these experiments was diluted 100 times with an inactive oil, the degree of calcification attending the administration of irradiated ergosterol should have been the same as that produced by cod liver oil. These observations indicate, however, that the administration of irradiated ergosterol in quantities which are theoretically equivalent to the vitamin D potency of cod liver oil does not result in a calcification of the bones comparable to that produced by the administration of cod liver oil.

SUMMARY.

The purpose of this study was to compare irradiated ergosterol or "Viosterol" and cod liver oil in a practical way.

The animals in each litter were divided into three groups of two animals each, one male and one female when possible, were placed on the purified diets, and the vitamins added. All of the animals received a quantity of yeast which was sufficient to promote growth when the other vitamins were present. The vitamins, in all cases, were placed directly in the animals' mouths.

Three diets were used, the first, *Diet B*, consisted of the purified ration, with yeast; the second, *Diet D*, consisted of the purified diet or ration, yeast, and $^1/_2$ drop of irradiated ergosterol daily; the third, *Diet C*, consisted of the purified ration, yeast, and $^1/_2$ drop of cod liver oil daily.

The quantity of irradiated ergosterol administered was reputed to contain the same antirachitic potency as the cod liver oil. However, this amount of irradiated ergosterol did not produce bones as well calcified as did the cod liver oil.

The animals which received no fat-soluble vitamins (*Diet B*) and those which received irradiated ergosterol (*Diet D*) developed all the reported symptoms of a fat-soluble vitamin A deficiency. The animals which received cod liver oil (*Diet C*) did not show any abnormalities.

CONCLUSIONS.

1. When animals are fed a purified diet adequate in proteins, inorganic salts, calories and vitamin B, they grow well for a short time, then the weight becomes stationary or falls off rapidly, and xerophthalmia develops. The upper respiratory

tract becomes inflamed; the liver and spleen become discolored, shrunken and infiltrated with fat; the kidneys become sand-colored; and the intestines are found to be thin-walled and filled with gas. In some cases the stomach, as well as the entire intestinal tract, are badly distended. There is no visible intraperitoneal fat present. The intestines vary in color from dull brown to a degree of inflammation which is almost red. The amount of calcium deposited in the bones is very small.

2. The addition of irradiated ergosterol ("Viosterol") to a diet like the foregoing increases the calcification of the bones, but does not prevent or cure the xerophthalmia, or increase the growth of the animal. Instead, the xerophthalmia appears to be aggravated, although it does not develop earlier. The condition of the upper respiratory tract is not improved, the intestines, kidneys, spleen and liver are found to be fully as bad, if not worse than the conditions found when no irradiated ergosterol is given.

3. The addition of cod liver oil to the foregoing (see 1. on page 1300) diet results in excellent growth of the animals, a better calcification of the bones, increased deposition of body fat, normal respiratory tracts, and the liver, kidneys, spleen and the intestines are found to be normal.

4. Irradiated ergosterol ("Viosterol"), therefore, did not demonstrate the power to stimulate growth and development of the body and vital organs, or to prevent infections of the upper respiratory tract, or to produce the same degree of calcification and growth of the bones as did cod liver oil.

5. Since "colds," malnutrition and intestinal inadequacies are more frequent in children than rickets, this study emphatically suggests that the apparently widespread substitution of irradiated ergosterol ("Viosterol") for cod liver oil in the diet of the child is not logical, and may result in an appreciable decrease of the child's strength and resistance to infections.

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LANTERN SLIDES.

Science is quoted in the following by P. E. Reid: "The advantages of a quick method for preparing lantern slides which may be simply done and without the usual cost are especially valuable in the case of taking diagrams from a text or journal, or for producing necessary slides on short notice.

"I have found the following preparation to have the above qualifications and to be very effective. Take a plain glass lantern slide, thoroughly clean it and allow to dry. When the slide has become quite dry apply a thin coat of albumin from a fresh egg and again allow the slide to dry. A smooth brush is essential in getting the coat of albumin evenly placed to avoid a streaked appearance.

"As soon as the albumin has completely dried, place the plate with its coated side uppermost, over the diagram or other copy, and trace on the slide with India ink. The width of the lines may be varied by using pens of different sizes. Colored effects may be added in the same manner except that inks made from aniline dyes (such as the common writing fluids) should be used. The pigmentation in colored India inks make them all appear black.

"Mount in the usual manner after the ink has dried by placing the newly made plate face down on another clean slide and fastening together with the usual lantern slide material or with adhesive tape.

"These slides are not temporary as might be supposed but may be left in the lantern for long periods of time without injury in spite of the intense heat of some lanterns. I have used this preparation for the past five years and it is now being used by others."