

SCIENTIFIC SECTION

BOARD OF REVIEW OF PAPERS.—*Chairman*, L. W. Rowe, George D. Beal, F. F. Berg, C. O. Lee, E. V. Lynn, John C. Krantz, Jr., Heber W. Youngken.

ABSORPTION OF VITAMIN D FROM THE SKIN.*

BY FLORIN J. AMRHEIN.

HISTORICAL INTRODUCTION.

For many years the workers in the field of nutrition have recognized the fact that certain food factors were responsible for many changes that occur in the human body. Many experiments were conducted with various food materials. It was found that pure carbohydrates, pure proteins, pure fats and pure mineral salts were not sufficient to maintain proper body needs and that the presence of other substances in food was apparently necessary.

To Mellanby (1) belongs the credit of the discovery of the specific antirachitic factor, now known as vitamin D. Mellanby, in his reports of 1918 and 1919, gave to the world the first accounts of experimental rickets developed in animals. He used the dog and he showed that rickets could be cured by dietary means.

Hess and Unger (2) in 1919–1920, believed that in the presence of another vitamin (now known as vitamin A), that rickets sometimes developed in infants receiving large amounts of milk which they believed contained large amounts of these fat-soluble vitamins.

Sherman and Pappenheimer (3) in 1921 in their experiments showed that rickets may be caused by a disturbance in the mineral constituents of the foods eaten. In their diets they used vitamin-deficient foods of all types. Their experiments were not as conclusive as they might have been due to the fact that the animals used had not been completely depleted of their vitamin reserve. It has been shown experimentally that the body is capable of storing vitamins for future use.

About this time we find that Shipley, Park, McCollum and Simmonds (4) reported that the phosphate ions may have something to do with the apparent deficiency of calcium in the bones of the animals. In 1921–1922 these same investigators made a comparison of the relative protective value of butter fat and cod liver oil when given to albino rats on a low calcium diet. They reported that cod liver oil contains an abundance of some substance which directs the bone development and that it enables the animals to develop bones on a low calcium diet, but that butter contains only slight amounts of the substance.

From this time up to the present we find that the literature on vitamin D has grown so that it is possible to find as many as one thousand references on the subject in all of its various phases. Among some of the workers in the field we find the following: Windhaus, Steenbock, Hess, Hoppert, Humphrey, Bogert, Trail, Nelson, Huldchinsky, Hyole, Poulsson, Bills, Holmes and Bliss.

It is a generally accepted fact that this fat-soluble vitamin, vitamin D, is necessary in the diet in order to insure proper calcium metabolism. If absent in the diet of infants and very young children it causes malformation of the skeletal structure

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of the body and the disease is known as rickets. In the adult its absence is said to cause the condition known as osteomalacia.

During all of this experimental work it was found that sunshine was a good source of vitamin D, although certain conditions were necessary in order to get optimum results. Wyman (5) in 1927, working at the Children's Hospital in Boston, found that much of the ultraviolet light, which is the source or activator of the vitamin, would be lost unless certain filters were used, and he also noted that certain types of glass completely absorbed the ultraviolet light and prevented its passage into the room.

Steenbock (6) reported that the vitamin could be produced in foods that were exposed to the light from a mercury arc. This process is now known as the process of activation of foods. The process is protected by United States patents, and persons using the process are licensed by the Alumni Association of the University of Wisconsin who own the patent.

Since the sun is nature's source of vitamin D it can be stated that before man covered his body completely with clothing he no doubt obtained much of the vitamin from the sun. However, with the over-population of the large centers and with the advent of the custom of completely covering the body with clothing much of the beneficial effects of the sun rays were lost and more and more the human race became dependent upon the food supply for this vitamin.

We may well ask what has all this to do with the calcium distribution in the human body? If we consider the structure of the skin we will find that in the sub-epidermal layer of the skin there are layers of fat cells which contain cholesterol. This cholesterol becomes activated by the ultraviolet rays of the sun and in this way the body is furnished with the vitamin which in turn is absorbed by the skin capillaries and carried into the blood stream. This complex reaction then is thought to cause calcium deposition.

As has been stated modern man living primarily in the canyons of the large cities is for the most part deprived of nature's way of getting vitamin D. This causes the various conditions previously described and therefore the medical profession recognizing this fact has attempted to overcome these natural inequalities by adding to the diet substances rich in vitamin D, such as cod liver oil and other fish oils.

OBJECT OF THE INVESTIGATION.

The object of this investigation is to determine under what conditions can vitamin D be absorbed from the skin. This work was suggested by the work done by Hume, Lucas and Smith (7) in 1927. These investigators irradiated cholesterol and applied an oil solution to the test animals after first removing the hair of the animals and covering the spot so that the animals could not lap off any of the material. They found that some vitamin was absorbed from the skin. The author experienced some difficulty in using their technic on white rats, so during the course of the work a new method was developed which will be described in another place.

DEVELOPMENT OF EXPERIMENTAL RICKETS.

In order to produce experimental rickets the diet must be deficient in vitamin D, and it must also be abnormal in both calcium and phosphorus. It has been

found that when diets contain calcium and phosphorus in the ratio of 2 parts of calcium to 1 part of phosphorus that normal bone is formed, but when the ratio of calcium to phosphorus is very high, *i. e.*, 4 to 1 or higher, that they produce a type of rickets similar to that found in infants. Low calcium-high phosphorous diets will also produce rickets, but of a different type.

The standard ricket-producing diet of Steenbock, No. 2965, was used in the experiments. Its composition is as follows:

Diet No. 2965.

Yellow corn.....	76%
Wheat gluten.....	20%
Calcium carbonate.....	3%
Sodium chloride.....	1%

Calcium-phosphorus ratio: Ca:P = 4:1.

This diet is decidedly lacking in vitamin D and it has a high calcium to phosphorous ration, although by no means ideal in other respects it is sufficiently adequate to support growth and maintain satisfactory state of nutrition during the experimental period. The rats are placed on this diet from twenty-one to twenty-eight days or until they show definite symptoms of rickets as indicated by their gait, etc.

The animals are then lightly etherized and roentgenograms of the knee joints are taken. Care must be exercised not to give the animals an overdose of ether as it will kill them. This method of determining whether or not active rickets has developed in the animal checks each animal, thus assuring the rachitic condition of each animal, also the animal may be used for the repair diet. The latter and the former are advantages of this method over the line-test method. During the entire experimental period the animals are kept in darkened rooms, away from both daylight and sunlight.

THE REPAIR DIET.

Ordinarily the vitamin D containing material is either fed directly to the animals or it is mixed with the diet that is fed the animals. For the purpose of this work it is obvious that the material cannot be fed to the animals but must be applied to the skin and then absorbed through the skin if possible.

Among the materials used in this investigation are included the following: peanut oil, unirradiated cod liver oil concentrate in peanut oil containing 6000 A. D. M. A. units per Gm., cod liver oil, U. S. P., irradiated cholesterol in creams. The cream base consisted of vitamin D free substances as mineral oil, waxes and water. One cream contained 1000 A. D. M. A. units and the other 3000 A. D. M. A. units per 120 Gm., respectively.

AUTHOR'S RAT-TAIL METHOD.

Pieces of pyrex tubing of the proper diameter (this depends upon the size of the rat's tail) were cut in lengths so that when the rat's tail is inserted into the tube about seven-eighths of it is encased by the tube. The tube is sealed at one end and the other end is fire polished so that it will not cut the animal. The animal is

then weighed without the tube. The material is then spread on the rat's tail and the tail is then slipped into the tube. The tube is fastened to the animal by means of adhesive tape. The material is allowed to remain on the tail for twenty-four hours. Each day the animal is weighed and its weight noted, the tube being removed first and also any adhering material during the 10-day test period. The results obtained are of value only if the animal has consistently gained in weight during the repair diet period. All food fed the animals is weighed each day and the amount left by each animal is also noted.

Experiments were carried out on peanut oil in order to make sure that the peanut oil did not contain any vitamin D.

TABLE I.—RESULTS OF EXPERIMENT USING PEANUT OIL.

Series I, Rat No.	Weight of Rat Beginning Test.	Weight of Rat End of Test.	Gain in Weight.
462	50.7 Gm.	58.6 Gm.	7.9 Gm.
464	66.8 Gm.	72.4 Gm.	5.6 Gm.
465	58.5 Gm.	68.3 Gm.	9.8 Gm.
467	65.1 Gm.	74.0 Gm.	8.9 Gm.

From the above table it will be seen that these rats continued to gain in weight when peanut oil was added to their diet, although examination of the roentgengram photographs attached at the end of the tables shows that active rickets are still in progress and that the oil does not contain vitamin D.

RESULTS OF EXPERIMENT USING COD LIVER OIL CONCENTRATE DISSOLVED IN PEANUT OIL CONTAINING 6000 A. D. M. A. UNITS PER GRAM.

This material represents the unsaponifiable material from cod liver oil. Cod liver oil when treated with potassium hydroxide forms a soft soap. This soap when extracted with ether yields in the ether extract a fatty wax-like solid which is not soluble in water, but is soluble in alcohol, fats, oils and waxes. It is sterol in character and conforms to all of the requirements for a lipoid. It has a distinct fishy odor. The odor is fairly well disguised when dissolved in a bland oil-like peanut oil, although corn oil tends to accentuate the odor of the concentrate.

TABLE II.—COD LIVER OIL CONCENTRATE IN PEANUT OIL.

Series II, Rat No.	Weight of Rat Beginning of Test.	Weight of Rat End of Test.	Gain in Weight.
455	62.7 Gm.	66.6 Gm.	3.6 Gm.
457	58.1 Gm.	63.7 Gm.	5.6 Gm.
458	74.9 Gm.	81.5 Gm.	6.6 Gm.
459	55.9 Gm.	59.6 Gm.	3.7 Gm.
460	62.7 Gm.	70.1 Gm.	7.4 Gm.
461	57.3 Gm.	58.8 Gm.	1.5 Gm.

In this series excellent repair was noted as indicated by the photographs of the roentgengrams. The experiments show definitely that vitamin D can be absorbed from oil solution.

A comparison of the photographs of the roentgengrams taken of the hind legs of the animals used in Table III shows conclusively that cod liver oil, *i. e.*, the vitamin D from cod liver oil is absorbed from the skin.

TABLE III.—RESULTS OF EXPERIMENT USING COD LIVER OIL.

Series III, Rat No.	Weight of Rat Beginning of Test.	Weight of Rat End of Test.	Gain in Weight.
479	60.6 Gm.	69.9 Gm.	9.3 Gm.
480	50.3 Gm.	52.4 Gm.	2.4 Gm.
481	61.9 Gm.	66.8 Gm.	4.9 Gm.
482	62.4 Gm.	66.1 Gm.	3.7 Gm.
483	49.2 Gm.	53.5 Gm.	4.3 Gm.

The experiments to this point indicate that vitamin D can be absorbed from the skin. All of these tests, however, were performed on material of vegetable and animal origin. The author was interested in the effect of mineral oil on the absorption properties of the skin, and so several experiments were conducted on creams prepared from mineral oil, wax, water and borax. The results are indicated in the roentgengram photographs included in the report.

The technic followed is the same as that already described.

TABLE IV.—RESULTS OF EXPERIMENT USING MINERAL OIL BASE CREAM.
CREAM CONTAINED 1000 A. D. M. A. UNITS PER 120 GM.

Series IV, Rat No.	Weight of Rat Beginning of Test.	Weight of Rat End of Test.	Gain in Weight.
472	62.8 Gm.	72.2 Gm.	9.4 Gm.
473	61.3 Gm.	64.9 Gm.	3.6 Gm.

TABLE V.—RESULTS OF EXPERIMENT USING MINERAL OIL BASE CREAM.
CREAM CONTAINED 3000 A. D. M. A. UNITS PER 120 GM.

Series V, Rat No.	Weight of Rat Beginning of Test.	Weight of Rat End of Test.	Gain in Weight.
474	62.4 Gm.	72.0 Gm.	9.6 Gm.
475	58.1 Gm.	65.1 Gm.	7.0 Gm.

The experiments made on the absorption of vitamin D from the skin by Hume, Lucas and Smith (7) were performed on cholesterol that had been artificially irradiated. For sometime the author has been interested in the problem of sun irradiation. In his experiments he has found that several factors affect the success of the work. However, experiments have shown that the sun may be used as a source of vitamin D when the proper technic and apparatus is used.

The author has irradiated cholesterol and has incorporated this cholesterol in the cream base described above. The roentgengram photographs show that vitamin D may be obtained by the sun irradiation of cholesterol provided the cholesterol has not been highly purified. It was found that cosmetic creams containing irradiated cholesterol caused repair to take place in rachitic rats.

TABLE VI.—RESULTS OF EXPERIMENT USING SUN IRRADIATED CHOLESTEROL IN
COSMETIC CREAM.

Series VI, Rat No.	Weight of Rat Beginning of Test.	Weight of Rat End of Test.	Gain in Weight.
476	55.1 Gm.	62.4 Gm.	7.3 Gm.
477	56.6 Gm.	61.1 Gm.	4.5 Gm.
478	60.0 Gm.	71.9 Gm.	11.9 Gm.

PHOTOGRAPHS OF ROENTGENGRAMS.

This part of the paper deals with the photographs of the roentgengrams taken of the knee prints of the experimental animals. The photographs that appear on

the left of the page under the title, "Beginning of the Test," indicate the stage of rickets developed in the animals due to being fed a diet deficient in vitamin D. In each case it will be noted that marked rickets are present.

The photographs that appear on the right of the page under the title, "End of the Test Period," represent the condition of the bones after the animal had been on a diet containing antirachitic material. With the exception of the experiments with peanut oil it will be noted that in each case the X-ray pictures show that distinct repair had been going on and in some instances complete healing had taken place.

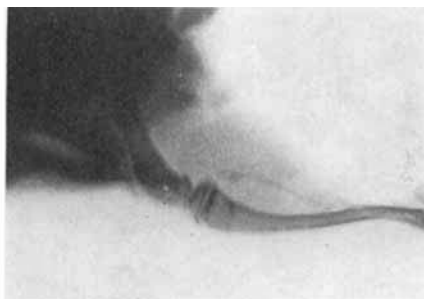
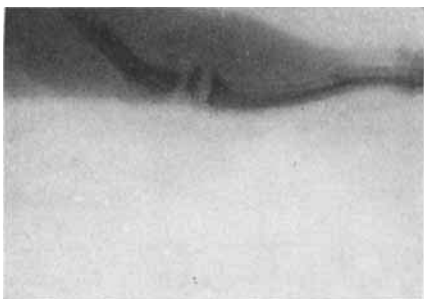
At the request of the publication committee the author has only submitted one roentgengram photograph of a rat in each series. Other photographs will be sent to anyone interested in same.

The author feels that it is entirely possible to use this method in quantitating the amount of vitamin D in material containing the vitamin and he is at present conducting said experiments.

Photographs of roentgengrams indicating that peanut oil does not contain any anti-rachitic substances since no repair has taken place in the rachitic bones after the oil was added to the diet.

Beginning of Test. Series I, 465.

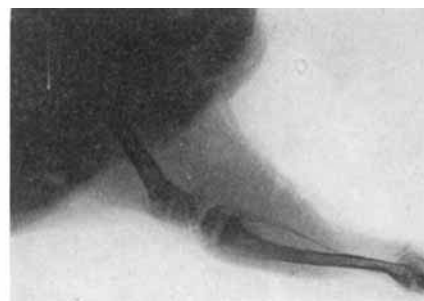
End of Test Period. Series I, 465.



Photographs of roentgengrams obtained in the experiments using cod liver oil concentrate in peanut oil, 6000 A. D. M. A. units per Gm.

Beginning of Test. Series II, 458.

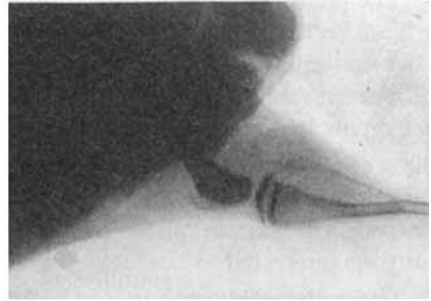
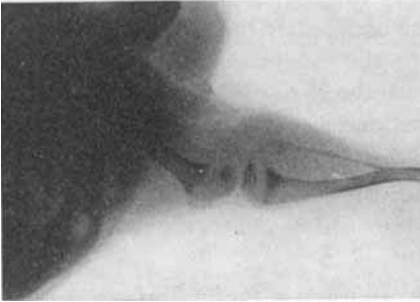
End of Test Period. Series II, 458.



Photographs of roentgengrams obtained in the experiments using cod liver oil, U. S. P. X.

Beginning of Test. Series III, 481.

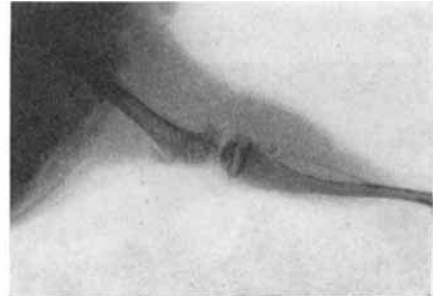
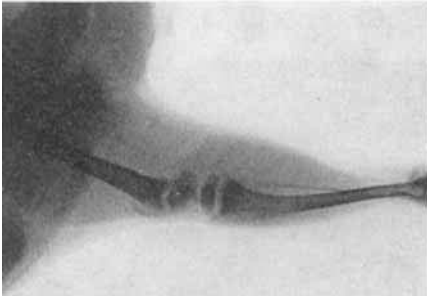
End of Test Period. Series III, 481.



Photographs of roentgengrams obtained using a mineral oil base cream containing 1000 A. D. M. A. units per 120 Gm.

Beginning of Test. Series IV, 472.

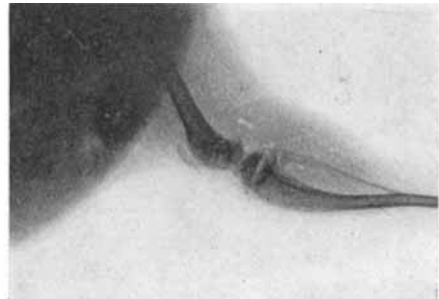
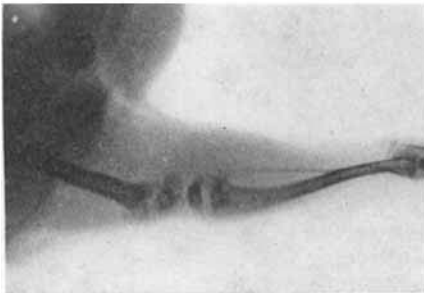
End of Test Period. Series IV, 472.



Photographs of roentgengrams obtained using mineral oil base cream containing 3000 A. D. M. A. units per 120 Gm.

Beginning of Test. Series V, 474.

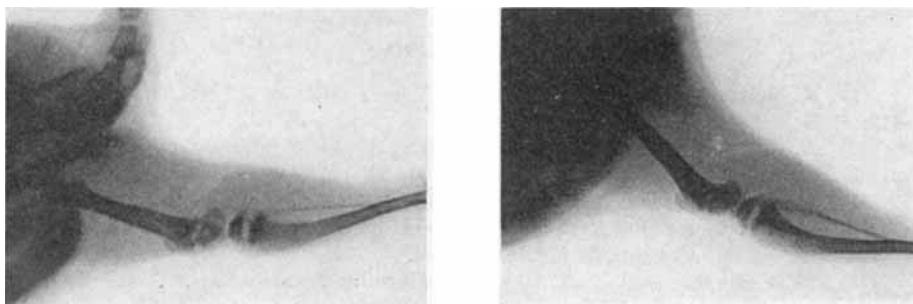
End of Test Period. Series V, 474.



Photographs of roentgengrams obtained using sun irradiated cholesterol in mineral oil cream base.

Beginning of Test. Series VI, 476.

End of Test Period. Series VI, 476.



PHOTOMICROGRAPHS.

In order to establish the extent of repair and also to approximate the extent of decalcification that had taken place in the experimental animal's bones the author felt that photomicrographs of the bone sections were necessary.

One of the hind legs of each animal was used for the purpose. After fixing in 10% formalin for 48 hours the legs were prepared for the "Celloidin Embedding" blocking process and sections of the bone were used to prepare microscopic mounts. Before mounting the sections in the usual routine manner, they were stained using "hematoxylin and eosin." Each photomicrograph represents about a 400 × magnification of the section.

Examination of the photomicrographs clearly shows the following: (1) the extent of decalcification that occurred in the bone during the development of experimental rickets, (2) the amount of repair or healing that took place during the ten-day repair period.

These photomicrographs also serve as a check on the roentgengram findings or interpretations. At the request of the editor of the *JOURNAL* and also the Committee on Publications these photomicrographs were omitted. However, anyone interested may receive them by writing to the author.

CONCLUSIONS.

1. The object of the thesis has been achieved, namely, that vitamin D can be absorbed from the skin. Also, the vehicle apparently has little or no effect in the absorption test, since the vitamin was absorbed from both vegetable and mineral oil bases.

2. In this investigation the author has developed a new method for the administration of vitamin D-containing substances, namely, the rat-tail method for the absorption of vitamin D.

3. That cosmetic creams may be used as a vehicle for carrying the vitamin substance has been demonstrated in two instances.

4. That the sun irradiation of cholesterol that has not been too highly purified is practical and that this material may be used as one of the sources of the vitamin.

5. Experiments are now under way for the quantitative estimation of vitamin D using the rat-tail method. This method may be of value in assay work.

6. It has been found that the presence of a small amount of vitamin A tends to produce more satisfactory results.

BIBLIOGRAPHY.

- (1) E. Mellanby, 1919, "An Experimental Investigation of Rickets," *Lancet*, 1 (1919), 407-412.
- (2) A. F. Hess and L. J. Unger, "The Rôle of the Fat-Soluble Vitamin in the Dietary of Infants," *Proc. Soc. Exptl. Biol. Med.*, 17 (1920), 49-50.
- (3) H. C. Sherman and A. M. Pappenheimer, "Experimental Rickets in Rats. I. A Diet Producing Rickets in White Rats, and Its Prevention by the Addition of Inorganic Salts," *Journal of Experimental Medicine*, 34 (1921), 189-198.
- (4) P. G. Shipley, E. A. Park, E. V. McCollum and N. Simmonds, "Studies in Experimental Rickets. III. A Pathological Condition Bearing Fundamental Resemblance to Rickets of the Human Being Resulting from Diets Low in Phosphorus and Fat-Soluble A. The Phosphate Ion in Its Prevention," *Bull. Johns Hopkins Hospital*, 32 (1931), 160-166.
- (5) E. T. Wyman, "The Prevention and Treatment of Rickets," *Boston Medical and Surgical Journal*, September 8, 1927.
- (6) H. Steenbock and A. Black, "Fat-Soluble Vitamins. XVII. The Induction of Growth-Promoting and Calcifying Properties in Fats in a Ration by Exposure to Ultraviolet Light," *J. Biol. Chem.*, 61 (1924), 405-422.
- (7) E. M. Hume, N. S. Lucas and H. H. Smith, "On Absorption of Vitamin D from the Skin," *J. Biochem.*, 21 (1927), 362-367.
- (8) H. C. Sherman and S. L. Smith, "The Vitamins," *American Chemical Society, Monograph No. 6*, 2nd Edition, 1931.
- (9) E. V. McCollum and N. Simmonds, "The Newer Knowledge of Nutrition," 4th Edition, 1929, MacMillan and Company.
- (10) H. C. Sherman, "The Chemistry of Food and Nutrition," 3rd Edition, 1927, MacMillan and Company.
- (11) M. S. Rose, "The Foundations of Nutrition," 1932, The MacMillan Company.
- (12) M. Luckiesch, "Artificial Sunlight, Combining Radiation for Health with Light for Vision," D. Van Nostrand Company, 1930.

MASSACHUSETTS COLLEGE OF PHARMACY.

FOOD AND DRUG LEGISLATION.

No very definite progress has been made in legislation before Congress on the various Food and Drug bills. The new Copeland bill, S. 2800, was voted a favorable report by the Senate Committee on Commerce after amending it in many particulars. The bill was reported to the Senate and has been printed in the Congressional Record for March 15th.

The bill contains a new provision for the discretionary appointment of advisory committees representing the several trades and the public, but no provision has been made for an administrative board of review, as strongly requested by the activities affected.

Other measures have not progressed and Senator Copeland is absent from Washington until about the end of March.