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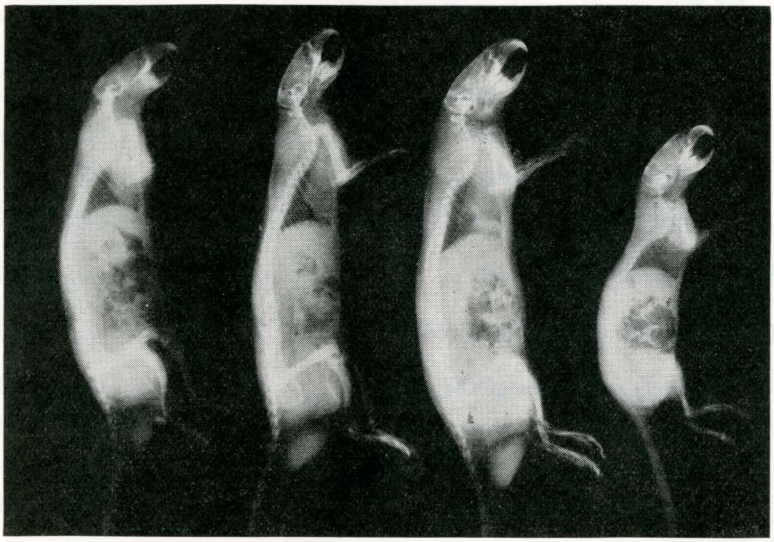

Rats 1 2 3 4
Rats 1, 2 and 3 received Rachitogenic Ration supplemented with 12 cc of cows milk daily. Rat 4 received the Ratchitogenic Ration only.

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By

T. M. Olson   G. C. Wallis

Milk still retains its enviable rating as our most nearly perfect food. It ranks alone as a superior food for infants, and is by no means of minor significance in the adequate nutrition of older children and adults. However, even with a liberal supply of milk, the problem of proper nutrition is not a simple one. A study of our public health statistics, and a consideration of the various factors stimulating refined nutritional research bear abundant evidence of the fact that our knowledge and practice of the science of nutrition is not as yet all that might be desired.

The place of vitamins in nutrition constitutes one of the important fields which is being studied. Research in this connection has shown that vitamin D is concerned with the proper retention and utilization of calcium and phosphorous in the building of strong bones and good teeth. A deficiency of this factor contributes to the improper mineralization of bones and results in the disease known as rickets. Borovsky (1933) has recently stated, "that rickets is the most common nutritional disease of childhood, variously estimated as present to some degree in 50 to 97 per cent of all infants is unhesitatingly admitted by all pediatricians." If this condition is to be improved upon, adequate amounts of vitamin D must be furnished to those most susceptible to rickets together with a diet that supplies a liberal amount of calcium and phosphorus.

Vitamin D has a very limited distribution among our common, natural food products. Milk, certain milk products, and eggs are about the only ones containing appreciable amounts, so the ordinary diet is likely to contribute but little to the supply of this factor. The normal supply of calcium and phosphorus in cow's milk makes this product of particular interest as one of the few natural food sources of vitamin D, for it simultaneously furnishes the minerals and the vitamin to assist in their proper retention and utilization in the body.

The fact that milk often makes up a large part, if not all, of the diet of the child during the time when it is most susceptible to rickets, indicates the importance of a knowledge of the factors influencing the amount of vitamin D in milk and its efficacy in preventing and curing rickets, and in promoting the adequate nutrition of the older child and adults. It is the purpose of this bulletin to discuss some of the information available on these topics and to present the results of experimental work bearing on certain phases of the problem.

Methods of Estimating the Amount of Vitamin D

A brief review of the methods used for estimating, and the units used for expressing the amount of vitamin D will undoubtedly prove helpful to a clearer understanding of the discussion to follow. Unfortunately, up to the present time, satisfactory chemical methods for quickly and economically estimating the vitamin D content of the various food and pharmaceutical preparations have not been perfected.. It is necessary to use laboratory animals for this purpose, the white rat being the most common, hence the process is called a bioassay. Three general types of procedure have been followed, namely, th line test, bone ash determinations, and X-ray studies.

The line test has been most widely used for quantitative determinations of vitamin D. For this purpose a colony of white rats must be maintained on a stock ration low in vitamin D, but adequate for the maintenance of vigor and health. The young animals are weaned at three to four weeks of age, and placed on a rickets-producing diet, the Steenbock ration No. 2965, composed of ground yellow corn, 76 per cent; wheat gluten, 20 per cent; calcium carbonate, 3 per cent; and sodium chloride (common salt) 1 per cent, being a common one. Severe rickets will develop usually, in from 21 to 25 days at which time they are ready for use in the test. The rats are given daily defiinite amounts of the material to be assayed together with the rickets-producing diet for eight days, and are then continued for two more days on the rickets-producing diet alone. The animals are killed at the end of the tenth day, the long bones of the hind legs are removed, split longitudinally through the knee, emersed in 10 per cent formaldehyde for a day or two, rinsed with distilled water, stained with a 2 per cent silver nitrate solution, developed by exposure to sunlight or artificial light, and examined for evidences of healing. A dark line across the wide band of metaphyseal cartilage which developed during the rickets-producing period, indicates the initiation of healing by the deposition of minerals in this uncalcified area. That amount of test material which just produces a narrow, continuous line of healing, when fed during this ten day test period is said to contain one Steenbock unit of vitamin D.

In the bone ash, or prophylactic, type of determination the rats are handled up to weaning time as outlined for the line test. They are then put on a rickets producing diet, as the Steenbock ration No. 2965, together with a daily allowance of the material to be tested. Negative controls, receiving only the rickets-producing diet, and positive controls receiving an adequate supply of vitamin D are carried along with the test animals. At the end of four or five weeks on the test diets the animals are killed, the bones of the hind legs removed, and the percentage of ash in the dry, fat-free bones is determined. It is desirable to obtain an ash percentage for the test diets approximately half way between the positive and negative controls. The relative antirachitic potency of the test materials is indicated either by the amounts required to give aproximately the same percentage of bone ash, or by noting the differences in bone ash percentage produced by the same amount of the test material. This method may be made quantitative for determining the units of vitamin D in any material by ascertaining the amount of test material required to give a bone ash percentage equivalent to that produced by a definite amount of a vitamin D standard of known strength.

X-ray studies may be used to estimate the relative antirachitic potency of various materials either by noting the healing induced in the bones of rachitic animals by comparing pictures taken before and after feeding of definite amounts of the test materials, or by observing the degree of protection afforded normal animals by the test diet, over a period of time. These studies may be made quantitative by ascertaining the amount of test material required to give a degree of healing or protection comparable to that given by a definite amount of a standard vitamin D preparation of known potency.

For determining the amount of vitamin D in milk, either the whole milk or the cream, or butterfat obtained from it may be used. Since the calcium and phosphorus content of whole milk is likely to be disturbing to the accuracy of the tests for vitamin D, the pure butterfat is more commonly used. To justify this procedure it is generally considered as stated by Mitchell (1932) Light, at the Medical Milk Commissioners Meeting (1932), and Smith (1934) that the fat-soluble vitamin D is practically all, if not wholly, associated with the butterfat fraction of the milk. Crawford, Golding, Perry, and Zilva (1930) have presented experimental data which in a measure, substantiate this conclusion.

Recently the United States Pharmacopoeia (1934) has adopted a new unit for expressing the vitamin D potency of foods and drugs, which became official on January 1, 1935. The new U. S. P. unit is equal in antirachitic potency for the rat, to one International Unit of vitamin D, which is defined as the vitamin D activity of one mg. of the international standard solution of irradiated ergosterol. For comparative purposes the U. S. P. vitamin Advisory Board states that 1 Steenbock unit of vitamin D is approximately equivalent to 2.7 International, or U. S. P. X. (1934) units, and that 1 International, or U. S. P. X. (1934), vitamin D unit is approximately the equivalent of 3.25 A. D. M. A. units.

Factors Influencing the Amount of Vitamin D in the Milk as Produced by the Cow.

Vitamin D Content Under Approximately Normal Conditions.—Some information is available as to the vitamin D content of milk produced under approximately normal conditions. Bechtel and Hoppert (1933) found that three grams of butterfat collected from the Michigan Station herd on August 20, 1932 contained 1 Steenbock unit of vitamin D, which was equivalent to 12.55 units per quart of 3.8 per cent milk. The same authors stated that Mitchell, Eiman, Whipple and Stokes, found 6 units per quart of normal milk but the exact conditions under which it was produced are not stated. Krauss, Bethke and Wilder (1933) maintained a check lot of cows on alfalfa hay, beet pulp, and a good grain mixture, with outside exercise on favorable days from January to May and reported a potency of approximately 2.76 Steenbock units of vitamin D per quart, the exact potency not being determined because the rats would not regularly consume enough butterfat. In other experiments Krauss, Bethke, and Monroe (1932) tested the antirachitic potency of butterfat from two Holstein cows kept under good winter conditions. The animals were giving a good flow of milk and received alfalfa hay, silage, and a good grain mixture with outdoor exercise in favorable weather. They reported 0.17 Steenbock units of vitamin D per gram of fat, which approximates 5.8 units per quart of 3.5 per cent milk as calculated by the writer. While studying the irradiation of milk, Supplee, Hanford, Dorcas, and Beck (1932) found that 150 to 200 cc. of the normal milk gave healing representative of 1 Steenbock unit of vitamin D. This would be approximately five units per quart of milk.

In the course of studying the vitamin content of typical English butter, Kon and Booth, (1933) found the vitamin D potency so low that 1.5 grams fed daily for the ten day test period was not sufficient to give a positive reading and the rats used for the assay would not consume larger amounts. Rough calculations indicate that this milk must have contained less than 2.6 Steenbock units per quart of 4 per cent milk. The following winter definite healing was initiated at the 1.5 gram level which would indicate a potency of approximately 2.6 units per quart for this milk. Steenbock, Hart, Riising, et al. (1930) kept three Holstein cows in the barn on a good winter ration and found that 120 cc. of the milk produced under such conditions contained 1 Steenbock unit of the antirachitic vitamin. This would be about 7.88 units per quart. The potency remained practically the same after the cows had been exposed to June sunshine for three weeks, the ration remaining the same. At the close of the pasture season, however, 60 cc. of milk from the same cows contained 1 Steenbock unit, or a potency of about 15.75 units per quart. In earlier work, Steenbock, Hart, Hoppert, and Black (1925) reported that 120 cc. of milk from stall-fed cows getting alfalfa hay, silage, and a grain mixture with outdoor exercise during February contained 1 Steenbock unit of vitamin D. Calculating to the quart basis, this would again be 7.88 units.

Hess, Lewis, McLeod, and Thomas (1931) stated that it took eight grams of butterfat obtained from cows kept inside on a ration of alfalfa hay, silage, and grain mixture to produce a 2+ to 3+ healing in rachitic rats. On this basis there would be at least 5 units per quart of 4 per cent milk.

Russell (1933) reported that milk from cows on summer pasture tested at New Jersey contained less than 16 units per quart. Later, Russell, Wilcox, Waddell, and Wilson (1934) reported that milk from a check lot of cows maintained indoors on a ration of beet pulp, silage, alfalfa, and grain contained less than 8 units per quart.

Effect of Vitamin D during the Growth of the Animal—It has been definitely shown by the work of Rupel, Bohstedt, and Hart (1931) (1933) Bechdel, Landsburg, and Hill (1933), Huffman (1931), and Wallis, Palmer, and Gullickson (1935) that young growing calves have a definite requirement for vitamin D to promote normal growth and well-being. A deficiency of vitamin D may result in a rachitic-like condition as shown in Figure 1, which is manifested by a reduction in growth, stiffness, progressive emaciation, deformity of the bones, humping of the back, and enlargement of the joints; by a reduction in the level of calcium and inorganic phosphorus in the blood serum or plasma; and by a reduction in the percentage of total ash in the dry, fat-free bone. The work of these investigators has also shown that a vitamin D deficient ration may be effectively supplemented with such materials as alfalfa hay, timothy hay, prairie hay, oat straw, cod liver oil, oxidized cod liver oil, irradiated ergosterol, and also by exposure of the calves to sunshine, and the irradiation of either the calves or their rachitogenic diet with ultra-violet light.

Although calves have been shown to have a definite vitamin D requirement, just what effect, if any, the early history of the animal with respect to vitamin D may have on the potency of the milk subsequently produced can not be definitely stated from the information now available. However, some experimental work at this station studying the effect of sunshine on the growth of dairy heifers and the vitamin D potency of the milk subsequently produced may be of interest in this connection.

Eight grade Holstein heifers, three to five weeks of age, were purchased from farmers in July of 1927. They were divided into two groups of four

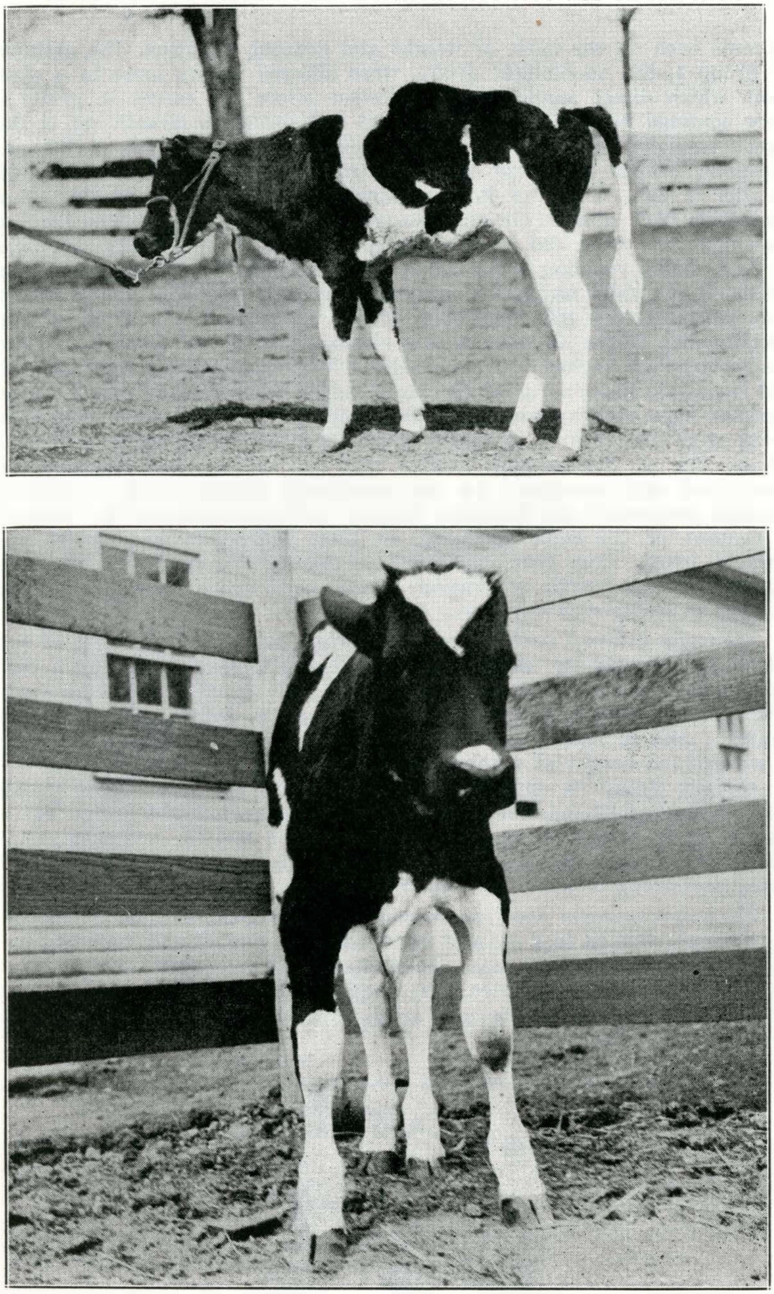

Fig. 1—Two views of a calf showing typical rachitic-like condition.

animals each on the basis of weight and general condition. The animals of group 1, the no-sunlight group, were allowed to run loose in a shed from which direct sunlight was excluded ,while the calves in group 2 were confined in a paddock in the yard and forced to remain out in the direct sunlight, summer and winter, throughout the course of the experiment, which continued until the animals were approximately 30 months of age. A shelter in one corner of the paddock provided some protection from rain. In all other respects the two groups received similar treatment and were fed identical rations, as to both quantity and kind of feed. After weaning from skimmilk at six months of age the ration consisted of alfalfa hay and a suitable grain mixture. This type of ration was continued for the remainder of the experiment. A record of the weights and height at withers was obtained at 10 day intervals. When the heifers had reached approximately 30 months of age the total calcium and inorganic phosphorus of the blood serum were determined on single samples from each animal after which representative animals from each group were slaughtered to obtain bones for breaking strength determinations. One of the sunlight heifers and two of the no-sunlight group were bred and continued on the prescribed experimental conditions through pregnancy and lactation to make further studies on the vitamin D content of the milk produced. In fact, representatives of the no-sunlight group have been continued on experiment until we now have animals of the fourth generation in the herd which have never been exposed to sunlight at any time.

Appendix tables 1 and 2 give the detailed data for the growth of the calves as indicated by weights and heights at withers. Each figure represents the average for the four animals in each group. This information with the corresponding normal in each case is shown graphically in Charts 1 and 2. Both groups of calves made better than normal gains in weight and height at withers with a slight advantage in favor of the no-sunlight group in each case. The calves in the no-sunlight group showed more gloss and bloom than did those in the sunlight group. These results are as might be expected in view of the fact the sunlight group was forced to stay outside during the cold winter weather with the food intake restricted to the same amount as given to the no-sunlight group.

When the animals were approximately 30 months of age the breaking strength of a six inch span of the femurs and humeri of representative heifers from each group was determined on a Materials Testing Machine of fairly large capacity. The results given in tabular form below indicate no significant differences in the breaking strength of the bones form the two groups.

Heifer No.	Breaking Load Femur	Breaking Load Humerus
	Lbs.	Lbs.
	No Sunlight group	
9103	1900	4400
	Sunlight group	
9102	2400	6800
9105	1300	4665
9106	1675	4670

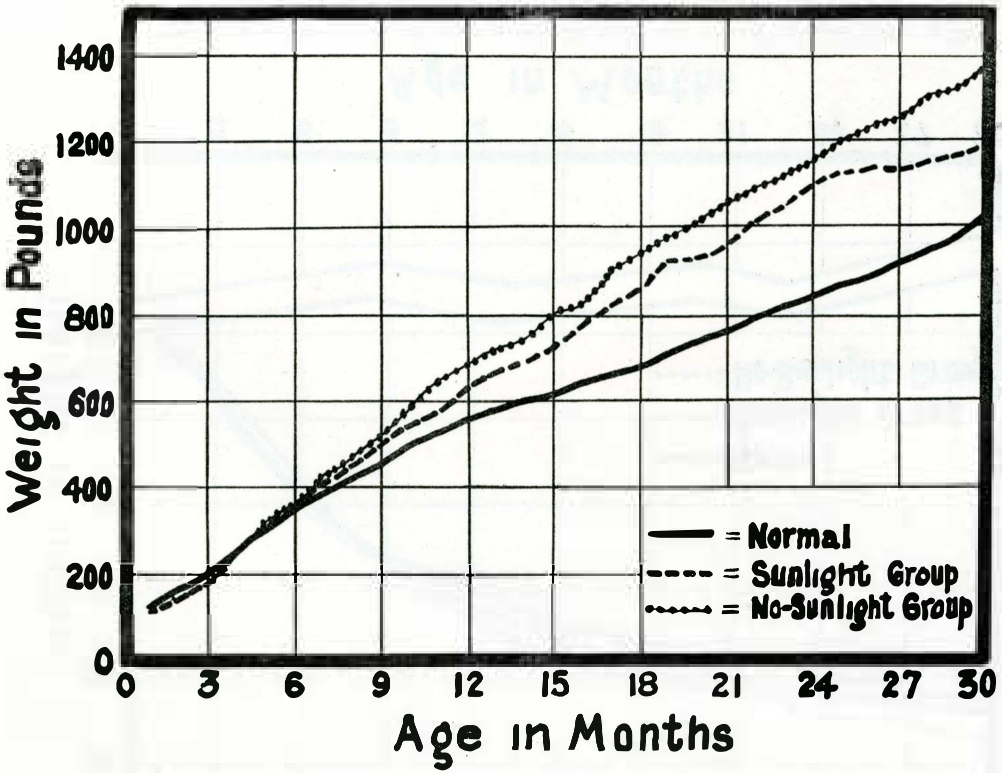

Chart 1—A graph showing the average monthly weight of the four calves in the sunlight and no sunlight groups as compared with normal weight of the calves.

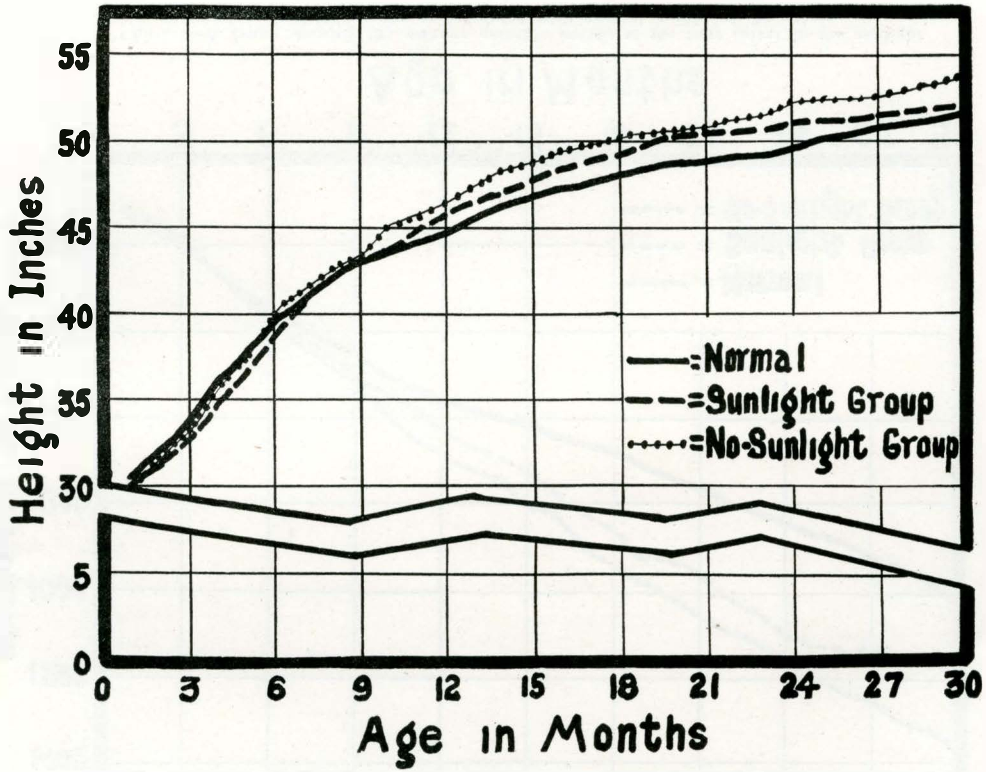

Chart 2—A graph showing the average monthly height at withers of the four calves in the sunlight and no sunlight groups as compared with normal height of calves.

Previous work at this station has shown the breaking strength of the bones of rachitic calves to be much inferior to that of normal calves. For instance the breaking load of a six inch span of the femur of a rachitic calf was 700 pounds, and for a four inch span of the humerus, 920 pounds, while corresponding values for a normal calf of the same age were 4000 and 2875 pounds. The above data for the heifers on this experiment give no such evidence of abnormalities of a rachitic nature.

The results of the blood analyses made at the close of this part of the experiment when the calves were about 30 months of age are shown below.

	Blood Analysis	
Animal Number	Ca per 100 cc. serum	P per 100 cc. serum
	mgm.	mgm.
	(no-sunlight group)	
9103	14.55	3.64
9107	11.96	6.82
9108	11.91	4.37
9109	11.76	8.83
Ave.	12.545	5.915
	(sunlight group)	
9102	14.29	5.43
9104	12.53	7.97
9105	14.54	8.49
9106	13.85	7.08
Ave.	13.802	7.242

The amount of data is too meager to be of much significance, although two of the animals in the no-sunlight group show slightly less than the normal amount of inorganic phosphorus in the blood serum and the average inorganic phosphorus for the no-sunlight group is somewhat less than for the sunlight group, which might possibly be a reflection of the more rachitogenic conditions under which they were kept.

A review of the information gathered indicates that the calves in both groups made normal growth or better with no definite evidence of any differences attributable to the lack of sunlight. In view of the work already referred to it would seem likely that the requirement for vitamin D in the calves of the no-sunlight group was met sufficiently well by the vitamin D supplied in the roughage portion of the ration to allow for the essentially normal responses. These results are in agreement with those obtained by Gullickson and Eckles (1927) who found that calves remained normal and made good growth when raised on essentially normal rations even though they were housed in complete darkness to avoid any antirachitic effects of exposure to direct sunlight. Similar results were also reported by Morrison and Rupel (1927) who noted no differences between calves exposed to sunlight and those on the same normal ration but kept continuously indoors. Furthermore, animals at this station have been continued under the no-sunlight conditions through gestation and lactation until the fourth generation has appeared with no evidences of abnormal conditions developing.

As previously stated, two of the heifers from the no-sunlight group and one from the sunlight group were continued through gestation and lactation under their respective experimental conditions. The milk from these animals was fed to young growing pigs under winter conditions and note taken of any development which might indicate a difference in the vitamin D potency of the two lots of milk. This work was carried on in cooperation with Professor T. R. H. Wright of the Animal Husbandry Department of South Dakota State College.

Three lots of pigs were selected for these trials. Each lot was self-fed a basal ration made up 75 parts of white corn, 15 parts of oil meal, and 10 parts of wheat middlings. A mineral mixture consisting of 28 pounds of steamed bone meal, 50 pounds of ground limestone and 20 pounds of salt was available at all times in a separate compartment of the self-feeder. Water was supplied ad libitum. The basal ration was supplemented by four pounds of milk per pig per day from the sunlight cow for Lot 1, four pounds of milk per pig per day from the no-sunlight cows for Lot 2, and a pint of cod liver oil per hundred of grain mixture was mixed with the grain for Lot 3. The pigs were confined to pens in a hog house and thus kept out of direct sunlight. The pens had a cement floor and were provided with a straw-covered bed made of lumber and raised six inches from the floor. The pigs were weighed at about the same time of day at weekly intervals and other notations made as to their condition. The experiment started on February 1, 1930 and continued for approximately five months.

By March 22, about seven weeks after the beginning of the experiment, several pigs in Lot 2 and one pig in Lot 1 seemed sore on their feet and showed signs of stiffness, which was particularly noticeable as they passed over a cinder path and cement floor while being driven to the scales for weighing. The pigs in Lot 3 were active and playful while those in Lots 1 and 2 were reluctant to move.

On March 23, the milk supplement of Lots 1 and 2 was increased from four to eight pounds per head daily. This seemed to check the development of abnormal symptoms in Lot 1 and to alleviate the condition somewhat in Lot 2, although the pigs in the latter group still continued to develop stiffness, soreness of feet, stilted gait, swollen joints, and other indications of a rachitic-like condition.

By April 10, a Poland sow in Lot 2, receiving the milk from the no-sunlight cows, showed market symptoms of a rachitic-like condition, and on April 11 had to be carried to the trough for her milk. On April 22 a picture (Figure 2) of this group was taken which shows the Poland sow lying in the foreground unable to stand. Several of the other pigs exhibited milder symptoms. At this time the Poland sow was removed from the lot, continued on the same ration, but moved out into the sunlight. In about ten days she was able to walk, and by May 17, she appeared normal except for the crooked hock as may be seen in Figure 3. The remainder of the pigs in Lot 2 were continued on their prescribed experimental program until May 19, during which time more and more of the pigs exhibited the development of rachitic-like symptoms with increasing severity. Figure 4 shows the condition of the group at this time. Note particularly the condition of the Duroc sow on the extreme left and the Chester barrow on the right.

Meanwhile, the pigs in Lot 3, receiving the cod liver oil supplement had continued to react normally in every respect, while those in Lot 1, receiving the supplement of milk from the sunlight cows, developed no market difficulties, yet were not as active and vigorous as the pigs in Lot 3.

After May 19, the same grain ration was continued but the milk and

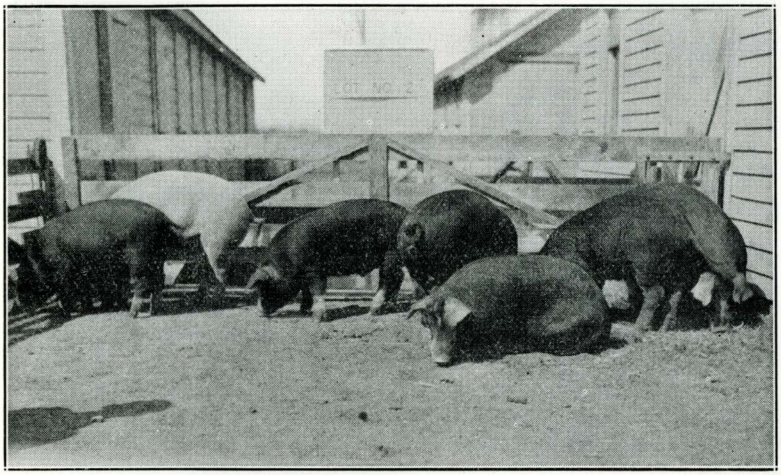

Fig. 2—Picture of lot 2 taken April 22, 1930—seven weeks after trial started. Several pigs showing symptoms of rickets. Poland sow not able to stand.

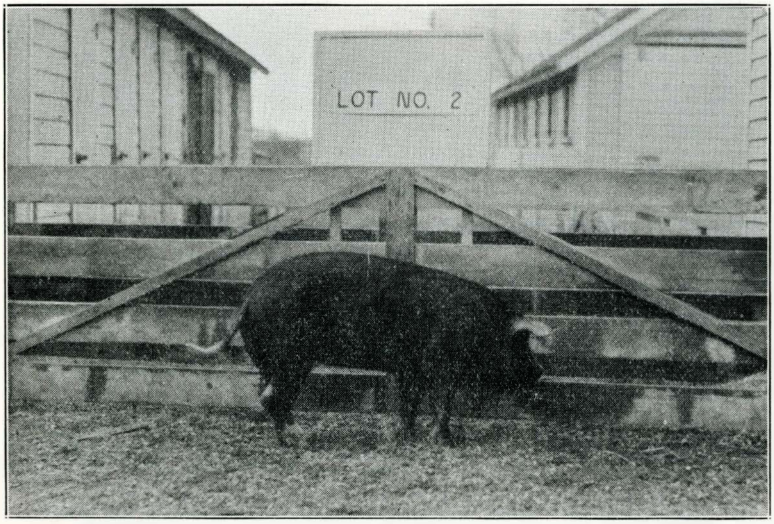

Fig. 3—A picture of the Poland sow from lot 2, showing her improved condition, after sunlight exposure from April 22 to May 17. The ration remained the same.

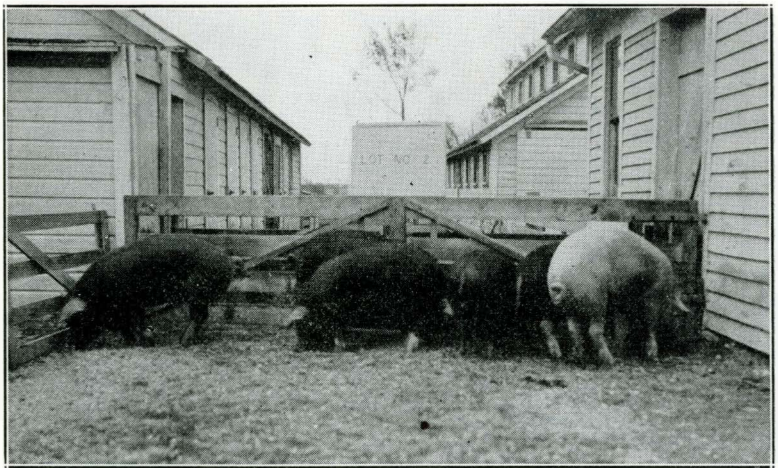

Fig. 4—A picture of lot 2 taken May 19, showing condition of the pigs on the day on which milk feeding was discontinued. Note especially the condition of duroc sow on extreme left and Chester barrow on extreme right.

Fig. 5—Picture of lot 2 taken on May 28, 9 days after milk feeding was discontinued. Duroc sow and Chester barrow unable to rise without assistance.

cod liver oil supplements were taken away from all of the groups. The pigs in Lot 2 grew rapidly worse as may be seen by comparing Figure 4 with Figure 5, which was taken May 28, nine days after the milk was removed from the ration. The Duroc-Jersey sow and the Chester White barrow were unable to stand without assistance. Figure 6, taken at the same time shows another view of these two pigs after they had been assisted to their feet. For comparative purposes, a picture of the pigs in Lot 1 and 3 taken on May 28, nine days after the removal of the supplements and when Lot 2 was at its worst, are shown as Figures 8 and 9 respectively. Although the picture of Lot 1, Figure 8, shows the pigs still on their feet, seemingly quite normal, yet it was very apparent that they walked with more difficulty and were noticeably worse since the milk was taken away. The pigs in Lot 3, Figure 9, appeared and acted essentially normal in all respects.

Starting on May 29, all the pigs were allowed access to runways on the south side of the hog house and were forced to stay in the sunlight most of the time. All the pigs showing rachitic-like difficulties responded favorably to this treatment. An idea of the improvement may be gained by comparing the condition of the Chester White barrow, Lot 2, as shown in Figures 5 and 6 taken on May 28, before sunlight exposure, with his condition on June 16, after 18 days of sunlight exposure as shown in Figure 7. The Duroc Jersey sow had died from pneumonia previous to the time of this last picture. As indicated in Appendix table 3, which gives the detailed growth and feed data for the lots of pigs on this experiment, Lot 2 consumed less feed and consequently made slowed gains which is undoubtedly attributable to their more severe rachitic-like condition.

In analyzing the results of this experiment, it should be remembered that the two kinds of milk used as supplements to the basal ration for Lots 1 and 2, were produced by animals that had been fed and handled throughout the period of growth and lactation under identical conditions except that one group was forced to remain out in the sunlight at all times and the other confined in a shed continuously to avoid sunlight exposure. The ration was made up largely of alfalfa hay and a suitable grain mixture, neither group having any access to green feeds or pasture. The pig feeding experiment, was started on Feb. 1, when the antirachitic action of the sun's rays on the sunlight cow had been steadily decreasing to its lowest season level. It was continued through the late winter and spring months during which time the sunlight cow was forced to stay outdoors where she would be exposed to the increasingly more potent antirachitic activity of the sun's rays. Although a study of the basal ration fed to the pigs during this trial indicates that the supply of vitamin A is not over abundant, yet there is no definite evidence of vitamin A deficiency complications. On the other hand, the two lots of pigs given the milk supplements developed rachitic-like symptoms during the course of the experiment which the check lots receiving cod liver oil did not do. The vitamin D deficiency symptoms in Lot 1 receiving the milk from the sunlight cow were mild, while those in Lot 2 receiving the milk from the no-sunlight cows were rather severe. Both lots of pigs exhibited notable improvement when exposed to the antirachitic action of spring and early summer sunlight. This was especially marked in the case of the Poland-China sow of Lot 2. In so far as the responses of these lots of pigs may be taken to indicate a difference in the vitamin

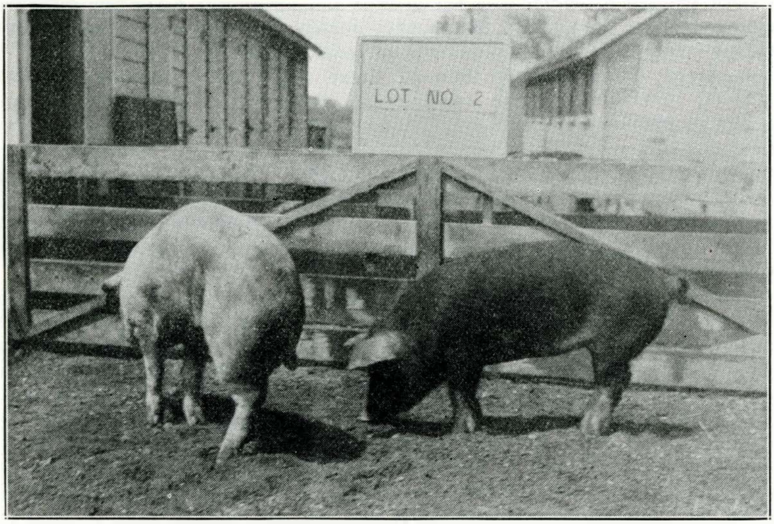

Fig. 6—Picture taken on May 28, of Chester barrow and Duroc sow immediately after they had been assisted to their feet.

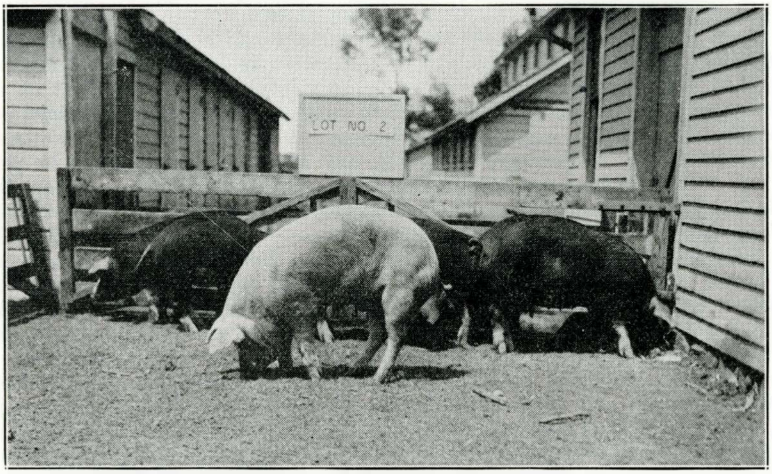

Fig. 7—Picture of lot 2, taken June 16, showing condition of pigs after 18 days of sunlight exposure. Note the improved condition of Chester barrow in foreground.

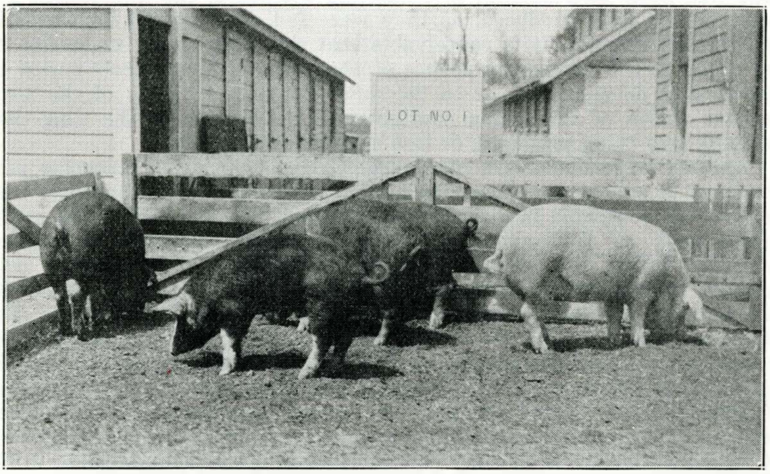

Fig. 8—Picture of lot 1 taken May 28, 9 days after milk feeding was discontinued. All the pigs were able to stand but walked with increasing difficulty, especially after the milk feeding was discontinued. Compare with Figures 5 and 9

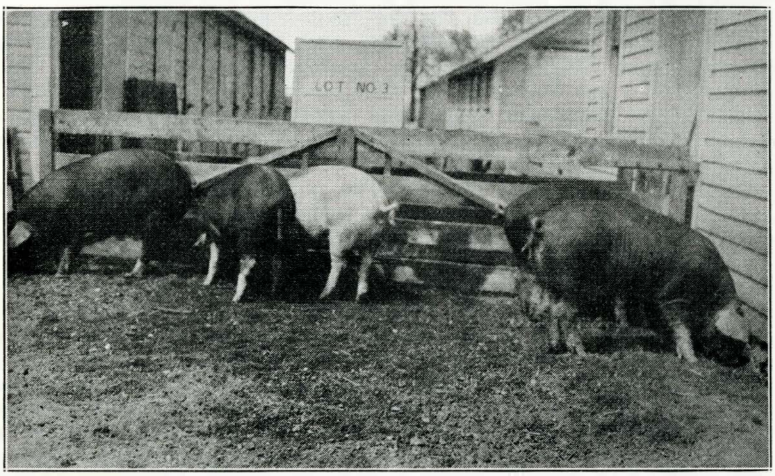

Fig. 9—Picture of lot 3 taken May 28, 9 days after the cod liver oil had been removed from the ration, showing the normal condition of the pigs. Compare with Figures 5 and 8, showing the condition of lot 1 and 2 at the same time.

D potency of the milk produced by these two groups of cows, it would seem logical to assume that the sunlight exposure of the one group of cows had been instrumental in enhancing somewhat the antirachitic potency of the milk produced, as they were fed and handled otherwise in an identical manner. It is conceivable that this may be an expression of the effect of the sunlight exposure of the animals during their period of growth and development, or to the more immediate effects of the antirachitic action of the spring sunlight on the cow during the time that the milk was being produced, or to a combination of both.

Another experiment has been conducted in which the butterfat produced by a second generation animal raised under the no-sunlight conditions was compared in vitamin D potency with that produced by a cow giving milk of approximately the same fat percentage and handled normally during the summer pasture period. The samples from both animals were collected between October 4 and 13 just at the time the cow handled normally was completing the summer pasture period. The cream was churned and the melted butter oil separated out by means of a separatory funnel for placing in cold storage to be assayed for vitamin D. Credit is due to Dr. N. B. Guerrant and Professor R. Adams Dutcher of Pennsylvania State College, who kindly consented to assay these samples of butterfat for vitamin D in the laboratories of the Department of Agricultural and Biological Chemistry of that institution. These workers, using the standard line test technique found that the butterfat from the normal cow at the close of the pasture season contained 227 Steenbock units of vitamin D per pound, while the sample from the no-sunlight cow contained only 64.8 units per pound. These results indicate that an animal which has not been exposed to sunlight is able to secure sufficient vitamin D, presumably, from the dry roughage portion of the ration to promote normal growth, reproduction, and lactation, the milk having about the same vitamin D potency (approximately 5.6 Steenbock units per quart of 4 per cent milk) as that produced by normal cows under average winter conditions. The animal exposed to sunlight under pasture feeding conditions produced butterfat with a vitamin D potency three and one-half times as great as that of the no-sunlight cow.

Sunshine and Seasonal Effect.—The antirachitic properties of the ultra-violet portion of sunshine is now well known. Luce (1924a) has reported some early investigations studying the influence of diet and sunlight upon antirachitic properties of cow's milk. A Jersey cow was maintained under varying conditions of diet and exposure to sunshine throughout five different periods. Milk representative of each set of conditions was tested for its antirachitic potency using histological studies, calcium depositing ability, and protective doses for young rats on McCollums rachitogenic diet 3143 as criteria. In the first experiment a dry ration deficient in vitamin D was used while the animal was exposed to summer sunlight. When the cow was confined in a dark stall on the same ration for period two there was a marked decrease in the antirachitic property of the milk, in fact, milk of the lowest potency reported was obtained under these conditions. There was a slight improvement when fresh green feed was substituted for the dry feed (period three) with the cow still confined in the dark stall. When the green feed was continued with the sow turned out into summer sunshine (period four) there was a decided improvement, so that milk of the highest antirachitic potency reported was obtained under these summer pasture conditions. Upon again substituting the dry feed for the green portion of the ration and leaving the cow out in the late fall and winter sunshine the antirachitic potency of the milk decreased but was still superior to that of the milk obtained on dry feed while the cow was in the dark. From these observations the author concluded that the antirachitic value of the milk depends on the diet of the cow and possibly also on the degree of illumination to which she is exposed. Milk from a pasture fed cow has a definite and high antirachitic value; the same animal when stall fed in the dark, yielded a milk much inferior in antirachitic properties.

Luce (1924b) continued this work, leaving the cow on dry fodder and sunshine exposure for January to July, but noted no marked increase in the vitamin D potency of the milk to result from the effects of the summer sunshine of the advancing season, so that the antirachitic value of the milk thus produced on dry fodder and sunshine exposure was not nearly as great as for that previously produced by green feed and sunshine exposure. It was, however, slightly superior to that produced on dry fodder in the dark. These observations led the author to again state that the diet of the cow seems to be the main factor in determining the antirachitic value of her milk. However, a careful study of the data presented indicates that the evidence for the separate effects of diet and sunshine exposure on the antirachitic value of the milk produced is not as clear cut as might be desired. For instance, as between periods three and four when the cow was on green feed and was moved from the dark into summer sunshine, there was a decided improvement in the antirachitic potency of the milk which could be interpreted to indicate that sunshine played a major role rather than the diet as suggested by the author. In fact, Boas and Chick (1924) used some of this same milk to study its effect on the calcium and phosphorus retention of rats. When fed at a 5 cc. level, rats receiving milk obtained after the animal had been on dry fodder in a dark stall for six months showed defective calcium and phosphorus retention as compared to controls. The defective retention still continued when the milk was obtained two months after green food had been substituted for the dry fodder. Rats receiving milk produced after the cow had been out in the summer sunshine for two months with the green food continued showed an increase in calcium and phosphorus retention, so that it compared favorably with the retention of positive controls. These differences are attributed by the authors to the effect of sunlight on the cow.

The above work of Luce (1924a) (1924b) was repeated by Chick and Roscoe (1926) who made use of some refinements in technique. These authors concluded that the insolation of the cow was the important factor determining the antirachitic properties of the milk, yet was not the whole truth for the milk from the cow on summer pasture was more potent than from winter feed in the open sunshine.

Dutcher and Honeywell (1927) found that butter from cows subjected to sunlight showed superior calcifying potency when fed to rats, to that of cows fed in the dark. The antirachitic and calcifying properties of dry milk prepared from summer milk was compared to that prepared from winter milk by Supplee and Dow (1927) whose data indicate a slightly higher ash percentage in each case for the bones of rats on the summer-prepared milk at the various levels tried, thus showing a somewhat greater potency for this milk.

After keeping three Holstein cows in the barn over winter on a good winter ration Steenbock, Hart, Riising, Hoppert, et al. (1930) tested the milk for its antirachitic potency and observed that it required about 12 cc. daily to give a positive line test on rachitic rats. Exposing the cows to June sunshine for three weeks while continuing on the same ration produced no appreciable change in the antirachitic value of the milk. After the cows had been on pasture all summer, 6 cc. of the milk daily were sufficient to initiate healing, indicating about twice the potency of the winter milk, but here again, there was a change in the diet as well as in the amount of sunshine exposure so the effect of each can not be clearly ascertained.

Comparisons of the vitamin D potency of butterfats produced under typical winter and summer conditions have also been made at this station. The summer butterfat was obtained from the cream delivered by farmers to the creamery of South Dakota State College during the month of August, 1932. The winter butterfat was secured on January 20, 1933 from cream produced by the Experiment Station herd.

The bone ash, or prophylactic, type of experiment was used in studying the vitamin D potency of the two fats. Five lots of four rats each were used. Lot 1 received the Steenbock rachitogenic ration No. 2965 only. Lots 2 and 3 received a supplement of 5 and 10 per cent of summer butterfat, while Lots 4 and 5 were fed 5 and 10 per cent of the winter butterfat, respectively. The fat was mixed with the ration for feeding, thorough mixing being effected by melting the fat and warming the ration. Only a sufficient amount for a few days feeding was mixed at one time. There was some scratching out of feed in the various lots so the daily food intakes indicated below may not be exactly absolute but serve to show that the relative intakes were of about the same order for the rats receiving the butterfat supplements.

Basal—rachitogenic diet only—4.82 grams daily.
Summer butterfat—5 per cent—6.39 grams daily.
Summer butterfat—10 per cent—6.16 grams daily.
Winter butterfat—5 per cent—5.91 grams daily.
Winter butterfat—10 per cent—5.85 grams daily.

The rats were on the experiment for approximately five weeks. At the end of this time one rat from each lot was selected for X-ray photographing, after which the femur and tibia of both legs were used for ash determinations, the results of which are shown in Table 1 below together with the average ash percentage for all the rats in each group.

TABLE 1—The Ash Percentage of Rats Fed Various Amounts of Summer and Winter Butterfat.

(First Trial)

Lot Number	Ash Percentage One-X-rayed rat	Ash Percentage Average for group
1—Basal only	25.52	27.06
2—5% summer butterfat	44.88	44.28
3—10% summer butterfat	50.14	47.37
4—5% winter butterfat	30.89	30.17
5—10% winter butterfat	35.28	34.56

The X-ray photographs of typical animals from each lot shown in Figures 10-13 verify the bone ash data and indicate that the summer produced butterfat is much more potent than the winter butterfat, also that the 10 per cent level in each case gives appreciably higher results than the 5 per cent level.

A second trial was run using the same summer butterfat but a different sample of winter butterfat, the latter being collected in March of 1933. The results for this trial as shown in Table 2 are essentially the same as for the first trial except that this sample of winter butterfat gives evidence of being somewhat more potent than the first sample of winter butterfat.

TABLE 2—The Ash Percentage of Rats Fed Various Amounts of Summer and Winter Butterfat.
(Second Trial)

Ration	No. of Rats	Average percentage of ash
Basal (Steenbock 2965)	5	28.83
Basal plus 5% summer fat	5	43.44
Basal plus 10% summer fat	5	46.56
Basal plus 5% winter fat	5	37.06
Basal plus 10% winter fat	5	40.62

The various pieces of experimental work just reviewed show quite uniformly that milk produced under summer conditions has a vitamin D potency somewhat superior to that of milk produced under typical winter conditions. There is some evidence to indicate that the diet of the cow and the beneficial effects of summer sunshine both have a part to play in increasing the antirachitic value of the summer milk but just how much should be attributed to each can not be defiinitely stated from the information available at the present time.

Ultra-violet Irradiation of the Cow.—Almost simultaneously Steenbock and Black (1924), and Hess and Weinstock (1924), reported from work in their respective laboratories that the irradiation of quite a variety of materials with artificially produced ultra-violet light would impart new or addition antirachitic properties. The possibilities of enhancing the vitamin D potency of the milk by subjecting the producing animal to such irradiations were soon investigated. Steenbock, Hart, Hoppert and Black (1925) were quite enthused over the demonstration that the ultra-violet irradiation of two goats resulted in a three fold and six fold increase, respectively, in the vitamin D potency of the milk produced. The results obtained from the irradiation of cows have been somewhat disappointing however, and have not been uniformly favorable.

Favorable results were obtained by Gowen, Murray, Gooch, and Ames (1926) as indicated by the fact that rachitic chicks receiving milk from two Holstein cows that had been irradiated 15 minutes daily for one month, and 30 minutes daily for another month were in good condition with no apparent rickets at the end of 50 days, whereas chicks on control milk moved toward more severe clinical and X-ray rickets. The experiment was repeated with two other lots of chicks with approximately the same results. Preliminary investigations by Mitchell, Eiman, Whipple, and Stokes (1932) using rats, as well as curative and protective studies

Rachitogenic Ration

Fig. 10—X-Ray picture of negative control rats on rachitogenic ration. Note the depressed condition of chest cavity, lack of density of the bony structure, and the wide band of cartilage on the ends of the long bones, indicating a severe rachatic condition.

on infants, suggested a definite increase in the vitamin D potency of milk from irradiated cows over that from control cows. Twenty infants were protected over a period of six to eight months and a small number were cured by this agent alone. Rat assays on the milk from the irradiated cows showed approximately 22 units of vitamin D per quart.

On the other hand, Bunger (1931) was unable to show any distinct increase in the vitamin D concentration in the milk from irradiation of the cow. Perhaps the most extensive investigation of this problem is that of Steenbock, Hart, Riising, Hoppert, et al. (1930). In series two of their work it was found by the use of curative tests on rachitic rats

Five Per Cent Winter Butterfat **Five Per Cent Summer Butterfat**
W **S**

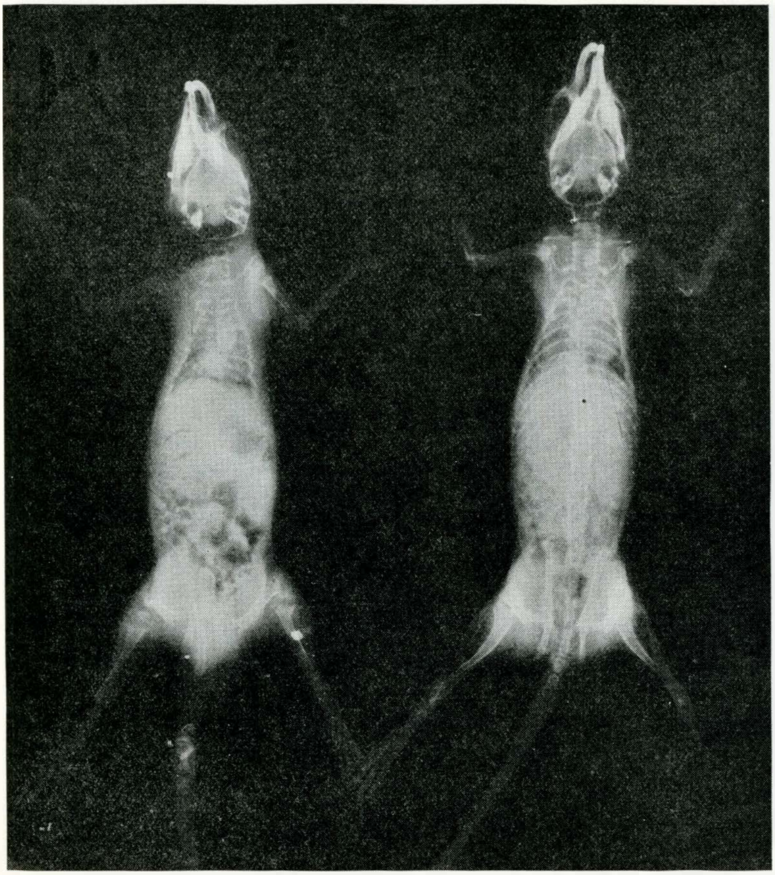

Fig. 11—X-Ray of rat (W) receiving a supplement of 5 per cent of winter butterfat, and rat (S) receiving 5 per cent of summer butterfat. Note the better formed chest, the greater density of the bony structure, and the narrower bands of cartilage at the ends of the long bones in the knee and wrist joints of rat (S) indicating the greater antirachitic potency of the summer butterfat.

that the milk from artificially irradiated cows was not appreciably more potent than that of non-irradiated control cows. Using the prophylactic, or preventive, method in another series, negative results were also obtained. Contrary to expectations, the rats receiving milk from two cows before they were irradiated showed a higher ash percentage in the bones in both cases than did those receiving the milk after the cows were irradiated. The authors suggested that this may have been due to the

Ten Per Cent Winter Butterfat
W

Fig. 12—X-Ray of two rats receiving a supplement of 10 per cent of winter butterfat. Note the lack of density of the bony structure as compared with the rats in Fig. 13 and rat (S) in Figure 11, indicating less potency for the winter butterfat.

fact that the rats receiving the milk from the irradiated cows were slightly younger. Two other series of tests were run using milk from cows before and after irradiation of the animal in both the curative and prophylactic type of test, but in no case was it possible to demonstrate any appreciable increase in the vitamin D potency of the milk by the irradiation of the cow producing it. These same authors, however, note in their review of literature that Falkenheim, Voltz, and coworkers found the milk of an irradiated cow to be antirachitically active both prophylactically and curatively in contrast to milk from a non-irradiated cow, while sunlight did not produce definite effects. Also, that Bruck,

Ten Per Cent Summer Butterfat
S

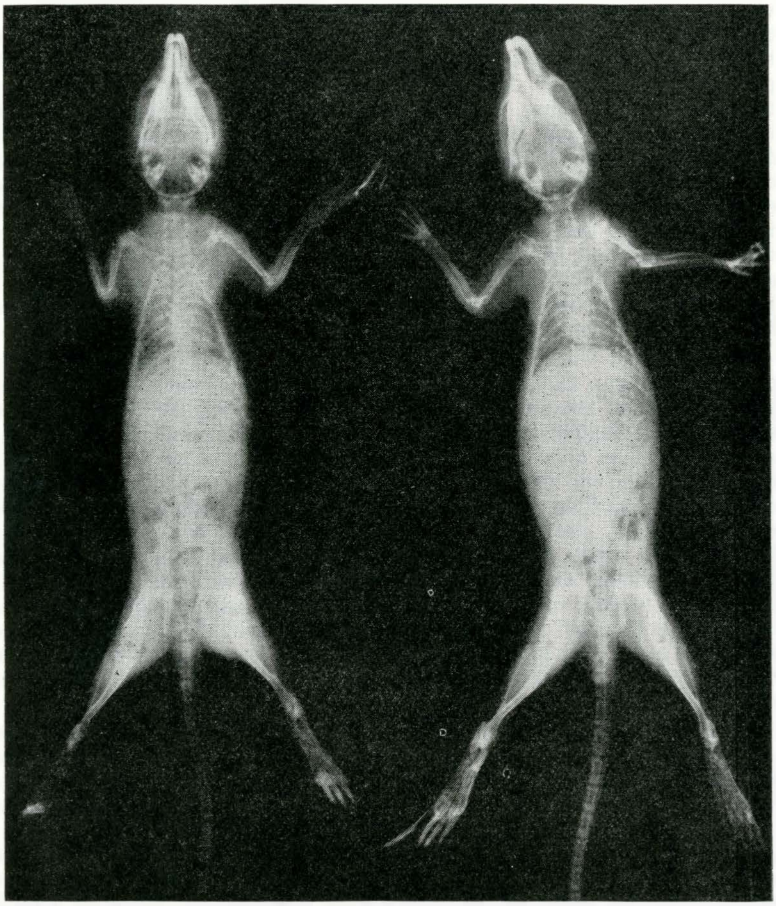

Fig. 13—X-Ray of two rats receiving a supplement of 10 per cent of summer butterfat. Note the density of the bony structure, well-formed bones and narrow bands of cartilage at the ends of the long bones, indicating a relatively high degree of protection from rickets.

Biesok, Pirquet and Wagner found milk from irradiated cows to prevent rickets in normal children, but failed to cure florid cases of rickets.

Consideration of the information available forces the conclusion that an intelligent statement as to the exact effect of artifically irradiating a cow with ultra-violet light on the vitamin D potency of the milk produced can not be made at the present time. Results to date are confusing. While some investigators have been able to demonstrate an appreciable enhancement of the antirachitic properties of the milk by irradiating the animal, others have been unable to do so.

Feeding Vitamin D Concentrates.—It was not long after vitamin D was identified as a separate food factor with an important role to play in the prevention of rickets and proper mineralization of the developing bones of the young that attempts were made to increase the antirachitic properties of milk by feeding the cow materials comparatively rich in this substance. Golding, Soames, and Zilva (1926) studied the antirachitic properties of milk from a group of Shorthorn cows on a basal ration deficient in vitamin D; another on the basal ration plus kale, and another on the basal plus cod liver oil. Judged by the ability of the different milks to promote normal mineralization of the bones of young rats the inclusion of cod liver oil in the ration increased the vitamin D potency of the milk produced, but the addition of kale failed to do so. The authors also noted that the cod liver oil depressed the fat production of this group of cows by about 15 per cent, a characteristic of cod liver oil which has also been reported by other investigators.

Golding and Zilva (1928) continued their studies using four groups of cows; one on a rigorous basal ration consisting of oat straw, roots, and a meal mixture, the animals being kept inside during the winter months; another on a control basal ration consisting of hay, silage, and a meal mixture to make a good winter ration, with outside exercise on favorable days; a third on the rigorous basal plus cod liver oil to excess(six to eight ounces daily; and the fourth on the rigorous basal ration plus two ounces of cod liver oil daily. Vitamin D studies with laboratory animals on butters made from the various groups showed that the good winter ration plus winter sunshine was much better than the rigorous basal ration. The feeding of two ounces of cod liver oil daily produced no better results than the good winter ration, while feeding of six to eight ounces resulted in a marked increase in the antirachitic action of the milk, but again depressed the fat percentage of the milk produced which the two ounce level did not do.

Kon and Henry (1935) tested the vitamin D potency of cacao shell and found 35 International Units per gram. Four cows were then used to determine the effect on the vitamin D potency of the milk when this material was substituted in the ration. Two cows on a good winter ration of hay, grain, and mangels in a preliminary period produced fat with a potency of 0.17 International Units per gram. A month after two pounds of cacao shell (32,000 I. U. of Vitamin D) had been introduced into the ration daily the butterfat contained 0.51 International Units of vitamin D per gram. The potency of the butterfat from two cows used as a control group was 0.20 I. U. per gram for the preliminary period and 0.21 I. U. for the sample taken one month later. Thus the cacao shell effected a three fold increase in the vitamin D potency of the butterfat as compared with the butterfat of the same cows in a preliminary period, and a two and one-half fold increase as compared with control cows carried along on the same winter ration as used in the preliminary period. The authors pointed out that this increase is approximately the same as observed by them when changing cows from typical winter to typical summer conditions in their locality. For purposes of comparison the 0.17 International Units per gram of butterfat approximates 2.46 Steenbock units, and the 0.51 amounts to about 7.38 Steenbock units per quart of 4 per cent milk.

After it had been demonstrated that yeast could be made highly antirachitic with ultra-violet irradiation, Steenbock, Hart, Hanning and Humphrey (1930) studied the possibilities of increasing the vitamin D in milk by feedng irraidated yeast to two cows. The ash percentage in the bones of young rats receiving a rickets-producing diet plus 4 cc. of milk daily from the preliminary period before feeding irradiated yeast was 37 and 34.2 per cent respectively for the two cows. Similar groups of rats receiving milk produced after the cows had been fed 200 grams of irradiated yeast daily showed an average ash of 51.7 and 52.3 per cent respectively, thus indicating a decided increase in the antirachitic action of the milk. In fact 2 cc. daily for five weeks were sufficient to give normal calcifications. A slight improvement in the antirachitic action of the milk was noted from feeding 10 grams of irradiated yeast to the cows daily with an increasing effect from feeding 50 to 200 grams daily. Feeding 180 cc. of cod liver oil produced results similar to 10 grams of of irradiated yeast.

Krauss, Bethke, and Monroe (1932) studied the effect on the milk of feeding irradiated ergosterol to cows. This material is one of the most potent sources of vitamin D. In a preliminary trial 5, 10, 25, and 100 mg. were fed daily to a Jersey cow in separate trials. The antirachitic value of the butterfat was not appreciably enhanced by the 5 and 10 mg. levels, the 25 mg. level gave a slight indication of enhancement, and the 100 mg. gave a marked increase. The work was continued using two Holstein cows giving about 35 pounds of milk daily and being kept under good winter conditions. Irradiated ergosterol was fed at various levels for periods of three weeks and butterfat samples collected for study during the last five days of each period. The regular line test procedure was used for determining the vitamin D potency of the various samples, and these results were checked later by the use of the prophylactic, or bone ash, method. Some of the more pertinent results are shown in tabular form below.

Fat Sample	Units of vitamin D fed cows daily	Steenbock rat Units per gram of fat
1	0	0.17
2	7,500	0.29
3	15,000	0.50
4	0	0.83
5	100,000	1.67
6	200,000	2.50
7	0	0.40

The data show that as the vitamin D intake of the cows increased the antirachitic potency of the butterfat increased correspondingly. A sixteen fold increase was obtained in sample 6 by feeding 200,000 rat units of vitamin D to the cows daily in addition to the good winter ration which they received when sample 1 was produced. Prophylactic tests on rats corroborated the essential features of the above observations. Gerstenberger and Horesh (1932) fed one pint daily of the sample 6 milk to each of two rachitic infants to test its efficacy in this way, and observed slow but definite signs of healing and recovery as indicated by X-ray, clinical, and blood composition evidence.

Krauss, Bethke, and Wilder (1933) have studied the effect of feeding a commercial cod liver oil concentrate, Vitex, on the vitamin D content of the milk. Two groups of cows were kept under good winter conditions which a suplepment of Vitex added during various periods the results of which are shown below.

Period	Group	Rat Units fed to cows daily	Calculated Rat Units per quart of milk
1	1	none	2.76
	2	none	2.76
2	1	6,000	5.31
	2	15,000	11.00
3	1	none	2.98
	2	none	3.08
4	1	40,000	19.81
	2	60,000	30.35
5	1	none	5.53
	2	none	6.02

There was a uniform increase in the potency of the butterfat with increasing doses of vitex fed to the cows, there being an eleven fold increase when 60,000 units were fed. Even this amount is relatively low for a vitamin D fortified milk and does not compare very favorably with the results obtained by feeding irradiated yeast or ergosterol to the cows.

Both irradiated yeast and irradiated ergosterol were used by Thomas and McLeod (1931) as supplements to the basal winter ration of 21 Holstein cows divided into seven experimental groups. One group was continued on the basal ration while three others received daily 10,000, 30,000 and 60,000 rat units respectively of vitamin D in irradiated yeast and the other three a daily supplement of 15,000, 45,000 and 135,000 rat units of vitamin D in irradiated ergosterol, respectively. An assay of the butter oil obtained after four weeks on the experimental rations showed that the milk from the groups receiving irradiated yeast contained two, eight and 16 times the vitamin D potency of the control group, while the groups receiving irradiated ergosterol showed a two, four and 16 fold increase in potency, respectively. These results indicate that the irradiated yeast was used more efficiently in increasing the vitamin D potency of the milk than was the irradiated ergosterol. Hess, Lewis, McLeod, and Thomas (1931) made observation on 102 infants fed 750 cc. daily of milk from groups of cows receiving a good winter ration supplemented with 100,000 and 200,000 rat units of vitamin D in irradiated ergosterol daily, and 30,000 and 60,000 units in irradiated yeast, respectively. The authors stated that of the four milks tested all except the weaker "yeast milk" were found, almost without exception, to prevent as well as cure rickets. The larger amounts of viosterol (irradiated ergosterol) had approximately the same efficacy in regard to both prevention and cure of rickets as the larger yeast milk. The milks were also tested biologically with rats using the standard line test technique. The authors interpreted their results to indicate approximately comparable healing from feeding eight grams of butter fat from the check lot, 0.5 gram from the supplement of 100,000 units of irradiated ergosterol, or 30,000 units of irradiated yeast, and 0.25 gram from 200,000 units of irradiated ergosterol, or 60,000 units of irradiated yeast. Thus the irradiated yeast was three times as effective as the irradiated ergosterol in increasing the vitamin D potency of the milk. This work has been criticized by Russell, Wilcox, Waddell, and Wilson (1934) who pointed out that too high a degree of healing was used in the biological assays to obtain sensitive measurements. They also reported further studies of their own on the relative values of irradiated yeast and irradiated ergosterol in the production of vitamin D milk. They carefully assayed the irradiated yeast and ergosterol used for supplemeting the winter ration, and employed other refinements in technique including the use of hydroquinone in some cases to check the effect of oxidation in the case of ergosterol. Some of their results are indicated below.

Daily Supplement to cows	Steenbock Rat Units per quart Lab. A	Lab. B	Ave.
Steenbock Units		SERIES I	
60,000—irrad. yeast	33	39	35
60,000—irrad. ergosterol	35	43	39
60,000—irrad. ergosterol plus hyrdo-quinone	65	65	65
120,000—irrad. ergosterol	49	65	56
No suplement		Less than 8	
		SERIES II	
120,000—irrad. ergosterol plus hyrdo-quinone	104	——	104
180,000—irrad. ergosterol	142	111	125
180,000—irrad. ergosterol plus hydro-quinone	142	120	230
180,000—irrad. yeast	173	142	156
300,000—irrad. ergosterol	142	142	142

A study of the above results shows that at the 60,000 unit level the irradiated ergosterol is approximately as efficient as the irradiated yeast for increasing the vitamin D potency of the milk. Yeast maintains a greater efficiency at the higher levels so as 180,000 units the relationship of the potencies is on the order of 1.25 to 1 in favor of the yeast. A supplement of 300,000 units of irradiated ergosterol gives a potency per quart about equal to 180,000 of irradiated yeast, or a relationship on the order of two to one on this basis. The greater increase in potency for the irradiated yeast at the higher levels than for the irradiated ergosterol would indicate that the maximum potency obtainable for yeast feeding was not being as closely approached as for the ergosterol feeding. At best, the recovery of the vitamin D in the milk from that fed the cows is not of a high order, as 1.7 per cent was the maximum in this study. There is some evidence of a beneficial effect of the hyroquinone, as an antioxidant especially at the lower levels.

Hess, Light, Frey and Gross (1932) obtained vitamin D potencies of a high order in the milk by feeding excessive doses of either irradiated ergosterol or yeast. Calculated to a quart basis, milk from cows getting 450,000 rat units of vitamin D daily as irradiated yeast showed a potency of 975 units, while milk from a cow getting 1,500,000 rat units of irradiated ergosterol daily contained approximately 1950 units per quart. These potencies are much higher than would have been obtained by Russell, Wilcox, Waddell, and Wilson (1934) assuming that the relationships found at the lower levels used would have prevailed at these higher levels. Hess, Light, Frey, and Gross (1932) also reported data showing that of 100,000 units of vitamin D fed a cow in irradiated yeast, 2.4 per cent was recovered in the 40 pounds of milk excreted, 27.4 per cent in the feces and none in the urine, the remainder presumably being destroyed or possibly stored to a limited extent. These investigators also studied the effect of high and low milk production, and high and low fat percentage on the vitamin D content of milk, and concluded that the lower the production of butterfat the higher the concentration of vitamin D in it, also that the greater the daily production of milk the greater the total vitamin D units excreted in it but the units per quart might be lower. They suggested that for economical production of vitamin D milk the herd, should be fed on the basis of the output of milk.

Light, Wilson and Frey (1934) found that the absorption of vitamin D into the blood begins in from one to two hours after ingestion, that the amount in the blood remains remarkably constant from the second to the fourth hours, that practically 100 per cent of the vitamin D appears in the blood, from where 2 to 3 per cent is excreted in the milk, about 27 per cent in the feces and the remainder destroyed. The milk showed the highest unitage per quart when the blood was richest in vitamin D.

Studies on the relation of such factors as the breed, fat percentage of the milk, individuality of the animal, etc. to the vitamin D potency of normal milk have not been reported. However, Standard Brands Inc. (1934) state from their extensive work relative to the production of milk enriched in vitamin D through the feeding of irradiated yeast to the cows that for the production of milk of a uniform potency the amount of irradiated yeast to be fed depends upon the milk production of the cow, and that "such factors as breed, butterfat, stage of lactation, geographical location, exposure to sunlight and basal ration have not direct effect on the transfer of vitamin D from the ration to the milk, acting indirectly only if they affect the volume of milk produced."

From a consideration of the above evidence it is quite apparent that the vitamin D potency of the milk produced by a cow can be materially increased by the feeding of various supplements rich in this factor.

Breast Feeding vs. Cow's Milk

It is commonly understood as pointed out by McCollum and Simmonds (1925), Hess and Weinstock (1927), Barnes, Cope, Hunscher, and Macy (1934), and many others that rickets is more prevalent in bottle fed than in breast fed infants. Hess and Weinstock (1927) stated that human milk stands preeminent in the prevention of this disorder and studied to determine if this was due to its superiority in the antirachitic factor. Young rats receiving a rickets-producing ration were protected from rickets by a daily supplement of 20-25 cc. of cow's milk, but the same amount of human milk failed to exert any appreciable protective influences, indicating that human milk contains even less of the antirachitic factor than the small amount contained in cow's milk. Various fractions of the milks were tested in an effort to determine which constituents were responsible for the protection afforded but no defiinite conclusions could be drawn.

Outhouse, Macy, and Brekke (1928) have also compared the antirachitic factor in human and cows' milk with about the same results. They employed the curative method using young rachitic rats. Cow's milk is considerably higher in mineral content than human milk and thus the addition of equal amounts of the two milks during the test period would change the calcium/phosphorus ratio more in one case than in the other. Any antirachitic property in the cow's milk would be favored by this shift in the calcium/phosphorus ratio so the authors adjusted the mineral content of the basal diets so that a calcium/phosphorus ratio of five to one could be maintained throughout the rachitogenic and test periods. The human milk was concentrated so that the rats could be given an equivalent of as much as 40 cc. daily during the test period, but even with these refinements there was no indication of antirachitic properties, whereas cow's milk under similar conditions gave definite healing in all cases at a level of 30 cc. daily. Mitchell (1932) and Friedman (1935) have also stated that cow's milk contains relatively more vitamin D than human milk, although a reference to the previous discussion reveals that the concentration of this factor in normal cow's milk is not very appreciable, being on the average from 5 to 10 Steenbock rat units per quart. As will be discussed in more detail later, this concentration of vitamin D in milk is not in itself sufficient to give infants adequate protection from rickets.

Incidentally, it may be of interest to note that goat's milk produced under winter feeding conditions has been found by Steenbock, Hart, Hoppert, and Black (1925) to have antirachitic properties of about the same potency as that of cow's milk produced under similar conditions. In the absence of direct comparisons, one would assume its efficacy for infant feeding to approximate that of normal cow's milk.

Attempts have been made to enhance the antirachitic potency of human milk by the ultra-violet irradiation of the mother, or by the inclusion of some material in the diet carrying vitamin D in a relatively concentrated form. Hess, Weinstock, and Sherman (1927) reported that 25 cc. of mother's milk given daily to rachitic rats for nine days failed to show any healing, whereas the same amount in a similar test after the mother had been irradiated for thirty days marked and even complete healing. Bunker, Harris and Eustis (1933) obtained very similar results by including in the mother's diet 24 ounces of vitaminized milk obtained from cows getting irradiated yeast or ergosterol. Barnes, Cope, Hunscher, and Macy (1934) reported results from feeding a mother daily in connection with a good diet, two quarts of cow's milk fortified with vitamin D by the addition of a cod liver oil concentrate to contain 150 Steenbock rat units per quart. The vitamin D milk feeding continued from the 19th week of pregnancy throughout lactation. Her own baby was protected from rickets during the rachitic season, but the potency of her milk was so low that three rachitic colored infants given 30-32 ounces daily for 11 to 43 days showed no signs of healing. When the same cod liver oil concentrate was given directly to the three colored infants in a cow's milk formula healing was evident in less than a month. Tests on rachitic rats showed only a trace of vitamin D in the mother's milk. A very good review and summary of the literature on the possibility of improving the antirachitic properties of mother's milk by such practices has been given recently by Friedman (1935), in which the results as a whole seem to be more or less favorable.

As cow's milk is higher in mineral matter than human milk and a review of the literature indicates that it is also more potent in vitamin D we have at the present time no adequate explanation for the fact that breast fed infants are less subject to rickets than are bottle fed babies.

Production of Vitamin D Milk

None of the commonly used articles of food are sources of abundant supplies of vitamin D. Nature originally provided the means of automatically taking care of the major portion of our requirements for this factor through the action, on the skin, of the ultra-violet rays of sunlight. However, as the distance from the tropics increases the protection is less efficient and is made even more so by our modern habits of life which require long hours of work in offices and factories in cities where much of the sunshine is cut off by high buildings, smoke, dust, fog, and other factors. Furthermore, the antirachitic rays of the sun are unable to effectively penetrate ordinary window glass and the clothing with which we cover our bodies. The increased use of artificially manufactured and highly refined articles of food has also tended to curtail the supply of vitamin D by eliminating in some cases the small, yet appreciable amounts associated with some of the more natural sources of food articles.

The increased use of such vitamin D carriers as cod liver oil and viosterol has not been able to accomplish the desired end of general rickets prevention, and there seems to be a growing feeling that under our modern mode of life there is a fundamental need for an automatic food source of vitamin D. As milk comprises the major portion of the diet of infants at the time when rickets is the most prevalent and also furnishes liberal quantities of minerals and other valuable nutritive factors it becomes the logical vehicle for supplying vitamin D automatically when and where it is most needed. In response to this situation three different methods of supplying milk with increased antirachitic properties have been developed on a commercial basis. There is abundant evidence of the protective and therapeutic value of such products as will be discussed in more detail later, so that its rise is meeting with increased favor from nutrition specialists, pediatricians, physicians, public health officials, commercial interests, and others concerned with individual and public health problems. From the foregoing, it would seem that vitamin D milk is destined to play an increasingly more prominent role in our nutrition and health problems in the future so the methods of producing vitamin D milk will be briefly discussed. The three common methods used are: **(1) the ultra-violet irradiation of the milk, (2) the addition to the milk of a concentrated vitamin D carrier, and (3) the feeding of concentrated vitamin D carriers to the cows as a supplement to their regular ration.**

Direct Irradiation of Milk.—As soon as it had been demonstrated that certain materials would develop antirachitic properties under the influence of ultra-violet irradiation, many materials were tested to learn the degree to which they might be activated. Because of their high nutritive value and wide usage, milk and dairy products were studied extensively. Steenbock and Daniels (1925) included milk and butter in their early list of activatable materials. Steenbock, Hart, Hoppert and Black (1925) reported a 12 fold increase in antirachitic properties after the irradiation of cow's milk, and a 24 fold increase after irradiating goat's milk. Hess and Weinstock (1925) reported experiments which lead them to believe that milk could be activated to such an extent that the property was evidenced in the behavior of infants receiving the milk; while Kramer (1925) furnished early direct evidence in this country that irradiated milk was effective against rickets in infants. Meanwhile, as Hess and Lewis (1932) have pointed out, similar evidence was accumulating in other countries. However, many scientific and commercial problems had to be solved before a dependable and satisfactory supply of irradiated vitamin D milk could be made available for the public. Supplee and his associates have made large and important contributions to the solution of these problems.

Supplee and Dow (1927a) laid the foundation for the commercial production of irradiated dry milk and noted that this could be done without undue destruction of vitamins A and C, or the impairment of keeping quality, if properly manipulated. The comparative antirachitic properties of irradiated milk and milk derivatives were studied by Supplee, Flanigan, Kahdenberg, and Hess (1931). They found that milk irradiated in fluid form and then dried exhibited marked antirachitic properties somewhat in proportion to the fat content and period of exposure, a few seconds being sufficient. Results of a positive nature were secured from the irradiation of natural milk, butterfat, and a fat-casein-albumin-free, water-soluble fraction, but the same was not true for several fractions of the non-saponifiable portion of the milk fat.

Up to this time there had been no careful study made of such problems as, the proper time of exposure, the effect of different sources of ultra-violet radiations, the energy required for a definite amount of activation, the amount of vitamin D that could be formed, the rate of its formation, the influence of the intensity and character of the radiations, and the many factors affecting the efficiency of the use of energy for activating milk. Supplee and associates carried on a very extensive piece of work to study these and related problems, the results of which have ben reported in a series of papers; Supplee, Dorca sand Hess (1932), Supplee, Hanford, Dorcas, and Beck (1932), and Supplee, Beck, and Dorcas (1932). For these studies separate, 1,000 pound lots of milk were irradiated under 238 different sets of conditions including the use of mercury vapor, and carbon arc lamps, the latter with various types of carbon. The intensity of the radiations was varied by altering the current strength, the use of reflected rays only from various reflectors, and the use of suitable screens. Exposures under these different conditions were made for varying lengths of time. The antirachitic and calcifying properties of the milks thus obtained were observed in biological tests on rats and in some cases by clinical studies on infants.

More recently, Supplee and Dorcas (1934), studied the depth to which ultra-violet radiations penetrated milk films and decided that synthesis of vitamin D takes place at or near the surface. Fat and casein were largely responsible for the opacity of milk to ultra-violet rays but other milk derivatives also absorbed substantial portions. Better results were obtained from short exposures at high intensities with the thicker (0.11-0.23 mm.) rapidly moving films bringing more of the milk to the surface momentarily than with slower, thinner (0.02 mm.) films. Supplee, Flanigan, and Bender (1934) reported that the fat content of the milk influences to a certain, but limited, degree the rate at which milk is activated, and further that although there is an increase in potency with an increased fat percentage, the degree is not proportional to the amount of fat present. Milks of little or no fat content were activated but not as quickly. They stated that milk of normal fat content can be activated to substantially maximum degree by an exposure of less than two seconds if suitable intensity is used with films of suitable thickness and flow characteristics.

Ansbacher and Supplee (1934) have reported a study of the relation of the cholesterol content of milk and various milk derivatives to the antirachitic activation of the milk and its constituents. About 18 per cent of the total milk cholesterol was associated with the milk proteins. This could be activated antirachitically, and lactalbumin after exposure to ultra-violet light showed substantial antirachitic potency.

Steenbock and Wirick (1930) and Hess and Lewis (1932) might be mentioned among others making contributions to the solution of problems associated with the production of irradiated milk, while a very good discussion of the fundamental relations of light to vitamin D and its application to the irradiation of milk has been given by Weckel (1934).

As a result of such studies methods have been developed for the commercial production of irradiated, fluid milk. A machine for irradiating the milk is available which consists essentially of a carbon arc lamp as the source of ultra-violet light, surrounded by a cylinder over the inside of which the milk flows in a very thin film so that all of it is exposed to the action of the ultra-violet rays for a few seconds which is sufficient for the production of appreciable amounts of vitamin D. The method of production and the automatic control devices on the machine are sufficiently sensitive to permit the production of a dependable product of relatively uniform vitamin D content. As commonly marketed at present irradiated milk contains 50 Steenbock units (135 U. S. P. X units) per quart. Under proper conditions this potency can be attained without imparting off-flavors or appreciable destruction of vitamins A and C. Producers of this type of milk must do so under license of the Wisconsin Research Foundation who hold the patent for this process.

Addition of a Cod Liver Oil Concentrate.—Research directed by Theodore F. Zucker of Columbia University resulted in the development of a method for preparing a concentrate of the antirachitically active portion of cod liver oil which was 1000 times more active than the original oil, and as East (1934) pointed out this can be refined so that it may be added to milk and other foods without altering the flavor. It is possible for dairies under license to obtain this concentrate which has been carefully assayed biologically before shipment and use it in the production of vitamin D milk. For this purpose the concentrate is first finely dispersed throughout a small quantity of milk, which is in turn thoroughly mixed with the total volume of milk to be treated. The milk is then pasteurized. As a definite quantity of vitamin D concentrate of known potency is added to a given volume of milk the potency of the final product is fixed in advance. At present, the standard potency of this type of milk is 150 Steenbock (405 U. S. P. X) units per quart. A good general discussion of this process has been given by East (1934).

The patent rights on the process for producing this concentrate were assigned to Columbia University which, in turn, formed an organization to administer it for the public good. The commercial manufacture of the concentrate is under the supervision of this organization, and dairies must be licensed to use it for the production of vitamin D milk.

The Feeding of Vitamin D Concentrates to the Cows.—The experimental work demonstrating the fact that milk can be greatly enriched in vitamin D by supplementing the ration of the cows with materials rich in this substance has already been discussed. Many problems still remained to be solved and many questions left to be answered before a detailed method satisfactory for the production of a vitamin D milk of uniform potency was developed. The Dry Yeast Department of Standard Brands Incorporated, in cooperation with the scientfic workers of experiment stations, physicians, and dairymen has been responsible for the making of many of the commercial adaptations. Up to the present, irradiated dry yeast has been accorded the most favor as the vitamin D concentrate to be fed to the cows. Russell (1932), Frey (1932) and Medical Milk Commissioners, et al. (1932) have discussed some of the early studies on such factors as, the cost of production by yeast feeding, the percentage of vitamin D recovered in the milk, the most efficient method of feeding yeast to produce a milk of the desired potency, the effect of the fat percentage of the milk, and the breed to which the cow belongs, the stage of lactation, the volume of milk produced, and the production of a yeast of uniform potency. A further discussion of the development of standardized feeding methods has been given by Woelffer (1933), who pointed out that the magnitude of the vitamin D secretion is primarly determined by the rate and volume of blood flow through the udder, so the efficiency of the cow in secreting vitamin D depends upon the volume of milk, with no direct relation to the fat content. The method now used for the production of vitamin D milk by the feeding of irradiated yeast has been given briefly by Smith (1934), who stated that the amount of yeast to be fed daily depends upon the volume of milk produced by the cow. The low producers require less yeast per animal, but the high producers are more efficient in transferring the vitamin D from the yeast to the milk. The yeast is fed at each feeding, the simplest way being to mix it with the grain and feed the yeast-grain mixture in proportion to the milk production of the cow. When starting to feed irradiated yeast it requires 15 to 20 days for the milk to reach the required potency. Ordinarily the milk reaches about 80 per cent of its maximum potency by the end of ten days and then increases in potency more slowly for the next five to ten days or more. Once the maximum potency has been reached, the potency will remain relatively constant, provided the yeast feeding is continued in proportion to milk production. It has been found that the ability of the cow to transfer vitamin D from the yeast to the milk does not depend upon the breed, the stage of lactation, or the fat percentage of the milk.

The process of producing vitamin D milk by the yeast feeding method is covered by a U. S. patent issued to Professor Harry Steenbock and assigned by him to the Wisconsin Alumni Research Foundation. As a safeguard to the production of vitamin D milk by this method, dairymen are required to meet certain regulations designed to protect them from dishonest competition and also to assist the health authorities in the proper control of the production and sale of such milk. A producer applying for a license must present a statement from his health officer that he is responsible, in good standing, and qualified to produce the milk under the proper conditions. He must agree to keep records of milk production and the amount of yeast fed, and at the end of each month to render a statement on simple printed forms showing the number of cows in his vitamin D herd, the total production of vitamin D milk, the average production per cow, the amount of yeast fed during the month, and such other information as may be helpful in determining whether the milk produced had the required vitamin D potency. As marketed at present, this type of milk contains 160 Steenbock units (432 U. S. P. X units) per quart.

Regulation and Control.—As previously indicated the production of all three types of vitamin D milk is covered by a U. S. Patent and producers must obtain a license from the proper authority before beginning operations. No unnecessary obstacles should be put in the way of a proper and natural development of the effort to make vitamin D milk generally available to the public, yet on the other hand, adequate safeguards must be employed to prevent the use of unscrupulous methods, unwarranted claims, and misleading statements, and to ensure the marketing of a dependable, safe food product. Unfortunately, the vitamin D potency of milk can be determined only through a biological test as previously outlined, which is time consuming and expensive so that the problem of control differs greatly from that of butterfat and milk solids. For this reason, it is important that only such a number of bioassays, or tests, be made as will give practical and effective control at a minimum cost. It is well to bear in mind that there are fairly effective control features inherent in the three methods now used for the production of vitamin D milk. Nevertheless, the health official should stand between the producer and the public and take such action as will assure the production of a milk of uniform, adequate and effective vitamin D potency. For this purpose, samples of vitamin D milk, as marketed may be collected by the proper official at irregular intervals and without the knowledge of the producer, and subjected to the proper tests. This measure is effective, not only because of any legal penalty, but also because of the possibility that the producer will lose the confidence and good will of the public. Until boards of health are more competent to take over the assaying, the patent holder or authorized assignee may continue to reserve the rights to assay the milk periodically. The producer may asure himself that his product meets the requirements by following closely the prescribed production methods and by arranging with a laboratory equipped for such work to make an assay at convenient intervals. This may often be accomplished through the laboratories maintained by the licensing authorities. For instance, Standard Brands Incorporated offer to make one free bioassay of the vitamin D milk for each licensee as soon as the yeast feeding has been under way long enough so that the milk is supposedly ready for the market.

It may prove helpful to mention that through usuage and common agreements, some of the terms used in connection with milk of enhanced antirachitic properties are taking on somewhat specific meanings. The term "Vitamin D Milk" is commonly used as a general expression in referring to any milk with increased antirachitic value irrespective of the method employed. Milk subjected to direct irradiation has come to be known either as "irradiated milk" or "irradiated vitamin D milk." Milk to which a vitamin D concentrate has been added is sometimes known as "fortified" milk, or as "fortified vitamin D milk." Milk produced by the yeast feeding method is spoken of as "metabolized," or "metabolized vitamin D milk."

Very good general articles covering many phases of the production, use, advantages, and control of the various types of vitamin D milk have been written by Standard Brands Incorporated (1934a), Brannon (1933), Krauss and Bethke (1933), Kon and Booth (1934), Borovsky (1933), Russell (1933), and Friedman (1935).

Efficacy of the Vitamin D Milks for Infants

As one of the important objectives in the use of vitamin D milk is the prevention and cure of rickets in infants, the relative efficiency and dependability with which the various types of vitamin D milks attain this objective becomes a question of utmost importance. The last chapter in the solution of this problem has not yet been written. The fact that the common antirachitics, when used at equal potencies, unit for unit, as determined by biological assay with the rat have not been reported to be equally efficacious for infants in preventing and curing rickets, and also the inherent difficulties involved in making the necessary clinical and laboratory studies on infants under comparable and controlled conditions have contributed greatly to the confusion that exists concerning the efficacy of the various antirachitics and their minimal effective dosages for infants. A review of the more important studies indicates that the issues are gradually being clarified.

One of the first studies reported is that of Hess, Lewis, McLeod, and Thomas (1931) who made observations on milk of four groups of cows fed either irradiated yeast at the rate of 60,000 and 30,000 units daily, or irradiated ergosterol (viosterol) at the rate of 200,000 and 100,000 units daily, respectively. Over 100 infants, divided into four groups, received the milks on both the curative and preventive basis. The authors stated that, "of the four milks tested all except the weaker 'yeast milk' were found almost without exception to prevent as well as cure rickets." Milk from the larger amount of viosterol feeding, 200,000 units daily, had aproximately the same efficacy as that from the larger yeast feeding, 60,000 units daily, although the rat units fed the cows were over three times as many in the case of the viosterol. Biological assays also indicated approximately equal potencies for these two milks, which the authors concluded to be 160 units per liter, while the two weaker milks are given at 80 units per liter. From these and other observations Hess concluded that one liter of yeast milk, 15 cc. of cod liver oil, or 10 drops of viosterol are protective for infants. The Steenbock units in these various amounts are given as 160, 200, and 830 or a ratio effectiveness in the order of 1:1.25:5.20 respectively. Unfortunately, the evidence supporting the indicated potencies of the irradiated yeast and ergosterol fed and that of the resulting milks, is not as complete and well controlled as might be desired. The lack of more adequate supervision and control of the infants and the absence of control groups also limit the definiteness of valid conclusions which may be drawn from these observations. However, it should be remembered that this is an early attempt in this field of work and it at least demonstrated that by feeding irradiated yeast or viosterol, cow's milk could be produced which exhibited market antirachitic properties. In a more carefully controlled investigation, Gerstenberger and Horesh (1932) found two rachitic infants to show slow but definite healing when given milk from cows fed irradiated ergosterol. Calculations from the data presented indicate a daily intake of from 45 to 50 Steenbock units of vitamin D which is somewhat less than the intake suggested by Hess to be effective in either of the two "viosterol milks" discussed above.

Hess and Lewis (1932) next studied milk irradiated under controlled conditions to contain about 50 Steenbock units per quart. The antirachitic properties were tested on 100 infants from the standpoint of both prevention and cure. Of 36 without rickets at the start all but one, a premature infant, remained free from roentgenologic rickets, but the milder forms indicated by craniotabes and beading could not always be warded off. Of 14 infants with moderate rickets as indicated by roentgenograms all showed healing in 4 to 6 weeks. The results with 48 showing only clinical rickets at the beginning were equally gratifying. The authors concluded that milk irradiated in this way proved to be highly satisfactory in the treatment of rickets both from a prophylactic and from a curative point of view. While a direct comparison was not possible the authors felt that this milk was as efficacious as the "yeast milk" used the previous year. (Hess, Lewis, McLeod and Thomas (1931) already reviewed). Another study of irradiated milk by Drake, Tisdall and Brown has been reviewed by Bunker and Harris (1934). Irradiated milk with 56 units per quart was fed to 141 infants during the five winter months in Toronto. Of those under four months of age, 38 received 20 ounces (35 Steenbock units) of this milk daily and 9 received 20-40 ounces (35-70 units), but failed to prevent the development of slight rickets in some cases. Similar groups four to eight months of age were protected and there were some cures of initial rickets in this older group.

In an appraisal of antirachitics in terms of rat and clinical units, Hess and Lewis (1933) compared irradiated milk stated to contain 56 units of vitamin D per quart and "yeast milk" (from feeding irradiated yeast to cows) containing 120 and 80 units per quart respectively, and also included some observations on cod liver oil and viosterol. The irradiated milk was fed at two levels, 24 and 16 ounces daily, to supply 42 and 28 units of vitamin D, while the yeast milks were fed at the rate of 24 ounces daily and thus supplied 90 and 60 units daily, respectively. Hess noted the best healing form the 42 units of irradiated milk and the 90 units of yeast milk and suggested from this a 2 to 1 ratio for the efficacy of the two types of vitamin D milk. However, the lower levels of both types of milk also gave good healing so that in neither case was the minimum effective dose ascertained as the milks were not compared on the same level, nor were minimum effective doses determined. The data obtained scarcely furnish a sufficient basis for the formulation of the conclusion stated by the authors as to the efficacy of the two milks and may be part of the of the reason, at least, why this relationship has not been substantiated by subsequent reports. From this study and other observations Hess summarized his study by suggesting that 35-40 Steenbock units of vitamin D in irradiated milk, 70 in yeast milk, 250 in cod liver oil, and 600-800 in viosterol would constitute a prophylactic dose for infants.

Three important and fairly well controlled investigations comparing the efficacy of irradiated milk and "yeast milk" have been reported since the above work of Hess and Lewis was published. Kramer and Gittleman (1933) used ten infants who showed active rickets, and no spontaneous healing in a preliminary period of 18-26 days, as indicated by roentgenograms, clinical observations and blood chemistry studies. The babies were between three and 22 months of age, were hospitalized, divided into four representative groups, and fed one quart of irradiated, or yeast milk adjusted to contain in each case either 55 or 40 Steenbock units of vitamin D. Each baby acted as its own control during the preliminary period and others in the same environment but not receiving vitamin D milk served as controls during the test periods. All three types of observations mentioned above were used to determine healing and the antirachitic potency was judged by the appearance of roentgenological healing and the time required for healing to be well advanced or complete. In each group healing began in from seven to 31 days and was well advanced in four to six weeks. At the levels fed, on an equal unit basis, there were no significant differences noted between the vitamin D milk perpared by direct irradiation and by feeding the cows irradiated yeast. Another very similar type of investigation has been reported by Wyman, Eley, Bunker, and Harris (1935) in which six rachitic infants were used under carefully controlled conditions. The infants were divided into two similar groups whereupon 26 to 32 ounces of vitamin D milk were substituted for a like amount of the ordinary milk in the diet. The irradiated milk contained 50 Steenbock units per quart and the milk from yeast fed cows 60-65 units as determined by periodic assays. All cases showed rachitic healing and there was no evidence of any difference between the two milks, unit for unit, in respect to their clinical effectiveness. The third report is that of Gerstenberger, Horesh, Van Horn, Krauss and Bethke (1935). The milks were supplied by Krauss and Bethke of the Ohio Experiment Station and repeated assays on both the curative and prophylactic basis showed the irradiated milk to contain 80 Steenbock units of vitamin D per quart and the yeast milk 55. The infants used had been previously observed in a preliminary period and the existence of active rickets established by means of roentgenograms, blood chemistry and phosphatase studies. Each milk was fed at two levels, 720 cc. and 480 cc. respectively, so that observations were made on four groups of infants. Thus the groups getting irradiated milk received about 60 Steenbock units daily (162 U. S. P. X units) and 40 Steen bock (108 U. S. P. X) units respectively while the yeast milk furnished 41 Steenbock (111 U. S. P. X) units and 27.5 Steenbock (74 U. S. P. X) units daily, respectively. Definite healing was obtained in all cases, but at the lower level of 27.5 units of yeast, or metabolized milk, the process was rather prolonged, which indicated that the lower level of effective dosage was being approached. From a study of the time and degree of healing in the various groups the authors concluded that there was for rachitic infants no practical difference in the antirachitic efficacy of the two types of vitamin D milk when the same amount of the antirachitic factor was administered as represented by an identical number of Steenbock rat units daily. These last three reports would seem to give a reasonable basis for concluding that, unit for unit, no appreciable differences in the antirachitic effectiveness of irradiated and yeast, or metabolized, vitamin D milks should be expected.

Two studies of the antirachitic effectiveness of milk fortfied with vitamin D by the addition of a cod liver oil concentrate prepared by the Zucker process have been reported. Barnes (1934) studied 38 infants prophylactically over the winter months. Eleven were under 4 months of age, the remainder were somewhat older ranging up to 12 months. Six had slight roentgenologic rickets in the fall but were cured during the test. Each infant received 50 Steenbock units daily of the Zucker cod liver oil concentrate in milk. Clinical observations and roentgenogram studies showed that none of the 38 developed rickets. As a control group, 25 infants having received no antirachitics, were picked at random from similar surroundings in April of the same year. Of these 56 per cent were rachitic, thus indicating that the antirachitic value of vitamin D milk fortified by the addition of the Zucker cod liver oil concentrate was of somewhat the same order as that of irradiated vitamin D milk. However, the results obtained by Wilson (1934) are not quite so favorable. Observations were made on 33 infants for a period of about four months. Twenty-three were under six weeks of age, when started and eight others under eight weeks. Two thirds were negroes and Italians. As indicated by monthly roentgenograms and clinical examinations, 14 of this group remained normal, 17 developed slight roentgenologic rickets, and two developed moderate rickets. A study of the rather complete set of data collected, indicated that the babies developing slight rickets grew faster than those remaining normal, but they did not receive more milk. The two developing moderate rickets took less milk and grew still faster. Coincident with a later slowing in growth rate there occurred an increase in the number of units of vitamin D taken daily per 100 grams of weekly increase in weight and as a result, healing of the rachitic process took place in these two infants. The milk fed contained 150 Steenbock (405 U. S. P. X) units per quart, so that the daily intake varied from 103 to 136 units depending on the age of the infant. The authors felt that if vitamin D milk was to be the sole source of vitamin D during the most critical stage between one and one-half and eight months, the average amount consumed by babies of this age should contain a sufficient amount for complete protection of all normal infants, but that further study was required to determine the amount required for various ages, growth rates and other such factors. According to their results the vitamin D milk fortfied by the addition of cod liver oil concentrate as commonly marketed at 150 units per quart is not sufficient.

The lack of agreement in these two reports is quite evident. The differences in the initial ages of the infants in the two studies may be one of the many variables that might account for some of the discrepancy. It will be noted that the babies in Wilson's study were younger than those used by Barnes, and it is well understood that on the average the protection of a young rapidly growing infant is a more severe test of an antirachitic substance than is the cure of an older one. In fact, it seems quite probable that the lack of uniform, standard, and well controlled observations for the clinical evaluation of antirachitics is responsible for much of the confusion in regard to their effectiveness and relative merits. This problem has been discussed at some length by Eliot and Powers (1934) who have presented and discussed a proposed set of conditions to meet these requirements, including such variables as the race and age of the infant, time of year, duration of the test period, methods of observation, kinds and amounts of antirachitics used, other foods permitted, outdoor exercise, number of infants, accurate measurements of infants, complete records of food consumption and other such items, taken under the direction of trained pediatricians, and supplemented with adequate follow-up work. It is hoped that before long all the common antirachitics will be tested simultaneously under a set of standardized and well controlled conditions so that their relative efficacies may be more definitely and adequately determined.

Bunker and Harris (1934) studied the clinical status of vitamin D milks and summarized the literature on the question up to the time of publishing their article. They state, "In appraisal of the available evidence concerning the value of vitamin D milks, in respect to their antirachitic effect when fed to infants, it is our opinion that yeast milk and irradiated milk, as today obtainable on the market, have both been proved to be valuable in curing and in preventing rickets, when used in the amounts and in the manner usually indicated for milk in infant feeding; that the more carefully controlled experiments fail to show any distinct difference between the relative efficacies of the two types of milk mentioned, in terms of rat unit equivalent potencies, and that in view of this fact it is reasonable to expect more antirachitic benefits from that type of milk which offers the larger unitage per quart.

"Evidence is still needed concerning the apparent unusual requirement of the very young infant in respect to antirachitics; concerning the minimum antirachitic potency of milk required to protect surely all infants against rickets; concerning the potencies required, not only to protect infants or to cure rickets, but to insure the most desirable rapidity of the healing process."

Stability of Vitamin D in Milk

Fortunately, vitamin D is a relatively stable product so that in the ordinary processes of handling and preparing vitamin D milk for consumption little, or no, destruction of its antirachitic potency need be lost. Wyman and Butler (1932) found that vitamin D milk obtained from cows receiving irradiated yeast was still effective in curing rickets in three infants after pasteurization and for a fourth following boiling for five minutes in addition to the pasteurization. More definite evidence has been supplied by Krauss, Erb, and Washburn (1933) who, tested samples of butterfat and cream obtained from whole milk before and after pasteurization. Line tests on the cream and butterfat at various levels, showed no difference in the antirachitic potency following pasteurization at 145 degrees F. for 30 minutes. Bone ash determinations on the same samples of butterfat substantiated these observations as did also line tests on cream of high antirachitic potency obtained from cows receiving irradiated yeast. While testing the antirachitic potency of cow's milk as influenced by various factors, Steenbock, Hart, Riising, Hoppert, et al. (1930) tested the same milk raw and after heating at autoclaving temperatures for periods of 30 and 120 minutes and found only slight evidence of a decrease in one case and none in the other. Honeywell, Dutcher and Dahle (1927) found evaporated milk to be lowered somewhat in calcifying potency by commercial methods of evaporation and sterilization.

Ordinarily, fluid milk is used in a comparatively short time so there would be little opportunity for deterioration of the antirachitic factor during storage. Furthermore, vitamin D has been found to be quite stable under favorable storage conditions. Steenbock and Wirick (1930) stored irradiated butterfat from July until the following March at room and refrigerator temperatures and found the antirachitic factor to be stable to such storage conditions. Clouse (1932) pointed out that irradiated dried milk kept for six months in a cupboard under ordinary conditions lost but little of its potency, and even after a year it had deteriorated only moderately. Frey in the discussion following the meeting of the Medical Milk Commission (1932) reported limited experience with one or two samples of vitamin D milk received for assay in a sour condition in which the activity was found to be still satisfactory.

From a consideration of the above information and the practices commonly observed where vitamin D milk is being used effectively, it would seem that there is little probability of any appreciable destruction of the antirachitic properties of vitamin D milks when ordinary care is used in following accepted practices in the handling and preparation of such milks for use.

Summary

Briefly, attention has been called to the importance of milk in the adequate nutrition of children and adults, especially because it contains a generous amount of calcium and phosphorus which gives added significance to its content of the antirachitic factor. The vitamin D present is usually considered to be concentrated in the fat fraction and can be measured by means of biological tests requiring the use of white rats. These tests have been described and the common units used for expressing the potency have been defined. As milk is one of the few naturally occurring foods containing appreciable amounts of vitamin D and is also an important constituent of the diet of infants at the time when they are most susceptible to the development of rickets, our knowledge of the factors influencing the concentration of vitamin D, the antirachitic factor, has been reviewed.

Cow's milk produced under essentially normal conditions has been found to contain small, yet appreciable amounts of vitamin D varying from five, or less, to about fifteen Steenbock units per quart. Milk produced under summer pasture conditions is ordinarily higher in antirachitic value than milk produced under typical winter feeding conditions, the former usually having a potency in the upper half of the indicated range while the latter is usually in the lower half. Differences in the amount of sunshine received by the cow and in the amount of vitamin D in the ration consumed undoubtedly have a part to play in influencing the relative potencies of summer and winter milk but the exact influence of each can not be stated at the present time. Neither is it known to what extent the past history of the animal with respect to its intake of vitamin D may influence the concentration of this factor in the milk. The possibilities of enhancing the vitamin D content of milk by irradiating the cow with ultra-violet light have been investigated but the results are not in agreement. Some workers have reported measurable increases while others have been able to demonstrate any positive effects. The feeding of vitamin D concentrates to cows in sufficiently large amounts has resulted, uniformly, in increasing the content of this factor in the milk. Irradiated yeast is the most favored material for this purpose at the present time and seems to be somewhat more efficient for the transfer of vitamin D to the milk than does irradiated ergosterol, especially at the higher levels. Cod liver oil depresses the fat percentage when used for this purpose.

Although breast fed babies are less subject to rickets than bottle fed babies, investigators have been unable to demonstrate appreciable amounts of vitamin D in breast milk, it being inferior to cow's milk in this respect. The reason for its superior antirachitic properties is not known. On the whole, the ultra-violet irradiation of the mother or the

inclusion of vitamin D concentrates in her diet seems to have some beneficial effects on the antirachitic potency of her milk.

Because of the widespread occurrence of rickets among infants even in our present age there seems to be a need for an automatic food source of vitamin D and milk seems to be the logical vehicle for this purpose. Three types of vitamin D milk have been produced and are finding their way to our markets in increasing amounts. One type, irradiated vitamin D milk, is produced by directly irradiating the fluid milk so that it contains 50 Steenbock (135 U. S. P. X) units per quart. Another is fortified vitamin D milk, produced by adding a cod liver oil concentrate to the milk in such amounts as to give it a potency of 150 Steenbock (405 U. S. P. X) units per quart. The third is called metabolized vitamin D milk and is produced by feeding irradiated yeast to the cows in such amounts as to produce 160 Steenbock (432 U. S. P. X) units of vitamin D per quart. All three types of vitamin D milks are produced under the license of the proper authority and control measures are practiced to insure a dependable product of proper potency.

The value of all three types of vitamin D milk in preventing and curing rickets has been demonstrated. Although considerable confusion still exists and our information is not as definite in many particulars as we might desire, there seems to be a reasonable amount of evidence to indicate that, unit for unit, the vitamin D potency of irradiated milk and of yeast, or metabolized, milk are approximately equally efficacious for the control of rickets in infants. Information as to the effectiveness of vitamin D milk fortified with a cod liver oil concentrate is somewhat limited but indicates the possibilities of results of somewhat comparable nature. There are numerous instances of the prevention and cure of rickets on milk intakes sufficient to furnish 40 to 50 Steenbock (108-135 U. S. P. X) units per day, which may indicate the approximate lower limits of effectiveness on the average, with the strong probability that somewhat larger amounts will be necessary for the complete protection of the younger, more rapidly growing infant.

Fortunately, vitamin D is a relatively stable product and there is little likelihood of the deterioration during the short holding period before fluid milk is consumed, or in the destruction of appreciable amounts by the common methods of processing this product for consumption.

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Appendix

Appendix Table 1.—Average monthly weight of the four calves in the sunlight and no-sunlight groups, compared with normal weight of calves.

Age	Normal Weight	Av. Wt. Sunlight Group	Av. Wt. No-Sunlight Group	Date
Months	lbs.	lbs.	lbs.	
1	121	108	110	July 20, 1927
2	157	133	135	Aug.
3	200	183	185	Sept.
4	249	242	248	Oct.
5	302	302	308	Nov.
6	349	361	360	Dec.
7	389	407	415	Jan. 1928
8	425	445	475	Feb.
9	466	506	521	Mar.
10	501	544	586	Apr.
11	529	580	652	May
12	558	654	690	June
13	574	669	715	July
14	596	691	750	Aug.
15	612	729	806	Sept.
16	643	775	816	Oct.
17	660	826	906	Nov.
18	686	873	958	Dec.
19	715	937	993	Jan. 1929
20	746	938	1020	Feb.
21	774	982	1062	Mar.
22	796	1015	1092	Apr.
23	824	1052	1135	May
24	841	1097	1176	June
25	869	1117	1198	July
26	893	1136	1232	Aug.
27	925	1134	1251	Sept.
28	966	1151	1308	Oct.
29	994	1172	1347	Nov.
30	1021	1192	1391	Dec.

Appendix Table 2.—Average monthly height at withers of the four calves in the sunlight and no-sunlight groups compared with normal height of calves.

Age	Normal Height	Av. Ht. Sunlight Group	Av. Ht. No-Sunlight Group	Date
Months	Inches	Inches	Inches	
1	30.2	29.9	29.8	July, 1927
2	32.3	31.6	31.9	Aug.
3	34.2	32.8	33.2	Sept.
4	36.2	34.7	36.1	Oct.
5	38.0	36.6	37.8	Nov.
6	39.7	38.9	40.1	Dec.
7	40.9	40.6	41.5	Jan. 1928
8	42.2	42.0	42.7	Feb.
9	42.9	43.1	43.2	Mar.
10	43.8	43.8	45.1	Apr.
11	44.3	45.1	45.8	May
12	44.8	46.0	46.8	June
13	45.6	46.5	47.4	July
14	46.2	47.0	48.6	Aug.
15	46.8	47.6	48.8	Sept.
16	47.4	48.3	49.5	Oct.
17	47.7	48.9	49.8	Nov.
18	47.9	49.3	50.3	Dec.
19	48.3	49.6	50.4	Jan. 1929
20	48.7	50.4	50.6	Feb.
21	48.9	50.4	51.0	Mar.
22	49.2	50.4	51.4	Apr.
23	49.5	50.8	51.8	May
24	49.8	51.2	52.7	June
25	50.2	51.4	52.8	July
26	50.5	51.4	52.8	Aug.
27	50.9	51.5	52.8	Sept.
28	51.1	51.8	53.0	Oct.
29	51.3	52.1	53.4	Nov.
30	51.5	52.1	53.8	Dec.

Appendix Table 3.—Results of Feeding Trial with Pigs.

	Lot I Sunshine Milk	Lot II No-Sunshine Milk	Lot III Cod liver Oil
Number of pigs	[illegible]	7	[illegible]
Number of days fed	142	142	142
Average initial weight per pig-lbs.	63.6	65.8	60.0
Average final weight per pig-lbs.	218.8	184.7	218.0
Total gain per pig-lbs.	155.2	118.9	158.0
Average daily gain per pig-lbs.	1.09	.84	1.11
Total milk consumed per pig-lbs.	905	905	-----
Average milk consumed per pig daily-lbs.	6.37	6.37	-----
Total basal ration consumed per pig-lbs.	487.2	387.4	528.5
Total mineral consumed per pig-lbs.	4.8	2.9	5.5
Basal ration consumed per 100 lbs. gain lbs.	313.9	352.8	344.4
Mineral consumed per 100 lbs. gain-lbs.	3.09	2.44	3.48
Milk consumed per 100 lbs. gain-lbs.	583.1	761.1	-----
* T. D. N. consumed per 100 lbs. gain lbs.	342.2	383.4	267.3

* T. D. N.—Total Digestible Nutrients.