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A DISCUSSION OF THE SOURCES OF VITAMIN D

A THESIS

PRESENTED TO THE FACULTY OF
THE COLLEGE OF MEDICINE OF
THE UNIVERSITY OF NEBRASKA
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REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF MEDICINE

WILLIAM Y. BAKER

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A DISCUSSION OF THE SOURCES OF VITAMIN D

Within the last few years medical literature has been flooded with articles dealing with the various sources of the antirachitic vitamin, their relative merits, deficiencies, and comparative values. Many of these reports are conflicting and at the end leave the reader in a maze and at sea as to the actual state of our knowledge of the subject.

Many investigators have based their opinions and conclusions upon data obtained from laboratory animals, while others have shown that these views are not applicable to the human patient in all phases. In the final analysis the prophylactic or therapeutic value of any agent must be determined by its action upon the human patient. There is no question but that we owe a great debt to the rat, chick, and dog for the rapid advancement in this field, but before any set standard of therapeutic dosage can be made or a rule of pharmaceutical action can be laid down, clinical evidence of the agent in question must be obtained and understood.

Quoting from the J. A. M. A. (1930) editorial, we find it stated, "At present the pathogenesis of rickets seems to be fairly well established and defined; few of the advances in the science of nutrition have been marked with as striking an outcome as has been the case with this disease. The relationship of the inorganic constituents of the diet, the specific effect of codliver oil, the curative effect of natural and artificial ultra-violet light, and the activation of ergosterol to anti-rachitic potency are milestones along the road of Progress, elucidating the etiology and biochemical relationship of rickets.

The novelty of producing a therapeutic agent by photochemical means, together with the tremendous potency of viosterol, has led to the undue canonization of the material by the physician. Without detracting from its merited value in the treatment of rickets, certain observations raise the question as to the simplicity of the pathogenesis of rickets implied in the current facile use of viosterol. If there exists a complacent belief among some investigators that rickets is due to absence of Vit. D alone, there are others who do not accept such a simple relationship."

Vitamin D, although it is the fourth of the essential food factors to be discovered, has far surpassed its fellows A, B, and C in the amount and extent of research, clinical use, and exploitation it has created in the eleven years it has been recognized. The knowledge of this vitamin has developed along two lines which converged in 1925 with Hess's demonstration of activation of the skin by sunlight. In 1918-1919 Dr. E. Mellanby, working with dogs and various diets for rickets, found that relatively small doses of codliver oil protected his puppies from the disease. His work was extensive and thorough, but in his conclusions he gave Vit. A., which was a known constituent of codliver oil, the credit for the antirachitic powers. The use of codliver oil in rickets was by no means new. The Norsemen and people of the northern fishing countries for years had used the various fish oils for diseases of the bone and growth, according to Schlutz ('32), and the Italian and Greek physicians also noted that the poor classes, who took the various drugs administered in a vehicle of codliver oil, suffered less from rickets than the richer class, who used almond oils as vehicles.

Mellanby's (1918) explanation failed to satisfy American investigators and was sharply refuted when Hess (1922) of New York City showed that babies fed on high cream diets, hence Vit. A, were more prone to rickets than babies who were fed skimmed milk. This field of investigation grew, and soon McCollum (1922) of Johns Hopkins proved the coexistence of a new factor in codliver oil by oxidizing the Vit. A with steam, proving its absence experimentally, and then curing rickets with the remaining codliver oil. This factor was called X and later Vit. D.

The second path of development started about the same time (1919) with the work of Huld~~s~~chinsky of Berlin. This man noted the prevalence of rickets in the children of the war generation. By use of the quartz mercury vapor lamp irradiation, he showed immediate improvement in all of his cases. From this he went to the use of sunlight and found the same thing to be true, both for prophylaxis and cure of the disease.

These two lines developed and were hard to correlate. Both cured rickets; both prevented the disease and yet were of totally different sources. The answer to the confusion was given by Hess (1924) and Steinbock (1924) at almost the same time. Hess found that irradiated cotton seed oil was actively antirachitic, and Steinbock prevented rickets in rats on a rickogenic diet by irradiation of the diet prior to feeding. Only one conclusion could be drawn, that irradiation produces or activates some provitamin to produce the active Vit. D. What the substance was remained in question. Cholesterol was the first source (Rosenheim, 1925), and then on purification a higher alcohol, ergosterol, which had been considered an impurity, was found to be the provitamin (Hess, 1927).

Prior to this time ergosterol had been given no biological significance, but later work showed that it was present in minute quantities in nearly all animal and many plant tissues. Ergot and yeast are the richest sources, and are at present the source of commercial for the material. With the discovery by Hess of ergosterol in the skin, the two lines of development join. The ultra-violet rays activate this provitamin in the skin and it is then carried by the blood to the tissues. This is supported by still other evidence. Hume, Lucas, Smith, and Blunt ('31) prevented rickets in rats by applying irradiated ergosterol to the skin and excluding all sunlight. More recent work by Hart and Steinbock, in which the Vit. D content of eggs was increased markedly by irradiation of the hens (Schlutz, '32) proves the activation of ergosterol in the living tissue.

With this background of the development of our knowledge of the presence of Vit. D, let us consider briefly its physiological action in the body. As clearly shown by the conditions resulting from the lack of Vit. D and by the remarkable action it exerts when supplied to deficient organisms, Vit. D plays a major role in the mineral metabolism of the body. Exactly where Vit. D fits into the picture is by no means clearly understood; but in a broad sense we know that Vit. D has to do first with deposition of calcium and phosphorus in the bones, second with the concentration of calcium and phosphorus in the blood, and third with the retention of calcium and phosphorus in the body. The second and third actions are questioned by some investigators. Brown and Shole ('30) attach more importance to the calcium and phosphorus content of the diet as the factor regulating the blood content and body retention of these minerals than to the action of Vit. D, which they believe has to do only with the calcification

of bone. Schlutz ('32) states, "The impression prevails in some quarters that if enough Vit. D is given and an adequate amount of light provided, the quantity and proportion of calcium and phosphorus becomes of little relative importance. It has been definitely shown that increasing the quantity of calcium and phosphorus in the diet will increase the storage of these elements in both infants and adults.....A good ratio is essential for a good diet, 2 to 1 or 1 to 1 calcium/phosphorus, if in an alkaline medium, has proven the best in animal organisms. The types of foods that make up the child's dietary have considerable influence on calcium and phosphorus retention. Food and food mixtures vary greatly in the readiness in which they give up their calcium and phosphorus to the body. This can be largely overcome by irradiation with ultra-violet light or addition of sufficient Vit. D to the ration. Some sugars such as lactose elevate calcium retention when taken in large amounts."

Clous ('32) on the other hand states, "Unquestionably one of the effects of small or moderate amounts of the antirachitic factor is to increase the amount of calcium and phosphorus retained in the body." Most of this dietary work has been carried out in Germany even prior to the discovery of Vit. D, and it was shown that the rachitic infant stores much less calcium and phosphorus than the normal infant, also that codliver oil increased this retention. Orr and others have more recently shown that ultra-violet light and other antirachitic agents produce the same retention.

This same phenomenon is found in the nonrachitic patient, i. e., on the administration of an antirachitic agent, the calcium and phosphorus retention is increased, but the mechanism

of this has not been satisfactorily explained. Whether this mechanism be increased absorption, decreased secretion, or increased utilization is not definitely known. Clous ('32) favors the theory of increased absorption because of the fact that in rachitic patients fecal calcium is high and urinary calcium is low. Upon administration of Vit. D the fecal calcium drops and urinary calcium rises. The work of Workany ('27) also seems to bear out the increased absorption theory. He found, in a series of carefully controlled experiments, that following the ingestion of a given amount of phosphorus the blood content of this element was doubled or trebled over the normal if Vit. D had been given prior to the ingestion.

Jones and Rapaport ('31) repeated Workany's work, using calcium, and obtained similar results. Unfortunately, however, neither these men nor Workany measured the fecal or urinary output carefully, although they did show that urinary excretions of calcium and phosphorus were markedly increased with administration of Vit. D.

Regardless of the exact action of the element, Vit. D has been shown to be a necessary factor. The question now arises and is often asked by both parent and physician, "How can this essential element be best supplied?"

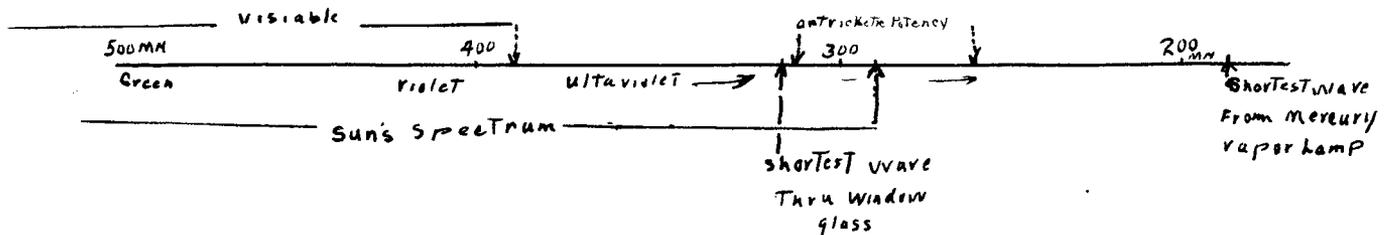
According to present day information and present knowledge, the natural distribution of Vit. D is rather limited. Certain foods and medical substances in common use contain it in considerable amounts. The commonest of these sources are sunlight, ultra-violet light, fish oils, egg yolk, irradiated ergosterol, and irradiated foods, which we shall now discuss in the order given.

Sunlight

The greatest natural source of Vit. D is in the action of the ultra-violet rays of sunlight upon the skin. The history of this source of the essential element has been briefly outlined in the preceding pages. Let us now consider the application, the advantages, and the disadvantages of this source of Vit. D. To do this let us review a few significant points in the physics of light (Blunt, '31). First, as to the spectrum. Sunlight passing through a quartz prism is broken up into the familiar color spectrum made up of radiations of different wave lengths. Only part of this spectrum is visible. The visible part ranges from red to violet and consists of radiations measuring from about 800 millimicrons at the red end to about 390 millimicrons at the violet end. The longer rays, i. e., above 800 millimicrons, are invisible and compose the heat, sound, and electrical waves, while the waves shorter than 390 millimicrons are evidenced by their action upon photographic plates as the ultra-violet rays and X-rays. These are the chemical or actinic rays. Laurens ('28) states, "Solar radiation as it reaches the earth extends from 290 millimicrons to 5 microns, although there is very little energy longer than 2 microns, and is often described as extending from .3 microns to 3 microns. On entering the earth's atmosphere 5% of the total radiation is ultra-violet, 52% visible, and 43% infra-red. Owing to extinction and absorption, by the time the radiations reach the earth's surface the relative amounts have changed, and at average heights of the sun, with a total radiation intensity of 1.0 cal. per sq. cm., the distribution is: ultra-violet, 1%; visible, 40%; and infra-red, 59%. The total intensity is primarily dependent upon height above sea level, as well as

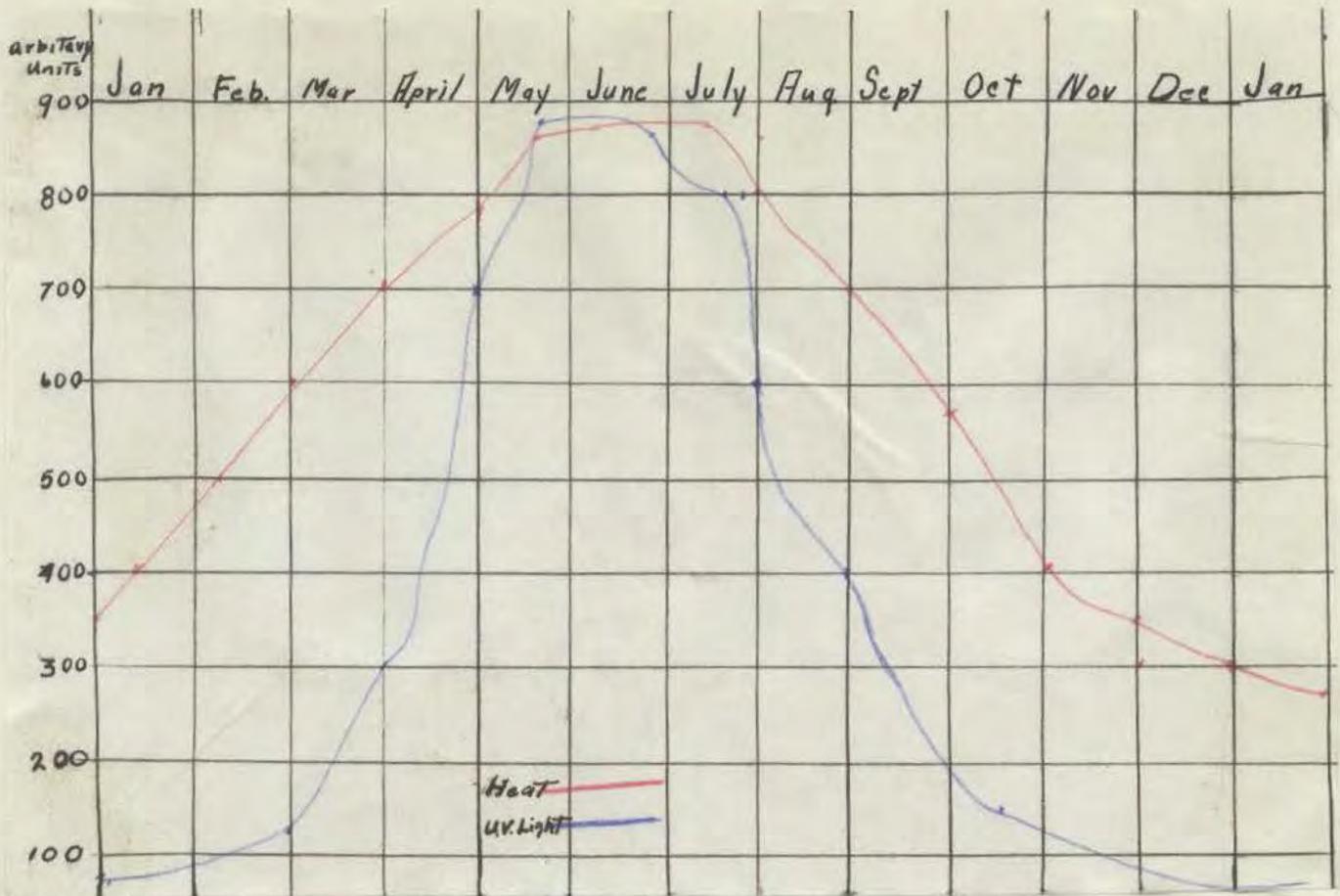
upon seasonal and daily variation, which particularly influence the ultra-violet. Abbot has shown that only 75% of the total solar radiation (the solar constant) reaches a level of 1800 meters and only 50% to sea level. Spring sunlight is the strongest and richest in infra-red, the autumn sun by comparison being richer in ultra-violet, the maximum content of which is reached in the summer."

Blunt and Cowan ('31) also present the following table, which summarizes these facts.



The question of wave length of light is only part of the problem. To know the value of a given source of light, we must also know its intensity. The intensity of the ultra-violet of the sun's radiation varies with time of day, season, local atmospheric conditions (i. e., fog, smoke, or dust) and altitude even to greater extent than do the longer light or heat waves. Hess and Lundagem ('22) have charted the seasonal variation of solar heat and ultra-violet radiation of a given locality. This chart is given on the following page.

The effect of altitude upon the concentration is due to the greater absorbability of the ultra-violet rays. Measurements at sea level, intermediate point, and on a mountain top, say at 100, 5000, and 10,000 feet, gives a ratio of 40-61-91, or over twice the ultra-violet at 10,000 feet as at sea level. This



is also intensified by the reflection of the ultra-violet by the snow upon the mountain peak, for snow reflects about 90% of the light falling upon it. This explains the success of the Swiss sanitariums and the marked cases of "glacier burn" acquired in mountain climbing.

Latitude also is an important factor. In the tropics the direct rays of the sun are of greater ultra-violet potency than in the temperate zones. Local atmospheric conditions also play an important role, often cutting the ultra-violet content of tropical sunlight below that found in temperate climates, but in the equatorial belt sunlight alone is sufficient to prevent rickets (Blunt, '31). This phase has been studied carefully, especially in Chicago, Toronto, New York, and Washington. The Chicago investigators, Dr. Bunderson ('27), Lemon, and others have shown that the winter sunlight is not sufficient to prevent

rickets and that the ultra-violet content is still further reduced by the smoke and soot from the manufacturing districts. Measurements elsewhere have reported a 50% reduction of ultra-violet light in the city compared with the adjacent country (Baltimore, '29). Washington and Toronto found that the natural sunshine there had antirachitic properties for rats. The Boston chickens were protected, although Hess of New York does not report satisfactory results in children treated by heliotherapy.

Trusdall and Brown ('29) made an interesting study of the relation of the sun's altitude and rickets. They report as follows, "Rickets occurs today largely in Europe and North America between the latitudes of 40° and 60° . The minimal seasonal altitude of the sun at 40° north is 26° . In Glasgow, Scotland, the minimum seasonal altitude is 11° , and for six months of the year the altitude is below 35° ." These men summarize their work as follows:

1. "A marked increase occurs in the antirachitic effect of sunshine when the sun reaches 35° or more.
2. "A study of the geographic distribution of rickets shows that rickets is uncommon in or exists in a mild form only in those places where the minimal seasonal altitude of the sun is above 35° .
3. "Conversely, severe rickets is chiefly encountered in those cities where the altitude of the sun is below 35° for some months of the year.
4. "The period of the year during which rickets will probably develop can be calculated for any city in the world. The duration of this period may be altered, however, by the of exposure of patients to highly effective sunshine on account of inclement spring weather or other factors."

Measurements of the ultra-violet content of light of any source is relatively simple. Dr. Janet H. Clark ('24) has devised a method which though rough is sufficient for general use. Chemically pure zinc sulphide is made into a paste with a saturated solution of lead acetate and exposed to the light in question until the color matches a standard. This is taken as "one zinc sulphide unit of ultra-violet energy." This same method is used in the "lithropone units" determination. Lithropone is a photosensitive paint containing zinc sulphide. Both are based upon the sensitivity of zinc sulphide to light of a wave length between 350 and 310 millimicrons.

Ultra-Violet Light

While upon the subject of ultra-violet radiation, we shall discuss the use of the quartz mercury lamp or carbon arc as a source of the ultra-violet energy when used in direct irradiation of the skin. As mentioned in the opening pages, Huldshinsky ('19), Berlin, was the first to use this source of light in healing of rickets. His lead was taken up by investigators all over the world, and today the literature available upon this subject has become very voluminous. Briefly, however, both carbon arcs and mercury arcs give off shorter radiations than are contained in the solar spectrum, and the short radiations are much more intense, that is, the lamps give far more powerful ultra-violet rays than does sunshine. Sharp comparison of them with sunlight is not easy, however, especially for the mercury arc, which emits a discontinuous spectrum in contrast with the sun's continuous one. Again the intensity of all lamps varies with the strength and type of current employed. The shortest wave length from the quartz mercury vapor lamp is 185

millimicrons, from the white flame carbon 218 millimicrons, and as forestated, in sunlight 290 millimicrons is the shortest.

Bunderson et al. ('27) state that in their work with incandescent lamps, "Incandescent lamps (of 300 watts) emit radiation in the ultra-violet region which are of physiologic interest, but do not place them in the class with carbon arc or mercury vapor lamps." Coblentz ('29) did not find this true in some lamps he tested.

As with all other notable advancements in science, the "fad" stage is now on with the ultra-violet lamps and actinotherapy. The market is overrun with sunlamps and apparatus for providing one and all with sunshine and a coat of tan without leaving the privacy of one's bedroom. How far can this go? What are the limitations and dangers? E. E. Free ('29) writes, "Fortunately many of the alleged 'sunlamps' and 'raylamps' of dozens of kinds and titles now being hurried out on the American market will damage nothing but the purchaser's pocketbook. This is especially true of the cheapest ones." This sums up the subject very well.

The therapeutic value of the short light waves is by no means limited to rickets and related diseases; these rays have bactericidal, stimulating, and tissue-building effects not to be discussed here. Whereas the sunlight has these same properties when available, the lamps provide a controllable, available source of the agent for its use in many fields.

What are the effects of over-irradiation? This question has been ably answered by Laurens ('28). In his reviews of all articles on the effect of irradiation, his conclusions are:

1. "The first reaction is an erythema of the skin which

may go on to blistering if continued. This erythema is followed by a secondary erythema due to the action upon the blood vessels by the ultra-violet rays, causing a dilation and atonia. This is thought to be due to the release or activation of a histamine-like substance which is followed by a dilatation of the vessels and subsequent drop of blood pressure (8 to 30 millimeters of mercury in most cases). After this secondary erythema pigmentation takes place in all but red-headed individuals. This pigment is formed by the action of the rays longer than 290 millimicrons because those shorter than 290 are absorbed before reaching the basal layer of the skin where pigmentation takes place. This pigment protects the skin from the action of the longer rays, but in no way prevents the action of the short waves, as they are absorbed in the outer layers of the skin."

2. Effect on the eye. "These are of great interest from a pathological point of view, since inflammation and injury to the conjunctiva, cornea, and lense, as well as to the retinae, may take place when the eye is exposed to strong radiation from arc lamps, molten glass, light reflected from snow, water, or ice, etc., unless protective glasses are used.

"Ultra-violet of wave lengths less than 295 millimicrons produces severe conjunctivitis and if long continued corneal ulcers. Sunlight ordinarily is harmless, but when increased by reflection from large areas of water or snow produces inflammation and blindness. Artificial illuminants which emit a large amount of radiation of wave lengths less than 295 millimicrons, such as the quartz mercury and carbon arcs, are extremely injurious and the eyes should be carefully protected."

3. Effects on general metabolism. "Although there is a general belief that sunlight is beneficial, observation has

suggested the possibility that man and animals can live in darkness for a relatively long period of time without serious functional disturbance. The statement that light increases metabolism is insufficiently supported by experimental data."

4. Effect on the nervous system. "Some mention should be made of the action on the nervous system with particular reference to the psychic influences, since sunlight is unquestionably one of the various factors having to do with the sensations of bodily and mental well being. Striking results have also been reported following irradiation by mercury and carbon arc lamps. Even after a dose so mild that it does not produce erythema, there is a feeling of exhilaration expressing itself in joy of work and of living. Hausmann describes feeling as if he had been on a mountain trip. Hasselback describes his sensation following a two-hour irradiation with a strong carbon arc lamp producing a marked erythema, 'Instead of the usual evening fatigue with frequent yawning, he felt lively and indefatigable. The feeling of exhilaration and of desire and ability to work usually lasted two days, the "light mania" being followed by a deeper depression than usual.' Hasselback suggests that "light mania" may be a kind of immunity against depression. The action on the nervous system is evidently indirect and depends upon improvement in circulatory and metabolic conditions.

"The effect of an overdose of radiation, well-known and personally familiar to most of us, is accompanied by feelings of unrest, more or less vague apprehensiveness, sleeplessness, and so forth. In many people these symptoms appear long before the erythema."

Priese ('31) points out that in these cases, antirachitic

treatment should not be commenced too abruptly. The metabolic balance in rickets is very labile and too vigorous institution of antirachitic therapy may cause alkalosis and produce active tetany. Ultra-violet frequently produces alkalosis and tetany, while viosterol is least dangerous from this standpoint.

The British Medical Research Council in its report for 1927 and 1928 issues a very adverse criticism to the use of ultra-violet light in therapy of any kind, and bases its work on a group of experiments conducted under its direction. Of this criticism Blunt and Cowan ('31) make this statement, "On the other hand report after report of carefully controlled experiments seem favorable. Caution is still necessary; much of the use is still largely experimental, but a useful method does seem available."

Peyrer ('31), in considering the use of ultra-violet light in comparison with other forms of antirachitic treatments, states that he believes that mothers bring their children more faithfully for ultra-violet radiation than for internal medication. In his opinion sunlight comes first in order of importance as a preventive measure against rickets. Next in importance he places ultra-violet radiation and lastly substances to be taken internally.

Fish Oils

The second most common sources of Vit. D are oils from fish livers. Bills ('27), in a study of twenty-eight varieties of animal and vegetable oils to determine their antirachitic properties by use of the line test, found the following oils were the most active. In the following list the average activity of Newfoundland medicinal oil was used as 100 units for comparison:

Halibut	2000
Puffer fish liver	1500
Codliver, Newfoundland (500 samples)	100
Goosfish liver, Boston	100
Herring, Newfoundland	100
Sardine, California	100
Shark liver	75
Muddy catfish	45
Coalfish	40
Channel catfish, visceral fat	6
Seal blubber	0
Salmon trimmings	20
Commercial hydrogenated codliver oil	0

Bills ('27) concludes this study with the following facts: Vit. D occurs in the livers of many species of fish, but the quantity present varies widely for different species.

How the fish livers obtain their high potency is not definitely known, but Bills ('28) and Hess ('32) maintain that it is by symphysis in the cod, although all efforts to prove this have not been conclusive.

Although many other fish oils, as shown, contain Vit. D, codliver oil is the one in common use. The first record of its use for ailments of bone and joints is found in Dr. Thomas Percival's (1798) Medical Essay (Vol. II, 1798, p. 354), in which he states it was used by Dr. Keys in the Manchester Infirmary from 1752 to 1784.

As with all other natural products, codliver oil varies greatly, depending upon source, season, method of extraction, and method of storage. Drummond and Hilditch ('32) in their extensive work on comparison of codliver oils state, "It is obvious that there is wide variation in the medicinal value of

codliver oil expressed in the terms of Vit. A and D content, and it is apparent that it is advantageous to choose an oil of high vitamin value for use in the treatment of human malnutrition. At the present moment, a considerable proportion of the codliver oil sold in this country (England) is relatively low in medicinal value--largely because the pharmaceutical trade has a preference for pale oils mainly of Norwegian origin." As a method of determining the medicinal value, i. e., the vitamin content, these men suggest a standard. "We are of the opinion that a reliable index of the medical value of codliver oil is the intensity of the blue color reaction with antimony trichloride. This test is simple to carry out and will give an indication of the order of Vitamin A potency of the oil. Whilst it is probably true that Vit. A and D activities do not always run parallel, we have no hesitation in expressing our opinion that any oil giving a strong antimony trichloride reaction will be found rich in both Vit. A and Vit. D."

The technic of the antimony trichloride test is given in detail on page 32 of the above article.

As to physical properties, Drummond and Hilditch list the following:

"Color of Medicinal Oil: It is apparent that the public must be educated to the fact that the yellow color of some oils probably indicates higher vitamin potency." They also mention experimental work of Dr. Alimad in which the yellow color has been successfully removed without lowering of the vitamin potency.

"Taste and Odor: Codliver oil when newly prepared from fresh livers possesses a very slight "fishy" smell and is not

unpleasant to the palate, apart from its "oiliness." Even a few hours' delay between removal of the livers and extraction of the oil markedly increases the "fishy" or "meaty" flavor. Innumerable attempts have been made to remove the constituents of codliver oil which are responsible for the taste and odor, but none appear successful and at the same time leave the medicinal properties unimpaired." Hydrogenation, a common method of making codliver oil more palatable, has been shown by several investigators, Nelson ('32), Drummond ('30), and Claus ('32), not only to render the codliver oil entirely negative as to vitamin content but also to leave the toxic elements unaffected.

As to the comparative value of codliver oil from various sources, Drummond and Hilditch ('30), using the antimony trichloride test described above, found that the oil from the Newfoundland cod, although as a rule more yellow in color, gave a much higher (two to eight Lavibord Units) reading than Norwegian, Scottish, or Icelandic oils. They state, "Zalva and Drummond were the first to draw attention to the high vitamin content of the oil prepared from the Newfoundland cod, an observation which has been repeatedly confirmed.....The Vit. D tests also reveal the relative high value of Newfoundland oil."

As to the season at which the oil is of greatest potency, these men find that the vitamin content is highest in July and August, which is just after spawning. "The richest vitamin oils will, therefore, be obtained in areas where abundant food supplies for the fish are available and at seasons when oil content of the livers is low.....This we believe is the explanation for the undoubtedly high vitamin value of the oil yielded by the Newfoundland cod....."

"Methods of extraction: Steam extraction under heavy pressure (100 pounds per sq. in.) for a prolonged period is the accepted method of extraction.

"Storage is also another important factor. The casts must be clean, air-tight, and light-proof to preserve all the properties of the oil. After refining, the product should be dispensed in dark glass bottles, filled to the top to exclude oxygen and closed with a tight cork or screw cap and plainly labeled to store in a dark cool place."

In a study of forty-two brands of codliver oil in this country, Nelson and Walker ('32) found five to be adulterated, none of which contained the standard (U. S. P. X) amount of Vit. D. "Of the remaining thirty-seven samples, only thirty-two showed a variation of 25% from standard, the majority showing only negligible variation."

We have already spoken briefly of the effect of treatment of the codliver oil upon the medicinal effect. Let us now consider the value of the so-called tasteless, purified, concentrated, or tablet forms of codliver oil which are today flooding the market. Claus ('32) states, "The value of these concentrates appears doubtful. For best results they probably should be dissolved in oil or taken with a meal rich in fat. For children their use is certainly not recommended."

TABLETS. Nelson and Walker ('32) examined 29 samples of codliver oil tablets, representing 17 different brands, for their vitamin D potency. They state, "Practically all the tablets examined in the early part of the work were devoid of the well-recognized therapeutic principals of codliver oil. As this became apparent, most of our tests were confined to Vit. D, since this vitamin was considered to be more stable than Vit. A

under the conditions to which the concentrate was subjected in the process of making tablets.....Of the seventeen brands of tablets tested only two were found to contain therapeutic quantities of Vit. A and D in the doses prescribed."

CAPSULES. Seven brands of capsules of codliver oil concentrates were examined by these men, and only two contained therapeutic quantities of Vit. A and D.

HYDRO-ALCOHOL. Hydro-alcohol preparations alleged to contain codliver oil concentrates or extracts were also examined. "Of twenty-three brands, not one was found to contain the vitamins of codliver oil in significant quantities."

CONCENTRATES. "Seven concentrates, each representing a different brand, were tested for vitamin potency. Of this number four were found to contain sufficient Vit. D and A to warrant them being classed as concentrates. Three contained less than 10% the Vit. D potency of codliver oil." Nelson goes on to state that since this work was done, the manufacturers have changed their products and these results may be inapplicable to present-day products.

STABILITY. The stability of Vit. D in codliver oil is of real interest to the purchaser of the oil because considerable time must necessarily elapse before this product reaches the retail market. Due to the fact that 90% of the codliver oil of this country is imported and this is done by three or four large concerns which buy and ship in large quantities, the oil as obtained is greatly blended and of uniform potency as a rule, but due to the widespread retail system these oils may remain for a year or more before sold and administered to the patient. Claus ('32) states that the Vit. D content of the oil is markedly stable and will resist action of oxygen and light for

several years. If stored in a dark, cold place, Paulsson has found that samples have retained their total Vit. D potency for twenty-five years at least.

TOXICITY. It has been known for a long time that codliver oil from decomposed livers was toxic and nauseating. Drummond ('30). This source has been eliminated by the carefully supervision of manufacturers by the Governmental Drug Regulations.

It is known that large doses of codliver oil or its concentrates produce a typical Vit. D intoxication, as described by numerous investigators, Drummond, ('30), Claus ('32), Seel, ('30), and Schlutz ('32), when given in massive doses. Hess also reports that with doses of thirty to forty times the minimum antirachitic dose, high blood calcium values and loss of weight can be produced in rats. He also states, "The reason hypercalcemia rarely if ever is produced in infants as results of codliver oil medication is simply that one cannot give more than about six to eight teaspoonfuls daily."

Others believe a toxic factor, separate from Vit. D, is present in codliver oil. This has not been proven nor disproven; hence this question awaits further investigation (Claus, '32).

Egg Yolk

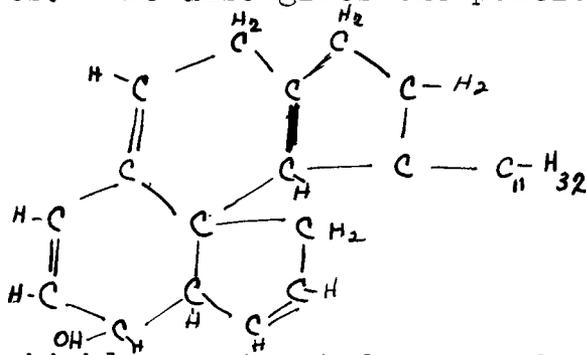
The third great natural source of Vit. D. is the yolk of eggs. This was one of the earliest known sources of antirachitic therapy, as was found by Mellanby ('18), Hess ('18), Kramer and Cusparis, and Schlutz ('32), who found that if egg yolk be added to the rickogenic diet it would prevent rickets and even cure them. This has been substantiated by the work of clinical men on children in whom rickets have been cured by the administration of one to three yolks daily. Egg yolk is, however, a better prophylactic than a cure for this disease.

The Vit. D content of the egg is remarkably constant. Boiling or poaching does not affect the potency; neither does long storage at low temperatures or even liquid preservation as carried on in the Orient (Claus, '32). The potency of egg yolk varies also with season, locality, and diet of the hen. Hess ('30) has increased the Vit. D potency ten times by irradiation of the hen. Similar results are noted when codliver oil is fed to the flock and a marked rise is noted in the summer months or in sunny climates.

This source of Vit. D is good, easy to obtain, is non-toxic and cheap, and as a prophylactic measure should rank high in antirachitic therapeutics.

Irradiated Ergosterol

The discovery and history of irradiated ergosterol was briefly discussed in the opening pages. Its discovery and utilization has been one of the outstanding forward steps in the science of medicine in the last decade. Ergosterol has been known to the chemist since 1889, when Tauret (France) discovered it in fungi and yeast, and in 1909 when he succeeded in purifying and extracting it. Windous ('31) states, "In its pure form ergosterol is a white crystalline solid, melting at 160° to 183° , depending upon the degree of hydration. It is sparingly soluble in alcohol and other organic solvents and readily recrystallizes." He also gives the possible structural formula as:



This shows a highly unsaturated compound, sensitive to oxidiz-

ation, even by oxygen of the air, hence capable of forming esters of acids, both organic and inorganic. It is levorotary and has an "absorption spectrum" in the ultra-violet range, showing strong absorption at 260, 270, 280-2, and 293.5 millimicrons. Many isomers of ergosterol exist, some naturally, some being made "in vitro" by actions of acids. Some of these are activatable, but as yet no method has been devised for commercial use of the synthetic active products.

When ergosterol is irradiated, several changes take place in its chemical and physical properties. The irradiated product is more soluble, has a lower melting point, and is almost neutral in its rotary power. The absorption properties change from a marked increase early in the process at 270-280 millimicrons to a gradual decrease to all wave lengths over 250 millimicrons with prolonged irradiation. This disappearance of the absorption of the longer waves is coincident with the disappearance of the antirachitic properties.

Extensive work has been done and many theories have been promulgated as to just what is the action of the light upon the ergosterol. At present the most accepted one is that of Windous ('31), in which he states that the light breaks the ergosterol into a series of different products (five or six) and that one of these is Vit. D, which may exist in several forms.

This element or elements has been separated by several investigators (Angus et al., Windous, Reerink, Bills, and others), all the products being obtained by different methods, and although similar in many respects, vary markedly in other respects. Claus ('32), in summarizing this field, states that "although none of these may be Vit. D in pure state, their prep-

aration is a marked advance toward the isolation of Vit. D."

POTENCY OF IRRADIATED ERGOSTEROL. The potency of the various preparations of ergosterol is known to vary markedly, and the maximum limit of potency has perhaps never been reached (Claus, '32). The English standard, irradiated in alcohol, is effective in doses of 0.0001 milligrams, but preparations have been made which show antirachitic activity in doses of 0.00002 milligrams. Compared with codliver oil, these products vary from 200,000 or less to as high as 700,000 times the average commercial oil. This variation is due first to the solvent in which the ergosterol is placed prior to irradiation, second to the wave length and intensity of the irradiating light, and third to the length of exposure of the ergosterol to light. The presence of oxygen also retards the complete activation, hence is a factor which is considered.

Ergosterol may be irradiated dry or in a solution of alcohol, ether, hexane, benzene, or any of the vegetable oils, but the potency obtained varies with the solvent used. Bills, Honeywell, and Cox ('31) have found that the oils are very satisfactory and of greater convenience and stability than other solvents, although other solvents, especially ether, give a much greater potency under the same conditions.

The effect of wave length and intensity has been discussed earlier, but the effects of over-irradiation must now be impressed. Hess, Bills, Steinback, and others have all found that excessive irradiation of any photoactive product is not only unnecessary but is actually harmful in many cases. With ergosterol prolonged irradiation reduces the antirachitic potency (Windaus, '31) and also increases the toxic effect by

production of some intolerable by-product of the reaction.

STANDARDIZATION. Because of the wide variation in potency, it is necessary to set some standard by which to regulate the action of the products.

In June, 1931, under the auspices of the permanent committee on Biological Standardization of the Health Organization of the League of Nations, a conference of experts was held in London with the object of preparing and adopting an international unit of Vit. D potency for both codliver oil and viosterol. The recommendation of this conference to the Bureau of Standards for Vit. D were as follows:

"The conference recommends that the standard solution of irradiated ergosterol which has been issued from the National Institute of Medical Research at Hampstead, England, for the past two years be adopted as the international standard." Because there is but a limited supply, the institution furnished all countries samples from which to produce a supply sufficient to meet the needs of the world (Lancet, '32). This unit is called the International Unit of Vit. D, and is defined as, "The antirachitic potency of a quantity of this preparation corresponding to 0.0001 milligrams of the ergosterol used in its preparation." The test is carried out on rats, the results computed by "line test," chemical analysis of bone, or the X-ray method.

The American Unit as defined by the Wisconsin Alumni Research Foundation (Steinback) and accepted by the Council on Pharmacy and Chemistry of the A. M. A. is as follows: "That amount of Vit. D when uniformly distributed into the standard Vit. D deficient diet--ration 2965, J. Biol. Chem.; 64:263, 1925--will produce a narrow continuous line of calcium deposits

on the metaphysis of the distal ends of the radii and ulnae of standard rachitic rats." This is used for codliver oil, and viosterol is standardized against a codliver oil which contains 13.33 rat units per gram, or one rat unit per seventy-five milligrams of oil. This seventy-five milligrams makes up the total amount fed during the test (Claus, '32).

The other units found in literature today are: "American Pharmaceutical Unit (Paulsson) is defined as the minimum amount which given daily for six days will cure severe rickets."

Windous of Germany uses as a unit "the minimum amount of the substance which will exactly protect young growing rats from rickets." In summing up the standardization of these products the following table shows the relative value of the mentioned units.

German (Holtz & Windaus)	-----	15000 rat units per cubic centimeter
New and Nonofficial Remedies (Steenbock)	300D potency-----	
		400 rat units per gram*.
British Official Unit (1930)	---10,000	rat units per c.c.
International Units	-----	10,000 rat units per c.c.
Oslo (Paulsson)	-----	12000 rat units per gram.
Patch (Holmes)	-----	26,500 rat units per gram.

* 1 c.c. = 1.09 gram.

THERAPEUSIS. The use of viosterol as a prophylactic and curative measure in rickets, tetany, and related diseases has been a very fertile field for argument and discussion since the advent of viosterol into general use. Hess ('29) in his book on these diseases states that ergosterol is "by far the most potent antirachitic" available and that "it is reliable in the cure as well as the prevention of rickets." This view is

sustained by McCollum et al., ('29), Strong ('29), Moore ('30), Kugelmas ('31), Bills ('30), Seel ('30), Enfinger ('30), Dugiud ('30), and others.

On the other hand, Collazo ('29) gives warning of hyperphosphatemia, hyperazotemia, albuminuria, cylinduria, and hyperossification with its uncontrolled use. De Sanctis ('30) believes that the standard dose of viosterol is too small to be of protective value in rickets, or that some factor which viosterol lacks is essential in antirachitic metabolism. Barnes ('30) also finds that viosterol is lacking in proficient prophylaxis.

As to the toxicity of viosterol, a review of the work will show that in most cases in which toxicity was produced the doses were massive, exceeding the usual therapeutic dose from 1,000 to 50,000 times before the effects were deleterious. As an example, Bills ('30) found first signs of toxicity at 1,000 times the therapeutic dose. Brown and Shohl ('30) exceeded the therapeutic dose by 2500 times to gain their negative results, while Haendel and Malet ('30) exceeded the usual dosage by 12,500 to 50,000 times.

Garely ('30) and Gordon ('32) both report an apparent idiosyncrasy to viosterol which is evidenced by a typical chain of symptoms. Gordon ('32) in his review of two hundred cases from private practice found that fifteen showed this sensitivity. He gives the following as the typical symptoms. "The ill effects in order of their frequency were: loose bowel movements, vomiting, loss of appetite, colic, and stationary weight or loss of weight, and in a few instances an eruption of uticularial nature." De Sanctis and Craig ('30) in their comparative study of codliver oil and viosterol believed that as a preventive codliver oil is the better because of the presence of Vit. A

and other factors. Brown and Shohl ('30) have shown clearly that Vit. D is concerned with bone calcification alone, whereas calcium and phosphorus retention in the body is largely due to the amount and ratio of these elements in the diet, irrespective of the presence or absence of Vit. D.

Harris ('30) has shown that bone growth is composed of three phases: first, growth of cartilage; second, calcification of the cartilage; and third, true bone formation. Hence, as rickets is a disease affecting all the stages of bone formation from cartilage to true bone and as Vit. D alone has to do with calcification only, there must be other factors as yet unrecognized which play a part in prevention and cure of this disease. The presence of these factors in codliver oil may explain the apparent better results obtained from codliver oil by the various investigators. De Sanctis ('31) finds that as a protective measure ten times the unit dose of codliver oil in form of viosterol gives 5% less protection to the child than does codliver oil. Barnes et al. ('30), in a study of 219 cases, conclude "that codliver in equivalent Vit. D units is a much better therapeutic agent than viosterol."

Steinback et al. ('32), working on chickens, conclude that viosterol and yeast, that is, Vit. A and D in pure form, were not so efficient antirachitics as codliver oil unless given in doses of 40 to 160 times the minimum dose of codliver oil, here again indicating the possibility of unknown factors as spoken of by Barnes ('30).

Thus far in this discussion it has been assumed that the toxic symptoms are due to an excess of Vit. D. Opinion on this point is, however, divided, and certain investigators, notably

Windous ('31), believe that the toxic effect may be produced not by Vit. D but by other products of the irradiation of ergosterol.

The reason for this they base upon the following facts.

In general the toxicity of viosterol preparations runs parallel to their antirachitic potency, and generally those agents destroying the antirachitic potency likewise destroy the toxicity. This latter statement is not true in all cases. Windous by hydrogenation and heat has removed the antirachitic properties and left the toxicity unaltered. He has also shown that the antirachitic potency and toxicity are not destroyed at the same rate by other agents.

To refute these findings, it has been pointed out that although the antirachitic properties may be destroyed without alteration of the toxicity the reverse has never been accomplished; hence the action of heat and hydrogenation may only alter the Vit. D so as to produce the toxic factor. It has also been shown that the crystalline Vit. D product "Calciferol," prepared by British investigators, is equally as toxic as the original irradiated product from which it was derived.

In summing up the subject of the toxicity of Vit. D, one may state that although evidence for a toxic factor not related to Vit. D is good, it has nevertheless not been proven, and we must await further work on this subject.

The amount of viosterol to be administered is very much in question. At present the standard dosage as advised by the manufacturers (ten drops a day) is thought too small by many investigators, De Sanctis ('31), Harris ('30), Brown ('30), Barnes et al. ('30), and Steinback et al. ('32), Michel et al. ('32),

Robertson ('32), Hess et al. ('30), and Schlutz ('32).

The Wisconsin Alumni Foundation has devised a standard generally accepted in this country, and postulates a potency for irradiated ergosterol preparations to 250 D, by which is meant a preparation 100 times as potent as good codliver oil, or 250 times the Wisconsin Standard. Of this product twenty drops equal one teaspoonful of good codliver oil. The Council on Pharmacy and Chemistry has accepted the irradiated ergosterol as prepared by the Wisconsin Foundation under the name of "viosterol", which signifies irradiated ergosterol in oil. The daily prophylactic dose for the average infant is approximately ten drops; for the premature and rapidly growing child it is much higher and variable; and the curative dosage in severe rickets is twenty to thirty drops.

Irradiated Foods

Reference has been made in the introduction to the almost simultaneous announcements by Hess and Steinback of successful activation of foods by irradiation with ultra-violet light. Further work in this field has shown that many foods may be made powerful antirachitic agents by this radiation.

The foods which have been irradiated compose a long list: numerous oils and fats, olive, cottonseed, corn, cocoanut, lard, butter, etc.; cereals and their products, refined wheat flower, whole wheat flower, shredded wheat, and even cornstarch; meat, milk, whole and dry, yeast, orange juice, and ice cream. Egg yolk has been irradiated with an increase of twenty to fifty times the original potency and butter fat from fifteen to twenty times.

Fearing commercial exploitation of this procedure, which is endowed with possible harm, Steinback in 1920 patented the pro-

cess of the irradiation of food and medicals by ultra-violet rays. This patent he then turned over to an organization of the Wisconsin University Alumni, "The Wisconsin Alumni Research Foundation," which was founded to receive it. This Foundation sells licenses to reputable concerns upon a royalty basis and controls the product and even the advertising of these firms. The profits of these licenses go to the Foundation, which in turn gives 15% of the total to the inventor (Hyde, '32).

The irradiation of materials is a simple, cheap, and short procedure. Steinback ('30) states, "We have found an exposure of a fraction of a second with a battery of quartz mercury vapor lamps is entirely satisfactory for cereals." This is also true for other dry substances. For milk, butter, and other fat-containing foods, three to ten minutes was found to be the average time of radiation.

The antirachitic property developed in foods by irradiation appears to be a stable property. Cereals retain their potency for eighteen months at 60° C. Irradiated dry milk kept in a cupboard at room temperature lost but little potency in six months. Butterfat retained total potency after six months of refrigeration. Ordinary cooking does not destroy the antirachitic potency as a rule; commercial baking may do so if the temperature is allowed to go too high.

The antirachitic value of the irradiated cereals on the market today is not sufficient to prevent rickets, but the main value lies in the fact that the irradiation has changed the cereals from ricket producing to ricket preventing foods and reduces the amount of antirachitic agents of medicinal nature necessary.

The wisdom of the general use of irradiated foods is

believed by Steinback to be of little if any danger and of a marked though small advantage.

CONCLUSIONS

We are now ready to attempt to answer the question asked previously, "How can this essential element be best supplied?" The following are our own opinions and are derived only from the brief review of the literature of this paper.

1. We believe that codliver oil at the present time is the best and most reliable prophylactic agent for rickets and associated diseases.

2. Viosterol has its place as a curative agent in cases of developed rickets, tetany, and associated diseases, and in those cases where the Vit. D requirement is so great that sufficient codliver oil cannot be tolerated either because of the fat content or the size of the recipient.

3. Sunlight or ultra-violet irradiation from artificial sources are of highest value if they are available and correctly controlled.

4. The use of irradiated foods is to be recommended in most cases but must be supplemented by other sources of Vit. D.

5. At the present time the use of codliver oil concentrates, pills, tablets, and so forth is to be avoided until evidence has been accumulated to prove their value.

6. The use of fish oils other than codliver oil is justifiable from a therapeutic view but from an economic and practical point of view they are not at this time accessible nor definitely regulated as to potency.

Summary

The history of the discovery and development of the value of the essential food factor Vit. D has been traced from its advent in 1918 to the present date. The physiological action of the Vit. D in the body was briefly outlined.

The present sources have been discussed as to comparative value, accessability, and toxicity of each.

An attempt has been made to answer the oft asked question, "How may Vit. D be best supplied?"

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