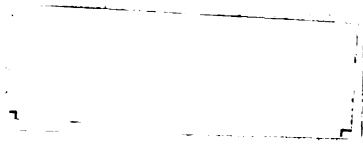




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EFFECT OF MINERAL OIL,
FATTY ACIDS, AND LECITHIN ON
VITAMIN D UTILIZATION IN THE RAT

Thesis for the Degree of M. S.
MICHIGAN STATE COLLEGE
Lawrence Valentine Hanks
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This is to certify that the

thesis entitled

Effect of Mineral Oil
Fatty Acids, and Lecithin on
Vitamin D Utilization in the Rat

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**EFFECT OF MINERAL OIL, FATTY ACIDS, AND LECITHIN
ON VITAMIN D UTILIZATION IN THE RAT**

by

LAWRENCE VALENTINE HANKES

A THESIS

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Introduction

In surveying the literature on the effect of mineral oil on the utilization of vitamin D, one finds more or less conflicting results. This situation is undoubtedly due to the selection of arbitrary procedures, some of which were far removed from stimulating the ordinary use of mineral oil as a laxative. Therefore, it was decided to carry out further studies in which particular attention was given to the dosage and the method of administering the mineral oil.

The second phase of the thesis deals with a study of the effect of various fatty acids and lecithin on the utilization of vitamin D from subcutaneously injected oils. It was thought that such additions to the oil might influence its dispersibility and thus accelerate the absorption of the vitamin D.

Historical

Studies of the effect of mineral oil on vitamin D absorption have led to somewhat contradictory conclusions. In 1927, Dutcher, Ely and Honeywell, reported that cod liver oil mixed with mineral oil promoted calcification in rachitic rats to the same degree as an equal amount of cod liver oil without mineral oil (7). The data they obtained was based on x-ray pictures and the line test developed by McCollum, Simmonds, Shipley and Park (3).

Almost simultaneously with the above publication, Burrows and Farr (6) presented experimental evidence which contradicted the above results. They found that mineral oil, unlike vegetable oil, tends to remove fat soluble vitamins from the food and produces other toxic effects. The procedure in the latter experiments involved mixing the oils in the feed, whereas in the previous work, the oils were fed separately. This difference in procedure may be responsible for the difference in results. The toxic effect of the oil was believed by these investigators to be due to the fact that it acts on the mucus membrane of the intestines by removing the fat soluble vitamin reserve.

Further information on this subject was published in 1929 by Hawks, Levene, Stucky and Oser (9). The reported that mineral oil interfered with the utilization of vitamin D in cod liver oil. Approximately three to four times as much vitamin D was required to produce recalcification when mineral oil was substituted for cotton seed oil as a solvent. In the

interpretation of their results, they used x-rays and the line test.

Later work by Jackson in 1933 (16) agreed essentially with that obtained by Dutcher, Ely, and Honeywell. He found that mineral oil administered separately from irradiated ergosterol did not interfere with the utilization of vitamin D. Jackson's procedure differed from that of previous workers in that he fed the oils to the animals separately with an eye dropper. The other men incorporated either the vitamin oil or the mineral oil in the feed, and fed the other oil separately. The difference in the methods of procedure may have been responsible for some of the variations in the results. Jackson was the first to administer a dose of mineral oil that was comparable to a normal human dose of 30 milliliters. This dosage equivalent was worked out by Jackson in 1937 (13), and his results were confirmed by Rowntree in the same year (14).

The most recent work on this problem was that done in 1940 by Smith and Spector (18). They incorporated the mineral oil in the basal ration at five and ten per cent levels. Therefore, the mineral oil was proportional to the food intake and lubrication was continuous. According to their calculations, there was a daily ingestion of .5 milliliter of mineral oil. They fed .05 milliliter of cod liver oil separately daily with a pipette during a ten day period. The amount of cod liver oil was such that they obtained a line with a +2.4 healing.

In all work previous to that of Smith and Spector, the animals were kept on the diet too long to establish whether or not there were differences in utilization. This was especially true in the work by Dutcher, Ely and Honeywell (7). They held the animals on the vitamin D supplement for twenty-one days. One would expect that unless the mineral oil interfered completely with vitamin D utilization, healing during this period might be considerable with or without the mineral oil. Beginning with Jackson's work, the official line test procedure was used and interpreted according to a scale developed by Bills (12), which in turn was based on the original description of the D line by McCollum, Simmons, Shipley and Park (3).

In the experiments previously mentioned, there was no attempt made to conform to customary human practice in the use of vitamin D and mineral oil. Therefore, particular care was taken in the present studies to simulate as closely as possible the ordinary methods of taking mineral oil and vitamin D preparations.

The second part of this study deals with the effect of fatty acids and lecithin on vitamin D absorption by the subcutaneous route. There is very little to be found in the literature concerning the assimilation of oils injected into the body. In 1925, Burrows and Johnston published some extensive work on the effect of subcutaneous injected oils on body tissues (4). They injected corn oil in such a way that it formed one large droplet which subsequently broke up into numerous drops of various sizes, each drop then becoming

firmly encapsulated, so that a multiple cystic tumor was formed. The oil had no stimulating action on the growth of the cells, but acted only to disrupt the normal organization of the tissue, and to build densely cellular tissues relatively free from blood vessels and intercellular substances. These men pointed out that this action delayed the absorption of the oil. Subsequent histological inspection of tissues revealed that the drops were cut off from the surrounding fibrous tissue by a layer of cells. All of the tissue was filled with cells migrating toward the oil. With the movement of the cells toward the oil drop, tiny droplets of oil were continuously breaking off and moving out into the tissue. In a few of the animals used, the workers did report that the oil was completely absorbed and the tumor collapsed, whereupon, the cells returned to a normal state. It might be expected that the assimilation of the oil could probably be accelerated or assisted, if the oil injected was more dispersed or rendered more easily dispersable by the addition of other agents such as fatty acids. There is also the possibility that the essential nature of a fatty acid may exert an influence. The tissues might show a preference to take up vitamin D from oils containing the essential acid. In 1930, Burr and Burr (11) found that rats suffering from a low fat disease were not benefited by the ingestion of saturated acids (lauric and myristic), but were cured by linoleic acid. In 1932, Burr, Burr and Miller (15) added

further information to this subject in that they found oleic acid was among those ineffective in curing the disease, whereas linolenic acid behaved similar to linoleic acid.

This dependency of the body on outside sources for certain fatty acids might give an increased assimilation of the vitamin D oils containing these acids, in spite of the fact that there is a disturbance caused by the presence of the oil as demonstrated by Burrows and Johnston. If the non-essential acids gave an increase in the vitamin D absorption, the increase might be said to be due to a simple physico-chemical phenomenon, that is, greater dispersibility due to soap formation. A greater increase with the essential acids could be said to be due to the fact that the body shows a preference for these acids, indicated by a more rapid utilization of the vitamin D. If all of the effects obtained were negative, the idea of cell function interference proposed by Burrows and Johnston could be applied in interpreting the results (4).

Because of the quantitative nature of the vitamin D line tests, vitamin D containing oils were used to determine the rate of assimilation of the injected oils.

**EFFECT OF MINERAL OIL, FATTY ACIDS, AND LECITHIN ON
VITAMIN D UTILIZATION IN RATS**

**Effect of Mineral Oil on Vitamin D Absorption in the
Intestine**

Experimental Methods

Preparation of Animals

In these tests, three to four week old rats weighing 45-60 grams were used. The animals were placed in pairs in wire cages and fed a diet that was a slight variation of Steenbock's basal rachitogenic diet (5). The Steenbock diet was modified by the substitution of table cornmeal for ground whole yellow corn and the introduction of oatmeal and brewers yeast to compensate for the losses in food value occasioned by the use of the refined product. The animals were maintained on this diet for a period of three weeks, at the end of which time they had developed the desired degree of rickets.

Conduct of Test

The rachitic animals were placed in individual cages and fed supplements containing a known number of units of vitamin D. The supplement was incorporated uniformly in 45 grams of feed. The animals were placed on the vitamin D supplemented ration and were fed mineral oil by means of a small syringe. In the initial experiments, the syringe was provided with a blunt needle to prevent injury to the animals' throats. However, it was found that some throat injuries were caused due to unavoidable movement of animals. Consequently, a small piece of number ten catheter tubing was rounded off at one end and slipped over the blunt needle. The rubber tubing was attached in such a way that it protruded over the end of the needle about one quarter of an inch to provide a little flexibility when inserting it. The

tube could be slipped down the animal's throat a small distance without any harm being done, and by having the tube in the animal's throat, the animal was unable to regurgitate the oil as happened frequently previous to the adoption of this technique.

The mineral oil was fed every morning, different volumes being fed to different groups. In addition to varying the amount of oil fed, several levels of vitamin D were also used.

Line Test

At the end of a ten day period, the animals were killed by etherization, the fore-paws removed with a pair of scissors, and the distal ends of the radii and ulnae with about one-quarter of an inch of the shaft retained for the line test. The flesh was stripped from the bones, and the bones placed in 95% ethyl alcohol for a period of at least twenty-four hours. Then according to Shipley's procedure, the bones were halved, immersed in 2% silver nitrate for about two minutes, and the freshly cut surfaces exposed to light. This technique revealed the calcification induced by the vitamin D in the rachitic metaphysis and the extent of calcification was evaluated by using a modification of Bill's scale.

Results

In the initial experiments, supplements of 2.7 and 5.4 U.S.P. Units of vitamin D were employed. These doses were obtained by diluting a U.S.P. standard reference oil with Wesson (cotton seed) oil. The administration of one milliliter of mineral oil daily proved to be excessive because after five days, the rats lost considerable weight and showed very soft stools. It was then found that this dosage corresponded to twice that prescribed for human usage so that for subsequent studies, smaller dosages were employed.

A second series of animals was used with the same number of units of vitamin in the feed. Three levels of mineral oil were used in this phase of the study, namely three-fourths, one-half, and one-quarter milliliter daily. All of the animals on the three-quarter milliliter level lost weight, and a few of them died before the tests were completed. These animals were unsatisfactory for the line tests, because animals losing weight are always rejected in official vitamin D assays. Most of the animals on the one-half and one-quarter milliliter levels gained weight and were, therefore, satisfactory for the test. The line tests showed definite interference with the assimilation of vitamin D, the amount of interference varying directly with the amount of mineral oil administered. The half milliliter level used corresponds to a 30 milliliter dose for humans. This amount still seemed excessive for the rats, so that subsequent studies were carried out using one-quarter and

one-eighth milliliter doses of mineral oil daily.

In the next series, the animals were fed supplements containing 2.7 and 5.4 units of vitamin D. One group was fed one-quarter of a milliliter of oil and the other one-eighth of a milliliter. Further evidence was obtained to indicate that there is an increased interference with the higher level of mineral oil ingestion.

Additional experiments were carried out to determine what the effect of using a higher intake of vitamin D might be. The amount of vitamin D in the feed was raised to 8.1 units, and mineral oil fed at one-quarter and one-eighth milliliter levels. The results indicated as before that increased injection of mineral oil interferes to a greater extent with the vitamin D absorption.

Another method of conducting the test was to incorporate both the vitamin D and mineral oil at various levels in the basal rachitogenic diet. The mineral oil was included in the feed at levels of one, two and three milliliters per 50 grams of ration, and the vitamin D kept constant at 6.7 U.S.P. Units. This was the method used by Hawk, Levene, Stucky and Oser (9). The results obtained with these animals were similar to those found by using the previous technique. The results of all of these tests are summarized in Table I.

Table I
The Effect of Varying Doses of Mineral Oil
on the Responses of Rachitic Rats
to Measured Intakes of Vitamin D

Ml. of Mineral Oil Fed Orally	Units of D	Average D Line	Time on Test	No. of Animals	Ml. of Oil Incorporated in Feed
1/8	2.7	.15	10 days	5	None
1/8	5.4	1.00	10 days	2	None
1/8	8.1	1.87	10 days	4	None
1/4	2.7	.29	10 days	12	None
1/4	5.4	.72	10 days	11	None
1/4	8.1	1.50	10 days	4	None
1/2	2.7	.60	10 days	5	None
1/2	5.4	.70	10 days	5	None
None	6.7	1.60	10 days	4	1 ml.
None	6.7	.75	10 days	4	2 ml.
None	6.7	.25	10 days	4	3 ml.
None	6.7	2.12	10 days	4	None

Discussion

The results of these experiments agree with those obtained by Burrows and Farr in 1929 (6), who observed that the ingestion of mineral oil limits the assimilation of the fat soluble vitamins from the intestinal tract. As mentioned previously, Smith and Spector (18) made similar observations. It is rather reasonable to expect some decrease in the assimilation of fat soluble vitamins during periods when mineral oil is ingested. These vitamins are soluble in mineral oil, and in as much as the latter is indigestible and not assimilated itself, the oil would obviously prevent the effective extraction of such vitamins by the digestive juices. That this interference with the assimilation of fat soluble vitamins is considerable, although not complete, is indicated by the fact that some utilization of vitamin D can be demonstrated when mineral oil containing this vitamin is fed to rachitic rats. Mineral oil exerts an effect also, because it coats the walls of the tract and interferes with the normal contact of digested food with the absorbing tissues of the digestive tract.

It may be of interest to mention here that at least one distributor of mineral oil preparations has taken note of the interference with the assimilation of vitamin A. A certain amount of vitamin A is added to the mineral oil product in order to compensate for the estimated loss occasioned by the use of the oil.

With regard to the conflicting results which are to be found in the literature on this subject these may be easily

accounted for. The length of the experimental period is of considerable importance. If one uses a fairly long period of feeding both vitamin D and mineral oil it is possible to get complete healing in rachitic rats with fairly small doses of vitamin D. This is clearly indicated by the results obtained in the present studies in which the experimental period was varied from 5 to 15 days. Differences in line test responses, which were fairly conspicuous in ten day animals, were no longer evident in fifteen day animals.

**EFFECT OF MINERAL OIL, FATTY ACIDS, AND LECITHIN ON
VITAMIN D UTILIZATION IN RATS**

**Effect of Free Fatty Acids and Lecithin on the Absorption
of Vitamin D, Subcutaneously**

Experimental Methods

Standard rats such as previously described were used in these tests. The solutions of vitamin D for injection were made up in 10 milliliter flasks using the official reference oil as the source of vitamin D, Wesson oil as the diluent, and relatively pure fatty acids and lecithin as special additions. Subcutaneous injections were made by means of a hypodermic needle. To avoid losing any of the solution by leakage from the site of injection, the needle was inserted at the base of the hind leg and passed subcutaneously to a spot on the abdomen opposite the left front leg. This procedure also assured getting the fluid in the same area in every animal, so that the same tissues would be involved in the absorption of the fluid.

The fatty acids used in these studies came from a variety of sources. The lauric and myristic acids were student preparations of a fairly high degree of purity. The oleic and linoleic acids were from a supply of C.P. grade of commercial origin. Linolenic acid and lecithin were freshly prepared from linseed oil and egg yolk respectively; their preparation will be described later in the paper. The various compounds were added on the basis of one gram per hundred milliliters of solution. All solutions used were made up fresh before each experiment.

In making up the various solutions, the vitamin D standard, fatty acids and lecithin were weighed in volumetric flasks and made to volume with Wesson oil. To insure complete solution of the solid acids, namely myristic and lauric, the

flasks were placed in an oven at 42 degrees and left there until a clear solution was formed. In the case of lecithin, it was found convenient to prepare a stock solution in Weason oil in order to avoid decomposition. Because of the difficulty in effecting direct solution, a double volume of ether was used. The ether was then allowed to evaporate and the resulting solution used as a diluent in making up the preparations which were intended to contain lecithin.

After injection, the animals were placed in separate wire cages on the basal diet for periods which varied from five to fifteen days. Most of the tests involved the conventional ten day period. The bones were examined according to the usual procedure for the line test. A five day test was used in order to determine whether there might be more noticeable differences in the effect of the various preparations which were injected. As in previous studies, animals which lost weight during the experimental period were discarded, because of the fact that spontaneous healing or calcification is usually observed. This would obviously make it difficult to evaluate any effect induced by the vitamin D.

In addition to the other preparations, an emulsion of the vitamin D containing oil was also used for injection. It was prepared by adding a solution of castile soap to the oil and passing the mixture through an emulsifier. The emulsion obtained was quite stable and suitable for injection.

Preparations

1. Lecithin

a) Cadmium Salt (Preparation and Purification)

In order to make a fairly pure preparation of lecithin, the best features of several methods were combined. The yolks of two dozen eggs were stirred into a homogeneous mass by means of a mechanical stirrer, and then poured into a beaker of hot 95% ethyl alcohol. The extract was filtered and the residue on the filter paper extracted several times with additional hot alcohol. The filtrates from all extractions were combined and the solution allowed to cool. A cold solution of cadmium chloride in 95% methyl alcohol (10) was added to the combined extracts. The precipitate was filtered off on a fluted filter paper, washed with ethyl alcohol a few times and then with ethyl ether (2).

The precipitate was suspended in 400 milliliters of petroleum ether and then extracted repeatedly with 75 milliliter portions of 80% ethyl alcohol. This extraction was repeated fourteen times, and occasionally petroleum ether added to keep the volume of the extract constant (19). This petroleum ether-alcohol extraction gives a better separation of the lecithin from the ether soluble substances. The first alcohol and ether washing removes most of the cephalins and sphingomyelins.

b) Liberation and Purification of Lecithin

The lecithin-cadmium salt was suspended in chloroform (400 ml./100 gms. salt) and the lecithin separated by

adding a cold solution of ammoniacal-methyl alcohol (2). The solution was centrifuged and the liquid siphoned off. The chloroform-ammonia-methyl alcohol treatment was repeated on the cadmium salt residue. All of the liquors thus obtained were concentrated under vacuum at 35 to 40 degrees centigrade. The residue was extracted with absolute ethyl alcohol to separate lecithin from any contaminating cephalin.

In order to achieve further purification the lecithin was treated with the cadmium chloride-methyl alcohol reagent and then filtered. The precipitate was extracted several times with anhydrous ethyl ether to remove any impurities that might still be present (8). Then the precipitate was suspended in chloroform and treated with ammoniacal-methyl alcohol solution to free the lecithin. The solution and residue were treated in the same manner as before, and the resulting combined extracts concentrated under vacuum (17). The residue was then dissolved in a minimum of ethyl alcohol and poured into anhydrous acetone to precipitate the lecithin. The product was finally dried on a watch glass in a vacuum oven at 38 degrees centigrade and stored under nitrogen in a test tube. The test tube was kept in a refrigerator to prevent decomposition. The final treatment with acetic acid as usually performed was omitted, because according to Maltaner, a pure product may be obtained without it (10). The acetic acid treatment generally yields a brown product, and reduces the yield.

2. Linolenic Acid (1)

a) Preparation of the Hexabromide

One hundred twenty grams of linseed oil was mixed with a solution of 50 grams solid sodium hydroxide, 150 ml. water, and 100 ml. of ethyl alcohol. This mixture was stirred thoroughly, and heated such that it boiled without spattering. As the alcohol and water boiled away, more was added to keep the volumes constant. After about forty minutes of heating, the saponification was complete, and the hot solution poured into about 3000 ml. of a filtered saturated salt (sodium chloride) solution. The precipitated soap was filtered off with suction, and washed twice with 50 ml. of ice-cold distilled water. Three hundred grams of soap obtained was put into 600 ml. of glacial acetic acid in a large erlenmeyer flask, which was subsequently surrounded with fine ice and salt. The erlenmeyer flask was fitted with a mechanical stirrer and a cold bromine-glacial acetic acid solution added by means of a dropping funnel. The solution obtained was allowed to stand over-night in a refrigerator, and the precipitate obtained, filtered off. The product was recrystallized from 400 ml. of benzene, and the recrystallization repeated until a melting point of 180-182 degrees Centigrade was obtained.

b) Preparation of the Methyl Ester

Twenty-eight grams of the hexabromide was mixed with an equal weight of zinc powder, and this mixture dissolved in anhydrous methyl alcohol. The latter was prepared (20) by treating methyl alcohol with magnesium turnings,

and after the initial vigorous reaction had subsided, it was refluxed for two hours. The anhydrous product was then distilled off into a cotton stoppered flask. A part of the anhydrous alcohol was saturated with dry HCl, which was prepared by adding concentrated HCl in a dropping funnel to concentrated sulfuric acid. This gas was dried by passing it through a series of bottles containing concentrated sulfuric acid. The bromine-zinc-alcohol solution was heated for a half hour before the HCl-alcohol solution was added dropwise over the course of one hour. This mixture was then refluxed over-night.

In the morning, the solution was chilled with ice to obtain the maximum yield of the methyl ester. The solution was extracted with ethyl ether, and the ether layer drawn off. The ether solution was treated with water to remove the excess methyl alcohol present and then dried with anhydrous sodium sulfate. Subsequently, the ether was distilled off in an atmosphere of carbon dioxide on a steam bath, and the ester then vacuum distilled in the presence of CO₂.

c) Isolation of Linolenic Acid

Seven grams of the methyl ester were mixed with 70 ml. of a 5% solution of sodium hydroxide in 95% ethyl alcohol, and allowed to stand over-night. To this solution, 70 ml. of dilute sulfuric acid were added to free the linolenic acid. The solution was then shaken and extracted several times with ether. Residual methyl alcohol was removed from the ether solution by several extractions with water.

The ether was distilled off in the presence of carbon dioxide, and the yellow oily residue containing the fatty acid was subsequently distilled under reduced pressure, likewise in a CO₂ atmosphere. The product which distilled over at about 225 degrees Centigrade was sealed under nitrogen before storing in a refrigerator.

Results

Preliminary injections of vitamin D containing oils indicated that there was some assimilation of the vitamin D by the subcutaneous route. Therefore, further tests were made in which the following factors were varied: (1) the amount of vitamin D, (2) the volume of the injected oil, and (3) the length of the test period. This was done to obtain the best conditions for the subsequent studies with fatty acids and lecithin. These exploratory studies also included a comparison between the vitamin D containing oil, with and without myristic acid.

The ten day test with a one milliliter injection indicated that myristic acid accelerated the assimilation of the injected vitamin D. Although the effect was not very great, it was consistent for levels of vitamin D in this volume of oil. When the volume of the injected oil was reduced to one-quarter of a milliliter, the resulting line tests showed considerable variability. The best condition for comparison appeared to be a half milliliter injection of a solution containing 10.8 units of vitamin D per milliliter. Fairly good results were obtained by injection of a half milliliter of a solution containing 16.2 units per milliliter. However, the differences in the lines were not as noticeable as in the series in which 10.8 units of vitamin D was used. Apparently, a fairly uniform distribution of oil is obtained with half-milliliter injections.

The experiments involving the time factor resulted as one would expect--the longer the test period, the stronger

was the line obtained. For the levels of vitamin D used in these tests, a ten day period revealed the greatest differences in utilization and was subsequently adopted as the standard procedure. At fifteen days, the differences were less pronounced as may be seen in tables II and III. The five day tests gave very low or negative responses, which indicated the animals had not yet assimilated enough vitamin D to produce noticeable healing. In general, the addition of a fatty acid to a solution of vitamin D increased the rate of absorption of the vitamin by the subcutaneous route. These increases were relatively large in the case of myristic, oleic, and linolenic acids. The test with the castile soap emulsion also showed slightly stronger responses indicating that previous dispersion of the oil accelerates the assimilation of the vitamin D.

With the preparations containing lecithin, weaker lines were obtained than without the lecithin. This decrease in utilization of the vitamin is not readily explained, although it may have been due to decreased dispersion of the oil in the subcutaneous tissues. All of the tests were carried out over a ten day period except those noted in the tables.

Table II

The Effect of the Amount of Vitamin D,
the Volume of the Injected Oil
and the Duration of the Experimental Period
on the Line Test Responses of Rachitic Rats

Units of D per ml.	Volume of Injection	Acid Used	Time on Test	No. of Animals	Average D Line
5.4	1 ml.	None	10 days	4	1.06
8.1	1 ml.	None	10 days	7	1.47
10.8	1 ml.	None	10 days	10	1.37
10.8	1/2 ml.	None	10 days	21	.69
16.2	1/2 ml.	None	10 days	4	1.12
21.6	1/4 ml.	None	10 days	5	.75
32.4	1/4 ml.	None	10 days	6	2.29
8.1	1 ml.	None	5 days	3	.75
8.1	1 ml.	None	15 days	3	2.00
32.4	1/4 ml.	None	5 days	4	1.75
32.4	1/4 ml.	None	15 days	3	2.50
5.4	1 ml.	Myristic	10 days	4	1.37
8.1	1 ml.	Myristic	10 days	7	1.74
10.8	1 ml.	Myristic	10 days	10	1.47
10.8	1/2 ml.	Myristic	10 days	21	1.57
16.2	1/2 ml.	Myristic	10 days	5	1.9
21.6	1/4 ml.	Myristic	10 days	5	.6
32.4	1/4 ml.	Myristic	10 days	5	1.55
8.1	1 ml.	Myristic	5 days	3	.5
32.4	1/4 ml.	Myristic	5 days	3	.83
10.8	1/2 ml.	Myristic	5 days	4	.24

Table III

The Effect of the Addition of Various Fatty Acids
and Lecithin on the Assimilation of Vitamin D
from Subcutaneously Injected Oils

Lecithin Used	Other Treatment	Acid Used	Time on Test	No. of Animals	Average D Line
None	None	Lauric	10 days	7	1.13
None	None	Oleic	10 days	8	1.16
None	None	Linoleic	10 days	5	1.00
None	None	Linoleic	10 days	6	1.37
1%	None	None	10 days	3	.25
None	Emulsified	None	10 days	3	1.00
None	None	Lauric	5 days	4	.43
None	None	Oleic	5 days	4	Neg.
None	None	Linoleic	5 days	4	Neg.
None	None	Linoleic	5 days	4	.81
None	Emulsified	None	5 days	4	.42
1%	None	None	5 days	4	.15
1%	None	Myristic	10 days	8	.09
1%	None	Oleic	10 days	8	1.16
1%	None	Linoleic	10 days	5	.35
1%	None	Linoleic	10 days	7	.57
1%	None	Lauric	10 days	6	.81
1%	None	Lauric	5 days	4	Neg.
1%	None	Myristic	5 days	3	Neg.
1%	None	Oleic	5 days	4	.13
1%	None	Linoleic	5 days	4	Neg.

* Injection - 1/2 ml. of oil - 10.8 units of D per ml.

Discussion

The results obtained in these experiments indicate that the fatty acids in some way accelerate the assimilation of vitamin D from the subcutaneous tissue. This may be due to a greater ease of dispersion of the oil, since it is well known that the presence of free fatty acids in fats or oils greatly facilitates their dispersion or emulsification.

Just what effect the lecithin has on the assimilation of the oil, whether it be physico-chemical or bio-chemical in nature, was not determined. Lecithin does, however, definitely reduce the utilization of the vitamin D. That lecithin or any of the other compounds used do not destroy the vitamin D in the oil was indicated by the fact that when the various oils were incorporated in the basal diet according to the standard method of vitamin D assay, the expected response was obtained. There seemed to be no specific difference between the effects of the saturated and the unsaturated fatty acids, although there were variations to be found among the members of each group. The idea of the oils being dispersed in the tissues due to soap formation is a possible means of explaining the results. However, no studies were made to determine whether or not this idea is tenable.

Summary and Conclusions

1. When mineral oil was fed to rachitic rats at a level corresponding to the laxative dose for humans, there was marked interference with the absorption of vitamin D.
2. The addition of certain fatty acids to subcutaneously injected vitamin D-vegetable oils tends to increase the rate of assimilation of the vitamin D as determined by the line test responses.
3. The addition of lecithin alone or with certain fatty acids to subcutaneously injected vitamin D-vegetable oils tends to retard the assimilation of vitamin D.
4. Emulsification of a vitamin D containing oil, previous to injection resulted in more rapid assimilation of the vitamin D.
5. The effects of adding fatty acids is probably due to increased dispersion of the injected oils.
6. Whether or not the presence of lecithin in oils affects the ease of dispersion was not determined in these experiments. It is possible that some other mechanism may be responsible for the retarding effect of lecithin.

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