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SOME EFFECTS ON ANIMAL NUTRITION OF THE INGESTION OF MINERAL OIL

By

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By Margaret Cammack Smith and Harry Spector

INTRODUCTION

Mineral oil, while it possesses many of the physical properties of edible oils, has one unique property which is the basis for its varied application. This property is its ability to pass through the gastrointestinal tract without being absorbed. It is this property which accounts for its action as a lubricant and its widespread use in the alleviation of constipation. Advantage is also taken of the fact that mineral oil is nonabsorbed and without fuel or food value to the animal body. For this reason it is commonly substituted for the food oil in mayonnaise and salad dressings for use in diets in which the restriction of calories or of fats is necessary—that is, in so-called "reducing diets" and in diets for those whose fat tolerance is low resulting, for example, from gall bladder disturbances.

On the other hand, disadvantages of the continued use of mineral oil have been suggested. Jorstad (1) found that mineral oil introduced into the tissues directly caused cancer in rats, and Burrows and Farr (2) have gone so far as to point to the possibility of a relationship between the great prevalence in America of cancer of the gastrointestinal tract and the extensive use of mineral oil as a laxative.

The postulation that ingested mineral oil, due to its solvent property, might logically be expected to dissolve and retain some of the fat-soluble material with which it comes in contact in the gastrointestinal tract finds some factual support in the literature. More especially, the assimilation of the fat-soluble vitamins A and D in the presence of mineral oil in which they are soluble has received consideration; Dutcher, Ely, and Honeywell (3) have confirmed their previous preliminary findings that when butterfat is diluted with mineral oil, a considerable portion of its vitamin A becomes unavailable. Rowntree (4) concluded from her experiments with rats that the effect of mineral oil on the utilization of vitamin A is "dependent upon the amount of vitamin A in the diet." She pointed out that "when the quantity of mineral oil given to rats is comparable to the human therapeutic dose and the vitamin is inadequate, the rats are unable to withstand the effects of mineral oil, but if the amount of vitamin A in the diet is more nearly optimum, mineral oil can be given without noticeable effects."

Jackson (5) objected to the mixing of the mineral oil with "the entire daily vitamin A ingestion" as being "contrary to human practice," and he repeated the experiments of Dutcher *et al.* (6)

except that he fed the mineral oil separately. He concluded that "although mineral oil when mixed with butterfat prior to ingestion caused a considerable loss of vitamin A, the administration of mineral oil separately caused only a slight diversion of vitamin A and that a moderate increase of butterfat intake protected the animal against deficiency even when mineral oil was given."

Available evidence shows more conclusively that mineral oil ingestion interferes with the utilization of vitamin A when present in its precurser form, carotene, as it occurs in plant tissue. Jackson (5) reports that even the separate administration of mineral oil seriously interferes with the utilization of the carotene of yellow corn. Mitchell (7) reported that from ten to twelve times as much vitamin A fed as spinach was necessary to induce a certain rate of weight gain when mineral oil was given.

In explanation of the action of mineral oil, Dutcher postulates that the hydrocarbons of the unassimilated mineral oil possess a greater solvent effect on carotene than is possessed by the lipids of the digestive juices, thereby preventing intestinal absorption. Conversely, it is suggested that the sterols and lipids of the digestive juices possess a preferential solvent action on vitamin A per se, thereby at least partially promoting utilization by removing this vitamin from the unassimilated oil.

Very recently Curtis and Kline (8) working with humans have found that the feeding of crystalline carotene in vegetable oil caused a rise in blood carotene but a similar rise did not occur when mineral oil was given at meal time along with the carotene. Likewise, Anderson (9) reported that administration of 15 cc. of paraffin oil to patients receiving 7,000 units of vitamin A resulted in a fecal excretion of from 500 to 1,750 units, whereas none was excreted on the same level of intake when no oil was given. He pointed out further that in control experiments vitamin A was excreted in but very small amounts or not at all when a purgative was used. This finding gives support to the view that the increase in excretion of vitamin A when mineral oil is given is due to its solubility in the oil rather than to any acceleration of intestinal evacuation.

Despite the fair abundance of somewhat conflicting reports which appear in the literature concerning the effect of mineral oil ingestion on the utilization of vitamin A, little investigation has been made of its effect on the fat-soluble vitamin D, and what few there are, are not in agreement. Dutcher and his co-workers (3) have observed that cod-liver oil diluted with mineral oil promoted calcification in rachitic rats to the same degree as an equal amount fed without the mineral oil. However, Hawk *et al.* (10) briefly reported that both in preventive and curative experiments mineral oil was found to interfere with the utilization of vitamin D in cod-liver oil when it was used as a diluent in place of cottonseed oil. Jackson criticized their experiments, objecting as before to the mixing of the oil with the vitamin D supply as being contrary to human practice. Jackson reported no difference in

"line test" and "bone ash" findings in rats given vitamin D as viosterol when mineral oil was fed separately. However, he used dosages of vitamin D which were, as he said, "at least 5 times the amount required to produce the maximum effect observed."

Clinical observation of two cases of rickets in children receiving supposedly protective doses of cod-liver oil to whom mineral oil was given daily for correction of constipation has led the authors to investigate further the question of the effect of mineral oil ingestion on animal nutrition.

EXPERIMENTAL PROCEDURE

GENERAL PLAN

In order to obtain information as to the effect of mineral oil ingestion on the nutrition of animals, experiments were conducted along the following lines:

1. Investigation of the effect upon the growth and reproduction of rats caused by the inclusion of mineral oil in the ration.
2. A comparison of the stores of vitamin A in the livers of rats and dogs reared on diets with and without mineral oil.
3. Measurement of the liver reserves of vitamin A in young rats whose mothers had been reared on a stock ration in which mineral oil was included.
4. A comparison of the antirachitic potency of cod-liver oil when fed to rachitic rats both in the presence and in the absence of mineral oil.
5. Calcium and phosphorus metabolism studies in young dogs fed an adequate ration with and without incorporated mineral oil.

AMOUNT AND METHOD OF ADMINISTRATION OF MINERAL OIL[1]

In order that the results might have practical significance, consideration of the therapeutic dose for humans was made in the selection of the amount of mineral oil to be fed to the experimental animals. The difficulty, however, of arriving at a comparable therapeutic dose for rats is apparent when the difference in size, food intake, eating habits, and rate of growth and development are considered.

Jackson (5) and Rowntree (4) independently estimated that 0.5 cc. of mineral oil daily per rat was comparable to an average human therapeutic dose of 30 cc. Rowntree based her estimate on the percentage of the dry diet, and Jackson used the comparative caloric consumption of man and the rat as a basis for his estimate. As to the method of administration of the oil, Dutcher used it as a diluent for the source of vitamin A fed—i.e., butterfat—and Rowntree mixed the mineral oil with the basal diet. Jackson stressing the importance of separation of the mineral oil from the source of the vitamin gave three daily feedings of min-

[1] The mineral oil used throughout was a heavy California liquid petrolatum, product of E. R. Squibb and Sons.

eral oil in order to insure a continuous stream flowing through the intestinal tract, but care was taken to give the fat-soluble vitamins as supplements in the interval between the mineral oil feedings.

Before deciding upon any dosage or method of administration of the mineral oil for this investigation, certain preliminary experiments were carried out. After trials in separate feedings of varying amounts of mineral oil by pipette given at different times of the day and noting the animal response, effect upon rate of intestinal evacuation, etc., the procedure of incorporation of the mineral oil in the basal ration at two different percentage levels was adopted as the most logical. In this way, the amount of mineral ingestion was automatically proportional for the food or caloric intake. Lubrication is continuous as is desired and the method of administration is simpler and more quantitative. The precaution was taken to give the vitamin supplements separately with cottonseed oil used as a diluent. In control experiments no difference in response between inclusion of mineral oil in the diet and separate mineral oil feedings given in the same amount was observed. The effect of variation in the time of day to give the vitamin supplements was also investigated. In comparing the antirachitic effect of standard cod-liver oil when mineral oil was and was not ingested, no difference in results was obtained when the cod-liver oil was fed at 8 a.m. or at 8 p.m. Since the rat usually consumes most of his food at night, this procedure allowed maximum and minimum contact with the mineral oil in the digestive tract without varying the ultimate results. A 5 and 10 percentage level of incorporation of mineral oil in the ration was adopted as reasonable, though akin to minimum rather than maximum human practice. Food records showed an average daily intake of from 0.2 to 0.5 cc. of the mineral oil. This amount of mineral oil caused no diarrhea; the feces were bulkier and softer, but as shown in Table 1 even the higher level of mineral oil ingestion produced slight if any acceleration in the rate of

TABLE 1.—COMPARATIVE RATES OF INTESTINAL EVACUATION IN RATS IN THE PRESENCE AND ABSENCE OF MINERAL OIL.

	Basal diet	Basal diet plus 5% min. oil	Basal diet plus 10% min. oil
	Hours	*Hours*	*Hours*
Trial 1, after fasting 43 hours	11.0	13.0	11.0
	12.0	13.2	10.5
Trial 2, after fasting 12 hours	14.0	14.0	13.0
	13.0	11.0	11.2
Trial 3, after no fasting	11.0	11.6	10.5
	10.5	14.3	11.0
	10.0	13.5	10.0
	14.0	8.75	11.0
Average	11.9	12.4	11.0

elimination. In the experiments testing this point, carmine was used to mark the feces. In some cases the animals were fasted before the test was made so as to insure more uniform intestinal conditions when the mineral oil was administered.

A day and night vigil for the first appearance of the "marker" was kept. In general there appeared more variation in time of elimination between individual rats in the same group than between the levels of mineral oil ingestion (Table 1). Food records were kept throughout.

EFFECT OF MINERAL OIL INGESTION UPON GROWTH AND REPRODUCTION OF RATS

In order to determine the effect upon the growth and reproduction, of the habitual ingestion of mineral oil by animals reared on the usual stock ration used in this laboratory, twenty-seven young male and twenty-seven young female rats were treated as follows. At weaning time, which is 3 weeks of age in this laboratory, the young rats were separated from their mothers which had been raised on Sherman's Diet B composed of one third whole milk powder, two thirds ground whole wheat, and 1.6 per cent sodium chloride and continued on this same ration except that nine males and nine females received 5 per cent mineral oil incorporated in the basal ration and nine males and nine females received the higher level of 10 per cent mineral oil. Litter and sex mates were distributed evenly throughout the three groups of animals. The animals were kept in square metal cages each containing three males and three females. The basal ration and tap water were given ad libitum.

All of the rats were weighed weekly for the duration of the experiment (1 year), and records were kept of the food intake for each cage lot during the first 4 months. The usual procedure for the separation of the pregnant females and their care during the gestation and lactation periods adopted in this laboratory was followed in all cases.

As a basis for comparison of the three lots of rats, records were kept of the number and size of the litters, the weight of mother and of the young at birth, and of the young again at weaning time. Data on these significant points considered as a basis for comparison of the three groups of animals are presented in Table 2.

It may be seen that there was no significant difference between the rates of growth of the three groups of animals. However, the fertility and reproductive records are distinctly adversely affected by the feeding of mineral oil at the higher level of 10 per cent. Not only were the litters fewer in number, but the young had less chance of survival to weaning age when their mothers had been subjected to a daily ingestion of mineral oil at the 10 per cent level.

The 10 per cent oil group of females produced only one third as many litters as the control group, and only 50 per cent of these

TABLE 2.—COMPARATIVE GROWTH AND REPRODUCTIVE RECORDS OF RATS ON AN ADEQUATE BASAL RATION WITH AND WITHOUT MINERAL OIL.

	Basal ration alone	Basal ration plus 5% min. oil	Basal ration plus 10% min. oil
Av. maximum gain of males (gm.)	323.6	336.9	336.3
Av. maximum gain of females (gm.)	242.8	219.7	243.0
Av. no. of litters per female	3.9	4.1	1.3
Av. no. of young born	22.6	22.1	4.7
Av. per cent of young raised to weaning age	78	81	50
Av. weight of young at birth (gm.)	5.1	5.2	4.8

young survived the lactation period. Food consumption records for the first 4 months of the test show that the average actual daily mineral oil consumption was less than 0.5 gram, which is probably less than even a small therapeutic dose for humans.

The continuous ingestion of mineral oil at the 5 per cent level did not appear to affect either growth or fertility adversely. Experiments which follow, however, suggest that the condition of the female rats was not optimum.

EFFECT OF MINERAL OIL INGESTION UPON THE VITAMIN A RESERVES OF MALE AND FEMALE ANIMALS

Further light upon the action of continuous ingestion of mineral oil has been obtained by measuring the reserve of vitamin A in animals which have been raised on adequate rations in which mineral oil was included. This study included the measurement of the vitamin A content of the livers of all the rats used in the experiments just described and also of four experimental dogs, three of which had received basal rations including mineral oil and one serving as the control without mineral oil additions to the ration. Vitamin A occurred in the ration of the rats chiefly in the whole milk powder. The dogs received a separate feeding of 400 U.S.P. units of vitamin A per kilogram of body weight per day in the form of an oil solution of carotene.

At the termination of the experimental feeding period, the animals were killed, the rats by anesthesia and the dogs by injection of chloroform directly into the heart.[2] The livers of all the animals were then dissected free from extraneous tissue, washed free from blood, weighed, and prepared for analysis of their vitamin A content. The method used was essentially that of Davies (11) modified for use in this laboratory (12) as follows:

The livers were first ground and mixed thoroughly, samples weighed for analysis in small Erlenmeyer flasks, and covered with 20 cc. of 5 per cent KOH. The corked flasks were then

[2] The assistance of Dr. William J. Pistor, of the Department of Veterinary Science, is gratefully acknowledged.

stored in the refrigerator until there was time for subsequent digestion. It has been found that under these conditions no destruction of vitamin A occurred in 2 weeks. The remaining liver was preserved by freezing until the assays were satisfactorily completed. The liver tissue was then digested in the KOH solution by heating in an electric oven maintained at 94 to 97 degrees C. for from 4 to 6 hours. Preliminary tests showed that a digestion period of this length allowed for maximum extraction of vitamin A and that some loss occurred during longer extractions. At this point the mixture which was still turbid was transferred to a separatory funnel, shaken strongly with about one half its volume of 95 per cent ethyl alcohol, and then with an equal volume of ether. To prevent troublesome emulsions at this point, a mixture of ethyl and petroleum ethers was used in this extraction as previously done in this laboratory by Bradfield and Smith (12) at the suggestion of the SMA Corporation. The liver tissue-KOH layer was allowed to separate out and was discarded, and the ether fraction was washed free from alcohol two or three times with water. It was then filtered by suction through a layer of anhydrous sodium sulphate in a sintered glass funnel into a wide mouthed flat bottomed Soxhlet flask, the ether rapidly evaporated off on a water bath, and the residue dried by suction.

Vitamin A assay procedure

For measurement of the vitamin A content, the solid residue was dissolved in anhydrous chloroform which had been washed free from alcohol and dried over freshly heated potassium carbonate and subsequently distilled to remove all traces of water which would interfere with the test. Except in cases of unusually high vitamin content of the liver samples, the chloroform solutions of the solid residues were made up to 5 cc. volumes and 0.5 cc. aliquots taken for colorimetric determination of their vitamin A values. Two cc. of antimony chloride prepared by the method of Notevarp and Weedon (13) were used in developing the blue color charactertistic of the antimony trichloride reaction for vitamin A. The colors were then matched in a Lovibond tintometer, care being taken to match them at the time of maximum color development—i.e., 20 to 30 seconds after the addition of the reagent. The average of three or more samples checking within 0.5 of a blue unit irrespective of any yellow, red, or neutral color used in matching the colors was taken as a measure of the vitamin A content of the sample under test. These data are presented in Table 3 for both species of animals.

It is obvious that one specific effect of continuous mineral oil ingestion is its interference with the availability of vitamin A at least for storage in the liver. In both rats and dogs the liver reserves of vitamin A were much lower when mineral oil was present in the ration. Although the basal ration contained ample vitamin A for normal development and permitted storage of an excess above daily needs in the control animals, the inclusion of

TABLE 3.—COMPARATIVE VITAMIN A RESERVES IN LIVERS OF ANIMALS FED ADEQUATE RATIONS WITH AND WITHOUT MINERAL OIL.

Test animals	Vitamin A content of livers in blue units		
	Basal diet alone	Basal diet plus 5% min. oil	Basal diet plus 10% min. oil
9 female rats on each diet	130	16	8
9 male rats on each diet	73	11	6
Dog 35 (negative control)	1,389	.....	.....
Dog 36 (positive control)	2,953	.....	.....
Dog 37	.....	0.0	.....
Dog 38	.....	.....	0.0

mineral oil in the ration served to reduce the availability of the vitamin A in the ration or that fed separately to such an extent that but little if any liver reserves were possible even when the lesser amount of mineral oil was fed. It is quite probable that the inferior reproductive performances of the mineral-oil-fed female rats, hitherto referred to in this paper, were due to this interference in the utilization of vitamin A.

It is interesting to note that in every case the females showed higher vitamin A reserves than the males, although in all cases the same proportionate reduction in the liver reserves occurred as a result of mineral oil ingestion.

Further evidence of mineral oil interference with the utilization of vitamin A by female rats was observed by determining the response of their young to a vitamin A deficient regime after weaning. Following the growth technique used by Sherman for the quantitative measurement of vitamin A, young rats taken at weaning age from mothers who had been reared on rations both with and without mineral oil were placed upon a diet devoid of vitamin A but complete in every other respect. The rats were weighed weekly until death from vitamin A deficiency causes occurred.

Some pertinent findings, as presented briefly below, show that there was a marked difference in the ability of the young rats to withstand a deficiency of vitamin A in their ration, thus giving proof of a difference in the amount of this vitamin which the mother rat passed on to her young. Not only did the young of the mothers who had received no mineral oil grow for a longer period of time before responding to a lack of vitamin A in their diet, but they gained more and survived longer than their mates who were the offspring of mother rats which were reared on the same basal

ration with mineral oil additions. Thus a deleterious effect of the addition of even the lower level of mineral oil feeding which was not evidenced before was obtained. In every case the young of mothers who had had the ration including 5 per cent mineral oil showed an earlier break in resistance to the infections which always result from an inadequate intake of vitamin A.

TABLE 4.—GROWTH AND SURVIVAL OF YOUNG RATS ON VITAMIN A DEFICIENT DIET AS AFFECTED BY MINERAL OIL INGESTION OF THE MOTHERS.

Diet of mothers	No. of rats	Maximum gain in weight (gm.)	First appearance of break in resistance to infection (days)	Survival time (days)
Basal diet	26	59.7	26.3	54.3
Basal diet +5% mineral oil	31	40.0	20.0	30.0

EFFECT OF MINERAL OIL INGESTION UPON THE ANTIRACHITIC POTENCY OF COD-LIVER OIL

The potency of standardized cod-liver oil in curing rickets in rats which were likewise receiving mineral oil has been investigated following the curative procedure for testing for vitamin D activity developed by McCollum, Simmonds, Shipley, and Park (14). Young rats weighing from 55 to 65 grams were placed on Steenbock's high calcium-low phosphorus rickets-producing diet Number 2965 composed of yellow corn, 76 per cent; wheat gluten, 20 per cent; calcium carbonate, 3 per cent; and sodium chloride, 1 per cent. When the precaution was followed of keeping the rats in a dark room from which all direct light was excluded, rats in this laboratory regularly developed severe rickets in 21 days. On the twenty-first day a representative rat from each litter was chloroformed, and the left tibia was dissected free from flesh and prepared for the "line" test of McCollum and co-workers (14) as modified by Bills *et al.* (15) by exposing to direct illumination longitudinally sectioned halves of the proximal ends of the tibias covered with 0.5 per cent silver nitrate solution. If a wide metaphysis in which no calcium salts were deposited indicative of severe rickets was found, the rest of the litter was considered ready for the test. Each rat was then placed in an individual cage with a raised screen bottom and given the same rickets-producing ration but with mineral oil additions, except that for control purposes at least two rats from each litter were continued on the base ration without mineral oil. A standardized solution of cod-liver oil of previously tested potency was fed separately by pipette directly into the mouths of the rats. The cod-liver oil had been so diluted with corn oil that a daily feeding of 0.05 cc. produced a narrow continuous line of calcification at the zone of provisional calcification in the rachitic metaphysis of rats prepared as de-

scribed above in a 10 day test period. This amount of healing identified as two positive by Bills is according to definition the degree of healing produced by 1 Steenbock unit of vitamin D.

Graded amounts of this cod-liver oil were given to the mineral-oil-fed rachitic rats, and the degree of calcification of the rachitic lesions after a 10 day test period as shown by the "line" test was noted in each. The extent of the healing which resulted from the feeding of the cod-liver oil was graded from one to four positive according to the method of Bills *et al.* (15). Care was taken to make significant comparisons within the litter and match sex where possible in order to eliminate litter variation in response. Records were kept of the food intake and gain in weight of each rat in order to guard against erroneous interpretation of results caused by the so-called "spontaneous healing" resulting from the liberation of phosphorus which accompanies loss in weight.

In certain instances corroboration of the comparative "line" test findings was obtained by the determination of the ash content of the right tibias. The bones were extracted in 95 per cent alcohol by refluxing over a water bath for two 9-hour periods. A third extraction using anhydrous ethyl ether followed, and the bones were then dried and ashed at dull red heat to constant weight to determine the percentage of ash in the dry fat-free bone.

The interference of mineral oil ingestion with the utilization of the vitamin D of cod-liver oil is plainly shown by the data presented in Table 5.

TABLE 5.—SUMMARIZED "LINE" TEST FINDINGS SHOWING THE EFFECT OF MINERAL OIL INGESTION UPON THE UTILIZATION OF THE VITAMIN D OF COD-LIVER OIL BY RACHITIC RATS.

Amount of cod-liver oil fed	Basal diet alone		Basal diet plus 5% min. oil		Basal diet plus 10% min. oil	
cc.	No. of rats	Av. "line" test	No. of rats	Av. "line" test	No. of rats	Av. "line" test
0.05	36	2.4 healing	11	no healing	12	no healing
0.1	....	.....	12	1.8 healing	7	no healing
0.15	....	.....	9	2.4 healing	13	1.6 healing
0.25	....	.....	4	2.8 healing	13	2.0 healing
0.50	....	.....	3	3.5 healing	3	2.8 healing

These "line" test findings show that the amount of cod-liver oil which produces a continuous line of calcification in the metaphysis of rachitic rats on the basal ration produces no healing whatsoever when either 5 or 10 per cent of mineral oil is incorporated in the ration. When the amount of cod-liver oil fed was increased, some healing occurred, but it may be seen that at least three times as much vitamin D fed as cod-liver oil was necessary to produce the same degree of calcification when as little as 5 per cent mineral oil was present in the ration, and between five and ten times as much was necessary to compensate for the ingestion of the

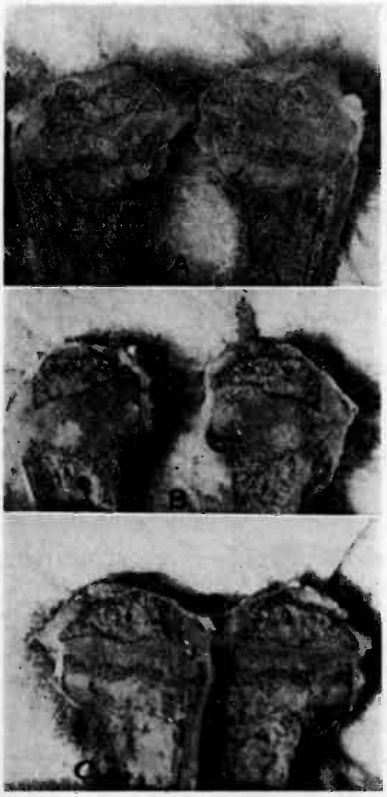

Plate I.—*A*, Calcification of rachitic metaphysis produced by 0.05 cc. cod-liver-oil solution in absence of mineral oil in the ration. *B*, Lack of calcification in rachitic metaphysis produced by 0.1 cc. of same cod-liver oil solution when ration contained 10 per cent mineral oil. *C*, Partial calcification of rachitic metaphysis of mineral-oil-fed rats when five times as much cod-liver oil was fed.

mineral oil at the higher level. Plate I shows typical cases. Records of the food consumption of these rats showed an average food intake of less than 50 grams during the 10 day period of the test so that it is plain that the mineral oil ingested even at the higher level of incorporation in the ration was below a comparable therapeutic dose recommended for humans.

Practical proof of the interference of mineral oil with the utilization of vitamin D and therefore a decrease in the antirachitic potency of cod-liver oil when mineral oil was ingested was thus afforded.

EFFECT OF MINERAL OIL INGESTION IN DOGS UPON THEIR CALCIUM AND PHOSPHORUS METABOLISM

Although rats have been used most extensively for the study of factors affecting the production of rickets and specifically for the measurement of vitamin D, Mellanby (16) has been most interested in the study of experimental rickets in dogs, believing that the course of rickets in puppies more closely resembles that in human beings. In dogs, as in children, a deficiency of vitamin D alone is sufficient to produce rickets without any distortion of the diet in other respects, whereas in rats the calcium and phosphorus in the ration must be abnormal as well.

A study of the effect of mineral oil ingestion by puppies receiving a ration normal in other respects including optimum calcium and phosphorus content and ample provision of vitamin D which regulates the deposition of calcium and phosphorus in the formation of bone has therefore been made. In this investigation the effect of mineral oil ingestion upon the actual metabolism of calcium and phosphorus has been studied by means of continuous

calcium and phosphorus "balance experiments" for approximately a 3 month period during the time of rapid growth and development.

Methods and materials

Four mongrel puppies weaned at 4 weeks of age were placed upon a ration similar to that of Morgan's (17) modification of the Karr-Cowgill laboratory ration for dogs; vitamin A and D supplements were given separately. This diet has been used in this laboratory for several years and has been proved adequate for normal development of the experimental dogs used in previous studies (12). The composition and amount of this diet fed daily is as follows:

Food	Gm./kg. of body weight
Casein (commercial)	19.2
Sugar	20.7
Salt mixture (O and M)	1.0
Brewer's yeast	1.2
Agar	0.4
Lard	5.0

The constituents of this ration were thoroughly mixed and then thinned with distilled water to a mushy consistency at the time of feeding.

When the puppies had become accustomed to their basal ration and were developing normally, they were housed in individual wire-mesh metabolism cages which were designed to permit collection of both the urine and feces. The cages were protected against strong sunlight by enclosing them in heavy brown paper and keeping them in the laboratory after the experimental regime was begun.

Dog 35 was placed on the vitamin A supplemented basal ration without vitamin D, serving as the negative control.

Dog 36 was given the basal ration with both vitamin A and D supplements fed separately and served as the positive control.

Dog 37 was given the basal ration in which 5 per cent mineral oil was incorporated. Vitamins A and D were fed separately.

Dog 38 was given the basal ration in which 10 per cent mineral oil was incorporated. Vitamin A and D supplements were fed separately in the same amount as given to the other dogs.

All the puppies received distilled water ad libitum. They were fed once a day in the morning and the vitamin supplements were given in the late afternoon. In most cases the food was quickly consumed but in instances in which part of it was refused after allowing a reasonable length of time for its consumption, it was returned to the refrigerator and fed the next day before a fresh allotment was given. Record of the food consumed by each dog was kept for the duration of the experimental period.

Vitamin supplements

Vitamin A was fed daily as Smaco, a commercial carotene-in-oil

solution, in the more than adequate amount of 400 U.S.P. units per kilo of body weight. The previous findings of this laboratory (12) have shown that normal growth of puppies was provided for, and a slight storage of vitamin A in the liver was permitted when 200 U.S.P. units of vitamin A per kilo of body weight were administered daily. At this level of supplementation, carotene appeared to be equally as well utilized as cod-liver oil as a source of vitamin A. The effect of mineral oil ingestion upon the reserves of vitamin A found in these dogs at the conclusion of the experiment has been discussed previously in this paper.

Supposedly adequate amounts of vitamin D were given to all the dogs except the negative control (as supplemental feedings of standard cod-liver oil). A level of feeding of 1 U.S.P. unit of vitamin D per kilo of body weight daily was selected on the basis of the findings of Kozelka, Hart, and Bohstedt (18). They reported this amount to be the minimum protective dose to prevent rickets when the diet was optimum in all respects save vitamin D. Although 1 U.S.P. unit per kilo in this study was found not to afford complete protection against rickets, for the positive control dogs later showed some evidences of rickets as enlarged knee and ankle joints, fortunately the effect of mineral oil ingestion was not obscured.

Collection methods

In the first part of this study, attempts were made to make quantitative collections of the urine and feces separately. The urine was collected after filtering through glass wool directly into a bottle containing a layer of the preservative toluene. The feces were collected daily and stored moistened with a 1:1 solution of hydrochloric acid in sealed containers in the refrigerator. At the end of each 7 day balance period, each cage was washed with distilled water and the wash water added to the total collection of urine. In the first period the urine and feces were analyzed separately, the urine being evaporated to dryness on a water bath and ashed; the feces were dried and ashed *in toto*. However, this procedure was tedious and did not lend itself to accuracy because of the great volume of material involved. It was found more satisfactory from every viewpoint to combine the urine and feces in an electric stirrer until a homogeneous mixture resulted, dilute to a known volume, and to take triplicate aliquots for analysis. These aliquots were then dried on a water bath, moistened with hydrochloric acid and alcohol, dried in a constant-temperature oven at 90 degrees, and subsequently ashed at dull red heat in an electric muffle. The ash was then dissolved in a 1:1 hydrochloric acid solution and made up to volume with distilled water. Calcium was precipitated in aliquots of this solution according to the method of McCrudden (19) and titrated with standard potassium permanganate (approximately 0.01*N*) solution in a microburette. Phosphorus was determined colorimetrically by the method of Fiske and Subbarow (20) using amino-naphthol-sulphonic acid

to reduce the ammonium phosphomolybdate to the blue compound.

Samples of the basal ration were analyzed by the same methods and these analyses in combination with the food consumption records gave the basis for data concerning the calcium and phosphorus intakes of the dogs during each 7 day balance period.

Data dealing with the intakes, outputs, and retentions of calcium and phosphorus of both the control and the mineral-oil-fed puppies during the first 6 weeks when vitamin D was fed at the minimum protective level of 1 U.S.P. unit per kilo per day are presented in Tables 6 and 7.

TABLE 6.—COMPARATIVE INTAKES AND EXCRETIONS OF CALCIUM BY DOGS FED AN ADEQUATE BASAL RATION WITH AND WITHOUT MINERAL OIL.

Dog number	Weight of dog (kg.)	Food intake (gm.)	Calcium intake (gm.)	Calcium excreted (gm.)	Calcium retained (gm.)	% of Ca intake retained	Gm. retained per kg.
Series I—dogs received minimum protective dose of vitamin D (1 U.S.P. unit per kg.)							
Period 1—age 65 to 71 days							
Dog 35, negative control, no. vit. D	5.4	795	3.58	2.73	0.85	23.8	0.0229
Dog 36, positive control	4.0	606	2.73	0.79	1.94	71.1	0.0692
Dog 37, 5% mineral oil	4.1	296	.....	.....	.....	.....	.....
Dog 38, 10% mineral oil	4.2	469	1.90	0.74	1.16	61.2	0.0396
Period 2—age 72 to 78 days							
Dog 35, negative control	5.8	1,157	5.22	2.49	2.73	52.2	0.0674
Dog 36, positive control	4.2	1,081	4.87	0.77	4.10	84.1	0.1379
Dog 37, 5% mineral oil	3.9	1,000	4.28	1.32	2.95	68.9	0.1094
Dog 38, 10% mineral oil	4.2	868	3.52	1.34	2.18	61.8	0.0736
Period 3—age 79 to 85 days							
Dog 35, negative control	6.4	1,673	7.54	3.04	4.50	59.6	0.1003
Dog 36, positive control	5.1	1,411	6.36	0.73	5.63	88.4	0.1550
Dog 37, 5% mineral oil	4.6	1,623	.....	.....	.....	.....	.....
Dog 38, 10% mineral oil	4.7	925	.....	.....	.....	.....	.....
Period 4—age 86 to 92 days							
Dog 35, negative control	7.3	1,337	6.03	2.86	3.16	52.5	0.0622
Dog 36, positive control	5.9	1,456	6.56	0.93	5.64	85.9	0.1365
Dog 37, 5% mineral oil	5.0	906	3.88	1.93	1.95	50.3	0.0524
Dog 38, 10% mineral oil	5.1	977	3.96	2.21	1.76	44.3	0.0492

TABLE 6.—COMPARATIVE INTAKES AND EXCRETIONS OF CALCIUM BY DOGS FED AN ADEQUATE BASE RATION WITH AND WITHOUT MINERAL OIL—*Continued.*

Dog number	Weight of dog (kg.)	Food intake (gm.)	Calcium intake (gm.)	Calcium excreted (gm.)	Calcium retained (gm.)	% of Ca intake retained	Gm. retained per kg.
Period 5—age 93 to 99 days							
Dog 35, negative control	7.7	1,842	8.31	3.68	4.63	55.7	0.0862
Dog 36, positive control	6.6	1,765	7.96	1.19	6.77	85.1	0.1470
Dog 37, 5% mineral oil	6.1	543	2.33	0.99	1.33	57.4	0.0371
Dog 38, 10% mineral oil	5.4	945	3.83	1.85	1.98	51.7	0.0525
Period 6—age 100 to 106 days							
Dog 35, negative control	9.0	1,738	7.84	4.13	3.71	47.4	0.0587
Dog 36, positive control	7.8	1,621	7.31	0.70	6.61	90.4	0.1215
Dog 37, 5% mineral oil	5.4	537	2.30	1.29	1.01	44.0	0.0268
Dog 38, 10% mineral oil	5.8	707	2.87	1.78	1.09	38.1	0.0270
Series II—dogs received five times minimum protective dose of vitamin D (5 U.S.P. units per kg.)							
Period 1—age 107 to 113 days							
Dog 35, rachitic control	9.2	2,076	9.4	3.25	6.10	65.2	0.0953
Dog 36, positive control	8.2	1,731	7.8	0.32	7.49	95.9	0.1309
Dog 37, 5% mineral oil	5.4	1,183	5.1	1.46	3.61	71.24	0.0948
Dog 38, 10% mineral oil	5.8	867	3.5	1.65	1.87	53.1	0.0461
Period 2—age 114 to 120 days							
Dog 35, rachitic control	10.4	2,036	9.2	0.51	8.67	94.4	0.1221
Dog 36, positive control	9.4	1,781	8.0	0.49	7.54	93.9	0.1144
Dog 37, 5% mineral oil	6.4	1,642	7.0	1.45	5.58	79.4	0.1244
Dog 38, 10% mineral oil	6.4	1,298	5.3	1.62	3.65	69.3	0.0821
Period 3—age 121 to 127 days							
Dog 35, rachitic control	10.4	2,008	9.0	0.43	8.62	95.2	0.1180
Dog 36, positive control	9.5	1,940	8.7	0.40	8.35	95.4	0.1250
Dog 37, 5% mineral oil	7.0	1,912	8.2	0.83	7.77	94.9	0.1491
Dog 38, 10% mineral oil	6.6	1,855	7.5	2.12	5.42	71.9	0.1171

TABLE 6.—COMPARATIVE INTAKES AND EXCRETIONS OF CALCIUM BY DOGS FED AN ADEQUATE BASE RATION WITH AND WITHOUT MINERAL OIL—*Continued.*

Dog number	Weight of dog (kg.	Food intake (gm.)	Calcium intake (gm.)	Calcium excreted (gm.)	Calcium retained (gm.)	% of Ca intake retained	Gm. retained per kg.
Period 4—age 128 to 134 days							
Dog 35, rachitic control	11.37	2,005	9.0	0.62	8.42	93.1	0.1058
Dog 36, positive control	10.72	1,849	8.3	0.41	7.93	95.1	0.1056
Dog 37, 5% mineral oil	8.22	1,933	8.3	1.66	6.62	79.9	0.1150
Period 5—age 135 to 141 days							
Dog 35, rachitic control	12.2	2,499	11.3	0.56	10.71	95.0	0.1252
Dog 36, positive control	11.2	2,118	9.5	0.52	9.02	94.5	0.1146
Dog 37, 5% mineral oil	8.8	1,880	8.0	2.18	5.88	73.0	0.0949
Dog 38, 10% mineral oil	8.1	1,813	7.4	3.55	3.81	51.8	0.0671

TABLE 7.—COMPARATIVE INTAKES AND EXCRETIONS OF PHOSPHORUS BY DOGS FED AN ADEQUATE BASE RATION WITH AND WITHOUT MINERAL OIL.

Dog number	Phos. intake (gm.)	Phos. excreted (gm.)	Phos. retained (gm.)	% of intake retained	Gm. retained per kg.	Ca/P retention ratio
Series I—dogs received minimum protective dose of vitamin D (1 U.S.P. unit per kg.)						
Period 1—age 65 to 71 days						
Dog 35, negative control	4.37	3.88	0.49	11.3	0.0108	2.08
Dog 36, positive control	3.35	2.18	1.17	35.0	0.0422	1.65
Dog 37, 5% mineral oil	2.32	1.72	0.60	24.5	0.0204	1.94
Period 2—age 72 to 78 days						
Dog 35, negative control	6.36	4.36	2.00	31.5	0.0495	1.36
Dog 36, positive control	5.95	3.04	2.91	48.9	0.0977	1.41
Dog 37, 5% mineral oil	5.22	1.08	4.14	79.3	0.1534	0.71
Dog 38, 10% mineral oil	4.30	2.24	2.06	47.9	0.0695	1.06
Period 3—age 79 to 85 days						
Dog 35, negative control	9.20	5.08	4.12	44.8	0.0909	1.09
Dog 36, positive control	7.76	1.82	5.94	75.5	0.1635	0.95
Period 4—age 86 to 92 days						
Dog 35, negative control	7.35	4.64	2.71	36.8	0.0533	1.17
Dog 36, positive control	8.00	5.60	2.41	30.1	0.0583	2.34
Dog 37, 5% mineral oil	4.73	3.50	1.23	26.1	0.0330	1.58
Dog 38, 10% mineral oil	4.83	3.57	1.26	26.0	0.0353	1.39
Period 5—age 93 to 99 days						
Dog 35, negative control	10.13	6.11	4.02	39.7	0.0750	1.15
Dog 36, positive control	9.71	3.06	6.64	68.4	0.1443	1.02
Dog 37, 5% mineral oil	2.84	2.20	0.63	22.3	0.0176	2.11
Dog 38, 10% mineral oil	4.67	2.96	1.71	36.7	0.0454	1.16
Period 6—age 100 to 106 days						
Dog 35, negative control	9.56	7.35	2.21	23.1	0.0350	1.68
Dog 36, positive control	8.91	3.96	4.95	55.6	0.0919	1.32
Dog 37, 5% mineral oil	2.80	2.52	0.28	10.2	0.0075	3.55
Dog 38, 10% mineral oil	3.50	2.70	0.80	22.8	0.0197	1.37

TABLE 7.—COMPARATIVE INTAKES AND EXCRETIONS OF PHOSPHORUS BY DOGS FED AN ADEQUATE BASE RATION WITH AND WITHOUT MINERAL OIL—*Continued.*

Dog number	Phos. intake (gm.)	Phos. excreted (gm.)	Phos. retained (gm.)	% of intake retained	Gm. retained per kg.	Ca/P retention ratio
Series II—dogs received five times minimum protective dose of vitamin D (5 U.S.P. units per kg.)						
Period 1—age 107 to 113 days						
Dog 35, rachitic control	11.41	6.56	4.85	42.5	0.0968	0.98
Dog 36, positive control	9.52	3.22	6.30	66.2	0.1102	1.19
Dog 37, 5% mineral oil	6.18	2.82	3.36	54.4	0.0382	1.07
Dog 38, 10% mineral oil	4.29	2.34	1.95	45.4	0.0482	0.96
Period 2—age 114 to 120 days						
Dog 35, rachitic control	11.20	4.48	6.72	60.1	0.0924	1.32
Dog 36, positive control	9.79	4.04	5.75	58.7	0.0874	1.31
Dog 37, 5% mineral oil	8.58	3.80	4.78	55.7	0.1065	1.17
Dog 38, 10% mineral oil	6.42	3.28	3.14	48.9	0.0707	1.16
Period 3—age 121 to 127 days						
Dog 35, rachitic control	11.04	5.08	5.96	54.0	0.0817	1.44
Dog 36, positive control	10.67	4.64	6.03	56.5	0.0904	1.38
Dog 37, 5% mineral oil	9.99	4.28	5.71	57.1	0.1161	1.28
Dog 38, 10% mineral oil	9.18	4.32	4.86	53.0	0.1053	1.11
Period 4—age 128 to 134 days						
Dog 35, rachitic control	11.03	3.22	7.81	70.8	0.0984	1.08
Dog 36, positive control	10.17	3.06	7.11	69.9	0.0946	1.12
Dog 37, 5% mineral oil	10.10	4.14	5.96	59.0	0.1035	1.11
Period 5—age 135 to 141 days						
Dog 35, rachitic control	13.74	2.94	10.80	78.6	0.1263	1.00
Dog 36, positive control	11.65	2.76	8.89	76.3	0.1129	1.02
Dog 37, 5% mineral oil	9.82	3.52	6.30	64.2	0.1018	0.93
Dog 38, 10% mineral oil	8.98	3.82	5.15	57.4	0.0908	0.74

In every balance period, the mineral-oil-fed puppies retained less calcium and phosphorus as seen in the absolute amount of these elements retained, in the amount retained per kilo of body weight, and in the percentage of the ingested calcium and phosphorus which was retained in the body. In no balance period did the puppies receiving the mineral oil retain more, and they usually retained even less calcium and phosphorus, than did the negative control dog which was given no cod-liver-oil supplement of vitamin D, thus indicating complete interference with the utilization of this vitamin as the result of mineral oil ingestion. The summarized results of the calcium and phosphorus metabolism studies which appear in Table 8 show that in the 6 week period of observation the mineral-oil-fed puppies retained only about a third as much calcium and phosphorus as did the positive control dog given the same basal ration and the same amount of the vitamin D supplement. Expressed on the basis of body weight, the positive control dog each day retained 0.0181 gram of calcium and 0.0142 gram of phosphorus per kilo as compared with an average daily retention of 0.0069 gram of calcium and 0.0054 gram of phosphorus by the litter mate dog whose ration contained 10 per cent mineral oil. Thus, in spite of supposedly adequate intakes of both calcium and phosphorus and of the vitamin which

regulates the metabolism of these mineral elements, the mineral-oil-fed dogs were not able to make satisfactory use of these essential dietary constituents.

TABLE 8.—SUMMARIZED RESULTS SHOWING THE EFFECT OF MINERAL OIL INGESTION UPON THE CALCIUM AND PHOSPHORUS METABOLISM OF DOGS RECEIVING A MINIMUM PROTECTIVE DOSE OF VITAMIN D DURING A SIX WEEK BALANCE STUDY PERIOD.

Dog number	Calcium			Phosphorus		
	Average daily retention		Average % of intake retained	Average daily retention		Average % of intake retained
	gm.	*gm./kg.*		*gm.*	*gm./kg.*	
Dog 35, negative control	0.49	0.0094	45.2	0.36	0.0123	31.2
Dog 36, positive control	0.73	0.0181	84.8	0.57	0.0142	62.4
Dog 37, 5% mineral oil	0.26	0.0080	55.2	0.22	0.0075	34.5
Dog 38, 10% mineral oil	0.23	0.0069	51.6	0.21	0.0054	23.6

This inability was also clearly reflected in the general nutritive condition of the puppies as shown in Plate II. The characteristic clinical evidences of severe rickets were apparent in the negative control dog which was given no vitamin D, and even more severe symptoms were noted in the mineral-oil-fed puppies which received 1 U.S.P. unit of vitamin D per kilo of body weight daily in the form of cod-liver oil. These puppies showed the flat-footed condition, bowing of the forelegs, rachitic posture of the hind legs, swelling of the wrist and ankle joints, general weakness and inability to support the weight of the body, which are the characteristics of severe rickets. In addition they became very timid, displayed signs of pain, and developed thin straggly hair; the latter is also characteristic of vitamin A deficiency. The negative control dog was decidedly bowlegged but was more active and had a thicker, better-nourished fur coat than did its mineral-oil-fed litter mates.

In a second series of balance studies on the same dogs, the level of feeding of vitamin D was increased fivefold—i.e., 5 U.S.P. units per kilo were given daily. In order to have a basis for comparison of the curative effect of this increased dosage in the presence and absence of mineral oil in the ration, the negative control rachitic Dog 35 was given the same relatively high dose of cod-liver oil as the mineral-oil-fed dogs, and their comparative metabolism of calcium and phosphorus was observed as before. The results are tabulated in Table 9, and photographs of the dogs at the completion of the test period are shown in Plate III.

Plate II.—The effect of feeding mineral oil to dogs for 6 weeks: *Dog 35* (basal diet alone), bowing and flat-footed condition of forelegs, knobs at wrist joint, and inability to support body weight; *Dog 36* (basal diet plus vitamin D), positive control; *Dog 37* (basal diet plus vitamin D plus 5 per cent mineral oil), marked spread and flat-footed condition of forelegs, inability to support body weight, and ruffled condition of hair; *Dog 38* (basal diet plus vitamin D plus 10 per cent mineral oil), marked spread and flat-footed condition of forelegs, inability to support body weight, and ruffled condition of hair.

When vitamin D as cod-liver oil was given to Dog 35, which previously served as the negative control, the retention of both calcium and phosphorus immediately increased. The average daily

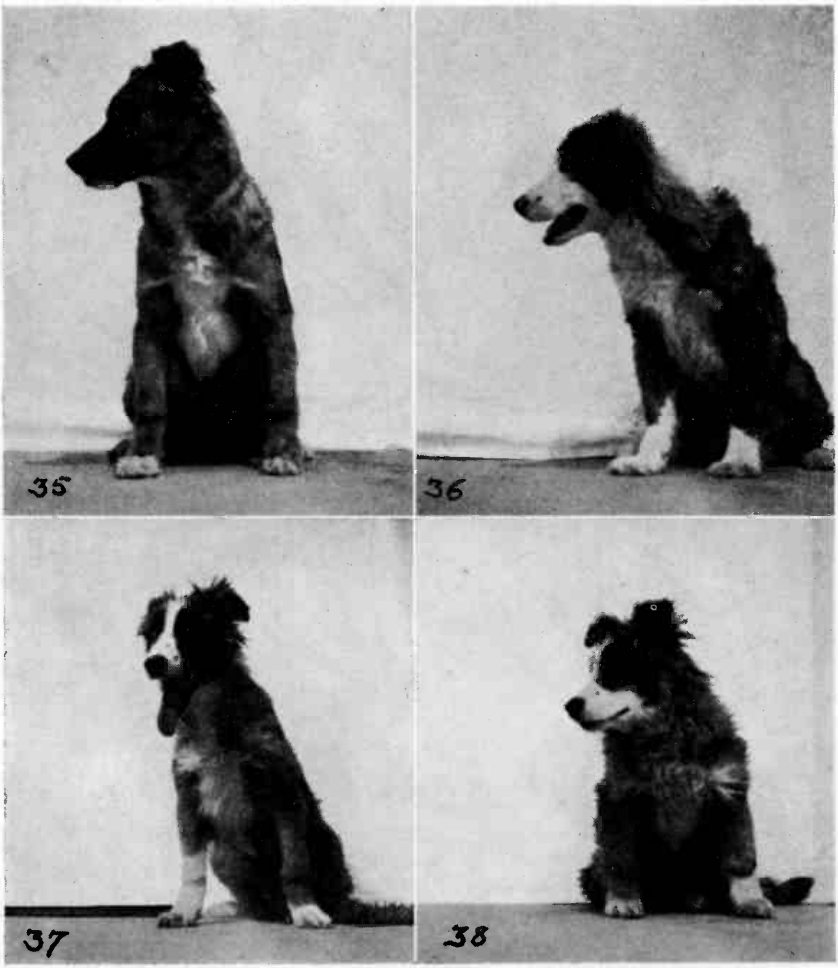

Plate III.—Improvement in dogs after increasing the dosage of cod-liver oil: *Dog 35* (basal diet plus vitamin D), improved condition of forelegs and firm stance; swelling of wrists is only sign of former rachitic condition; *Dog 36* (basal diet plus vitamin D), forelegs are normal except for slight swelling of wrists; *Dog 37* (basal diet plus vitamin D plus 5 per cent mineral oil), bowing of forelegs, swelling of wrists, ruffled condition of fur, and leanness of the dog persist in spite of improvement shown; *Dog 38* (basal diet plus vitamin D plus 10 per cent mineral oil), same rachitic characteristics persist in spite of improvement.

retention of calcium increased from 0.0094 gram per kilo in the first period when no cod-liver oil was given to 0.0162 gram in the second period. The percentage of ingested calcium which was retained increased from 45 to 88 per cent, and there were accompanying parallel increases in the retention of phosphorus. Dog

TABLE 9.—SUMMARIZED RESULTS SHOWING THE COMPARATIVE CURATIVE EFFECT OF COD-LIVER OIL UPON THE ABNORMAL CALCIUM AND PHOSPHORUS METABOLISM OF RACHITIC DOGS WITH AND WITHOUT MINERAL OIL DURING A 5 WEEK PERIOD.

Dog number	Calcium			Phosphorus		
	Average daily retention		Average % of intake retained	Average daily retention		Average % of intake retained
	gm.	*gm./kg.*		*gm.*	*gm./kg.*	
Dog 35, rachitic control	1.21	0.0162	88.6	0.83	0.0141	61.2
Dog 36, positive control	1.15	0.0168	94.9	0.97	0.0141	65.5
Dog 37, 5% mineral oil	0.84	0.0165	79.6	0.74	0.0147	58.1
Dog 38, 10% mineral oil	0.42	0.0111	61.0	0.55	0.0112	51.2

35 was retaining, therefore, practically as much of these mineral elements as Dog 36, which had always served as the positive control. These changes were reflected in the outward characteristics of Dog 35 which changed from an inactive, unsteady, timid animal with severely bowed forelegs to a lively animal which appeared normal except for the enlarged wrist and ankle joints which gave evidence of his previous rachitic condition. Thus, ample evidence that the base ration was rendered complete by the addition of vitamin D as cod-liver oil was afforded.

Again it may be noted, however, that the retention of calcium and phosphorus by the mineral-oil-fed dogs was not optimum. This was especially true in the case of Dog 38 which received the base ration containing 10 per cent mineral oil. Increasing the amount of cod-liver oil fed to this dog fivefold, although it resulted in a greatly increased retention of the bone-forming minerals and a decided improvement in the nutritive condition of the animal, did not make optimum retention possible. Dog 38 in the second period retained somewhat less than two thirds the amount of calcium and phosphorus retained by the rachitic negative control dog which was given the same dosage of vitamin D. Here again, as in the case of the rachitic rats previously discussed, the apparent potency of cod-liver oil in healing rachitic lesions was lessened by the presence of mineral oil in the alimentary canal.

SUMMARY AND CONCLUSIONS

Some effects of the continuous ingestion of mineral oil by experimental animals are as follows (in all cases the mineral oil was incorporated in the basal ration at either the 5 or 10 per cent level):

1. During the period of observation, mineral oil ingestion was found to have no effect upon the rate of growth of rats but to

markedly affect the reproductive performance of the females. At the 10 per cent level of feeding, the females showed a shortened period of fertility, produced only one third as many litters as their non-oil-fed litter mate sisters and were less successful in raising their young to weaning age.

2. Vitamin A reserves in the livers of both the rats and dogs receiving mineral oil, if present at all, were lower than found in the litter mate animals given no mineral oil.

3. The young of female rats reared on rations containing mineral oil possessed smaller stores of vitamin A and were correspondingly less able to withstand subsequent deprivation of vitamin A as indicated by an earlier break in resistance to respiratory infections and shorter survival periods on a vitamin-A-free regime.

4. Mineral oil ingestion interfered with the utilization of vitamin D fed separately as cod-liver oil to such an extent that three times as much cod-liver oil was necessary to induce healing of the rachitic lesions of rats when the base ration contained 5 per cent mineral oil, and somewhere between five and ten times as much was needed when 10 per cent mineral oil was incorporated in the base ration.

5. "Balance studies" showed that mineral oil ingestion by young dogs interfered with the retention of both calcium and phosphorus so seriously that normal calcification of the bony structure was not possible. The mineral-oil-fed dogs showed the characteristics of severe rickets even though they received adequate amounts of calcium and phosphorus and were given a supposedly minimum protective dose of cod-liver oil. Increasing the amount of cod-liver oil fivefold did not provide for optimum retention of the mineral elements in the dog receiving the ration containing 10 per cent mineral oil.

These findings lead to the conclusion that the continuous ingestion of mineral oil in amounts probably not greater than the corresponding therapeutic dose for humans seriously interferes with the utilization of vitamins A and D in rats and dogs and thus adversely affects animal nutrition.

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