THE RELATION OF FAT TO THE UTILIZATION OF VITAMIN A IN THE BODY

by

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INTRODUCTION

Recent research has led some people to believe that there is an increased demand for vitamin A in fat utilization, with a definite existing relationship. Some accepted A-free diets used for vitamin investigation contain fat while others are comparatively fat free. For this reason it seemed of interest to plan experiments to study the relation of vitamin A to fat utilization in the body. In addition to securing information of value in planning vitamin A experiments, it appeared advisable to study the relation of fat in the diet to calcium storage.

REVIEW OF LITERATURE

A search has been made of the literature for references to fat utilization as connected with vitamin A and calcium.

Mallon, Jordon, and Johnson (1) found with two women subjects that variation in the amount of fat in the diet has no effect on the calcium balance. Holt, Courtney, and Fales (2) with their work on the relation of calcium to fat excretion in the stools of infants and children, found that in older children who were taking a mixed diet there was a

great variation in the intake of fat per kilogram and generally no close relation between the fat intake and the calcium absorption. When the fat intake was high, frequently the calcium intake was also high, and as a result there was a good assimilation of calcium. There was no evidence that a very large intake of fat was followed by an excessive excretion of calcium.

The best absorption of calcium oxide occurred when the intake of fat exceeded 3.0 grams per kilo, and when, at the same time, for every gram of fat there was in the diet from 0.03 to 0.05 grams of calcium oxide. The excretion of calcium in the stools was not at all related to the excretion of total fat, but bore some relation to the excretion of fat as soap. The calcium lost as soap in the stools of normal children given a mixed diet was, in most cases, an insignificant part of the calcium intake. Cod liver oil increased the absorption of calcium, except in cases in which the intake of calcium or of fat was very low. The substitution of vegetable fats for milk fat did not affect the calcium metabolism of children taking a mixed diet.

Nakahara (3) in working with rats found that vitamin A helps in the assimilation of foreign fat in the body.

Irwin, Brandt, and Nelson (4) made a statistical study on the data of vitamins A and B feeding experiments com-

paring the Sherman and Munsell vitamin A-free diet (practically devoid of fat) with the Osborne and Mendel A-free diet (containing fat in the form of Crisco), and the Sherman and Spohn B-free diet with the Osborne and Mendel B-free diet. The chief difference between the two vitamin A-free diets and between the two vitamin B-free diets is in the amount of fat contained. It was found that during the experimental period the gain in weight on the Sherman and Munsell fat-free diet was greater than on the Osborne and Mendel diet containing fat. They say, "Whether the difference in gain is due to the difference in caloric intake or to some metabolic property of the fat itself is a question. Takahashi states that vitamin A plays an important part in the combustion of fat consumed and vitamin B functions in proportion to the amount of carbohydrate consumed."

Crisco is a hardened vegetable fat and has been considered suitable for use in A-free diets. Drummond (5) used whale oil for experimental work and found that the "hardening process had destroyed all power to act as a source of fat-soluble A." He reported vitamin A as "completely destroyed." Among those who report the use of Crisco in A-free diets are: Morgan and Osburn (6),

Morgan (7), Huston, Lightbody and Ball (6), and Karshan, Krasnow and Harron (9).

METHOD

The experiments here described were designed to study rations of known and constant vitamin A content, differing widely in fat, but comparable as regards calories, protein, minerals and other vitamins.

At the present time there are a few recognized vitamin A-free diets.

Sherman and Munsell (10) have developed a diet consisting of:

A-free casein	20 per cent
Starch	70 " "
Yeast	5 " "
Osborne and Mendel salt mixture	4 " "
Sodium chloride	1 " "

The Osborne and Mendel A-free diet used by Irwin, Brandt and Nelson (4) consists of:

A-free casein	18 per cent
Starch	51 " "
Yeast	5 " "
Osborne and Mendel salt mixture	4 " "
Crisco	22 " "

Steenbock and Nelson (11) have prepared a basal ration free from vitamin A which consists of:

A-free casein	18 per cent
Salt 32	4 " "
Agar	2 " "
Yeast	2 " "
Dextrin	74 " "

Drumond and Coward A-free diet consists of:

Purified caseinogen	18 per cent
Purified rice starch	52 " "
Refined hydrogenated vegetable oil	15 " "
Yeast extract	5 " "
Orange juice	5 " "
Salt mixture	5 " "

To compare diets differing in amount of fat, the Sherman A-free diet was used as a basis for the four diets, with Crisco to make diets more comparable to that of Osborne and Mendel. The experimental diets developed are:

A-Free Diet I — PRACTICALLY DEVOID OF FAT (Sherman and Munsell Diet)

Grams		Calories
1300 cornstarch	x 3.5	4550.0
400 casein	x 4.0	1600.0
200 yeast	x 4.0	800.0
80 Osb salts		
20 Na Cl		
		6950.0

A-Free Diet II — 25 PER CENT OF CALORIES FROM FAT

Grams		Calories
193 fat	x 9.0	1737.0
804 cornstarch	x 3.5	2814.0
400 casein	x 4.0	1600.0
200 yeast	x 4.0	800.0
80 Osb salts		
20 Na Cl		
		6951.0

A-Free Diet III 45 PER CENT OF CALORIES FROM FAT

Grams			Calories
348	fat	x 9.0	3132.0
405	cornstarch	x 3.5	1417.5
400	casein	x 4.0	1600.0
200	yeast	x 4.0	800.0
80	Osborne and Mendel salts		
20	Na Cl		
			6949.5

A-Free Diet IV 65 PER CENT OF CALORIES FROM FAT

Grams			Calories
502	fat	x 9.0	4518.0
9	cornstarch	x 3.5	31.5
400	casein	x 4.0	1600.0
200	yeast	x 4.0	800.0
80	Osborne and Mendel salts		
20	Na Cl		
			6949.5

The diets were planned so that for each 100 calories any animal would consume, he would receive a constant amount of casein, yeast, Osborne and Mendel salt mixture, sodium chloride and vitamins.

Calories were calculated using figures of Rose (13). Since no figures were available for the calorie value of one gram of the yeast, an estimated value of four calories per gram was used. In any event, this figure would have no effect upon final results since the same amount of yeast was used in each lot of diet.

Vitamin A was removed from the casein according to the Sherman and Munsell method (10).

Patch's cod liver oil was used to furnish the vitamin

A in the diet. At the beginning of the experiments a sufficient supply was purchased to use throughout the period. The bottle of cod liver oil was stored in a box in an electric refrigerator. In preparing a diet, the weighed amount of cod liver oil was first thoroughly distributed through the casein and then this was well mixed with the remaining ingredients.

Vitamin D was supplied by means of cod liver oil and irradiated food. Cornstarch for the foreperiods and casein for the experiments were irradiated by means of an Alpine Sun Lamp, voltage 110, using five amperes. The material to be irradiated was finely sifted on a tray and placed under the lamp for five minutes at a distance of 12 inches. The tray was removed, the material resifted and returned for another five minutes of irradiation.

Diet I, the Sherman and Munsell A-free diet, was used throughout these experiments for the foreperiod to deplete the supply of vitamin A stored by the animals.

Since these experiments were to study vitamin A as connected with fat utilization, it was necessary to add a known amount of vitamin A to the carefully prepared A-free diets. Cod liver oil was chosen as a convenient and potent source of vitamin A.

A preliminary experiment was run to determine the

amount of cod liver oil to be used in the diets. The amount first tried was 1.2 grams of cod liver oil to 6950 calories of diet. The rats grew so large that the amount was reduced to 0.6 grams of cod liver oil for the same amount of food. Less cod liver oil was used so that the animals would grow more slowly, for as Sherman (14) said, "a definite small gain in weight furnishes the best basis for quantitative comparisons."

Albino rats from the Wistar Colony were used for stock rats in this laboratory.

The rats used for breeding were kept on a diet suggested by Sherman and Crooker (15). It consisted of:

Dried whole milk 1/3

Ground whole wheat 2/3

Sodium chloride, 2 per cent of weight of wheat

The diet was fed ab libitum and there was access to distilled water at all times. Complete growth and birth records were kept. At four weeks of age young rats were numbered and those that weighed 35 to 55 grams were used for these experiments.

Following the vitamin A technique of Sherman (10), the test period to find the relation of fat to the utilization of vitamin A in the body was divided into two periods,

the foreperiod and the test period. The purpose of the foreperiod was to deplete the store of vitamin in the body of the animal. This was determined when the rat had been stationary in weight for one week, or when xerophthalmia developed. At this time the animal was put on one of the four diets. Distribution was made according to the litters, and also to males and females.

Each animal was kept in a cylindrical cage, 10 inches in diameter and 12 inches high made of wire screen three meshes per inch, with raised flooring of the same material. Food and distilled water were fed in pint fruit jars. Each day the cages were cleaned and fresh water was provided. The cages and jars were sterilized each week for 20 minutes in a bath of boiling water.

Animals and food were weighed weekly on a torsion balance to the nearest gram. The amount of gain or loss, the number of grams of food eaten during the week, and points noted in close observation of the animals were recorded.

The animals were housed in a south room having three windows. The walls, ceiling and floor were of cement which assisted in controlling the temperature, prevented invasion of vermin, and facilitated cleaning. Steam radiators

were used in cold weather and the temperature was maintained at 70° F. or above. Ventilation was provided without a draft. All food was kept cold until fed.

The experiments were terminated at eight weeks according to the Sherman and Munsell method (10). At this time the animals were killed, except for three females from each diet which were allowed to live until they were 24 weeks of age in order to observe any new conditions. Several males and females from each of the four diets upon completion of the test period were ashed to determine the calcium and phosphorus content of their bodies. The animals were killed, the alimentary tracks removed and the carcasses weighed to obtain the net body weight. The procedure was according to Sherman and MacLeod (16).

Determinations of calcium in the bodies of the animals were made by the McCrudden Method (17) from samples ashed in a muffle furnace. For phosphorus determinations the volumetric method used was the Official and Tentative Methods of Analysis by the Association of Official Agricultural Chemists (18), with magnesium nitrate for ashing.

An autopsy was also made if the animal died before the end of the test period. The general condition of the rat, whether or not it had xerophthalmia, the condition of the

bladder, kidneys, lungs, and the presence or absence of pus in the throat and under the tongue were noted, together with the gain in weight, amount of food eaten, condition of fur and how it behaved during life.

DISCUSSION

From the growth records of the experimental animals composite curves were prepared. These are shown in Figure 1 which gives the composite curves for the males and females on each of the four diets during the eight experimental weeks.

Food records were kept for all experimental animals. The four diets were well consumed, resulting in good growth as indicated by the composite curves. Since the diets had been carefully planned to provide uniform amounts of cod liver oil per 100 calories, it is evident that the rats on each of the different diets always had cod liver oil in a constant ratio to the fat. Thus variation in food intake could bring about no change in the ratio of cod liver oil to fat for any one diet.

The animals on Diet I grew well but those on the diets containing fat made better gains. Animals receiving fat in the form of Crisco did not seem to require addition-

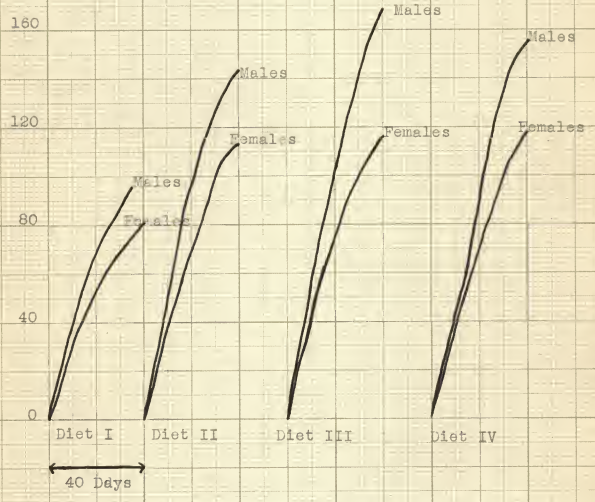

Figure 1

Growth Curves for Animals at Termination of 8 Weeks Experiment

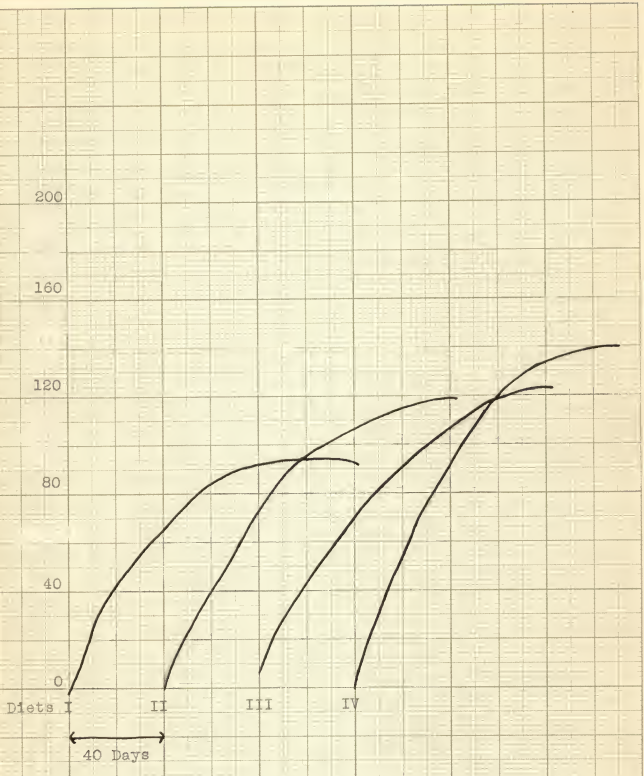

Figure 2

Growth Curves for Females - 24 Weeks Old

al vitamin A to utilize this fat.

Figure 2 gives composite curves of females showing the rate of growth during twenty-four experimental weeks on the four different diets. The animals receiving fat in their rations grew as well, if not better, than the animals whose diet was practically devoid of fat.

Table I gives the average grams of gain of all the animals on the diets for eight experimental weeks, and it also gives the number of experimental animals used. It is definitely shown that animals with fat in their diets grew not only as well as, but better than animals on the diet nearly devoid of fat. They did not need additional vitamin A in order to utilize the fat in the diet.

The sexes were not as evenly distributed as would be desirable, but this was unavoidable using the animals ready for the experiment. On account of this it seemed interesting to make Table II showing the average grams of gain of the females. The conclusions are the same as noted above. These findings are somewhat contrary to those of Irwin, Brandt and Nelson (4) previously quoted.

Table I

Average Grams of Gain of All Animals on Diets for Eight Experimental Weeks

	Average Total Gain of Rats on			
	Diet I	Diet II	Diet III	Diet IV
Males	108	143	166	156
Females	80	104	111	109
	Number of Animals Used for Averages Above			
Males	7	3	7	4
Females	15	15	12	13

Table II

Average Grams of Gain of Most Comparable Females on Diets for Eight Experimental Weeks

Weight at Beginning of Eight Week period	Average Total Grams of Gain for Females on			
	Diet I	Diet II	Diet III	Diet IV
41 to 49 grams	82	125	131	100
50 to 59 grams	67	94	104	108
	Number Animals Used for Averages Above			
41 to 49 grams	5	4	4	5
50 to 59 grams	5	9	7	6

Table III

Number of Animals at Beginning and End of Experimental Period Observed to have:

	No. on Experiment	Sore Nose	Sore eyes		Scaly tails		Nervous Symptoms		Sneezes		Neck Enlarged		Bad Fur		Fat Deposits			
		Begin.	Begin.	End	Begin.	End	Begin.	End	Begin.	End	Begin.	End	Begin.	End	None	Small	Average	Large
															Total Number			
Diet I																		
Males	7	1	4	1	4	6	2		2				1					
Females	16	1	3	2	6	4	1	1	1	1	2		2		1	13	3	3
Diet II																		
Males	3		2	2	1	1												
Females	15	1	7	1	11	11	4	1	3	3			3	1		6	6	3
Diet III																		
Males	7		1		5				1				2					
Females	12	1	4		10	8			1	1						3	9	4
Diet IV																		
Males	4	2	2		1	1	2	1	1									
Females	13		2		111	5	3		3	1			3	1		2	8	4
							Older Animals											
Diet I																		
Females	3				3	3		2		3					3			
Diet II																		
Females	3					3												3
Diet III																		
Females	3																1	2
Diet IV																		
Females	3																	3

Plate I

Photographs of typical rats taken at the termination of the eight week experimental period.

Fig. 1 - Typical rat from Diet I
(almost devoid of fat)

Fig. 2 - Typical rat from Diet II
(25 per cent of calories from fat)

Plate I

Fig. 1

Fig. 2

Plate II

Photographs of typical rats taken at the termination of the eight week experimental period.

Fig. 1 - Typical rat from Diet III
(45 per cent of calories from fat)

Fig. 2 - Typical rat from Diet IV
(65 per cent of calories from fat)

Plate II

Fig. 1

Fig. 2

Plates I and II show photographs of typical males from the four diets, taken at the termination of the eight week experimental period. These were typical of all the animals regardless of sex and are presented to show that the rats were in good condition and had no apparent abnormalities.

During the progress of the experiment notes were taken particularly at the time of weighing concerning the general condition of the animal. These have been assembled and presented in Table III. Comparisons of records made of observations during life of the rats showed among animals on the four diets fairly equal distribution of sore eyes, scaly tails, signs of nervousness, sneezing, and other symptoms.

Comparisons of records made at autopsy showed that animals fed on diets containing fat had distinctly more stored body fat at the termination of the experiments than the animals fed a diet practically devoid of fat. This storage of body fat on diets containing Crisco was especially marked in the animals kept on the diets until 24 weeks of age. On Diet I, only 30 per cent of the animals stored an average amount or more of body fat; on Diet II, 60 per cent stored an average amount or more; on Diet III, 84 per cent of the animals and on Diet IV, 86 per cent of

the animals stored an average or more than average amount of body fat.

The animals kept until 24 weeks of age on Diet I stored small amounts of body fat, but the animals on Diets II, III, and IV stored large amounts of body fat except for one animal on Diet III, which stored an average amount.

Table IV

Average Per Cent of Minerals Based Upon Wet Body Weight

	Calcium		Phosphorus	
	Males	Females	Males	Females
Diet I	1.06	1.14	0.64	0.66
Diet II	0.86	1.11	0.57	0.64
Diet III	0.87	1.40	0.56	0.66
Diet IV	0.95	0.98	0.54	0.50

Table IV shows the per cent of calcium and phosphorus, based on wet body weight for the animals ashed from the different diets at the end of the eight week experimental period. It may be noted that the per cent of calcium for the females for every diet is as great or greater than that of the males on the same diet. Much the same may be said for the phosphorus. In every case except for Diet IV the females had a higher per cent of phosphorus.

The males on Diet I had a higher per cent of calcium than on any other diet and the females were second highest and larger than those on two of the other diets. The males on Diet I had a higher per cent of phosphorus than had animals on any of the other diets, and the females were as high or higher than those on the other three diets.

Sherman and MacLeod (16) found that 90 day old male rats had 0.93 per cent of calcium in the body, and that 120 day old rats had 1.01 per cent of calcium. According to this the males of Diet I only had an abundance of calcium at the age of 105 days, when the experiment was terminated.

For normal females which had borne no young, 1.09 per cent at 90 days and 1.07 at 120 days are the figures given. According to this, all the animals except those on Diet IV were normal.

Sherman and Quinn (19) give figures for the phosphorus content of normal rats. For normal males 0.82 per cent at 92 days of age and 0.85 per cent at 119 days of age are the figures given. According to this only the males of Diet I would be normal.

For females that bore no young 0.88 per cent at 90 days of age and 0.89 per cent at 120 days are the figures presented. According to this, those animals killed at 105

days of age would all have slightly less than the normal per cent of phosphorus.

Table V

Average Grams of Minerals Present in the Body of the Rat

Diet	Calcium		Phosphorus		Weight of the Males	Weight of the Females
	Males	Females	Males	Females		
I	1.620	1.473	0.974	0.851	153.4	129.4
II	1.640	1.463	1.091	0.843	190.0	131.8
III	1.933	2.042	0.783	0.966	220.1	146.1
IV	1.898	1.370	1.074	0.717	199.9	144.7

Table V shows the average grams of calcium and phosphorus present in the animal body, together with the average net weights of the males and females ashed for analysis. As already shown, the males of Diet I stored more calcium in proportion to carcass weight than did animals from other diets. The females of Diet I had a good store of calcium, while the females on the other diets were larger, only one group stored more calcium. The males on Diet I stored more phosphorus compared with the others, and although the other animals were much larger they did not have much more phosphorus. The females on Diet I had a good store of phosphorus in proportion to their weight, and only one group had more grams of phosphorus per rat.

SUMMARY AND CONCLUSION

Four diets were designed using Sherman's A-Free diet as a basis, to study the relation of fat to the utilization of vitamin A in the body, and to study the relation of fat in the diet to the calcium and phosphorus storage.

The diets were planned so that for each 100 calories of food an animal consumed he would receive a constant amount of casein, yeast, Osborne and Mendel salt mixture, cod liver oil, sodium chloride and vitamins A and D.

Diets	Calories in the Diet Furnished by Fat (Crisco)
Diet I	Practically devoid of fat
Diet II	25 per cent
Diet III	45 per cent
Diet IV	65 per cent

Animals that weighed from 35 to 55 grams at four weeks of age were put on Sherman's A-Free diet to deplete their bodies of vitamin A. Distributions of the animals on the four different diets was then made according to litters and to sexes.

At the end of eight weeks the experiment terminated and the animals were killed, except for three females from each diet allowed to live until they were 24 weeks of

age to observe any new conditions appearing. Several males and females from each of the four diets when they completed the test period were ashed to determine the calcium and phosphorus content of their bodies.

An autopsy was made if an animal died before the end of the experiment and also when killed, and careful records were kept during the study.

From the data collected the following points were outstanding:

1. Animals fed on diets differing widely in fat content (Crisco) but comparable as regards vitamin A, protein, minerals, and other vitamins per 100 calories, showed by their growth that those receiving fat grew at least as well as those receiving a diet almost devoid of fat.

2. With Crisco used as the fat an increase of fat in the diet does not demand an increase in the amount of vitamin A in order that the animal may utilize the fat.

3. Comparisons of records made of observations during life of the rats showed fairly equal distribution among animals on the four diets of sore eyes, scaly tails, signs of nervousness, sneezing, and other symptoms.

4. Comparisons of records made at autopsy showed that animals fed on diets containing fat had distinctly

more stored body fat at the termination of the experiments than the animals fed on a diet practically devoid of fat. This storage of body fat on diets containing Crisco was especially marked in the animals kept on the diets until 24 weeks of age.

5. Comparisons of figures for ash determinations showed that the males and also the females fed the diet practically devoid of fat contained more calcium, calculated as per cent of calcium in the carcass, than the majority of the animals fed diets containing fat. Comparing with the normal figures of Sherman and MacLeod (16) it is shown that only the males of Diet I had a normal percentage of calcium. All of the females except on Diet IV had normal percentages of calcium.

6. Similar comparisons of figures for phosphorus determinations showed that the animals fed a diet practically devoid of fat had stored in their bodies approximately as much phosphorus calculated as per cent of the weight of the carcass, as had any of the animals receiving fat. According to Sherman and Quinn only the males of Diet I had a normal percentage of phosphorus. All the females had slightly less than the normal amount of phosphorus.

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