RETENTION OF FLUORINE IN THE SKELETON OF THE RAT RECEIVING DIFFERENT LEVELS OF FLUORINE IN THE DIET [1]

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(Received for publication June 24, 1954)

An accepted criterion for the evaluation of the utilization of dietary fluorine is the uptake of fluorine by the skeleton. This is suggested in the findings of Sharpless and McCollum ('33) and Evans and Phillips ('39) in which the femurs or tibias were used as a measure of the dietary fluorine uptake under various experimental conditions. The latter authors estimated the fluorine level of their experimental diet to be 1.6 p.p.m. They stated that when rats received this diet over various periods of time there was little change in the fluorine concentration in the skeleton, although in one instance their data indicated a considerable decrease in fluorine concentration after 7 months on the diet. The total amount of fluorine in the bones was not indicated. When sufficient fluorine was fed (as sodium fluoride) to increase the concentration in the diet by 0.1 p.p.m., 72 p.p.m. of fluorine were found in the femurs after 4 months and 44 p.p.m. after 7 months. These findings corroborate those of Sharpless and McCollum ('33). Not until 1.0 p.p.m. fluorine (as sodium fluoride) was added to the basal diet did Evans and Phillips ('39) find that the dietary fluorine level kept the concentration in the femurs from decreasing with age.

[1] This investigation was supported in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-12.

In addition to the decrease in fluorine concentration in the femurs when the fluorine intake continues to be low, a sex difference in the metabolism of fluorine is apparent in the data of Sharpless and McCollum. However, they did not comment on the difference and apparently it was not believed to be significant.

The purpose of this investigation was to prepare a diet as free from fluorine as possible and then determine the essentiality of fluorine for the rat by comparing animals receiving the fluorine-low diet with those ingesting the same diet without removing the fluorine. The comparison was with respect to weight, growth and reproduction, as well as to the relation of age to the uptake and storage of fluorine in the femurs of both males and females. All of the fluorine was present as a constituent of the basal diets or as a contaminant, since no fluorine compound was added to any of the diets used in the study.

EXPERIMENTAL

A total of 222 rats of the McCollum strain were used. The composition of the diets is given in table 1. The rats on diets I, II and III were born of breeders receiving the same three diets respectively. Group IV rats were fed substantially the same diet as those in group III with the exception that all of the components besides those very low in fluorine were carefully purified to reduce the fluorine to a very low level. The rats on this latter diet were born of breeders on diet III and were placed on diet IV at 30 days of age. This procedure was followed because reproduction was not as good on diet IV as on diet III. Redistilled fluorine-free (F = 0.005 μg per ml) drinking water was used for the animals of group IV. Ordinary distilled water containing 0.09 p.p.m. of fluorine was given to those of groups I, II and III.

The vitamin mixture used in diets II, III and IV was composed of commercial thiamine HCl, 50 mg; riboflavin, 50 mg; pyridoxine HCl, 50 mg; calcium pantothenate, 250 mg; nicotinic acid, 250 mg; inositol, 500 mg; p-aminobenzoic acid,

500 mg; folic acid, 25 mg; menadione, 25 mg; biotin in 25 mg "fluorine-free" starch, 2.5 mg; vitamin B_{12} (crystalline) in 200 mg sodium chloride, 0.20 mg; choline chloride, 6000 mg; "fluorine-free" starch 42.0 gm.

TABLE 1

The composition of the experimental diets

COMPONENT	DIET			
	I	II	III	IV[1]
	%	%	%	%
Ground yellow corn	64	..	..	
Powdered whole milk	30	..	..	
Alfalfa leaf meal	3	..	..	
Irradiated yeast	2	..	..	
Sodium chloride	1	..	..	
Sucrose	..	72	..	
Casein	..	18	20	20
Wesson oil (cottonseed oil)	..	5	..	..
Vitamin mixture	..	1	1	1
Salts 12 a	..	4	4	4
Percomorph oil, 20 drops per kilogram of diet where used	..	+	+	+
Starch	..	..	60	60
Butterfat	..	..	15	15
Fluorine, p.p.m.	3.1	2.2	1.8	< 0.1

[1] All the components except the percomorph oil and the vitamins in the vitamin mixture were purified to reduce the fluorine content to a very low level.

The salt mixture used in diet II (salts 12a) was prepared by mixing sodium chloride, 293 gm; potassium dihydrogen phosphate, 817 gm; magnesium sulfate, 120 gm; calcium carbonate, 801 gm; ferric citrate, 69 gm; manganese sulfate, 9.4 gm; zinc sulfate, 2.5 gm; cupric sulfate pentahydrate, 1.0 gm; and potassium iodide, 1.7 gm. The salt mixture for diet III was the same except that "fluorine-low" calcium oxide [2] was substituted for calcium carbonate, and iron oxide prepared in this laboratory was used instead of ferric citrate.

[2] Secured from Fischer Scientific Company, Pittsburgh, Pennsylvania. The fluorine content was 1.8 p.p.m. as determined by analysis in our laboratory.

The iron oxide was prepared by dissolving pure iron metal in hydrochloric acid. It was precipitated as the hydroxide by the addition of ammonium hydroxide. Following this it was dried. The salt mixture for diet IV was the same as that used for diet III except that all the other components were purified by recrystallization.

Fluorine was determined by a standard method (Smith and Gardner, '48). The procedure involves the steam distillation of the sample from perchloric acid and silver perchlorate. Titration of the unknowns was made with thorium nitrate, using alizarin red S as the indicator.

RESULTS AND DISCUSSION

Table 2 shows the fluorine uptake in the femurs of the rats receiving the various experimental diets as a function of age when the feeding was ad libitum. These data confirm the findings of previous investigators (Sharpless and McCollum, '33 and Evans and Phillips, '39) in demonstrating a decrease in fluorine concentration in the skeleton with age when the animal receives a diet containing approximately 3 p.p.m. of fluorine or less. This occurred even though three different types of experimental diets were used. In the group of animals receiving diet I the uptake of fluorine was the greatest, and increased until the animals were between 50 and 90 days old, after which time a decrease in concentration occurred, as indicated by the analytical data for rats 160 days old. Similar findings were observed in the animals receiving diet II. There was an increase until about 80 days, after which the concentration in both males and females decreased.

The highly purified diet IV contained less fluorine than has been reported for any diet that is moderately adequate. The rats receiving this diet also gave results similar to those described above, but in this instance the decrease in fluorine concentration began to occur earlier. At 51 days the animals (combined sexes) had approximately 31 p.p.m. of fluorine while at 240 days only 23 p.p.m. were found.

TABLE 2

Fluorine storage in the femurs of rats receiving diets containing varying levels of naturally occurring fluorine

DIET	DAYS ON DIET	NO. OF RATS		AV. BODY WEIGHT		TOTAL ASH		FLUORINE CONCENTRATION		TOTAL FLUORINE	
		M	F	M	F	M	F	M	F	M	F
				gm	*gm*	*mg*	*mg*	*p.p.m.*	*p.p.m.*	*μg*	*μg*
I	30	6	8	67	66	53	51	88 ± 18[1]	86 ± 5	4.5 ± 0.91[1]	4.5 ± 0.99
(3.1 p.p.m. F)	51	6	10	159	120	133	113	186 ± 27	165 ± 22	25.1 ± 2.16	20.0 ± 1.90
	93	15	11	240	190	252	222	191 ± 24	160 ± 25	48.2 ± 4.25	39.6 ± 3.65
	160	8	10	294	215	376	281	131 ± 21	144 ± 25	49.5 ± 5.48	39.2 ± 3.22
II	40	6	4	130	90	106	77	51 ± 3	48 ± 14	4.4 ± 1.05	3.9 ± 1.06
(2.2 p.p.m. F)	54	5	5	192	110	122	98	70 ± 8	60 ± 9	8.9 ± 1.08	8.3 ± 1.20
	68	4	7	203	150	128	114	98 ± 19	83 ± 13	13.0 ± 1.37	9.9 ± 1.20
	82	14	6	258	196	213	169	78 ± 11	70 ± 10	18.0 ± 2.55	13.0 ± 1.29
	96	6	10	264	203	221	208	57 ± 5	63 ± 8	19.6 ± 1.66	14.4 ± 1.51
IV	30	4	5	49	42	42	39	tr.	tr.	tr.	tr.
(0.1 p.p.m. F)	51	6	6	152	140	140	137	39 ± 4	23 ± 2	5.1 ± 0.66	3.0 ± 1.02
	240	3	3	357	209	385	256	19 ± 1	26 ± 2	9.3 ± 1.36	8.9 ± 1.04

[1] Standard deviation.

In no instance did the total amount of fluorine decrease in either sex. This is in contrast to the findings of Jackson et al. ('50), who reported that the total fluorine content of the rat was decreased when a diet containing 4.6 p.p.m. of fluorine was fed over a period of 18 months. At 6 months they found 3.22 mg of fluorine per rat carcass, at 12 months, 4.25 mg, and at 18 months, 3.05 mg. However, they stated that, "the total fluorine found per carcass was fairly constant and was independent of of length of time on the diet." It was a constant finding in the present study that the concentration of fluorine in the females exceeded that in the males after the concentration in both sexes had begun to decline. A possible explanation for this may be that when the fluorine level of the diet is quite low, the available fluorine cannot keep pace with the increasing size of the skeleton. This observation was suggested by Savchuck and Armstrong ('51) when they stated, "... skeletal accretion would have the effect of diluting the fluorine present in the skeleton." When a stock corn diet containing 8.1 p.p.m. of fluorine was fed, the males always had a higher concentration of fluorine than the females. Thus, at this level of dietary fluorine, the concentration in the femurs continued to increase with age. This indicates that the decrease in concentration noted when diets contained less than 3 p.p.m. of fluorine could be due to the skeleton being formed faster than the food dietary can furnish fluorine.

One possible explanation is that the two sexes consume different amounts of food. However, Zipkin and McClure ('53) in studies on fluorine storage in rats have reported that the capacity of the rat to deposit fluorine is not affected by semi-starvation *per se* but is directly related to the fluorine intake per unit of tissue mass. Under normal conditions the male rat eats more food than the female.

To investigate this more thoroughly a paired feeding experiment was conducted. Four litters of McCollum strain rats were separated as to sex, litter mates, weight, general appearance, et cetera and were placed at 30 days of age on a diet

similar to diet III, except that sucrose was used instead of starch. The fluorine concentration of this diet was 1.8 p.p.m. The paired feeding technique was designed to supply identical amounts of food to each male and female. This was accomplished by accurately determining the amount of food eaten by the female and providing the paired male with the same amount. Table 3 summarizes the results of this experiment.

Under conditions of paired-feeding it was again demonstrated that after about 70 days of age both the males and the females began to show a decrease in the fluorine concentration of the femurs. From about 70 days the females had a greater concentration than the males. The data on total amount of fluorine under these conditions appear to confirm the work of Jackson et al. ('50). After about 70 days both the males and females began to lose stored fluorine, and from about 72 days the females contained considerably more fluorine in their femurs than the males. This is also similar to the sex difference in the metabolism of iron. It indicates that the storage of fluorine in the skeleton is not only dependent upon the amount of food ingested, but also upon the level of fluorine in the diet and the age and sex of the animals.

A word is necessary concerning the accuracy of the method for determination of fluoride. It has been routinely possible to determine fluorine in food and in calcified tissues which contain less than 20 μg F/gm with an accuracy of about 0.5%. The data presented in table 2 indicate that there is a significant difference in both sexes (probability of 0.002) between the total fluorine found in the femurs of the animals receiving diet IV at 51 and 240 days. In order to further strengthen these fluorine storage data, pooled samples from each age group and for each sex were examined and they confirmed the analytical values obtained for the animals receiving both diets II and IV.

One of the primary objectives of this study was the preparation of a diet totally devoid of fluorine. However, even with exceptionally careful purification the diet still contained traces (< 0.1 μg/gm). Although the actual amount of fluorine was so low as to make an accurate estimate impossible, it is

TABLE 3

Comparison of the concentration and total amount of fluorine in the pooled femurs of pair-fed males and females at various ages

Each value represents the mean of six litter-mate rats [1]

AGE	CONSUMED TOTAL FOOD		TOTAL ASH		FLUORINE CONCENTRATION		TOTAL FLUORINE		TOTAL FLUORINE INGESTED	
	M	F	M	F	M	F	M	F	M	F
days	*gm*	*gm*	*mg*	*mg*	*p.p.m.*	*p.p.m.*	*μg*	*μg*	*μg*	*μg*
42	210	209	113	110	72.5 ± 9.1 [2]	70.6 ± 6.2	8.2 ± 0.19	7.8 ± 0.19	378	376
56	348	348	170	207	53.7 ± 5.0	39.0 ± 1.9	9.1 ± 0.20	8.1 ± 0.15	626	626
70	519	520	196	147	73.4 ± 7.5	90.5 ± 8.7	14.4 ± 0.32	13.3 ± 0.41	934	936
84	651	651	194	159	25.0 ± 0.8	59.0 ± 4.4	trace	trace	1171	1171

[1] Diet used in this experiment was identical with diet III except that sucrose replaced starch. The fluorine concentration of this diet was 1.8 p.p.m.

[2] Standard deviation.

known that some fluorine must have been present since fluorine was recovered in the skeleton. Thus, any comments relative to the essential nature of this element cannot be made as a result of this study. However, certain facts were obvious, the conclusions from which may or may not be related to the essentiality of fluorine. The most common finding was related to the difficulty of obtaining young from rats receiving the highly purified diet. After many attempts to obtain second generation rats, a different diet had to be used. Attempts to determine if this was due to the highly purified nature of the diet or to the lack of fluorine were inconclusive, and this fact obviously needs to be studied further. Although the animals receiving the highly purified diet seemed to grow slowly at first, the final weights were not sufficiently different from those of the controls receiving the similar unpurified diet to warrant any definite conclusions. The skeleton, the teeth and other organs were not markedly different from those of the controls, as judged both from gross observations and histological examinations. However, in connection with the suggested role of fluorine in dental health, an increase in dental caries would not have been expected, since the animals did not receive a cariogenic diet. In short, this study, which was conducted with a diet very low in fluorine, does not indicate either the essentiality or non-essentiality of fluorine. Further study is in order especially in regard to the effect of fluorine in cellular enzyme systems, on calcification and its suggested role with respect to certain endocrine organs (Muhler and Shafer, '54).

SUMMARY

The storage of fluorine was determined in the femurs of rats fed three diets containing different amounts of naturally occurring dietary fluorine. On diets containing 3 p.p.m. of fluorine or less the concentration of fluorine decreased after about 75 days of age. Diets containing about 8 p.p.m. of fluorine appeared to permit the skeleton to acquire increasing concentrations of fluorine.

Females had lower concentrations of fluorine in their femurs as long as the concentration was increasing, but after the concentration had begun to decrease the males had lower concentrations. This was observed in animals fed ad libitum and in those in which the males were restricted to the same food intake as the females. The sexual difference may be dependent upon the rate of skeletal growth.

ACKNOWLEDGMENT

The author wishes to thank Professor Harry G. Day for his constant academic stimulation, and his suggestion of initiating this study, as well as his helpful suggestions throughout the investigations and the preparation of the manuscript.

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FAT EXCRETION

THE INFLUENCE OF DIETARY FAT ON FECAL FAT EXCRETION [1, 2]

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(Received for publication May 18, 1954)

The presence of fat in the feces of fasting animals and of animals on a fat-free diet is well known. The early work of Hill, Bloor, Sperry and Angevine, cited by Bloor ('43) provides the strongest available evidence in support of the hypothesis that fat is secreted into the intestinal lumen from the blood either directly or indirectly. The term "endogenous fecal fat" originated with this group of workers and refers to fat which does not have as its source the unabsorbed dietary fat, and which is measured by the fecal fat excretion of animals on fat-free diets.

Wollaeger et al. ('47), compiling data from the literature, found a linear relationship between the amount of dietary fat and the amount of fecal fat and concluded that, "unabsorbed dietary fat may account for a larger proportion of the fecal fat than is commonly supposed, and that it is necessary to use standard test diets in which the amount and kind of fat (and other foodstuffs) are kept constant in order to detect small degrees of abnormal fecal fat loss (steatorrhea)."

Wollaeger et al. ('53) conducted a study, the results of which became known during the course of the present investi-

[1] Taken from the dissertation submitted by Leonard N. Norcia ('52) in partial fulfillment of the requirements for the degree of Doctor of Philosophy to the Faculty of the Graduate School, University of Minnesota.

[2] Hormel Institute publication no. 101.

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gation, concerning the metabolism of simple triglycerides in the human. They found that upon changing the subjects from a fat-free diet to a diet containing triolein as the sole fat, a relatively large quantity of material analyzing as saturated fatty acids appeared in the feces. These workers report obtaining evidence to indicate that the "saturated" acids appearing in the fecal fat were produced by saturation of oleic acid by the intestinal flora.[4] Our experiment was expected to yield a different kind of evidence that would either corroborate this explanation or support an alternative possibility that the presence of dietary fat had an effect on the amount of endogenously excreted fat. Apart from its fundamental importance, the latter would be a consequence of importance in the determination of the digestibility of fats by the conventional methods.

In this research with rats, using simple triglycerides as the sole dietary fat as a means of labeling dietary fat, it was found that the presence of fat in the diet was without effect on the nature or quantity of excreted non-dietary fatty acids, and that changing the body fats by long-term ingestion of different conditioning diets was not reflected in the nature of the endogenously excreted fat.

The effects of the several dietary fats on the composition of rat body fats were also studied and will be reported in a subsequent publication.

EXPERIMENTAL

Plan of the experiments. Two preliminary experiments were performed; one with 11 rats on a lard conditioning diet (exp. A) and one with three rats on a tripalmitin conditioning diet. The former was useful in standardizing methods, and also in providing control data, since it was felt that the body

[4] More recent work by these workers (Chipault, '52, '53) indicates that these "saturated" acids are probably hydroxy fatty acids formed by action of the intestinal flora on dietary oleate, and that the main reason for the high fatty acid excretion during the triolein regime appears to be due to the manner in which the triolein was ingested.

fats of rats on the lard diet would be similar to those of rats maintained on an ordinary mixed diet. The experiment involving the diet containing tripalmitin was done primarily to ascertain whether the animals would eat and maintain health while taking such a diet.

Following these preliminary studies two main experiments were performed using 25 rats each. In one experiment weanling male rats were conditioned for 30 days on a diet containing 15% tripalmitin (exp. B) as the sole fat and in the other 15% olive oil (exp. C). Following the 30-day conditioning period, 5 rats from each group were sacrificed by etherization and the pooled body tissues used for study of the effects of conditioning on body fats. The remaining 4 sets of 5 animals from each group were placed on the following dietary regimens for a 10-day period: fat-free diet (dextrose substituted isocalorically for fat), 15% tripalmitin diet, 15% triolein diet, and 15% tripalmitin-trilinolein diet (1:1 mixture, weight basis). During the last 7 days of the 10-day period, feces were collected and pooled from each set of animals. The animals were sacrificed by etherization at the end of the 10-day period and various tissues samples were obtained from each set. The fats extracted from the feces and the tissue samples were characterized analytically.

Composition of diets. The composition of the diets used is given in table 1. The carbohydrate was a 1:1 mixture of dextrose and corn starch for experiment A. For experiments B and C, dextrose was used exclusively. Except during those periods when some groups of animals received isocaloric quantities of dextrose in place of fat, all of the animals received 15% fat as previously described. The fat soluble vitamins A, D, and E were supplied in the dietary fat in amounts exceeding accepted minimum requirements by 43 to 100%. 2-Methyl-1,4-naphthoquinone was dissolved in the dietary fat in amounts of 10 mg/1000 gm of basal mixture.

The animals were caged individually in a manner avoiding access to spilled food and feces. A special non-scatter type of feeding cup was used. The spilled food, as measured by

TABLE 1

Diets used

BASAL MIXTURES	Exp. A	Exp. B	Exp. C	SUPPLEMENTS OTHER THAN FAT SOLUBLE VITAMINS	Exp. A	Exp. B	Exp. C
	%	%	%		*gm/kg basal mixture*		
Carbohydrate	54	54.5	54.5	Methyl cellulose (Dow methocel, 4000 cps)	8	50	70
Purified casein [1]	22.5	20.5	20.5				
Fat	15	15	15	Nordihydroguaiaretic acid (dissolved in dietary fat)	0.015	0.015	0.015
Salt mixture [2]	5	5	5				
Yeast extract [3]	2.5	4	4	Ascorbic acid	0.5	0.5	0.5
Liver extract [4]	1	1	1	Choline hydrochloride	none	1	1

[1] Reprecipitated technical casein exhaustively extracted with methyl alcohol and ether.

[2] Hubbell, Mendel and Wakeman ('37) salt mixture modified to contain trace amounts of Zn and Co.

[3] Fleischmann's type 41 obtained from Standard Brands, Inc., New York, N. Y.

[4] Wilson's Liver Fraction L obtained from The Wilson Laboratories, Chicago, Illinois.

collecting on paper trays and weighing, was found to be about 3.5% of the food fed. Food loss for the different experiments was uniformly small. From the food intake data of the preliminary studies, a feeding schedule was prepared for experiments B and C slightly below the ad libitum levels. Thus, the food intake for experiments B and C was closely controlled and was virtually the same for the two experiments.

Extraction of fecal and tissue fat. Each sample of pooled feces (about 50 gm) was ground with a mortar and pestle, acidified with 6 ml of concentrated HCl, extracted twice with 75 ml portions of boiling absolute ethanol, and then extracted 5 to 7 times with 75 ml portions of boiling 1:1 ethanol-diethyl ether. The pooled extracts were reduced to small volume by evaporation under reduced pressure and extracted with petroleum ether (30 to 60°C.). The fecal lipids were recovered by complete removal of the solvent from the petroleum ether extract under reduced pressure.

The pooled tissue samples were minced in a Waring Blendor, extracted twice with boiling absolute alcohol, and then extracted three times with boiling 1:1 ethanol-diethyl ether. The lipids were obtained from the pooled extracts in the same manner as that described for the fecal lipids.

All lipid samples so obtained were stored in stoppered vials under refrigeration at about —15°C. until analyzed.

Measurements and analyses. Measurements and analyses made on the samples obtained included total lipid, total nonlipid dry matter, unsaponifiable matter, total fatty acids, melting point (capillary tube), neutralization equivalent, iodine value (½ hr. Wijs), fatty acid composition (alkali conjugation-spectrophotometric method), and peroxide values. In addition, the total wet and dry weights of the feces were obtained.

RESULTS

Growth rates. The growth curves of the animals are compared in figure 1 with a growth curve obtained by others

using a more normal diet. It is evident that the diets used in these experiments did not produce optimum growth. The food intake data suggest that the apparent inadequacy is

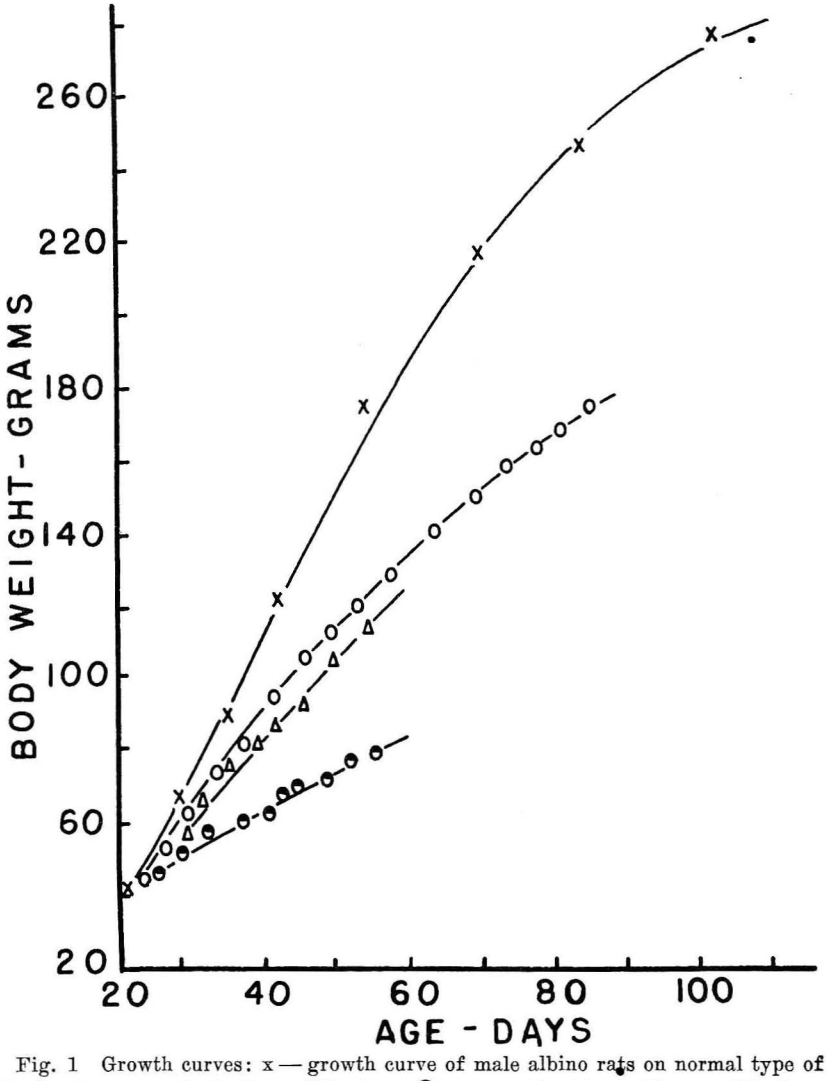

Fig. 1 Growth curves: x — growth curve of male albino rats on normal type of diet[1]; ○ — exp. A, lard conditioning; ◓ — exp. B, tripalmitin conditioning; △ — exp. C, olive oil conditioning.

[1] Taken from Hawk, P. B., B. L. Oser and W. H. Summerson, Practical Physiological Chemistry, Blakiston Co., Philadelphia, 12th ed., p. 1264 (1947). Courtesy of The Blakiston Company.

probably attributable largely to lack of palatability. Other contributing factors could include a lack of essential fatty acids in the tripalmitin conditioning diets, plus caloric insufficiency owing to the poor absorption of tripalmitin, and a possible deficiency of unknown dietary essentials in all diets.

TABLE 2

Fecal fat analysis, experiment A (lard conditioning) and analytical values for lard

AVERAGE VALUES OF THE POOLED WEEKLY FECES OF 11 RATS OVER A 6-WEEK PERIOD			VALUES FOR LARD [2]
		Std. Dev.[1]	
Total lipid, grams/week/11 rats	4.9	0.89	
Total fatty acids, grams/week/11 rats	3.9	0.80	
Unsaponifiable matter, per cent	9.5	1.2	0.2
"Melting point" of fatty acids	56–59°C.	..	36–42°C.[3]
Neutralization equivalent	292.3	1.4	285.9 [4]
Iodine value, fatty acids	22.0	0.28	46–70 [5]
Monoethenoic acid, % of acids	17.7	0.49	50.4
Diethenoic acid, % of acids	2.41	0.11	6.0
Triethenoic acid, % of acids	0.38	0.072	
Tetraethenoic acid, % of acids	0.21	0.042	
Saturated acid, % of acids	79.4	0.39	41.5
Conjugated diene, as % octadecadienoic acid	0.45	0.046	

[1] Standard deviation calculated by the formula $s_x = \sqrt{\frac{(x-\bar{x})^2}{n}}$ in which s_x is the standard deviation, $(x-\bar{x})$ is the deviation of each result from the mean, and n is the number of individual results.

[2] From Bailey ('45).

[3] Titer of lard.

[4] Saponification equivalent of lard.

[5] Iodine value of lard.

Fecal fats of rats on diet containing lard. Feces collection was begun after the weanling rats had been on the lard diet for 10 days and the pooled fecal output for a full week from all 11 rats was collected for 6 consecutive weeks. The mean values obtained in analysis of the weekly samples of fecal lipids is shown in table 2. Comparison of these data with a typical analysis of lard reveals that the fecal lipid differed

from the dietary fat mainly in being more saturated and containing more unsaponifiable matter.

The absolute quantities of unsaponifiable matter in the fecal lipids remained essentially constant during the 6-week period. At the same time, the absolute quantities of total fecal lipids and fecal fatty acids decreased from 6.17 and 5.20 gm respectively in the first week to 4.20 and 3.27 gm in the 6th week. This indicated an increasing efficiency in fat absorption in the growing animals, or, alternatively, a decreasing production of endogenous fecal fat. Results of the subsequent experiments make the former appear more likely.

Fecal fats of rats in experiments B and C. The analytical data for the fecal fats of experiments B (animals given preliminary conditioning on tripalmitin) and C (preliminary conditioning on olive oil) differ in several ways (table 3). The principal findings are as follows:

1. The total lipid excreted was greater in the fat-free C group than in the fat-free B group. This quantitative difference was evident in both the fatty acid and the unsaponifiable moieties. Qualitatively, the lipids were the same in spite of differences in body fat composition induced by the previous conditioning diets, as will be discussed later. Therefore, since all of the C groups consisted of larger animals than the B groups, the difference in total lipid excretion appeared to be related directly to difference in animal size, although it is possible that the difference is within the limits of experimental variation.

2. Quantitatively and qualitatively, the fecal fats from triolein and fat-free groups of tripalmitin-conditioned animals were not significantly different. In the case of olive oil-conditioned animals, the rats of the triolein group excreted some 0.22 gm more fatty acid than the fat-free group. This is accounted for largely by an increase of 0.12 gm monoethenoic acid and 0.09 gm saturated acid excretion for the triolein group as compared to the fat-free group. The increased monoethenoic acid very likely represents unabsorbed oleate while the increased saturated acid may have originated

TABLE 3

Fecal lipid analysis, experiments B and C
(Tripalmitin and olive oil conditioning, respectively, for 30 days)

Each sample represents the pooled feces of 5 rats for the last 7 days of the 10-day diet

ANALYSIS	EXP.	10-DAY DIET			
		Fat-free	Tripalmitin	Triolein	Tripalmitin-trilinolein
Total lipid, grams	B	0.743	20.38	0.651	13.17
	C	0.902	33.51	1.231	12.04
Total fatty acids, grams	B	0.284	19.34	0.257	11.23
	C	0.335	29.6	0.549	9.83
Unsaponifiable matter, grams	B	0.218	0.492	0.183	0.303
	C	0.271	0.469	0.282	0.275
Unsaponifiable matter, % of total fat	B	29.2	2.4	28.1	2.3
	C	30.0	1.4	22.9	2.3
Melting point of fatty acids, degrees cent.	B	37–45	59–62	35–45	60–62
	C	36–48	61–63	36–45	61–63
Neutralization equivalents	B	340	278	381	272
	C	347	255	325	255
Iodine values, fatty acids	B	43.2	1.65	40.1	7.6
	C	48.75	0.90	51.2	3.1
Monoethenoic acids, % of acids	B	36.0	0.10	34.6	0.00
	C	34.7	0.79	43.6	1.94
Diethenoic acids, % of acids	B	3.72	0.41	3.68	3.74
	C	5.02	0.19	3.48	0.64
Triethenoic acids, % of acids	B	0.91	0.30	0.72	0.64
	C	(—0.13)	0.07	0.05	0.07
Tetraethnoic acids, % of acids	B	0.47	0.00	0.09	0.00
	C	2.52	0.00	1.65	0.00
Saturated acids, % of acids	B	58.9	99.2	60.9	95.6
	C	57.8	98.95	51.2	97.35
Conjugated dienes, as % octadecadienoic acid	B	1.82	0.12	2.02	2.61
	C	3.33	0.07	1.34	0.32
Peroxide values, m.eq./1000 grams	B	0.0	0.0	0.0	
	C	89.0		66.0	

through conversion of dietary oleate by the intestinal flora to a material analyzing by difference (spectrophotometric) as saturated acid as suggested by Wollaeger et al. ('53). In any case, the differences are small and the findings indicate virtually complete absorption of dietary triolein by the rats, irrespective of conditioning, and further appears to indicate the absence of any influence of the concurrently-fed triolein on endogenous fecal fat.

3. The fatty acid composition of the fat-free and triolein groups indicates the qualitative similarity of these fats, as mentioned previously. The triolein C group shows higher monoethenoic acid and lower saturated acid percentages than the remaining fat-free and triolein groups. This difference is accountable on an absolute basis as mentioned in section 2 above. The saturated acids of the fat-free and triolein groups approach 60%. Rat body fats containing 60% of saturated acids have been obtained only with trilaurin or coconut oil conditioning by a number of other workers. Thus the fatty acid composition of the fecal fats from these groups is evidence of the large difference in these fats from animal body fat. The fecal fatty acids of the groups receiving tripalmitin were composed almost entirely of saturated acids, representing unabsorbed palmitate.

4. The neutralization equivalents of the fat-free and triolein groups are excessively high for mixed fatty acids of animal origin, indicating the presence of nonfatty acid material in these extracts. Neutralization equivalents for tripalmitin conditioned animals receiving tripalmitin-containing test diets were higher than those for olive oil-conditioned animals receiving the same test diets. The significance of this observation is not apparent.

5. The unsaponifiable matter in the fecal lipids of the tripalmitin groups is significantly higher than in the fat-free and triolein groups. When fat is being poorly absorbed from the bowel, it seems likely that lipid and lipid soluble material entering the bowel with the gastrointestinal juices and glandular secretions will also be poorly absorbed. This may account

for the greater amount of unsaponifiable material for the tripalmitin groups.

6. The absorption of tripalmitin by the rats was poor and much palmitic acid therefore appeared in the fecal fat. Particularly noteworthy, however, is the finding that the animals of the tripalmitin B group (tripalmitin conditioned) even though smaller in size absorbed more tripalmitin than the corresponding tripalmitin C group (olive oil conditioned) animals. This would indicate that under some conditions, at least, the digestibility or absorption of a fat may depend markedly on the type of fat consumed during the previous dietary history.

7. In the tripalmitin-trilinolein groups, the tripalmitin-conditioned animals showed no better absorption of tripalmitin than the olive oil-conditioned group. Apparently the presence of appreciable amounts of unsaturated triglycerides assisted in the absorption of tripalmitin by rats in the C group to an extent that overcame any advantage gained by the B group through tripalmitin conditioning.

8. In comparing the fecal fat excretion of animals receiving a lard containing diet (table 2) to that of animals on a fat-free diet (table 3), it is seen that the presence of lard in the diet caused a marked increase in the fecal lipid, corroborating the findings of Wollaeger et al. ('47, '53) that unabsorbed dietary fat may account for a larger proportion of the fecal fat than is commonly supposed. The fatty acid composition of fecal fats from rats on a lard diet, plus the findings (a) that animals on a triolein diet had a fat excretion similar to that of animals on a fat-free diet, while (b) animals receiving tripalmitin had very large amounts of fecal fat, suggest further that the amount of fecal fat excretion over and above the endogenous fecal fat excretion is directly related to the absolute amounts of dietary long chain saturated fatty acids. Mattil ('46) has shown that the general limiting factor of digestibility of fat is the amount of saturated fatty acids present.

Another evidence of the lack of influence of dietary fat on the endogenous fecal fat is present in these data. In table 4 are given the sums of all fatty acids (calculated on a mg/rat/day basis) found in the fecal lipids of the various groups receiving dietary fat, exclusive of those fatty acids that were present in the fat of the diet. These values are therefore an arbitrary measure of the endogenous fecal fat.

TABLE 4

Comparison of "Endogenous" fecal fat excretion of animals on fat-free and fat-containing diets

EXPERIMENT	"ENDOGENOUS" FECAL FATTY ACIDS, MILLIGRAMS/RAT/DAY					
	Dietary regimen					
	Tripalmitin	Fat-free	Triolein	Fat-free	Tripalmitin-trilinolein	Fat-free
B	4.4	3.4	4.8	5.2	2.1	3.0
C	8.9	4.0	8.9	6.3	5.7	3.6

TABLE 5

Ratio of saturated acids to monoethenoic acids for fecal and depot fats

Experiments B and C

EXPERIMENT	FECES	PERIRENAL		MESENTERIES		HAMS	
	Fat-free	Control	Fat-free	Control	Fat-free	Control	Fat-free
B	1.64	0.731	0.598	0.805	0.646	0.427	0.461
C	1.67	0.468	0.563	0.483	0.503	0.446	0.451

They are compared with the corresponding sums for the fat-free groups. Conparisons are also made of the effects of the two types of conditioning on these values.

The excretion of non-dietary fatty acids is slightly greater in the C groups, again a reflection of animal size. There appear to be no consistent differences produced by the various fat-containing diets compared with the fat-free diets. The endogenous fat in these experiments therefore appeared not to have been significantly affected either quantitatively or qualitatively by conditioning or by the contemporary dietary

fat, within the limits of experimental variation due to uncontrolled factors.

Relationship of body fat to fecal fat. As a means of comparing body fats with endogenously excreted fat, the ratios of saturated to monoethenoic acids were calculated for depot fats and endogenously excreted fats of experiments B and C.

Certain limitations in these comparisons must be pointed out. First, fat from the hams is not primarily a depot fat as is the case with the perirenal and mesenteric fats. Second, when the animals are placed on fat-free diets the fat depots undergo relatively marked changes in composition in the 10-day fat-free dietary period. For this reason, ratios of saturated to monoethenoic acids are given for both the control (animals sacrificed after conditioning period) and fat-free (animals sacrificed after fat-free dietary period) groups. Thus the recorded values may be regarded as the extremes of a range of ratios that prevail in the fat-free dietary period. Third, if the composition of the fecal fat was related to body fat composition, the ratios for the fecal fat would likewise be undergoing change; hence the ratio obtained represents an average value for the 7-day period of feces collection.

It is seen in table 5 that the ratio of saturated acids to monoethenoic acids in the fecal fats is virtually the same irrespective of whether the animals have been tripalmitin conditioned (B) or olive oil conditioned (C). The ratios for the true depot fats, on the other hand, are considerably greater for the B groups than for the C groups, particularly for the control animals. During the 10-day fat-free dietary period, the depot fats underwent changes such that the ratios for the B group decreased in all cases except the hams sample, and increased for the C group. At the end of the period the differences were no longer as great as might be desired but nevertheless persisted. Moreover the ham fat, which was least representative of a fat depot and therefore more susceptible to rapid turnover, was least like the fecal fat in fatty acid composition. These results are interpreted to mean that the composition of the endogenous fecal fat is independent

of the composition of, or changes in the body fats. This, therefore, appears to be independent evidence in support of the view that the major portion of the endogenous fecal fat does not originate from a secretion of fat into the intestinal lumen or from a desquamation of cells from the mucosal epithelium of the intestines, and suggests strongly that the endogenous fecal fat is synthesized by intestinal microorganisms.

To explain our data in terms of the theory of origin of endogenous fecal fat from body fat would necessitate postulating one or more of the following events: alteration of structure of the fatty acids by the animal organism during transit from the fat depots to the intestinal lumen, alteration of structure of the fatty acids in the intestinal lumen, selective or differential secretion of fatty acids into the intestinal lumen, selective or differential reabsorption of fatty acids previously secreted into the intestinal lumen, or selective or differential assimilation and catabolism of fatty acids by the intestinal flora. While any one or a number of the above possibilities are feasible it is difficult to visualize how frankly different body fats of differently conditioned animals would be altered in such a manner as to produce virtually identical endogenous fecal fat unless the alterations in the two cases were entirely fortuitous. In any case, the origin of endogenous fecal fat through synthesis by the intestinal flora should be investigated further by more direct methods.

Another point of interest in the data of table 5 is that the ratio of saturated to monoethenoic acids for ham fats is less for the tripalmitin-conditioned than for the olive oil-conditioned animals, and further that the former ratio increases and the other ratios for the tripalmitin-conditioned group decrease during the fat-free dietary period. The significance of these findings is not known, but may be related to the deficiency of essential fatty acids throughout the dietary history of the tripalmitin-conditioned group.

The ratios in table 5 are of further interest in that they are quite different from the findings of Eckstein ('25) who

reported, "The ratio of liquid to solid fatty acids of the subcutaneous fat from the abdomen of man is similar to that reported by others for the fat of the blood and feces." Our findings are in accord with those of Wollaeger et al. ('53) who showed no similarity between blood lipids and fecal lipids in the human.

TABLE 6

Digestive coefficients

DIETARY REGIMEN	EXP.	FATTY ACID INGESTED AS TRIGLYCERIDE	FATTY ACID EXCRETED	DIGESTIBILITY OF FATTY ACID
		gm fatty acid	*gm*	*%*
Fat-free	B		0.102 Monoethenoic a. 0.0106 Diethenoic a. 0.167 Saturated a.	
	C		0.116 Monoethenoic a. 0.0168 Diethenoic a. 0.194 Saturated a.	
Tripalmitin	B	38.4 Palmitic a.	19.2 Saturated a.	50.5
	C	44.8 Palmitic a.	29.3 Saturated a.	35.1
Triolein	B	39.4 Oleic a.	0.089 Monoethenoic a.	100
	C	43.2 Oleic a.	0.239 Monoethenoic a.	99.6
Tripalmitin-trilinolein	B	21.5 Palmitic a.	10.73 Saturated a.	49.2 (Palmitic a.)
		21.6 Linoleic a.	0.419 Diethenoic a.	98.0 (Linoleic a.)
	C	22.8 Palmitic a.	9.56 Saturated a.	58.7 (Palmitic a.)
		22.9 Linoleic a.	0.0624 Diethenoic a.	99.8 (Linoleic a.)

Digestive coefficients of ingested fats. The data obtained on food intake and fat excretion for animals of experiments B and C when they were placed on the various test diets were used to determine the digestibilities of the several dietary triglycerides used. The data obtained are given in table 6. It should be observed that the digestive coefficients were calculated on a fatty acid basis.

The data of table 6 show that the digestibility of tripalmitin following tripalmitin conditioning was 50.5 and 35.1% follow-

ing olive oil conditioning. This suggests, as pointed out earlier, an increased tolerance of a difficultly digestible fat following conditioning upon it.

Mattil and coworkers (Longenecker and Mattil, '42; Mattil and Higgins, '45) in studying the digestibility of tristearin in rats found that tristearin was almost completely indigestible when mixed with triolein but that tristearin was partially utilized by the rat when it was the only fat in the diet. They suggested that a selective utilization may exist. Our data show that tripalmitin was not more poorly absorbed when fed as a mixture with trilinolein than when tripalmitin was fed alone. On the contrary, as pointed out earlier, the data from rats in group C show that the absorption of tripalmitin was facilitated by the presence of trilinolein; however, this is not the case with rats in group B.

SUMMARY AND CONCLUSIONS

Fat excretion was studied in two groups of rats following a 30-day period of conditioning during which the animals took diets containing either 15% tripalmitin or 15% olive oil as the only lipid. The two groups were then divided into subgroups, each of which was placed on one of 4 test diets for 10 days, during the last 7 of which feces were collected for analysis. The test diets included: a fat-free diet, a tripalmitin diet, a triolein diet, and a tripalmitin-trilinolein diet. The animals were sacrificed and analyses of the lipids in various body tissues were made. The lipid analysis of the samples obtained included: unsaponifiable matter, total fatty acids, melting point, neutralization equivalents, iodine value, fatty acid composition, and peroxide value.

From the data obtained it is concluded that: The endogenous fecal fat was unaffected either in quantity or composition by the dietary fats used in these experiments. When tripalmitin-containing diets were fed, however, a considerable portion of the excreted fat represented unabsorbed dietary fat, whereas triolein appeared to be completely absorbed.

Although the depot fats of rats were markedly different depending on whether they had been conditioned on tripalmitin or olive oil feeding, the composition of the depot fats, and changes in composition induced by dietary changes, exerted no influence on the composition of the endogenous fecal fat.

Both of these findings support the view that endogenous fecal fat does not result from secretion of fat into the intestinal lumen, or from desquamation of epithelial cells of the intestinal mucosa, and hence are consonant with the view that endogenous fecal fat is synthesized by intestinal bacteria.

The digestibility of tripalmitin when present as the sole fat in the diet was found to be increased by conditioning on a tripalmitin diet.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. J. R. Chipault, Hormel Institute, for supplying all the pure triglycerides used in these studies.

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BODY FAT DEPOSITION

THE INFLUENCE OF LARD, OLIVE OIL AND SOME SIMPLE TRIGLYCERIDES ON RAT BODY FATS [1, 2]

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(Received for publication May 18, 1954)

In a recent study of endogenous fecal fat excretion by the authors (Norcia and Lundberg, '54), simple triglycerides were used as sole dietary fats primarily for the purpose of labeling dietary fat. In this study rats were conditioned on lard, olive oil, or tripalmitin. The effects of these several types of conditioning on rat body fats are reported here. Also, groups of olive oil and tripalmitin-conditioned animals were subdivided and the subgroups placed on several new dietary regimens differing from the conditioning diet in the fat component. The changes that occurred in the body fats with the new dietary regimens were observed during a 10-day period.

EXPERIMENTAL

The plan of the experiments, composition of diets, extraction of tissue fats, and measurements and analyses were described in detail in a previous publication (Norcia and Lundberg, '54).

In the study of the effects of conditioning on body fats three different types of conditioning diets were used: a 15%

[1] Taken from the dissertation submitted by Leonard N. Norcia (1952) in partial fulfillment of the requirements for the degree of Doctor of Philosophy to the Faculty of the Graduate School, University of Minnesota.

[2] Hormel Institute publication no. 102.

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lard diet (exp. A), a 15% tripalmitin diet (exp. B), and a 15% olive oil diet (exp. C). The diets of experiments B and C differed from that of experiment A by containing 1 gm per kilogram of choline hydrochloride. The dietary source of choline in experiment A was the yeast and liver extracts which were present in all diets.

In experiment A, 11 weanling (23 days old), male albino rats (Sprague-Dawley) were individually caged and placed on a diet containing 15% lard for 63 days, following which the animals were allotted to two groups of 5 and 6 animals each and sacrificed. Tissues were removed from all animals and pooled for each group. The tissue lipids were extracted and characterized analytically.

Similar procedures were followed in experiments B and C, except that these experiments employed 25 animals each for a 30-day conditioning period. Following the 30-day conditioning period of experiments B and C, 5 animals from each experiment were sacrificed and pooled tissues were obtained for study of the effects of conditioning. The remaining animals of each experiment were placed in groups of 5 for 10 days on the following diets: fat-free, 15% tripalmitin, 15% triolein and 15% tripalmitin-trilinolein (1:1 mixture, weight basis). After the 10-day period on the new diets, all animals of experiments B and C were sacrificed and tissues were removed and pooled for each 5-animal group for study of the body fats.

RESULTS AND DISCUSSION

Effects of conditioning on body fats. The effects of the several different types of conditioning on the nature of the body fats of the animals are given in tabular form, table 1. The values given for lard conditioning are values obtained from one of the groups on lard conditioning.

Since it has been established that the nature of animal body fat varies with the nature of the dietary fat, it was felt that the body fats of rats receiving lard as the dietary fat would more nearly approach the usual type body fat

of rats on ordinary mixed diets than would be the case for rats receiving tripalmitin or olive oil as dietary fat. Hence, the effects of conditioning with tripalmitin or olive oil containing diets on body fats are referrable to the body fats of rats receiving lard as the dietary fat.

Prior to making such comparisons, certain uncontrolled factors in the experiments which may have contributed to the nature of the body fats for the several experiments should be pointed out. Since body fat originates through both synthesis from carbohydrate and protein precursors and deposition of dietary fat as body fat, it is desirable that growth rates of the animals be normal for the several experiments in the belief that synthesis will then account for more nearly the same quantities of body fat in the different experiments, provided intestinal absorption of the diets is the same in all experiments. As was pointed out in a previous publication (Norcia and Lundberg, '54) the diets used in these experiments did not produce normal growth, and this inadequacy was more apparent for the animals on tripalmitin conditioning. Unquestionably, the absence of essential fatty acids from the tripalmitin-conditioning diets contributed to the changes in the body fats as well as to the growth rates of these animals.

Also to be considered are several sources of error in the fatty acid composition analyses. First, hexaenoic and pentaenoic acids were not measured; the content of these in the fats examined, however, is known to be small. Second, autoxidation of some samples prior to analysis occurred to some extent. Nonetheless, it is felt that the data are useful in that they show qualitative changes in the body fats under the various experimental conditions.

In comparing the body fats of the differently conditioned animals, a number of findings are of interest.

1. The iodine values for body fats of the differently conditioned animals corroborate numerous similar findings in this and other species by other workers that feeding of a hard fat results in decreased iodine values and elevations of

TABLE 1

Effects of conditioning on body fats

MEASURED VALUES AND TYPE OF CONDITIONING	TYPE OF FAT				
	Liver	Organ [1]	Perirenal	Mesenteric	Ham
% unsaponifiables					
Lard	9.4	7.2	0.6	1.1	1.7
Tripalmitin	8.7	11.8	4.1	3.0	3.3
Olive oil	11.8	9.0	1.5	. .	2.7
"Melting point" of fatty acids, °C.					
Lard	38–41.5	40–43	39–42.5	40–43.5	38–42.5
Tripalmitin	39–43	37–43	34–41	41–45	38–44
Olive oil	35–37	36–40	35–39	37–40	33–36
Neutralization equivalents					
Lard	287	287	273	272	278
Tripalmitin	335	306	280	275	283
Olive oil	287	304	267	267	274
Iodine values of fatty acids					
Lard	103	76	58	59	68
Tripalmitin	85 [2]	73 [3]	55 [3]	50	68
Olive oil	114	90	70	70	78
Monoethenoic acids, % of acids					
Lard	37	45	51	50	50
Tripalmitin	79 [2]	71 [3]	57 [3]	55	68
Olive oil	50	71	64	63	61
Diethenoic acids, % of acids					
Lard	13	8.7	5.8	6.4	9.2
Tripalmitin	6.2 [2]	4.2 [3]	1.5 [3]	0.8	2.7
Olive oil	11	8.5	6.0	6.1	9.8
Triethenoic acids, % of acids					
Lard	0.0	0.0	0.4	0.3	0.0
Tripalmitin	0.4 [2]	0.0 [3]	0.3 [3]	0.1	(—0.1)
Olive oil	(—5.1)	(—0.5)	0.5	0.4	(—0.1)
Tetraethenoic acids, % of acids					
Lard	15	6.0	0.1	0.4	2.1
Tripalmitin	0.6 [2]	0.3 [3]	0.1 [3]	0.0	0.7
Olive oil	14	3.2	0.0	0.3	1.7
Saturated acids, % of acids					
Lard	35	40	43	43	39
Tripalmitin	14 [2]	24 [3]	41 [3]	44	29
Olive oil	25	17	30	30	28
Conjugated dienes, as % octadecadienoic acid					
Lard	1.0	1.3	0.2	0.2	0.5
Tripalmitin	4.0 [2]	2.8 [3]	0.8 [3]	0.5	1.4
Olive oil	1.6	3.5	0.4	0.3	0.3

[1] Pooled hearts, lungs, kidneys, and spleens.

[2] Peroxide value above 600 m. eq./kg. Analytical value likely to be in error from this cause.

[3] Peroxide value not determined. Also, no peroxide values were determined for the samples of the lard group.

melting points of body fats while feeding of a soft fat results in increased iodine values and lowered melting points of body fats.

2. The neutralization equivalents of the samples from tripalmitin-conditioned animals are not lower than those for lard-conditioned animals, indicating that no appreciably large amounts of dietary palmitate were deposited in tissues as palmitate.

3. The melting points of the body fats of tripalmitin-conditioned animals are not appreciably different from those of lard-conditioned animals, while those of the olive oil-conditioned animals are distinctly lower than those of the lard-conditioned animals.

4. A preliminary study with three rats conditioned on tripalmitin gave somewhat different analyses for body fats than was obtained in experiment B (tripalmitin conditioning). In the preliminary experiment, iodine values were at least 7 to 9 points lower, monoethenoic acids 9 to 17 percentage points lower, and saturated acid 8 to 15 percentage points higher. Experiment B differed from the preliminary experiment in that the diet of experiment B was supplemented with choline hydrochloride as previously mentioned. It appears that these differences in the body fats for the two experiments are correlated to the amounts of dietary choline.

5. Since measurement of total body fat per unit body weight was not made, the total amount of fat (petroleum ether solubles) extracted from the several tissues removed from each 5-animal group is used as a rough index of whether body fat depletion took place or not. These data are shown in table 2. While the amount of total fat is not correlated to total tissue weight, the data nonetheless indicate that body fat was depleted in the tripalmitin-conditioned animals, calculated either on the basis of fat per rat or fat per unit body weight. The depletion of body fat on tripalmitin conditioning may be accounted for by one or a combination of several factors: (a) caloric insufficiency; the tripalmitin-conditioned animals absorbed less fat. (b) Absence of essential fatty

acids from the diet. Holman and Witten (Holman, '51a, '51b; Witten and Holman, '52a) find that generally there is a depletion of body fat in animals receiving essential fatty acid-deficient diets; however, in one research these workers (Witten and Holman, '52b) found no apparent depletion of body fat in essential fatty acid deficiency. Kummerow et al. ('52) found that female rats which had grown to maturity on fat-free diets were deficient in arachidonic acid and not deficient in fat *per se*. In any case, in our experiment, the rats were not deprived of essential fatty acids sufficiently long to produce the usual grossly observable deficiency symptoms, and hence it is a moot point whether or not the fat

TABLE 2

Total fat of tissue sources[1]

CATEGORY OF INTEREST	EXPERIMENT		
	A	B	C
CONDITIONING	Lard	Tripalmitin	Olive oil
Total fat, grams	9.2	2.4	8.3
Average weight of animals, grams	175	78	115
Age of animals, days	86	54	54

[1] The tissue sources were the pooled livers, organs, mesenteries, perirenal fat, and hams of 5 rats for each experiment.

depletion can be attributed to this cause. (c) The body fats of animals conditioned on tripalmitin may have been depleted in a manner suggested by Campbell et al. ('49). These workers point out that if palmitic acid provides a large proportion of the energy value of the food supplied to the body, there is evidence indicating that elevation of the iodine number to about 80 is obligatory for combustion by the liver and other body tissues. This elevation of the iodine value could occur in two ways; by desaturation or by blending with unsaturated fatty acids of the body tissues. Campbell et al. ('49) interpret their findings with regard to depletion of body fat during palmitic acid feeding, liver lipid accumulation, and liver triglyceride iodine numbers as favoring the latter possibility.

Thus, while there may have been several factors operative in causing the apparent fat depletion of animals on tripalmitin conditioning in our experiment, it is not possible with the available data to determine the relative importance of each of these several factors.

6. In comparing (table 1) the relative amounts of monoethenoic acids of experiment B (tripalmitin conditioning) to those of experiment A (lard conditioning), one finds relatively much larger amounts of monoethenoic acids and much smaller amounts of saturated acids in the livers, pooled organs, and hams samples of experiment B. The relative amounts of these fatty acids of the perirenal and mesenteries samples are not very different for the two experiments. These differences in monoethenoic and saturated acids are most apparent in those samples known to contain much phospholipid. It appears that this increase in monoethenoic acid is correlated with the depletion of diethenoic and tetraethenoic acids. This agrees in part with the findings of Reiser ('51) who reported, "oleic acid replaces polyunsaturated fatty acids in the neutral fat of eggs produced by hens on a fat-free ration, the saturated acids remaining comparatively constant."

7. Following 30 days of tripalmitin conditioning, the relative value of liver monoethenoic acids was 79% (table 3). By continuing the animals for 10 more days on tripalmitin, a value of 82% monoethenoic acids was obtained. However, by placing another group of tripalmitin-conditioned animals on triolein for 10 days, no significant relative increase of monoethenoic acids was noted for liver whereas relative increases of monoethenoic acids were evidenced in depot fats (perirenal and mesenteric). This suggests that a value of about 82% of monoethenoic acids for the liver may be the upper limiting value obtainable for rat liver fats by dietary conditioning. However, Mattson et al. ('51) report obtaining 93% monoethenoic acids for perirenal fats of rats conditioned for 10 weeks on a diet containing 25% triolein.

TABLE 3

Body fat analysis, experiments C (tripalmitin conditioning) and D (olive oil conditioning). Each sample represents the pooled tissues of five animals

MEASURED VALUES	DIETARY REGIMEN	EXP.	TYPE OF FAT				
			Liver	Organ [1]	Perirenal	Mesenteric	Ham
Unsaponifiable matter, % of total fat	Control [2]	B	8.7	11.8	4.1	3.0	3.3
		C	11.8	9.0	1.5	..	2.7
	Fat-free	B		10.1	1.5	1.9	3.1
		C	10.0	12.0	0.8	1.1	2.3
	Tripalmitin	B	8.6	8.3	1.9	1.6	2.0
		C	9.3	13.1	1.2	1.6	3.5
	Triolein	B	13.0	8.2	1.7	1.0	2.9
		C	13.1	11.6	0.9	1.0	2.3
	Tripalmitin-trilinolein	B	9.9		1.3	1.3	2.1
		C	10.7	11.0	1.7	1.0	2.1
"Melting point" of fatty acids, °C.	Control [2]	B	39–43	37–43	34–41	41–45	38–44
		C	35–37	36–40	35–39	37–40	33–36
	Fat-free	B	38–47	40–43	39–44	42–45	38–43
		C	40–42	37–41	37–40	40–43	35–38
	Tripalmitin	B	39–44	39–42	41–45	41–45	40–43
		C	38–42	42–45	41–44	42–45	38–41
	Triolein	B	34–37	34–36	31–38	35–38	32–37
		C	32–34	36–40	36–39	37–41	34–36
	Tripalmitin-trilinolein	B	40–42	40–43	39–43	38–44	37–42
		C	32–39	40–43	41–43	41–44	37–39
Neutralization equivalents	Control [2]	B	335	306	280	275	283
		C	287	304	267	267	274
	Fat-free	B	294	302	273	274	277
		C	305	292	275	273	274
	Tripalmitin	B	297	295	274	276	279
		C	292	298	270	269	274
	Triolein	B	295	304	279	287	288
		C	292	303	275	272	276
	Tripalmitin-trilinolein	B	294	311	275	279	282
		C	313	313	265	275	279
Iodine values, fatty acids	Control [2]	B	85 [3]	73 [4]	55 [4]	50	68
		C	114	90	70	70	78
	Fat-free	B	86 [3]	53	58	57	65
		C	97	89	64	64	75
	Tripalmitin	B	87	73 [3]	56	58 [4]	65
		C	114	87	61	62	72
	Triolein	B	85	81	68	63	69
		C	97	88	64	68	77
	Tripalmitin-trilinolein	B	116	74	82	71	83
		C	122	82	75	71	85
Monoethenoic acids, % of acids	Control [2]	B	79 [3]	71 [4]	57 [4]	55	68
		C	50	71	64	63	61
	Fat-free	B	81 [3]	53	62	60	67
		C	46	51	61	63	63
	Tripalmitin	B	82	72	58	59 [4]	58
		C	51	46	60	58	57
	Triolein	B	79	64	71	67	69
		C	65	53	66	67	67
	Tripalmitin-trilinolein	B	79	69	35	37	71
		C	23	37	42	39	44

[1] See table 1.

[2] The control group represents those animals sacrificed after the 30-day conditioning period.

[3] See table 1, footnote 2.

[4] See table 1, footnote 3.

TABLE 3 *(continued)*

Body fat analysis, experiments C (tripalmitin conditioning) and D (olive oil conditioning). Each sample represents the pooled tissues of five animals

MEASURED VALUES	DIETARY REGIMEN	EXP.	TYPE OF FAT				
			Liver	Organ [1]	Perirenal	Mesenteric	Ham
Diethenoic acids, % of acids	Control [2]	B	6.2 [3]	4.2 [4]	1.5 [4]	0.8	2.7
		C	11	8.5	6.0	6.1	9.8
	Fat-free	B	4.6 [3]	3.0	0.8	0.7	1.6
		C	8.7	7.5	4.0	3.2	6.4
	Tripalmitin	B	5.1	3.8 [4]	1.2	1.5 [4]	2.5
		C	11	8.0	3.5	3.7	6.9
	Triolein	B	4.3	4.9	1.1	1.0	1.7
		C	5.8	6.9	2.1	2.9	5.5
	Tripalmitin-trilinolein	B	7.2	5.6	23	19	9.2
		C	33	18	20	19	21
Triethenoic acids, % of acids	Control [2]	B	0.4 [3]	0.0 [4]	0.3 [4]	0.1	(—0.1)
		C	(—5.1)	(—0.5)	0.5	0.4	(—0.1)
	Fat-free	B	0.5 [3]	(—0.1)	0.4	0.2	0.3
		C	(—2.9)	(—2.3)	0.4	0.2	(—0.3)
	Tripalmitin	B	0.9	0.4 [3]	0.6	0.5 [4]	0.0
		C	(—3.7)	(—2.0)	0.2	0.2	(—0.6)
	Triolein	B	1.3	1.2	0.5	0.3	0.6
		C	(—0.3)	(—2.4)	0.3	0.4	(—0.4)
	Tripalmitin-trilinolein	B	(—0.8)	(—0.2)	0.5	0.9	0.3
		C	(—3.6)	(—1.3)	0.5	0.4	(—0.5)
Tetraethenoic acids, % of acids	Control [2]	B	0.6 [3]	0.3 [4]	0.1 [4]	0.0	0.7
		C	14	3.2	0.0	0.3	1.7
	Fat-free	B	1.2 [3]	0.1	0.0	0.4	0.2
		C	12	8.7	0.1	0.1	1.9
	Tripalmitin	B	0.6	0.0 [3]	0.1	0.2 [4]	2.3
		C	15	9.1	0.0	0.8	2.5
	Triolein	B	0.6	3.4	0.0	0.0	0.8
		C	8.6	8.4	0.0	0.4	2.1
	Tripalmitin-trilinolein	B	9.6	0.4	0.3	0.2	0.6
		C	12	4.7	0.1	0.2	2.0
Saturated acids, % of acids	Control [2]	B	14 [3]	25 [4]	41 [4]	44	29
		C	25	17	30	30	28
	Fat-free	B	13 [3]	44	37	39	31
		C	33	33	35	34	29
	Tripalmitin	B	11	24 [3]	40	39 [4]	37
		C	23	37	36	37	34
	Triolein	B	15	27	27	32	28
		C	21	32	32	29	25
	Tripalmitin-trilinolein	B	4.2	25	38	43	19
		C	32	40	37	41	33
Conjugated dienes, as % octadecadienoic acid	Control [2]	B	4.0 [3]	2.8 [4]	0.8 [4]	0.5	1.4
		C	1.6	3.5	0.4	0.3	0.3
	Fat-free	B	2.7 [3]	1.8	0.2	0.2	0.7
		C	3.4	2.4	0.3	0.6	0.3
	Tripalmitin	B	3.3	2.4 [3]	0.2	0.4 [4]	0.3
		C	2.8	2.8	0.2	0.2	0.2
	Triolein	B	2.3	2.0	0.1	0.2	0.4
		C	0.8	2.4	0.3	0.2	0.2
	Tripalmitin-trilinolein	B	5.5	3.8	0.3	2.2	2.4
		C	4.1	4.5	1.7	2.6	1.8

[1] See table 1.

[2] The control group represents those animals sacrificed after the 30-day conditioning period.

[3] See table 1, footnote 2.

[4] See table 1, footnote 3.

Effects of new diets on body fats of conditioned animals. The effects on body fats of animals conditioned for 30 days on either tripalmitin or olive oil and transferred to the new diets for a 10-day period are shown in table 3. The complete lipid analyses are included here for all samples because they characterize a number of different body fats of rats on different dietary fat regimens.

The values for unsaponifiable matter and neutralization equivalents indicate that these constants are not correlated with the nature of the different fats fed in these experiments. Of particular interest is the fact that dietary palmitate was not reflected in the neutralization equivalents of the body fats.

The melting points and iodine values show clearly the influence of conditioning, and that a 10-day period on new dietary regimens is adequate to produce rather large changes in these values.

The fatty acid composition analyses yield information on the influence of dietary fat on body fat, the influence of essential fatty acid deficiency on body fat, and the effectiveness of a 10-day period on a new diet for alteration of animal body fat. Since autoxidation of some of the samples occurred prior to the time of analysis care must be exercised in interpreting the fatty acid composition data. This is particularly true for the liver and pooled organs samples which showed the most autoxidation. Also, negative values were frequently obtained for triethenoic acids. The spectrophotometric method of Brice et al. ('45), modified for small samples, was used to determine fatty acid composition. It has been shown by Herb and Riemenschneider ('53) that when such a method is applied to samples containing pentaenoate and hexaenoate that one obtains negative values for the trienoate. It is significant that negative values were obtained only in those samples containing much phospholipid. The magnitude of the negative values for triethenoate are indicative of the magnitude of error incurred through neglecting pentaenoate and hexaenoate in the analysis, assuming triethenoate to be absent or present in small amount only.

A number of examples of the influence of dietary fat on body fat are apparent. Monoethenoic acids of the body fats of tripalmitin-conditioned animals were increased when the animals were placed on triolein. Saturated acids were increased when olive oil-conditioned animals were placed on tripalmitin or tripalmitin-trilinolein. Diethenoic acids were increased when animals were placed on tripalmitin-trilinolein. The fat-free diet resulted in a decreased proportion of unsaturated fatty acids in the olive oil-conditioned animals and in little change in the tripalmitin-conditioned animals. This finding, in conjunction with the iodine value and melting point data, indicates the "hardening" effect of the fat-free diet on the body fats of the rats conditioned on olive oil substantiating the suggestion of Anderson and Mendel ('28) that the "harder" type of fat produced with a high carbohydrate diet includes a relatively higher proportion of saturated fatty acids. However, our findings in this regard are complicated by the fact that the fat-free diet, although fed for only a short term, did not contain any essential fatty acids. Changes in the body fats of animals placed on new diets were not observed in all tissues studied. For example, tripalmitin-conditioned animals placed on triolein showed increases of monoethenoic acids for perirenal and mesenteries samples only.

Data obtained from olive oil-conditioned animals placed for 10 days on the fat-free diet indicated that the diethenoic acids are depleted before the tetraethenoic acids, while data obtained from tripalmitin conditioned animals on the tripalmitin-trilinolein diet indicate that tetraethenoic acids recover rapidly in the liver. This finding is in agreement with that of Rieckehoff et al. ('49) who reported deposition of tetraethenoic acids upon feeding corn oil acids to essential fatty acid-deficient rats. This was interpreted as synthesis of arachidonic acid from linoleic acid. Our finding in feeding tripalmitin-trilinolein to olive oil-conditioned rats that diethenoic acids rose to high levels in the body fats while the tetraethenoic acids did not increase indicates that, whereas

it is well established that interconversion of linoleic acid to arachidonic acid occurs in the essential fatty acid deficient animal, when body arachidonic acid is at a normal level interconversion of linoleate to arachidonate does not occur to a significant extent, or at a rate not greater than the rate of catabolism of arachidonate and possible interconversion of arachidonate to other fatty acids.

Changes in body fats of differently conditioned animals by use of new diets for short periods is of interest because of possible application of the findings to the preparation of nonruminant meat animals for market. Shorland ('50) has indicated that in ruminants, diet has little effect on body fats. Our studies show that effects of previous conditioning on body fats were not erased in a 10-day period, since differences were shown in the groups on the different test diets depending on the previous conditioning. It is to be noted, however, that the major differences remaining were present in the livers, pooled organs, and hams samples indicating that dietary lack of essential fatty acids in experiment B (tripalmitin-conditioning) played a major role in the body fat differences remaining between tripalmitin- and olive oil-conditioned animals which had been placed on the several test diets for 10-day periods.

SUMMARY AND CONCLUSIONS

Fat deposition was studied in two groups of rats by conditioning the rats for 30 days on diets containing either 15% tripalmitin or 15% olive oil. Each of these groups was then divided into subgroups and put on various test diets for 10 days. The animals were then sacrificed and analyses of the lipids in the various body tissues were made.

The test diets included a fat-free diet, a tripalmitin diet, a triolein diet, and a tripalmitin-trilinolein diet. The lipid analysis of the samples obtained included: unsaponifiable matter, total fatty acids, melting point, neutralization equivalent, iodine value, fatty acid composition and peroxide value.

From the data obtained, it is concluded that:

1. The effect of tripalmitin conditioning with a diet deficient in essential fatty acids was manifest in rat body fats by: a depletion of fat reserves, a decrease of iodine value of body fats, a depletion of diethenoic and tetraethenoic acids, a relative increase of monoethenoic acids of liver, pooled organs, and hams samples with relative decreases of saturated acids for the same samples. The relative amounts of monoethenoic acids in such an experiment appear to be correlated with the amount of dietary choline.

2. Diethenoic acids increased markedly in all body fats of rats conditioned on olive oil and transferred to a tripalmitin-trilinolein dietary regimen. Diethenoic acids increased markedly only in depot fats (perirenal and mesenteries) of rats conditioned on a tripalmitin diet deficient in essential fatty acids and placed on a tripalmitin-trilinolein dietary regimen.

3. Following depletion of tetraethenoic acids in rats by tripalmitin conditioning with a diet deficient in essential fatty acids, the tetraethenoic acids of the liver showed a substantial increase when the animals were placed on a tripalmitin-trilinolein dietary regimen for 10 days.

4. The effects obtained on body fats of differently conditioned animals by placing groups of the animals for 10 days on several new diets differing from the conditioning diets in the fat component is discussed.

ACKNOWLEDGMENT

Grateful acknowledgment is made to Dr. J. R. Chipault, Hormel Institute, for supplying all the pure triglycerides used in these studies.

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STUDIES IN CALCIUM METABOLISM. EFFECT OF FOOD PHYTATES ON CALCIUM^{45} UPTAKE IN CHILDREN ON LOW-CALCIUM BREAKFASTS [1,2,3,4,5]

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(Received for publication June 4, 1954)

Although many investigators have studied the influence of the inositol hexaphosphates (commonly called phytates) on the uptake of calcium by human beings, the mechanism of the phytate effect is not well understood. This is partly due to the metabolic balance technique used by these workers

[1] Presented at the Annual Meeting of the American Institute of Nutrition, Chicago, Illinois, April 6–10, 1953.

[2] Contribution No. 205 from the Department of Food Technology, Massachusetts Institute of Technology.

[3] Partial support to this study was provided by a grant of the Quaker Oats Company to the Department of Food Technology and by Contract AT(30–1)–952 of the Atomic Energy Commission with the Department of Physics.

[4] The data in this publication are taken from the thesis (1952) presented by Felix Bronner to the Department of Food Technology, Massachusetts Institute of Technology, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[5] Authorization for the use of restricted doses of radiocalcium (Ca^{45}) in patients institutionalized for mental inadequacy was granted through the Subcommittee on Human Applications by the Isotope Division of the Atomic Energy Commission. The radiocalcium was obtained from the Oak Ridge National Laboratory.

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(McCance and Widdowson, '42a, b; Krebs and Mellanby, '43; Walker et al., '48; Nicolaysen and Njaa, '51), with all the difficulties inherent in the interpretation of results (Hegsted, '52). A new approach to this problem became possible when radioisotopes were made available for biochemical research.

Isotopes were used by Sharpe et al. ('50) in a study of the effect of phytates on the absorption of iron in human subjects. In the investigation reported here, radioactive calcium (Ca^{45}, half-life 180 days [7]) was used to study the effect of phytates on the uptake and excretion of calcium in a similar group of adolescent boys.

As in the earlier study (Sharpe et al., '50) use was made of the "one meal technique," in which the isotope is administered in a single test meal. Since the mass effect of the isotope is negligible, no adjustment period is needed and the study can be terminated as soon as the effects under investigation have been observed. This technique has the real advantage of permitting conditions which are quite similar to those prevailing under normal living, and thus largely circumvents the influence of seasonal factors (McCance and Widdowson, '43). On the other hand, the technique cannot be used to study the adjustment on the part of the body to unusual conditions of intake, for it tends to dramatize effects which over long periods of time may be of minor significance.

In the present investigation the effect of a phytate-rich cereal (oatmeal) on the uptake of calcium was compared with that of a phytate-free cereal (farina). Milk, commonly taken in conjunction with cooked breakfast cereals, was used as the principal source of calcium. The effect of food phytates was further compared with that of soluble sodium phytate which was added to farina. In order to neutralize any effect due to the solids content of the food (Sharpe et al., '50), all

[7] In the absence of reliable information, the half-life of Ca^{45} was considered to be 180 days. Shortly after the experimental part of this investigation was concluded, Delaney and Poole ('53) published a half-life value of 163.5 ± 4 days (maximum error). Because of the use of an experimental conversion factor, as discussed in the section of Analytical Methods, the data presented here do not depend on knowledge of the half-life of the isotope.

meals were equalized to a common solids content by the addition of farina.

The general procedure was to mix the radiocalcium intimately with the milk of the test meal and then to measure the calcium and radiocalcium content of the serum, urine and feces at intervals following ingestion of the test meal of which the milk was a part.

A criss-cross design was used, that is, all individuals were given two test meals three weeks apart, to permit both paired and group comparisons. A three-week interval was chosen because preliminary studies had indicated that with an intake of 0.85 μc Ca^{45} the level of radiocalcium in blood, urine and feces was negligible after that time. This finding was again confirmed in these studies.

EXPERIMENTAL

Subjects

Nineteen adolescent boys, of inadequate intelligence but otherwise normal, who were institutionalized in a state school under uniform nutritional and environmental conditions, volunteered for these experiments (table 1). The subjects were prepared for the study by giving each a daily supplement of one quart of milk and one multivitamin tablet [8] during one month prior to the start of the experiment, and until it was concluded.

Experimental meals

Three meals were given: an oatmeal breakfast (meal I) with phytic phosphorus naturally present in the oatmeal; a farina breakfast (meal II) which contained no phytate; and a farina breakfast with added sodium phytate (meal III). The composition of these meals is shown in table 2. Radiocalcium (0.85 μc Ca^{45} or 1.85×10^5 c.p.m.) as $Ca^{45}Cl_2$ (1 mg. Ca), was added to the milk which was then mixed intimately into the cereal of each meal.

[8] Vi-Penta Perles, generously donated by Hoffman-LaRoche Inc.

TABLE 1

Characteristics of experimental subjects

DIET GROUP		AGE IN YEARS		WEIGHT IN POUNDS		MENTAL AGE IN YEARS		I. Q.		NO. OF SUBJECTS
Exp. A	Exp. B	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	
Oatmeal (I)	Farina (II)	15.5	15.0–17.3	114.4	80–141	8.5	6.3– 9.1	57.8	47–75	5
Oatmeal (I)	Farina + phytate (III)	15.5	14.3–16.5	117.0	89–144	8.7	8.0– 9.8	59.0	55–67	4
Farina (II)	Oatmeal (I)	15.5	13.5–16.3	116.8	99–133	8.6	7.2–11.4	60.0	46–75	5
		15.5 [1]	13.5–16.3	114.5 [1]	99–133	8.9 [1]	7.2–11.4	61.0 [1]	46–75	4 [1]
Farina + phytate (III)	Oatmeal (I)	15.3	14.3–16.6	117.0	88–166	8.8	7.7–10.2	60.8	55–75	5
		15.6 [1]	14.6–16.6	124.3 [1]	94–166	8.5 [1]	7.7– 9.3	57.3 [1]	55–59	4 [1]

[1] The elimination of one subject in this group in experiment B altered the characteristics as indicated.

TABLE 2

Composition of test meals

GROUP	EXP.	TYPE OF FOOD	QUANTITY		SOLIDS	Ca	P	PHYTIC PHOSPHORUS	RATIO OF TOTAL Ca TO TOTAL P
			Wet weight	Dry[1] weight					
					gm	*mg*	*mg*	*mg*	
I	A	Milk + Ca^{45}	55 ml	..	5	63[2]	46	0	
		Oatmeal	180 gm	34 gm	31	21[3]	161[3]	118[3]	
		Farina	235 gm	33 gm	29	9	30[3]	0	
		Total			65	93	237	118	0.39
I	B	Milk + Ca^{45}	55 ml	..	6	61[2]	46	0	
		Oatmeal	180 gm	30 gm	28	19	124	113[3]	
		Farina	235 gm	31 gm	28	8	28	0	
		Total			62	88	198	113	0.44
	A + B	Av. total			64	91[2]	218	116	0.42
II	A	Milk + Ca^{45}	60 ml	..	5	69[2]	50	0	
		Farina	410 gm	57 gm	51	16[3]	51[3]	0	
		Total			56	85	101	0	0.84
II	B	Milk + Ca^{45}	60 ml	..	6	66[2]	50	0	
		Farina	410 gm	55 gm	49	15	48	0	
		Total			55	81	98	0	0.83
	A + B	Av. total			56	83	100	0	0.83
III	A	Milk + Ca^{45}	60 ml	..	5	69[2]	50	0	
		Farina	410 gm	57 gm	51	16[3]	51[3]	0	
		Na phytate	7 ml	..	1	0	78	78	
		Total			57	85	179	78	0.47
III	B	Milk + Ca^{45}	60 ml	..	6	66[2]	50	0	
		Farina	410 gm	55 gm	49	15	48	0	
		Na phytate	7 ml	..	1	0	78	78	
		Total			56	81	176	78	0.46
	A + B	Av. total			57	83	178	78	0.47

[1] Dry weight basis: farina 89.5% — solids; oats — 91.5% solids.

[2] Includes 1 mg of inert calcium due to the radiocalcium dose.

[3] Based on the analysis of the dry, uncooked cereal. All other analyses made on an aliquot of the cooked cereal as fed.

Sample collection

Blood was obtained by venipuncture at the antecubital fossa, usually about two and one-half hours following ingestion of the test meal. Earlier studies had shown that the peak level of calcium in the blood under the conditions of these experiments occurred about two to three hours postprandially. The blood was placed in a 50 ml centrifuge tube and allowed to clot. Following centrifugation the serum was removed, refrigerated and later analyzed for total calcium, and for Ca^{45} content. The activity in the red blood corpuscles was presumed to be negligible (Minder and Gordonoff, '52).

Urine specimens were collected directly in two-quart screwcap jars during the periods 0 to 1, 1 to 2, 2 to 3 and 3 to 5 days. Urine samples for the first three days were collected separately to assure complete collection and were then pooled in the laboratory before analysis. Earlier work had shown that with the low amount of Ca^{45} used here the measurable level of activity approached background in 5 days. The urine samples were preserved by the addition of glacial acetic acid, 2% by volume.

Feces. Five-day pooled samples of feces were collected directly in 4-quart screwcap glass jars placed inside a commode (Bronner, '52). The samples were preserved with toluene which was added in 50 to 100 ml quantities.

Analytical procedures

Analysis for calcium. Calcium analyses were carried out using a modification of the method of Salomon, Gabrio and Smith ('46). The serum and urine samples were analyzed by directly precipitating the calcium as the oxalate in 15 ml centrifuge tubes. The aliquot used contained approximately 0.05 mg of calcium. After standing overnight the samples were centrifuged three times at 1500 r.p.m. for 15, 10 and 10 min., respectively. After each period of centrifugation the supernatant liquid was decanted (Clark and Collip, '25). The precipitate was washed with 3 ml of 2% ammonium hydroxide

(freshly prepared with redistilled H_2O) after the first and second centrifugations. After the third centrifugation, the oxalate was dissolved in 2N perchloric acid ($HClO_4$) and titrated against perchloratoceric acid [$H_2Ce(ClO_4)_6$ in 2N $HClO_4$] with nitro-o-phenanthroline ferrous sulfate indicator. Each sample was analyzed in triplicate.

The coefficient of variation [9] of triplicate analyses was usually 3% or better. Analyses were repeated when the precision was not satisfactory. Recovery of added calcium in a series of serum samples was 97% and in a series of urine samples was 99%.

Feces samples were prepared by making a slurry of feces with toluene and water until a creamy, homogeneous emulsion was obtained. The slurry was then sampled into moisture dishes and crucibles. The moisture determinations served as checks on the homogeneity of the slurry and as a basis of comparison when an ashing had to be repeated. The slurry in the crucibles was dried overnight, charred over a Bunsen burner, dry-ashed overnight in a muffle furnace (540°C.) and wet-ashed with a mixture (1:1) of conc. HNO_3 and $HClO_4$ (Salomon, Gabrio and Smith, '46). The resulting ash solution was analyzed for calcium content by precipitation of the calcium as the oxalate and titration with perchloratoceric acid. The coefficient of variation of duplicate feces samples, each analyzed in triplicate, generally ranged from 3 to 5%. The recovery of added calcium in test experiments approximated 95%.

Analysis for radiocalcium. Radiocalcium analyses were carried out by a modification of methods originally developed at the Radioactivity Center, Massachusetts Institute of Technology.

Serum. The calcium of 5 ml serum samples was precipitated directly as the oxalate and isolated by the centrifugation procedure given above. The wash volume, however, was

[9] Coefficient of variation:

$$\frac{100[\Sigma(x_i - \bar{x})^2]^{\frac{1}{2}}}{[\bar{x}(n-1)]^{\frac{1}{2}}}$$

5 ml and the supernatant fluid was removed by a hooked capillary (Assoc. Official Agricultural Chemists, '46), in order to avoid losses that might occur when the larger (50 ml) tubes are inverted. After the oxalate was dissolved in 2N $HClO_4$, calcium chloride was added as a carrier in amounts sufficient to bring the total calcium to 4.0 mg; then the calcium was reprecipitated. After standing overnight, the solution was poured through a two-section sintered glass filter apparatus, and the calcium oxalate was collected on a 24 mm disc of filter paper (Schleicher and Schuell, No. 576). The filter paper was glued on a copper planchet and the calcium^{45} content of the sample determined by counting in an automatic apparatus (Tracerlab) equipped with a Geiger-Muller thin end-window (1.2 mg/cm^2) counter. Suitable Ca^{45} standards which had been prepared under identical conditions were employed in all counting runs. In addition, electroplated Co^{60} standards served to monitor the apparatus (Maletskos, C. J., and J. W. Irvine, Jr., unpublished). Samples were counted in three cycles, to an accumulation of 3072 counts.

Urine samples were prepared by direct precipitation. The precipitated calcium oxalate was removed by filtration (Schleicher and Schuell, red ribbon paper) when more than 25 ml of urine were required to prepare a sample containing 4 mg of calcium or slightly less; otherwise centrifugation was employed. The procedure used for preparing and counting planchets of urine samples was identical with that employed with the serum samples. When necessary, a carrier was added to bring the calcium content to 4.0 mg. When urine samples were not clear, the urine was ashed by a procedure similar to that used for the feces samples, and the resulting ash solution was then used in the preparation of samples to be counted.

Ashed solutions of the *feces* were analyzed for radiocalcium content by pipetting out a quantity containing 4 mg of calcium or slightly less. Where necessary, carrier was added to bring the calcium content to 4.0 mg. The calcium was then precipitated with ammonium oxalate and the procedure from

then on was the same as that employed for the analysis of serum samples.

The coefficient of variation of the net activity of the samples counted in duplicate was usually below 10%, depending on the activity of the sample, and often below 5%. The recovery of radiocalcium added to serum, urine and feces samples was generally between 90 and 95%.

All counting data were converted to a common time base and a common counter efficiency. Decay factors were determined experimentally with the aid of the radiocalcium standards. The results on radioactivity measurements are reported in terms of total activity (per cent dose administered) and of specific activity (per cent dose administered per milligram of Ca).

Analysis for phosphorus. Following ashing in a muffle furnace, food samples were wet-ashed with HCl (HCl + H_2O, 1:1) and the resulting ash solution was reduced to dryness twice. The solution was filtered through ashless filter paper which in turn was oxidized over a Bunsen burner and the resulting ash was again taken up in 1:1 HCl, filtered and added to the ash solution. This solution was analyzed for inorganic (total) phosphorus by the method of Lowry and Lopez ('46).

Analysis for phytic phosphorus. The method of Earley ('44) was used, except that the analysis of iron was carried out according to the procedure of Hahn ('45) which in turn was modified by the use of ascorbic acid as a reducing agent.

Detailed descriptions of all procedures are given by Bronner ('52).

EXPERIMENTAL RESULTS

The experimental results are presented in table 3. Because there was poor duplication (coefficient of variation $> 10\%$) of counts in serum samples (experiment A, table 3), further replicates were prepared by ashing the serum samples. An analysis of variance showed that counts obtained on ash samples did not differ significantly ($P > 0.05$) from counts obtained by direct precipitations. To avoid bias, all counts

TABLE 3

Ca^{45} levels in serum, urine and feces. Dose: 0.85 μc (1.85×10^5 c.p.m.)

Serum

SUBJECT NO.	EXPERIMENT A			EXPERIMENT B		
	Group	Ca content	Ca^{45} content	Group	Ca content	Ca^{45} content
		mg/ml	*% dose/mg*		*mg/ml*	*% dose/mg*
36	I	0.100	0.0152	II	0.102	0.0329
37	I	0.0962	0.0166	II	0.103	0.0356
38	I	0.100	0.0108	II	0.102	0.0275
39	I	0.0870	0.0197	II	0.0930	0.0405
40	I	0.0994	0.0103	II	0.0984	0.0351
41	I	0.0956	0.0242	III	0.0990	0.0205
42	I	0.0965	0.0210	III	0.101	0.0190
43	I	0.0922	0.0211	III	0.101	0.0133
44	I	0.0877	0.0228	III	0.0933	0.0129
45	II	0.0948	0.0343	I	0.0962	0.0220
46	II	0.0942	0.0312	I	0.0974	0.0184
47	II	0.0994	0.0439	I	0.0981	0.0291
48	II	0.0972	0.0316	I	0.103	0.0267
49	II	0.0948	0.0164	I	..	..
50	III	0.0942	0.0121	I	0.0848	0.0159
51	III	0.0924	0.0128	..	..	..
52	III	0.0958	0.0104	I	0.0980	0.0196
53	III	0.107	0.0125	I	0.0988	0.0224
54	III	0.0984	0.0133	I	0.0993	0.0169

Urine

SUBJECT NO.	EXPERIMENT A				EXPERIMENT B			
	Group	Ca output	Ca^{45} content	Total Ca^{45} excreted	Group	Ca output	Ca^{45} content	Total Ca^{45} excreted
		mg	*% dose/mg*	*% dose*		*mg*	*% dose/mg*	*% dose*
36	I	454	0.00648	3.24	II	421	0.00657	2.70
37	I	587	0.00627	3.66	II	199	0.00739	1.45
38	I	262	0.00524	1.37	II	366	0.00442	1.61
39	I	443	0.00762	3.36	II	354	0.00885	3.05
40	I	63.2	0.00491	0.31	II	99.2	0.00416	0.41

TABLE 3•*(continued)*

41	I	444	0.00638	2.82	III	270	0.00338	0.91
42	I	911	0.00843	7.03	III	733	0.00361	2.64
43	I	103	0.00461	0.47	III	35.7	0.00261	0.09
44	I	669	0.00708	4.70	III	622	0.00205	1.28
45	II	397	0.00924	3.86	I	366	0.00576	2.09
46	II	25.7	0.00374	0.09	I	28.9	(0.00053)[1]	(0.01)[1]
47	II	265	0.00762	2.23	I	291	0.00636	1.83
48	II	296	0.00891	2.63	I	422	0.00668	2.78
49	II	46.5	0.00440	0.20	..	..	..	..
50	III	112	0.00313	0.35	I	106	0.00891	0.93
51	III	375	0.00247	0.93	..	..	..	..
52	III	467	0.00344	1.61	I	427	0.00619	2.64
53	III	671	0.00422	2.26	I	630	0.00529	3.31
54	III	247	0.00300	0.75	I	143	0.00311	0.46
				Feces				
36	I	3487	0.0160	55.8	II	3073	0.0105	32.1
37	I	384[2]	0.0101	3.87[2]	II	1837	0.00630	11.4
38	I	6481	0.00875	58.1	II	5607	0.00636	35.2
39	I	2897	0.0129	38.6	II	3071	0.00837	25.5
40	I	2749	0.0173	46.5	II	6224	0.00587	36.0
41	I	2835	0.0183	50.2	III	2438	0.0299	72.4
42	I	2319	0.0[3]	0[3]	III	2871	0.0208	57.3
43	I	3117	0.0133	41.7	III	2024	0.0312	62.4
44	I	2246	0.0149	34.1	III	2630	0.0236	63.5
45	II	3173	0.00702	22.2	I	2445	0.0142	35.1
46	II	1431	0.0202	29.2	I	3082	0.00989	30.2
47	II	2900	0.00482	14.1	I	1475	0.00684	10.7
48	II	2182	0.0134	29.2	I	2897	0.0171	49.4
49	II	2737	0.00740	16.1	..	..	..	..
50	III	3806	0.0189	69.7	I	4569	0.0140	64.8
51	III	4898	0.0160	80.5	..	..	..	..
52	III	2663	0.0299	79.9	I	4222	0.0140	59.3
53	III	4007	0.0172	69.2	I	4653	0.0120	55.1
54	III	2411	0.0156	38.0	I	3549	0.0172	60.5

Notes: I Oatmeal
II Farina
III Farina plus Phytate

Serum samples obtained 2.5 hours post-prandially
Urine samples are 72-hour samples
Feces samples are 120-hour samples

[1] Count did not differ significantly from background.
[2] Collection probably incomplete.
[3] No activity recovered in feces.

obtained by both methods were utilized in the statistical analysis (table 4).

The primary data were subjected to an analysis of variance to determine whether the treatment resulted in significant effects in experiments A and B, separately and combined, and whether the experiments represent good replication. In this analysis the procedure outlined by Snedecor ('46) for non-orthogonal data was followed.

Serum. There was an appreciable difference in the serum specific activity of the three groups; the highest level was observed in the individuals on the farina breakfast, while those who had received the oatmeal exhibited only 60% of the specific activity level of the farina group. The individuals who had received the sodium phytate had the lowest radiocalcium level, 40% that of the farina group. Statistical analysis (table 4) revealed that these treatment effects were extremely significant ($P < 0.01$) both separately ($F_A = 44.4$; $F_B = 38.6$) and when pooled ($F = 77$). It is true that a combined analysis of variance indicated that the experiments differed from each other significantly ($F = 7.09$); the higher calcium[45] levels observed in experiment B would lead one to expect this. However, this does not detract from the significance of the treatment proper, particularly since the F-value associated with the treatment effect is very nearly 11 times larger than that associated with the difference between experiments.

Urine.[10] It is to be expected that the specific activity of the urine would be similar to that of the serum. The specific activity was highest in the urine of the group on farina breakfast, was lowest in the urine excreted by the group which received sodium phytate in the meal; an intermediate level of isotope concentration was observed in the urine excreted by the oatmeal group. An analysis of variance performed on the data (table 4) indicated a highly significant difference

[10] Urine samples were collected for 5 days, but only the data of the first three days were utilized in the statistical analysis, as samples of the last two days exhibited very low activity. Qualitatively the trend of the data of the last two days was similar to that of the first three days.

TABLE 4

Statistical analysis of specific activity in counts per minute (c.p.m.) per milligram of calcium [1]

GROUP	STATISTIC	SERUM Experiment A	SERUM Experiment B	URINE [2] Experiment A	URINE [2] Experiment B	FECES Experiment A	FECES Experiment B
		Summary of data					
I	n#	25	16	19	16	34	32
	Sx	857.2	670.5	208.1	165.0	877.6	770.1
	Sx^2	32,156.34	29,177.37	2,471.21	1,849.08	23,750.08	19,757.43
	S.S.	2,761.25	1,079.62	191.97	147.52	1,097.68	1,224.49
	Mean	34.29	41.91	10.95	10.31	25.81	24.07
II	n#	14	10	10	10	20	17
	Sx	868.2	633.4	125.5	114.6	334.0	234.8
	Sx^2	59,229.52	40,897.70	1,753.09	1,420.46	6,957.58	3,445.76
	S.S.	5,396.16	7,781.44	178.06	107.14	1,379.78	202.76
	Mean	62.01	63.34	12.55	11.46	16.70	13.81
III	n#	19	8	10	8	23	18
	Sx	403.7	237.1	60.3	42.3	805.8	850.2
	Sx^2	8,877.09	7,283.99	375.19	235.61	30,364.36	41,431.46
	S.S.	299.49	256.95	11.58	11.95	2,133.33	1,273.68
	Mean	21.25	29.64	6.03	5.29	35.03	47.23

n# = number of variates, i.e., number of observations in the experimental group, *not* number of subjects; Sx = sum of the variates; Sx^2 = sum of the squares of the variates; S.S. = $Sx^2 - (\bar{x})\ Sx$ where $\bar{x}$ = mean.

Completed analysis of variance

SOURCE OF VARIATION	SERUM N**	SERUM SUM OF SQUARES	SERUM MEAN SQUARE	SERUM F–RATIO	URINE N**	URINE SUM OF SQUARES	URINE MEAN SQUARE	URINE F–RATIO	FECES N**	FECES SUM OF SQUARES	FECES MEAN SQUARE	FECES F–RATIO
Between experiments:	1	871.74	871.74	7.09*	1	11.28	11.28	1.17	1	213.97	213.97	1.77
Dietary effect:	2	18,953.88	9,476.94	77.13*	2	429.72	214.86	22.22*	2	13,033.32	6,516.66	53.98*
Interaction:	2	151.54	75.77	0.62	2	0.66	0.33	0.034	2	1,450.21	725.11	6.01*
Individuals	86	10,567.23	122.87		67	648.22	9.67		138	16,660.53	120.73	

N** = degrees of freedom. *F–ratio significant at 5% probability level.

[1] Data reported in this table have not been converted from counts per minute to percent dose, because the statistical analysis is independent of the units employed.

[2] Data on urine samples of subject no. 46 excluded from statistical analysis, because count did not differ significantly from background.

between the diet groups when the experiments were analyzed separately ($F_A = 11.3$; $F_B = 11.1$) and in combination ($F = 22.2$). This indicates that more calcium was excreted, and presumably taken up, by the group which received the farina breakfast than by the groups on the oatmeal or sodium phytate meals.

A corresponding analysis of the total output of calcium[45] in the urine (table 5) reveals a very different picture. Here the oatmeal group in experiment A had the largest total output of calcium[45]. As a result the analysis of variance revealed a significant interaction, a significant dietary effect and a significant difference between experiments A and B. In other words, the data do not justify the assumption that the experimental subjects represent a homogeneous group as far as total urinary output of calcium[45] is concerned. This is not surprising. A 10-fold variation in the urinary output of calcium by members of an apparently homogeneous group is not unusual (see table 3). For this reason the standard deviation of the different group means (table 5) in all cases exceeded 50%. In the case of the other results (specific activity of serum, urine and feces; total calcium[45] output in feces) the standard deviation was usually 25% or less.

Feces. The total quantity of radiocalcium excreted in the feces of the subjects is analyzed in table 5 [11], while the specific activity of the feces is analyzed in table 4. In table 6, the fecal output is presented as the group average, expressed as the percentage of the total intake. In contrast to the observations on the urine, the relation between the fecal specific activities of the three groups (table 4) corresponds to that between the total fecal output of the groups (table 5). The probable explanation is that the interindividual variations in

[11] Data on subject No. 37 in experiment A are excluded because only one small stool was obtained from him in the 5-day collection period. On the second day he passed a stool with a specific activity within the expected range; however, his total output was only a fraction of that excreted in the feces by all other subjects. Subject No. 42 also passed only one stool in the entire period and it contained no detectable activity. We have no explanation for this occurrence. The data of experiment B indicate that in all likelihood this was not a metabolic event.

the level of the calcium output in the feces for the 5-day period were not nearly as great as those of the urinary calcium output (table 3). As a result, analysis of either total output or specific activity indicated group differences.

TABLE 5

Statistical analysis of total calcium^{45} output in urine and feces (c.p.m. $\times 10^{-3}$)[1]

GROUP	STATISTIC	URINE Experiment A	URINE Experiment B	FECES Experiment A	FECES Experiment B
		Summary of data			
I	n#	17	17	42	32
	Sx	98.14	58.59	3,278.4	2,678.6
	Sx^2	787.9250	262.7533	287,879.5	258,934.0
	S.S.	221.3680	60.8246	32,164.3	17,029.7
	Mean	5.773	3.446	78.0	83.7
II	n#	10	10	20	24
	Sx	31.92	34.18	850.4	1,165.9
	Sx^2	168.0678	148.2102	38,511.1	63,582.9
	S.S.	66.1783	31.3830	1,364.8	6,919.3
	Mean	3.192	3.418	42.5	48.6
III	n#	10	9	23	18
	Sx	23.89	18.26	2,914.6	2,090.2
	Sx^2	83.1185	64.0262	286,924.1	245,017.0
	S.S.	26.0453	26.9877	17,644.3	2,298.8
	Mean	2.389	2.029	126.7	116.1

n# = number of variates, i.e., number of observations in the experimental group, *not* number of subjects; Sx = sum of the variates; Sx^2 = sum of the squares of the variates; S.S. = $Sx^2 - (\bar{x})\,Sx$ where $\bar{x}$ = mean.

SOURCE OF VARIATION	URINE N**	URINE Sum of squares	URINE Mean square	URINE F–ratio	FECES N**	FECES Sum of squares	FECES Mean square	FECES F–ratio
			Completed analysis of variance					
Between experiments:	1	11.3680	11.3680	6.24*	1	109	109	1
Dietary effect:	2	72.9531	36.4766	20.0 *	2	123,369	61,685	121.9*
Interaction:	2	24.0605	12.0303	6.60*	2	2,038	1,019	2.01
Individuals:	67	122.1506	1.8231		153	77,421	506	

N** = degrees of freedom. *F–ratio significant at 5% probability level.

[1] Data reported in this table have not been converted from counts to per cent dose because the statistical analysis is independent of the units employed.

Variations in fecal calcium[45] output induced by differences in the endogenous calcium output would not have been expected under the experimental conditions. Unpublished results with intravenously injected calcium[45] indicate that less than 5% of the calcium absorbed from the intestinal tract is lost in the feces within 5 days. This loss is less than the deviation of the results reported in tables 4 and 5.

TABLE 6

Average per cent dose of Ca^{45} excreted in feces

GROUP	EXPERIMENT A	EXPERIMENT B	MEAN % DOSE OF Ca^{45} UPTAKE [2]
I [1]	46.4	41.9	55
II	22.2	26.1	76
III	67.5	63.9	34

[1] Excluding subjects 37 and 42 in experiment A.

[2] Ca^{45} uptake = 100 — fecal output.

I — Oatmeal breakfast.
II — Farina breakfast.
III — Farina plus phytate breakfast.

DISCUSSION

The agreement between the data on the feces and serum samples shown in table 7 is of interest. Data on serum and feces samples [12] were obtained independently, and yet the ratios of the mean specific activity of the serum data are the same as the inverse ratios of the feces data. This may be taken as evidence that, under the conditions of this experiment, the fecal calcium[45] was essentially unabsorbed cal-

[12] At present, it is not permissible to exclude the possibility that calcium exchange takes place between the intestinal tract and the plasma (Norris, '52). Because the quantities of stable and radioactive calcium and the food solids content administered to the experimental groups were the same, the experimental effect, whatever its nature, in all likelihood was induced by the phytate molecule. The assumption that all of the calcium[45] not recovered in the feces represents calcium uptake merely magnifies the absolute phytate effect; its relative effect in all probability would be unchanged, whatever the true uptake.

cium[45], and that unimportant amounts of metabolic calcium were excreted in the feces.

The quantity of calcium in these meals was so chosen that there was present just sufficient calcium to combine with all of the food phytate to form pentacalcium phytate (Hoff-Jørgensen, '44; Møllgaard et al., '46). The finding that the group fed sodium phytate actually took up 35% of the calcium ingested (table 6) indicates that possibly only a part of the calcium reacted with the phytate, that the calcium in calcium phytate is partially available for absorption, or

TABLE 7

Comparison of mean specific activity ratios

RATIO	EXPERIMENT A			EXPERIMENT B		
	Serum	Urine	Feces	Serum	Urine	Feces
I/II [1]	0.55 [2]	0.87 [3]	2.09 [4] (0.47) [5]	0.67	0.90	1.49 (0.67)
III/I	0.62	0.55	1.45 (0.69)	0.71	0.51	1.53 (0.65)
III/II	0.34	0.48	3.04 (0.33)	0.47	0.46	2.27 (0.44)

[1] I = Oatmeal breakfast, II = farina breakfast, III = farina + phytate breakfast.

[2] Serum values obtained two and one-half hours following ingestion of Ca^{45}.

[3] Urine values obtained on 72-hour samples.

[4] Feces values obtained on 120-hour samples.

[5] Values in parentheses are the reciprocals.

that calcium compounds other than pentaphytates are formed in the intestinal tract (Gosselin and Coghlan, '53).

It is interesting to note that the phytate naturally present in oatmeal is less reactive with food calcium than sodium phytate, since 78 mg of phytic phosphorus in the form of sodium phytate permitted much less calcium to be taken up than 116 mg of phytic phosphorus in the form of oat phytate (34 versus 55%).

The data presented would lead to the conclusion that less calcium was taken up when sodium phytate or when a phytate-containing cereal was fed than when the phytate-free farina was ingested. Taken together with the studies by earlier

investigators (McCance and Widdowson, '49) these findings leave little room for doubt that phytate does interfere with calcium uptake, and that less calcium enters the body when phytate-containing cereals are eaten than when phytate-free cereals are ingested.

On the other hand, the absolute quantity of calcium rendered unavailable as a result of the action of the food phytate was so small (15 mg) that phytates cannot be very significant in the usual U. S. diets. This will be discussed in greater detail elsewhere (Bronner, Harris, Maletskos and Benda, to be published).

SUMMARY AND CONCLUSIONS

1. Nineteen adolescent boys, of inadequate intelligence, but otherwise normal, who were institutionalized under uniform nutritional and other environmental conditions, were given three types of breakfast low in calcium: (I) oatmeal, (II) farina, and (III) farina and sodium phytate. These meals contained, respectively: 55, 60, and 60 ml of milk; 91, 83, and 83 mg of calcium; 116, 0, and 78 mg of phytic phosphorus, and 0.85, 0.85, and 0.85 μc of radioactive calcium (Ca^{45}). Calcium uptake was studied by measuring the Ca^{45} content of serum, urine and feces for a period of 5 days. Individual variations were controlled by placing each subject on two different Ca^{45}-labeled breakfasts, 30 days apart.

2. The uptake of Ca^{45} by the boys on the oatmeal breakfast was 74% as great as that of the boys on the farina breakfast. Similarly, the uptake of Ca^{45} by the boys on the farina plus phytate meal was 45% that of farina meal. These differences proved to be statistically significant on a 5% probability level.

3. Significantly less Ca^{45} was taken up in the presence of sodium phytate than in the presence of an equivalent quantity of phytic phosphorus supplied by oats.

ACKNOWLEDGMENT

The technical assistance of Mrs. Alice J. Liu Chen and Miss Lee H. Weiss is gratefully acknowledged. Thanks are due to Dr. Malcolm J. Farrell, Superintendent, and the staff and personnel of the Walter E. Fernald State School, for assistance in carrying out the research. Thanks are also due to the boys themselves for their willing cooperation.

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PHYSICAL MEASUREMENTS OF IOWA SCHOOL CHILDREN [1, 2]

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ONE FIGURE

(Received for publication June 5, 1954)

Physical measurements are frequently used to assess the growth and well-being of children. Standards for evaluation may be selected with little concern for their applicability to the particular children involved or for the nutritional status of the children represented in the standards. The present study was planned to reveal certain aspects of the physical growth of a state-wide sample of Iowa school children, about whom considerable information concerning food habits, nutrient intake, and nutritional status was available. The nutritive value of the diets has been described in the first paper of this series (Eppright et al., '54).

Since 1930 two other mass studies have been made of body measurements of Iowa school children. The first, conducted at the experimental elementary and high schools of the State University of Iowa in Iowa City, was designed to establish norms of growth approaching "the optimum for general pediatric practice" (Jackson and Kelly, '45; Stuart and Meredith, '46).

[1] Contribution no. 11, Subproject 2 — "The Nutritional Status of School Children: The School Lunch as an Influencing Factor" of the North Central Region Cooperative Project NC-5, "Nutritional Status and Dietary Needs of Population Groups." (a) Human Nutrition Research Branch, Agricultural Research Service, Washington, D. C. (b) Iowa Agricultural Experiment Station, Ames, Iowa, as Journal Paper no. J-2446, Project 1021.

[2] Supported in part by a grant and other assistance from General Mills, Inc., as part of its nutrition education program.

The children were examined between 1930 and 1945 by trained clinicians and the "standards" developed were considered applicable to "physically normal" children. The second was conducted by the Bureau of Human Nutrition and Home Economics ('41) for the purpose of obtaining body measurements with which to standardize patterns and sizes of children's garments. Between February 8, 1937, and June 30, 1939, measurements were made of 8,644 children, of whom 7,921 were located in one county (Polk). Most of the remaining children were located in 6 other counties.

The present report presents means and standard deviations of 5 body measurements of approximately 1,200 children ranging in age from 6 to 18 years. Observations were distributed throughout the school years of 1948 to 1952; fewest measurements were made in September, December, and January. The selection of the random sample of 61 schools and of the children within the schools has been described in the foregoing report.

METHODS

Methods of measurement were essentially the same as those used in the two previous studies of Iowa children. Girls wore undergarments, and boys athletic trunks. Portable scales frequently checked against standard weights in the Physics Department of the Iowa State College were used. Weights were recorded to the nearest pound. The children were weighed during the mid-morning, immediately after the bladder was emptied. Most of the children were measured during the week of the dietary study at the time blood samples were taken.

For measuring height, a centimeter scale made at the University of Iowa was mounted on a portable standard rigidly attached to a platform on which the child stood. Leg girth, hip width (bi-iliac diameter), and chest breadth were also measured. The research-team leader was trained to take the measurements according to the techniques used at the University of Iowa. She in turn trained the three technicians who assisted in this phase of the study.

TABLE 1

Mean physical measurements of Iowa school boys

AGE IN YEARS	AVERAGE AGE, MO.	NO.		HEIGHT	WEIGHT	CHEST BREADTH	BI-ILIAC DIAMETER		LEG GIRTH		
				cm	*kg*	*cm*	*cm*		*cm*		
	A [1]	A		A	A	A	A	B [2]	A	B	C [3]
6	78	38	x [4]	117.5	22.6	18.6	19.1	19.5	24.0	24.1	23.3
			S.D. [5]	4.74	2.87						
7	89	57	x	124.1	25.0	19.0	19.6	20.3	25.3	25.2	24.2
			S.D.	5.22	3.52						
8	101	53	x	130.9	28.6	20.4	21.1	21.1	26.2	26.3	25.2
			S.D.	5.50	4.79						
9	116	55	x	135.6	32.1	20.9	21.5	21.7	27.3	27.3	26.2
			S.D.	6.22	5.61						
10	125	60	x	139.5	33.7	21.2	22.0	22.3	27.7	28.1	27.2
			S.D.	5.76	4.55						
11	137	50	x	144.1	37.9	21.8	23.0	23.1	29.3	29.0	28.3
			S.D.	6.56	7.89						
12	148	90	x	148.3	40.8	22.6	23.6	24.1	29.9	30.1	29.1
			S.D.	7.12	8.74						
13	161	44	x	156.5	47.9	24.2	25.6	25.2	32.2	31.6	30.6
			S.D.	5.28	8.87						
14	174	40	x	160.7	51.4	25.2	25.8	26.3	32.3	32.9	32.0
			S.D.	10.11	10.59						
15	185	32	x	171.1	61.6	27.0	28.2	27.1	34.4	33.8	
			S.D.	6.51	7.08						
16	196	34	x	170.4	63.6	27.6	28.7	27.6	35.0	34.4	
			S.D.	7.32	9.76						
17	209	21	x	172.8	63.8	27.7	28.9	27.9	34.6	34.8	
			S.D.	7.52	3.48						
18	223	18	x	172.4	66.1	28.2	29.4		35.0		
			S.D.	8.82	9.55						

[1] A — State-wide sample 1948–1951.
[2] B — Iowa City children.
[3] C — Children measured by Bureau of Human Nutrition and Home Economics.
[4] Mean.
[5] Standard deviation.

TABLE 2

Mean physical measurements of Iowa school girls

AGE IN YEARS	AVERAGE AGE, MO.	NO.		HEIGHT	WEIGHT	CHEST BREADTH	BI-ILIAC DIAMETER		LEG GIRTH		
				cm	*kg*	*cm*	*cm*			*cm*	
	A [1]	A		A	A	A	A	B [2]	A	B	C [3]
6	78	50	x [4]	117.8	22.0	17.5	18.6	19.2	24.2	24.3	23.4
			S.D. [5]	4.85	3.44						
7	89	48	x	122.6	24.8	18.5	19.7	20.1	25.4	25.3	24.4
			S.D.	6.44	4.92						
8	101	44	x	126.7	26.3	19.0	20.3	20.9	24.9	26.3	25.4
			S.D.	5.48	4.43						
9	114	64	x	133.4	30.7	19.8	21.5	21.8	27.0	27.3	26.3
			S.D.	6.64	6.53						
10	125	61	x	140.8	35.3	20.7	22.5	22.9	28.3	28.3	27.3
			S.D.	7.34	7.77						
11	137	58	x	146.0	40.1	21.8	23.9	24.2	29.5	29.5	28.5
			S.D.	7.56	7.93						
12	149	82	x	151.4	45.6	22.8	25.1	25.5	30.7	30.7	29.8
			S.D.	7.09	11.41						
13	161	44	x	153.2	46.8	23.4	25.9	26.5	32.0	31.6	30.7
			S.D.	7.48	8.78						
14	173	37	x	159.4	51.3	24.2	27.1	27.2	32.6	32.3	31.8
			S.D.	5.14	7.24						
15	186	38	x	161.2	56.6	24.8	28.2	27.8	33.9	32.9	
			S.D.	6.66	8.04						
16	197	37	x	160.5	57.2	25.0	27.9	28.2	34.2	33.3	
			S.D.	5.59	8.93						
17	209	26	x	162.5	57.5	25.3	28.4	28.4	32.9	33.5	
			S.D.	4.79	9.22						
18	222	12	x	163.2	54.1	24.7	28.5		33.1		
			S.D.	6.54	4.38						

[1] A — State-wide sample 1948–1951.
[2] B — Iowa City children.
[3] C— Children measured by Bureau of Human Nutrition and Home Economics.
[4] Mean.
[5] Standard deviation.

Tables 1 and 2 present the mean measurements with standard deviations and mean ages in months for the boys and girls of the state-wide sample, together with some of the data obtained from the two earlier studies. Figure 1 enables a comparison to be made of the heights and weights obtained in the three mass studies of Iowa children.

COMPARISON OF MEASUREMENTS MADE IN THREE MASS STUDIES OF IOWA CHILDREN

Differences in mean measurements of successive age groups may not be identical with differences observed when a single group of children is measured at successive years. However, a cross-sectional study based on an adequate sample of children will show how children as a group are growing and may reveal worthwhile information regarding the expected changes in physical development.

Figure 1 shows that the mean weights of the children of the present study approximated or exceeded the Iowa City norms (50th percentiles), at most ages. They were also consistently larger than the corresponding means observed by the Bureau of Human Nutrition and Home Economics 10 years earlier, with the single exception of the 8-year-old girls. As regards stature the Iowa City children tended to be tallest; the children measured by the Bureau of Human Nutrition and Home Economics shortest; the children of the present study, intermediate. The Iowa children of the present study tended to approximate, or even to exceed, the weights of the heavier of the two groups previously studied, but to compare closely with the shorter of the two groups in mean heights. Marlatt et al. ('52) have reported that the 9-, 10-, and 11-year-old children measured in Ohio and Kansas, as well as those in Iowa, tended to be taller and heavier than those previously measured in those states by the Bureau of Human Nutrition and Home Economics.

The measurements of maximum leg girth, sometimes considered an index of the muscular mass (Stuart and Meredith, '46), were similar at each yearly age level in all three Iowa

studies (table 2). These data indicate that this measurement increases as age increases, and that over the period of time represented by the three studies in Iowa, children have not changed appreciably in this physical characteristic. Increases in leg girth were similar in trend to those in height and weight. Year-to-year increases in leg girth as well as in other body measurements were "spurt-like" for boys but fairly steady for girls.

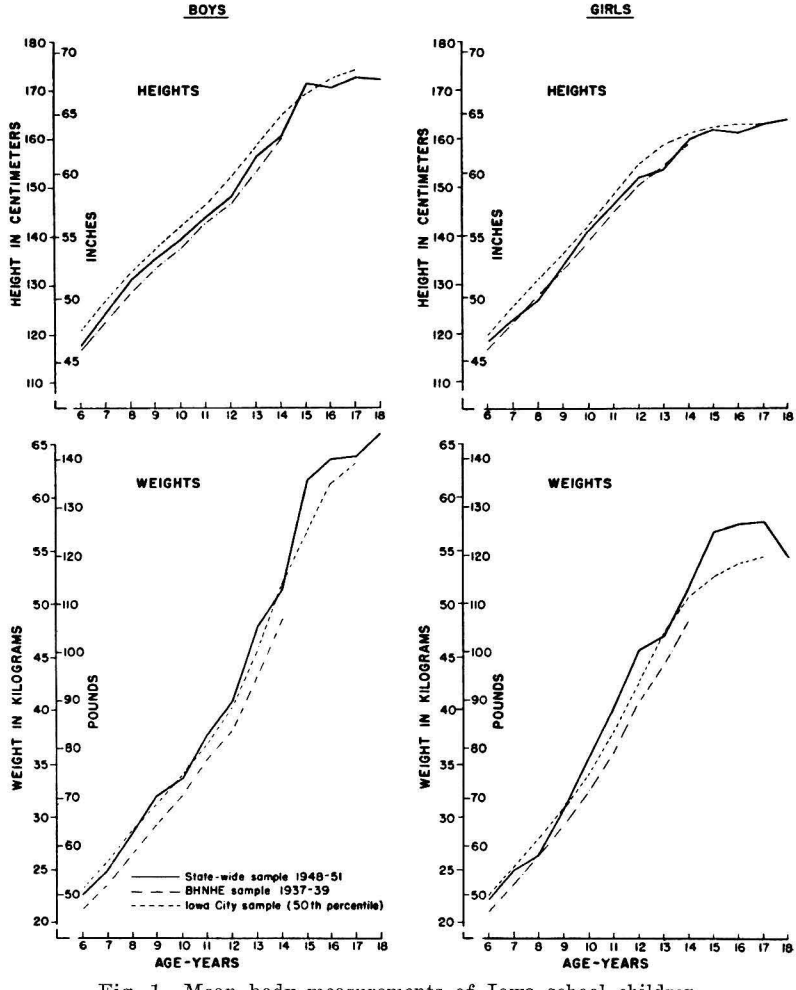

Fig. 1 Mean body measurements of Iowa school children.

At each age all comparable body measurements of the three groups of Iowa children were similar, but of the three groups of data, those of the Bureau of Human Nutrition and Home Economics tended to be the lowest, for both boys and girls. Ninety-one per cent of the children studied by the Bureau lived in one county in Iowa and were mostly from Des Moines. Many were probably from families of lower socio-economic levels than those represented in the Iowa City study. The physical development of the children was shown in the Bureau study to be related to the socio-economic level of the family. Because of the nature of the sampling, it is unlikely that the results of the present study are biased by economic level. The economic level of the children of the present sample may have been higher than that of the children of the Bureau study. This condition may account in part for the differences noted between the children of the present study and those observed by the Bureau.

DIFFERENCES IN WEIGHT AND HEIGHT OF SUCCESSIVE AGE GROUPS

Mean weights and heights of the Iowa boys and girls at successive years increased more or less regularly from age 6 through 15, after which increases, if any, were small. Mean weights of 15-year-old boys exceeded those of 6-year-old boys by 39 kg with approximately 99% confidence limits of $\pm$ 3.4 kg. The differences in weight between the 6- and 15-year-olds was 9 kg greater than expected according to the computations of Watson and Lowrey ('51, p. 62) from data obtained from a number of studies. Increments in weight were smallest for boys between the ages of 9 and 10 and largest between 14 and 15. These increases were 1.6 and 10.2 kg respectively. At other ages yearly differences in mean weights of boys were from 3 to 4 kg.

Mean weights of the girls from age 6 through 15 years differed by 34.6 kg with approximately 99% confidence limits of $\pm$ 3.5 kg. According to the compilation by Watson and Lowrey the expected difference for girls for this period

of growth was 29.3 kg, and the actual differences were 5.3 kg more than the expected. The mean weights of Iowa girls as well as boys increased significantly more than the expected from 6 through 15 years. The year-by-year increments of mean body weights of girls were more regular than those of boys. From 8 through 15 years the annual increase in mean weights of girls was approximately 4.5 kg except between 7 and 8, and 12 and 13, when it dropped sharply to 1.5 and 1.2 kg respectively.

Mean heights of boys of age 6 through 15 increased 53.6 cm, an amount which is in close agreement with the 53.0 cm given by Watson and Lowrey. Mean heights of girls increased 43.4 cm with 99% confidence limits of $\pm$ 3.3 cm. This difference in mean height of girls during the period was 5.4 cm less than that expected. Therefore Iowa girls from 6 through 15 years made slightly less than the expected gains in height, according to Watson and Lowrey. These comparisons suggest that Iowa school children as studied cross-sectionally at the mid-century were exceeding expected gains in weight but not in height. This observation together with the comparisons with the other mass studies in Iowa may indicate a trend in the development of Iowa children which needs careful scrutiny.

Differences in mean measurements at certain yearly intervals were of special interest. For boys, these periods were as follows: 9 to 10 years, when increases in mean weight were the smallest and increases of all measurements were small; and 14 to 15 years, when increases in weight, height, bi-iliac diameter and chest breadth were largest and increase of leg girth was next largest. For girls, there were no outstanding "spurt-like" periods but from 7 to 8 and from 12 to 13 years the differences in body measurements were conspicuously small.

COMPARISON WITH STANDARDS

Physical fitness of the Iowa boys and girls at all ages was evaluated by the Pryor ('37) and the Baldwin-Wood stand-

ards (Taylor and MacLeod, '49). Evaluations by the two methods presented different pictures of the group tendencies at almost every age. Their usefulness in classifying Iowa children may therefore be questioned.

According to both standards, more Iowa children tended to have ratings of 10% or more above the standards than 10% or more below. More of the younger than of the older children were within the middle range. At 6 years of age approximately 85% of the boys and 70% of the girls were rated within the middle range; at 16, approximately 70% of the boys and 60 to 70% of the girls were within this range. The 12-year-old children had the smallest proportion of ratings within this range. At most ages more of the boys than of the girls were within the middle range, and more of the girls than of the boys were 10% or more above the standards.

Although the propriety of using the Wetzel Grid for cross-sectional data involving single measurements has been questioned, thus applied it may suggest trends of development in groups and may afford a convenient means of classification. Its applicability to mass studies merits further study. The mean heights and weights of the children were plotted in the Wetzel Grid. These evaluations indicated that the Iowa school children of the state-wide sample were progressing slightly below the A_1 channel and along the 15th percentile on the scale of auxodromes. The Iowa boys and girls reached the 140th and 130th iso-developmental levels at approximately the ages noted by Spies ('53) for boys and girls without nutritive failure. The smallest differences in mean developmental levels were between 9 and 10 for boys and between 12 and 13 for girls.

The percentage distribution of the children according to physique channel and auxodrome is presented in table 3. For this comparison the children were divided into 5 groups as shown in the table. At all ages the majority of children were in the group described as having good physique and good growth. For both sexes 12-year-olds had the smallest proportion of children with good physique and satisfactory growth

TABLE 3

Percentage distribution of children according to physique channel and schedule of development in the Wetzel Grid

AGE IN YEARS	BOYS [1]					GIRLS [1]				
	I Channel A_4	II B_1 and above 67% auxodrome [2]	III B_1 and below 67% auxodrome [3]	IV B_2 and above 67% auxodrome [2]	V B_2 and below 67% auxodrome [3]	I Channel A_4	II B_1 and above 67% auxodrome [2]	III B_1 and above 67% auxodrome [3]	IV B_2 and above 67% auxodrome [2]	V B_2 and below 67% auxodrome [3]
	%	%	%	%	%	%	%	%	%	%
6	7.9	92.1	0	0	0	6.0	82.0	0	10.0	2.0
7	6.9	75.9	1.7	15.5	0	10.4	72.9	0	14.6	2.1
8	9.4	66.0	0	24.5	0	4.5	68.2	4.5	13.6	9.1
9	7.3	65.4	3.6	21.8	1.8	7.8	64.1	7.8	10.9	9.4
10	6.7	71.7	1.7	16.7	3.3	6.6	60.6	1.6	21.3	9.8
11	8.0	64.0	2.0	18.0	8.0	6.9	65.5	3.4	12.1	12.1
12	8.9	57.8	5.6	20.0	7.8	15.8	56.1	3.6	11.0	13.4
13	9.1	65.9	0	20.4	4.5	11.4	63.6	4.5	11.4	9.1
14	7.5	60.0	12.5	10.0	10.0	8.1	78.4	0	8.1	5.4
15	0	90.6	0	6.2	3.1	21.0	73.7	0	0	5.3
16	8.8	70.6	5.9	11.8	2.9	13.5	70.3	10.8	5.4	0
17	4.8	61.9	14.3	14.3	4.8	15.4	61.5	15.4	3.8	3.8
18	11.1	61.1	16.7	0	11.1	0	83.3	8.3	8.3	0

[1] I Obese, II Good physique and good growth, III Good physique but slow growth, IV Thin body build but good growth, V Thin build and slow growth.

[2] Two per cent to 67%.

[3] Sixty-seven per cent to 98%.

rates (group II); many of the boys of this age, though thin, were growing well (group IV), whereas many of the girls of this age group were either obese (group I), or thin and growing slowly (group V). The percentage of obese girls was highest from 12 to 17 years inclusive, and the percentage of thin girls was highest from 8 to 13 inclusive. The percentage of obese boys was fairly uniform throughout the school years.

These comparisons with the three standards focus attention on the age of 12 as a turning point in body size. Girls seemed more likely than boys to be classified at the extremes of physical-fitness ratings. Weight control may indeed be a more difficult problem for girls than boys.

NUTRITIONAL CHARACTERISTICS AND RELATIONSHIPS TO PHYSICAL MEASUREMENTS

Biochemical data indicative of nutritional status will be presented in later reports. For comparison of the general characteristics of the children in this respect with physical status the following summary of the blood findings is given. Hemoglobin concentrations were predominantly "fair" and "good," according to the Bessey-Lowry ('47) rating. Only 3% of 578 girls had values below 11 gm per 100 ml of blood, and only 5% of the 583 boys had values which might be classified as low (i.e., below 11 gm at ages less than 13; below 11.5 gm at 13 and 14; or below 12 gm above 14 years of age). Ascorbic acid nutrition was perhaps less satisfactory as shown by the fact that 26% of 329 boys and 24% of 326 girls had serum concentrations below 0.40 mg of ascorbic acid per 100 ml or less, while 30% of the boys and 32% of the girls had values of more than 1.0 mg. Approximately one-fourth of the children tested had serum carotenoid concentrations lower than 75 μg per 100 ml, but few had serum vitamin A values below 20 μg per 100 ml. Only about 5% had serum alkaline phosphatase values of 8 nitrophenol units or higher.

Medical examinations of a subsample of the children revealed no symptoms of drastic malnutrition but dental ex-

aminations showed a startling prevalence of defective tooth surfaces.

Mean increments in body measurements of the boys tended to increase in "spurt-like" fashion. The nutrient consumption, except for calcium, increased regularly from year to year. The two largest spurts of growth, observed at 14 to 15, and 12 to 13, were accompanied by increments in the mean intake of most nutrients. On the other hand, between the ages of 9 and 10, when mean increments in weight were only 1.6 kg, the nutrient intake decreased or remained the same. With boys trends of mean body measurements and of nutrient intake were roughly comparable, except in calcium, yet the regularity of increments in intake in relation to the irregularity of increments in body size raises an interesting question regarding the utilization and storage of nutrients during growth.

For the girls increments in mean body measurement proceeded rather regularly until 16. The nutrient intakes followed an irregular course, and for the most part failed to increase after 12. The period 12 to 13, marked by small changes in mean body measurements, was accompanied by a notable decrease in mean consumption of most nutrients. Between 12 and 16 years the mean weights of girls increased by 11.6 kg and mean heights by 9.1 cm. Concurrently the mean nutrient consumption remained the same or actually decreased. A very low intake was noted at 16 years, the age at which the mean weights of girls reached a plateau.

These year-to-year comparisons of mean nutrient intake of girls with mean body measurements raise a number of questions. Is the decrease in mean intake between 12 and 13 related, by cause or effect, to retardation in growth noted at that time? Do girls of this age adopt food practices which are not readily changed in accordance with their physiological needs? Nutrition education and other efforts to improve the nutrition of girls, such as the school lunch, may thus appear to be particularly important at the age of 12. A further question is whether or not the nutrient intake of teen-age

girls suffices to sustain the bory growth and at the same time to provide adequately for the stores needed in early adulthood. The diminishing calcium intake and the low levels of dietary iron of girls, whose growth has evidently not ceased, suggest the possibility of a poor quality of growth for girls during the teens.

Although the diets of the teen-age girls tended in all respects, including the food-energy value, to be poorer in comparison to the Allowances than those of the younger girls, more of the older girls tended to be obese or overweight by most standards of comparisons, and more of the younger girls tended to be thin. Also, in spite of the considerable superiority in nutrient intake of boys over girls after 12, more boys than girls tended to be thin or underweight. More information is needed concerning the interplay of nutrition with other factors in determining the body size of children, particularly of girls after 12.

SUMMARY

Five body measurements were made of a state-wide sample of Iowa school children, whose nutrient intakes and nutritional status were studied concurrently. The means at successive ages, 6 through 18, agreed closely with norms based on a partially longitudinal study of Iowa City children.

Mean weights at most ages tended to be larger than those observed in a mass study conducted in Iowa about 10 years ago, but mean heights were about the same. There was some evidence of a trend toward relatively greater gains in weight than in height.

By most standards of comparison fewer girls than boys were in the so-called normal range. At 12, fewest children were within the normal range. Overweight was more frequent among girls than boys, and among the older than the younger girls.

Major differences in mean body measurements of children of successive ages were compared with mean nutrient intake. Of special interest were the periods of 9 to 10 and 14 to 15

for boys, and 12 to 13 for girls. Increments in mean body weight from year to year were irregular for boys and fairly regular for girls despite the opposite trend in nutrient intake. Moreover, in the teen-age, mean weights and heights of girls tended to increase although mean nutrient consumption apparently failed to increase or in some cases, notably calcium, actually decreased with age.

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RELATIONSHIP OF ESTIMATED NUTRIENT INTAKES OF IOWA SCHOOL CHILDREN TO PHYSICAL AND BIOCHEMICAL MEASUREMENTS [1,2]

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THREE FIGURES

(Received for publication June 5, 1954)

The relationship of the levels of nutrient intake of children living at home and eating self-selected diets to their physical growth and nutritional status needs clarification. Adequate exploration of this problem by experimental procedures is difficult, since children cannot be subjected deliberately to suboptimal conditions and since results obtained with controlled diets may not simulate those with the self-selected type. Mass studies of well-sampled groups may provide data suitable for such a study.

The purpose of the present analysis was to find out whether differences in level of nutrient intake were associated with differences in body measurements and nutritional status among the children themselves in the state-wide sample of Iowa school children described in the foregoing reports (Eppright et al., '54; Eppright and Sidwell, '54). Furthermore, in view

[1] Contribution no. 12, Subproject 2 — "The Nutritional Status of School Children: The School Lunch as an Influencing Factor" of the North Central Region Cooperative Project NC-5, "Nutritional Status and Dietary Needs of Population Groups." (a) Human Nutrition Research Branch, Agricultural Research Service, Washington, D. C. (b) Iowa Agricultural Experiment Station, Ames, Iowa, as Journal Paper no. J-2445, Project 1021.

[2] Supported in part by a grant and other assistance from General Mills, Inc., as part of its nutrition education program.

TABLE 1

Mean daily nutrient consumption of two groups of Iowa children classified according to dietary adequacy

AGE	GROUP	NO.	FOOD ENERGY VALUE	PROTEIN	CALCIUM	IRON	VITAMIN A VALUE	THIAMINE	RIBOFLAVIN	NIACIN	ASCORBIC ACID
			Cal.	*gm*	*mg*	*mg*	*I.U. × 100*	*mg*	*mg*	*mg*	*mg*
					Boys						
6	I [1]	17	2397	75	1285	10	66	1.2	2.2	12	95
	III [2]	4	1974	59	728	9	52	.9	1.4	11	49
7	I	22	2387	74	1234	10	69	1.1	2.2	12	91
	III	13	1940	58	776	9	43	.9	1.4	11	42
8	I	23	2550	80	1315	12	82	1.3	2.3	13	94
	III	12	1875	57	819	8	36	.9	1.5	10	53
9	I	20	2760	88	1324	14	110	1.4	2.5	16	102
	III	10	2025	57	753	9	46	.9	1.4	10	62
10	I	9	2904	91	1383	13	109	1.4	2.6	15	99
	III	18	2163	64	798	10	48	1.0	1.6	12	56
11	I	12	2950	96	1430	14	115	1.5	2.7	15	112
	III	13	2420	67	824	10	45	1.0	1.6	13	61
12	I	17	3567	101	1645	17	116	1.8	3.0	19	128
	III	35	2312	71	926	11	50	1.1	1.8	12	54
13	I	5	3592	110	1789	17	121	1.7	3.3	19	167
	III	24	2598	77	904	12	59	1.2	1.8	14	76
14	I	4	3742	106	1576	17	165	1.7	3.1	18	104
	III	19	2873	84	881	14	72	1.4	1.9	16	87
15 +	I	12	4121	126	1775	20	121	2.1	3.3	21	160
	III	36	3082	88	1013	14	72	1.4	2.1	15	75

TABLE 1 *(continued)*

					Girls						
6	I	11	2153	69	1129	10	83	1.1	2.0	11	89
	III	14	1639	53	684	8	37	.8	1.3	9	47
7	I	13	2213	71	1110	10	70	1.2	2.1	11	107
	III	14	1720	49	535	8	31	.9	1.1	9	61
8	I	13	2316	74	1224	11	81	1.2	2.2	12	111
	III	10	1729	51	963	7	35	.8	1.4	9	46
9	I	11	2604	80	1207	13	96	1.3	2.3	13	97
	III	16	2069	60	722	9	48	1.0	1.4	12	63
10	I	8	2745	80	1200	13	87	1.4	2.3	14	128
	III	22	2010	57	685	10	70	1.0	1.5	11	64
11	I	7	2889	94	1433	14	94	1.5	2.7	16	104
	III	23	1990	59	778	9	46	1.0	1.5	11	67
12	I	9	3101	97	1427	15	108	1.5	2.5	16	117
	III	32	2344	70	865	11	73	1.1	1.7	13	62
13	I	3	3531	105	1406	16	102	1.6	2.4	18	139
	III	26	2122	62	781	10	61	1.0	1.5	11	60
14	I	2	3290	100	1348	15	79	1.7	2.5	17	126
	III	16	2136	62	692	11	48	1.1	1.5	12	70
15 +	I	5	3072	106	1464	15	122	1.6	2.6	17	132
	III	75	2273	65	705	11	56	1.1	1.5	12	78

[1] I Children whose mean daily diets conformed fully to the Allowances.

[2] III Children whose mean daily diets contained at least one nutrient in amounts less than 67% of the Allowances.

of the frequency with which the Recommended Allowances of the National Research Council are used in the evaluation of diets of groups of children, it seemed important to find out whether the children whose diets conformed fully to the Allowances differed from those whose diets were lacking in at least one respect.

Classification of children according to estimated nutritive value of their diets

For the present analysis the children were divided into three categories: I, those with diets in which the mean daily nutrient content was equivalent to 100% or more of the Allowances in all respects; II, those with diets in which some nutrients were present in less than 100% of the Allowances, but none in less than 67%; III, those with diets in which at least one nutrient was present in less than 67% of the Allowances (1953 revision). This classification was chosen because it has been widely used with various modifications in mass dietary studies (Tucker et al., '52; Storvick et al., '51; Moschette et al., '52). Of the 586 Iowa boys, 24% had diets in group I, 45% in group II, and 31% in group III. Of the 602 Iowa girls, 14% had diets in group I, 45% in group II, and 41% in group III. For this report, groups I and III were selected for comparison because they represent contrasting nutrient levels which might result in differences in the children. Diets providing less than two-thirds of the amounts of the Allowances of one or more nutrients may not be poor, but this method of screening faciliated the selection of two groups of children with contrasting levels of nutrient intake. Group I consisted of 223 children and group III of 432.

RESULTS

The mean daily food-energy value and nutrient content of the diets are shown in table 1 for the two groups of children by age and sex. The children of group I had liberal diets in every respect. Their mean daily intakes far exceeded the

Allowances at all ages and for both sexes. The group III diets averaged less than the Allowances in most items, except for vitamin A value, the B-vitamins, and iron for a few of the age-sex groups. Mean intakes of these nutrients approximated the Allowances. The mean daily intakes of the children of group I exceeded those of group III by 25 to 50% for most nutrients.

Calcium was the nutrient most frequently lacking in the diets of the Iowa children. Shortage of dietary calcium alone was responsible for the classification in group III of 29% of the 184 boys and 32% of the 248 girls included. An additional 31% of the boys and 40% of the girls had a calcium shortage combined with shortages in some other nutrient, notably ascorbic acid. Forty percent of the boys and 28% of the girls of this group had less than 67% of the Allowances of some nutrient other than calcium. Of the diets adequate in calcium, shortage of ascorbic acid was most often responsible for the classification in group III, though for girls iron and riboflavin shortages were almost as frequent as those of ascorbic acid. Only 5% of the boys and 13% of the girls in group III had diets with protein content of 67% of the Allowances or less.

Physical characteristics of two groups of children with different levels of nutrient intake

Figure 1 shows graphically by age and sex the mean values of weight, height, developmental level, and leg girth for the children of groups I and III. Data for the children 15 years and above were combined because of the small numbers in group I and because above that age increments in body size were small.

At most ages the children of group I were, on the average, heavier, taller and larger in leg girth than the children of group III. They also exceeded those in group III in developmental levels and in the auxodromic ratings on the Wetzel Grid The latter were observed but are not shown in the figure. Differences were also observed in mean measurements of bi-

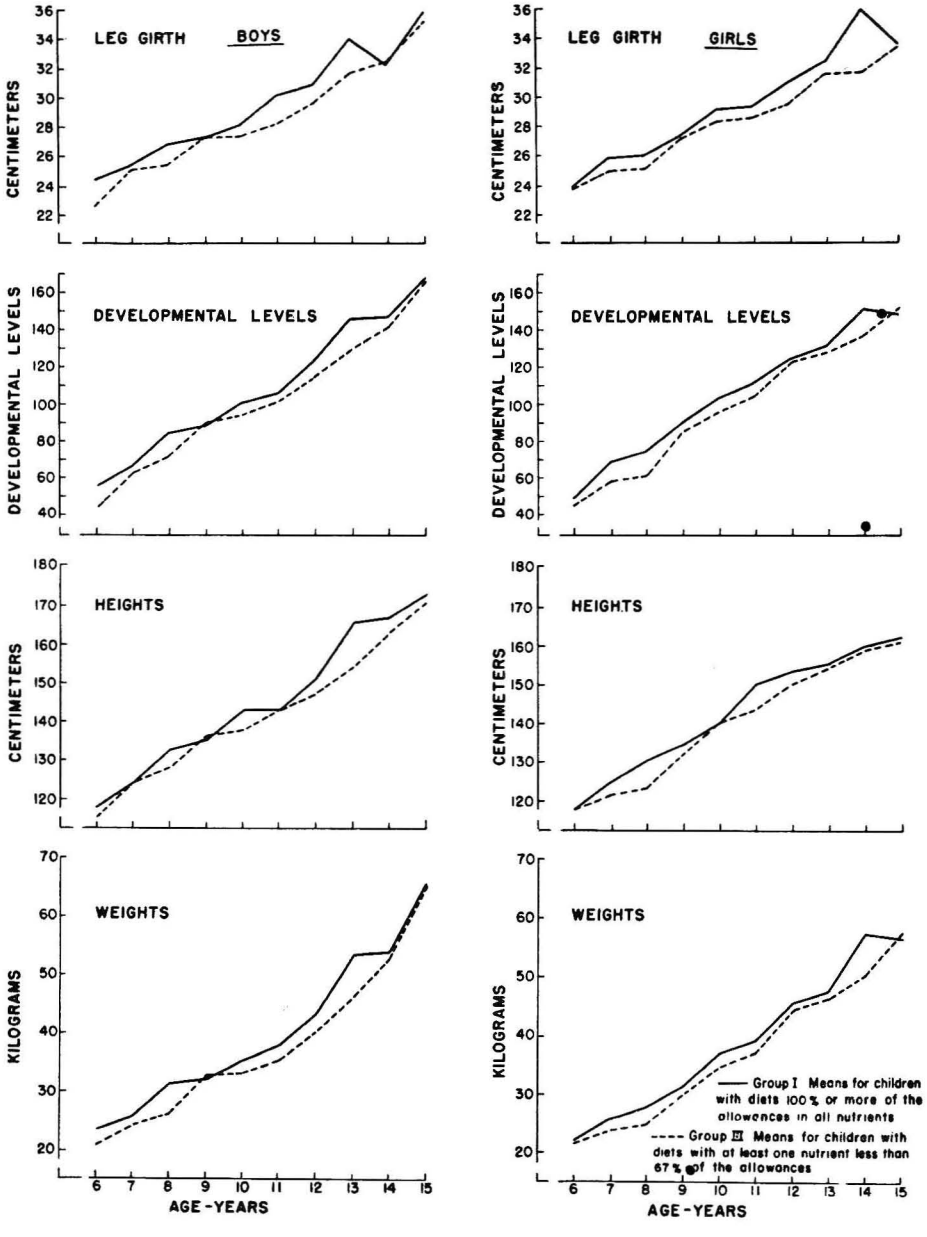

Fig. 1 Physical measurements of two groups of Iowa school children with diets of different nutrient levels.

iliac diameter and chest breadth. The differences were small but because of their persistent occurrence in all measurements and throughout the age groups they are considered significant.

Further analyses of the data were made in an effort to ascertain whether these differences in body measurements were associated with differences in specific nutrients, particularly calcium. Mean weights, heights, developmental levels, and measurements of leg girth were calculated for the children of group III, subdivided according to the following nutrient shortages: (a) calcium only; (b) calcium plus other nutrients; (c) nutrients other than calcium. The numbers in some of the age-sex groups were small and the results of the analysis were not conclusive. The deviations in mean body measurements of the children in the smaller groups (a) and (c) from the means of group I were about equally pronounced. However, when calcium shortages were accompanied by low intakes of other nutrients (b), the body measurements tended to deviate more from those observed in group I than when the dietary shortage was in calcium only (a) or was in one or more nutrients other than calcium (c).

The deviations in body measurements of children of group III from those in group I noted in this study apparently resulted from moderate dietary inadequacies, as indicated by the Allowances, mainly in calcium and ascorbic acid. The levels of mean daily calcium intake between which these small differences in body measurements were noted were 600 to 900 mg for the low group and 1200 mg for the high. The corresponding levels of intake of ascorbic acid were 42 to 87 mg and 89 to 167 mg (see table 1).

The boys and girls of group I were found to be at the 140th and 130th developmental levels of the Wetzel Grid at the ages noted by Spies ('53) for children "without nutritive failure." The boys and girls of group III at these same developmental levels were older than those of group I. They were, however, not so retarded as the children reported by Spies "with nutritive failure." Apparently, according to these cross-sectional data, the diets of the children of group

III were sufficiently low in nutrients to retard growth slightly but they were considerably above the levels leading to nutritive failure.

Hemoglobin concentrations

Hemoglobin concentrations have been described as valuable indicators of nutrition as a whole and have been regarded as useful in assessing dietary adequacy (Kaucher et al., '48). It would therefore seem of interest to ascertain whether differences in hemoglobin concentrations were evident between groups I and III. The mean concentrations were computed for boys and girls subdivided into 4 age groups and further divided according to place of living, rural or urban.

The mean hemoglobin concentrations of all groups were reasonably good and there were no statistically significant differences between the concentrations of hemoglobin in the blood of either boys or girls of groups I and III. This observation is not surprising since the diets of the children of group III, though lower than those of group I in protein, iron, and the B-vitamins, did not deviate far from the Allowances for these nutrients for most groups, except possibly for the older girls. In 6 of the 8 comparisons, boys of group I had higher hemoglobin concentrations in the blood than had corresponding boys of group III, whereas in only three of the 8 comparisons did the girls of group I exceed the corresponding girls of group III with respect to hemoglobin concentrations. These results suggest that under these conditions the hemoglobin concentrations of boys may reflect dietary conditions more than do those of girls. This difference in response may be expected in view of the greater complexity of factors affecting the hemoglobin concentrations of girls than of boys.

Serum alkaline phosphatase concentrations

Determinations of the concentration of serum alkaline phosphatase were made of a subsample of 134 children of group I and 243 children of group III. The mean values obtained for ages 6 through 13 are presented in figure 2. After 13, too few

data were available in group I to permit comparisons. With both groups of boys mean serum alkaline phosphatase concentrations revealed characteristic increases between 11 and 13 years (Harrison et al., '48; Clark and Beck, '50; Bessey and Lowry, '47) and there were no consistent differences in this serum constituent for the two groups of boys.

Except at ages 6 and 9 mean concentrations of serum alkaline phosphatase were larger for girls of group III than

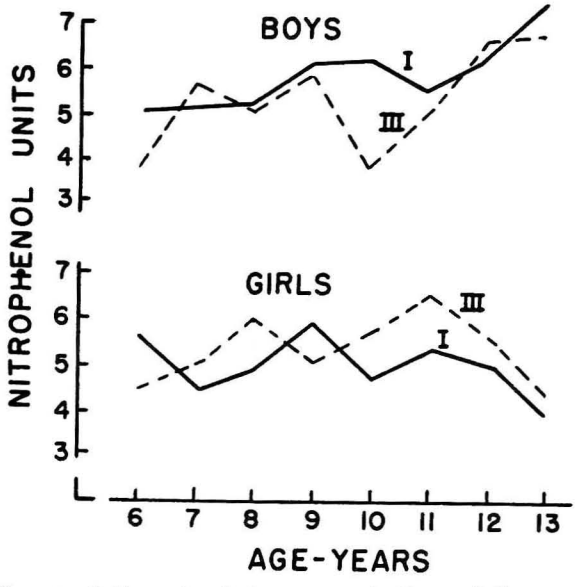

Fig. 2 Serum alkaline phosphatase concentrations of Iowa school children with diets of different nutrient levels.

for those of group I. The mean serum alkaline phosphatase concentrations of the girls of group I, excluding the 6-year-olds, attained what might be construed as a peak at 9 and those of group III, at 11. Moreover the concentrations seemed to be descending toward adult levels at later years for the girls of group III than for those of group I. For girls the differences generally were in the direction which may be expected with differences in calcification or growth rate, although the numbers at each age were too small to offer conclusive evi-

dence. In view of the suggested relationships of serum alkaline phosphatase concentrations to calcification it may be of interest that the calcium intakes of the girls of group III seemed more inadquate than those of the boys of group III (see table 1). Few in either group had supplementary vitamin D. The possible reflection of the dietary differences as noted in this study in the serum alkaline phosphatase concentrations of the girls, but not of the boys, merits further study.

Serum ascorbic acid and carotenoid concentrations

The serum ascorbic acid and serum carotenoid concentrations of subsamples of approximately 50 girls and 80 boys in group I and 100 girls and 90 boys in group III are shown in figure 3. The mean daily intakes of ascorbic acid by the boys of group I ranged from 90 mg daily for the 6- to 8-year olds to 137 mg for the 12- to 14-year olds. Corresponding intakes for girls of group I were 102 to 116 mg. Boys and girls of group III had intakes which on the average were roughly one-half as large as those of group I at the same ages. The mean vitamin A value of the daily intakes of group I was 7500 I.U. or larger for each age-sex group; corresponding values for group III were about one-half as large as those of group I. Approximately one-half of the Vitamin A supplied by the diets was derived from vegetables and fruits.

The mean serum concentrations of both ascorbic acid and carotenoids reflected the wide differences in intakes of these substances. However, the influence of other factors was suggested by differences in trends of concentrations in the serum of boys and girls of the two groups at the various ages.

For girls the concentrations of serum ascorbic acid and of carotenoids decreased with age from 6 to 8 years to 12 to 14 years whether the level of intake was high or relatively low. This change occurred in spite of a general trend toward higher intakes as the girls became older. The rate of decrease in serum concentrations of both substances with age was noticeably less for girls in group III than for those in group I. Un-

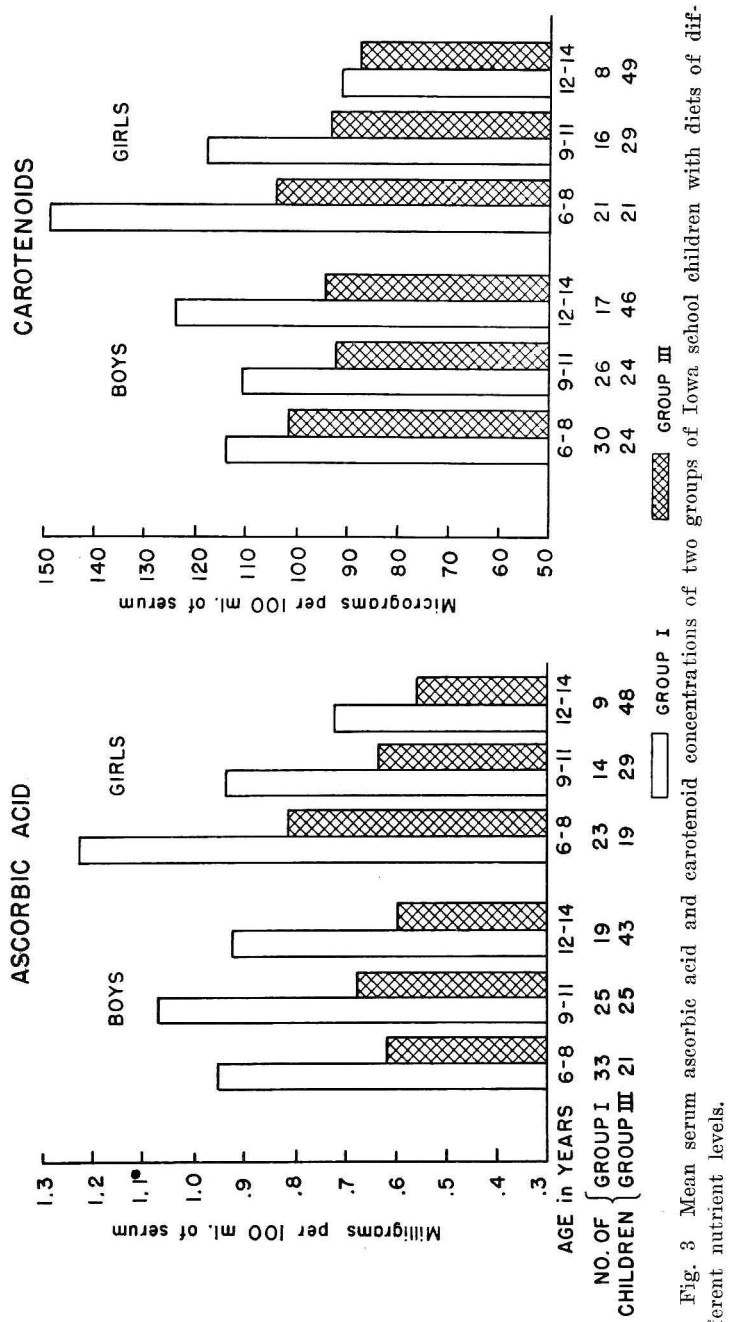

Fig. 3 Mean serum ascorbic acid and carotenoid concentrations of two groups of Iowa school children with diets of different nutrient levels.

like girls, boys with liberal intakes did not show these marked changes of serum concentrations of these two substances with age. Among boys and girls with liberal intakes the differences observed in the serum concentrations of these two substances suggest that the two sexes utilize these substances differently as they grow older.

The complexity of factors involved in plasma carotenoid concentrations was suggested by Szymanski and Longwell ('51) who stated that these concentrations may be related to growth rate, and that in view of sex differences which they noted, a slight change in endocrine balance could influence the plasma carotene, or an increased rate of growth might be related to the more rapid conversion of carotenoids to vitamin A. Further study is needed of the serum concentrations of ascorbic acid and carotenoids of boys and girls with different levels of intake and different rates and stages of growth.

SUMMARY

Relationships between levels of nutrient intake of groups of school children and their development as revealed by body measurements, hemoglobin concentrations in blood, and serum concentrations of alkaline phosphatase, ascorbic acid and carotenoids have been studied.

Comparisons were made between 223 children with liberal intakes of food energy and 8 nutrients and 432 children with diets considerably lower in nutritive value. These children were selected from a state-wide sample of public school children. Their nutrient consumption represented the upper and lower extremes found in a fairly well-fed school population. Dietary information was obtained by calculation of 7-day records. The N.R.C. Allowances were used as a basis for the dietary classifications.

Children with the liberal diets which conformed fully to the Allowances, tended to be slightly taller, heavier, and larger in leg girth than the children with diets at the other extreme, which on the average were somewhat below the Allowances.

No significant differences in hemoglobin or serum alkaline phosphatase concentrations were noted, though certain trends worthy of further study were observed. The mean serum concentrations of ascorbic acid and carotenoids reflected the intakes of these two substances by the two groups of children, but other influences on the serum concentrations were evident. Age-sex differences in the serum concentrations of these two substances were noted among the children with liberal intakes.

Commonly used dietary study methods may detect group differences in nutrient consumption which in turn may be reflected in the nutritional status of the children. Larger differences might be expected in a more heterogeneous population than is found in Iowa. The differences in nutritional status of children noted in this study were small, but they are indicative of trends which need to be scrutinized for the large groups of children whose diets are not drastically poor, but may be suboptional in nutrient content. The significance of these differences to the long-time health and well-being of children should be assessed through longitudinal studies.

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EFFECT OF INJECTING AND FEEDING VITAMIN B_{12} TO HENS ON CONTENT OF THE VITAMIN IN THE EGG AND BLOOD

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ONE FIGURE

(Received for publication July 6, 1954)

Although it has been definitely established that vitamin B_{12} is transferred from the hen to the egg, there are very few quantitative data on this transfer. Reports by Halick and Couch ('51), Milligan et al. ('52), and Yacowitz et al. ('52) show that a correlation exists between the quantity of vitamin received by the hen and that deposited in the egg. Recently Halick et al. ('53) have presented some quantitative data. They found 4.6 μg of B_{12} per yolk when the hens were fed about 12 μg of the vitamin daily and 12 μg per yolk after hens had been injected on 4 successive days with 250 μg of the vitamin per day.[2]

The present paper is concerned with the B_{12} content of eggs, chicks and blood when hens were injected or fed different levels of crystalline vitamin B_{12}. A preliminary report of the work has been presented by Denton et al. ('53a).

EXPERIMENTAL

Hens that had been on a B_{12}-deficient diet for about 6 months were injected or fed successively 3, 5, 10, 25, and 50 μg

[1] Deceased.

[2] The B_{12} values were calculated on the basis of an 18 gm yolk.

of crystalline B_{12} per bird per day. The same hens, except for one group which was injected only with 50 μg were used for all the levels, with each level being administered until the B_{12} activity of the eggs became a fairly constant value. Three hens were injected and two were fed the vitamin up to the 25 μg level. Five hens were injected at the 50 μg level.

Eggs were saved for incubation during the periods of such stabilized values following the administration of 5 and 25 μg of the vitamin. Blood samples were obtained by heart puncture at the end of each such period. After the administration of the 25 μg level the hens were allowed to go through a 14-week depletion period. During this time the B_{12} content of the eggs was estimated periodically. The procedure for the extraction of B_{12} described by Denton and Kellogg ('53) was used and the B_{12} activity was estimated by the U. S. Pharmacopeia Method of Assay ('50).

RESULTS AND DISCUSSION

The curves in figure 1 show the rate and extent of B_{12} deposition in the eggs when 3 μg of the vitamin were administered per bird per day. Eggs from the injected hens reached their maximum activity after 10 days. In contrast, when the hens were fed the vitamin, the highest B_{12} content of the eggs was not reached until the 18th day. From these results it would seem that it took twice as long to get one-half as much B_{12} into the egg when the vitamin was fed. However, this did not hold true for all of the other levels of administration.

A summary of the average B_{12} activity of eggs and day-old chicks when the different levels of the vitamin were injected or fed is shown in table 1. At the 3 and 25 μg levels, the injected hens deposited about twice as much B_{12} in the egg as did the hens that were fed the vitamin. However, at the 5 and 10 μg levels, there were increases of only 15 and 31% respectively, when B_{12} was injected. The value of 11.8 μg per egg which resulted from the injection of 50 μg is approximately the same as that obtained by Halick et al. ('53) when

250 μg of B_{12} was injected daily for 4 days. The deposition of B_{12} in the eggs decreased from 45% of the daily injection at the 3 μg level to 24% at the 50 μg level. At the three inter-

TABLE 1

Effect of administration of different levels of vitamin B_{12} on the B_{12} content of eggs and day-old chicks

B_{12} ADMINISTERED PER HEN, DAILY	AVERAGE B_{12} ACTIVITY OF EGGS	
	Injected	Fed
μg	*μg*	*μg*
0	.08 (3)[1]	.08 (2)
3	1.25 (3)	.66 (2)
5	1.62 (3)	1.41 (2)
10	3.25 (3)	2.47 (2)
25	7.78 (3)	3.53 (2)
50	11.8 (5)	. . .
	AVERAGE B_{12} ACTIVITY OF CHICKS	
5	1.63 (3)	1.25 (2)
25	6.17 (3)	3.30 (2)

[1] Figures in parentheses indicate the number of hens in each group.

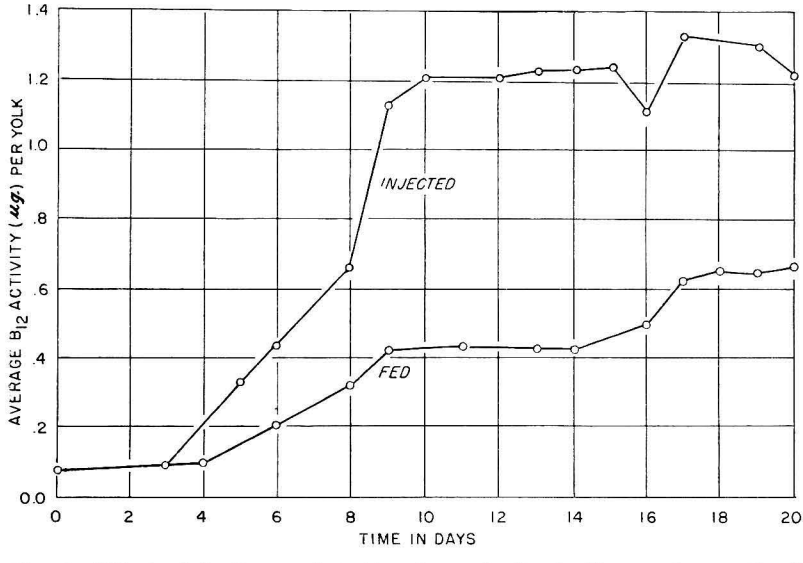

Fig. 1 Effect of feeding or injecting 3 μg of vitamin B_{12} per day on the B_{12} activity of eggs.

mediate levels about 32% of the quantity injected was transmitted to the egg. About 25% of the vitamin fed was deposited in the egg at the lower levels of administration as compared with 14% at the 25 μg level.

These results do not appear to be consistent with those of Milligan et al. ('52). They found a decrease in efficiency of transfer of the vitamin to the egg when the daily dose was increased from 0.5 to 2 μg, whereas our data indicate no loss in efficiency of transfer resulting from an increase of 3 to 10 μg. In addition, when 0.5 μg was fed, they observed about the same quantity of the vitamin in the egg as our results show when 5 μg were fed.

This discrepancy in results may be explained by the fact that Milligan and co-workers used the chick assay procedure which does not give the true B_{12} content of eggs. The nonspecificity of the chick assay for B_{12} in egg was demonstrated by Denton et al. ('54), who found that chick growth is stimulated by factors in egg yolk other than B_{12}.

As the egg production of the hens was relatively uniform during each of the experimental periods very little information was obtained on the effect of the rate of production on the deposition of B_{12} in the egg. However, during a 20-day period when 25 μg per day were being fed, one hen laid 6 eggs which had an average B_{12} content of 3.30 μg, whereas another hen laid 12 eggs which had an average value of 3.50 μg. These rather meager data did not indicate any relationship between the rate of egg production and the quantity of B_{12} in the egg when large quantities of the vitamin were fed.

It may also be seen in table 1 that the average B_{12} values for the day-old chicks are in agreement with those obtained for the eggs at the corresponding levels of vitamin administration.

According to Yacowitz et al. ('52), B_{12} is not normally present in egg white. In these studies the whites of the eggs contained a measurable quantity when 25 μg of the vitamin

were injected into the hens. The average of the individual whites assayed was 0.05 μg.

When the supplementary B_{12} was withdrawn, the B_{12} content of the eggs dropped rather rapidly for the first two weeks and thereafter at a much slower rate (table 2). The activity of the eggs of the injected group decreased a little faster during the first part of the depletion period, but, in general, the rate of decrease in the two groups was essentially the same. The depletion is a rather slow process, for by the

TABLE 2

Effect of withdrawal of supplementary B_{12}

TIME ELAPSED AFTER WITHDRAWAL OF B_{12}	AVERAGE B_{12} ACTIVITY OF EGGS	
	Injected	Fed
weeks	*μg*	*μg*
0	7.78	3.53
1	5.91	2.30
2	3.10	1.80
4	1.58	.97
6	1.44	.70
14	.37	.22

end of the 14th week the eggs from the injected group still contained about 4 times more activity than did the eggs before administration of the vitamin.

The results of the assay for B_{12} activity of blood at different levels of B_{12} administration are shown in table 3. The values for blood from the B_{12}-depleted hens, compared with reported values for normal chickens, are lower than those observed by Rosenthal and Brown ('54) but are higher than those reported by Couch et al. ('50) and Pfander et al. ('52).

The increase in the activity of the blood in both groups at the 3 and 5 μg levels seems reasonable. There is approximately twice as much activity in the blood of the injected hens, which is proportional to the activity found in the eggs. When 10 and 25 μg of the vitamin were fed, only slight increases in

blood level resulted, but large increases were obtained in the blood of the injected hens, particularly at the 25 μg level. These increases were not reflected in the B_{12} activity of the eggs.

Doctor and Couch ('52), using the bioautograph procedure, observed that chicken blood contains, in addition to B_{12}, three other growth factors for *L. leichmannii*. These factors were identified as desoxyribosides which were liberated by blood

TABLE 3

B_{12} activity of blood

B_{12} ADMINISTERED PER HEN, DAILY	B_{12} ACTIVITY PER ML OF BLOOD				
	Injected			Fed	
	Hen no. 3	Hen no. 6	Hen no. 39	Hen no. 9	Hen no. 26
μg	*mμg*	*mμg*	*mμg*	*mμg*	*mμg*
0	3	4	..	3	4
3	28	21	..	10	8
5	32	33	39	17	26
10	99	109	142	14	48
25	475	606	517	23	48

enzymes when the blood was autolyzed. When fresh blood was autoclaved it contained only one growth factor which by its position on the chromatogram was presumed to be B_{12}

In the present work the samples were autoclaved immediately after collection, therefore eliminating the possibility that the B_{12} values were influenced by the presence of desoxyribosides.

SUMMARY

A more rapid and greater deposition of vitamin B_{12} in eggs resulted when the vitamin was injected rather than fed to hens.

An average of the quantities of B_{12} deposited at the different levels of injection showed that about 33% of the daily dose was deposited in the egg. When the vitamin was fed, about 20% of the quantity ingested was deposited. A decrease in the efficiency of transfer of B_{12} to the egg was apparent

at the higher levels of administration. Newly hatched chicks showed about the same B_{12} activity as the eggs.

At the lower levels of administration of the vitamin, the blood of the injected hens showed, on the average, about twice as much activity as did the blood of hens fed B_{12}. When injected at higher levels, a large increase in the B_{12} activity of the blood resulted. This increase was not reflected in the B_{12} content of the eggs.

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THE EFFECT OF THIAMINE ANALOGS ON EMBRYONIC DEVELOPMENT AND GROWTH OF THE CHICK[1]

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TWO FIGURES

(Received for publication July 6, 1954)

INTRODUCTION

Neopyrithiamine and oxythiamine are antagonistic to thiamine in certain animals and microorganisms (Soodak and Cerecedo, '44; Wilson and Harris, '49; Daniel and Norris, '49; Eusebi and Cerecedo, '49; Woolley, '50; Ulrich and Fitzpatrick, '51; Cerecedo, Eusebi and Soodak, '52), but relatively little information is available about their mode of action. Suggestions made include the possibility that oxythiamine may displace thiamine from the body (Frohman and Day, '49) and that it might inhibit the phosphorylation of thiamine in the tissues (Cerecedo, Soodak and Eusebi, '51). Evidence largely from non-animal materials indicates that the phosphorylated antivitamins may be the active antagonists (Eich and Cerecedo, '54). Woolley ('51) has shown that neopyrithiamine inhibits the formation of cocarboxylase from thiamine by chicken blood, but the amount of neopyrithiamine required in this system was very large compared to that which

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affects the living animal adversely. Cocarboxylase was more effective than thiamine in prolonging the survival of mice given a large dose of neopyrithiamine. Woolley and Merifield ('52) observed that neopyrithiamine produced neurological symptoms of thiamine deficiency in mice without affecting the levels of blood pyruvate or liver cocarboxylase. Mice fed oxythiamine, on the other hand, did not develop any gross symptoms of thiamine deficiency although the levels of blood pyruvate were raised, while the concentrations of cocarboxylase in the tissues were depressed. As a result of these observations Woolley and Merifield ('52) suggested a new metabolic function for thiamine not mediated through cocarboxylase.

The present experiments deal with contrasting quantitative effects of neopyrithiamine and oxythiamine on the development of the chick embryo and on the growth of the young chick. The former is a phase of the life cycle during which fat and protein metabolism are paramount (Needham, '31, '42); the latter a phase during which carbohydrate metabolism is of major importance.

EXPERIMENTAL

Chick embryo experiments. The procedures followed for the injection and incubation of eggs were identical with those described by Cravens and Snell ('49) except that solutions for injection were made from non-sterile compounds in previously sterilized water and equipment. The small volume and the instability of the completed solutions prevented further sterilization. All solutions were injected immediately after preparation at a total volume of 0.1 ml per egg. Ten eggs were used in each experimental group.

Chick growth experiments. Day old New Hampshire × Single Comb White Leghorn crossbred chicks of both sexes were reared in electrically heated batteries with raised screen floors. Food and water were supplied ad libitum. Ten chicks were used for each experimental group. The thiamine-deficient ration had the following composition in grams per

kilogram: sucrose 600, hot alcohol-extracted casein 180, gelatin 100, DL-methionine 3, soybean oil 50, salts V (Briggs, Luckey, Elvehjem and Hart, '43) 60, fish oil (300 I.C.U. vitamin D; 1500 I.U. vitamin A) 5, and choline chloride 2. Vitamin supplements were as follows in milligrams per kilogram: riboflavin 6.0, niacin 50.0, calcium pantothenate 20.0, pyridoxine·HCl 4.0, biotin 0.2, folacin 4.0, α-tocopherol acetate 3.0, 2-methyl-1,4-naphthoquinone 0.5, p-aminobenzoic acid 100.0, and m-inositol 1000.0. Vitamin B_{12} was supplied in crude form.[4]

Supplements were given daily by subcutaneous injection after a 4-day depletion period, except in an experiment testing the effect of route of administration. A single dose of 20 μg of thiamine per 100 gm of body weight was given to all chicks on the 5th day to compensate for differences in body stores of the vitamin and on the 6th day experimental treatment with vitamin or antivitamin or both was started. Body weights were recorded daily and used to determine the size of the dose to be administered. Solutions required for injection were prepared immediately before use.

Thiamine, cocarboxylase, neopyrithiamine, and DL-thioctic acid were obtained from commercial sources. Oxythiamine was prepared by the method of Rydon ('51). The preparation contained 0.001% of thiamine as measured by the standard thiochrome assay. The melting point and the ultraviolet absorption spectrum coincided closely with reported values.

RESULTS

Embryo experiments. Preliminary additions of neopyrithiamine or oxythiamine to incubating eggs indicated that the compounds could prevent normal embryonic development, and that this toxic effect could be counteracted by the simultaneous injection of thiamine. Variations in dosage showed that both the hatchability of eggs and the subsequent survival of the chicks decreased with increasing dose of neopyri-

[4] Merck and Co., vitamin B_{12} concentrate no. 3 was added to supply 50 μg of B_{12} per kilogram of ration.

TABLE 1

Effect of neopyrithiamine on the developing chick embryo

COMPOUND INJECTED (amount per egg)	INCUBATION PERIOD PRIOR TO INJECTION											
	None			5 days			10 days			15 days		
	No. of eggs	% [1] Hatch	% [1] Survival	No. of eggs	% [1] Hatch	% [1] Survival	No. of eggs	% Hatch	% [1] Survival	No. of eggs	% Hatch	% [1] Survival
None	20	75	75									
0.1 ml water	20	60	60	60	90	90	20	100	100	20	95	95
0.25 mg neopyrithiamine	10	50	50	10	40	30	10	70	70	10	90	90
0.5 mg neopyrithiamine	10	20	0	10	0	0	10	70	60	10	80	10
1.0 mg neopyrithiamine	10	0	0	10	0	0	10	40	10	10	60	10
2.0 mg neopyrithiamine	10	0	0	10	0	0	10	30	0	10	20	0
1.0 mg neopyrithiamine + 1.0 mg thiamine	10	40	40	10	80	80	10	100	100	10	100	100

[1] Percentage of total eggs surviving a 72-hour holding period immediately following hatching.

TABLE 2

Effect of oxythiamine on the developing chick embryo

COMPOUND INJECTED (amount per egg)	INCUBATION PERIOD PRIOR TO INJECTION											
	None			5 days			10 days			15 days		
	No. of eggs	% Hatch	% [1] Survival	No. of eggs	% Hatch	% [1] Survival	No. of eggs	% Hatch	% [1] Survival	No. of eggs	% Hatch	% [1] Survival
None	20	75	75									
0.1 ml water	20	60	60	60	90	90	20	100	100	20	95	95
0.5 mg oxythiamine	10	40	40	10	30	30	10	100	100	10	100	100
1.0 mg oxythiamine	10	0	0	10	10	10	10	100	100	10	90	90
2.0 mg oxythiamine	10	0	0	10	0	0	10	100	100	10	100	100
4.0 mg oxythiamine	10	0	0	10	0	0	10	10	10	10	30	30
2.0 mg oxythiamine + 1.0 mg thiamine	10	20	20	10	70	70	10	90	90	10	100	100

[1] Percentage of total eggs surviving a 72-hour holding period immediately following hatching.

thiamine (table 1), and that the embryos appeared to be relatively more sensitive to the antivitamin when injected during the first 5 days of incubation. When administered during the later stages of development, neopyrithiamine permitted almost normal hatchability, although the chicks did not survive a subsequent 72-hour holding period and all exhibited symptoms of polyneuritis including ataxia, head retractions, loss of equilibrium and paralysis. Thiamine almost completely reversed the effects of neopyrithiamine at all stages of incubation.

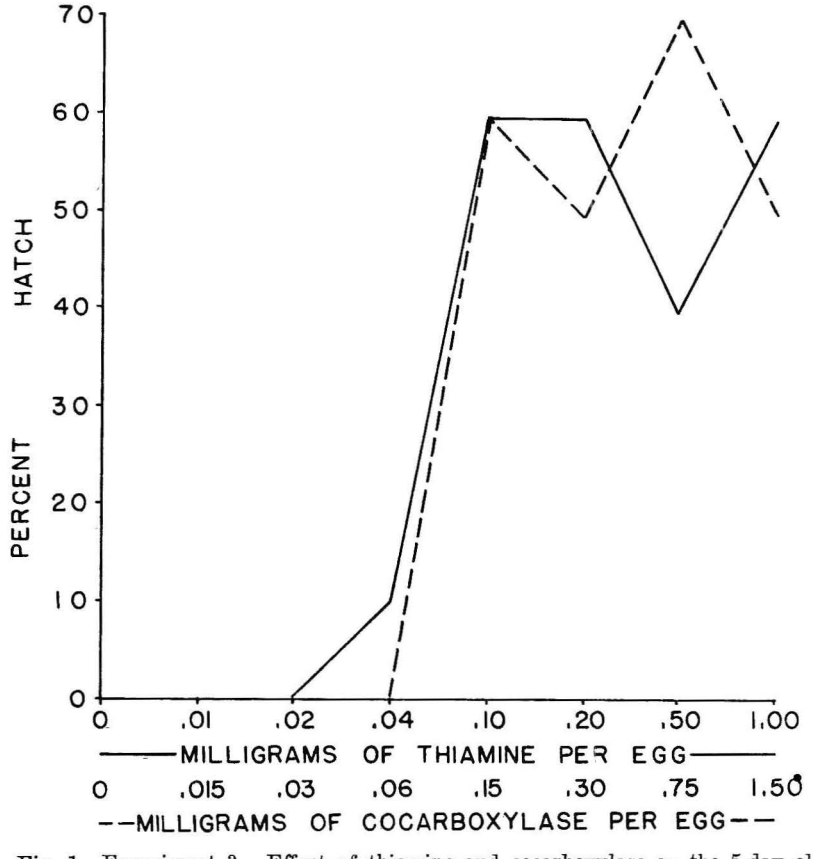

Fig. 1 Experiment 3. Effect of thiamine and cocarboxylase on the 5-day old chick embryo simultaneously given one milligram of neopyrithiamine.

The sensitivity of embryos to oxythiamine was more dependent upon age than the sensitivity to neopyrithiamine. Young embryos were sensitive to 0.5 or 1.0 mg of oxythiamine whereas 10- and 15-day old embryos were almost completely resistant to this dosage (table 2). Chicks from eggs injected with oxythiamine survived the 72-hour holding period and none developed polyneuritis. Embryos dying during the later stages of incubation showed a high incidence of body hemorrhage, edema and abdominal hernia. In general 2 to 4 times as much oxythiamine as neopyrithiamine was required to produce similar embryonic mortality.

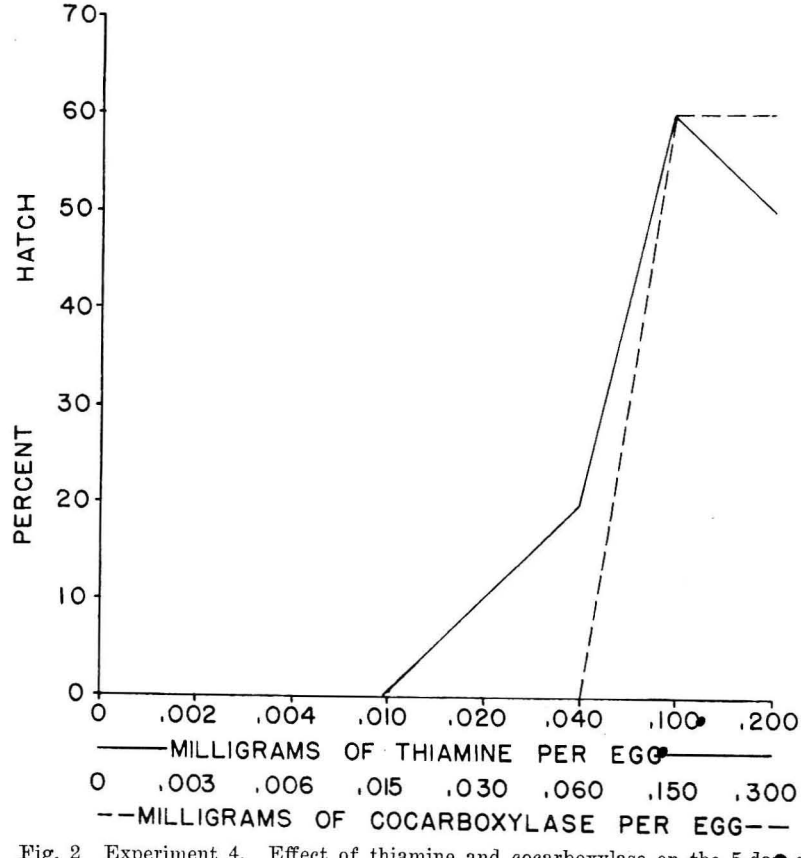

Fig. 2 Experiment 4. Effect of thiamine and cocarboxylase on the 5-day old chick embryo simultaneously given two milligrams of oxythiamine.

The minimum amounts of antagonist for 100% mortality of 5-day old embryos were 1.0 mg of neopyrithiamine and 2.0 mg of oxythiamine, and the amounts of thiamine required to reverse this effect completely lay between 40 and 100 µg. This is approximately equal to the amount of thiamine originally present in the egg (Cheldelin and Williams, '42; Scrimshaw, Porter and Scrimshaw, '49).

Cocarboxylase versus thiamine. Five-day old embryos were given either 1.0 mg of neopyrithiamine or 2.0 mg of oxythiamine and these doses resulted in a complete failure of hatchability. Certain embryos also received equimolar quantities of thiamine and cocarboxylase with the antivitamins. Figure 1 illustrates hatchability plotted against the level of thiamine and cocarboxylase given with neopyrithiamine. Under these conditions cocarboxylase was no more effective than thiamine in reversing the effect of neopyrithiamine. An inhibition ratio calculated from the minimum quantity of vitamin required to give maximum hatchability showed the ratio to be 1/10 for both thiamine/neopyrithiamine and cocarboxylase/neopyrithiamine on a molar basis.

Cocarboxylase was also equivalent to thiamine in reversing the effect of oxythiamine (fig. 2), the inhibition ratio being 1/20. The results suggest either that the two antivitamins do not prevent the formation of cocarboxylase from thiamine or that the coenzyme is hydrolyzed in the egg before it reaches its site of action.

Experiments with thioctic acid showed that 0.006 to 0.6 mg of this compound failed to counteract the effect of 1.0 mg of neopyrithiamine or 2.0 mg of oxythiamine when injected at 5 days of incubation.

Chick growth experiments. Because of the instability of free thiamine in purified rations thiamine was administered in various ways. Chicks grew better when 20 µg of thiamine per 100 gm of chick were injected subcutaneously or deposited directly into the crop (average weights at three weeks, 163 and 168 gm) than when given intramuscularly (average weight 55 gm). When neopyrithiamine was injected sub-

cutaneously together with either 20 or 40 μg of thiamine, a graduated reduction in growth resulted (table 3). From these data it was possible to estimate the amount of thiamine blocked by neopyrithiamine. Chicks receiving 20 μg of thiamine and 40 μg of neopyrithiamine (group 7) grew as well as those getting only 10 μg of thiamine (group 3) and hence about 10 μg of thiamine given to group 7 appeared to be blocked

TABLE 3

Effect of thiamine and neopyrithiamine on chick growth

GROUP NO.	SUBCUTANEOUS INJECTION (μg/100 gm body wt./day)		SURVIVORS AT 3 WKS.	AV. BODY WT. AT 3 WKS.	THIAMINE EQUIVALENT OF GROWTH SHOWN IN PREVIOUS COLUMN	THIAMINE BLOCKED BY NEOPYRITHIAMINE	GROWTH INHIBITION RATIOS
	Thiamine	Neopyrithiamine					THIAMINE: NEOPYRITHIAMINE
			no.	*gm*	*μg*	*μg*	
1	0	0	0/10	..	..	..	..
2	5	0	0/10	..	..	..	..
3	10	0	17/20	70	10	0	..
4	20	0	20/20	120	20	0	..
5	40	0	10/10	136	40	0	..
6	20	20	18/20	93	15	5	1:4
7	20	40	17/20	68	10	10	1:4
8	20	80	1/10	47	?	..	..
9	20	160	0/10	..	..	..	..
10	40	40	10/10	128	30	10	1:4
11	40	80	10/10	94	15	15	1:3.2
12	40	120	7/10	67	10	30	1:4
13	100	40	9/10	140	..	..	..
14	100	80	9/10	137	..	..	..

by the 40 μg of neopyrithiamine. The growth inhibition ratio would therefore be 1/4. This ratio remained relatively constant over the entire range of levels of both thiamine and neopyrithiamine that resulted in submaximum growth (table 3). That thiamine was capable of completely counteracting the effect of the antivitamin was demonstrated by the maximum growth of groups 13 and 14. Typical symptoms of polyneuritis were observed in groups 1, 2, 3, 7, 8, 9 and 12; partial recovery from these symptoms was noted during

periods of several hours after the daily administration of thiamine and neopyrithiamine solutions.

When 1000 to 4000 μg of oxythiamine were injected together with 20 μg of thiamine, growth was depressed (table 4) and the data indicated the inhibition ratio for growth to be 1/200. Because of the large quantities of oxythiamine required, the effect of this inhibitor at higher levels of thiamine was not determined. Symptoms of polyneuritis were observed

TABLE 4

Effect of thiamine and oxythiamine on chick growth

GROUP NO.	SUBCUTANEOUS INJECTION (μg/100 gm body wt./day)		SURVIVORS AT 3 WKS.	AV. BODY WEIGHT	THIAMINE EQUIVALENT OF GROWTH SHOWN IN PREVIOUS COLUMN	THIAMINE BLOCKED BY OXYTHIAMINE	GROWTH INHIBITION RATIOS
	Thiamine	Oxythiamine					THIAMINE: OXYTHIAMINE
			no.	*gm*	*μg*	*μg*	
1	0	0	0	..	..	..	..
2	5	0	6	46	5	0	..
3	10	0	10	71	10	0	..
4	20	0	10	143	20	0	..
5	40	0	10	181	40	0	..
6	20	1000	10	114	15	5	1: 200
7	20	2000	8	70	10	10	1: 200
8	20	4000	0		..	..	..
9	200	2000	8	161	..	..	..

in groups 1, 2 and 3 but not in any of the groups injected with oxythiamine; instead, some local and general edema was observed during the last week of the experiment.

DISCUSSION

The changing sensitivities of the chick to the two antagonists is illustrated by the following minimum protective ratios: thiamine/neopyrithiamine, 1/10 for the 5-day old embryo and 1/4 for the chick; thiamine/oxythiamine, 1/20 and 1/200 respectively. The activity of neopyrithiamine was only moderately dependent on the age of the chick, and the increase

in sensitivity observed with age appeared to be associated primarily with the 5-fold increase in metabolic rate which occurs on hatching (Lussana, '06). Neopyrithiamine administered after the 10th day of embryonic development did not exert any very frequent adverse effects until the chick began to hatch. Oxythiamine, on the other hand, was most deleterious early in embryonic life, and sensitivity to this antagonist diminished markedly after the 5th day, when the embryo ceases to depend upon carbohydrate for growth and development, and relies instead upon fat and protein (Needham, '31, '42). Sensitivity to oxythiamine continued to diminish after hatching, when chicks subsist under the combined influences of a fixed metabolism and a higher metabolic rate.

In all probability the changing potencies of the antagonists with age, as well as the differences in the deficiency symptoms elicited, reflected the capacities of oxythiamine and neopyrithiamine to inhibit different biochemical reactions of thiamine in a more or less selective way. In the presence of oxythiamine there were no neurological symptoms, but pyruvic acid accumulated (Woolley and Merifield, '52), and edematous areas developed near the site of injection. A number of explanations might be offered for the decreasing potency of this antagonist with age. The older chick might be able to destroy the antagonist, or it might be supplied with a substantial excess of the enzyme-coenzyme systems that involve pyruvate metabolism. Another possibility is that the metabolic demands of the older chick may be met by reactions which do not involve pyruvic acid as an intermediate. The details of a pathway alternate to the Embden-Meyerhof reactions have recently been published by Horecker et al. ('54). Observations with neopyrithiamine likewise point to the importance of some such alternate system, since this antagonist is more toxic than oxythiamine, yet it does not elicit an accumulation of pyruvate in the tissues (Woolley and Merifield, '52). Presumably, then, polyneuritic symptoms could be the result of a defect in the "alternate" system. In this connection it is of interest that symptoms of arsenic poisoning resemble

those of polyneuritis (Porter, '39; Abbott, Bird and Cravens, '54). Arsenite inhibits a number of enzyme systems including those responsible for oxidative decarboxylation of pyruvate and α-ketoglutarate (Baldwin, '52).

SUMMARY

Neopyrithiamine and oxythiamine injected into eggs increased embryonic mortality and decreased hatchability. Oxythiamine was less toxic during the later stages of incubation, whereas neopyrithiamine remained highly toxic during the entire incubation period. The inhibitory action of both antagonists was prevented by the simultaneous administration of thiamine or cocarboxylase. Inhibition ratios of vitamin/antivitamin determined for the 5-day old embryo were 1/10 for thiamine/neopyrithiamine and cocarboxylase/neopyrithiamine; and 1/20 for thiamine/oxythiamine and cocarboxylase/oxythiamine. Polyneuritis was observed in chicks hatching from eggs injected with neopyrithiamine during the later stages of incubation. Oxythiamine failed to produce polyneuritis but caused edema in some embryos that died prior to hatching.

Growth inhibition ratios for young chicks were 1/4 for thiamine/neopyrithiamine and 1/200 for thiamine/oxythiamine. As in the embryo experiments, neopyrithiamine brought forth polyneuritis and oxythiamine caused some local and general tissue edema. The changing effectiveness of the two antivitamins with age is consistent with known changes in developmental metabolism and with the hypothesis that the two antivitamins affect different enzyme systems.

ACKNOWLEDGMENTS

Neopyrithiamine used in these experiments was kindly supplied by Dr. Laurent Michaud of Merck and Company, Rahway, New Jersey and DL-thioctic acid was kindly supplied by Dr. E. L. R. Stokstad of the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, New York.

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DIETARY PROTEIN AND TUMOR-HOST RELATIONSHIP IN THE RAT [1, 2]

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(Received for publication June 25, 1954)

The work of Devik et al. ('50) and Algire and Chalkley ('45) emphasizes the sequence of events which takes place before the growth of a transplanted tumor becomes evident, events which include an initial inflammatory reaction, and development of the primary vascularization process after which the growth of the tumor can be measured. Tannenbaum and Silverstone ('53), in their recent review, discussed the delay associated with a low-protein diet in the development of these processes, pointing out also that the responses, in general, of tumors to dietary protein parallels the response of the host. Further work is needed to compare optimum dietary protein for induction and growth of the tumor with the optimum for growth of other tissues of the body. There is need also to correlate more adequately the effect of diet and various forms of treatment on the growth of transplanted tumors. The studies of Elson and Lamerton ('49) and of Devik et al. ('50) have shown that the response of tumors to x-irradiation is influenced by dietary protein. Preliminary results in our laboratories have suggested that the ethylenimines, compounds which reduce the growth rate of some tumors (Crossley et al., '53), may have a specific effect upon food and protein utilization. The following experiments were

[1] Supported by an Institutional Grant from the American Cancer Society.

[2] Presented before the meeting of the American Chemical Society, Kansas City, Missouri. April, 1954.

performed, therefore, to measure the optimum dietary casein intake for tumor and carcass growth in the presence and absence of N, N′, N″-triethylenephosphoramide (TEPA) and to determine the effect of supplementation with DL-methionine.

TABLE 1

Basal diet for rats

INGREDIENTS	AMOUNT	VITAMINS	AMOUNT
	gm		*mg/2400 gm agar diet*
Casein	180	Thiamine	4.8
Sucrose	134	Riboflavin	7.7
Dextrose	202	Nicotinic acid	96.0
Dextrin	159	Calcium pantothenate	96.0
Lard	232	Para-amino-benzoic acid	96.0
Wesson's salt mixture ('32)	40	Pyridoxine	3.9
Agar	33	Choline	2400.0
Cod liver oil	10	2-Methyl naphtho-quinone	0.5
α tocopherol (1%) in fat [1]	10	Biotin	0.5
Vitamin solution	25	Folic acid	0.5
Water	1375	Inositol	240.0
	2400		

[1] Crisco.

METHODS

Male Wistar rats, in groups of 10 were fed a semi-synthetic diet, which is recorded in table 1. Where the casein content was varied, the dextrose and sucrose were replaced isocalorically with the protein.

The food was prepared as follows: The agar and approximately two-thirds of the water were heated until the agar was in solution. The lard was added, the mixture cooled slightly, and the dry ingredients were mixed in by the use

of a mechanical stirrer. The alpha tocopherol, dissolved in fat,[3] the cod liver oil, and the water soluble vitamins, together with water to make up to weight were then added and mixed by beating. The slightly warm diet was poured into pans to cool and gel. The food was kept under refrigeration.

The tumor used in these experiments was the sarcoma R-1 studied also by Babson ('54). The tumor was transplanted subcutaneously in a lateral position by a trocar technique. The rats were fed ad libitum, daily food intakes being recorded. For each diet, there were two groups of animals. On the 5th day after transplantation, one group received daily intraperitoneal injections of N, N′, N″-triethylenephosphoramide (TEPA). Each rat was injected by weight so that all received 1 mg/kg of body weight of the drug. The other group provided the tumor control. The feeding of all diets was started at the time of transplantation.

The tumors were measured in width and length with a caliper and the weight of tumor calculated according to the following expression: $(0.796\ a^2b) \times$ specific gravity of the tumor tissue where a = the mean width and b = the length of the tumor. The average specific gravity of this sarcoma was determined to be 1.023. The correlation coefficient between actual weight and the calculated weight was 0.996 with a probability much less than 0.01.

The tumor weight was subtracted from the total weight of the tumor-bearing rat to calculate the weight of the carcass.

RESULTS

Preliminary results in our laboratory demonstrated that this sarcoma was always largest, after two weeks of growth, in rats fed the semi-synthetic diet containing 12% of casein. Further experiments revealed that this relatively rapid development was the result of the short time in which the tumor was established. The rate of growth of the tumor after it was established was relatively independent of the

[3] Crisco.

protein content of the diet. A straight line was obtained by plotting the logarithm of the weight of the tumor against time, the slopes of these lines being taken as a measure of the rate of growth of the tumor. These growth rate constants are recorded in table 2. Increasing the casein content of the diet to 25 or 35%, or supplementing the 12% of casein with DL-methionine, did not alter them significantly. The number

TABLE 2

Induction periods (days to develop tumor = 0.5 gm), growth rate constants, and tumor weights at the end of 14 days in rats with and without TEPA

DIET	INDUCTION PERIOD	GROWTH RATE K	TUMOR WT.	TUMOR WT. (TEPA)
	days		*gm*	*gm*
		Experiment 1		
Casein 12%	1.4 ± 0.4 [1]	0.23 ± 0.02	40.5 ± 5.6	29.7 ± 2.8 [2]
+ methionine	3.7 ± 0.6	0.27 ± 0.05	25.7 ± 4.5	19.7 ± 1.9
		Experiment 2		
Casein 12%	4.0 ± 0.8	0.17 ± 0.01	19.5 ± 2.1	4.7 ± 0.8 [3]
+ methionine	5.8 ± 0.4	0.19 ± 0.02	14.2 ± 1.0	4.2 ± 0.5
Casein 25%	5.6 ± 0.7	0.21 ± 0.03	9.0 ± 1.5	5.3 ± 0.6
Casein 35%	7.1 ± 0.3	0.20 ± 0.01	10.0 ± 0.8	4.6 ± 0.5
		Experiment 3		
Casein 12%	7.3 ± 0.7	0.20 ± 0.01	23.1 ± 3.7	6.3 ± 1.3
+ methionine	9.2 ± 0.6	0.16 ± 0.02	10.3 ± 1.8	....
Casein 25%	8.8 ± 0.5	0.20 ± 0.02	17.0 ± 2.6	4.7 ± 1.1
+ methionine	9.0 ± 0.2	0.22 ± 0.01	16.3 ± 1.6	....

[1] Standard error.
[2] Dosage = 0.4 mg/kg/day.
[3] Dosage = 1.0 mg/kg/day.

of days required to develop a tumor equal to 0.5 gm was calculated as the induction period, such a tumor being well established. The induction periods recorded in this table increased, if the 12% of casein was supplemented with DL-methionine, or if the casein content of the diet was raised. Adding methionine did not, however, lengthen the induction period of the tumor in rats fed the higher casein diet. The data in table 2 also illustrate the larger tumor size at the

end of two-weeks growth in animals fed 12% of casein. Survival time was shortest in these animals although all of the rats eventually died as the tumor increased in size. The last column in the table illustrates the retarding effect of TEPA on the growth of this sarcoma, the drug being injected intraperitoneally (1 mg/kg body weight/day) 5 days after implantation of the tumor. Survival time is lengthened by this type of therapy but survival time is not only a function of the size of the tumor, it is also a function of the condition of the carcass, the carcass here being used to refer to all tissues in the body except tumor.

TABLE 3

The relative change in weight of carcass with respect to tumor, with and without TEPA therapy, together with food efficiency of the carcass (△ carcass/100 gm food intake). The data were calculated 14 days after implantation

DIET	Δ CARCASS / TUMOR		Δ CARCASS/100 GM FOOD INTAKE	
	Diet alone	+ TEPA [1]	Diet alone	+ TEPA
Casein 0%	— 1.3	— 10	— 3.5	— 11.1
Casein 6%	— 1.9	0.8	4.5	— 1.2
Casein 12%	2.2	3.5	15.5	4.8
+ methionine	4.1	8.5	18.3	12.7
Casein 25%	5.8	6.4	16.9	12.7
Casein 35%	8.3	5.9	24.9	10.8

[1] 1 mg/kg body weight/day.

The relative increase in weight of the carcass with respect to tumor, as dietary casein increased, is illustrated by the ratios recorded in table 3, ratios which were calculated 14 days after implantation of the tumor. Thus the animals fed a high casein diet or 12% of casein supplemented with methionine developed a larger carcass than did those fed 12% or less of casein. The effect of diet on the development of the carcass is particularly striking in those animals given TEPA, a drug that not only reduces the development of the tumor but also that of the carcass. Thus tumor-bearing animals treated with TEPA and fed a protein-free diet had a ratio of — 10 which can be compared with — 1.3 determined in

untreated animals. Supplementing 12% of casein with methionine or feeding 25% of casein increased the ratio to values as high or higher than those recorded for untreated tumor-bearing controls. Both tumor and carcass were reduced in these animals given TEPA, a condition which even in the presence of reduced carcass size, favored longer life and regression of some tumors. Relative survival, for example, at the end of 4 weeks of growth of the tumor was 20% in tumor-bearing controls fed 12% of casein, 65% in corresponding animals fed 25% of casein but in animals given TEPA the survivals were increased to 80 and 100% respectively.

The reduction in the growth of both tumor and carcass in animals given TEPA may be, in part, a result of reduced food utilization. The data in table 3 demonstrate that changes in the carcass weight per 100 gm of food intake were reduced below those in tumor-bearing controls in animals given TEPA, and that supplementing the 12% casein diet with DL-methionine, or increasing the protein content, improved the growth of the carcass with respect to food intake. A reduction in food utilization was observed also in normal animals given TEPA. Normal rats gained 3.7 gm per gram of nitrogen. This protein efficiency ratio was reduced to 1.5 in rats given TEPA (0.4 mg/kg body weight), results which can be interpreted to mean that the drug affects protein anabolism (Allison et al., '54a).

The data presented in this paper suggest that the control of the growth of the tumor has some endogenous origin where the effect of dietary protein is minimized. Possibly part of this control is reflected in the liver where tissue protein is maintained and not depleted as rapidly as in other tissues in the tumor-bearing rat (Allison et al., '54b). There could be a flow of amino acids from the soft tissues of the body to the liver to the tumor, thus maintaining and promoting the growth of the tumor, a flow which could represent a balance between the control of protein anabolism by tumor and by host. This balance is normally positive toward the tumor, essentially uni-directional, but the data presented here em-

phasize that diet and chemotherapy can make that balance less positive. The data demonstrate that TEPA reduced the growth of the body as well as the tumor, reducing the utilization of food, possibly altering protein anabolism more or less directly. A high protein intake favored the development of the body, helping to make possible a more positive balance toward the body, leading to regression of some tumors. The protective effect of methionine suggests that this amino acid may play some specific role in the relationship between growth of the body and TEPA.

SUMMARY

The time for the establishment of a transplanted sarcoma in the rat was at a minimum in animals fed 12% of casein. Supplementing 12% of casein with 0.67% of DL-methionine, or increasing the casein content of the diet to 25 or 35% lengthened this induction period. However, the rate of growth of the tumor after it was established was the same on all diets.

Feeding the methionine-supplemented diet or the high-casein diet favored the development of the carcass of the tumor-bearing rat, thereby reducing the depleting effect of the growing tumor.

N, N′, N″-triethylenephosphoramide reduced the development of both tumor and carcass; food utilization by the carcass was also reduced in the presence of this drug.

Supplementing 12% of casein with methionine, or feeding high protein diets, favored the development of the carcass in the presence of TEPA, resulting in a more favorable condition for long survival time and regression of the tumor.

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THE RELATION OF VITAMIN B_{12} TO EGG YOLK STORAGE OF FOLIC ACID [1]

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(Received for publication June 7, 1954)

An interrelationship between vitamin B_{12} and folic acid in the chick was first noted by Nichol et al. ('49). Dietrich and co-workers ('49) reported that the addition of vitamin B_{12} and vitamin C to a synthetic chick ration produced an increase in the folic acid content of the liver, and that folic acid stimulated the synthesis of B_{12}, as measured by liver storage of that vitamin. Broquist et al. ('51) reported that the urinary excretion of the citrovorum factor (CF) in man was increased about three-fold when ascorbic acid was fed simultaneously with folic acid, as compared to the excretion when folic acid was fed alone. These authors also found that the conversion of folic acid to CF by rat liver homogenates in vitro was increased by the presence of ascorbic acid. Dietrich et al. ('51) reported that the intramuscular injection of 0.1 to 0.3 μg of B_{12} per day increased the storage of folic acid in the liver while the injection of 0.5 to 1.0 μg of B_{12} per day suppressed folic acid storage. The oral administration of vitamin B_{12} increased liver levels of both folic acid and CF.

The purpose of these investigations was to determine the relationship between vitamin B_{12} and folic acid in the laying

[1] This work was supported in part by Lederle Laboratories, Pearl River, New York, and the U. S. Public Health Service, National Institutes of Health, Bethesda, Maryland, under grant no. RG-1862.

[2] Ralston Purina Fellow during part of the period when this study was carried out.

hen. The criteria used in the evaluation were egg production and hatchability, and vitamin B_{12}, folic acid and CF contents of the egg yolk.

EXPERIMENTAL

The Single-Comb White Leghorn pullets used in this experiment were reared on range and were placed at approximately 6 months of age in individual laying cages with raised wire floors. The birds were fed a practical ration, adequate in vitamin B_{12}, prior to being placed on experiment. This ration consisted of 65% corn, 25% soybean oil meal, 2% oyster shell, 1.5% defluorinated rock phosphate, 0.5% salt, 0.0125% manganese sulfate, 2% whey, and 4% fish meal. In addition, the following were added in the specified number of milligrams per kilogram of feed: riboflavin, 4.4; calcium pantothenate, 11.0; niacin, 27.6; and aureomycin, 11.0. Vitamin B_{12} was added at the level of 13.2 μg per kilogram of feed.

After a pre-experimental period of 6 weeks, during which time individual egg production and hatchability records were kept, 12 groups of 6 hens each were selected for the experiment reported herein. The birds had access to feed and water at all times. The birds were artificially inseminated twice weekly with pooled semen from New Hampshire cockerels. Eggs were collected daily and marked with both the hen number and the date. At weekly intervals, eggs from each group were collected for microbiological assays. All other eggs laid by the hens were set and hatchability data ascertained. The eggs were candled on the 7th, 14th, and 18th days of incubation. Those eggs not containing viable embryos were broken out and the age of the embryo determined.

The diet used in this study consisted of 22.5% isolated soybean protein,[3] 69.5% glucose,[4] 5.0% salts IV [5] and 3.0% refined soybean oil. The following were added in the specified number of milligrams per kilogram of diet: thia-

[3] Drackett 220, obtained from the Drackett Company, Cincinnati, Ohio.

[4] Cerelose.

[5] Hegsted et al. ('41).

mine HCl, 4.0; riboflavin, 6.0; calcium pantothenate, 15.0; niacin, 1000.0; pyridoxine HCl, 4.0; *p*-aminobenzoic acid, 20.0; biotin, 0.2; 2-methyl-1,4-naphthoquinone, 0.5; α-tocopheryl acetate, 6.0; inositol, 1000.0; and choline chloride, 2000.0. Vitamins A and D were each supplied at the levels of 10,000 I.U. and 2,000 I.C.U. per kilogram, respectively.

The chicks in the first group received the basal diet unsupplemented. The second and third groups received 30 and 500 μg of vitamin B_{12} per kilogram of diet, respectively. The 4th group was supplemented with 2 mg of folic acid per kilogram of diet. The 5th and 6th groups received 2 mg of folic acid plus 30 and 500 μg of B_{12} per kilogram, respectively. The 7th group received 100 mg of folic acid per kilogram. The 8th and 9th groups received 100 mg of folic acid plus 30 and 500 μg of B_{12} per kilogram, respectively. The 10th group received 400 mg of folic acid per kilogram of diet. The 11th and 12th groups received 400 mg of folic acid plus 30 and 500 μg of B_{12} per kilogram, respectively.

At weekly intervals, eggs were collected from individual hens. The yolks were separated from the whites and the latter discarded. The yolks from each group were pooled and thoroughly mixed. A one-gram portion of the pooled sample of yolk was then weighed out for each of the vitamins determined. The vitamin B_{12} assays were carried out according to the procedure of Skeggs et al. ('50) with *Lactobacillus leichmannii* 4797 (American Type Culture Collection number) as the test organism. The B_{12} was released from the egg yolks by autoclaving for 30 minutes at pH 4.6 in an acetate buffer according to the procedure of Halick and Couch ('51), modified to the extent that 50 μg of KCN were added to each flask, as suggested by Soars and Hendlin ('51), to protect B_{12a} from destruction during autoclaving. The folic acid determinations were carried out with *Lactobacillus casei* 7469 as the test organism, according to the procedure of Couch et al. ('49). The folic acid was liberated from the yolk by incubating with chick pancreas at pH 7.2. Citrovorum factor (CF) determinations were carried out according to the pro-

cedure of Steele et al. ('49) using *Pediococcus cerevisiae* 8081, previously known as *Leuconostoc citrovorum* 8081. CF was liberated from the yolk by incubating at pH 4.5 in a sodium acetate buffer overnight at 37°C. Acid production at 72 hours was used as the index of growth in all determinations.

TABLE 1

The effect of vitamin B_{12} and folic acid on egg production, hatchability, and the deposition of B_{12}, folic acid, and CF in egg yolk

(Fifth through 14th week)

SUPPLEMENT TO BASAL [1]	B_{12}	CF	FA	EGG PRODUCTION	HATCHABILITY
	mμg/gm	mμg/gm	mμg/gm	%	%
None	2.5	36	176	52	64
B_{12} (30)	46.0	48	278	43	78
B_{12} (500)	154.0	47	529	57	83
Folic acid (2)	4.9	59	430	40	61
Folic acid (2) + B_{12} (30)	50.0	67	393	43	84
Folic acid (2) + B_{12} (500)	154.0	89	833	42	73
Folic acid (100)	4.3	90	805	40	66
Folic acid (100) + B_{12} (30)	65.0	96	1149	36	91
Folic acid (100) + B_{12} (500)	144.0	108	1164	45	79
Folic acid (400)	3.7	70	1103	51	67
Folic acid (400) + B_{12} (30)	56.0	89	1169	37	88
Folic acid (400) + B_{12} (500)	142.0	94	1239	43	82

[1] Vitamin B_{12} added in micrograms per kilogram, shown in parentheses. Folic acid added in milligrams per kilogram, shown in parentheses.

RESULTS AND DISCUSSION

Folic acid and citrovorum factor contents of egg yolk

The addition of 30 and 500 μg of vitamin B_{12} to the basal diet increased the folic acid content of the egg yolk from 176 to 278 and 529 mμg per gram of yolk, respectively (table 1). The CF content was increased from 36 to 48 and 47 mμg per gram, respectively, when 30 and 500 μg of B_{12} were added.

When the folic acid level in the diet was raised to 2 mg per kilogram, the B_{12} was effective only at the 500 μg level. The addition of B_{12} at this level produced an increase in the folic acid content of the yolk from 430 to 833 mμg per gram of yolk. The CF content was also increased from 59 to 89 mμg. Vita-

min B_{12} was effective in increasing the folic acid content of the yolk even when the folic acid level in the diet was raised to 100 or 400 mg per kilogram. At the 100 and 400 mg levels of folic acid, the 30 and 500 µg levels of vitamin B_{12} were equally active. At the 100 mg level of folic acid, the addition of 500 µg of B_{12} increased the folic acid in the egg yolk from 805 to 1164 mµg per gram. The CF content was increased from 89 to 108 mµg per gram of yolk. When 400 mg of folic acid were added to the diet, the addition of 500 µg of B_{12} increased the folic acid in the yolk from 1103 to 1240 mµg per gram. The CF content was raised from 70 to 94 mµg per gram. These values are lower than the corresponding values in the group fed 100 mg of folic acid.

When the basal diet was supplemented with folic acid alone, there was a significant increase in the folic acid and CF contents of the yolks. This increase was rather uniform with each increase in the folic acid level in the diet. The folic acid in the yolk was increased from 176 to 430 mµg per gram of yolk by the addition of 2 mg of folic acid per kilogram of diet. Increasing the folic acid level of the diet to 100 and 400 mg per kilogram increased the folic acid in the egg yolk to 805 and 1103 mµg per gram, respectively. This increase in the folic acid content of the yolk agrees with the earlier work of Couch and German ('50), although the values recorded herein were somewhat lower than those obtained by Couch and German. The values reported here agree more closely with those reported recently by Evans et al. ('53). These workers found that the normal folic acid content of the egg yolk ranged from 182 to 276 mµg per gram of yolk. The addition of 2 mg of folic acid per kilogram of diet increased the CF content of the yolk from 36 mµg per gram to 59 mµg per gram. Increasing the folic acid content of the diet to 100 and 400 mg per kilogram increased the CF content of the yolks to 90 and 70 mµg per gram, respectively.

The data obtained on the folic acid content of the egg yolks agree with the work of Dietrich et al. ('51) in which it was reported that the feeding of vitamin B_{12} increased the storage

of folic acid in the livers of chicks. Also, these data indicate that vitamin B_{12} is involved in the synthesis of CF from folic acid, either directly as vitamin B_{12}, or the physiological constituent of that vitamin, or indirectly, through an influence upon the storage of folic acid in the egg yolks.

Vitamin B_{12} content of egg yolk

Supplementation of the basal diet with vitamin B_{12} alone resulted in an increase in the vitamin B_{12} content of the egg yolks as measured by *Lactobacillus leichmannii* 4797 (table 1). The quantity of B_{12} in the egg yolk varied from 2.5 to 154 mμg per gram of egg yolk and was directly related to the vitamin content of the diet. These data are in complete agreement with the earlier work of Halick et al. ('53) in which the B_{12} content of the yolk was increased from 3.5 mμg per gram of yolk by feeding 50 μg of B_{12} per kilogram of diet up to 46.2 mμg of B_{12} per gram of yolk by feeding 1,750 μg of B_{12} per kilogram of diet. The values obtained in this experiment were somewhat higher than those reported by Halick et al. ('53), possibly due to the fact that KCN was used to protect B_{12a} from destruction (Soars and Hendlin, '51) during the liberation of the vitamin from the samples by autoclaving. These data also agree with the work of Denton and co-workers '53).

The addition of folic acid to the diet did not influence the B_{12} content of the egg yolk, even when fed at the level of 400 mg per kilogram of diet.

Hatchability

The omission of B_{12} from the hens' diet resulted in a general decrease in the hatchability of the fertile eggs. Supplementation of the diet with vitamin B_{12} resulted in an increase in the hatchability of eggs from all groups where this vitamin was added (table 1), irrespective of folic acid addition to the diet. The average hatchability of eggs from those groups

receiving B_{12} was 82.1%, as compared to 64.1% for the eggs from those groups not receiving B_{12}. The highest level of B_{12} fed had no deleterious effect on hatchability. This is in agreement with the results of Halick et al. ('53).

The rather high hatchability observed in those groups receiving no vitamin B_{12} (64.1% average for the 5th through the 14th week) is at variance with the earlier results reported by Olcese et al. ('50), where hatchability was decreased to zero per cent when the hens were fed a diet low in B_{12} for three to 6 weeks. This variation in results may be traced to the fact that the pre-experimental diets varied greatly with regard to B_{12}. The birds used in the present study had been fed a diet containing 4% fish meal plus 6.6 µg of vitamin B_{12} per kilogram of feed. The pre-experimental diet probably influenced the hatchability through a carry-over of B_{12} and other factors obtained during the growing period.

SUMMARY AND CONCLUSIONS

The addition of vitamin B_{12} to the synthetic diet increased the deposition of folic acid and CF in the egg yolks. The increase in the folic acid content of the yolk due to B_{12} was more apparent when the lower levels of folic acid were fed. The B_{12} content of the yolk increased only when the vitamin was added to the diet.

Hatchability was increased by the addition of vitamin B_{12} to the diet.

ACKNOWLEDGMENTS

Cerelose was supplied through the courtesy of the Corn Products Refining Company, Argo, Illinois. Folic acid was supplied by Lederle Laboratories, Pearl River, New York; biotin by Hoffman-La Roche, Inc., Nutley, New Jersey, and the rest of the B-vitamins by Merck and Company, Rahway, New Jersey. Methionine was obtained from Dow Chemical Company, Freeport, Texas.

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THE FAILURE OF THYROXINE AND HIGH-FAT DIETS TO MODIFY THE RATE OF THIAMINE LOSS FROM THE BODY [1]

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THREE FIGURES

(Received for publication July 9, 1954)

The so-called "thiamine sparing" action of fat (Evans and Lepkovsky, '29) has received relatively little study in recent years. Although Kemmerer and Steenbock ('33) failed to find differences in the thiamine content of tissues of rats fed high- or low-fat diets deficient in thiamine, Evans and Lepkovsky ('35) reported that fat has a sparing action on the vitamin B content of rats' tissues. Gruber ('50) concluded from studies on pigeons that a large consumption of carbohydrate clearly causes more rapid depletion of the vitamin from certain tissues. In discussing the physiology of thiamine, Cowgill ('39) cited the three possibilities given by Westerbrink ('34) to explain the sparing action of fat. "(1) When fat is fed, the organism uses in its metabolism less vitamin B_1 than it does when carbohydrate is fed." This is the view favored by Evans and Lepkovsky ('35). (2) "Whether the diet is high carbohydrate or high fat, the vitamin is used at the same rate, but the presence of much fat in some unknown manner affects the time of onset of the polyneuritis. (3) Under the two sets of dietary conditions vita-

[1] This work was supported in part by grants-in-aid from the Nutrition Foundation, Inc., New York, N. Y.; Merck and Company, Rahway, N. J.; Kellogg Company, Battle Creek, Mich., and the J. M. Kaplan Fund, Inc., New York, N. Y.

min B_1 is used at the same rate, but when carbohydrate is metabolized, a toxic metabolite arises which in the absence of the vitamin is not removed and which therefore induces the polyneuritis."

The effect of thyroid administration, exercise, etc., (Cowgill, '39) upon increasing the thiamine requirement, shortening the time required to produce polyneuritis, or increasing the effects of thiamine deficiency has received little fundamental study. It is apparently intellectually satisfying to many workers to conclude that since thiamine is required in

TABLE 1

Composition of thiamine-deficient diets used

	LOW FAT	HIGH FAT
	gm	*gm*
Sucrose	70.7	55.7
Casein	20.0	20.0
Corn oil	4.0	19.0
Cod liver oil	1.0	1.0
Salts IV [1]	4.0	4.0
Choline	0.3	0.3

Vitamin supplements: 8 mg riboflavin, 40 mg niacin, 20 mg calcium pantothenate, 4 mg pyridoxine, 1 mg folic acid, 0.2 mg biotin, 1 mg menadione per kilogram of ration.

[1] Hegsted et al. ('41).

the metabolism of carbohydrate, when more carbohydrate is metabolized the thiamine requirement will be increased. On the contrary, a review of the known metabolic functions of thiamine (Reed, '53) offers no explanation since there is no reason to believe that thiamine is "worn out" by participation in metabolic reactions. Also, since thiamine is believed to be required for the oxidative decarboxylations in the citric acid cycle (Reed, '53), a need for thiamine is not by-passed by the oxidation of fat.

Quantitative vitamin requirements have to date been simply descriptive, i.e., how much is required by an animal to prevent certain symptoms or biochemical changes. An under-

standing of the mechanisms requiring the vitamin explains the need for the vitamin but does not greatly contribute to an explanation of the amount needed. It would appear that final understanding of requirements would require an explanation of those factors which determine the rate of loss from the body, that is, excretion or destruction of the nutrient.

EXPERIMENTAL

Three experiments are reported. In the first, a group of young adult male mice [2] which had received the low-fat diet (table 1) containing 4 mg of thiamine per kilogram for one week's time were given the same diet without thiamine and killed at intervals as indicated in the table and figures. The livers and the remaining carcasses were weighed separately and homogenized in water. Sulfuric acid was added to approximately 0.1 N and the samples were autoclaved for 30 minutes at 5 pounds pressure. They were then brought to pH 4.5 with sodium acetate, takadiastase was added (0.5%) and the samples were incubated at 37°C. overnight under toluene. Thiamine determinations were run using a thiamine-requiring *E. coli* mutant (National type culture collection No. 9723C). Samples and standards were dried upon small circles of filter paper which were then placed upon a large seeded plate of agar media as described for other vitamin assays by Williams et al. ('52). The determinations were made by comparing the diameter of the growth area with standards on the same plate. Reasonably satisfactory assays were obtained, but at low concentrations it was difficult to get sufficient material on the filter paper circle.

In experiment II, three groups of 18 adult male mice were given the diets shown in table 1 and the low-fat diet plus 25 mg of thyroxine per kilogram of diet. These animals, as in experiment I, received the low-fat diet plus 4 mg of thiamine for two weeks prior to the start of the experiment, and this was raised to 10 mg/kg for the last 4 days. Animals

[2] Obtained from Carworth Farms.

were killed upon the first day and at intervals thereafter. The samples were prepared as described above. Thiamine was determined by applying the thiochrome method of Bessey et al. ('52) to tissue extracts prepared as described above.

In the third experiment two groups of mice were used. These received either the low-fat diet (table 1) or the high-fat diet. In this experiment the high-fat diet was modified to contain 23.7% casein at the expense of sucrose in order to make it isonitrogenous with the low-fat diet. Evans and Lepkovsky ('34) have stressed the importance of a satisfac-

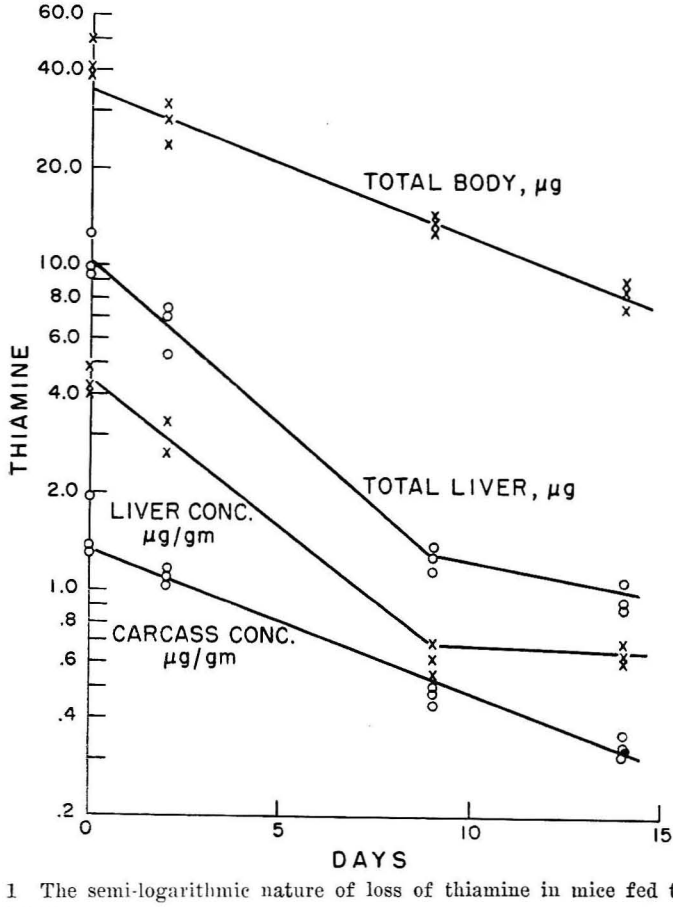

Fig. 1 The semi-logarithmic nature of loss of thiamine in mice fed thiamine-deficient diets.

tory protein level in studies dealing with the thiamine-sparing action of fat. Nine mice were sacrificed at the beginning of the experiment and 9 from each group were taken on the 10th and 20th days. Liver and carcass cocarboxylase contents were determined by a modification of the method of Ochoa and Peters ('38).

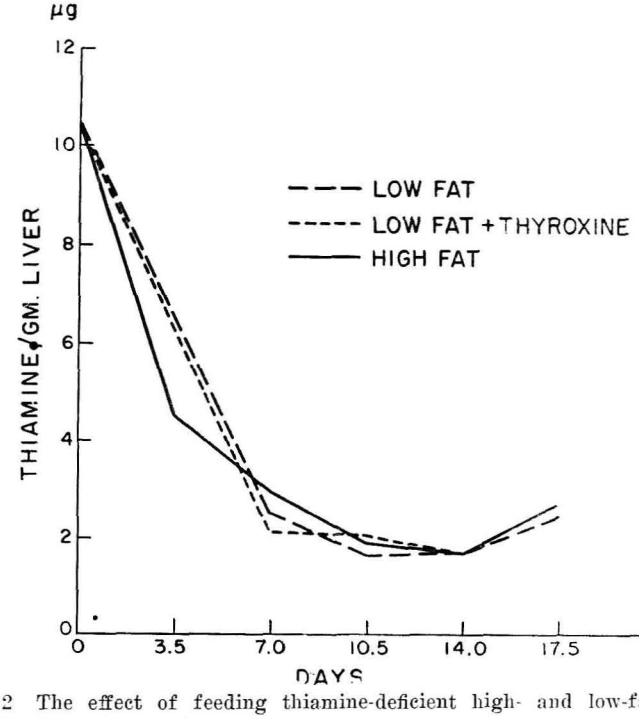

Fig. 2 The effect of feeding thiamine-deficient high- and low-fat diets on liver thiamine concentration.

RESULTS

In the first experiment the animals maintained their weight or gained slightly for approximately 10 days. A scatter diagram of the results of the thiamine determinations plotted on a semi-logarithmic scale is shown in figure 1. The rate of thiamine loss appeared to be semi-loragithmic with respect to time until the animals began to lose weight both in the liver and the remaining carcass. The liver concentration ap-

parently reached a minimum on about the 9th day. Some deaths occurred on the 12th day and all animals were dead on the 17th day.

Changes in the thiamine concentration of the liver and carcass of the animals receiving the three diets in experiment II are shown in figures 2 and 3. No difference in the rate of loss was observed although all animals receiving thyroxine were dead on the 14th day and no animals receiving the low-fat diet were available after the 18th day. As in the previous experiment the animals receiving the low-fat diet generally maintained their weight until the 10th day. Those

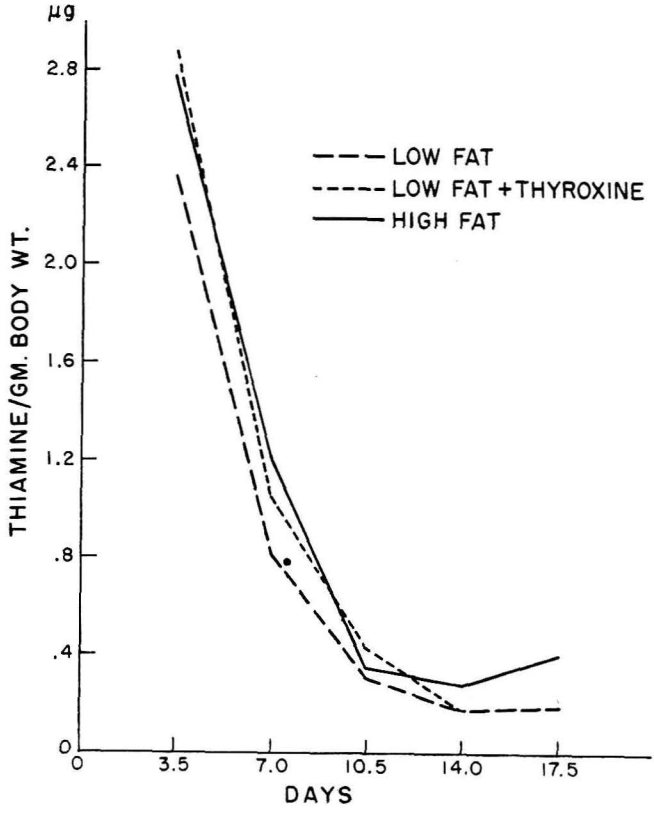

Fig. 3 The effect of feeding thiamine-deficient high- and low-fat diets on body stores of thiamine.

receiving thyroxine had all lost some weight by the third day and this continued whereas the animals on the high-fat diet maintained their weight until the 14th day and none died of thiamine deficiency by the time the experiment was terminated.

TABLE 2

The effect of high- and low-fat thiamine-deficient diets on mouse liver and carcass cocarboxylase

	0 days	TIME ON DEFICIENT DIETS	
		10 days	20 days
	μg Cocarboxylase/gm liver [1]		
High-fat diet	5.65 ± 1.85	1.85 ± .76	.69 ± .33
Low-fat diet		1.65 ± .69	.76 ± .60
	μg Cocarboxylase/gm body weight		
High-fat diet	2.31 ± .22	.68 ± .25	.21 ± .13
Low-fat diet		.60 ± .18	.31 ± .20

[1] Mean plus standard deviation.

The results of the cocarboxylase determinations in experiment III are shown in table 2. Although the beneficial action of the high-fat diet was evident from the condition of the animals, no differences in cocarboxylase contents were found.

DISCUSSION

These studies confirm the many reports in the literature of the thiamine-sparing action of fat and the opposite effect of thyroxine administration. Much of this has been reviewed by Williams and Spies ('38). Our data confirm the early conclusion of Kemmerer and Steenbock ('33) that the tissue level of thiamine is not higher in the animals fed the high-fat diets. It would appear that the rate of thiamine loss from the body is a function of the thiamine content of the tissues and essentially independent of the diet fed. The data suggest

that the body weight is maintained until such time as the thiamine level reaches a minimum concentration and then body tissue is lost, presumably to provide thiamine. The results fail to explain the earlier deaths of the animals receiving the thyroxine-containing diets or the prolonged survival of the animals fed the high-fat diets.

In Gruber's first experiments ('50) he fed pigeons three thiamine-deficient diets for 13 days. Very large differences in cocarboxylase content of the liver, heart, and cerebrum were found, in each case higher in the high-fat group. In later studies ('52) the animals were allowed to die of the deficiency. At this time the cocarboxylase of the heart and liver was significantly higher in the animals receiving no carbohydrate while that of the breast muscle and cerebrum was significantly higher in the animals which received no fat. Thus for the cerebrum the ratio of the thiamine content of the high-fat/low-fat animals was reversed at death compared to the ratio at 13 days of deficiency. Although there is no obvious way to reconcile the difference between the results shown in this paper and those given by Gruber, the extreme diets fed by Gruber may be suggested. No fat was supplied by his low-fat diet and no carbohydrate was present in the high-fat diet.

Gruber speculates that death occurs when the thiamine pyrophosphate content of some particular center attains the lethal level. He does not believe that carbohydrate has a toxic effect. While our results neither confirm nor deny the supposition, if we are correct in that the overall rate of thiamine loss is independent of the diet, it places the protective action of fat at the tissue level of some particular susceptible center. Gruber concludes from the determination of thiamine in the excreta that intestinal synthesis is practically ruled out as a contributing factor.

The semi-logarithmic or "self-catalytic" nature of the loss of thiamine from the body deserves some emphasis. Although there are as yet insufficient data for generalization, it is probable that this is not unusual. Vitamin A losses from the

liver follow a similar pattern (Frey and Jensen, '45). Indeed this is simply a reversal of the usual log dose-response curve which is probably valid for all vitamins (Bliss, '51). In any event the thiamine requirement is clearly related to the level of thiamine desired in the tissues. Doubling the thiamine intake will produce a relatively small increment in tissue concentration since, if the response is proportional to the log of the dose, a 10-fold increase is required to double the tissue concentration.

We are disturbed by the difference in slope of the thiamine-depletion curve as determined by the various methods. Measurement of thiamine by the *E. coli* mutant or of cocarboxylase by the yeast method yields a rate of loss approximating 10% of the body thiamine per day. This is essentially the same as the rate of loss in the rat as estimated by Lowry ('52). On the other hand, the thiochrome method gave a slope nearly twice as high. Since the rates of loss obtained with the two biological methods are in agreement and compare with the rate of thiamine loss in rats reported by Lowry, we are inclined to place more reliance on these values, particularly those obtained by the cocarboxylase method. Van der Mijll Dekker ('52) has concluded that during bread making thiamine is destroyed by the yeast to form a compound which is measurable by the thiochrome method but destroyed by heat during the baking process. Holt [3] has also concluded that a "pseudothiamine" occurs in urine which is measurable as thiochrome. Whether the difference in results can be explained in this manner is problematical.

SUMMARY

Young adult mice were fed thiamine-free diets containing 5 and 20% fat as well as the 5% fat diet supplemented with 25 mg of thyroxine per kilogram of diet. Liver and total body thiamine and cocarboxylase were determined at intervals. Although the thyroxine hastened weight loss and time

[3] Personal communication.

of death and the high-fat diet showed the reverse effect, no difference in the rate of loss of thiamine from the liver or carcass was found. It is concluded that the rate of thiamine loss is dependent upon the thiamine content of the body or tissues and essentially independent of the diet fed.

ACKNOWLEDGMENT

The authors wish to thank Doris Hedrick, Miriam Gargill and Ruth Sullivan for their technical assistance.

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ANTIBIOTICS, GROWTH, FOOD UTILIZATION AND THE USE OF CHROMIC OXIDE IN STUDIES WITH RABBITS[1,2]

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ONE FIGURE

(Received for publication July 1, 1954)

Although the use of the rabbit as a meat animal promises to increase on a world-wide basis because it can convert grasses from small areas into useful food for man, too little is known of its basic nutrition and physiology. The present studies were undertaken to determine the effect of antibiotics upon growth and digestibilities of different nutrients in this species of animal. Overall utilization studies were also made to compare the chromic oxide technique with the conventional balance method.

Some claim has been made by commercial rabbit growers that antibiotics are useful especially when sanitary standards are low. In limited studies with terramycin, however, no evidence was found for favorable effects upon growth (Lawrence and McGinnis, '52).

The rabbit requires niacin in its diet (Wooley and Sebrell, '44), but may obtain other water-soluble B vitamins by its well known practice of ingesting its own excreta (Olcese et al., '48; Kulwich et al., '53). Thus, there is an opportunity for

[1] This research was supported by the China Institute in America, New York, New York.

[2] The authors are indebted to Charles Pfizer and Company, New York, for supplying the terramycin for this experiment.

synthesis of essential vitamins within the cecum. Little attention has been given to such synthesis or to absorption from the cecum before the excreta are taken back into the stomach. Due to this unique method of nutrition, one cannot anticipate the effect of antibiotics.

EXPERIMENTAL

In all studies the Silver Marten strain of rabbit was used. At maturity these animals weigh from 6 to 8 pounds.

Litter-mate weanling rabbits 4 to 6 weeks of age were used. In the first three trials, they were kept in false-bottom cages during the growing period and fed in groups of two to 6 each according to litter size. In the later trials they were fed individually.

The basal ration in experiments I to IV was a commercial mixture of the following percentage composition: bran 12.5, middlings 17.5, wheat germ 8.5, soybean oil meal 15.0, ground oats 20.0, corn meal 12.7, alfalfa meal 11.0, limestone 1.0, dicalcium phosphate 0.75, and irradiated yeast 0.025.

DISCUSSION OF RESULTS

The antibiotic trials

In experiments I to III the growth of the rabbits receiving this basal ration was compared to that of animals receiving the basal ration supplemented with either (1) terramycin, (2) aureomycin, (3) a commercial form of terramycin or (4) a commercial form of aureomycin and vitamin B_{12}. The results, as well as the levels of supplements are given in table 1. No evidence was found for increased growth as a result of feeding these supplements.

Using the same basal diet, an attempt was made to determine the effect of (1) sulfathalidine and (2) sulfathalidine plus terramycin on the growth of the young rabbits. This also yielded negative results (table 1, exp. IV). The growth of the rabbits was retarded on both diets containing sulfathalidine. The addition of terramycin neither counteracted

TABLE 1

Growth of rabbits fed natural rations with and without antibiotics

EXP. NO.	RATIONS	NO. ANIMALS	LENGTH OF EXP.	INITIAL WT.	FINAL WT.	AV. DAILY GAIN [1]
			weeks	*gm*	*gm*	*gm*
I	1. Basal	15	11	758	2403	21
	2. Basal + 0.5% Aurofac [2]	15	11	737	2275	20
II	1. Basal	11	8	1106	2481	26
	2. Basal + 0.5% Bicon [3]	12	8	972	2220	24
III	1. Basal	4	6	1394	2290	22
	2. Basal + 100 p.p.m. Aure.[3]	4	6	1395	2138	22
	3. Basal + 50 p.p.m. Terra.	4	6	1373	2335	23
	4. Basal + 100 p.p.m. Terra.[4]	4	6	1415	2229	24
IV	1. Basal	5	9	471	1461	23
	2. Basal + 0.5% sulfathalidine	5	9	476	1274	20
	3. Basal + 0.5% sulfathalidine + 33 p.p.m. Terra.	5	9	486	1252	20

[1] The differences noted in this column were judged to be insignificant by the "t" test at the 5% level of probability.

[2] Aurofac contained 1.8 gm of aureomycin and 1.8 mg of vitamin B_{12} per pound of supplement.

[3] Bicon contained 5 gm of terramycin per pound of supplement.

[4] Parts per million of either terramycin or aureomycin.

nor increased the toxic effect of sulfathalidine. Attempts were then made to feed a semi-purified diet in order to determine if terramycin would stimulate the slower growth observed commonly when rabbits are fed such diets. These diets consisted of crude casein 25.0% or soy protein 24.7% plus 0.3% methionine, corn sugar 27.0%, corn meal 20.0%, cellulose 10.0%, cottonseed oil 4.0%, mineral mixture 4.0% [3] and vita-

[3] The following minerals were added in grams per hundred grams of U.S.P. salt mixture no. 13, copper sulfate 0.09, cobaltous nitrate 0.09, manganese sulfate 0.035, and potassium iodide 0.008.

min supplement [4] (table 2, exp. V). Diets of lower casein content, ranging from 10, 15, and 20% were also tested substituting corn sugar for part of the casein (table 2, exp. VI). The results of these two experiments afforded no evidence that

TABLE 2

Growth of rabbits fed synthetic rations with and without antibiotics

EXP. NO.		RATIONS	NO. OF ANIMALS	LENGTH OF EXP.	INITIAL WEIGHT	FINAL WEIGHT	AV. DAILY GAIN
				weeks	*gm*	*gm*	*gm*
V	1.	24.7% soy protein plus methionine	6	4	538	738	7.1
	2.	24.7% soy protein plus terramycin [1]	6	4	548	757	7.2
	3.	25% casein	6	4	532	693	5.7
	4.	25% casein plus terramycin	6	4	570	714	5.1
VI	1.	10% casein [2]	4	4	560	681	4.3
	2.	10% casein plus terramycin	4	4	547	695	4.4
	3.	15% casein	4	4	573	798	8.0
	4.	15% casein plus terramycin	4	4	585	800	7.7
	5.	20% casein	4	4	585		[3]
	6.	20% casein plus terramycin	4	4	550		[3]
VII	1.	Basal plus niacin [2]	4	6	670	1004	8.0
	2.	Basal plus niacin and terramycin	4	6	680	932	6.0
	3.	Basal	4	6	630	585	— 1.1
	4.	Basal plus terramycin	4	6	695	650	— 1.0

[1] Terr. = 33 parts per million of terramycin was added.

[2] Twenty per cent of corn meal was replaced by 20% of corn sugar.

[3] Two rabbits from each group died of unknown causes during the 4th week.

[4] Vitamins were added in the following amounts per 100 gm of feed: thiamine 0.7, riboflavin 0.7, niacin 20.0, choline chloride 200.0, pyridoxine 0.7, calcium pantothenate 0.7, vitamin C 5.0, vitamin B_{12} 0.015, biotin 0.001, P-A-B-A 15.0, menadione 0.075, inositol 10.0, folic acid 0.05, vitamin E 3.0 mg, vitamin A 400 I.U., and vitamin D at 40 I.U.

terramycin influenced the inferior growth in these studies. The rate of growth appeared to be a reflection of the protein level. Also, 25% casein seemed inferior to the 24.7% soybean protein plus 0.3% methionine. In one experiment the terramycin also did not improve the growth of the rabbits which were fed the 25% casein ration and collared to prevent coprophagy.

In experiment VII (table 2) the essential vitamin, niacin, was omitted in a basal ration similar to that containing 20.0% casein, used in the previous experiment. The rabbits receiving no niacin lost in body weight during the 6 weeks' trial while those receiving niacin gained weight gradually. But the antibiotic terramycin failed to improve the growth of these rabbits, fed with or without niacin.

It has been postulated that antibiotics are effective in rats or pigs only when these animals receive an imbalanced ration (Cunha et al., '50; Lih and Baumann, '52). In the present experiments when terramycin was added to rations either low in niacin or protein, no growth response was observed in either case.

The utilization of nutrients by the whole body and various parts of the intestinal tract

In these studies adult rabbits were fed the same commercial mixture whose composition was given at the beginning of this report. A comparison was first made between rabbits with and without collars. Collars was fitted to half the animals to prevent consumption of excreta. For this purpose, 12 rabbits were divided into two groups. After a 7-day preliminary period in individual metabolism cages, a 5-days' collection was made and the feed intake recorded. In the animals without collars there was significantly better utilization of protein and of dry matter than in those in which coprophagy was prevented (table 3). This indicates that the passage of ingested excreta through the rabbit leads to a more efficient usage of food materials.

The use of an insoluble indicator such as chromic oxide permits the determination of the absorption of nutrients from the whole or parts of the intestinal tracts of animals (Schurch, Lloyd and Crampton, '50; Carroll et al., '53).

In one experiment, 6 adult rabbits were fed the same commercial mixture supplemented with 1.0% of chromic oxide. The collection periods lasted for 11 days. Daily feces were collected for each individual rabbit and stored separately after 7 days of preliminary feeding of the basal ration.

TABLE 3

Digestibility of nutrients by rabbits[1]

NUTRIENTS	RATIONS			
	Basal	Basal + collar	Basal + 1.0% Cr_2O_3	Basal + 1.0% Cr_2O_3
	Method of fecal collection			
	Conventional	Conventional	Conventional	Indicator
	%	%	%	%
Dry matter	74 ± 2 $P < 0.01$[2]	69 ± 3	69 ± 5	68 ± 4
Protein	76 ± 6 $P < 0.01$	68 ± 11	72 ± 11	71 ± 6
Fat	68 ± 9	72 ± 10		
Carbohydrate	75 ± 6	73 ± 8		
Ash	59 ± 16	53 ± 13		

[1] Six animals in each group.

[2] "P" represents the probability of chance occurrence of the difference observed between the test and control groups.

The data indicate that the chromic oxide method is satisfactory for such studies with rabbits and has the advantage that samples collected at random can be taken instead of quantitative fecal collections (table 3). However, the values from the conventional method were slightly higher than those obtained by the indicator method (1.9% higher for dry matter and 1.1% for protein). A study of the daily excretion of chromic oxide after the initial feeding indicates that a 5-day preliminary period is needed (fig. 1).

To determine the relative absorption of nutrients from different segments of the gastrointestinal tract, 5 adult rabbits were collared and fed for 6 days upon the stock diet containing 1% chromic oxide until the individual daily feed intakes were constant. They were then sacrificed by cervical dislocation and their gastrointestinal tracts were segmented into stomach, small intestine, cecum, and large intestine.

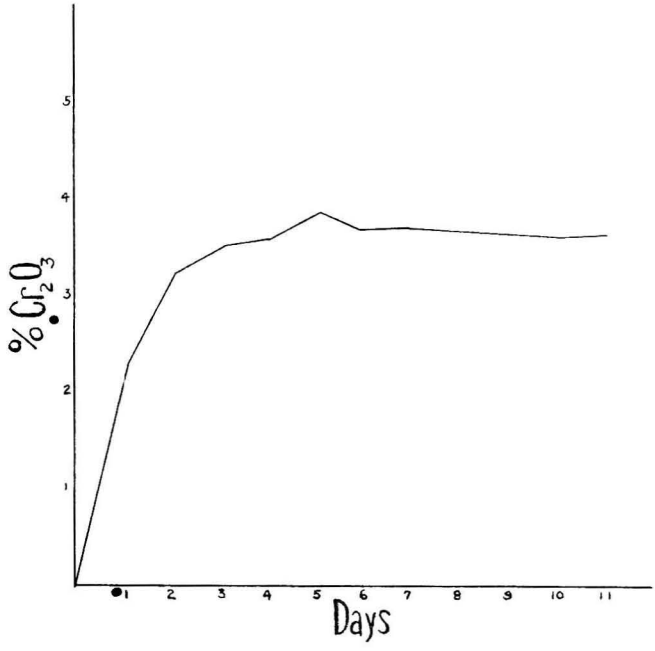

Fig. 1 The daily variation of the percentage of chromic oxide in the feces of rabbits receiving 1.0% chromic oxide in their diet.

The rate of absorption of different nutrients was determined by using chromic oxide as an indicator. Vitamin B_{12} contents were also determined for all these segments as well as for meat and liver.

Contrary to the common belief, the data in table 4 indicate that the small intestine of the rabbit absorbs only part of the nutrients. A greater portion of the nutrients disappears from the cecum and large intestine. This disappearance

might be due to the formation of some gases which in turn pass out of the body unabsorbed. However, since the small intestine of the rabbit in our trials absorbed only one-third of the total digestible nutrients, it is rather doubtful that the remaining two-thirds of the nutrients could be lost in the form of gases. The cecum utilizes most of the fat and crude fiber while the large intestine absorbs most of the protein.

TABLE 4

Absorption of nutrients from different sections of gastro-intestinal tract (5 animals)
All values are percentages

NUTRIENTS	STOMACH	SMALL INTEST.	CECUM	LARGE INTEST.	SOFT FECES	HARD FECES
Dry matter	6	19	56	61	49	66
Protein	3	14	30	57	27	80
Fat	..	10	58	61	37	65
Crude fiber	..	..	42	11	39	20
Ash	..	..	21	21	26	51
Vitamin B_{12} [1]	..	11	4944	1906	2006	347

[1] Millimicrograms per gram of dry matter (liver and meat, 382 and 72 respectively).

TABLE 5

Comparison of the composition of cecum contents, soft feces and hard feces (Dry basis)

ITEMS	PROTEIN	FAT	ASH	CRUDE FIBER
	%	%	%	%
Cecum contents	36.4	1.8	15.4	13.4
Soft feces	37.8	1.5	14.3	14.3
Hard feces	14.8	1.8	14.8	27.8

It was observed also that the cecum contents and soft feces were quite similar in composition. The pH values of the two were about the same, 6.65 and 6.29. It is believed, therefore, that the soft feces are residues of cecal contents passing through the large intestine too rapidly to lose much water by absorption. However, the possibility of a special origin of soft feces should not be overlooked. The data show also that vitamin B_{12} was increased from 11 mμg per gram of material

in the small intestine to 4,944 per gram of cecal contents. The concentration of this vitamin decreased in the large intestine. It seems evident therefore that the synthesis of vitamin B_{12} and probably other B vitamins occurs in the cecum of the rabbit.

It has been observed also by other workers (Kulwich et al., '53) that the rabbit consumes almost all of its soft feces (26.8% of the total feces in our experiment) but negligible amounts of the hard feces. On the basis of the data in tables 4 and 5, it is possible that the ingestion of the soft feces improves the utilization of protein and vitamin B_{12}, since these two substances were present in the soft feces in significantly greater amounts than in the hard feces. This improvement of protein utilization is in accordance with that observed in the previous digestion trial. The protein in the soft feces may originate in the cecum bacteria and require digestion in the small intestine before it can be absorbed.

Feed conversion by rabbits

Since limited data are available upon the amount of feed needed to produce a given live weight of rabbit, especially in relation to breed, a study was made using young, growing rabbits during the 12 weeks after weaning. During the first 6 weeks it required 2.7 ± 0.7 gm of dry feed to produce one gram of live body weight. During the whole 12 weeks 3.7 ± 0.7 gm of feed were needed to produce a gram of live weight. Since the dressing percentage of swine is about 75%, and that of rabbits, 60%, the conversion of feed seems somewhat superior for the swine but final comparisons must await careful analyses of rabbit carcasses.

SUMMARY

When terramycin or aureomycin was fed at levels ordinarily recommended for other species of animals, no growth improvement was shown for young rabbits fed a pelleted natural ration. Terramycin did not improve growth when the diet

contained 0.5% sulfathalidine. This antibiotic also was ineffective in improving the growth of rabbits which received a semi-purified ration containing either 25% casein or 24.7% soy protein, plus 0.5% methionine. Purified rations consisting either of 10% casein plus niacin or 20% casein without niacin were observed to be inadequate for the growth of rabbits. Nevertheless, terramycin did not improve these rations.

The chromic oxide method was found to be applicable in digestion trials with rabbits. However, a preliminary feeding of 5 days appeared necessary.

Rabbits appear to improve the utilization of protein and dry matter by consumption of their own excreta. The cecum apparently functions in the digestion of crude fiber and the synthesis of vitamin B_{12} while both the cecum and large intestine seem to absorb appreciable amounts of other nutrients.

The rabbits required 2.7 ± 0.7 or 3.7 ± 0.7 gm of dry feed to produce one gram of live body weight from weaning to 6 or 12 weeks of age respectively.

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THE EFFECT OF TERRAMYCIN OR FISH SOLUBLES, OR BOTH, ON THE GROWTH, ADRENAL GLANDS AND GONADS OF THE RAT

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(Received for publication July 2, 1954)

A large amount of data has been accumulated in recent years concerning the results of feeding certain growth-promoting agents. Among these factors are antibiotics and fish products. However, the effect of these growth-promoting substances on the endocrine glands has not been fully explored. Considering the close relationship between growth and the endocrine system, information of this nature might be of value in the elucidation of the mechanism of action as well as the resultant effects of these promotants.

Barnard ('52, '53) has reported that the so-called "mycin" antibiotics have profound adrenocorticomimetic effects when administered orally. He mentions that this effect is seen principally in seriously ill individuals. Selye ('51) includes terramycin in his list of non-specific stressors. Meites ('51) was able to overcome cortisone growth inhibition by using antibiotics and Ershoff ('50) found a marked adrenal hypertrophy in rats following the feeding of aureomycin. However, Baxter and Campbell ('52) failed to demonstrate adrenal changes following a 9-day feeding trial with aureomycin.

[1] This article is part of a dissertation presented by the senior author to the faculty of the graduate school of the State College of Washington in partial fulfillment of the requirements for the degree of Master of Science.

Very little has been published with regard to the effect of antibiotics on sex organs or on sex difference in response. Meites and Shay ('51) could show no significant change in the testes of the rat following the feeding of antibiotics. Palafox and Rosenberg ('52) found a consistently better response in male chicks but Almquist and Merritt ('51) were unable to demonstrate a sex difference in turkeys. Atkinson and Couch ('51) reported that male turkeys responded better to a growth factor found in fish solubles. Stern and coworkers ('51) confirmed this observation in rats, using fish meals.

Since an increase in weight can often be due merely to the deposition of fat or to water retention, some means of measuring skeletal growth should be used when studying the action of growth factors. The tail growth was decided upon since it reflects the actual increase in long bone length and has the further advantage of being relatively easy to measure.

EXPERIMENTAL

Experiment 1. Weanling rats, 21 days old, of the Sprague-Dawley and Long-Evans strains were used in this study. Since there was no difference in their response, data from both strains will be combined. The rats were distributed at random into 4 experimental groups: (1) practical-type basal diet composed of 36.8% ground whole wheat, 18% ground yellow corn, 39% soybean oil meal, 3.3% dried brewer's yeast, 1.0% choline chloride, 4000 I.U. of Vitamin A and 800 I.U. of Vitamin D; (2) practical-type basal diet plus 50 mg/kg of terramycin hydrochloride; (3) practical-type basal diet modified to include 5% fish solubles at the expense of 2.5% of the casein and 2.5% of the sucrose; (4) practical-type basal diet modified to include 5% fish solubles plus 50 mg/kg of terramycin hydrochloride. Four animals per diet constituted a replicate and 6 replicates were established. The rats were fed and watered ad libitum throughout the experiment. Weights were recorded at the time of weaning, each week thereafter, and at the termination of the experiment. Chloroform was used

for euthanasia. The rats were fed the experimental diets for a period of three weeks post weaning. At the termination of the experiment, the adrenal glands and gonads (testicles and seminal vesicles in the male and combined ovaries and uterus in the female) were removed, freed of fat, blotted on moistened filter paper, and weighed on a Roller-Smith torsion balance. Both adrenal glands from a single animal were weighed together and both gonads were also weighed together. The tissues were placed in formalin for future microscopic study. All the data were analyzed statistically using standard methods.

Experiment 2. This experiment differed from experiment 1 in the following ways: (1) a semi-purified diet consisting of 50% ground yellow corn, 18% casein, 30% sucrose, minerals (Hubbell et al., '37), 0.1% choline chloride, 0.1% DL-methionine, and vitamins (Stern and McGinnis, '50) was used as the basal diet; (2) the duration of the feeding trial was 7 weeks post weaning rather than three weeks; (3) sexes were separated resulting in 4 experimental groups; (4) four animals of each sex per diet constituted a replicate and three replicates were established; (5) the tail length was measured from the tip of the tail to the edge of the hairline near the base of the tail. Tail lengths were measured simultaneously with weighing; (6) at necropsy, a vaginal smear was taken from all females to determine the stage of estrous.

Experiment 3. This experiment was designed to test the response of castrates to the growth stimulants under study. The same 4 diet groups were used as in experiment 2 but there were 4 sex categories: (1) male (2) castrate male (3) female (4) castrate female. Three animals of each sex group were placed in each of the 4 diet groups; this constituted a replicate. Two replicates were established. With 16 treatment groups, a total of 96 rats were used. The animals were castrated at 31 to 44 days of age. Mock surgery under general anesthesia was performed on the non-castrates. Weekly weights and tail lengths were taken thereafter and the experiment was terminated after 11 weeks on the experimental

diet. All other techniques and procedures were identical with those of experiment 2. However, in addition to these procedures, an attempt was made to assess the functional integrity of the adrenal gland by testing the eosinophil response to injected epinephrine. In this test, an eosinophil count was made and the animal injected subcutaneously with epinephrine; another eosinophil count was taken 4 hours later. A 50% reduction in total eosinophils was used as the criterion of a normal response in this experiment; if the initial count was 125 or less, the gland was considered normal and a second count was unnecessary.

TABLE 1

Summary of results from experiment one using practical-type basal diet supplemented with terramycin or fish solubles or both

	21 DAY WEIGHT GAIN	ADRENAL GLAND WEIGHT
	gm	*mg*
Males:		
Basal (11)[1]	91.4 ± 4.3[2]	28.4 ± 2.4
Basal + terramycin (10)	88.6 ± 6.3	29.5 ± 3.7
Basal + fish solubles (13)	100.7 ± 4.0	33.8 ± 3.8
Basal + terramycin (17) + fish solubles	102.5 ± 2.8	34.2 ± 2.9
Females:		
Basal (12)	75.2 ± 4.4	35.6 ± 3.7
Basal + terramycin (13)	81.4 ± 2.5	31.6 ± 3.1
Basal + fish solubles (10)	83.7 ± 1.8	35.7 ± 4.0
Basal + terramycin + fish solubles[3]		

[1] Number of animals in group.
[2] Standard error of the mean.
[3] Too few animals for valid interpretation.

RESULTS

Experiment 1. The results of experiment 1 using the practical-type basal diet are summarized in table 1. Terramycin appeared to have very little effect on the weight gain of the male rat when added to the practical-type basal diet for a 21-day feeding trial; there was a slight, but not significant response in the female rats. Fish solubles, on the other

hand, stimulated significant ($P < 0.05$) increases in weight gains compared to those of the unsupplemented rats. This was true for either sex. Fish solubles caused a significant ($P < 0.05$) increase in the weight of the adrenal glands of the male rats. Neither terramycin nor fish solubles affected the weight of the female or male sex organs.

Experiment 2. The results of this experiment using a semi-purified basal diet are summarized in table 2. In this experiment, terramycin stimulated weight gains of both sexes slightly, but not significantly. This effect reached its maximum at about 21 days. Fish solubles stimulated increased weight gains in both sexes at either the 21-day period or at the 49-day period. This effect was very significant ($P < 0.01$). Both fish solubles and terramycin gave significant ($P < 0.05$) increases in adrenal weights when compared to those of the unsupplemented animals. Fish solubles very significantly ($P < 0.01$) increased tail lengths after 49 days of supplementation; it is probable, therefore, that fish solubles contain a true growth factor for the rat. In this experiment, supplementation with fish solubles resulted in a significant ($P < 0.05$) increase in the weight of the female sex organs. On the other hand, terramycin caused a definite decrease in the weight of the ovaries + uterus. Neither supplement had an effect on the number of animals in heat as measured by vaginal smears. Neither of the supplements had a significant effect on the male sex organs. While the increases in the weights of the adrenal glands and sex organs could be merely reflections of the general body weight increase obtained with the use of fish solubles, calculation of the data on the basis of milligram/100 gm of body weight failed to support this idea.

Experiment 3. The results of this experiment are summarized in table 3. Examination of the weekly weights and tail lengths revealed that the growth response to fish solubles or terramycin or both had reached its maximum at the 49-day weighing period. For this reason, only 49-day and final (77-day) data are given. There was a significant growth response at 49 days from either supplement in all 4 sex groups.

TABLE 2

Summary of results from experiment two using semi-purified basal diet supplemented with terramycin or fish solubles or both

	21-DAY GAINS	21-DAY TAIL INCREASE	49 DAY GAIN	49-DAY TAIL INCREASE	WEIGHT OF ADRENAL GLANDS	OVARIES + UTERUS WEIGHT OF	NO. OF ANIMALS IN ESTRUS
	gm	*mm*	*gm*	*mm*	*mg*	*mg*	
Males:							
Basal (11)[1]	93.0 ± 5.8[2]	62.8 ± 1.6	149.0 ± 4.9	91.4 ± 2.2	33.1 ± 1.7		
Basal + T[3] (12)	99.3 ± 4.7	68.3 ± 1.8	158.5 ± 7.4	92.9 ± 1.6	40.0 ± 2.7		
Basal + F (12)	102.5 ± 4.7	68.8 ± 2.2	183.6 ± 8.3	98.8 ± 2.1	39.6 ± 5.0		
Basal + T + F (11)	95.7 ± 4.9	68.6 ± 2.4	176.1 ± 12.3	99.8 ± 3.1	40.9 ± 5.2		
Females:							
Basal (14)	72.8 ± 2.8	62.9 ± 1.4	106.6 ± 4.4	81.6 ± 1.4	40.4 ± 2.6	259.9 ± 20.5	6
Basal + T (13)	77.6 ± 2.3	63.3 ± 1.4	112.5 ± 3.5	82.7 ± 2.1	45.2 ± 2.0	243.5 ± 19.1	6
Basal + F (13)	83.5 ± 2.0	64.9 ± 1.5	127.8 ± 5.3	87.6 ± 2.0	48.5 ± 2.6	320.6 ± 23.7	6
Basal + T + F (14)	84.1 ± 3.0	62.3 ± 1.4	131.4 ± 5.1	85.8 ± 1.6	45.8 ± 2.5	287.4 ± 18.2	6

[1] Number of animals in group.

[2] Standard error of the mean.

[3] T — Terramycin. F — Fish solubles.

This was true for both weight gains and tail length increases. The response was demonstrated to a greater degree in the intact and castrate males than in either female group. The castrate females appeared to be stimulated slightly more by either supplement than the intact females. There was little difference between fish solubles and terramycin as to the degree of response and factorial analysis revealed no interaction. The 49-day weight gains and tail length increases were somewhat lower than those in experiment 2. In all probability the surgery caused a slight setback.

The mean difference between basal and supplemented animals after 77 days on the experimental diets were relatively unchanged from the 49-day differences. This would indicate that no increase in growth response to fish solubles or terramycin took place during the last 4 weeks.

As anticipated, the castrate females were significantly heavier than intact females; this was true in all diet groups.

In this experiment only a slight gain in adrenal weight was found from supplementation with fish solubles; the terramycin-supplemented animals were normal in this respect. Fish solubles gave this effect in both sexes and in both castrate and intact animals. Fish solubles caused an increase in female sex organ weight; this increase showed a trend towards significance ($P < 0.1$) but valid interpretation would be difficult as 4 animals were in estrus in this group as contrasted with three in the basal group. This increase was seen only in the intact female. Neither supplement affected the weight of the male sex organs. The eosinophil screening test for adrenal cortical function resulted in normal eosinopenia in the same percentage of animals in all groups. (About 72% of the animals responded normally.)

DISCUSSION

In this series of experiments, terramycin did not give a growth response in normal healthy rats when added to a practical-type or semipurified basal diet. However fish solubles gave very good growth promotion in the two shorter

TABLE 3

Summary of results from experiment 3 using a semi-purified basal diet supplemented with terramycin or fish solubles or both

	49-DAY GAINS	49-DAY TAIL INCREASE	77-DAY GAIN	77-DAY TAIL INCREASE	WEIGHT OF ADRENAL GLANDS	WEIGHT OF UTERUS [1]	NO. OF ANIMALS IN ESTRUS
	gm	*mm*	*gm*	*mm*	*mg*	*mg*	
Males:							
Basal (6)[2]	124.3 ± 10.4	65.0 ± 2.0	190.3 ± 11.4	81.2 ± 2.9	34.0 ± 2.3		
Basal + T [3] (6)	143.2 ± 5.1	70.8 ± 2.4	202.3 ± 8.5	80.7 ± 3.0	35.2 ± 2.4		
Basal + F (6)	154.2 ± 8.1	70.3 ± 3.7	219.5 ± 15.7	82.0 ± 4.9	38.4 ± 2.4		
Basal + T + F (6)	165.8 ± 9.3	72.0 ± 3.5	222.5 ± 8.6	83.8 ± 3.9	38.7 ± 4.0		
Castrate males:							
Basal (6)	119.0 ± 11.3	68.0 ± 2.9	182.7 ± 14.2	80.7 ± 3.1	39.4 ± 5.1		
Basal + T (6)	149.3 ± 9.6	75.0 ± 3.2	204.5 ± 14.8	75.0 ± 7.8	42.4 ± 5.8		
Basal + F (6)	141.3 ± 6.9	73.5 ± 3.9	198.3 ± 11.0	86.7 ± 3.6	44.2 ± 3.3		
Basal + T + F (6)	142.3 ± 5.8	72.6 ± 4.2	207.5 ± 7.4	79.7 ± 6.3	43.3 ± 5.6		
Females:							
Basal (6)	100.0 ± 5.7	61.5 ± 2.5	127.6 ± 10.1	70.5 ± 3.3	53.0 ± 3.8	250.3 ± 21.3	3
Basal + T (6)	111.0 ± 3.9	65.8 ± 2.4	135.7 ± 4.0	68.3 ± 4.7	54.2 ± 4.2	252.9 ± 18.7	4
Basal + F (6)	113.7 ± 3.0	63.2 ± 3.1	142.8 ± 4.4	66.3 ± 4.4	57.9 ± 5.7	289.8 ± 29.1	4
Basal + T + F (6)	111.0 ± 3.9	65.5 ± 4.7	132.7 ± 3.3	88.8 ± 4.6	57.2 ± 3.6	248.4 ± 23.5	3
Castrate females:							
Basal (6)	105.3 ± 5.2	62.5 ± 4.5	172.0 ± 4.4	76.4 ± 3.8	49.1 ± 5.1	26.0 ± 2.2	
Basal + T (6)	128.7 ± 6.8	65.6 ± 3.2	194.2 ± 12.8	80.5 ± 3.1	48.9 ± 1.2	28.7 ± 2.4	
Basal + F (6)	122.8 ± 10.2	66.3 ± 1.9	185.8 ± 15.0	82.8 ± 2.5	56.9 ± 6.8	20.3 ± 2.2	
Basal + T + F (6)	149.0 ± 8.7	67.8 ± 1.8	197.7 ± 16.1	76.5 ± 2.6	55.3 ± 4.9	31.3 ± 4.1	

[1] Only the uterus was weighed so that results from intact animals could be compared with those from castrates.
[2] Number of animals.
[3] T — Terramycin F — Fish solubles.

experiments. Apparently the action of fish solubles is exerted at an early age for, after 77 days on the diet without supplementation, the animals had made practically comparable gains. Tail length measurements reveal that this growth stimulus was, at least in part, true body growth. The growth stimulation from terramycin at 49 days in experiment 3 cannot be explained entirely satisfactorily. However, since all of the conditions were similar to those of experiment 2 except the application of surgery about two weeks following weaning, it is possible that terramycin acted as a growth stimulant only in an animal subjected to stress. It is interesting in this connection to recall the work of Meites ('51) in overcoming cortisone-induced growth inhibition with the use of antibiotics. In addition, this explanation could be reconciled with the observations of Coates et al. ('51) that antibiotics failed to stimulate growth when chicks were raised in new, clean quarters.

Inspection of the data reveals a very high correlation of adrenal weight with general body weight gains. Of course, this could be entirely due to the body weight increases themselves. Correlation analysis between body weight and adrenal weight using the error terms to eliminate treatment effects gave a coefficient of + 0.2194. This is not significant, indicating to a slight extent that adrenal weight increases were independent of overall body growth. However, it is felt that microscopic observations must be made and histochemical techniques taken into account before conclusions can be reached.

The significant increase in weight of the female sex organs seen as a result of supplementing a semi-purified basal diet with fish solubles in experiment 2 raises the possibility of estrogenic action or an increased sensitivity of the female organs to estrogen. This latter phenomenon would be more logical since female sex organ response has been related to nutritional levels (Hertz and Tullner, '49; Kline and Dorf-

man, '51). A direct estrogenic action from fish solubles is not considered to be a very strong possibility since: (1) this would theoretically lead to depression rather than stimulation of general body growth and (2) the castrate females in experiment 3 failed to show a response.

SUMMARY

1. Fish solubles gave a significant ($P < 0.05$) growth response at 21 days post weaning with a practical-type basal diet. With a semi-purified basal diet, fish solubles gave a very significant growth response at 21 and 49 days but not at 77 days post weaning. This growth response was measured by weight gains and by the increase in tail length.

2. Terramycin failed to stimulate growth of the normal healthy rat to a significant degree. In an experiment in which surgery (castration or sham-castration) was performed 14 to 20 days following weaning, significant ($P < 0.05$) growth stimulation was obtained after 49 days on the experimental diet. It is suggested that terramycin acted as a growth stimulant only to the animal subjected to stress.

3. There was some indication that both terramycin and fish solubles caused an increase in the weight of the adrenal gland in the growing rat.

4. Neither supplement affected the weight of the male sex organs of the growing rat.

5. The addition of fish solubles to a semi-purified basal diet caused a significant ($P < 0.05$) increase in the weight of the ovaries and uterus of the growing rat at 49 days post weaning. After 77 days on the experimental diets, fish solubles caused an increase ($P < 0.1$) in the weight of the uterus of intact females, but not in castrate females.

ACKNOWLEDGMENTS

The authors express their appreciation to Chas. Pfizer and Co. Inc., and Oceanic Fisheries for furnishing some of the materials used in this investigation.

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OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000.00 established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

Nominations may be made by anyone. Nominations for the 1955 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 1, 1955.

Chairman, Nominating Committee:

DR. FLOYD S. DAFT
Institute of Arthritis and Metabolic Disease
National Institutes of Health
Bethesda, Maryland

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The formal presentation will be made at the annual meeting of the Institute in the spring of 1955. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 1, 1955. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

Chairman, Nominating Committee:

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