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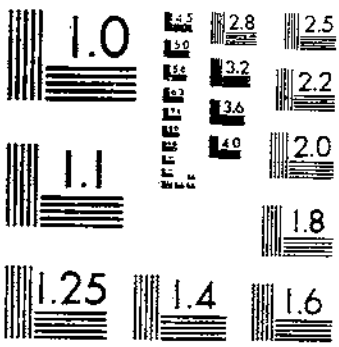
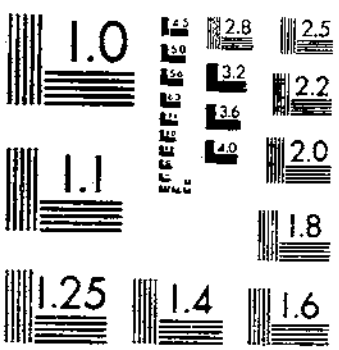


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A



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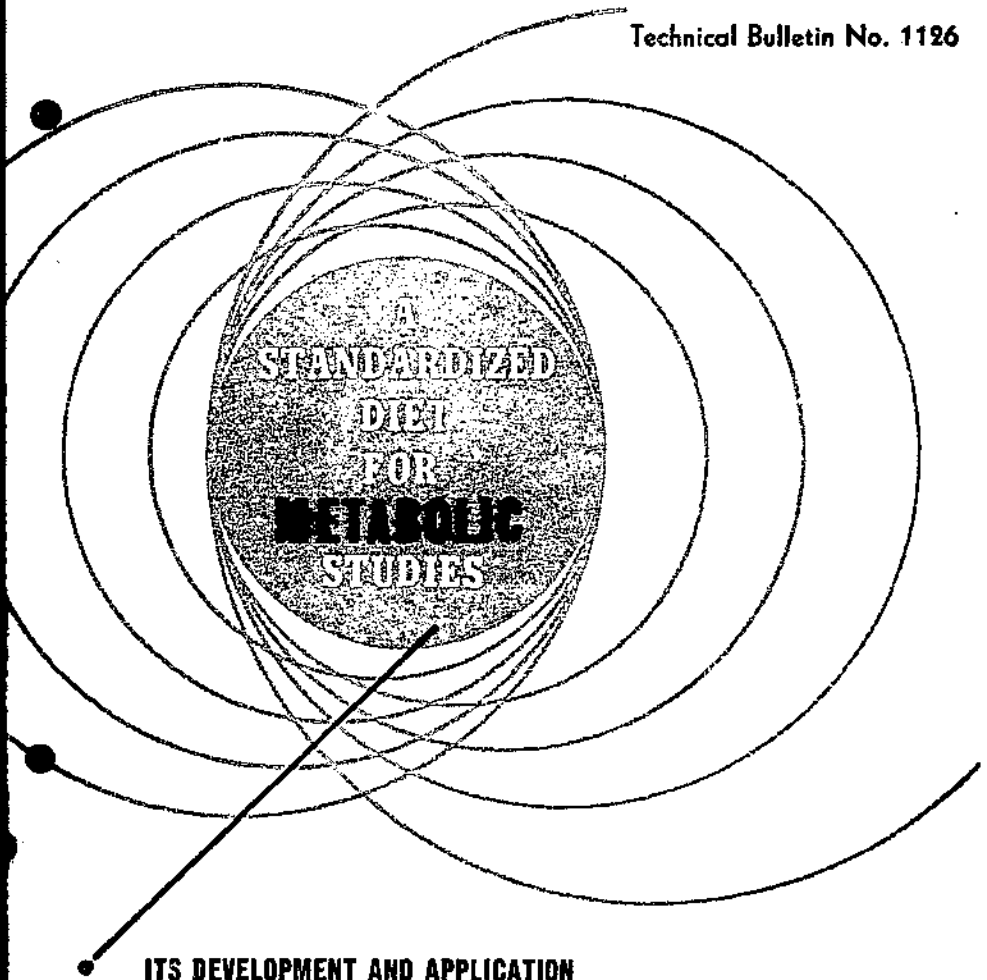

ITS DEVELOPMENT AND APPLICATION

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U. S. DEPARTMENT OF AGRICULTURE
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A STANDARDIZED DIET FOR METABOLIC STUDIES . . .

Its development and application[1]

By FRIEDA L. MEYER, MYRTLE L. BROWN, HARRIET J. WRIGHT, and MILICENT L. HATHAWAY, *Human Nutrition Research Branch, Agricultural Research Service*

SUMMARY

To meet the needs for a uniform basal diet to be used in studying human nutritional requirements, utilization of various nutrients, or interrelationship of nutrients, a standardized diet was developed by the Human Nutrition Research Branch of the Agricultural Research Service. The diet is composed of a core group of foods, which remains constant and supplies amounts of most nutrients at restricted or deficiency levels, and complements I and II, which provide sources of nutrients to bring the intakes of all nutrients to reference levels during equalization periods and for alteration in level of single nutrients during experimental periods. It is believed to be applicable to the study of most nutrients, except protein and amino acids.

The diet was tested in the spring of 1 . and was well accepted by a group of six University of Maryland omen students, aged 19 to 23 years, during a 40-day metabolic study. Wide variation was found among these subjects in their metabolic response to the levels of intake in the standardized diet. Changing the fat content of the diet from 76 gm. (34 percent of the total calories) to 24 gm. (11 percent of the calories) for 3 subjects during the last 20 days had no apparent effect on nitrogen, calcium, magnesium, and phosphorus retention, on thiamine and riboflavin excretion, on fecal lipid excretion, or on the proportion of fecal lipid as fatty acids, neutral fat, or unsaponifiable material.

NEED FOR A STANDARDIZED DIET

Diets used for the study of nutritional requirements are usually designed to be adequate in all dietary essentials except the nutrient under study. Such diets are seldom applicable to the study of more than one nutrient at a time. Even when the same nutrient is under study, basal diets and procedures vary from laboratory to laboratory. There are differences, for example, in types of cereal products, proportions and kinds of fruits and vegetables, use of accessories such as condiments, flavorings, and soft drinks, and method of changing the level of intake through the use of synthetic supplements and the introduction or substitution of foods. Such variations make it difficult to compare results from one laboratory with those from another, or to study the interrelationship of nutrients.

As part of the research reported here, a standardized diet was developed, which is believed to be applicable to the study of the requirements and utilization of a large number of nutrients. It was

[1] Submitted for publication February 23, 1955.

planned so that, in general, levels of nutrients other than those under study could be maintained practically unchanged under various experimental conditions.

The diet, as presented, was used in a study to test its acceptability by young women subjects, to obtain information on the range of their metabolic response to the levels of nutrients selected, and to test the application of the diet to a study of the possible effect of change in the fat level on the metabolism of selected minerals and water soluble vitamins. Modifications primarily in caloric intakes would, of course, be needed if the subjects were young men or were chosen from other age groups.

Before this standardized diet was planned a review was made of the more recent metabolic studies for each nutrient, with special attention to the diets used, probable deficiency levels, and levels which appear to provide "adequate" intakes. The data from the literature which were used as a basis for the selection of the levels of nutrients for the diet are presented in section IV.

SECTION I—THE DIET, ITS NUTRITIVE VALUE AND APPLICATION

PLAN OF THE DIET

To devise a single diet to be applicable to the study of every nutrient known at present would require an entirely synthetic diet to which there would be obvious objections. A diet made up of a combination of foods and synthetic and purified products has therefore been planned to provide palatable and "normal" meals and to be applicable to the study of many nutrients. (See Possible Applications of the Diet, p. 9.) In the discussion of this diet, the term "restricted" is used to describe levels which have been used for deficiency phases in metabolic studies, and "reference" level for intakes selected as reasonable levels for equalization periods, from which deviations can be made for experimental purposes. These reference levels probably meet ordinary physiological needs, although the margin of safety may be considerably greater for some than for others. It seemed advisable to use only moderate levels for all nutrients, since there is evidence that excesses of some nutrients will alter the metabolism of others.

The diet consists of three parts—the core, and complements I and II. The core, made up of natural and refined foods, forms a nucleus for the meals and provides most nutrients at levels sufficiently restricted to permit this portion of the diet to remain unchanged for deficiency phases. Complement I, composed of refined foods, and complement II, composed of mineral salts and synthetic or purified vitamins, provide additional sources of nutrients for reference levels, and also provide a relatively simple means of adjusting the level of any single nutrient for experimental purposes. Levels of protein, fat, and carbohydrate can be controlled by increasing or decreasing the amounts of selected foods in complement I, and levels of minerals and vitamins by changing the amounts in complement II.

THE CORE.—The choice of foods for the core was necessarily limited in order to keep this portion of the diet constant and low enough in nutritive value to be used unaltered in the restricted, or

deficiency, phase of metabolic studies for most nutrients. The following fruits and vegetables were selected because of their low vitamin content and general acceptability: Apples, pears, peaches, green beans, celery, lettuce, and dehydrated potatoes. Cereals were all unenriched. Small amounts of beef, haddock, evaporated milk, and a specially prepared tomato puree were included to provide palatable and reasonably normal menu patterns. Foods such as vitamin C-rich fruits and vegetables, carrots with their variable carotene content, beets with their high pigment content, and corn products because of their relation to niacin metabolism, were omitted.

COMPLEMENT I.—Foods in complement I were selected to provide additional sources of food energy and protein with a minimum increase in the vitamin and mineral content of the diet, so that alterations in the proportions of carbohydrate, fat, and protein could be made. The following foods were chosen for this purpose: Cake flour, gluten flour, vitamin-free casein, gelatin, sugar, low-vitamin jellies, and fat. These foods were used primarily in the preparation of baked products or as spreads. Through the proportioned use of cake and gluten flour in the rolls, the protein content was about twice as high and the mineral and vitamin content approximately the same as would have been obtained through the use of only unenriched all-purpose flour. Casein and gelatin were used as additional sources of protein.

COMPLEMENT II.—In order to control the levels of individual minerals and vitamins in the diet it was necessary to provide them in as simple forms as possible and, therefore, additional amounts needed for reference levels were supplied in synthetic or purified forms. Problems encountered in the selection of specific chemical forms for minerals are discussed in section III under Methods of Administering Vitamins and Mineral Salts (p. 60).

NUTRITIVE VALUE OF THE DIET

An outline covering the estimated nutritive value of the standardized diet as planned for adults, presenting distribution of nutrients in the core and complements I and II, is shown in table 1. The essential nutrients have been divided into three groups. Group A includes the nutrients given in most tables of food composition and used in calculating diets. Group B includes minerals and group C, vitamins, about which much less is known both as to amounts present in foods and as to human requirements. For the purpose of discussion the amounts of foods in complement I have been adjusted to add approximately 1,450 calories and 40 gm. protein to the amounts in the core (to give a total of 2,000 calories and 60 gm. protein).

GROUP A NUTRIENTS.—The approximate amounts of calcium (200 mg.), phosphorus (600 mg.), thiamine (0.3 mg.), riboflavin (0.5 mg.), and ascorbic acid (10 mg.) in the core and complement I are in the range used by most laboratories for the deficiency phase in metabolic studies and can therefore be used for restricted levels. The 6 mg. iron is generally considered low for women, but may be sufficient for men. The vitamin A value (about 1,000 I. U.) is considered low but is higher than that used for depletion to deficiency levels in vitamin A studies. The 7 mg. niacin, however, is probably sufficient for the reference level in the presence of the protein used.

TABLE 1.—*Estimated nutritive value of the standardized diet as planned*

Nutrient [1] and unit	Core	Complement I [2]	Subtotal: Core+complement I	Complement II	Total: Reference [3]
GROUP A					
Food energy—calories	550	1,450	2,000	0	2,000
Protein—grams	20	40	60	0	60
Fat—do	10	70	80	0	80
Carbohydrates—do	100	150	250	0	250
Calcium—milligrams	150	50	200	500	700
Phosphorus—do	300	300	600	400	1,000
Iron—do	4	2	6	4	10
Vitamin A value—International Units	1,000	(3)	[3] 1,000	[3] 3,000	4,000
Thiamine—milligrams	0.15	0.15	0.3	0.5	0.8
Riboflavin—do	0.3	0.2	0.5	0.5	1.0
Niacin—do	5	2	7	0	7
Ascorbic acid—do	10	----------	10	50	60
GROUP B					
Copper—milligrams	0.6	0.2	0.8	0	0.8
Iodine—do	0.090	0.015	0.105	0	0.105
Magnesium—do	90	30	120	100	220
Manganese—do	1	1	2	0	2
Potassium [4]—do	900	200	1,100	1,000	2,100
Sodium [4]—do	300	trace	300	2,100	2,400
Zinc—do	3	1	4	0	4
GROUP C					
Chlorine—milligrams	120	80	200	100	300
Cobalamin (B_{12})—micrograms	1	----------	1	4	5
Folic acid—do	25	25	50	50	100
Pantothenic acid—milligrams	2	1	3	1	4
Pyridoxine—do	0.3	trace	0.3	0.5	0.8
Vitamin D—International Units	----------	----------	----------	400	400

[1] Group A nutrients are those included in most tables of nutritive value; group B minerals and group C vitamins are those about which much less is known both as to amounts present in foods and human requirements.

[2] Amount of foods in complement I adjusted to give a total of approximately 2,000 calories and 60 gm. protein.

[3] Vitamin A value in complements I and II depends on amount and forms of fat used.

[4] Potassium and sodium values shown for the core and complement I are the estimated amounts for the foods only and do not include the mineral content of the sodium chloride and baking powder used in recipes.

As a reference level for protein an intake of 60 gm. may be liberal, but it seemed important to assure nitrogen equilibrium when other nutrients were to be studied. The core foods supply about 20 gm. and complement I foods about 40 gm. The complement I sources of protein were planned to provide some easily replaceable protein, so that a constant level of protein intake could be maintained when the diet was used for studies of the physiological utilization of foods containing fair amounts of protein. The fat was planned to constitute about 35 percent of the calories, a conservative estimate of the amount in usual American diets. Carbohydrate levels were roughly 50 percent of the total calories. Chiefly on the basis of data from the University of Illinois, an intake of 700 mg. calcium was selected for the reference level. The level of 1 gm. for phosphorus is about one and one-half times the calcium level. The level of iron, 10 mg., seemed necessary to assure the replacement of menstrual losses for most women.

Although vitamin A and carotene are stored in the body, a reference level of 4,000 I. U. was planned in order that low vitamin A would not be a factor affecting metabolism of other nutrients. The thiamine reference level of 0.8 mg. was selected as intermediate between 0.6 and 1.0 mg., two levels referred to as adequate, depending on the

criteria used. For riboflavin an intake of 1.0 mg. was chosen, since this amount seems adequate to prevent clinical signs of deficiency and to maintain work performance, although it may not be sufficient to maintain tissue saturation. Until the relationship between thiamine and riboflavin requirement and caloric intake is more clearly established, it seemed unnecessary to plan for variation in intake with the caloric value of the diet. On the basis of work by Goldsmith and associates (*66*) [2] the 7 mg. niacin and calculated 0.33 gm. tryptophan in the core and complement I seemed sufficient for use as a reference level. For ascorbic acid, 60 mg. was selected as the reference level on the basis of blood studies. This amount will usually give values between 0.5 and 0.7 mg. per 100 ml. of whole blood, plasma, or serum and will maintain white cell ascorbic acid values of 20 to 25 mg. per 100 ml.

GROUP B NUTRIENTS.—Nutrients in group B include essentia minerals for which very few data on human requirements are available. Since the amounts of most of these minerals in the core and complement I are below those found in the usual diet, some additional amounts were provided in complement II, although no provision was made for additional copper, iodine, manganese, or zinc. The reference level of 220 mg. magnesium, based on intakes that have been found to give equilibrium, was used so that a deficient intake of magnesium would not become a limiting factor in calcium and phosphorus studies. The reference level of 2.1 gm. potassium is near the lower level found in the usual American diet. In most studies, the sodium intake will not be controlled, since salt intake is not considered to affect the metabolism of nutrients other than potassium. If it is desirable to control the sodium intake, it should be noted that the levels of sodium chloride suggested for use in the recipes (see sect. III) will provide about 2.1 gm. more of sodium or a total sodium intake of 2.4 gm. This quantity is satisfactory as a reference level.

GROUP C NUTRIENTS.—Our knowledge of the quantitative human requirements of Group C vitamins is still very limited. Choline requirement is related to other sources of available methyl groups; cobalamin (B_{12}) and folic acid needs are related to each other and to ascorbic acid; reports of pantothenic acid deficiency in humans have not been found, but the usual diet contains more than is found in the core and complement I; pyridoxine metabolism is related to tryptophan and niacin metabolism and is important in various enzyme systems; and opinions differ as to the need of vitamin D for adults. Consequently reference levels were selected rather arbitrarily and they can easily be altered as new information is secured.

MEAL PATTERNS AND NUTRITIVE VALUE OF FOODS.—The foods in the core in 3 meal patterns and their content of food energy and 11 nutrients in amounts suggested for the diet are given in table 2. Both the choice of foods and the size of servings in the core were kept unchanged (except as indicated for studies of vitamin A, p. 12). The foods in complement I can be adjusted for different nutrient levels. Amounts as shown in table 2 are adjusted to add approximately 1,450 calories and 40 gm. protein. The items in complement I are listed separately for use with core foods in meal patterns 1 and 3 and

[2] Italic numbers in parentheses refer to Literature Cited, p. 71.

TABLE 2.—*Meal patterns and nutritive value [1] of foods [2] in the standardized diet*

Item	Quantity served	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Reduced ascorbic acid
CORE FOODS													
Meal pattern 1:													
Breakfast:	*Grams*	*Calories*	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*International units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Applesauce, canned	100	72	0.1	0.1	19.7	1	5	0.4	30	0.005	0.009	Trace	0.6
Farina, unenriched (dry weight)	20	74	2.2	.2	15.5	4	20	.2	0	.012	.004	0.2	[3]
Evaporated milk	25	34	1.7	2.0	2.5	64	49	Trace	100	.012	.122	Trace	[3]
Lunch:													
Rice, precooked (dry weight)	25	95	2.0	Trace	20.8	4	19	.2	0	Trace	Trace	Trace	[3]
Tomato puree	30	30	.5	2.2	2.3	4	10	.1	327	.024	.013	.2	3.0
Lettuce	20	3	.2	Trace	.6	4	4	.1	108	.010	.005	Trace	.5
Pears and juice, canned	100	68	.2	.1	18.4	3	7	.2	Trace	.007	.009	.1	2.2
Dinner:													
Beef (raw weight)	45	82	10.3	1.7	0	8	110	1.3	0	.036	.078	2.1	0
Potato, precooked and dried (dry weight)	25	89	2.4	.2	20.5	5	52	1.0	10	.002	.021	1.1	1.8
Green beans, frozen (cooked weight)	100	35	1.3	.2	7.7	47	24	1.1	450	.038	.081	.6	3.2
Total		582	20.9	6.7	108.0	144	300	4.6	1,025	.146	.342	4.3	11.3
Meal pattern 2:													
Breakfast:													
Applesauce, canned	100	72	.1	.1	19.7	1	5	.4	30	.005	.009	Trace	.6
Rice Krispies, unenriched	15	59	1.1	.1	13.2	3	17	.3	0	.003	.010	.1	[3]
Evaporated milk	25	34	1.7	2.0	2.5	64	49	Trace	100	.012	.122	Trace	[3]
Lunch:													
Spaghetti (dry weight)	25	94	3.1	.3	19.1	5	36	.4	0	.020	.004	.5	[3]
Tomato puree	30	30	.5	2.2	2.3	4	10	.1	327	.024	.013	.2	3.0
Lettuce	20	3	.2	Trace	.6	4	4	.1	108	.010	.005	Trace	.5
Dinner:													
Beef (raw weight)	45	82	10.3	1.7	0	8	110	1.3	0	.036	.078	2.1	0
Potato, precooked and dried (dry weight)	25	89	2.4	.2	20.5	5	52	1.0	10	.002	.021	1.1	1.8
Celery, frozen (thawed weight)	60	11	.4	.1	2.2	32	14	.3	0	.016	.017	.2	2.5
Peaches and juice, canned [4]	100	68	.4	.1	18.2	1	11	.4	450	.005	.015	.7	2.7
Total		542	20.2	6.8	98.3	127	308	4.3	1,025	.133	.294	4.9	11.1

Meal pattern 3:													
Breakfast:													
Applesauce, canned	100	72	.1	.1	19.7	1	5	.4	30	.005	.009	Trace	.6
Cheerios, unenriched	10	39	1.4	.7	6.8	19	39	.4	0	.009	.011	.1	(3)
Evaporated milk	25	34	1.7	2.0	2.5	64	49	Trace	100	.012	.122	Trace	(3)
Lunch:													
Rice, precooked (dry weight)	25	95	2.0	Trace	20.8	4	19	.2	0	Trace	Trace	Trace	(3)
Tomato puree	30	30	.5	2.2	2.3	4	10	.1	327	.024	.013	.2	3.0
Lettuce	20	3	.2	Trace	.6	4	4	.1	108	.010	.005	Trace	.5
Pears and juice, canned	100	68	.2	.1	18.4	3	7	.2	Trace	.007	.009	.1	2.2
Dinner:													
Haddock, frozen fillet (raw weight)	50	39	9.4	Trace	0	18	90	.3	----------	.023	.048	1.2	(3)
Potato, precooked and dried (dry weight)	25	89	2.4	.2	20.5	5	52	1.0	10	.002	.021	1.1	1.8
Green beans, frozen (cooked weight)	100	35	1.3	.2	7.7	47	24	1.1	450	.038	.081	.6	3.2
Total	----------	504	19.2	5.5	99.3	169	299	3.8	1,025	.130	.319	3.3	11.3
COMPLEMENT 1 FOODS													
For meal patterns 1 and 3:													
Rolls, 3.2 percent casein (weighed as dough)	250	642	36.0	12.5	94.5	55	255	1.2	(5)	.132	.165	2.1	(3)
Fat	45	398	(3)	45.0	0	0	0	0	(5)	(3)	(3)	0	(3)
Sugar	5	19	(3)	0	5.0	(3)	(3)	0	0	(3)	(3)	0	(3)
Jelly, grape	30	76	.1	0	19.5	2	1	.1	0	.004	.006	.1	.1
Gelatin	2	7	1.6	0	0	9	1	0	0	0	0	0	
Cookies (weighed as dough)	75	293	2.4	14.5	39.5	4	25	.1	(5)	.010	.005	.1	(3)
Total	----------	1,435	40.1	72.0	158.5	70	282	1.4	(5)	.146	.176	2.3	0.1
For meal pattern 2:													
Rolls, 3.2 percent casein (weighed as dough)	250	642	36.0	12.5	94.5	55	255	1.2	(5)	.132	.165	2.1	(3)
Fat	45	398	(3)	45.0	0	0	0	0	(5)	(3)	(3)	0	(3)
Sugar	5	19	(3)	0	5.0	(3)	(3)	0	0	(3)	(3)	0	(3)
Jelly, quince	30	76	.1	0	19.5	3	2	.1	0	.001	.001	.1	.3
Gelatin	2	7	1.6	0	0	9	1	0	0	0	0	0	
Dough for cobbler (peach)	65	98	2.5	4.8	13.2	5	27	.1	(5)	.010	.006	.2	(3)
Cookies (weighed as dough)	25	215	.8	10.0	29.1	1	8	Trace	(5)	.003	.002	Trace	(3)
Total	----------	1,455	41.0	72.3	161.3	73	293	1.4	(5)	.146	.174	2.4	0.3

[1] Values for protein, calcium, phosphorus, thiamine, riboflavin, and ascorbic acid are from laboratory analyses of the lot of foods used in a preliminary study; values for other nutrients are calculated primarily from U. S. Dept. Agr. Handb. No. 8 (*184*).

[2] Sources of special foods and procedures for preparation of the foods are given in sect. III.

[3] No analyses made.

[4] Used as cobbler.

[5] Depends on type of fat used.

TABLE 3.—*Nutritive values* [1] *of 100-gm. portions of foods in complement I*

Food	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Reduced ascorbic acid
	Calories	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*International units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Dough for rolls: [2]												
No casein	257	11.9	5.0	40.3	22	82	0.6	[3]	0.054	0.065	0.9	[4]
3.2 percent casein [5]	257	14.4	5.0	37.8	22	102	.5	[3]	.053	.066	.9	[4]
5.8 percent casein [5]	256	16.4	5.0	35.7	23	118	.5	[3]	.052	.065	.8	[4]
7.3 percent casein [5]	255	17.6	5.0	34.4	23	128	.5	[3]	.053	.065	.8	[4]
Fat	884	0	100	0	[4]	[4]	0	[3]	[4]	[4]	0	[4]
Sugar	385	0	0	99.5	[4]	[4]	0	0	[4]	[4]	0	[4]
Jelly, grape	252	.2	0	65.0	7	5	.3	10	.015	.020	.2	0.5
Jelly, quince	252	.2	0	65.0	9	6	.3	10	.002	.002	.2	1.0
Gelatin	335	81.6	.1	0	465	42	0	0	0	0	0	[4]
Dough for cobbler (peach) [2]	331	3.8	15.4	44.7	7	42	.2	[3]	.016	.009	.3	[4]
Dough for cookies [2]	391	3.2	19.3	52.7	6	33	.2	[3]	.013	.007	.2	[4]
Cake flour	364	8.7	.8	79.4	16	94	.5	0	.036	.020	.6	[4]
Gluten flour	354	40.1	2.0	43.0	66	169	1.1	0	.144	.066	1.6	[4]
Casein	340	89.9	0	0	33	713	0	0	.007	.018	Trace	[4]

[1] Values for protein, calcium, phosphorus, thiamine, riboflavin, and ascorbic acid are from laboratory analyses of the lot of foods used in a preliminary study; values for other nutrients are calculated primarily from U. S. Dept. Agr. Handb. No. 8 (*184*).

[2] Values obtained from nutritive value of ingredients and weight of dough before weighing individual portions (see p. 59).

[3] Depends on type of fat used.

[4] No analyses made.

[5] Casein content refers to grams of casein per 100 gm. dough.

those in pattern 2 in order to show that only slight changes in any nutrient result from the substitution of quince jelly for grape jelly and of a biscuit dough (in cobbler) for two cookies.

The nutritive values of 100-gm. portions of foods in complement I are given in table 3 in order to provide a basis for alterations in level of nutrients. The data indicate, for example, what changes will occur in other nutrients when food energy or protein levels are changed. The analyzed values for nitrogen, calcium, phosphorus, thiamine, riboflavin, and ascorbic acid shown in tables 2 and 3 were obtained from the lot of foods used during a preliminary test period (see sect. II).

POSSIBLE APPLICATIONS OF THE DIET

EQUALIZATION PERIODS.—In any metabolic study the previous dietary intake of the subjects may be reflected in their metabolic response to the experimental diet. In planning the standardized diet, the reference levels were selected for use in preliminary or equalization periods, regardless of type of study to be made, to determine the metabolic response of the subjects to these levels and to help overcome the effects of the self-chosen diet immediately prior to the experiment. The length of time needed for subjects to come to equilibrium on these reference levels will vary with the particular nutrient under study and the previous diet of the individual but probably will be at least 10 days and frequently longer. The nutritive value of the diet for the equalization periods is summarized in table 4, type A.

REQUIREMENT STUDIES.—When requirement for a specific nutrient is studied under controlled dietary conditions, the nutrient is often given at a markedly restricted level and subsequently graded amounts are added to the diet. The extent of restriction depends on the objectives and conditions of the experiment. The amount of calcium, phosphorus, iron (for women), thiamine, riboflavin, and ascorbic acid in the foods of the core and complement I generally correspond to the restricted levels commonly used. The standardized diet should therefore be useful in studying the requirement of these nutrients by omitting or altering the level of the respective nutrient in complement II (see table 4, type B).

For the study of amino acid and protein requirements, basal diets usually contain as little as 0.4 gm. nitrogen (*22*, *23*, *77*, *153*, *154*), whereas the core foods alone contain about 3 gm. Thus the standardized diet is not applicable to the study of requirements for these nutrients.

The diet might be applicable to the study of fat and fatty acid requirements, if a fat-free diet were not required (*25*). In a recent study reported by Hansen and Wiese (*70*), the blood levels of dienoic, tetraenoic, and hexaenoic acids were found to be significantly less in malnourished infants and children than in a group of well-nourished children previously studied (*186*) who were receiving about 3 percent of the total calories as linoleic acid. The foods in the core have a calculated linoleic acid content of only about 0.1 gm. (0.04 percent of the 2,000 calorie diet). By controlling the type of fat in complement I, for example, by the use of coconut oil, butterfat, or cottonseed oil—reported to contain no linoleic acid, and roughly 4 percent and 50 percent of linoleic acid, respectively (*46*)—the requirement for this essential fatty acid probably could be studied.

TABLE 4.—*Adaptation of the standardized diet for different types of studies*

Type of study and diet	Quantity served	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A value	Thiamine	Riboflavin	Niacin	Reduced ascorbic acid
	Grams	*Calories*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Milligrams*	*International units*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
A. Equilization period:													
Core (average values for a 5-day period)		550	20.3	6.5	102	142	303	4.3	1,025	0.138	0.318	4.3	11.2
Complement I [1]													
Rolls (3.2 percent casein)	250	642	36.0	12.5	94.5	55	255	1.2	(2)	.132	.165	2.1	
Fat	45	398		45.0	0	0	0	0	(2)			0	
Sugar	5	19		0	5.0			0	0			0	
Jelly, grape	30	76	.1	0	19.5	2	1	.1	0	.004	.006	.1	.1
Cookies	75	293	2.4	14.5	39.5	4	25	.1	(2)	.010	.005	.1	
Gelatin	2	7	1.6	0	0	9	1	0	0	0	0	0	
Subtotal		1,985	60.4	78.5	260.5	212	585	5.7	(2)	.284	.494	6.6	11.3
Complement II						[3] 500	[3] 388	4.0	(4)	.500	.500		50.0
Total		1,985	60.4	78	260	712	973	9.7	4,000	.784	.994	6.6	61.3
B. Requirement studies	Core and complement I as under A above. For complement II omit or alter the nutrient under study												
C. Interrelationship studies (e. g., effect of low protein):													
Core		550	20.3	6.5	102	142	303	4.3	1,025	.138	.318	4.3	11.2
Complement I [1]													
Rolls (no casein)	100	257	11.9	5.0	40.3	22	82	.6	(2)	.054	.065	.9	
Fat	20	177		20.0					(2)				
Sugar	20	77			20.0				0				
Jelly	30	76	.1		19.5	2	1	.1	0	.004	.006	.1	.1
Cookies, frosted (150 cookies, 48 frosting)	198	852	4.8	45.2	110.7	9	49	.3	(2)	.019	.010	.3	
Gelatin	0												
Subtotal		1,989	37.1	76.7	292.5	175	435	5.3	(2)	.215	.399	5.6	11.3
Complement II [5]						[3] 530	[6] 565	5.0	(4)	.570	.600	1.3	50.0
Total		1,989	37.1	77	292	705	1,000	10.3	4,000	.785	.999	6.6	61.3

D. Utilization studies (e. g., riboflavin in milk):													
Core		550	20.3	6.5	102	142	303	4.3	1,025	.138	.318	4.3	11.2
Complement I (with milk) [1]													
Milk	300	204	10.5	11.7	14.7	354	279	.3	480	.12	.51	.3	
Rolls (no casein)	250	642	20.8	12.5	101.0	55	205	1.5	[2]	.135	.162	2.2	
Fat	33	293		33.0					[2]				
Sugar	5	19			5.0				0				
Jelly	0												
Cookies	75	292	2.4	14.5	39.5	4	25	.1	[2]	.010	.005	.1	
Gelatin	0												
Subtotal		2,000	63.0	78.2	262.2	555	812	6.2	[2]	.403	.995	6.9	11.2
Complement II [3]						[3] 160	[6] 180	4.0	[4]	.400			50.0
Total		2,000	63.0	78	262	715	992	10.2	4,000	.803	.995	6.9	61.2

[1] Values for meal patterns 1 and 3.
[2] Values for complement I depend on amount furnished by fat used.
[3] From dicalcium phosphate.
[4] Values for complement II will be varied to maintain a constant daily intake.
[5] Appropriate substitutions need to be made in the complement II portion of the diet for the minerals and vitamins in groups II and III (table 1).
[6] From dicalcium phosphate and potassium dihydrogen phosphate.

Diets used for the study of vitamin A requirements have contained only around 100 I. U. (*20, 173*), whereas the vitamin A value of the foods in the core of this diet is about 1,000 I. U.—100 I. U. from vitamin A in milk and 900 I. U. from carotenoids in other core foods. The diet can be adapted for vitamin A studies by omitting the tomato puree and substituting skim milk for evaporated milk, wax beans for green beans, bleached lettuce for regular lettuce, and a second serving of apples for the peaches used in the cobbler. These alterations bring the vitamin A value down to about 100 I. U. Although the altered diet does not offer the variety and palatability found in diets commonly used for vitamin A studies, which include foods such as pork products and citrus fruits, such adaptations would permit the control and study of other nutrients simultaneously with vitamin A.

The diet as set up is not applicable to the study of niacin requirement. The calculated niacin content of the foods in the standardized diet is approximately 7 mg.—5 mg. in the core and 2 mg. in complement I. These values have been confirmed by analysis of composites containing the core foods and of individual foods in complement I. The calculated tryptophan content of the beef, milk, and rolls (without casein) is 0.26 gm., or approximately the minimum requirement (0. 25 gm.) tentatively suggested for this amino acid for men by Rose (*153*). The niacin and tryptophan content of the standardized diet can be lowered to about 6 mg. and 0.22 gm., respectively, by substituting cornbread for the wheat rolls and maintaining the protein level with gelatin instead of the gluten flour. These levels are not so low as the intake of 4.7 mg. niacin and 190 mg. tryptophan reported by Goldsmith and coworkers (*66*) as necessary to produce deficiency symptoms, but might be sufficiently restricted for study of physiological utilization of niacin from foods.

On the basis of information available, the amounts of other minerals and the more recently recognized B vitamins found in the core and complement I appear to be sufficiently restricted, so that the requirement for the nutrients can be studied by controlling the amounts of the corresponding nutrient in complement II.

Interrelationship Studies.—The level of single nutrients in a diet has been reported to affect the excretion and metabolism of other nutrients (*60, 67, 137*). In studying simple or multiple interrelationships among nutrients, therefore, it is desirable to alter the level of each nutrient independently with as little change as possible in the general composition of the diet. The greatest part of fat, carbohydrate, and protein in the standardized diet is found in complement I. By supplying these nutrients from rather highly purified sources, the intakes can easily be altered with only minor changes in other nutrients such as minerals and vitamins. For example, the core contains only about 6 gm. fat, so that by changing the kinds or levels of fat in complement I its possible effect on the requirement of various other nutrients can be studied. Changes in the level of fat will, of course, require changes in carbohydrate to make the two diets isocaloric. Or, as another example, the level of protein in complement I can be lowered from about 40 gm. (see table 4, type A) to about 16.5 gm. (table 4, type C) by omitting the gelatin and using 100 gm. of "no-casein" rolls instead of 250 gm. of 3.2-percent casein rolls. This change in rolls will also lower the nutritive value of

complement I primarily in food energy value, but this can be compensated for by adjustments in complements I and II as indicated in table 4, type C. The total protein in the diet can therefore be lowered from 60 to 37 gm., with a minimum of change in the amounts of other nutrients.

The effect of deficient or excessive amounts of selected minerals and vitamins on the utilization of other nutrients can readily be studied by altering the amount of the mineral salts or purified vitamins in complement II.

UTILIZATION STUDIES.—The diet can be used to study the utilization of nutrients from foods by substituting the test food for comparable amounts of selected nutrients in complements I and II and regulating the amounts of other nutrients so that insofar as possible the nutritive content of the diet will be unchanged. For example, to study the utilization of riboflavin from milk, the addition of 300 gm. milk to complement I would replace the 0.5 mg. riboflavin in complement II. This amount of milk would also contribute an appreciable amount of other nutrients. To maintain as nearly constant an intake as possible, adjustments in complements I and II illustrated in table 4, type D would need to be made. Compensations would also be made where possible for the lesser known vitamins and minerals. Foods may contain other nutrients, at present unknown, but by this plan, intakes of most of the recognized nutrients could be controlled.

SECTION II—METABOLIC RESPONSE TO THE STANDARDIZED DIET AND TO A LOW-FAT INTAKE

PRELIMINARY TEST OF THE DIET

Before the main study was made, a 10-day test was carried out to evaluate the acceptability of the diet. Three staff members served as subjects. As a result of this test, several changes were made which have been included in the diet as presented in section I. Complement I originally contained egg white, used for meringues and angel food cake. Since egg white provided more riboflavin than was anticipated, it was replaced by 2 gm. gelatin and additional cake flour to supply an equivalent amount of nitrogen. Although these proteins are of poorer quality than those in egg white, the calculated amounts of essential amino acids in the diet still meet requirements as suggested for men by Rose (*153*). Macaroni, used on alternate days for lunch, was replaced by spaghetti, since it was more acceptable with the limited amount of tomato puree used for flavoring. The recipe for tomato puree was modified to include a greater proportion of onion and celery to improve the flavor. Although suggested combinations of foods and recipes as used in the original diet had been tried and tested for palatability by a small untrained group before this preliminary test period, reactions to flavor and texture of some foods proved less satisfactory when full servings were eaten repeatedly.

Collections of foods and excreta during this preliminary test provided an opportunity for checking general collection procedures and adapting analytical methods to the equipment available. The "carmine" used at the beginning of this test was excreted in the urine and therefore was unsatisfactory as a feces marker. Carmine, alum lake No. 1239, from National Analine Division of Allied Chemical &

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Dye Corp., was found satisfactory. Experiences during the 10-day test in preparation of the meals, in collection and preservation of the samples, and in evaluation of the diet and of the analytical procedures proved invaluable as a preparation for carrying out the metabolic study reported here.

GENERAL PLAN OF THE STUDY

The diet as described in section I was used in a 40-day metabolic study in the spring of 1952 by the Human Nutrition Branch of the Agricultural Research Service, in cooperation with the Maryland Agricultural Experiment Station and the College of Home Economics of the University of Maryland. This study was undertaken to evaluate the acceptability of the diet by a group not associated with its planning, to obtain information on the metabolic response to the levels of nutrients in the diet selected as reference levels, and to test the use of the diet for a study of the effect of differences in fat intake on utilization of other nutrients.

The 6 subjects, selected from 15 volunteers, were home economics students of the University of Maryland; 3 were juniors, 2 were sophomores, and 1 was a special student. All were considered to be in good health by the examining physician. Age, weight, height, and calculated body surface of these subjects are shown in table 5. The range in age was from 19 to 23 years; in height, from 157.7 to 173.7 cm.; in weight, from 47.2 to 74.7 kg.; and in calculated body surface, from 1.51 to 1.89 square meters. According to Davenport's table of weight for height and age (*42*), subjects B, E, and F were within 10 percent of the expected weight; A was 18 percent, and D, 20 percent underweight; and C, 18 percent overweight. Subjects A and D were sisters.

TABLE 5.—*Physical measurements of subjects*

Subject	Age	Weight [1]	Height	Body surface [2]
	Years	*Kilograms*	*Centimeters*	*Square meters*
A	19	50.1	170.8	1.58
B	22	59.4	157.7	1.59
C	20	74.7	173.7	1.89
D	21	47.2	166.9	1.51
E	23	66.5	161.1	1.61
F	20	58.9	169.3	1.68

[1] Mean of weights for entire study taken at the beginning, middle, and end of each period. (See table 6 for trends.)

[2] Calculated from the formula of Dubois and Dubois (*51*): Wt. kg.$^{0.425}$ × Ht. cm.$^{0.725}$ × 71.84

A furnished house within walking distance of the University was rented in order to provide pleasant living quarters and adequate facilities for preparing and serving meals and for collection and temporary storage of metabolic samples. Preparation and serving of the meals and supervision of the collection of samples were carried out by four professional members of our staff. Two members were present at each meal and one was on duty at all times.

The experiment was divided into eight 5-day periods. During the first 4 periods, all 6 subjects were given the amounts of fat and carbo-

hydrate provided in the standardized diet; during the second 4 periods, 3 of the subjects were maintained on the same intake to serve as controls, and the other 3 were placed on a lower fat—higher carbohydrate intake. No preliminary period was used and no attempt was made to saturate body stores with vitamins and minerals, since it was of interest to follow the metabolic response of these subjects to intakes of the various nutrients in the standardized diet when they changed directly from their customary nutrient intakes and diet patterns. Test doses of thiamine, riboflavin, and ascorbic acid were given the last day of the experiment.

The foods in the core as listed in table 2 were used throughout the study. Since 5-day periods were desired, meal patterns 1 and 2 were repeated. The foods in complement I were served in essentially the same amounts as shown in table 2. Minor adjustments in sugar and jelly intake were made to take care of individual calorie requirements as indicated by weight changes. Procedures for the preparation of the diet and recipes for baked products are given in section III, pages 56 to 60. Vitamins and mineral salts in complement II were administered as described in section III, pages 60 and 61. Distilled water and sodium chloride were allowed ad libitum, but the amounts taken by each subject were recorded. A mixture of sodium chloride and sodium bicarbonate was used as a dentrifice.

The following plan was used to vary the fat level in the diet. During the first 4 periods when all subjects were maintained on the same intake, the estimated 78 gm. fat in the diet were from the following sources: Approximately 6 gm. fat from foods in the core, 22 gm. hydrogenated fat (10 in cooking, 12 in the rolls), and 50 gm. butterfat (15 in cookies, or cooky and cobbler, and 35 as table fat). During the last 20 days the 50 gm. butterfat were removed from the diet for subjects D, E, and F, and the estimated 28 gm. fat remaining in the diet were from the following sources: Approximately 6 gm. fat from foods in the core and 22 gm. hydrogenated fat (10 in cooking, 6 in the rolls, and 6 in cookies, or cooky and cobbler). The food energy content of the 50 gm. butterfat was replaced with isocaloric amounts of sugar and jelly. Each subject was allowed to choose the proportion of sugar (partly as fondant) and of jelly that she preferred. On the basis of the estimated 2,000 calories and intakes of 78 and 28 gm. fat, the fat would contribute about 35 and 13 percent of the total calories. Analyzed intakes of fat averaged 76 and 24 gm., so that the fat calories averaged nearer 34 and 11 percent. The higher of these two intakes is in the range found in average diets of nonobese women (*11*) and the lower intake in diets generally considered to be low in fat (*2*, *75*).

Procedures for the collection and preservation of samples of foods in the core and complement I, and of blood, urine, and feces are given on page 61, section III.

Thiamine, riboflavin, nitrogen, calcium, magnesium, and phosphorus analyses were carried out on food, urine, and feces samples; fat analyses on foods and feces samples; ascorbic acid on foods, urine, and blood samples; and creatinine on urine samples. Methods for analyses are given in section III, page 62.

RESULTS AND DISCUSSION

General Response to the Diet

The diet was well accepted by the 6 subjects during the 40-day study. Several days were required to adjust to the large proportion of cereal products and several of the subjects were not accustomed to using as much as 35 gm. table fat, but adjustments to these dietary changes were readily made.

The effect of the diet on intestinal motility was difficult to evaluate, because it varied among the subjects. Little or no effect was noted for subjects B, C, and F. It took longer for the carmine to appear in the stools for subject A toward the end of the study than during the first weeks. With time, motility also became slower for subjects D and E, but both subjects stated that it was not unusual for them to have intervals of several days between eliminations. These subjects were given agar in periods 7 and 8, but elimination was still slow.

The food-energy intake and the average body weight for the 6 subjects for each period are given in table 6. Subject C, the tallest and heaviest subject in the group, had the highest increase in food energy intake during the study (from 2,015 to 2,510 calories in the first 20 days), yet lost a total of 3.0 kg. of which 1.2 kg. was lost during the last 20 days on 2,510 calories. The others varied less than 0.5 kg. on intakes of 1,865 to 2,055 calories during this same period.

Creatinine Excretion

The daily creatinine excretion values are shown in figure 1. Mean values with standard deviations for 5-day periods and for the 40-day study are shown in table 7. Creatinine determinations were carried out on both halves of the urine samples (see sect. III, p. 62) to check the accuracy of separation, but only total values are reported. The results indicated that subjects A, D, E and F divided all urine samples satisfactorily and made complete collections throughout the 40-day study. Subject B apparently lost part of two samples. Subject C admittedly lost a number of samples toward the end of the study, including those following the test dose, and made unsatisfactory division of many samples. Daily variations in creatinine excretion for this subject were large and from casual remarks it was apparent that she did not appreciate the importance of accurately terminating 24-hour collections. Therefore, samples for the last period were discarded and urinary vitamin values are reported only by 5-day periods. Coefficients of variation [3] within 5-day periods for the other 5 subjects were 5 percent or less, except for subject A in period 4, B in period 5, and D in period 8, and in these cases the coefficients did not exceed 10 percent. Coefficients of variation for period means were all within 5 percent, including those for subject C. Daily variations in creatinine excretions for these subjects were not related to the menstrual cycle (figure 1).

[3] Standard deviation expressed as percent of the mean.

TABLE 6.—*Body weight* [1] *and calculated food energy intake of the subjects during the study*

5-day period	Subject A		Subject B		Subject C		Subject D		Subject E		Subject F	
	Weight	Food energy	Weight	Food energy	Weight	Food energy	Weight	Food energy	Weight	Food energy	Weight	Food energy
	Kilograms	*Calories*	*Kilograms*	*Calories*	*Kilograms*	*Calories*	*Kilograms*	*Calories*	*Kilograms*	*Calories*	*Kilograms*	*Calories*
1	50.6	2,015	59.7	2,015	76.5	2,015	47.0	2,015	56.0	2,015	58.7	2,015
2	50.2	2,015	59.8	1,995	76.1	2,315	47.0	2,015	55.8	2,015	58.7	2,015
3	49.9	2,015	59.3	1,920	74.9	2,315	47.0	2,015	56.1	2,015	58.6	2,015
4	49.7	2,015	59.0	1,920	74.3	2,390	46.9	2,015	56.3	1,995	58.7	2,015
5	50.1	2,015	59.0	1,920	74.6	2,510	47.1	2,055	56.8	2,035	59.0	2,055
6	50.2	2,015	58.9	1,920	74.1	2,510	47.4	2,055	57.1	1,925	59.1	2,055
7	50.3	2,015	58.8	1,920	73.8	2,510	47.4	2,055	57.2	1,865	59.0	2,055
8 [2]	50.1	2,015	58.5	1,920	73.5	2,510	47.6	2,055	57.0	1,865	59.1	2,055

[1] Mean of weights for entire study taken at the beginning, middle, and end of each period.
[2] 4-day period.

TABLE 7.—CREATININE: *Average daily excretion values*

5-day period	Subject A		Subject B		Subject C		Subject D		Subject E		Subject F	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	1.07	0.04	1.03	0.03	(1)	----------	1.02	0.02	1.02	0.03	1.14	0.03
2	1.10	.05	1.07	.02	1.31	0.07	1.06	.03	1.01	.04	1.12	.03
3	1.05	.04	[2] 1.05	.02	1.23	.09	1.01	.04	.97	.04	1.09	.04
4	1.11	.11	1.05	.05	1.18	.16	1.03	.01	1.00	.01	1.11	.02
5	1.15	.02	1.01	.07	1.14	.18	1.05	.02	1.02	.03	1.13	.04
6	1.13	.05	1.04	.02	1.19	.16	1.03	.03	1.03	.01	1.15	.02
7	1.09	.03	.99	.05	1.20	.20	1.07	.04	1.00	.02	1.14	.01
8	1.10	.03	1.04	.04	(3)	----------	1.08	.09	1.02	.02	1.13	.02
Mean (8 periods)	1.10	.03	1.04	.02	1.21	.06	1.04	.03	1.01	.02	1.13	.02
Mean (40 days)	1.10	.06	1.04	.04	1.21	.17	1.05	.04	1.01	.03	1.13	.03

[1] One sample lost before creatinine was analyzed.
[2] 3-day period; values for 2 days indicated incomplete collection.
[3] Collections known to be incomplete.

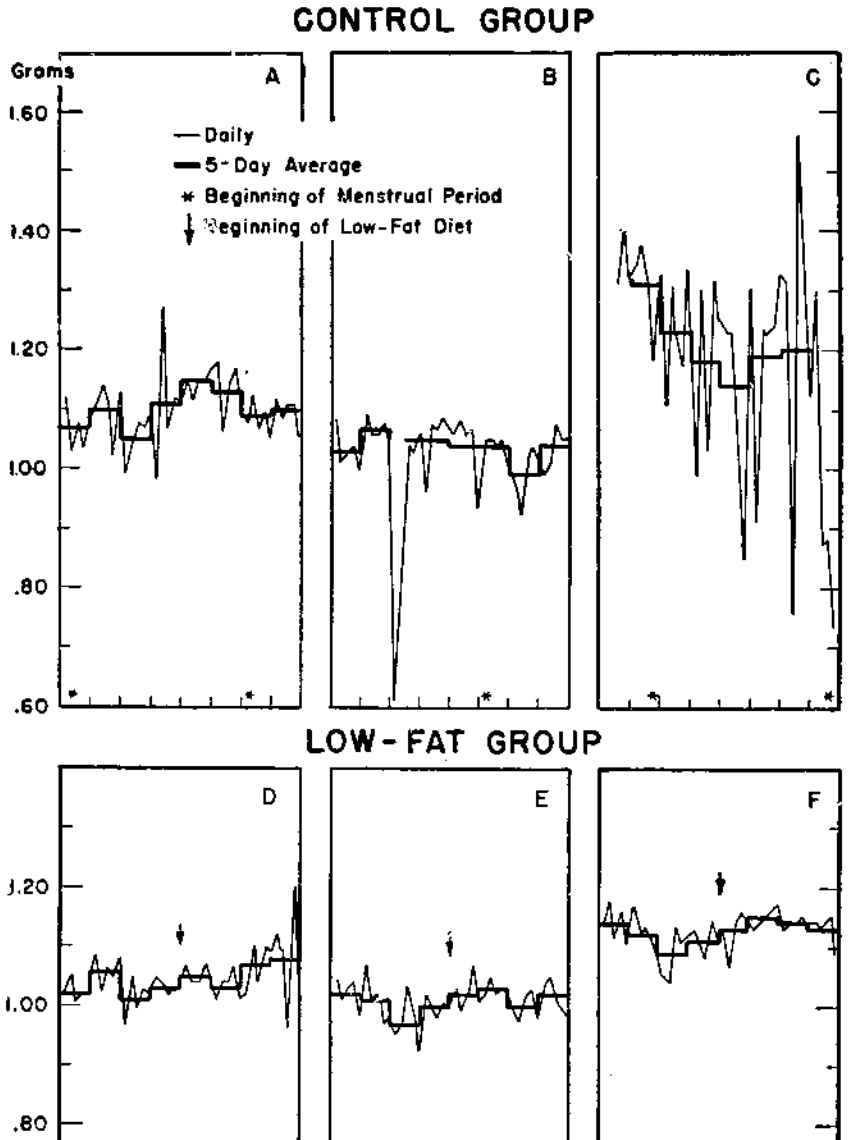

FIGURE 1.—CREATININE: Daily urinary excretions.

The creatinine and creatinine nitrogen coefficients (milligrams preformed creatinine or creatinine nitrogen per kilogram body weight) for the six subjects are shown in table 8. A detailed discussion of possible relationship of creatinine excretion to body weight, muscular mass, and muscular creatinine is given in the monograph on creatine and creatinine by Hunter (*83*).

TABLE 8.—*Average creatinine and creatinine nitrogen coefficients*

Subject	Creatinine coefficient based on—		Creatinine nitrogen coefficient based on—		Subject	Creatinine coefficient based on—		Creatinine nitrogen coefficient based on—	
	Actual weight	Ideal weight	Actual weight	Ideal weight		Actual weight	Ideal weight	Actual weight	Ideal weight
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
A	22	18	8.1	6.6	D	22	18	8.2	6.7
B	18	19	6.5	7.1	E	18	17	6.6	6.4
C	16	19	6.0	6.9	F	19	19	7.1	6.9

Nitrogen Metabolism

The data on nitrogen balance are summarized in table 9. The mean daily nitrogen intake for all subjects for the entire study was 10.99 gm., equivalent to 64.8 gm. protein.[4] An average of 3.11 gm. was obtained from foods in the core and 7.88 gm. from foods in complement I (7.09 gm. from rolls, 0.49 gm. from cookies and cobbler, and 0.30 gm. from gelatin). Approximately 27 percent of the nitrogen (3.02 gm.) was obtained from animal protein other than gelatin (1.57 gm. from beef or haddock, 0.27 gm. from milk, and 1.18 gm. from casein). This was distributed in the meals as follows: 0.51 gm. in the breakfast, 0.47 gm. in the lunch, and 2.04 gm. in the dinner. The nitrogen content of the rolls was higher than planned because of a marked variation in two lots of gluten flour. The lot used during the study contained 7.69 percent of nitrogen compared with 6.41 percent found in the lot used in the preliminary test. This resulted in an additional 5 gm. protein from the rolls.

Urinary nitrogen and nitrogen retention values indicated that subject C had been accustomed to a somewhat higher and the other five subjects to a slightly lower protein intake that than of the standardized diet. Excretions for all subjects except subject C were stabilized after the second 5-day period.

Fecal nitrogen ranged from an average of 0.39 gm. for subject E to 1.06 gm. for subject B. Wide ranges in fecal nitrogen among subjects on a constant intake have also been reported by Johnston and McMillan (*86*) and Toscani and Whedon (*180*).

Replacing 50 gm. fat with isocaloric amounts of carbohydrate apparently did not affect the nitrogen retention. As shown in table 9, retention values for the 3 subjects in the low-fat group receiving approximately 24 gm. fat during the last 4 periods of the study were not consistently different from those found during their control periods or from those of the 3 subjects in the control group receiving 76 gm. fat throughout the study.

[4] Protein conversion factor used was 5.90.

TABLE 9.—NITROGEN: *Average daily intake, excretion and retention values*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Group, subject, diet, and period	Intake	Excretion		Retention
		Urine	Feces	
CONTROL GROUP				
Subject A:				
Control diet:				
Period 1	*Grams* 11.08	*Grams* 8.83	*Grams* 1.17	*Grams* 1.08
2	11.03	8.78	.70	1.55
3	11.07	9.14	.91	1.02
4	10.85	8.89	[1] 1.81	1.15
5	10.85	9.95	[1] 1.81	.09
6	10.88	9.17	.80	.91
7	10.72	9.27	.83	.62
8	10.54	9.51	.69	.34
Subject B:				
Control diet:				
Period 1	11.08	7.94	.90	2.24
2	11.03	8.60	1.15	1.28
3	11.07	10.11	1.09	−.13
4	10.85	9.93	.99	−.07
5	10.85	9.08	.96	.81
6	10.88	9.36	1.16	.36
7	10.72	8.93	.94	.85
8	10.54	8.94	1.23	.37
Subject C:				
Control diet:				
Period 1	11.08	10.35	.64	.09
2	11.03	10.60	[1] 1.66	−.23
3	11.07	10.92	[1] 1.66	−.51
4	10.85	9.88	1.04	−.07
5	10.85	8.98	.84	1.03
6	10.88	8.84	.50	1.54
7	10.72	9.35	.75	.62
8		([2])		
LOW-FAT GROUP				
Subject D:				
Control diet:				
Period 1	11.08	8.11	.52	2.45
2	11.03	9.34	.44	1.25
3	11.07	9.33	.68	1.06
4	10.85	9.30	.75	.80
Low-fat diet:				
Period 5	11.20	9.65	1.02	.53
6	11.20	9.56	.63	1.01
7	11.20	9.48	.39	1.33
8	11.20	10.17	.64	.39
Subject E:				
Control diet:				
Period 1	11.08	8.70	.93	2.05
2	11.03	9.70	.50	.83
3	11.07	9.52	.27	1.28
4	10.85	9.68	.54	.63
Low-fat diet:				
Period 5	11.20	9.41	[1] .33	1.46
6	11.20	9.52	[1] .33	1.35
7	11.20	9.91	.25	1.04
8	11.20	10.09	.64	.47
Subject F:				
Control diet:				
Period 1	11.08	7.58	1.07	2.43
2	11.03	8.79	1.00	1.24
3	11.07	8.89	1.04	1.14
4	10.85	8.83	.88	1.14
Low-fat diet:				
Period 5	11.20	9.66	.75	.79
6	11.20	9.93	.70	.57
7	11.20	8.98	1.19	1.03
8	11.20	9.15	.85	1.20

[1] Average for 2 periods; separation between periods not satisfactory.
[2] Creatinine values indicated incomplete collection. (See p. 16.)

Protein metabolism in man has been reported to be affected adversely when almost all the carbohydrate in the diet was replaced by fat (*165, p. 199*) and favorably when fat or carbohydrate was superimposed on a diet already adequate in protein and food energy (*39*), or when a fat emulsion was used as a supplement to a diet deficient in protein and food energy (*181*). In the present study, in which the protein and food energy intake were adequate, no effect on protein metabolism was found as a result of the change from 250 gm. carbohydrate and 76 gm. fat to 360 gm. carbohydrate and 24 gm. fat.

The mean daily nitrogen retention values for the 6 subjects for periods after equilibrium was reached were as follows:

Subject:	Daily total [1]	Per square meter of body surface	Per kilogram of body weight
	Grams	*Grams*	*Milligrams*
A	0.69	0.44	14
B	.37	.23	6
C	.78	.41	10
D	.85	.56	18
E	1.04	.65	13
F	.98	.53	17
Mean	.78	.48	14

[1] Periods 4 to 7 for subject C, 3 to 8 for other 5 subjects.

The mean total retention value of 0.78 gm. for these subjects is comparable with the value of 0.72 gm. reported by Johnston and McMillan (*86*) for 6 women aged 20 to 31 years during their first 4-week study on a diet containing 10.72 gm. nitrogen. The mean retention value of 0.48 gm./m.^{2} is also similar to Johnston and McMillan's value of 0.47 gm./m.^{2} A mean retention value of 0.37 gm./m.^{2} was reported by Bricker and associates (*23*) for a group of women aged 19 to 30 years during a 10-week period on an intake of only 5.08 gm. nitrogen. Mitchell (*126*) found a small positive nitrogen balance during a long-time study in men aged 18 to 31, even after correction for dermal loss, which he considered to be required for growth of hair and nails.

Variation in average nitrogen retention values of from 0.37 to 1.04 gm. for the subjects in this study cannot be explained on the basis of age, weight, or surface area.

Dietary Fat and Fecal Lipids

As analyzed, the average daily intake of fat during the control periods was 76 gm. (34 percent of the calories) and during the low-fat periods 24 gm. (11 percent of the calories). The amounts of hydrogenated fat and butterfat used in the diet were discussed under the plan of the metabolism study (p. 15). The total amount of fat found by analysis was about 2 gm. lower than had been estimated.

Lipid excretion values for each subject are shown in table 10. The values during the low-fat periods were similar to those found for the same subjects during the control periods. That fecal lipid for the three subjects in the low-fat group was not affected by the change in dietary fat from 76 to 24 gm. was indicated also by analysis of variance. Fat balance values and digestibility coefficients were not calculated, since there was no significant change in fecal lipids with the change in intake. Mean excretion values for the 6 subjects during the study averaged 2.02 gm., with a standard deviation of 0.64.

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TABLE 10.—LIPIDS: *Average daily fecal excretion and partition values*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Group, subject, diet, and period	Excretion			
	Daily total	Fatty acids	Neutral fat	Unsaponifiable
CONTROL GROUP				
Subject A:				
Control diet:	*Grams*	*Percent*	*Percent*	*Percent*
Period 1	2.47	39	19	42
2	1.73	40	16	44
3	2.37	41	14	45
4	[1] 1.72	42	15	44
5	[1] 1.72	42	15	44
6	1.70	40	14	46
7	1.68	38	14	48
8	1.73	42	18	40
Subject B:				
Control diet:				
Period 1	1.65	36	25	39
2	1.94	33	15	52
3	2.17	36	20	44
4	2.22	36	20	44
5	1.70	40	16	44
6	2.35	32	22	46
7	1.96	36	20	44
8	2.65	33	25	43
Subject C:				
Control diet:				
Period 1	3.10	63	12	25
2	[1] 3.38	54	13	33
3	[1] 3.38	54	13	33
4	4.21	46	14	41
5	3.03	42	16	42
6	2.16	43	18	40
7	3.56	47	15	37
8	[2]			
LOW-FAT GROUP				
Subject D:				
Control diet:				
Period 1	1.37	43	26	31
2	1.55	49	14	37
3	1.64	52	12	36
4	1.80	44	15	41
Low-fat diet:				
Period 5	.90	41	20	40
6	1.76	44	18	38
7	1.46	50	16	34
8	2.47	50	17	32
Subject E:				
Control diet:				
Period 1	2.70	44	20	36
2	1.05	37	17	46
3	.80	40	21	39
4	1.56	40	17	42
Low-fat diet:				
Period 5	[1] 1.22	44	15	41
6	[1] 1.22	44	15	41
7	.86	38	29	33
8	2.41	35	15	50
Subject F:				
Control diet:				
Period 1	2.52	41	23	35
2	1.89	43	16	41
3	1.97	45	16	39
4	1.54	41	15	44
Low-fat diet:				
Period 5	1.48	37	22	41
6	1.42	36	28	36
7	1.08	32	21	46
8	1.44	35	24	40

[1] Average for 2 periods; separation between periods not satisfactory.
[2] Not analyzed.

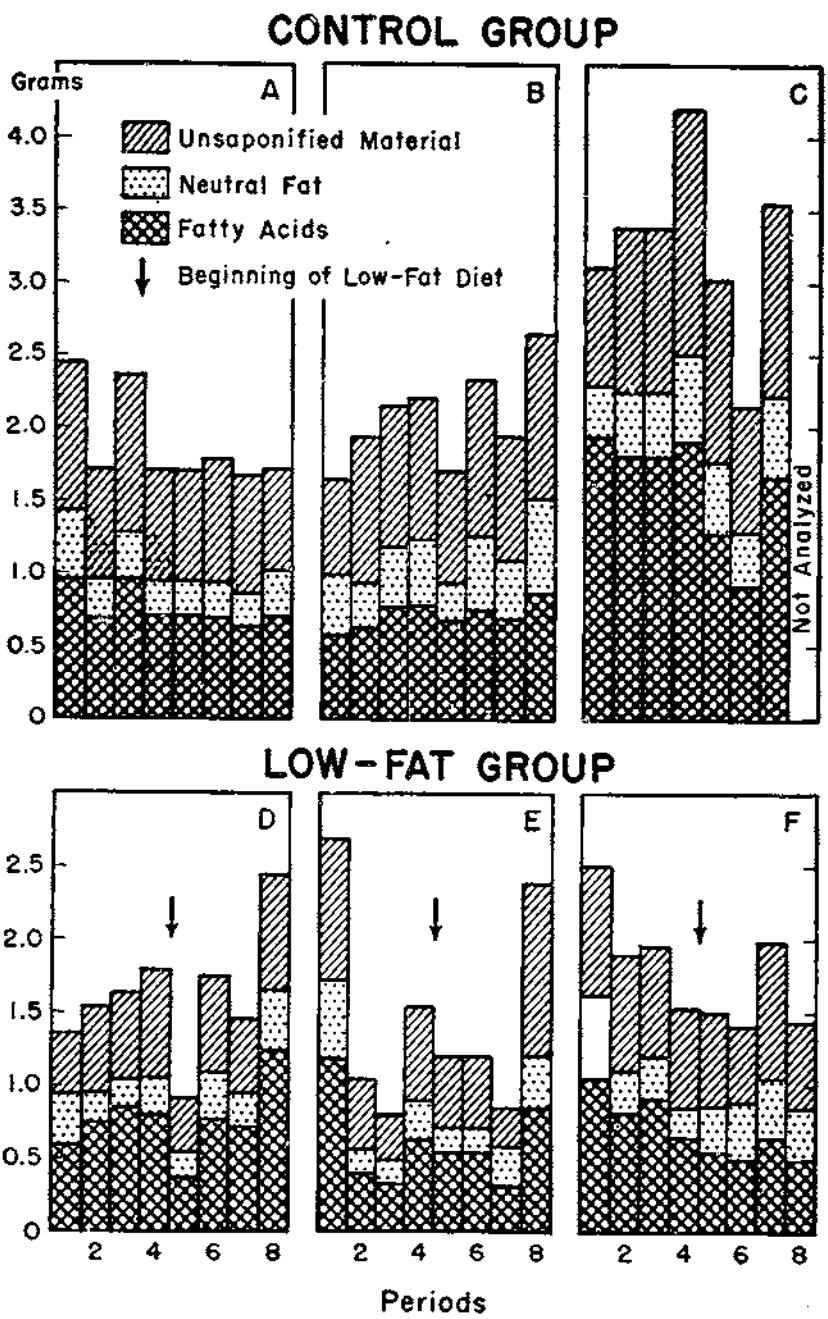

FIGURE 2.—LIPIDS: Average daily fecal excretions by periods.

Reports in the literature on the effect of fat level in the diet on the fecal lipids are controversial. Annegers, Boutwell, and Ivy (*3*), for example, using from 60 to 150 gm. hydrogenated fat or lard in addition to the fat in the basal foods, reported that differences in fecal lipids due to individual variations in fat excretion can be expected to be greater than differences due to the level or type of fat in the diet; Wollaeger, Comfort, and Osterberg (*191*), using diets containing 100 or 200 gm. fat (but not with the same subjects), reported increases in fecal lipids with higher intakes.

The average daily values for fecal lipids with amounts excreted as fatty acids, neutral fat, and unsaponifiable material are shown in figure 2, and are given as percentages of the total in table 10. Total fatty acids and unsaponifiable material were about equally distributed and together constituted about 80 to 85 percent of the total output; only about 15 to 20 percent of the output was in the form of neutral fat. The samples were not analyzed for soaps, as the glacial acetic acid used to preserve the samples for vitamin analyses had resulted in some hydrolysis.

Calcium, Phosphorus, and Magnesium Metabolism

CALCIUM.—The calcium intake, urinary and fecal excretion, and retention values for each subject are shown in table 11. The mean daily calcium intake during the study was 725 mg. An average of 144 mg. was obtained from foods in the core, 81 mg. from foods in complement I (64 mg. from rolls, 7 mg. from cookies and cobbler, and 10 mg. from gelatin), and 500 mg. as dicalcium phosphate from complement II. In addition, all subjects received from 1 to 4 mg. per day from jelly; subject E, 6 mg. from agar in period 7 and 18 mg. in period 8; and subject D, 6 mg. from agar in period 8.

Since the greater proportion of the calcium is usually excreted in the feces and unsatisfactory separation of feces between periods is often a source of error, evaluation of calcium metabolism data by single periods is difficult. However, since retention values for most of the subjects for period 1 were markedly different from those for succeeding periods, it has been considered as an adjustment period and values for period 1 were omitted in all averages. An adjustment period of 4 days to a week is usually considered adequate if the intake is not radically different from the subject's usual intake (*102, 172, 87, 21*). Nicolaysen and coworkers (*134*) have found marked differences among subjects in the length of time needed to adapt to intakes of half the original value.

The mean calcium intake, urinary and fecal excretion, and retention values with standard deviations for periods 2 to 4 and periods 5 to 8 are shown in table 12. Subjects in the low-fat group retained less calcium during the low-fat periods 5 to 8 than during the control periods 2 to 4. However, retention values for subjects A and B in the control group were also less during periods 5 to 8 than during periods 2 to 4. Analysis of variance indicated that differences in retention between periods 2 to 4 and periods 5 to 8 for the 6 subjects were not significant, and that calcium retention for the 3 subjects in the low-fat group was not influenced significantly by lowering the fat content of the diet from 76 to 24 gm. for 20 days.

TABLE 11.—CALCIUM: *Average daily intake, excretion and retention values*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Group, subject, diet, and period	Intake	Excretion		Retention
		Urine	Feces	
CONTROL GROUP				
Subject A:	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Control diet:				
Period 1	736	169	667	−100
2	733	132	383	218
3	720	128	545	47
4	728	181	[1] 458	89
5	720	154	[1] 458	117
6	742	190	490	62
7	734	163	468	103
8	718	147	436	135
Subject B:				
Control diet:				
Period 1	736	179	454	103
2	733	158	531	44
3	719	151	604	−36
4	727	227	552	−52
5	728	191	506	31
6	741	229	625	−113
7	[2] 733	193	522	18
8	717	203	608	−94
Subject C:				
Control diet:				
Period 1	736	134	464	138
2	736	174	[1] 552	12
3	725	190	[1] 552	−17
4	730	189	783	−242
5	731	102	608	−69
6	744	211	387	146
7	736	218	536	−18
8		(2)		
LOW-FAT GROUP				
Subject D:				
Control diet:				
Period 1	736	182	484	70
2	733	223	404	106
3	720	228	524	−32
4	726	244	483	1
Low-fat diet:				
Period 5	726	260	588	−122
6	725	284	516	−75
7	725	293	446	−14
8	731	261	594	−124
Subject E:				
Control diet:				
Period 1	736	176	596	−36
2	733	166	365	202
3	720	172	260	288
4	728	211	514	3
Low-fat diet:				
Period 5	726	235	[1] 357	134
6	725	300	[1] 357	68
7	731	215	243	273
8	743	200	645	−102
Subject F:				
Control diet:				
Period 1	736	189	681	−134
2	733	181	515	37
3	720	158	606	−44
4	726	159	506	63
Low-fat diet:				
Period 5	726	212	463	51
6	726	211	443	72
7	726	199	693	−166
8	726	204	479	43

[1] Average for 2 periods; separation between periods not satisfactory.
[2] Creatinine values indicated incomplete collection. (See p. 16).

TABLE 12.—CALCIUM: *Mean intake, excretion, and retention values for control and low-fat periods*

[Control diet, 76 gm fat; low-fat diet, 24 gm.]

Group, subject, and periods	Diet	Intake, mean	Excretion				Retention	
			Urine		Feces			
			Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
CONTROL GROUP		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Subject A:								
Periods 2–4	Control	727	147	30	462	81	118	89
5–8	do	731	164	19	463	22	104	31
Subject B:								
Periods 2–4	do	723	179	42	562	38	−15	51
5–8	do	740	204	18	565	60	−39	74
Subject C:								
Periods 2–4	do	731	184	9	629	133	−82	139
5–7	do	737	207	13	510	113	20	112
LOW-FAT GROUP								
Subject D:								
Periods 2–4	Control	727	232	11	470	61	25	72
5–8	Low-fat	727	275	17	536	70	−84	52
Subject E:								
Periods 2–4	Control	727	183	24	380	128	164	146
5–8	Low-fat	731	238	44	400	172	93	156
Subject F:								
Periods 2–4	Control	727	166	13	542	55	19	56
5–8	Low-fat	726	206	6	520	117	0	111

The mean urinary calcium values for the subjects in both groups were higher in periods 5 to 8 than in periods 2 to 4, but no consistent increase was shown in values for individual periods (see table 11). Analysis of variance indicated that the increase in urinary calcium values for the subjects in the low-fat group during the low-fat periods was not significant at the 5-percent level. The F value of 3.99, however, approached 4.21 necessary for such significance.

In 2 short-time studies on women (*62*, *114*) and 1 on men (*6*) changes in the level of fat were reported to have no effect on calcium retention. Basu and Nath (*10*) reported a short-time study on 4 men in which 4 of 5 fats tested favored absorption and retention of calcium and phosphorus. Steggerda and Mitchell (*172*) concluded from a long-time study on 13 men that the fat level used in the study had no influence on calcium metabolism. The source of calcium in all these studies was from foods. Levels of fat ranged from 5 to 105 gm. (lard) in the Mallon study (*114*) to as high as 200 gm. (butterfat and olive oil) in the study reported by Aub and associates (*6*). In these studies, the food energy (when reported) was kept constant primarily through substitution of sucrose for fat. That the type of carbohydrate in the diet might be an influencing factor in calcium retention was indicated by the study of Mills and associates (*125*), who reported that the substitution of 36 gm. lactose for an equivalent amount of sucrose increased the percentage of calcium retained by children. In the present study the diet was kept isocaloric during the low-fat periods through the use of increased amounts of jelly and sugar.

The mean daily calcium retention values for the 6 subjects were as follows:

Subject:	*Daily total* [1]	*Per kilogram of body weight*	*Per centimeter of height*
	Milligrams	*Milligrams*	*Milligrams*
A	110	2.20	0.64
B	−28	−.49	−.18
C	−32	−.43	−.18
D	−37	−.78	−.22
E	123	2.18	.75
F	8	.13	.05
Mean	24	.47	.14

[1] Periods 2 to 7 for subject C, 2 to 8 for other 5 subjects.

These mean retention values were not related to the subjects' weight, height, age (19 to 23 years), or previous calcium intakes on their usual diets (obtained by dietary history). Leitch (*103*) from her review of available data concluded that there was no correlation between calcium requirement and body weight. Steggerda and Mitchell (*171*) disagreed with Leitch's conclusion, but stated that "other factors are so much more potent in causing variation in calcium metabolism as to completely obscure the effect of variable body size."

Mean urinary calcium values among our subjects ranged from 156 to 256 mg., and fecal values from 392 to 570 mg. Leichsenring and associates (*102*) reported mean urinary calcium values of 18 and 232 mg. for 2 of their subjects and a corresponding difference in calcium balance. In the present study subjects A and D (sisters), who showed the lowest and highest mean urinary calcium values, had corresponding retention values of 110 and −37 mg. In the other 4 subjects mean urinary calcium values ranged only from 189 to 214 mg. and differences in retention were due to variations in fecal values.

Retention values for these 6 subjects (−37 to 123 mg.) are in the general range reported by the Illinois group (*24, 169, 170*) and also those reported by Patton and Sutton in Ohio (*144*). Drake and associates (*49*) and Johnston and associates (*87*) found somewhat larger negative balances among their subjects receiving intakes over 800 mg. than those reported in the Illinois and Ohio studies on intakes ranging from 725 to 754 mg., or in the present experiment on an average intake of 725 mg.

PHOSPHORUS.—The phosphorus intake, urinary and fecal excretion, and retention values for each subject are shown in table 13. The mean daily phosphorus intake during the study was 942 mg. An average of 271 mg. was obtained from foods in the core, 283 mg. from complement I (255 mg. from rolls, 28 mg. from cookies and cobbler) and 388 mg. as dicalcium phosphate from complement II. In addition, the subjects received from 3 to 13 mg. from jelly.

The first period was apparently needed for adjustment. Retention values were different from those for succeeding periods for at least three of the subjects (A, B, and D). Values for period 1 were therefore omitted from all averages.

TABLE 13.—PHOSPHORUS: *Average daily intake, excretion, and retention values*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Group, subject, diet, and period	Intake	Excretion		Retention
		Urine	Feces	
CONTROL GROUP				
Subject A:	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Control diet:				
Period 1	945	514	423	8
2	953	482	248	223
3	946	494	360	92
4	953	526	[1] 309	118
5	949	560	[1] 309	80
6	938	554	301	83
7	931	526	317	86
8	928	618	287	23
Subject B:				
Control diet:				
Period 1	945	382	382	181
2	953	450	440	63
3	943	470	471	2
4	950	502	458	−9
5	946	522	429	−5
6	935	530	532	−127
7	928	459	406	33
8	925	491	479	−45
Subject C:				
Control diet:				
Period 1	945	536	292	117
2	963	638	[1] 408	−83
3	936	628	[1] 408	−80
4	957	557	626	−226
5	953	472	490	−9
6	942	504	297	141
7	935	454	421	60
8		[2]		
LOW-FAT GROUP				
Subject D:				
Control diet:				
Period 1	948	446	317	183
2	948	540	381	107
3	948	594	381	−27
4	948	578	350	20
Low-fat diet:				
Period 5	954	640	420	−106
6	952	557	360	35
7	952	532	322	98
8	952	598	470	−116
Subject E:				
Control diet:				
Period 1	948	526	419	3
2	948	508	302	140
3	948	470	212	266
4	948	574	396	−22
Low-fat diet:				
Period 5	954	555	[1] 269	130
6	953	536	[1] 269	148
7	953	576	184	193
8	953	528	487	−62
Subject F:				
Control diet:				
Period 1	948	472	434	42
2	948	561	367	20
3	948	507	444	−3
4	948	479	394	75
Low-fat diet:				
Period 5	954	551	336	67
6	954	580	285	89
7	954	529	484	−59
8	954	633	286	35

[1] Average for 2 periods; separation between periods not satisfactory.
[2] Creatinine values indicated incomplete collection. (See p. 16).

The mean phosphorus intake, urinary and fecal excretion, and retention values with standard deviation for periods 2 to 4 and periods 5 to 8 are shown in table 14.

TABLE 14.—PHOSPHORUS: *Mean intake, excretion, and retention values for control and low-fat periods*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Group, subject, and periods	Diet	Intake, mean	Excretion				Retention	
			Urine		Feces			
			Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
CONTROL GROUP								
Subject A:		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Periods:								
2–4	Control	951	501	23	306	56	144	69
5–8	do	936	504	39	304	13	68	30
Subject B:								
Periods:								
2–4	do	949	474	26	456	10	19	39
5–8	do	924	508	21	462	56	−36	68
Subject C:								
Periods:								
2–4	do	959	608	44	481	126	−130	83
5–7	do	944	477	25	403	98	64	75
LOW-FAT GROUP								
Subject D:								
Periods:								
2–4	Control	948	571	29	344	40	33	68
5–8	Low-fat	953	552	47	393	65	−22	106
Subject E:								
Periods:								
2–4	Control	948	517	53	303	92	128	144
5–8	Low-fat	953	549	21	302	130	102	113
Subject F:								
Periods:								
2–4	Control	948	516	42	401	39	31	40
5–8	Low-fat	954	573	45	348	94	33	65

Analysis of variance indicated that differences in retention between periods 2 to 4 and periods 5 to 8 for the 6 subjects were not significant and that phosphorus retentions for the 3 subjects in the low-fat group were not influenced by lowering the fat content of the diet from 76 to 24 gm. for 20 days. Mean urinary phosphorus values for periods 5 to 8 were slightly higher for the 3 subjects in the low-fat group and for 2 of the subjects in the control group during periods 5 to 8 than during periods 2 to 4, but these differences were not significant and were not related to the change in the fat level of the diet.

The mean daily phosphorus retention values for the 6 subjects were as follows:

Subject:	*Daily total* [1]	*Per kilogram of body weight*	*Per centimeter of height*
	Milligrams	*Milligrams*	*Milligrams*
A	102	2.04	0.60
B	−12	−.20	−.08
C	−33	−.44	−.19
D	2	.04	.01
E	113	2.00	.69
F	31	.53	.18
Mean	34	.50	.20

[1] Periods 2 to 7 for subject C, 2 to 8 for other 5 subjects.

The mean retention of 34 mg. is higher than the mean of −27 mg. reported by Kunerth and Pittman (*99*) for 3 subjects receiving intakes, over a 45-day period, similar to those in the present study. However, their individual retention values of −27, −59, and +5 are comparable to the retention values of −12, −33, and +2 found for three of the subjects in the present study. An intake of 800 mg. was reported to be adequate for a group of subjects studied at Minnesota and Ohio, (*102*), but a later study carried out in the same laboratories (*145*) indicated that an intake of 800 mg. was marginal.

Variation in retention among the subjects of the present study of −33 to 113 mg. was not related to weight, height, or age (19 to 23 years). Mean urinary phosphorus values ranged from 493 to 577 mg. and fecal phosphorus from 303 to 459 mg. The 2 subjects excreting approximately 300 mg. through the feces retained about 100 mg., and the 2 showing fecal values of approximately 450 mg. lost small amounts of phosphorus. Urinary phosphorus values of about 540 mg. were found for 4 subjects, including those showing the highest and lowest retentions.

MAGNESIUM.—The magnesium intake, urinary and fecal excretion, and retention values for each subject are shown in table 15. The mean daily magnesium intake during the study was 182 mg. An average of 65 mg. was obtained from foods in the core and 117 mg. from complements I and II. The rolls contained 114 mg. (which included the 100 mg. magnesium incorporated as magnesium gluconate) and the cookies and cobbler contained 3 mg. The calculated intake, primarily from Sherman's table (*165, p. 682*) of mineral elements in foods, was about 90 mg. from foods in the core and 135 mg. from complements I and II, so that the actual intake was 43 mg. less than was expected. With the low intake of 182 mg. it is not surprising that 5 of the 6 subjects were in negative magnesium balance.

As with calcium and phosphorus, the subjects evidently needed period 1 for adjustment to the new intake, as retention values for at least three of the subjects, B, C, and D, were less negative than those for succeeding periods. Mean intake, excretion, and retention values with standard deviations for periods 2 to 4 and 5 to 8 are shown in table 16. The values during the low-fat periods were similar to those found during the control periods. That magnesium retentions for the 3 subjects in the low-fat group were not affected by the change in the fat level from 76 to 24 mg. was also indicated by analysis of variance.

TABLE 15.—MAGNESIUM: *Average daily intake, excretion, and retention values*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Group, subject, diet, and period	Intake	Excretion		Retention
		Urine	Feces	
CONTROL GROUP				
Subject A:	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Control diet:				
Period 1	187	86	150	−49
2	184	90	88	6
3	182	104	116	−38
4	178	100	[1] 94	−16
5	183	99	[1] 94	−10
6	186	102	94	−10
7	178	103	89	−14
8	168	108	82	−22
Subject B:				
Control diet:				
Period 1	187	46	115	26
2	184	70	157	−43
3	182	70	162	−56
4	178	73	149	−44
5	183	70	144	−31
6	186	78	184	−76
7	178	75	154	−51
8	168	64	158	−54
Subject C:				
Control diet:				
Period 1	187	60	97	30
2	184	72	[1] 139	−27
3	182	84	[1] 139	−41
4	178	75	178	−75
5	183	80	133	−30
6	186	78	86	22
7	178	72	121	−15
8		[2]		
LOW-FAT GROUP				
Subject D:				
Control diet:				
Period 1	187	68	105	14
2	184	100	97	−13
3	182	100	118	−36
4	178	106	103	−31
Low-fat diet:				
Period 5	183	110	131	−58
6	183	110	114	−41
7	183	104	97	−18
8	183	109	141	−67
Subject E:				
Control diet:				
Period 1	187	84	112	−9
2	184	108	67	9
3	182	104	51	27
4	178	115	90	−27
Low-fat diet:				
Period 5	183	117	[1] 62	4
6	183	103	[1] 62	18
7	183	109	47	27
8	183	111	129	−57
Subject F:				
Control diet:				
Period 1	187	100	104	−17
2	184	129	81	−26
3	182	119	101	−38
4	178	116	75	−13
Low-fat diet:				
Period 5	183	112	71	0
6	183	117	76	−10
7	183	111	124	−52
8	183	116	76	−9

[1] Average for 2 periods; separation between periods not satisfactory.
[2] Creatinine values indicated incomplete collection. (See p. 16).

TABLE 16.—MAGNESIUM: *Mean intake, excretion, and retention values for control and low-fat periods*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Group, subject, and periods	Diet	Intake, mean	Excretion				Retention	
			Urine		Feces			
			Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
CONTROL GROUP								
Subject A:		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Periods:								
2–4	Control	181	98	7	99	15	−16	22
5–8	do	179	103	4	90	6	−14	6
Subject B:								
Periods:								
2–4	do	181	73	3	156	7	−48	7
5–8	do	179	72	6	160	17	−53	18
Subject C:								
Periods:								
2–4	do	181	77	6	152	23	−48	25
5–7	do	182	77	4	113	24	−8	27
LOW-FAT GROUP								
Subject D:								
Periods:								
2–4	Control	181	102	3	106	11	−27	12
5–8	Low-fat	183	108	3	121	19	−46	22
Subject E:								
Periods:								
2–4	Control	181	109	6	69	20	3	27
5–8	Low-fat	183	110	6	75	37	−2	38
Subject F:								
Periods:								
2–4	Control	181	121	7	86	14	−26	13
5–8	Low-fat	183	114	3	87	25	−18	23

The mean daily magnesium retention values for the subjects were as follows:

Subject:	*Daily total* [1]	*Per kilogram of body weight*	*Per centimeter of height*
	Milligrams	*Milligrams*	*Milligrams*
A	−15	−0.30	−0.09
B	−51	−.56	−.03
C	−28	−.37	−.16
D	−38	−.80	−.23
E	0	0	0
F	−21	−.36	−.12
Mean	−26	−.45	−.10

[1] Periods 2 to 7 for subject C, 2 to 8 for other 5 subjects.

Again, as with calcium and phosphorus, the magnesium retention values were not related to the weight or the height of the subjects.

Leichsenring and associates (*101*) reported a mean daily magnesium retention of 11.6 ± 2.9 mg. for 9 women on an average intake of 261 mg. McCance and Widdowson (*118*) found small retentions of 3 to 16 mg. for 5 of 6 subjects on average intakes of 229 to 317 mg. The single negative retention (—26 mg.) was on an average intake of 243 mg. Tibbetts and Aub (*177*) reported that on intakes of 300 mg. medical students regularly showed magnesium retentions.

Leichsenring and associates (*101*) and Tibbetts and Aub (*177*) reported that about 40 percent of the magnesium was excreted in the

urine and 60 percent in the feces. In the present study subjects B and C excreted 30 to 40 percent of the total output through the urine, while subjects E and F excreted about 60 percent by this route.

Leichsenring and associates (*101*) reported a mean urinary magnesium value of 96.2 ± 3.2 mg. and a mean fecal value of 153.5 ± 3.8 mg. In the present study, on an intake about 80 mg. lower, the mean urinary value of 97 mg. (72 to 117 mg.) is similar to their figure, but the mean fecal value of 110 mg. (73 to 158 mg.) is considerably lower. The lowest and highest fecal excretion values were associated with the highest and lowest retention values, respectively.

CALCIUM-PHOSPHORUS-MAGNESIUM INTERRELATIONSHIP.—The mean calcium, phosphorus, and magnesium retention values were as follows:

Subject:	*Calcium* [1] *Milligrams*	*Phosphorus* [1] *Milligrams*	*Magnesium* [1] *Milligrams*
A	110	102	−15
B	−29	−12	−51
C	−32	−33	−23
D	−37	2	−38
E	123	113	0
F	8	31	−21

[1] Periods 2 to 7 for subject C, 2 to 8 for other 5 subjects.

Subject E had the highest retention of these three minerals, subject A the next highest, and subject F the third highest. For subjects B, C, and D the retentions were mostly negative, and their order of retentions of the minerals varied. Calcium and phosphorus retentions, in general, tended to fluctuate in the same direction from period to period but not in the same magnitude or proportion. For example, during period 2, subject A retained 218 mg. calcium and 223 mg. phosphorus (ratio 1:1) and in period 3 corresponding values were 47 and 92 mg. (ratio 1:2).

From a study carried out on subjects in Minnesota and Ohio (*102*) it was concluded that the amount of phosphorus in the diet appeared to be of considerable importance in determining the calcium utilization in adult subjects. Two levels of calcium (300 and 1,500 mg.) and two of phosphorus (900 and 1,400 mg.) were used. From a later study (*145*) in which three levels of calcium and of phosphorus were used, giving calcium-phosphorus ratios in the diet of 1:0.50 to 1:3.10, it was concluded that utilization of both minerals was more closely related to the levels of intake than to the ratios of calcium to phosphorus. In the present study the dietary calcium-phosphorus ratio was 1:1.3.

Variations in the retention of these three minerals among these subjects were highly significant and were not related to their age, weight, or height. There was also no relation between variation in mineral retention and variation in fecal lipids, either total or as fatty acids.

Thiamine Metabolism

The average daily intake of thiamine during the study was 789 μg. Of this amount, 156 μg. came from foods in the core, 141 μg. from foods in complement I (132 μg. from the rolls and 9 μg. from the cookies and cobbler), and 492 μg. from thiamine hydrochloride in complement II. An additional 3 to 9 μg. was obtained from jelly.

As shown in table 17 and figure 3, neither urinary nor fecal thiamine excretion appeared to be influenced by substituting approximately 110 gm. carbohydrate for 50 gm. butterfat during periods 5 to 8 for the 3 subjects in the low-fat group. Subject D in this group excreted less thiamine in the urine during low-fat periods 5 to 8 than during the control periods. This change was probably not related to the change in the diet, as excretion values for the other 2 subjects in this group were in the same range as during their control periods. Furthermore, subject C on the control diet throughout the study showed an even more marked drop in urinary thiamine in periods 5 to 7 than did subject D. Variations in fecal thiamine, free or combined, were not related to the change in carbohydrate-fat level.

Reports in the literature on the effect of the dietary level of fat and carbohydrate on the thiamine excretion are not in agreement. Cahill (*29*), for example, reported no change. Reinhold and coworkers (*151*) reported that urinary thiamine excretion was decreased in 5 of 6 subjects, but differences in free or total thiamine in the feces were not significant when the carbohydrate-fat ratio in the diet was increased. These studies were both carried out over short periods and so may well have been affected by adjustment of the subjects to the new levels of intake.

The daily urinary thiamine values for 5 subjects and 5-day averages for 6 subjects are given in figure 4 and table 18. With the exception of subject E, these subjects had apparently been accustomed to an intake higher than the 0.79 mg. in the standardized diet, as values for the preliminary day (106 to 194 μg.) were higher than those for succeeding days. Stabilization time on the 0.79-mg. intake apparently ranged from 0 days for subject E to at least 35 days for subjects C and D. Keys and associates (*95*) reported that 24-hour excretion values for thiamine became constant in less than a month in 2 groups of subjects receiving average intakes of 0.61 and 1.61 mg. In a later report from the Minnesota laboratory by Mickelsen and coworkers (*123*), they concluded from statistical analysis of their data that about 6 weeks were required for thiamine and pyramin urinary excretion values to come to equilibrium when the intake was increased from 1 to 2 mg.

The mean urinary thiamine values for the subjects in periods 7 and 8 (at which time all subjects appeared stabilized) ranged from 38 to 89 μg., with a group average of 67 μg. Data from Keys' group (*93*) showed a value of about 50 μg. for 4 subjects after a period of 6 weeks on an intake of 0.69 mg. (0.23 mg. per 1,000 calories), and was considered adequate. (Criteria of adequacy are reported on page 66, sect. IV.) As discussed by Mickelsen and associates (*122*), the wide differences in individual response to a given intake make it difficult to establish an arbitrary urinary excretion value as a criterion of adequacy. In the present study, after a period of 35 to 40 days, 2 subjects showed excretion values of approximately 90 μg.; 3 subjects, of approximately 60 μg.; and 1 subject, of only about 40 μg. The first 2 subjects showed the lowest and highest values on the preliminary day of the study. These variations in their response to the same level of intake cannot be explained on the basis of differences in weight (actual or ideal), surface area, or fecal thiamine excretions.

TABLE 17.—THIAMINE: *Average daily excretions on average intakes of 789 micrograms*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Group, subject, diet, and period	Excretion			
	Urine	Feces		
		Total	Free	Combined
CONTROL GROUP				
Subject A:	*Micrograms*	*Micrograms*	*Percent*	*Percent*
Control diet:				
Period 1	123	704	40	60
2	98	549	46	54
3	80	697	57	43
4	66	[1] 616	32	68
5	59	[1] 616	32	68
6	58	355	44	56
7	60	573	46	54
8	68	533	41	59
Subject B:				
Control diet:				
Period 1	89	345	22	78
2	76	548	24	76
3	62	497	19	81
4	69	453	17	83
5	67	490	15	85
6	64	521	28	72
7	63	308	15	85
8	58	597	15	85
Subject C:				
Control diet:				
Period 1	135	228	58	42
2	139	[1] 500	63	37
3	141	[1] 500	63	37
4	138	850	50	50
5	101	632	56	44
6	95	429	44	56
7	89	586	47	53
8		([2])		
LOW-FAT GROUP				
Subject D:				
Control diet:				
Period 1	113	425	50	50
2	108	344	59	41
3	90	523	47	53
4	90	575	44	56
Low-fat diet:				
Period 5	77	371	40	60
6	72	550	54	46
7	65	303	69	31
8	61	764	88	12
Subject E:				
Control diet:				
Period 1	91	650	39	61
2	98	343	48	52
3	95	192	60	40
4	91	385	57	43
Low-fat diet:				
Period 5	91	[1] 202	63	37
6	78	[1] 202	63	37
7	80	156	64	36
8	90	420	63	37
Subject F:				
Control diet:				
Period 1	63	610	89	11
2	46	614	46	54
3	46	650	47	53
4	42	571	54	46
Low-fat diet:				
Period 5	36	566	64	36
6	34	516	65	35
7	38	873	58	42
8	37	588	48	52

[1] Average for 2 periods; separation between periods not satisfactory.
[2] Creatinine values indicated incomplete collection. (See p. 10.)

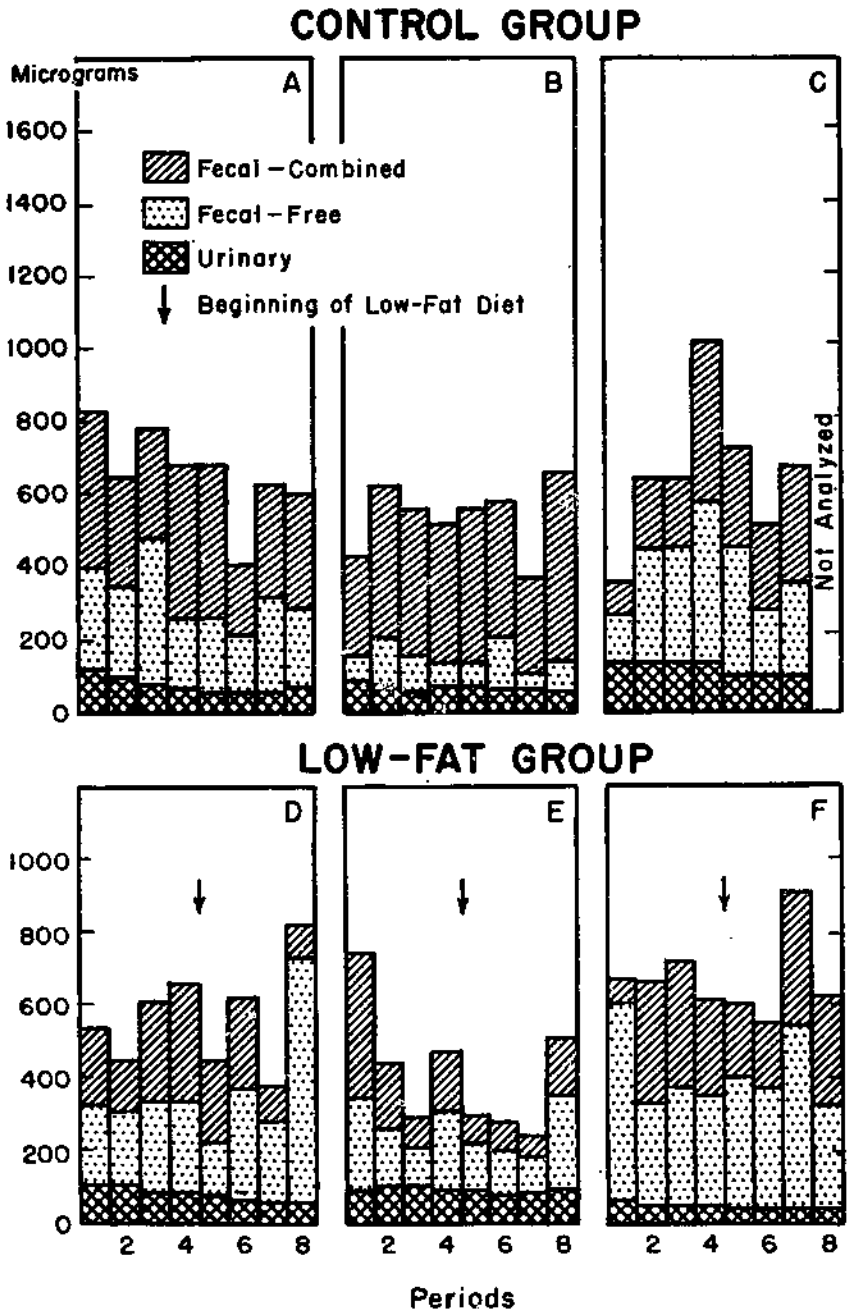

FIGURE 3.—THIAMINE: Average daily excretions by periods on average intakes of 789 micrograms.

TABLE 18.—THIAMINE: *Daily urinary excretions on average intakes of 789 micrograms*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Period and date	Control group			Low-fat group [1]		
	Subject A	Subject B	Subject C [2]	Subject D	Subject E	Subject F
	Micrograms	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*
Preliminary day: April 15, 1952	135	168	194	172	106	111
Period 1:						
April 16	157	99	----	134	92	87
17	129	78	----	108	83	66
18	128	83	----	113	96	61
19	106	91	----	100	93	55
20	96	94	----	105	93	48
Average	123	89	135	113	91	63
Period 2:						
April 21	116	94	----	131	109	44
22	94	94	----	95	98	46
23	88	59	----	96	95	46
24	91	60	----	111	91	44
25	99	63	----	108	96	48
Average	98	76	139	108	98	46
Period 3:						
April 26	87	(3)	----	88	87	46
27	75	(3)	----	92	99	49
28	77	60	----	96	100	44
29	79	59	----	92	92	42
30	84	66	----	84	90	47
Average	80	62	141	90	95	46
Period 4:						
May 1	70	72	----	93	93	42
2	(4)	(4)	----	94	86	45
3	65	60	----	86	102	41
4	67	70	----	84	96	41
5	61	75	----	95	78	42
Average	66	69	138	90	91	42
Period 5:						
May 6	50	70	----	86	98	40
7	52	78	----	74	91	31
8	60	71	----	70	100	35
9	57	68	----	86	91	31
10	75	48	----	68	77	44
Average	59	67	101	77	91	36
Period 6:						
May 11	62	59	----	68	68	37
12	54	61	----	66	69	32
13	57	60	----	76	80	34
14	60	72	----	71	87	31
15	57	66	----	81	86	37
Average	58	64	95	72	78	34
Period 7:						
May 16	62	61	----	68	81	35
17	59	61	----	70	74	35
18	59	64	----	62	101	53
19	62	59	----	56	81	33
20	60	72	----	68	91	35
Average	60	63	89	65	86	38
Period 8:						
May 21	75	61	----	69	95	41
22	65	58	----	61	99	38
23	63	53	----	57	87	36
24	70	64	----	65	79	34
Average	68	59	(4)	61	90	37

[1] Control diet, periods 1 to 4; low-fat diet, periods 5 to 8.
[2] Preliminary day April 17, only aliquots for each period were analyzed.
[3] Creatinine values indicated incomplete collection.
[4] Sample lost in storage before analysis.

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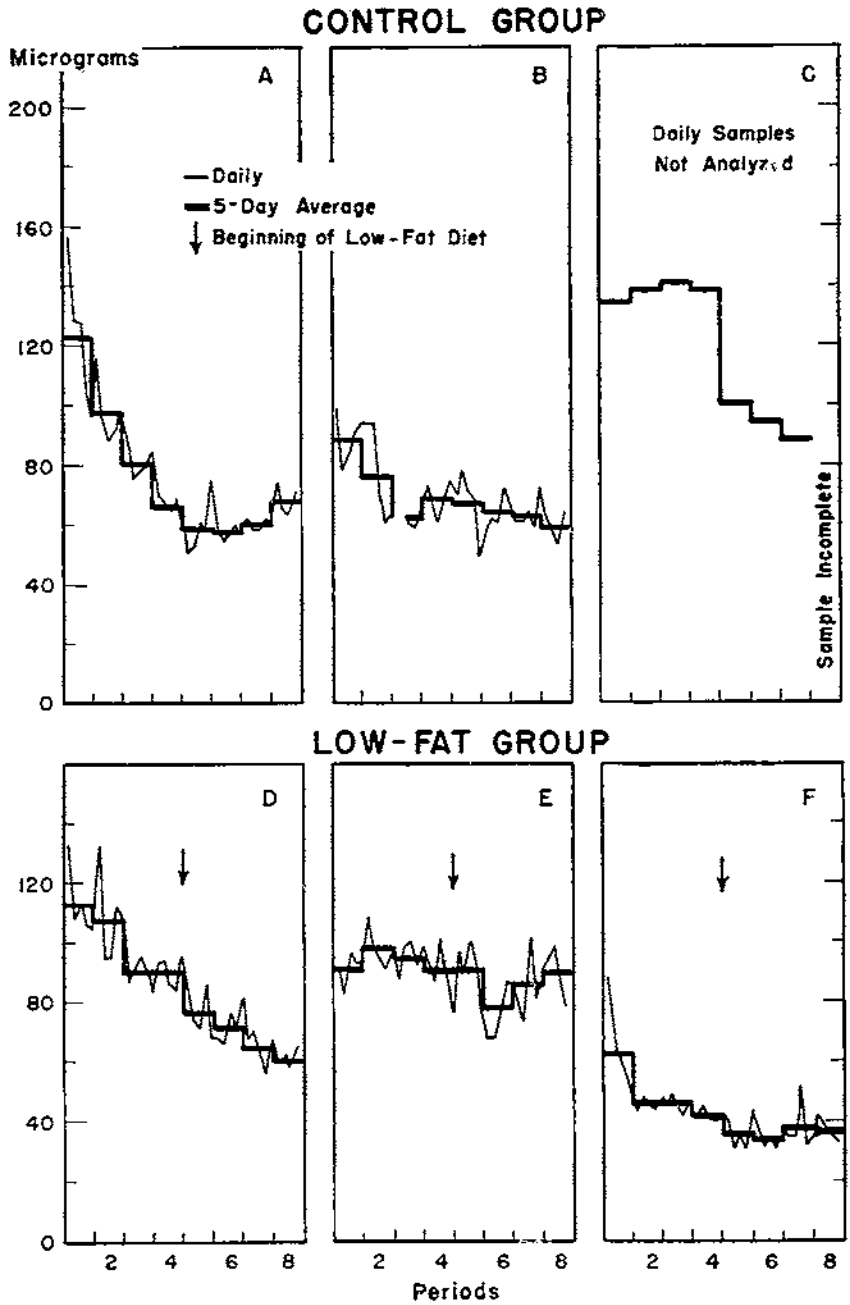


FIGURE 4.—THIAMINE: Daily urinary excretions on average intakes of 789 micrograms.

Mickelsen's group (*122*) also found no satisfactory explanation for the wide differences in excretion values among their subjects.

On the last day of the study a 1-hour fasting sample of urine was collected. Thiamine values for the 6 subjects ranged only from 1 to 3 μg. These values are lower than the values of 5 to 8 μg. reported by Oldham and associates (*136*), but the methods of analyses in these 2 studies were different. Coryell and associates (*36*) have shown that fasting values vary from day to day. Papageorge and Lewis (*143*) suggested that 4 μg. may be the critical 1-hour fasting value, based on the assumption that 24-hour values should exceed 100 μg. Holt (*78*) considered that a deficiency did not exist unless the fasting value was zero.

After the collection of the fasting urine sample, test doses of 1 mg. thiamine, 1 mg. riboflavin, and 400 mg. ascorbic acid were given with breakfast. Urine was collected separately for the first 4 hours and for the next 20 hours after the test doses.

The results of thiamine analyses of these samples are shown in table 19. The 4-hour excretion values cannot be compared directly with those in the literature because of differences in plans of the studies. Oldham's group (*136*), in reporting test dose returns, subtracted a 4-hour basal urinary excretion value. It seems of interest, however, that in the present study from 1 to 7 percent of the 1-mg. test dose (plus the 0.2 mg. in the breakfast) was excreted within 4 hours, while Oldham reported a range of 1 to 8 percent return in 5 subjects who have been receiving an intake of 0.74 mg. for a period of 45 days. Williams and coworkers (*189*) administered their 1-mg. test dose subcutaneously and concluded that a return of 50 μg. or less in 4 hours was indicative of severe deficiency.

The percentage of the test dose excreted during the 24 hours in general followed the percentage excreted during the last period of the study. Subject E, who excreted about 11 percent of the 0.79 mg. intake during period 8, excreted 16 percent of the test dose; subjects A, D, and F excreted 9, 8, and 5 percent, respectively, of the intake in period 8, and 7, 7, and 5 percent, respectively, of the test dose. Subject B, however, who excreted 8 percent of the dietary intake, excreted only 4 percent of the test dose. The 5 subjects excreted about twice as much thiamine in the 24 hours after the 1-mg. test dose as during period 8 (table 19). This indicates that on the 0.79 mg. intake the body stores were not depleted.

TABLE 19.—THIAMINE: *Urinary excretions in response to a 1-milligram oral test dose*

Subject	Excretion after test dose			4-day average excretion, period 8	Difference in excretion due to test dose	Proportion of test dose excreted
	4 hours	20 hours	24 hours			
	Micrograms	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*	*Percent*
A	34	101	135	68	67	7
B	16	84	100	59	41	4
C	38	[1]				
D	34	94	128	61	67	7
E	80	173	253	90	163	16
F	18	70	88	37	51	5

[1] Sample was incomplete.

Riboflavin Metabolism

The average riboflavin intake during the study was 972 μg. An average of 292 μg. was obtained from foods in the core, 167 μg. from foods in complement I (158 μg. from the rolls and 9 μg. from the cookies and cobbler), and 513 μg. as purified riboflavin from complement II. Jelly contributed an additional 2 to 5 μg.

The average daily urine and fecal riboflavin values for each 5-day period are shown in table 20 and figure 5. No apparent effect on urinary riboflavin was found when approximately 110 gm. carbohydrate (largely sucrose) were substituted for 50 gm. butterfat for the 3 subjects in the low-fat group during periods 5 to 8. Fluctuations in urinary riboflavin excretion during the last 20 days for the subjects in the low-fat group were similar to those for subjects in the control group. Fecal riboflavin values for the two groups also showed no consistent change. It is conceivable that fecal riboflavin excretion might have been affected had a different carbohydrate been used to replace the fat calories. It has been demonstrated in rats that more riboflavin is synthesized on diets containing dextrin, cornstarch, and lactose than on diets in which sucrose is used as the source of carbohydrate (*115*).

Since riboflavin excretion values apparently were not influenced by the change in the diet, the values for all 6 subjects will be discussed together. The daily urinary riboflavin values for 5 subjects and 5-day averages for 6 subjects are given in table 21 and figure 6. Apparently the subjects were accustomed to more liberal intakes of riboflavin than the 0.97 mg. used in this study. The urinary riboflavin values for the preliminary day ranged from 396 to 970 μg. and decreased within the first period to average values of 171 to 407 μg. A plateau was evidently reached for 5 of the subjects in 15 days, since values in succeeding periods fluctuated both above and below those of period 3. The average values for subject C, whose trend was consistently downward, indicate that she required from 20 to 25 days for stabilization.

Because of fluctuations in urinary riboflavin excretion and differences among subjects, a standard stabilization period is difficult to define. Hathaway and Lobb (*71*) found that their subjects were stabilized at the end of 15 days, and Davis and coworkers (*44*) after 10 days. Keys and associates (*94*) reported that about 6 weeks were required to stabilize the urinary riboflavin excretion of his male subjects. Horwitt's group (*79*) concluded that 11 weeks were required for the stabilization of their male subjects on an intake of 0.85 mg., but they also reported that on a 0.55 mg. intake the average excretion of the group dropped within 7 days to a value which persisted for many months.

The mean excretion values for subjects A, D, and E were in the general range reported by other authors for subjects on similar intakes given for 40 days (*71, 94, 187*), but values for subjects B, C, and F were somewhat lower. Urinary riboflavin values were not related to the amounts of riboflavin excreted in the feces. Subjects A and D, who excreted similar amounts of riboflavin in the urine, had average values in the feces of 866 to 1,365 μg. and 183 to 558 μg., respectively (table 20).

TABLE 20.—RIBOFLAVIN: *Average daily excretions on average intakes of 972 micrograms*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.]

Group, subject, diet, and period	Excretion			
	Urine	Feces		
		Total	Free	Combined
	Micrograms	*Micrograms*	*Percent*	*Percent*
CONTROL GROUP				
Subject A:				
Control diet:				
Period 1	407	1,365	90	10
2	274	992	82	18
3	184	1,236	83	17
4	158	[1] 1,184	90	10
5	227	[1] 1,184	90	10
6	390	1,265	77	23
7	162	1,160	83	17
8	174	866	98	2
Subject B:				
Control diet:				
Period 1	210	668	86	14
2	164	829	85	15
3	113	711	82	18
4	130	728	80	20
5	120	655	89	11
6	129	806	91	9
7	100	680	83	17
8	79	769	89	11
Subject C: [2]				
LOW-FAT GROUP				
Subject D:				
Control diet:				
Period 1	287	302	89	11
2	229	183	77	23
3	154	363	75	25
4	145	429	83	17
Low-fat diet:				
Period 5	173	305	89	11
6	173	558	73	27
7	178	207	82	18
8	142	416	85	15
Subject E:				
Control diet:				
Period 1	361	648	86	14
2	219	351	82	18
3	136	137	88	12
4	183	337	65	35
Low-fat diet:				
Period 5	108	[1] 145	78	22
6	85	[1] 145	78	22
7	109	119	87	13
8	121	369	70	30
Subject F:				
Control diet:				
Period 1	171	912	77	23
2	109	866	77	23
3	102	909	70	30
4	86	871	64	36
Low-fat diet:				
Period 5	92	906	91	9
6	102	564	87	13
7	93	930	91	9
8	70	731	75	25

[1] Average for 2 periods; separation between periods not satisfactory.
[2] Values omitted because of unsatisfactory fecal analyses.

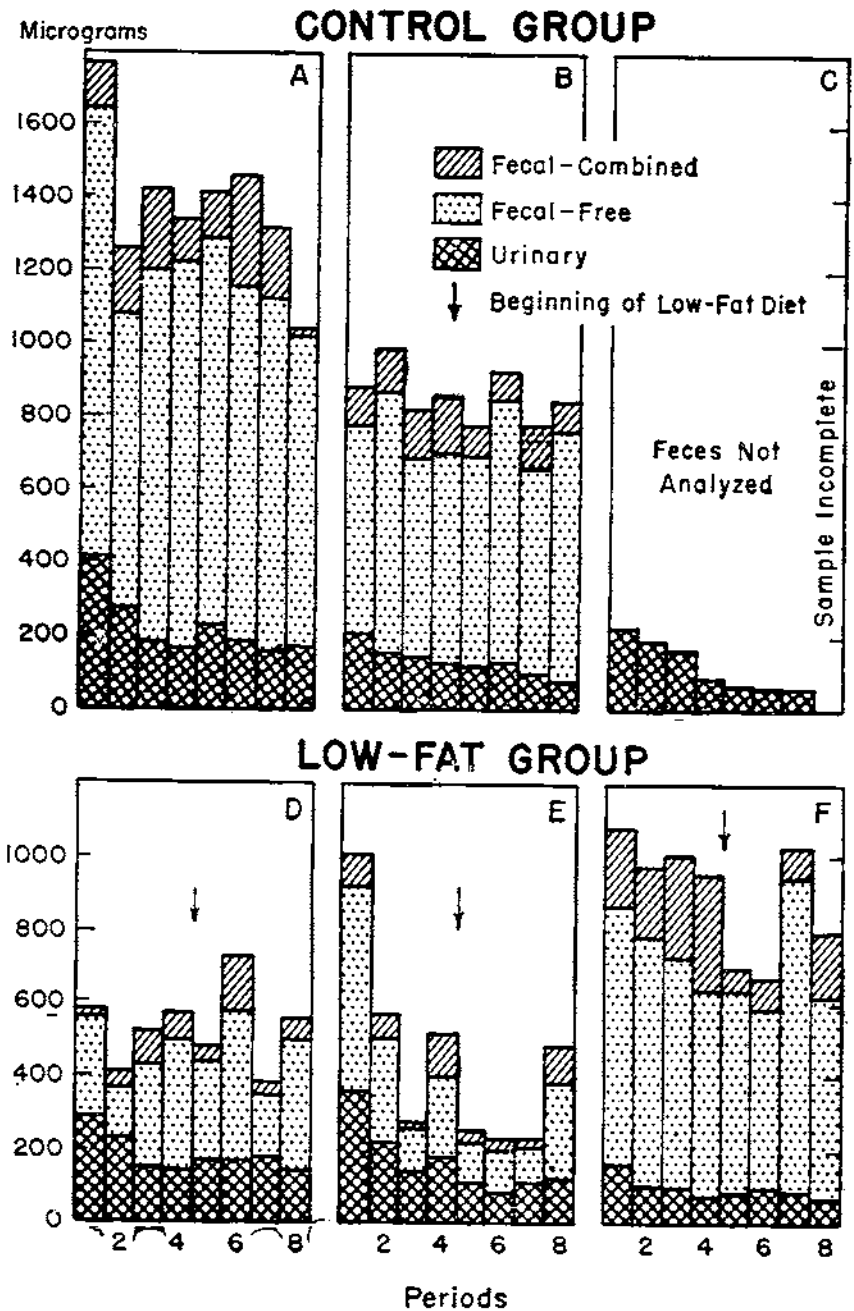


FIGURE 5.—RIBOFLAVIN: Average daily excretions by periods on average intakes of 972 micrograms.

TABLE 21.—RIBOFLAVIN: *Daily urinary excretions on average intakes of 972 micrograms*

[Control diet, 76 gm. fat; low-fat diet, 24 gm.

Period and date	Control group			Low-fat group [1]		
	Subject A	Subject B	Subject C [2]	Subject D	Subject E	Subject F
	Micrograms	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*
Preliminary day, April 15, 1952	706	396	474	970	406	414
Period 1:						
April 16	498	240		342	398	228
17	426	174		284	370	200
18	318	196		308	478	166
19	458	160		314	318	126
20	334	278		188	242	134
Average	407	210	226	287	361	171
Period 2:						
April 21	254	218		256	236	110
22	264	168		270	246	130
23	230	136		238	232	92
24	316	160		164	180	98
25	306	138		216	200	117
Average	274	164	186	229	219	109
Period 3:						
April 26	236	[3]		124	144	142
27	156	[3]		194	152	112
28	176	92		146	100	74
29	204	100		142	116	56
30	158	148		162	166	128
Average	184	113	172	154	136	102
Period 4:						
May 1	148	112		156	[4]	84
2	[4]	116		152	138	76
3	126	148		140	250	98
4	172	158		158	178	104
5	100	114		118	166	69
Average	158	130	90	145	183	86
Period 5:						
May 6	230	162		171	120	74
7	211	88		102	141	82
8	253	130		168	107	125
9	261	101		206	102	90
10	181	[4]		158	72	88
Average	227	120	60	173	108	92
Period 6:						
May 11	173	104		144	82	103
12	123	115		178	90	112
13	162	122		195	88	88
14	218	139		224	88	117
15	274	163		126	77	88
Average	190	129	65	173	85	102
Period 7:						
May 16	174	134		175	77	115
17	144	85		222	89	86
18	191	125		175	114	74
19	154	61		142	117	98
20	146	94		176	146	102
Average	162	100	62	178	109	93
Period 8:						
May 21	136	83		174	117	65
22	189	86		125	138	72
23	179	86		133	130	69
24	192	62		136	98	72
Average	174	79	[3]	142	121	70

[1] Control diet, periods 1 to 4; low-fat diet, periods 5 to 8
[2] Preliminary day April 17, only aliquots for each period were analyzed.
[3] Creatinine values indicated incomplete collection.
[4] Sample lost in storage before analysis.
[5] Sample not analyzed.

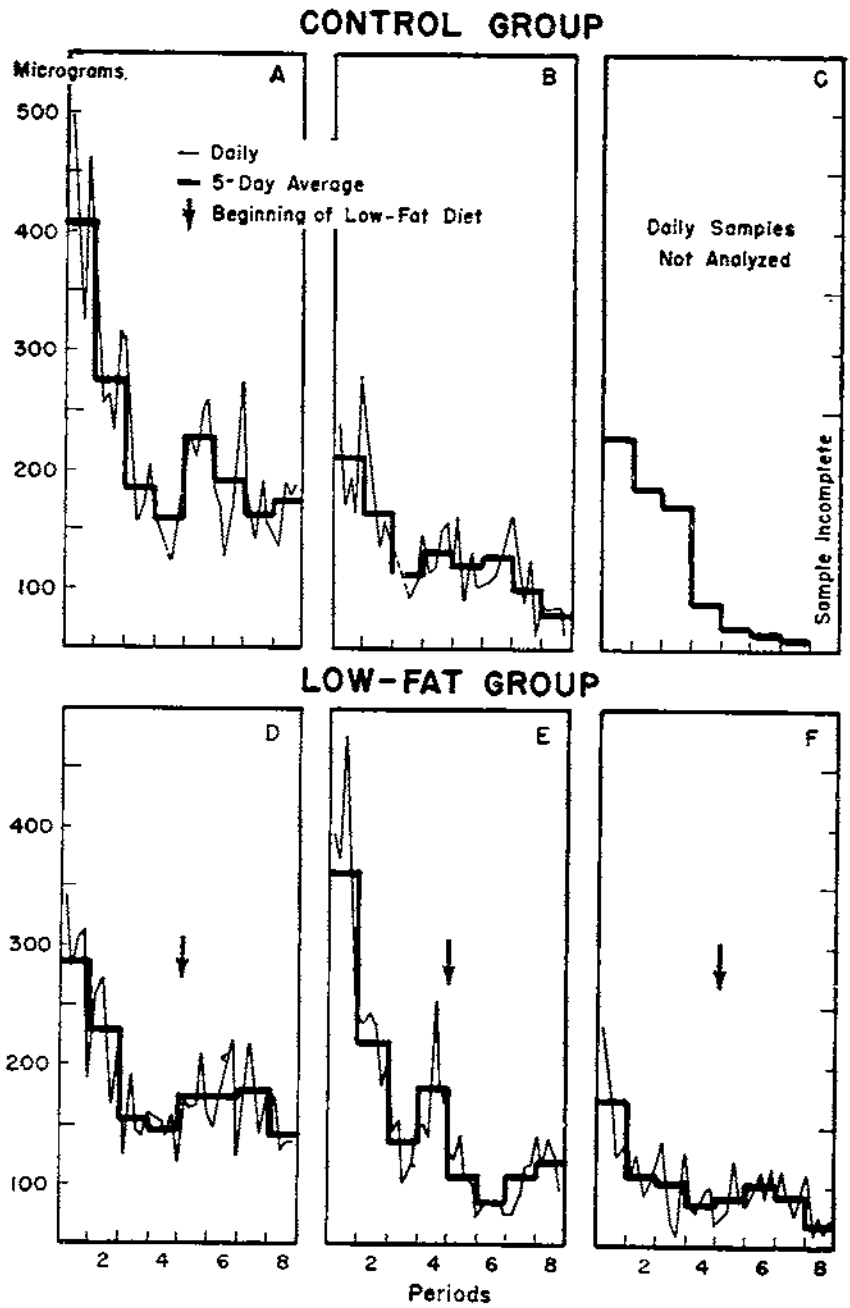


FIGURE 6.—RIBOFLAVIN: Daily urinary excretions on average intakes of 972 micrograms.

The 1-hour fasting urine sample collected on the last day of the study was analyzed for riboflavin content as well as for thiamine content. Riboflavin values of 5 to 11 μg. found for these subjects are similar to those of 6 to 8 μg. reported by Hathaway and Lobb (*71*) and 8 to 13 μg. reported by Davis and associates (*44*) on intakes of approximately 1 mg.

Results of the analyses of the 4-hour and 20-hour urine samples collected after the test dose of 1 mg. riboflavin are given in table 22. Subjects A, D, and E, who excreted over 100 μg. during period 8, excreted from 22 to 29 percent of the test dose in 24 hours, while subjects B and F, who excreted less than 100 μg. during period 8, excreted only 17 and 10 percent of the test dose.

Urinary riboflavin values after a test dose are difficult to compare with those in the literature because of differences in the size of the test dose and the methods of administration (*132*). It seems of interest, however, that the lowest 4-hour excretion value of 111 μg. for subject B is considerably higher than the average values of 81 and 57 μg. reported by Horwitt and associates (*79*) for 2 groups of male subjects given 1 mg. intravenously after maintenance for 13 weeks on an intake of 1.1 mg. of riboflavin. The average 24-hour test dose return of 16 percent in our subjects is also somewhat higher than the average of 9 percent reported by Keys and coworkers (*94*), but their subjects had been maintained on a diet containing about 1 mg. for 80 days at the time of the first test dose.

TABLE 22.—RIBOFLAVIN: *Urinary excretions in response to a 1-milligram oral test dose*

Subject	Excretion after test dose			4-day average excretion, period 8	Difference in excretion due to test dose	Proportion of test dose excreted
	4 hours	20 hours	24 hours			
	Micrograms	*Micrograms*	*Micrograms*	*Micrograms*	*Micrograms*	*Percent*
A	212	181	393	174	219	22
B	111	134	245	79	166	17
C	142	(1)				
D	244	186	430	142	288	29
E	221	125	346	121	225	22
F	125	48	173	69	104	10

[1] Sample was incomplete.

The fact that nearly twice as much riboflavin was excreted by all 5 subjects in the present study when a 1-mg. test dose was added to the 0.97 mg. of the diet indicates that on the 0.97-mg. intake body stores were not depleted.

Ascorbic Acid Metabolism

The average reduced ascorbic acid intake during the study was 57 mg. An average of 7 mg. was obtained from foods in the core and 50 mg. as crystalline ascorbic acid from complement II. Jelly in complement I contributed at most 0.3 mg.

The daily values for urinary reduced ascorbic acid for 5 of the subjects are shown in table 23 and figure 7. This table also shows the 5-day averages for the 6 subjects. The values for the subjects in the

TABLE 23.—REDUCED ASCORBIC ACID: *Daily urinary excretions on average intakes of 57 milligrams*

Period and date	Subject A	Subject B	Subject C[1]	Subject D	Subject E	Subject F
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Preliminary day, April 15, 1952	12	6	7	6	75	4
Period 1:						
April 16	15	9		13	38	6
17	20	12		12	27	8
18	16	12		16	24	5
19	18	13		13	23	6
20	14	14		10	20	4
Average	17	12	9	13	26	6
Period 2:						
April 21	11	17		12	15	8
22	12	16		10	12	10
23	13	13		8	12	7
24	15	15		11	14	10
25	11	16		12	9	8
Average	12	15	10	11	12	9
Period 3:						
April 26	14	(2)		8	15	8
27	13	(2)		11	10	11
28	11	14		11	12	10
29	10	15		11	10	10
30	12	15		10	11	10
Average	12	15	8	10	12	10
Period 4:						
May 1	10	14		12	10	8
2	14	13		13	12	10
3	12	14		20	11	9
4	10	16		16	11	9
5	10	15		12	10	9
Average	11	14	8	15	11	9
Period 5:						
May 6	10	14		11	9	10
7	12	16		11	10	10
8	11	15		9	9	10
9	12	16		12	9	10
10	12	12		13	8	8
Average	11	15	8	11	9	10
Period 6:						
May 11	13	14		22	8	8
12	10	14		10	8	8
13	12	13		16	6	10
14	13	14		16	9	10
15	14	13		13	8	10
Average	12	14	8	15	8	9
Period 7:						
May 16	11	13		21	6	9
17	14	14		15	9	10
18	12	14		16	8	10
19	11	14		12	9	10
20	12	13		11	8	9
Average	12	14	9	15	8	10
Period 8:						
May 21	11	12		12	8	9
22	13	13		16	10	10
23	13	14		14	9	10
24	14	12		15	8	10
Average	13	13	(2)	14	9	10

[1] Preliminary day, April 17; average values for each period. (See p. 16.)
[2] Creatinine values indicated incomplete collection.

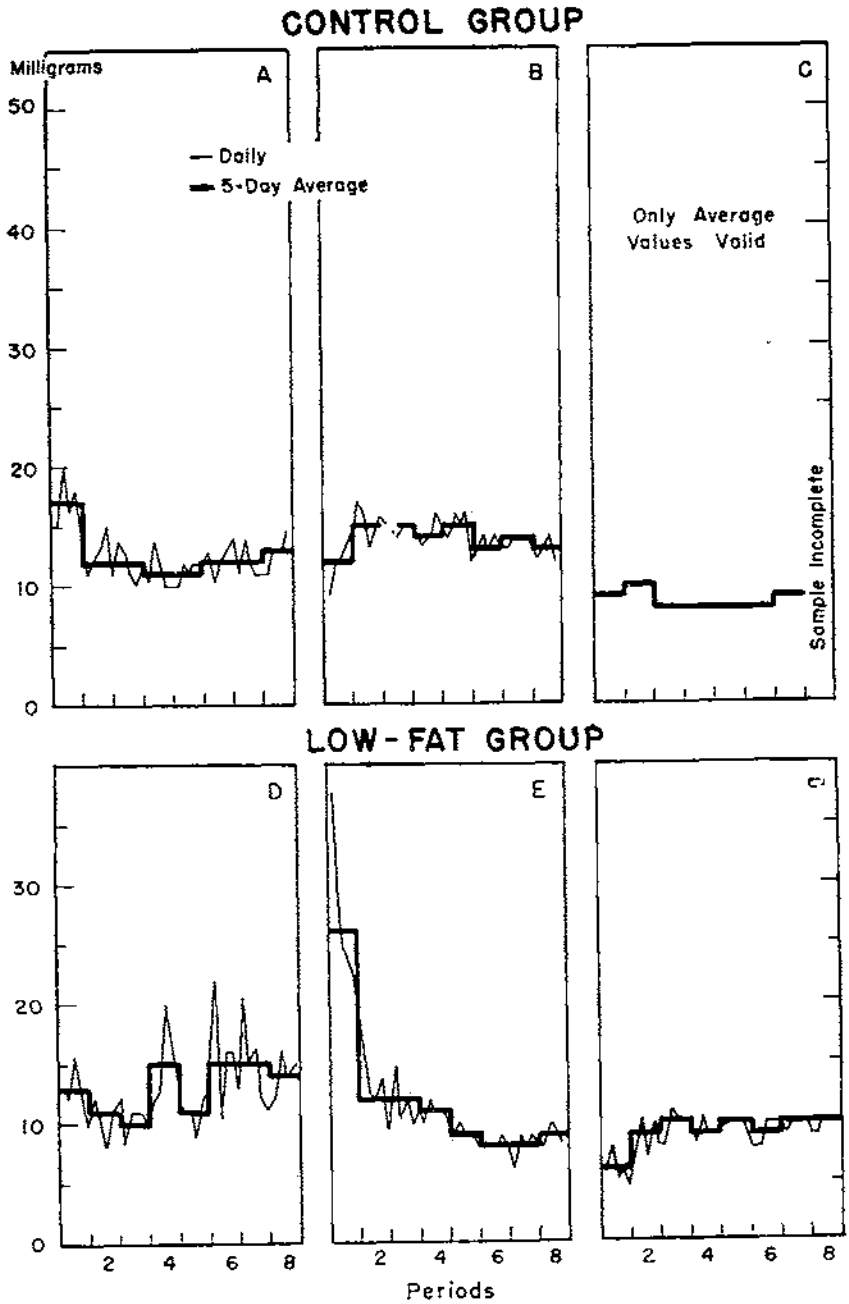


FIGURE 7.—REDUCED ASCORBIC ACID: Daily urinary excretions on average intakes of 57 milligrams.

2 groups are not separated for presentation, since, as was expected, no change occurred with the difference in carbohydrate-fat ratio in the diet. Values for the preliminary day ranged from 4 to 75 mg. At the end of 10 days, when excretion values appeared to be stabilized for all subjects except subject E, average values ranged from 9 to 15 mg. Subject E, who showed the highest initial value of 75 mg., apparently required from 20 to 25 days to stabilize at a level of about 9 mg. An apparent stabilization value of 12 mg. was reached within 10 days for this subject and was maintained for the next 2 periods, but after 20 days a new plateau of 8 to 9 mg. was reached and maintained for the rest of the study. After stabilization, daily fluctuations in ascorbic acid excretions in 4 of the 5 subjects were within 1 to 3 mg. Daily values for subject D varied as much as 12 mg.

Dodds, Price, and MacLeod (*48*), using a colorimetric method of analysis similar to the one used in the present study, reported an average reduced ascorbic acid excretion of 10.8 mg. on the 11th and 12th days for 24 subjects receiving an average intake of 57 mg. This is comparable to the 5-day averages of 8 to 15 mg. (mean 11 mg.) found in period 3 for our 6 subjects. Haines and coworkers (*69*), using the titration method, reported excretion values of 17 to 22 mg. during the sixth week of their study for 4 subjects receiving an intake of 53 mg. This range is higher than the 9 to 14 mg. found in the present study during the sixth week.

On the last day of our study a 400-mg. test dose of reduced ascorbic acid was given with the breakfast in addition to the 50 mg. given at this time as part of the diet. The excretion values during the next 24 hours for 5 of the subjects are given in table 24. Samples taken after the test dose for subject C were incomplete.

TABLE 24.—REDUCED ASCORBIC ACID: *Urinary excretions in response to a 400-milligram oral test dose*

Subject	Excretion 24 hours after test dose	4-day average excretion, period 8	Difference in excretion due to test dose	Proportion of test dose excreted
	Micrograms	*Micrograms*	*Micrograms*	*Percent*
A	161	13	148	37
B	27	13	14	4
C	(1)	(1)		
D	168	14	154	38
E	191	9	182	46
F	123	9	114	28

[1] Sample was incomplete.

Three of the subjects, A, D, and E, were near tissue saturation, if 50 percent excretion of the test dose is used as the criterion of saturation (*174*). Subject F excreted 28 percent and subject B only 3 percent. There is no apparent explanation for the low test dose return by subject B. Urinary excretion values during the study for this subject were consistently higher than those for subject E, who excreted 46 percent of the test dose. Only one study (*69*) has been found in which a test dose was administered after controlled intakes had been given for as long as 6 weeks. When the 4 subjects of that study were on the 53-mg. intake, they excreted only from 18 to 28 mg.

after the 400 mg. test dose, or only from 1 to 11 mg. more than their average excretions for the sixth week. This difference between the results of these two studies is greater than would be expected merely on the basis of variation in analytical method used or in subject response to a test dose.

Blood ascorbic acid values for our study were made on serum by the method of Bessey and associates (*15*) for total ascorbic acid. Davey, Wu, and Storvick (*43*) found that total ascorbic acid values were significantly higher than reduced ascorbic acid values in both serum and plasma, and that both were significantly higher in serum than in plasma.

Serum ascorbic acid values for our subjects are shown in table 25. Subject E, with the highest initial ascorbic acid excretion value (75 mg.), showed the highest initial serum ascorbic acid value, 1.4 mg. per 100 ml. Subject A, who had an initial serum value of 1.1 mg., excreted 12 mg. on the preliminary day. The other 4 subjects, with initial excretion values of 3 to 7 mg., showed serum values of approximately 0.5 mg. Values for 3 of the subjects with low initial values increased to over 1.0 mg. per 100 ml. by the end of the study, but in subject C the value increased to only 0.7 mg.

TABLE 25.—TOTAL ASCORBIC ACID: *Milligrams per 100 milliliters of serum on the the first day of each period*

Day of sampling	Subject A	Subject B	Subject C	Subject D	Subject E	Subject F
April 15–17	1.1	0.5	0.4	0.4	1.4	0.4
21	1.1	.5	.4	.6	1.1	.6
26	1.3	.7	.5	.8	1.2	.9
May 1	1.2	.7	.5	1.1	1.3	1.0
6	1.5	.9	.7	1.4	1.5	1.4
11	1.4	1.3	.9	1.3	1.4	1.4
16	1.4	1.1	.6	1.1	1.2	1.4
1	1.7	1.3	.6	1.3	1.4	1.3

Dodds and MacLeod (*47*) reported an average plasma reduced ascorbic acid value of 0.72 mg. per 100 ml., with a standard deviation of 0.21 mg. for the last 3 days of a 2-week study for 24 subjects receiving an intake of 57 mg. There appear to be only three studies in which ascorbic acid values in plasma or serum were followed for longer periods on intakes similar to the present study, and in two of them the intake was not rigidly controlled. Kyhos and coworkers (*100*) reported plasma reduced ascorbic acid values of 0.7 to 1.0 mg. per 100 ml. during the summer months for 45 subjects receiving a prison diet supplemented with 50 mg. ascorbic acid. Johnstone and associates (*89*) reported an average plasma reduced ascorbic acid value of 0.75 mg. per 100 ml., with a standard deviation of 0.30 mg. for 22 subjects maintained on an average intake of 62.5 mg. (found in the Canadian Air Force diet) for a period of 6 to 8 months. Haines and coworkers (*69*) reported plasma reduced ascorbic acid values of only about 0.5 mg. per 100 ml. during the sixth week of their study on 4 subjects receiving an intake of 53 mg. In the present study serum total ascorbic acid values at the beginning of the sixth week were 1.3 mg. per 100 ml., with a standard deviation of 0.19 mg. These variations in results cannot be explained entirely by differences in methods of analysis (*43*).

RÉSUMÉ OF THE NUTRITIVE VALUE OF AND THE METABOLIC RESPONSE TO THE STANDARDIZED DIET

The Nutritive Value of the Standardized Diet

The standardized diet used during the metabolic study is summarized in table 26. Values for food energy, fat, carbohydrate, and vitamin A in complements I and II are for the rolls, cookies (or cooky and cobbler), and table fat used during the control periods. (Jelly and sugar were added to adjust for individual food energy requirements and amounts were increased during the low-fat periods, see p. 15). The protein content of the diet was higher than planned because of variable nitrogen content of the gluten flour. The 14 gm. fat in the core includes 10 gm. hydrogenated fat which was used for cooking. Magnesium value for complement I includes the 100 mg. from magnesium gluconate, since it was incorporated in the rolls. Potassium values in complement II include the amount from baking powder as well as from potassium gluconate, and sodium values include the amounts from baking powder and sodium chloride used in cooking and baking. The iron results are not reported because of an error in the laboratory.

TABLE 26.—*Nutritive value of the standardized diet as used in control periods during the metabolic study*

Nutrient and unit	Core	Complement—		Total	Source of values [1]
		I	II		
GROUP A					
Food energy.....calories	550	1,370	.....	1,920	Calculation (*184*).
Protein.....grams	19	46	.....	65	Analysis.
Fat.....do	14	62	.....	76	Do.
Carbohydrate.....do	100	150	.....	250	Calculation (*184*).
Calcium.....milligrams	144	81	500	725	Analysis.
Phosphorus.....do	271	283	388	942	Do.
Vitamin A value.....International Units	1,000	1,500	1,500	4,000	Calculation (*184*).
Thiamine.....micrograms	156	141	492	789	Analysis.
Riboflavin.....do	292	167	513	972	Do.
Niacin.....milligrams	5	2	.....	7	Analysis (preliminary period).
Ascorbic acid (reduced).....do	7	.....	50	57	Analysis.
GROUP B					
Copper.....milligrams	0.6	0.2	.....	[2] 0.8	Estimation (*76, 165*).
Iodine.....do	0.090	0.015	.....	0.105	Estimation (*1*).
Magnesium.....do	65	[3] 117	.....	182	Analysis.
Manganese.....do	1.1	0.7	.....	1.8	Estimation (*76, 165*).
Potassium.....do	907	196	1,232	2,335	Estimation (*18*).
Sodium.....do	289	17	2,047	2,353	Do.
Zinc.....do	3.3	0.9	.....	4.2	Estimation (*66, 176, 178*).
GROUP C					
Chlorine.....milligrams	120	80	100	300	Estimation (*55*).
Cobalamin (B_{12}).....micrograms	1	.....	4	5	Estimation (*34, 108*).
Folic acid.....do	25	25	50	100	Analysis (preliminary period).
Pantothenic acid.....milligrams	2	1	1	4	Estimation (*91*).
Pyridoxine.....do	0.3	trace	0.5	0.8	Do.
Vitamin D.....International Units	.....	.....	400	400	

[1] Calculations based on U. S. Dept. Agr. Handbook 8 (*184*); and estimations based on data from sources as indicated.

[2] Analyses of several meal composites and rolls indicated that the total copper intake was about 6 mg. There was a small amount of copper in the distilled water used for cooking and making the composites but this did not account for the difference between estimated and analyzed values.

[3] Includes the 100 mg. magnesium from complement II (magnesium gluconate in the rolls).

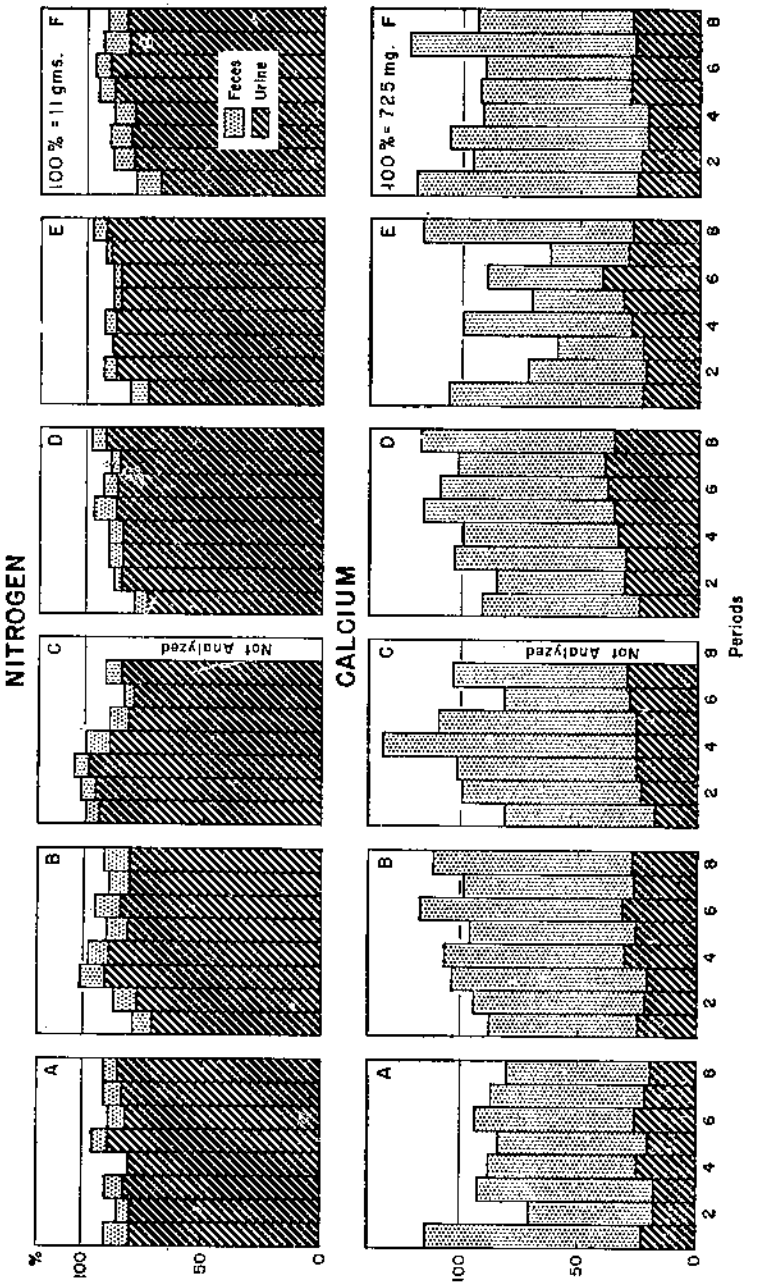
Metabolic Response

Table 27 presents a summary of the response of the six subjects to the levels of intake in the standardized diet after periods allowed for stabilization for each nutrient. Since a change in the fat level of the diet had no appreciable effect on the metabolism of nutrients studied, the values for the subjects in the control and low-fat groups have been combined to derive means. Mean retention values of 0.78 gm. nitrogen, 24 mg. calcium, 34 mg. phosphorus, and −26 mg. magnesium were found on intakes of 10.97 gm. nitrogen, 728 mg. calcium, 948 mg. phosphorus, and 182 mg. magnesium. Mean urinary excretion for thiamine was 67 μg., for riboflavin 116 μg., and for ascorbic acid 11 mg. on intakes of 795 μg. thiamine, 976 μg. riboflavin, and 57 mg. ascorbic acid.

As seen by comparison of individual values and confirmed by standard deviations of the means, wide variation was found in the response of the six subjects. Greater variation (in proportion to amount present) was usually found in excretion by way of the feces than by way of the urine. Greatest variation was found in retentions. The variation in individual responses was not related to height, weight (actual or ideal), age, or amount of urinary creatinine.

Total excretion values in terms of percent of the intake for nitrogen and minerals are shown in figure 8, and urinary excretion in percent of the intake for the three vitamins in figure 9. Subject E showed the highest retention of nitrogen, calcium, phosphorus, and magnesium and excreted the smallest amount of these elements through the feces. This subject also excreted the least thiamine and riboflavin through the feces (table 27), but her urinary thiamine and riboflavin values were not different from those of subjects who excreted large amounts through the feces. The apparent better absorption of nitrogen and minerals by subject E cannot be explained on the basis of slow intestinal motility, as the time required for carmine to appear was about the same for subjects D and E but subject D showed a small negative calcium retention. Aub and coworkers (*6*) reported that neither voluntary constipation nor diarrhea influenced fecal calcium excretion in two normal subjects.

Further work is obviously needed on factors that cause individual variation in response to identical intakes. Hormones, particularly parathormone, are recognized as influencing calcium metabolism. Estrone therapy has been found to depress calcium retention in normal girls at the age of puberty (*88*), but to restore positive calcium balances in women with postmenopausal osteoporosis (*149*). With improved methods suggested in the literature for the determination of hormone excretion in the urine, information can perhaps be obtained on calcium-hormone relationship as an explanation for individual variation in calcium metabolism and perhaps for metabolism of other nutrients.


NITROGEN
CALCIUM
%
100
50
0
A
B
C
D
E
F
Not Analyzed
100 % = 11 gms.
100 % = 725 mg.
Feces
Urine
2
4
6
8
Periods

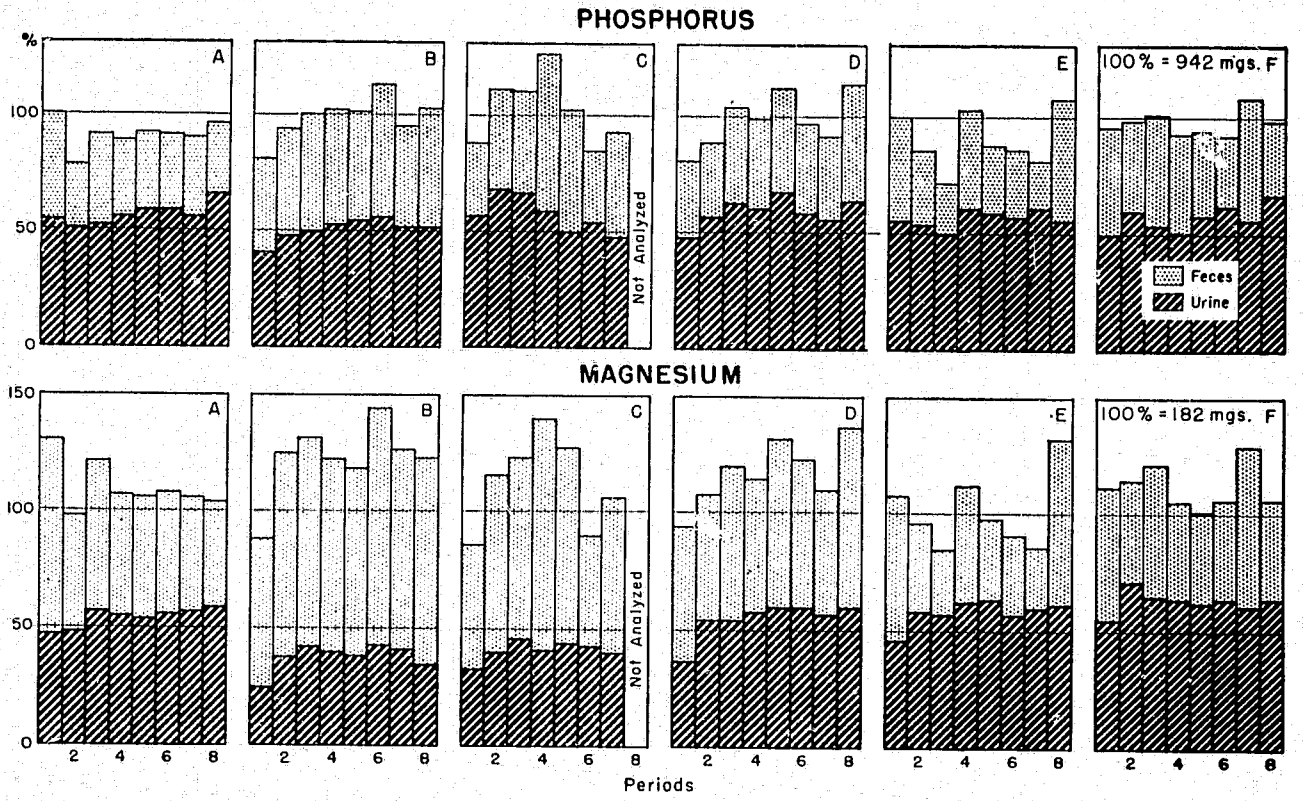


FIGURE 8.—SUMMARY: Excretions, as percentages of intakes, for nitrogen and minerals.

TABLE 27.—*Response (after stabilization) of subjects to the standardized diet*

Subject	Height	Weight		Age	Creatinine periods 1-8	Thiamine, periods 7-8				Riboflavin, periods 6-8				Ascorbic acid, periods 5-8		
		Actual	Ideal		Urinary excretion	Intake	Excretion			Intake	Excretion			Intake	Urinary excretion	Blood serum
							Urine	Feces			Urine	Feces				
								Total	Free			Total	Free			
	Centimeters	*Kilograms*	*Kilograms*	*Years*	*Grams*	*Micrograms*	*Micrograms*	*Micrograms*	*Percent*	*Micrograms*	*Micrograms*	*Micrograms*	*Percent*	*Milligrams*	*Milligrams*	*Milligrams per 100 milliliters*
A	170.8	60.1	62.1	19	1.10	792	64	553	44	974	175	1,097	85	57	12	1.5
B	157.7	59.1	54.4	22	1.04	789	61	452	15	972	103	752	88	57	14	1.2
C [1]	173.7	74.7	64.9	20	1.21	797	89	586	47	977	64	(2)	(2)	57	8	0.7
D	166.9	47.2	59.4	21	1.05	797	63	534	82	977	164	394	78	57	14	1.3
E	164.1	56.5	58.1	23	1.01	797	88	288	63	977	105	211	75	57	8	1.4
F	169.3	58.9	60.8	20	1.13	798	38	730	54	977	88	744	85	57	10	1.4
Mean					1.09	795	67	524	51	976	116	640	82	57	11	1.2
Standard deviation					0.07	4	19	147	22	2	44	345	5	0	3	0.3

Subject	Nitrogen, period 3-8				Calcium, periods 2-8				Phosphorus, periods 2-8				Magnesium, periods 2-8				Fat			
																	Periods 1-4		Periods 5-8	
	Intake	Excretion		Retention	Intake	Excretion		Retention	Intake	Excretion		Retention	Intake	Excretion		Retention	Intake	Fecal excretion	Intake	Fecal excretion
		Urine	Feces			Urine	Feces			Urine	Feces			Urine	Feces					
	Grams	*Grams*	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Grams*	*Grams*	*Grams*	*Grams*
A	10.82	9.32	0.81	0.69	729	156	463	110	943	537	304	102	180	101	194	−15	76	2.07	76	1.73
B	10.82	9.39	1.06	.37	728	193	564	−29	940	493	459	−12	180	72	158	−51	76	2.00	76	2.16
C [1]	10.82	9.26	.78	.78	734	196	570	−32	951	542	442	−33	182	77	133	−28	76	3.52	76	2.92
D	11.12	9.58	.69	.85	727	256	508	−37	951	577	372	2	182	106	114	−38	76	1.59	24	1.65
E	11.12	9.69	.39	1.04	729	214	392	123	951	535	303	113	182	110	73	0	76	1.53	24	1.43
F	11.12	9.24	.90	.98	726	189	529	8	951	549	371	31	182	117	86	−21	76	1.98	24	1.58
Mean	10.97	9.40	.77	.78	729	201	504	24	948	539	375	34	181	97	110	−26	76	2.11	-------	1.91
Standard deviation	0.16	0.18	0.23	0.24	3	33	68	74	5	27	66	61	1	18	32	18	0	0.72	-------	0.55

[1] Period 8 omitted in all averages; nitrogen values for periods 4 to 7 only. [2] Not analyzed.

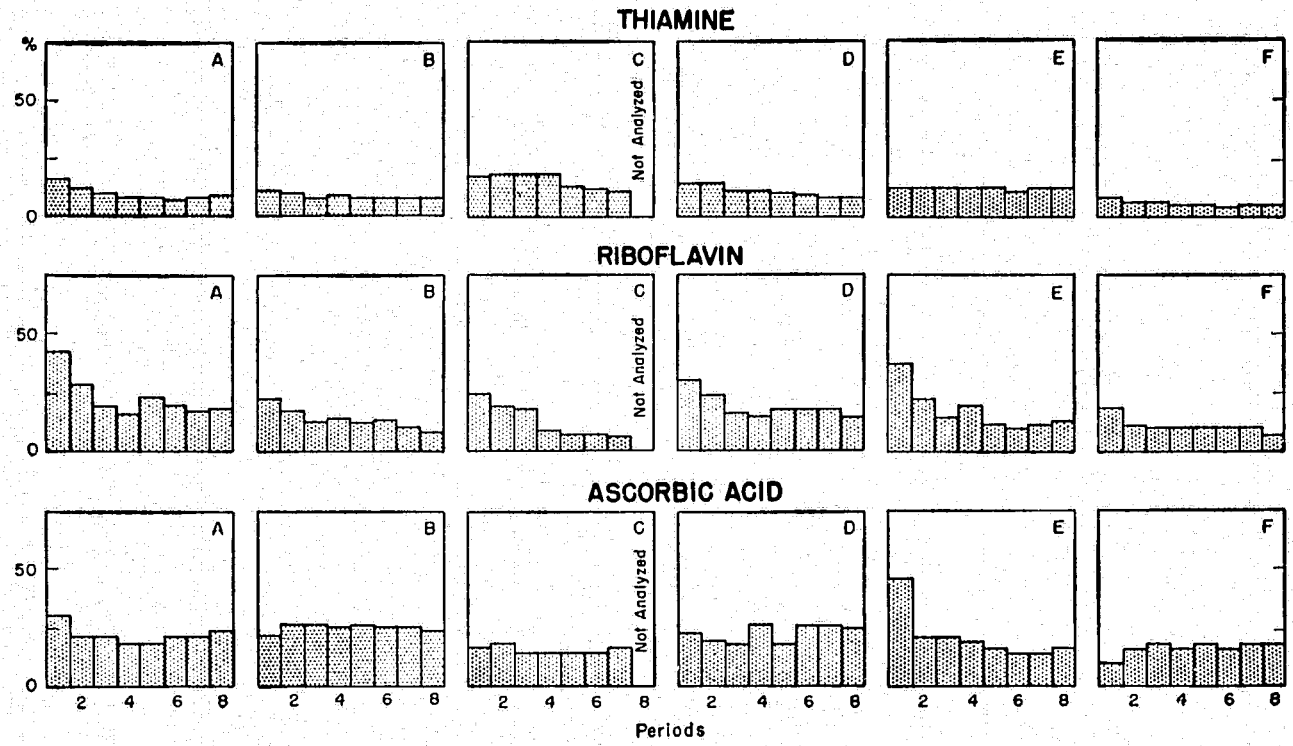


FIGURE 9.—SUMMARY: Urinary excretions, as percentages of intakes, for three vitamins.

Suggested Reference Levels for Future Studies

The 60 gm. protein, 700 mg. calcium, 1 gm. phosphorus, and 220 mg. magnesium, as planned for the standardized diet, should be satisfactory as reference levels at least for young women. As a group, the subjects retained small amounts of calcium and phosphorus and none of them showed consistently negative balances of these two minerals during the study. Although balances on 182 mg. magnesium were consistently negative for 4 of the 6 subjects, and generally for a fifth subject, it is conceivable that the mean negative balance of 26 mg. would be wiped out if the intakes of magnesium were increased by 40 mg. to the total of about 220 mg. as planned. An intake of 250 mg. might be tested with our diet, since 260 mg. gave positive balances on Leichsenring's diet (*101*), and the suggestion of 220 mg. had no strong basis (*52*).

Although urinary thiamine and riboflavin excretion values were low, excretion of these vitamins was about doubled after an oral test dose of 1 mg., an indication that the tissues were not depleted. Therefore, intakes of approximately 0.7 mg. thiamine and 1 mg. riboflavin appear satisfactory for reference levels.

Changes in the reference level are suggested for some of the other nutrients. In re-evaluating the vitamin A and D intakes of 4,000 and 400 I. U., respectively, they seemed high in relation to levels of other nutrients. Intakes of 3,000 I. U. and 300 I. U. of vitamin A and D, respectively, would seem sufficient to keep them from being limiting factors when other nutrients are being studied.

The reduced ascorbic acid intake of 60 mg. appears higher than desirable as a reference level for metabolism studies. Urinary excretions after a test dose showed that from 28 to 45 percent of a 400-mg. test dose was excreted by 4 of 5 subjects. Serum levels for the group averaged over 1 mg. per 100 ml. at the end of the study. Probably a reference level of 40 or 50 mg. ascorbic acid might be tested for this diet to see whether it will maintain a serum level of 0.4 to 0.6 mg. per 100 ml. and a white cell level of 14 mg. per 100 ml. or above (*113, 168*). This would be more in line with reference levels selected for other nutrients, and is still 10 mg. above the 30 mg. intake considered satisfactory by Canadian and British workers (*8, 147*).

SECTION III—PROCEDURES FOR THE METABOLIC STUDY

PREPARATION OF THE DIET

Most of the foods in the standardized diet (table 2) can be obtained from the open market. The special foods and the less common mineral salts used in this study were obtained from the following sources: [5]

Casein ("Labco" vitamin-free), The Borden Co., New York, N. Y.
Cereals (unenriched):
Cheerios and Farina, General Mills, Minneapolis, Minn.;
Rice Krispies, The Kellogg Co., Battle Creek, Mich.
Potato (French's Instant), R. T. French Co., Rochester, N. Y.
Rice (Minute), General Foods Corp., New York, N. Y.
Magnesium gluconate and potassium gluconate, Chas. Pfizer & Co., New York, N. Y.

[5] Mention of foods or manufacturers does not imply indorsement by the Department of Agriculture or discrimination against other products.

All foods, except lettuce, were obtained in single lots in amounts sufficient for the study.

Preparation of Foods Before the Study

BEEF.—All visible fat was removed from bottom round steak. After being ground and mixed for uniform sampling, the meat was packaged in freezer bags in amounts needed for each day and stored at −20° C. Since beef is the chief source of protein in the core, the nitrogen content was determined previous to the experiment, so that, if necessary, serving size could be adjusted to the desired protein level.

CELERY.—The stalks were cleaned, cut into ½-inch pieces, and mixed for uniform sampling. Quart lots were blanched for 2 minutes, immediately cooled in ice water, packed in freezer jars, and stored at −20° C.

COOKIES, TOMATO PUREE, ROLLS.—These foods were prepared in advance as described under Recipes (p. 58) in quantities needed for the entire study.

Preparation of Foods for the Meals

POTATO.—800 ml. distilled water containing 1 teaspoon salt (5 gm.) was heated to boiling. After being removed from the heat, 300 gm. dry potato was stirred in quickly and mixed until smooth. When cooled, 83-gm. portions, equivalent to approximately 25 gm. dry potato, were weighed, shaped into cakes, placed in casseroles with 10 gm. melted hydrogenated fat and the raw beef (or fish), and heated in an oven at 375° F. for 20 minutes. Meat and potato were turned after 10 minutes.

BEEF AND FISH.—After being thawed at room temperature, 45 gm. beef was shaped into cakes and placed in the casserole with the potato. The fish was cut in small pieces for uniform sampling and 50 gm. placed in casseroles.

CELERY.—60 gm. thawed, drained celery was weighed directly in pyrex cups, covered, and heated in an oven at 375° F. for 10 minutes.

GREEN BEANS.—Four 10-oz. packages of frozen beans were added to 480 ml. boiling distilled water containing 1 teaspoon salt (5 gm.). The blocks were separated with a fork to hasten thawing, and after the water was again brought to boiling, the pan was covered and the beans cooked for 5 minutes. After being drained and allowed to cool for 5 minutes, 100-gm. portions were weighed in pyrex cups. They were covered and heated in an oven at 375° F. for 10 minutes.

FARINA.—20-gm. portions of farina were cooked individually in glass casseroles containing 200 ml. boiling distilled water and 3 measures of salt (porcelain spoon used as measure, 3 measures=1 gram).

RICE.—25-gm. portions of rice, 2 measures of salt (approximately 0.7 gm.), and 65 ml. distilled water were heated to boiling in individual casseroles. They were then removed from the heat, covered, and allowed to stand 10 minutes. Thirty grams of tomato puree was then weighed directly into the casserole and mixed with the rice; the casserole was then covered and heated in the oven at 350° F. for 15 minutes.

SPAGHETTI.—150 ml. distilled water and 2 measures of salt (approximately 0.7 gm.) were heated to boiling in individual casseroles. Twenty-five gms. spaghetti was added and cooked at low heat until

just tender. After being removed from the heat, the water was poured off and when cool 30 gms. tomato puree was weighed directly into the casserole, mixed with the spaghetti, and the casserole covered and heated at 350° F. in the oven for 15 minutes.

PEACH COBBLER.—Individual portions of peach cobbler were prepared on the day of use (see recipe, p. 59).

TOMATO PUREE.—The day's supply of tomato puree was thawed at room temperature, then warmed to melt the fat particles; then it was mixed and individual portions were weighed.

ROLLS.—The rolls were removed from the freezer 15 minutes before mealtime and heated in an oven at 350° F. for 15 minutes.

COOKIES.—The frozen cookies were thawed at room temperature (reheating was not necessary).

GELATIN.—Weighed portions of gelatin were sprinkled over applesauce.

Recipes

BAKING POWDER. (Yield: Approximately 900 gm.)

Ingredients:	Grams
Potassium bitartrate	562.5
Sodium bicarbonate	250.0
Cornstarch	87.5

Mix in a ball mill for 1 hour for thorough mixing of ingredients.

COOKIES. (Yield: Approximately 3,000 gm. dough.)

Ingredients:	Control Grams	Low-fat Grams
Washed butter fat	595	------
Hydrogenated fat	------	185
Sugar	780	940
Cake flour	1,095	1,315
Baking powder (see recipe)	60	75
Salt	15	15
Water	470	580
Vanilla [1]	30	30

[1] Pure vanilla extract should be used if pyridoxine metabolism is to be studied (*159*).

1. Cream fat, salt, sugar, and vanilla in mixer at medium speed.
2. Sift cake flour and baking powder together three times.
3. Add sifted ingredients to creamed mixture alternately with water. Dough is of a soft, firm consistency.
4. Refrigerate dough.
5. Take out small lots of dough and weigh individual cookies (25 gm.) directly on squares of aluminum foil. Place on baking sheets. (The use of foil for baking eliminates sticking.)
6. Bake at 375° F. for 15 minutes.
7. Cool, wrap individual cookies in wax paper, and seal with Scotch tape. Place number of cookies needed per day in freezer bags, and store at −20° C.

FONDANT. (Yield: Approximately 480 grams.)

Ingredients:	Grams
Sugar	500
Water	240
Potassium bitartrate	0.6
Vanilla	3.2 (1 tsp.)

1. Combine sugar, water, and potassium bitartrate in saucepan and stir over low heat until sugar is dissolved.
2. Remove spoon and boil the mixture, covered, for about 3 minutes so that the steam may dissolve the crystals of sugar that collect on the sides of the pan.
3. Remove cover, attach thermometer, and continue boiling steadily. Wipe off any sugar crystals that may appear on the sides of the pan with a piece of wet cheesecloth. Remove pan from the heat as soon as the sirup has reached 238° F., take out the thermometer, and add vanilla.
4. Pour the sirup onto a cold platter. To avoid the introduction of sugar crystals do not scrape the last bit from the pan. Cool until the mixture feels only slightly warm to the touch.
5. Scrape the fondant from the edge of the platter toward the center with a wooden spoon or wide spatula. Work with the spoon until the mixture becomes white and creamy, then knead with the hands until smooth and free from lumps. Place in a tightly covered glass jar, and weigh out desired amounts as needed.

PEACH COBBLER. (Yield: Approximately 330 gm. dough for control cobbler; 300 gm. dough for low-fat cobbler.)

Ingredients:	*Control* *Grams*	*Low-fat* *Grams*
Washed butter fat	50	-------
Hydrogenated fat	------	20
Sugar	30	30
Cake flour	150	150
Baking powder (see recipe)	10	10
Salt	3	3
Distilled water	90	90
Peaches (from core).		

1. Cream fat and sugar.
2. Sift cake flour, baking powder, and salt together three times.
3. Add sifted ingredients to creamed mixture alternately with water. Dough should be light and soft but not sticky.
4. Weigh 100 gm. peaches in casserole; weigh 65 gm. dough directly into casserole, spreading it over the top of the peaches as it is weighed.
5. Bake at 425° F. for 35 minutes.

ROLLS. (Yield: Approximately 5,000 gm. dough for control rolls; 5,750 gm. dough for low-fat rolls.)

Ingredients:	*Control* *Grams*	*Low-fat* *Grams*
Cake flour	1,675	1,675
Gluten flour	1,420	1,420
Distilled water	1,800	1,800
Sugar	305	305
Casein	190	190
Fat (hydrogenated)	260	130
Magnesium gluconate [$Mg(C_6H_{11}O_7)_2 \cdot 2H_2O$]	42.6	42.6
Potassium gluconate ($KC_6H_{11}O_7$)	137.8	137.8
Ferric chloride ($FeCl_3 \cdot 6H_2O$)	.5566	.5566
Sodium chloride	70	70
Yeast	120	120

1. Sift the two flours together four times.
2. Measure out total water. (To keep yield and therefore composition constant from lot to lot, quantity of water for each batch must be kept the same.)

3. Blend sugar and casein.
4. Melt fat in saucepan after saving about 10 gm. for greasing bowl and dough.
5. Dissolve salts in about 800 ml. water.
6. Add sugar mixture and melted fat to dissolved salts. Mix well.
7. Dissolve yeast in about 150 ml. lukewarm water and add to above mixture.
8. Transfer mixture to a 30-quart mixing bowl (use rubber spatula for all transfers) and mix at low speed with paddle attachment (dough hook was not satisfactory for this type of dough).
9. Add part of the flour. Start mixing at low speed. Add the rest of the flour and water alternately. Total process takes 20 minutes, which amounts to approximately 15 minutes for mixing. Dough when finished will be smooth, satiny, and elastic, and will not stick to bowl.
10. Remove dough to large greased bowl. Form into smooth ball. Brush on all sides with fat. Cover and leave in refrigerator overnight.
11. Next morning remove bowl from refrigerator and set in pan of warm water for 2 minutes. Divide dough into small portions, punch down, and leave covered on bread board until dough comes to room temperature.
12. Weigh 50-gm. portions, shape into rolls, place on lightly greased pan, and put in proofing oven set at 28° to 30° C.
13. Let rise until increased 2 to 2½ times in volume (about 2 hours).
14. Bake at 400° F. for 12 minutes.
15. Cool, put quantity needed per meal in freezer bags, and store at −20° C.

TOMATO PUREE. (Yield: Approximately 7,000 gm.)

Ingredients:	Grams
Tomatoes (canned)	12, 250
Celery tops	500
Celery stalks	3, 000
Onions	1, 500
Fat (hydrogenated)	450
Cake flour	270
Salt	45
Sugar	70

1. Heat tomatoes, celery tops, celery stalks, and onions together slowly for about 40 minutes.
2. Strain.
3. Melt fat; blend in flour, salt, and sugar.
4. Add part of the strained juice, stirring until thick. Add remaining juice and bring to a boil.
5. Pour amounts needed per day in freezer jars and store at −20° C.

METHODS OF ADMINISTERING VITAMINS AND MINERAL SALTS

VITAMINS.—Thiamine, riboflavin, niacin, pyridoxine, and choline are reported to be stable in acid solutions (*155*). Pantothenic acid, folic acid, and cobalamin (vitamin B_{12}), however, are unstable at low pH ranges (*17, 152, 157*). A solution of calcium pantothenate and folic acid made up in sterile water and stored in the refrigerator was checked in our laboratories and found to be stable for at least 2 months. Facilities were not available to check the stability of cobalamin.

For the metabolic study a 0.02 N acetic acid solution was prepared, which contained 0.5 mg. each of thiamine, riboflavin, and pyridoxine, and 100 mg. choline chloride per 15 ml.; 5 ml. was given at each meal. A water solution was also prepared, which contained 1.09 mg. calcium pantothenate (equivalent to 1 mg. pantothenic acid), 50 μg. folic acid, and 4 μg. cobalamin per 3 ml.; 1 ml. was given at each meal.

The ascorbic acid (50 mg.) was weighed directly into No. 3 capsules and given with the breakfast.

The vitamin A concentrate as purchased contained 50,000 I. U. per gm. and the vitamin D concentrate contained 10,000 I. U. per gm. A mixture in cottonseed oil was prepared, 3 drops (90 mg.) of which contained 1,500 I. U. vitamin A and 400 I. U. vitamin D. This vitamin mixture was then measured into No. 5 capsules with a polyethylene dropper, designed for prescription use. The total vitamin A and carotene intake was estimated to be 4,000 I. U.—1,000 I. U. from foods in the core, 1,500 I. U. from the 50 gm. butterfat, and 1,500 I. U. from the vitamin A concentrate. In order to maintain a similar intake during the last 20 days, the vitamin A concentrate was increased to 3,000 I. U. for the 3 subjects receiving no butterfat. The capsule containing the vitamin A and D concentrates was given with the morning meal.

Mineral Salts.—Some of the minerals—iron, magnesium, and potassium—were incorporated in the bread. In the selection of mineral salts, both palatability and availability were considered. Blumberg and Arnold (*19*), who carried out a study on rats on the availability of iron in enriched bread, found both ferrous sulfate and ferric chloride available. Although ferrous salts may be better utilized by the human (*129*), both ferrous sulfate and ferrous chloride have a styptic taste, which is readily recognized when either is incorporated in the bread. Ferric chloride has no effect on the flavor and was therefore used as the iron supplement. Potassium in the form of gluconate is better tolerated than the chloride (*18*). Magnesium gluconate was selected, because it is easily dissolved in water and affects neither the flavor nor the texture of the baked product. These salts were added in such amounts that the intake of rolls per day contained 5 mg. iron, 0.10 gm. magnesium, and 1.0 gm. potassium.

Calcium diphosphate was administered by capsule. The salt is not readily soluble in water and if incorporated in bread may not be evenly enough distributed for a calcium-balance study. The salt was accurately weighed for the 5-day period for each subject as suggested by Kempster and associates (*92*) and then transferred in equal amounts to 10 capsule , size 00. One capsule was given at breakfast and the other at lunch. Calcium and phosphorus intakes per day from the salt were 0.500 and 0.388 gm., respectively.

By use of these procedures for administering the vitamins and mineral salts, preparation was possible prior to the study.

COLLECTION AND PRESERVATION OF SAMPLES

It was planned to carry out analyses for reduced ascorbic acid, thiamine, riboflavin, minerals, and nitrogen on the samples collected during the metabolic study. Since the same preservative is not satisfactory for all these determinations, the following procedures were used:

FOODS.—For ascorbic acid analyses, samples of single foods at serving time were weighed directly into a Waring Blendor, an equal weight of 2-percent oxalic acid was added (*5*), the contents were well blended, and analyses carried out the same day. At least two samples of each ascorbic acid-containing food were collected and analyzed during the study. Composites of core foods from the breakfast, lunch, and dinner were prepared on 25 days during the study for thiamine, riboflavin, nitrogen, and mineral analyses. These composites were prepared by a procedure essentially as described by Horwitt and associates (*81*), the composite being brought to weight rather than volume. Slurries of the 12 lots of rolls, 5 lots of cookies, and 6 of the 24 batches of cobbler dough were prepared with glacial acetic acid to give a final concentration of 1 percent. Samples were stored at —20° C.

URINE.—The subjects divided each urine sample into 2 equal parts, using graduated cylinders, and poured the samples into brown bottles, one of which contained 100 ml. 10-percent oxalic acid for ascorbic acid analyses, the other 20 ml. glacial acetic acid for all other analyses. These samples were stored in a refrigerator. At the end of each 24-hour period, the samples were brought to the laboratory, measured, and brought to a volume of 1 liter. Ascorbic acid analyses were carried out within 24 hours; samples preserved with glacial acetic acid were stored at —20° C.

FECES.—Samples of feces were collected in 1-quart glass storage jars placed in the frame of an ordinary strainer, which was attached to the underside of a toilet seat. Samples were kept under refrigeration until taken to the laboratory, where each specimen was weighed, transferred to a freezer jar, preserved with 10 percent by weight of glacial acetic acid, and stored at —20° C. Carmine (approximately 300 mg. in a No. 00 capsule) was given before breakfast at the beginning of each period. The samples for each 5-day period were pooled, mixed in a Waring Blendor, and brought to a known weight by the addition of distilled water. A portion of this sample was taken for thiamine, riboflavin, and fat analyses, and an acid digest of the remainder prepared for other analyses.

BLOOD.—Fasting blood samples for ascorbic acid analyses were taken by the finger-tip technique preceding the diet and at the beginning of each 5-day period. Sera were separated, trichloracetic acid filtrates prepared immediately according to the procedure outlined by Bessey, Lowry, and Brock (*15*), and the filtrates stored at —20° C.

ANALYTICAL METHODS

FOODS.—Thiamine and riboflavin analyses of the foods were made on the wet samples. Other analyses were made on air-dried samples which were ground in a Wiley mill with a 20-mesh screen. An acid digest was prepared for the jellies, since they could not be dried and ground satisfactorily. Nitrogen was determined by the Kjeldahl-Gunning-Arnold method (*4*), using mercuric oxide as a catalyst and distilling the ammonia into boric acid (*116*); fat, by the direct ether extraction method (*4*); calcium, by the method of Ingols and Murray (*84*); magnesium, by the method of Orange and Rhein (*140*); and phosphorus, by an adaptation of the Fiske and Subbarow method

(*58*). The dry ashing procedure was used for calcium and magnesium and wet ash for phosphorus. The wet ash used for phosphorus was not satisfactory for magnesium analyses.

Chemical procedures were used for thiamine and riboflavin analyses and were essentially those described in Methods of Vitamin Assay (*5*). The sulfuric acid extracts used for both thiamine and riboflavin were incubated overnight with takadiastase at 38° C. For removal of fluorescent substances which interfere in the riboflavin analyses, an aliquot was treated with 2 ml. 4-percent potassium permanganate for 1 minute and excess potassium permanganate removed with a minimal amount of 3-percent hydrogen peroxide; however, adsorption and elution were omitted (*158*). The increment technique was used for flourometric readings and calculations. Reduced ascorbic acid was determined by the xylene extraction method essentially as described by György and Rubin (*68*). Analyses for reductones were carried out on the cooked peaches, potatoes, and jellies by the formaldehyde method (*68*).

URINE AND FECES.—Reduced ascorbic acid in urine was determined daily by the Bessey method (*14*). Creatinine was determined on both fractions of each daily urine sample by the Jaffé reaction as described by Clark and Thompson (*33*). Nitrogen, calcium, magnesium, and phosphorus were determined on 5-day urine aliquots and feces samples by the methods listed for foods.

Analyses of fecal lipids were carried out essentially as described by Kamer and associates (*90*). Total fatty acids and neutral fat were determined as described, except that the petroleum ether extract was washed several times with distilled water to remove the acetic acid used to preserve the samples for riboflavin and thiamine analyses. It was not feasible to try to adapt the method to the determination of soaps, as the addition of acetic acid to the samples had resulted in their hydrolysis. Unsaponifiable matter was determined by treating the ether-extract residue with alcohol and petroleum ether and drying the extract to a constant weight.

An aliquot of the fecal samples was extracted with 0.1 N sulfuric acid for analysis for free thiamine and riboflavin and a second aliquot extracted and incubated with takadiastase for determination of total thiamine and riboflavin. Daily urine samples and both fecal extracts were analyzed for thiamine by the method of Mickelsen, Condiff, and Keys (*124*). For the riboflavin analyses the permanganate treatment and extraction procedure suggested by Najjar (*131*) were used, together with the internal standard and series of short successive exposures to sunlight for calculation of the unknown sample as suggested by Slater and Morell (*166*). The extracted samples were placed directly under a G. E. sunlamp at a distance of 26 cm. and fluorometric readings taken initially and after 3, 5, and 15 minutes of exposure.

BLOOD.—Only the ascorbic acid content of the serum was determined. The method of Lowry, Lopez, and Bessey (*112*) as modified by Bessey, Lowry, and Brock (*15*) was used for these analyses.

A Farrand fluorophotometer was used for the fluorometric analyses and a Beckman spectrophotometer for all colorimetric analyses.

SECTION IV—BRIEF REVIEW OF LITERATURE ON HUMAN REQUIREMENTS OF ESSENTIAL NUTRIENTS FOR ADULTS

Recent studies on the requirements of human adults for essential nutrients were reviewed prior to planning the amounts to be included in the standardized diet. Studies published since plans for the diet were completed have also been examined and pertinent ones are included in this bulletin.

PROTEINS

Bricker and associates (*22*, *23*), using women as subjects, replaced isocaloric amounts of a basic diet very low in nitrogen with single foods or food combinations in studies of nitrogen balance. In their 1945 study, protein needs for nitrogen equilibrium varied from 34 to 59 gm., depending upon the protein source (39 gm. average from mixed foods). In their 1949 study, using a diet containing 70 percent of cereal protein, the protein needs were from 27 to 42 gm. (average 32 gm.). Hegsted and associates (*74*) considered from 25 to 30 gm. protein as sufficient to maintain nitrogen balance for women (the calculated value being based on a surface area of 1.66 sq. meters), while Ohlson and coworkers (*135*) considered 45 gm. as necessary (based on a mean weight of 60 kg.).

MINERALS

Calcium.—Intakes of 300 mg. or less of calcium per day have resulted in negative balances for subjects in the United States (*21*, *24*, *102*, *162*, *169*, *170*, *171*). However, instances of adaptation of the human body to low calcium regimes have been reported by various workers in other areas (*9*, *73*, *133*, *142*, *148*).

An intake of 550 mg. calcium per day was estimated as the maintenance requirement for women by Leitch (*103*) from an analysis of data from the literature prior to 1937, but she considered that some additional allowance "for health" should be made. She found no correlation between the requirement for calcium balance and body weight. Mitchell and Curzon (*127*) concluded from available data that only 30 percent of the dietary calcium is utilized, and considered an intake of 9.75 mg. per kilogram necessary for equilibrium. Steggerda and Mitchell (*171*) reported a mean requirement for equilibrium by 43 subjects, studied at the University of Illinois, of 653 mg. or 9.99 mg. per kilogram but with coefficients of variation of 22.5 and 23.1 percent, respectively. They stated that although they believed calcium requirement was related to size, other factors (not listed) were much more potent in causing variation in calcium metabolism. Reference to the original data on these 43 subjects (*24*, *141*, *170*, *171*) revealed that individual subjects had requirements for equilibrium ranging from 222 to 1,018 mg. per day.

Phosphorus.—Few studies have been reported on the phosphorus requirements of adults. Sherman (*164*) suggested a minimum requirement of 0.88 gm. per day for a 70 kg. individual. Leverton and Marsh (*106*) concluded from their balance studies on self-chosen diets that a value of 0.6 gm. or less resulted in negative balances, that 1.03 gm. was the minimum requirement for equilibrium, and that 1.43

gm. was an optimal allowance. No more recent publications on phosphorus requirements of normal adults have been located.

IRON.—Studies on iron metabolism have demonstrated that iron losses from the body are primarily through blood losses rather than via the gastrointestinal tract or kidneys (*117*). Frenchman and Johnston (*61*) concluded that for women the iron requirement is primarily to replace that lost in the menses. They calculated that iron retentions needed for this purpose varied from 0.08 to 2.6 mg. daily, but that a retention of 0.71 mg. would be sufficient for 62 percent of the women studied, and 1.21 mg. for 86 percent. Moore and Dubach (*128*) found in studies with radioactive iron, that with rare exceptions less than 10 percent of food iron was absorbed, that iron-deficient subjects did not absorb food iron more efficiently than normal ones, and that ascorbic acid enhanced the assimilation of food iron.

An iron intake of 3.5 mg. was found by Leverton (*104*) to be insufficient to maintain equilibrium for 3 women, but 6.5 mg. was sufficient. Considering the need to replace menstrual losses, Johnston and associates (*85*) found intakes of 7 mg. borderline as to needs for their 5 subjects, but 10.4 mg. was ample. Only these 2 levels were used.

COPPER.—Leverton and Binkley (*105*) studied the copper metabolism of 65 subjects on self-chosen diets over short periods of 7 days and of 4 subjects on a constant diet for 75 to 140 days. Analysis of their data indicated that as the intake of copper was increased, 50 to 98 percent of the added copper was retained. They suggested an allowance of 2.0 to 2.5 mg. per day. De (*45*) confirmed 2 mg. as a minimum requirement. Cartwright (*30*) in a review on copper metabolism in human adults reported that on intakes of less than 2 mg., negative balances developed, but that there is no record in the literature of true copper deficiency in adults.

IODINE.—Curtis and Fertman (*38*) in their review on iodine requirement concluded that the optimum intake was about 200 μg. per day. Studies reviewed in their report, however, indicated that intakes of 50 to 70 μg. were sufficient to maintain equilibrium.

MAGNESIUM.—Duckworth and Warnock (*52*) from a review of the literature suggested a magnesium value of 220 mg. for women and 250 mg. for men. McCance and Widdowson (*118*) found that 5 of 6 subjects whose intakes ranged from 229 to 317 mg. per day were in balance for a period of 14 days. Leichsenring and associates (*101*) found average retentions of 1 to 39 mg. among 9 women subjects on intakes of 258 to 294 mg. per day over a 6-week period, but no lower intakes were used. The differences in retention were not associated with differences in calcium intake, phosphorus intake, or a combination of the two.

MANGANESE.—Kehoe and associates (*91*) reported that the mean daily intake of manganese in one normal adult was 4.28 $\pm$ 3.38 mg. and that the output was practically equivalent to the intake over a period of 28 days. Excretion was almost entirely via the feces. Hodges and Peterson (*76*) calculated that a normal diet contains only about 2.5 mg. De (*45*) concluded from a balance study on Indian adults that the requirement was 2.7 mg.

POTASSIUM.—The usual potassium intake has been estimated at 2 to 4 gm. (*41*). Reimer and coworkers (*150*) in a study of potassium metabolism reported that a normal adult male did not attain balance on an intake of 0.7 gm. over a 12-day period.

SODIUM.—The average sodium chloride intake amounts to about 8 to 15 gm. per day (*41*). Butler and Talbot (*28*) suggested 6 gm. sodium chloride as a maintenance allowance. This is equivalent to 2.4 gm. sodium.

ZINC.—The zinc content of a normal diet has been estimated to be from 12 to 20 mg. (*130*). McCance and Widdowson (*119*) found that their subjects were in equilibrium on an average intake of about 5 mg. zinc and that higher intakes merely increased fecal excretion.

VITAMINS

VITAMIN A.—Vitamin A and its precursors present a complexity of problems. Differences in availability of vitamin A in various carriers (*7, 107*) and of carotenoids from different foods (*20, 82*) and differences in vitamin A storage among subjects at the beginning of the experiment (*82*) are examples of these problems. Hume and Krebs (*82*) carried out the most comprehensive study to date on the human requirement of vitamin A and presented a good review of the literature with their report. Using blood values of vitamin A and carotene and dark adaptation as criteria, they found that 1,300 I. U. preformed A or 1,500 I. U. β-carotene daily was a minimum protective dose, and suggested 2,500 I. U. preformed A and 3,000 I. U. β-carotene as an estimate of the requirement to cover individual variations and leave a margin of safety. If carotenoids were from carrots, 12,000 I. U. were needed, if from spinach or cabbage, 7,500 I. U., or if β-carotene in fat, 4,000 I. U. If a single value is wanted for carotenoids in foods, they suggested three times the amount of preformed vitamin A, or 7,500 I. U. An intake of 5,000 I. U., with approximately two-thirds from carotenoids, has been recommended as a dietary goal by the National Research Council, the British Medical Council, and the Canadian Council on Nutrition (*147*).

THIAMINE.—Recommended levels of intake for thiamine are usually made in terms of milligrams per 1,000 calories, although the evidence for such a relationship is not satisfactory (*93*). For this review, intakes of thiamine per 1,000 calories were converted to total intakes per day (using the average caloric intakes reported in the studies), so that values could be compared on the basis of total intake.

Different criteria have been used by various research workers for the evaluation of thiamine requirements. An intake of around 0.20 to 0.30 mg. has been considered deficient regardless of criteria used (*40, 53, 81, 136, 189*). Holt (*78*), using absence of thiamine in 1-hour fasting urine samples as a criterion of deficiency, considered an intake of 0.47 mg. as adequate. However, intakes of 0.40 mg. were considered deficient by Horwitt and associates (*81*), 0.45 mg. by Williams and associates (*188*), and 0.53 mg. by Foltz, Barborka, and Ivy (*59*), when clinical observations were used as part of their evaluation. Oldham, Davis, and Roberts (*136*), using tissue saturation and blood levels as criteria, found that intakes up to 0.74 mg. were inadequate, while Keys and coworkers (*93*), using clinical tests, pyschomotor

tests, and blood pyruvate levels for evaluation, found that intakes of 0.69 mg. (0.23 mg. per 1,000 calories) were adequate.

An intake of 0.65 mg. thiamine was found by Elsom and associates (*53*) to be sufficient to prevent clinical symptoms of deficiency. Intakes of 0.63 mg. were considered adequate by Daum and associates (*40*) chiefly on the basis of "fitness" tests. Since only about 50 μg. were excreted on these intakes, they would be considered inadequate by the criterion of Williams and associates (*189*) that urinary thiamine excretions of less than 100±10 μg. indicate thiamine deficiency. Intakes of about 1 mg. were recommended by Williams and associates (*188*) and Foltz and associates (*59*) on the basis of subjective symptoms; by Melnick (*121*), Williams and associates (*189*), and Oldham and associates (*136*) chiefly on the basis of returns from test doses; and by Hathaway and Strom (*72*) on the basis of urinary excretions. In the recent report by Louhi and associates (*109*), intakes of 1 mg. thiamine per day for 14 to 31 days were considered adequate for their 8 subjects on the basis of 100 μg. urinary excretions, but borderline for 2 of them using 13 percent excretion of the intakes, or 150 μg. thiamine per gram creatinine as the criterion. For most of the subjects 0.6 mg. per day was inadequate by all 3 criteria.

Little information is available on blood thiamine values. Burch and associates (*27*) reported values of 3.4±0.11 μg. per 100 ml. of whole blood in frank and suspected beriberi, 3.7±0.12 in doubtful cases, and 4.0±0.18 in nonsymptomatic subjects in 1948; 4.2±0.13 in doubtful cases and 4.3±0.09 in nonsymptomatic subjects in 1950 in surveys in Bataan, Philippines. Values for Burch's own blood averaged about 6 μg. per 100 ml. Dubé and associates (*50*) reported ranges of 2.8 to 4.6 μg. on approximately 600 μg. intakes, and of 3.3 to 5.4 μg. on 1,000 μg. intakes. Differences between values on the 2 levels for individuals were significant for only 1 of the 3 groups of subjects studied. After 15 days on the 600 μg. intake, changes in values 1 hour after test doses of 5 mg. varied from −0.2 to +1.0 μg. per 100 ml. For the same subjects after 30 days on freely chosen diets, corresponding changes after the test doses varied from 0.3 to 1.0 μg. Lowry (*110*) in a review of earlier data based on other methods gave the following blood thiamine values as normal: 0.8 μg. per 100 ml. plasma, 8 μg. for red cells, and 70 μg. for white cells.

Riboflavin.—As with thiamine, recommended intakes of riboflavin are often given in terms of milligrams per 1,000 calories, although there seems to be no evidence to support such a relationship. For this discussion intakes have been converted to total milligrams per day on the basis of average caloric intakes reported for subjects of each study.

Values used for the deficiency or restricted phase of metabolic studies ranged from 0.013 to 0.35 mg. per 1,000 calories, or 0.05 to 0.70 mg. per day. The value of 0.35 mg. per 1,000 calories, or a total of approximately 0.7 mg., was considered deficient only because of decreased excretion (*187*). Levels of 0.5 to 0.6 mg. per day, administered over periods of 3 to 10 months, produced lesions considered due to riboflavin deficiency (*80, 163*).

Williams and associates (*187*) found that an intake of 1 mg. riboflavin was sufficient to maintain normal physiological and neurological reactions and that tissue stores were depleted only slightly. This same

level of intake was found by Davis and coworkers (*44*) by test dose returns and saturation tests, to be sufficient to supply minimum needs, but not for tissue saturation. Keys and associates reported that intakes of 0.29 mg. per 1,000 calories (*95*) and 0.31 mg. per 1,000 calories (*94*), equivalent to about 1 mg. for their subjects, appeared adequate on the basis of clinical examination, work performance tests, psychomotor tests, and urinary excretion values. Hathaway and Lobb (*71*) also found 1 mg. intake adequate on the basis of urinary excretion values. Horwitt and associates (*79*) considered that for riboflavin storage their subjects required between 1.1 and 1.6 mg., since the riboflavin excretions increased fourfold at the higher level. Intermediate levels were not studied. They reported that healing of lesions was retarded when excretions were less than 50 μg. per day. Their data showed that on intakes of 0.85 mg. per day excretions were maintained at 76±38 μg. for a period of over 2 years, and on intakes of 0.75 mg. excretions reached a plateau of 73±5μg. in 10 to 12 weeks. The length of time the subjects were maintained on these levels varied widely among these experiments, but none was shorter than 45 days.

Few data are available on blood riboflavin values. Burch, Bessey, and Lowry (*26*) reported normal serum values for free riboflavin—plus traces of flavin-mono-nucleotide (FMN)—of 0.3 to 1.3 μg. (average, 0.8) percent, flavin-adenine-dinucleotide (FAD) of 1.8 to 3.0 μg. (average, 2.4) percent and total riboflavin of 2.6 to 3.7 μg. (average, 3.2) percent; total riboflavin values for white cells and platelets of 227 to 293 μg. (average, 252) percent and for red cells of 18.0 to 26.2 μg. (average, 22.4) percent. Suvarnakich, Mann, and State (*175*), using the same methods, reported mean serum values of 0.84±0.71 μg. percent for free riboflavin+FMN, 2.32±0.42 μg. percent for FAD, and 3.16±0.87 μg. percent for total riboflavin for 141 well-nourished healthy subjects. Wu, Warren, and Storvick (*192*) reported serum values for 29 normal adults as follows: Free riboflavin+FMN, 0.10 to 3.87 μg. (average, 1.01) percent; FAD, 0.66 to 5.11 μg. (average, 1.89) percent, and total 1.58 to 5.67 μg. (average, 2.89) percent. Lowry (*110*) concluded that the concentration of free riboflavin is so low that it is difficult to measure with precision, that the total riboflavin in serum is probably of little value since it is largely FAD which appears to fluctuate to a considerable extent without relation to riboflavin intake (*167*), but that analysis of red cells may prove of real value since the concentration of flavin is higher in the red cells than in serum and is decreased in deficiency.

NIACIN.—Although the importance of niacin in the diet was recognized in 1937, early attempts to determine the human requirement for it were complicated until 1945 by lack of recognition of its important relationship to tryptophan (*98*). The recognition of the 6-pyridone of N′-methylnicotinamide as a metabolite of niacin and its isolation from urine (*96*) has further facilitated measurement of niacin needs.

In recent studies by Goldsmith and associates (*65*, *66*) symptoms of niacin deficiency were found in subjects receiving a corn diet containing approximately 200 mg. tryptophan and 4.7 mg. niacin for periods of 3 or 4 months. When wheat was substituted for corn, thereby increasing the tryptophan to 250 mg. and the niacin to 6 mg., no signs of deficiency were found in subjects after 3 months. These authors consider that 40 mg. tryptophan is equivalent to about 1 mg. niacin.

Tests in their laboratory (*156*) on the excretion of N′-methylnicotinamide and 6-pyridone of N′-methylnicotinamide resulted in much more marked increases in excretions of the pyridone than of the N′-methylnicotinamide itself on administration of test doses. On small test doses of 10 or 25 mg. the excretion of both metabolites was lower for pellagrins than for normal subjects. On 50-mg. test doses excretions of N′-methylnicotinamide were about the same for both groups, although differences were still found in pyridone excretions.

Ascorbic acid.—In most American studies on ascorbic acid needs, basic diets containing from 7 to 20 mg. have been used (*12*, *48*, *69*, *168*, *174*, *179*). A study by Crandon and coworkers (*37*) of experimental scurvy in which the diet was devoid of ascorbic acid is the exception.

In a study at Sheffield, England (*8*), a basic diet containing only 1 mg. ascorbic acid was used for a total of 17 months. Supplements of 10 mg., 50 mg., and 70 mg. for at least 6 weeks resulted in average fasting plasma values of less than 0.1 mg., 0.3 mg., and 0.6 mg. per 100 ml. and white cell values of 2.7 mg., 8.6 mg., and 16.6 mg. per 100 ml., respectively.

Dodds, Price, and MacLeod (*48*) illustrated that blood plasma values may show a carryover effect of previous intake, still evident at the end of 12 days. Data from Haines and associates (*69*) showed that starting from saturation levels, plasma values for their subjects on 70-mg. intakes reached a plateau of 0.5 to 0.9 mg. per 100 ml. by the third week, but on 33- to 53-mg. intakes the plasma values continued to drop slightly (roughly 0.1 mg. per week) through the fifth or even the sixth week to levels of 0.2 to 0.4 and 0.4 to 0.6 mg., respectively.

Lowry and coworkers (*111*) considered that the white cell content of ascorbic acid was a better measure of total body ascorbic acid than serum or plasma content. With an increase in serum concentrations from 0.5 to 2.2 mg. per 100 ml. the white cell concentrations increased from about 25 to 28 mg. to 34 mg. per 100 ml., but with serum values below 0.4 mg. the white cell values fell as low as 8 to 12 mg. Similar relationships have been reported from other laboratories (*8*, *168*, *190*).

Choline.—The human requirement of choline has been estimated from work on animals to be less than 500 mg. (*54*). No reports have been found in which the amount required by humans has been estimated directly. The intake on an average mixed diet ranges from about 250 to 600 mg. (*16*).

Vitamin B_{12} (Cobalamin).—Chow (*32*) estimated that the daily oral intake of B_{12} in what he considered an adequate diet ranged from 2 to 5 μg. Conley and associates (*35*) stated that no instance of recognized nutritional B_{12} deficiency had been encountered at Johns Hopkins hospital except in pernicious anemia. They found that in uncomplicated pernicious anemia 45 μg. B_{12} by injection every 6 weeks, equivalent to about 1 μg. per day, was satisfactory.

Folic Acid.—From comparative studies on monkeys, chickens, and turkeys, with caloric intake used as a basis for conversion, the human requirement for folic acid has been estimated as 0.1 to 0.2 mg. per day (*54*).

Pantothenic Acid.—Pantothenic acid is widely distributed in foods, and several studies have been made on urinary excretion of the vitamin (*146*, *161*). However, Krehl (*97*) commented that no clear-

cut evidence has been found to show that ill health in man is occasioned by lack of pantothenic acid in the diet. Sarett and associates (*160*) estimated the probable content of pantothenic acid in American diets as 3 to 18 mg. from analysis of restaurant foods and computation of values for possible meals consisting of various combinations of these foods. Elvehjem (*54*) suggested from work on animals that the human requirement cannot be above 5 mg.

PYRIDOXINE.—Experiments on animals indicate that the human requirement for pyridoxine may be about the same as that for thiamine; namely, about 1.5 mg. a day (*54*). Vilter and associates (*182*) recently demonstrated a series of clinical signs of pyridoxine deficiency in 34 of 50 subjects by feeding them the antimetabolite desoxypyridoxine. Symptoms of deficiency were found on intakes of 0.5 mg. of pyridoxine but not on 5 mg. Although they used no intermediate levels of intake, they estimated the requirement to be 2 to 3 mg.

PARA-AMINOBENZOIC ACID.—This compound may act indirectly by stimulating the production of unknown factors through intestinal synthesis, but there is no evidence that it must be supplied preformed in the human diet (*54*).

BIOTIN.—Oppel (*138*) showed that biotin excreted in urine of human subjects often exceeded the amount in the diet, and total excretion was often three to six times the intake. This work was confirmed by Gardner and associates (*63, 64*). In a later study Oppel (*139*) showed that avidin (in raw egg white) could effectively block urinary biotin excretion, but the large doses of sulfa drugs were required to inhibit intestinal synthesis. He also showed that biotin by rectum increased the urinary output, which indicates that biotin synthesized by intestinal flora is absorbed.

INOSITOL.—No definite symptoms of inositol deficiency have been produced in man (*185*).

VITAMIN D.—No reports have been found giving evidence of dietary needs for vitamin D in normal adults. McKay and coworkers (*120*) reported that the daily addition of 500 I. U. vitamin D as viosterol had no apparent effect on the retention of calcium. Nicolaysen, Eeg-Larsen, and Malm (*134*) considered it advisable to give adults at least 200 I. U. vitamin D per day. They based their recommendation on their finding that in older rats which had suffered substantial loss of calcium from the skeleton, vitamin D increased markedly the calcium absorption when they were put on a better diet.

VITAMIN E.—No evidence of dietary needs of vitamin E for normal adults has been found. Filer and coworkers (*57*) found that monkeys (*Macaca rhesus*) on vitamin E-deficient low-fat diets for over 2 years showed no symptoms of deficiency other than low plasma tocopherol (0.18 vs. 0.58 mg./100 ml. in controls) and slight changes in the R and T waves in electrocardiograms and pneumocardiograms.

VITAMIN K.—Statements that vitamin K is distributed widely in nature are included in reviews on the vitamins but quantitative data are scarce. According to Warner (*183*) intestinal synthesis apparently accounts for sufficient amounts to meet the needs of normal adults.

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