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GUINEA PIG
HAMSTER
MONKEY
MOUSE
RAT

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PREFACE

The previous publications of the Committee on Animal Nutrition have dealt, for the most part, with animals and poultry that are economically important in the production of meat, milk, eggs, hide, wool, and fur. The horse and dog are important as work and sporting animals and, in the case of the dog, as a household pet. Several of the species—dogs, poultry, rabbits, and swine—are also widely employed as laboratory animals.

This report deals with the nutrient requirements of six species that do not fall in any of the categories mentioned above. None of these species is commonly used, in the United States at least, for human food. Although some may occasionally be treated as pets, the predominant production and importation of these six species are for scientific experimentation, bioassay, and related uses.

We are indebted to many persons for reading and critically evaluating one or more of the chapters. Of particular assistance were the following: R. M. Forbes, A. E. Harper, B. C. Johnson, Q. R. Rogers, M. L. Scott, L. A. Witting, H. P. Morris, W. G. Hoag, P. L. Day, J. A. Gavan, L. D. Greenberg, L. E. Harris, D. M. Hegsted, G. R. Kerr, H. B. Lofland, L. H. Schmidt, G. van Wagenen, H. A. Waisman, B. L. O'Dell, F. Christensen, B. S. Schweigert, S. A. Miller, F. J. Judge, F. S. Doft, and J. D. Luckey.

NUTRIENT REQUIREMENTS OF THE CAT

Although there are more domestic cats than dogs in this country and the use of cats as experimental animals is extensive, until recently very little was known of their nutritional requirements. The main reason for this lack of knowledge probably is the reluctance of cats to accept experimental diets, particularly purified rations. In addition, controlling diseases in cat colonies and obtaining suitable and sufficient animals with which to work are prominent problems.

An excellent discussion of cats is available in Scott's book on the care and management of laboratory animals (Scott, 1967).

Few attempts have been made to set up nutritional standards for cats. Many authorities suggest a varied diet containing fresh meat, fish, milk, liver, and vegetables. This is undoubtedly good advice but affords the nutritionist little information concerning the nutritive requirements of cats. A publication of the National Academy of Sciences–National Research Council (Albrittan, 1953) lists the daily nutrient allowances for the cat. The values in this table provide little specific information concerning specific nutrients, since they consist entirely of levels of nutrients found in raw and canned cat feeds, admittedly inadequate for reproduction and lactation.

As with other species, estimations of the nutritional requirements of cats are complicated by a variety of genetic differences and undoubtedly differ with age, sex, and activity. For the most part, the limited studies of cat nutrition have not considered these factors, as evidenced by experimentation with animals of mixed breed.

It is impossible at this time to describe quantitatively the nutrient requirements for cats. However, this report will attempt to summarize the present knowledge of cat nutrition.

GROWTH

Expected growth rates are of interest to the nutritionist. Hall and Pierce (1934) presented data that show that kittens usually weigh about 100 g at birth and can be expected to gain approximately 10 g per day up to the age of 50 days. Table 1 shows the type of growth that has been obtained from cats fed semipurified diets in Gershoff's (unpublished data) laboratory. The values in this table are similar to those reported by Da Silva (1950b) from cats fed semipurified diets and by Waterhouse and Carver (1962) from cats fed fish-based commercial canned cat food.

TABLE 1 Growth of Cats Fed a Purified Diet

Initial Weight (g)	Males		Females	
	No.	g Gained/Day during Next 30 Days	No.	g Gained/Day during Next 30 Days
500	1	9.3	4	9.8 ± 1.8
600	3	13.8[a]	10	10.6 ± 2.0
700	11	11.0 ± 1.3[b]	13	9.6 ± 1.3
800	13	11.4 ± 1.4	13	12.7 ± 1.4
900	9	10.6 ± 1.8	13	12.1 ± 1.1
1,000	12	12.7 ± 2.0	12	12.1 ± 1.2
1,100	14	13.9 ± 1.7	13	11.9 ± 1.1
1,200	14	14.9 ± 1.5	13	8.7 ± 1.1
1,300	14	15.8 ± 1.5	10	9.0 ± 1.3
1,400	12	16.6 ± 1.5	9	9.6 ± 0.8
1,500	9	17.1 ± 1.6	8	10.6 ± 1.3
1,600	8	15.3 ± 1.9	8	9.3 ± 1.0
1,700	8	15.5 ± 2.2	7	9.7 ± 1.5
1,800	8	15.3 ± 2.5	7	8.6 ± 1.2
1,900	9	14.4 ± 2.3	7	8.4 ± 1.2
2,000	9	12.2 ± 2.4	5	8.7 ± 1.3

[a] Individual gains: 4, 17, 20.3 g.
[b] Mean ± standard error of the mean.

Da Silva (1950) and Greaves (1965) reported better growth in cats fed natural stock rations than semipurified diets. Spray and Widdowson (1950) have presented data on the effect of growth from birth to maturity on the body composition of cats with respect to protein, water, fat, Na, K, Ca, Mg, P, Fe, Cu, and Zn.

ENERGY

Krehl *et al.* (1955) reported a daily requirement of 65 kcal per kg of body weight per day for young adult cats. Miller and Allison (1958) found that the energy requirement of young kittens was about 250 kcal per day per kg of body weight, but decreased rapidly to approximately 134 kcal per kg of body weight at 30 weeks of age. The energy need became constant in animals kept in metabolism cages at about 60 kcal per day per kg of body weight at 50 weeks. Cats allowed to exercise in runs increased their energy intakes to about 80 to 90 kcal per day per kg of body weight. Da Silva (unpublished data) has observed a daily energy consumption of 159 kcal per kg of body weight per day by growing cats and 90 kcal by adults.

Greaves and Scott (1960) observed a tendency for adult cats to increase their caloric intake as the protein concentration in their diets decreased from above 32 percent to 19 percent.

FAT AND PROTEIN

Most cat nutritionists favor high-fat, high-protein diets (Da Silva, 1950b; Dickinson and Scott, 1956c; Gershoff *et al.*, 1957b; Krehl *et al.*, 1955). Purified diets containing 25–30 percent fat and 30–40 percent protein are commonly used. These high-fat rations appear to be more palatable than low-fat diets. Gershoff (unpublished data) obtained better growth in cats fed semipurified diets containing 26 percent fat than in those fed 5 percent fat, although the diets were isonitrogenous. Dickinson and Scott (1956) reported that satisfactory growth of cats in their laboratory could only be obtained with dietary protein concentrations greater than 30 percent. Miller and Allison (1958) found that 5-week-old cats retained about 1.7 g of nitrogen per day per kg of body weight for growth. This retention decreased to approximately 0.5 g per day per kg of body weight at 25 weeks, and nitrogen equilibrium was reached at about 55 weeks. Kittens under 10 weeks of age required $\geq$0.7 and adult cats approximately 0.5 g of nitrogen per day per kg of body weight, to maintain their body protein stores. These authors also found that the nitrogen balance indices of casein and gluten were higher for adult cats than for dogs, suggesting that adult cats may have lower requirements for lysine and sulfur-containing amino acids than other species. Greaves and Scott (1965) found that adult resting cats, when fed a protein source of mixed fish and liver having a biological value of approximately 52 for cats, remained in nitrogen balance when their diets contained 21 percent protein. This was equivalent to about 0.8 g of nitrogen per kg of body weight per day. Greaves (1965) has reviewed the literature on protein and calorie requirements of cats. He has calculated the theoretical net protein requirements for growth and maintenance in cats from birth to maturity based on available data for endogenous urinary and metabolic fecal losses and protein retained in the body with age. His data suggest that the minimum requirements of cats for protein vary from 19 g/kg body wt/day, during the first week of life, to 2 g/kg body wt/day, at maturity. In a previous report Greaves and Scott (1960) found that optimal mixed natural rations for cats contained 32 percent protein, supplying 29 percent of the calories. When he fed cats from 11 to 21 weeks of age casein-based semipurified diets, supplying 29 percent of calories as protein, the protein intakes were about twice the estimated protein required, indicating a utilization of casein in good diets for growth of about 50 percent.

CARBOHYDRATES

Carbohydrates have not been demonstrated to be required in the diets of cats. If diets are adequate in protein and fat, the balance of calories probably can be supplied by carbohydrates. Greaves and Scott (1963) observed increased food consumption in cats when dextrin, rather than sucrose, was used in their rations.

MINERALS

Few studies have been made of the quantitative requirements of cats for any of the mineral elements. Four to five percent of mixed salts, such as Salts IV (Hegsted *et al.*, 1941), have been used successfully in semipurified diets. Scott *et al.* (1961) fed kittens raw or cooked heart exclusively. This diet was particularly deficient in calcium and iodine. After about

7 weeks, the kittens showed signs of nervousness, ataxia, and finally paralysis of the hind limbs. Osteoporosis (osteitis fibrosa), but not rickets, was observed. Paresis was due to pressure following collapse of bony structures. Thyroids were hyperplastic and enlarged, and kidneys also were heavier than normal. These cats exhibited all the signs described by veterinarians as osteogenesis imperfecta. Supplementation of the diets with calcium entirely prevented the occurrence of signs referable to the skeleton and partially protected against thyroid hyperplasia. Fifty micrograms of iodine daily prevented gross enlargement of the thyroid and 100 μg daily completely prevented hyperplastic changes. Iodine supplementation delayed the onset of skeletal dystrophy. In a continuation of this work, Roberts and Scott (1961) have shown that supplementation of the heart-containing diet, with 100 μg of iodine per cat per day, reduced losses of calcium in urine and feces. It appears that there is a synergistic relationship in the requirements for calcium and iodine. The conclusion from these studies is that cooked or raw heart muscle with its associated fat, supplemented with calcium salts to give a Ca:P ratio of 1:0 and with iodine, provides an excellent diet for cats, supporting satisfactory reproduction. Additional morphologic, roentgenologic, and clinicopathologic findings in studies of Ca deficient cats have been described (Humphreys and Scott, 1964; Jowsey and Gershon-Cohen, 1964; Krook *et al.*, 1963; Scott *et al.*, 1963).

Cats are particularly prone to kidney and bladder stones. Morris (1953) suggested that high-mineral diets induce urinary calculi. Dickinson and Scott (1956a) and Gershoff (unpublished data) were unable to produce urinary calculi in kittens fed diets containing as high as 30 percent ash. In cats fed a commercial cat food high in ash, Gershoff *et al.* (1959b) found diffuse intratubular calcium oxalate deposits in the kidneys, which were caused primarily by vitamin B_6 deficiency.

FAT-SOLUBLE VITAMINS

Vitamin A

Vitamin A deficiency has been produced in kittens by Gershoff *et al.* (1957b). The first symptom of vitamin A deficiency in cats is a decrease in food consumption, followed by emaciation. Some animals show weaknesses of the hind legs with some signs of rigidity. Histologic examinations of vitamin A-deficient cats reveal the classic changes of squamous metaplasia in a number of organ systems, with bronchopneumonia being a common complication. Reproductive failure has also been reported in vitamin A-deficient cats (Scott and Scott, 1964).

Within reasonable limits, changes in the level of dietary fat have little, if any, effect on the absorption of vitamin A in rats (Burns *et al.*, 1951), hens (Russell *et al.*, 1942), or human beings (Wilson *et al.*, 1936). However, Gershoff *et al.* (1957b) have found that increasing dietary fat resulted in increased serum levels of vitamin A. It is obvious from this study that, in the preparation of cat food, sufficient fat should be included to ensure utilization of fat-soluble vitamins. This is particularly important in view of the high incidence of lung infection associated with the feeding of vitamin A-deficient diets to cats.

The studies of Ahmad (1931), Rea and Drumond (1932) and Gershoff *et al.* (1957b) demonstrated that for all practical purposes β-carotene is not utilized by the cat as a source of vitamin A, whether administered orally or parenterally.

Symptoms of Deficiency Morris (1965) and Scott *et al.* (1964) have reported blindness and degenerative retinopathy in cats fed casein-based purified diets containing amounts of vitamin A considered more than adequate for other species of animals. Scott *et al.* (1964) have suggested that the continual feeding of casein made it difficult for cats to utilize vitamin A. When they induced vitamin A deficiency in cats on a meat diet, no evidence of retinal damage was obtained, although conjunctivitis appeared. These observations have been confirmed in part by Gershoff (unpublished data), who has maintained cats on casein-based purified diets for periods up to 4 years and found that some developed similar eye pathology.

A crippling bone disease of cats fed diets containing a high proportion of raw liver has been reported. This disease is characterized by the development of exostoses in various parts of the skeleton resulting in postural changes and musculoskeletal deformities. Seawright and English (1965) and Seawright *et al.* (1967) have attributed this condition to hypervitaminosis A and have produced it experimentally in cats fed diets composed of meat and milk with daily oral supplements of 166 μg of vitamin A/g body weight.

Vitamin D

Gershoff *et al.* (1957a) produced vitamin D deficiency in cats by maintaining them on vitamin D

deficient purified diets containing different calcium: phosphorus ratios. Rickets was produced as shown by x-ray evidence, high serum alkaline phosphatase, and low bone ash values. More severe rickets was produced by a diet containing 1 percent calcium and 1 percent phosphorus than by one containing 2 percent calcium and 0.65 percent phosphorus. This may have been the result of what appeared to be the poorer growth of the cats fed the 2:0.65 Ca:P ratio.

A marked spontaneous improvement was observed in the condition of most of the cats surviving the acute rickets of the first year of the experiment. This probably indicates a very low vitamin D requirement for cats $\geq 1\frac{1}{2}$ years old.

Vitamin E

Cordy (1954) produced vitamin E deficiency in cats by feeding them commercial cat food high in fish content. The deficiency state was characterized by orange or orange-tan colored fat (steatitis). In some kittens anorexia occurred, followed by death. Degenerative muscle lesions and leg weakness were not observed. There was little edema, and hemorrhages were absent in the fat. Splenomegaly occurred in some of the kittens.

Gershoff (unpublished data) has observed similar changes in kittens fed commercial cat food high in fish content. These foods contained 18 mg of total tocopherol per kg of food on a dry-weight basis. These changes were not observed when other commercial pet foods containing twice as much tocopherol were fed. Munson *et al.* (1958) have reported steatitis in cats fed diets that included canned red tuna fish. They reported that improvement was brought about by α-tocopherol administration.

Gershoff and Norkin (1962) have administered purified diets, containing varying levels of vitamin E, with and without tuna oil as a source of high unsaturated fatty acids, for periods up to $13\frac{1}{2}$ months. Steatitis was observed only in cats receiving diets deficient in vitamin E and containing tuna oil. Vitamin E provided complete protection against steatitis in these experiments. Cats deficient in vitamin E, but not receiving tuna oil, showed relatively mild symptoms of deficiency, particularly muscle changes, after a year on the experimental diets.

Vitamin K

The dietary requirement of cats for vitamin K is probably very low. The feeding of diets containing irradiated beef with 6 μg vitamin K/100 g of dry diet did not result in prolonged prothrombin times in cats (Reber and Malhotra, 1961).

WATER-SOLUBLE VITAMINS

Ascorbic Acid

Ascorbic acid is ordinarily not included in purified diets for cats and is presumed to be synthesized in their tissues.

Biotin

No studies have been made on the biotin requirements of the cat.

Choline

Da Silva *et al.* (1959b) have produced choline deficiency in cats, which is characterized by weight loss and fatty livers. Hypoalbuminemia has also been reported in choline-deficient cats (Mansur Guerios and Hoxter, 1962).

Folic Acid

Da Silva *et al.* (1955) produced folic acid deficiency by feeding cats semipurified diets deficient in folic acid and containing sulfaguanidine or sulfathalidine. These authors were unable to obtain deficiency symptoms in cats when either sulfaguanidine or sulfathalidine was not added to their diets. The deficiency signs were weight loss, macrocytic anemia, and leukopenia. Weight responses were obtained with single doses of 1 mg of folic acid or two doses of 0.8 mg of folinic acid each. For hematological recovery, 2 mg of folic acid were sufficient. If folic acid was given with vitamin B_{12} or liver extract, better results were obtained than with folic acid alone.

Inositol

Scott (1965) has stated that 10 mg of inositol per day protected cats against fatty livers which may be produced in the absence of dietary inositol despite the addition of choline to semipurified diets. However, her data have not been published, and inositol often is not included in semipurified diets for cats.

Niacin

Niacin deficiency in cats has been described by Heath *et al.* (1940) and Da Silva *et al.* (1952). The

deficiency is primarily characterized by diarrhea, emaciation, and death. Da Silva's group found no buccal or skin lesions. Heath *et al.* reported an elevated body temperature and mouth lesions consisting of an ulcerative, reddish margin in the upper part of the palate close to the midline, a redness of the terminal portion of the tongue, thick saliva, and foul mouth odor. Da Silva found that as little as 1–3 mg of niacin given subcutaneously to niacin-deficient cats evoked a weight gain response of 100–200 g during a 4- to 8-day period. The cat's ability to store the vitamin appeared limited. Not one of the 45 cats lived more than 20 days on a niacin-free diet. Death was often preceded by respiratory disease.

These authors have also presented evidence that tryptophan is not converted to niacin. Tryptophan is not effective in promoting growth or in bringing about urinary excretion of *N*-methyl nicotinamide by niacin-deficient cats. Confirmatory data, supporting the observation that tryptophan is not converted to niacin, have been provided by Braham *et al.* (1962), who also observed that cats can utilize niacin from raw and lime-treated corn to an equal extent.

Pantothenic Acid

Gershoff and Gottlieb (1964) have produced pantothenic acid deficiency in cats. The deficiency was accompanied by loss of weight, fatty liver, histologic changes in the small intestine, and impaired ability to acetylate *p*-aminobenzoic acid. On the basis of maintenance data and values for urinary excretion of pantothenic acid and acetylation of *p*-aminobenzoic acid, 5 mg of calcium pantothenate per kg of diet appeared to meet the growing cat's requirement.

Riboflavin

Gershoff *et al.* (1959a) fed kittens isonitrogenous, semipurified diets, varying in riboflavin content and ratio of carbohydrate to fat. The symptoms of acute riboflavin deficiency were characterized chiefly by anorexia with resulting emaciation and death. In one experiment, acute riboflavin deficiency was accompanied by hair loss, particularly about the head. In a second experiment, alopecia was not observed. In chronically deficient cats, cataracts were observed. None of the chronically deficient cats showed hair loss. High carbohydrate diets partially protected cats against riboflavin deficiency even though the carbohydrate used was sucrose. Fecal and urinary riboflavin determinations indicated that this effect was due to increased intestinal synthesis on high carbohydrate diets. Three milligrams of riboflavin per kg of diet appeared adequate when the high carbohydrate diet was fed and 4 mg when the low carbohydrate diet was fed.

Thiamin

Thiamin deficiency in cats has been described by a number of groups (Everett, 1944; Odom and McEachern, 1952; Smith and Proutt, 1944; Toman, 1945), but no data are available concerning the cat's thiamin requirement. The deficiency is marked by anorexia, vomiting, ataxia, abnormal reflexes, convulsions, and cardiac disorders. Smith and Proutt (1944) produced thiamin deficiency in cats by feeding raw carp or herring, presumably because thiaminase is present in the tissues of these fish, but no deficiency occurred in cats when fed raw perch, catfish, butterfish, or spots. This is of some importance in that many people feed fish or fish products to their cats.

Vitamin B_6

Gershoff *et al.* (1959b) and Da Silva *et al.* (1959a) have shown that vitamin B_6 deficiency in cats is characterized by growth failure, emaciation, convulsions, anemia, kidney disease, and iron deposition in the liver. Gershoff *et al.* (1959b) have found that the kidney lesions in vitamin B_6-deficient cats are associated with the presence of large amounts of kidney and urinary endogenous oxalate. These workers found that cats fed diets containing 2 mg of pyridoxine hydrochloride per kg of diet did not develop signs of pathology associated with vitamin B_6 deficiency. However, since less oxalate was excreted by cats receiving 4 mg of pyridoxine hydrochloride per kg of diet than those receiving 2 mg, it has been suggested that the cat's requirement for vitamin B_6 may be set above 2 mg per kg of diet.

Vitamin B_{12}

No studies have been made on the vitamin B_{12} requirements of the cat.

MISCELLANEOUS

Two lengthy studies have been conducted on the comparative value of cooked and uncooked foods for cats (Mostyn, 1947; Pottenger and Sinonsen, 1939). In these experiments, consistently better growth, development, reproduction, and lactation were obtained

TABLE 2 Examples of Formulas of Stock Diets for the Growing Cat[a]

Ingredient	Dry Matter in Ingredient (%)	Diet A		Diet B		Diet C	
		Dry (%)	As Fed (%)	Dry (%)	As Fed (%)	Dry (%)	As Fed (%)
Casein, crude	90.7	13.2	10.0	17.0	10.0	—	—
Milk, dried whole	93.7	27.3	20.0	—	—	—	—
Liver, raw beef	26.0	13.3	35.0	—	—	—	—
Beef, raw lean muscle	23.3	—	—	8.7	20.0	30.3	33.3
Sardines, deboned and eviscerated	29.3	—	—	10.9	20.0	38.1	33.3
Oats, compressed, slightly cooked	91.7	40.1	30.0	31.6	20.0	—	—
Potatoes, cooked, mashed	24.3	—	—	6.8	15.0	31.6	33.3
Butter, lard, or vegetable oil	84.5	6.1	5.0	15.8	10.0	—	—
Cod liver oil	100.0	—	—	5.6	3.0	—	—
Bone meal	97.1	—	—	3.6	2.0	—	—
Total		100.0	100.0	100.0	100.0	100.0	100.0

[a] Adapted from Da Silva (1950a). See Table 4 for vitamins to be added to this diet.

when raw meat and milk were fed to cats than when the cats received cooked meat and milk. In evaluating these papers, characterization of the single or multiple-deficiency state produced by these diets has been difficult. It appears unlikely that the results can be ascribed to thiamine deficiency. Once this deficiency state was produced in kittens, it could not be reversed (Pottenger and Sinonsen, 1939). When deficient adult cats were returned to a raw diet, normal animals were not produced for several generations (Mostyn, 1947).

Hegsted *et al.* (1956) developed palatability tests for cats, and noted a number of factors that must be considered in tests of this kind. Some foods of high acceptability were found to be nutritionally inadequate when used over extended periods.

EXAMPLES OF ADEQUATE DIETS

Stock Diets

Much of the commercial cat food is nutritionally inadequate and must be supplemented with other foods. Three stock diets formulated by Da Silva (1950a) are presented in Table 2 as examples of adequate rations for the growing cat. Other successful diets have ben formulated (Dickinson and Scott, 1956b; Waterhouse and Carver, 1962).

EXPERIMENTAL DIETS

The composition of a semipurified diet, fed successfully by Gershoff (1959a), is presented in Table 3. The vitamins that were added to this diet are listed in Table 4. Gershoff's semipurified diet is similar to the one developed by Da Silva (1950b). However, Da Silva fed each cat vitamins emulsified with 2 ml of cod liver oil three times a week, instead of adding the vitamins directly to the diet. It should be pointed out that the stability of a number of nutrients in natural and semipurified diets is affected by the length and conditions of storage of the rations.

SUMMARY OF NUTRIENT REQUIREMENTS

A list of the nutrients presently known to be required by the cat is shown in Table 5. It is evident that much more research is needed before feline nutrition can be defined adequately.

TABLE 3 Examples of Satisfactory Semipurified Diets for Growing Cats

Ingredient	Dry Matter in Ingredient (%)	Diet	
		Dry (%)	As Fed (%)
Casein, purified	90.7	30.1	32.1
Sucrose	100.	38.9	37.6
Corn oil	100.	12.9	12.5
Fat, hydrogenated	98.	12.7	12.5
Cod liver oil	100.	1.0	1.0
Salt mixture[a]	100.	4.1	4.0
Choline chloride	100.	0.3	0.3
Vitamins[b]	100.	[b]	[b]
Total		100.0	100.0

[a] Hegsted's Salt mixture IV (Gershoff *et al.*, 1959a), which is composed of $CaCO_3$, 600; K_2HPO_4, 645; $CaHPO_4 \cdot 2H_2O$, 150; $MgSO_4 \cdot 7H_2O$, 204; NaCl, 335; Ferric citrate, 55; KI, 1.6; $MnSO_4 \cdot 4H_2O$, 10.0; $ZnCl_2$, 0.5; $CuSO_4 \cdot 5H_2O$, 0.6 parts by weight.

[b] See Table 4.

TABLE 4 Examples of Vitamin Supplements Used Successfully with Purified Diets for Growing Cats

Nutrient	Supplement I[a] Dry (mg/kg diet)	Supplement I[a] 90% Dry Matter (mg/kg diet)	Supplement II[b] (mg given orally 3 times/week)
Thiamin hydrochloride	4.44	4.0	0.75
Riboflavin	8.88	8.0	0.75
Pyridoxine hydrochloride	4.44	4.0	0.75
Niacin	44.44	40.0	2.50
Calcium pantothenate	22.22	20.0	2.50
Folic acid	1.11	1.0	0.50
Biotin	0.22	0.2	0.05
Inositol	—	—	30.00
p-Aminobenzoic acid	—	—	0.75
Menadione (Vitamin K)	1.11	1.0	0.75
α-Tocopherol	—	—	15.00
Vitamin B_{12}	0.11	0.1	—

[a] Vitamins A and D are provided in the diet by the inclusion of 1% cod liver oil.

[b] Suspended in 1 ml of water emulsified with 2 ml of cod liver oil.

TABLE 5 Summary: Nutrient Requirements of the Growing Cat

Nutrient		Dry[a] (kg)	90% Dry Matter[a]
Total protein	%	>33.3	>30.0
Minerals		Required	Required
Vitamin A	IU/kg	27,777.	25,000.
Vitamin D	IU/kg	1,111.	1,000.
Vitamin E	IU/kg	151. (122)	136. (110)[b]
Vitamin K		—	[c]
Thiamin	mg/kg	4.4	4.
Riboflavin	mg/kg	4.4 (2.7)	4. (2.5)
Vitamin B_6	mg/kg	2.2 (1.1)	2. (1.0)
Niacin	mg/kg	44.	40.
Pantothenic acid	mg/kg	5.5 (3.3)	5. (3.0)
Biotin	mg/kg	—	[c]
Folic acid		—	[c]
Ascorbic acid		—	[c]
Choline	mg/kg	3,333. (1,111)	3,000. (1,000)
Vitamin B_{12}		—	[c]
Inositol	mg/kg	222.	200.

[a] The values not in parentheses are estimated from various adequate rations; in some cases they may be considerably in excess of the actual requirement. Values in parentheses represent the highest levels fed reported not to meet the cat's requirement and, thus, are below the actual need.

[b] These values are obtained on a diet containing a high level of unsaturated fish oil provided by 15 percent lard and 6 percent fish oil. When 20 percent lard and 1 percent of fish oil were fed, 3.4 IU of vitamin E per 100 g of diet provided complete protection against deficiency signs.

[c] No information is available on a dietary requirement under normal feeding conditions.

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NUTRIENT REQUIREMENTS OF THE GUINEA PIG

The guinea pig is a valuable animal for nutritional research because of its unusually high requirement for certain vitamins and amino acids. Among the commonly used laboratory animals, only it and the primates require a dietary source of ascorbic acid. Considerable progress has been made in determining its nutritional requirements, and fuller knowledge should increase its usefulness as an experimental animal.

One of the most useful methods for evaluating the adequacy of a diet is to measure weight changes of growing animals. Figure 1 shows growth curves of male and female animals of a mongrel strain. However, as Mannering (1949) has pointed out, one should not accept an absolute value for maximum growth at this time. More extensive vital data, which may be of use to nutritionists, have been presented by Reid (1958).

PROTEIN

A 25 percent level of a well-balanced mixture of proteins is adequate to meet the growth requirements of the guinea pig. Commercial rations of good quality contain about this amount, largely from plant sources. It is the supply of specific essential amino acids that is important rather than the protein as such, since these animals grow fairly well on an amino acid mixture (Reid, 1958). If a single protein, such as casein or purified soybean protein, is fed without amino acid supplements, a 35 percent level is required for maximal growth (Reid, 1963). If a 20 percent level of dietary casein is supplemented with L-arginine (1.0 percent of the diet) and the same level of purified soybean is supplemented with DL-methionine (1.0 percent of the diet), a good rate of growth—though not maximal—is obtained (Reid and Mickelsen, 1963).

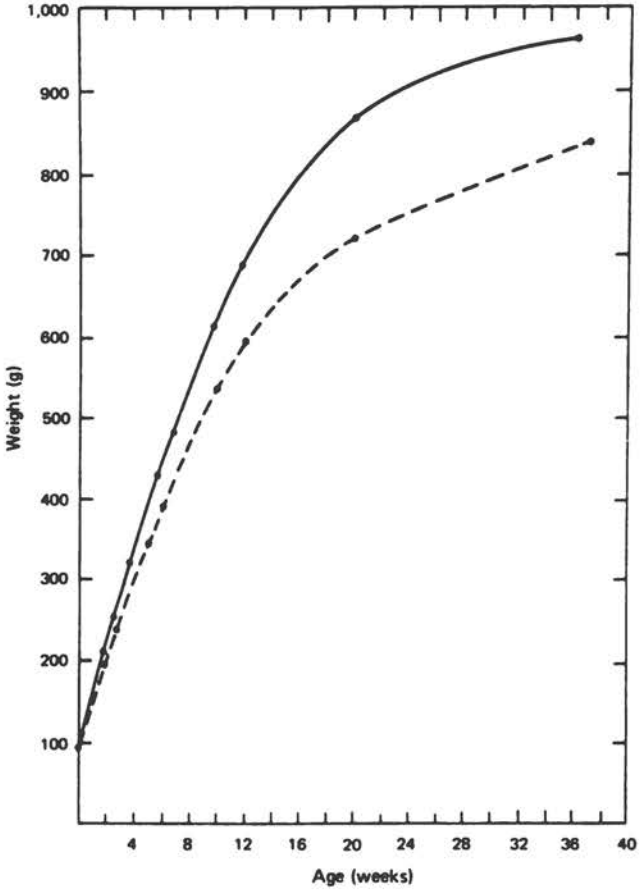

FIGURE 1 Growth curves for guinea pigs from the colony of the allergens station, U.S. Department of Agriculture. Solid line: males; dotted line: females. (Studies by Dr. E. J. Coulson.)

Maximal growth results when a 30 percent level of casein supplemented with 0.3 percent L-arginine–HCl is fed, as when a 30 percent soybean protein diet is supplemented with 0.5 percent of DL-methionine. The protein requirements for reproduction and lactation

have not been specifically determined. Some of the presently available commercial rations, containing 26 percent or more of protein adequately supplied with ascorbic acid, appear to be fairly satisfactory for reproduction and lactation.

Arginine

The apparently high protein need of the guinea pig is chiefly a consequence of a high arginine requirement (Heinicke *et al.*, 1955, 1956). The arginine requirement with a 30 percent level of dietary casein appears to be approximately 1.56 percent of the diet (Reid and Mickelsen, 1963). It is considered doubtful that more than 70 percent of the arginine in casein is available to the young guinea pig (Heinicke *et al.*, 1956).

Tryptophan

A study was made of the tryptophan requirement of the guinea pig using a diet containing protein that was adequate in all essential amino acids other than tryptophan (Reid and Von Sallmann, 1960). After 6–8 weeks on the deficient diet, the eyes showed advanced cataractous changes and some corneal vascularization. Growth was somewhat retarded. Alopecia was seen in most of the animals. The requirement for tryptophan to prevent eye damage is greater than that for maximal growth. The requirement for complete eye protection and for good growth is more than 0.16 percent but possibly somewhat less than 0.2 percent. The D-isomer appeared to have from one fourth to one third the growth-promoting activity of the L-form.

Sulfur-Containing Amino Acids

In a diet prepared with a 30 percent level of purified soybean protein, the total sulfur-containing amino acid requirement has been reported to be 0.71 percent, with 0.36 percent being derived from cystine and 0.35 percent from methionine (Reid, 1966). D-Methionine was found to be less active than the L-isomer.

Other Amino Acids

The requirement of the guinea pig for other amino acids has not been determined.

CARBOHYDRATES

There is no indication that any particular carbohydrate is essential for the guinea pig. It seems likely that a mixture of carbohydrates may be desirable since the guinea pig's natural diet of vegetables contains a combination of sugars, dextrin, starch, hemicellulose, cellulose, and lignin. The nitrogen-free extract content of stock diets commonly ranges from 45 to 48 percent and of purified diets from 38 to 55 percent. Looseness of the diet, with as little tendency as possible to packing, is desirable. Diets prepared with sugars as the chief source of carbohydrates are especially susceptible to packing.

FAT

The guinea pig has a definite requirement for a dietary supply of unsaturated fatty acids (Reid, 1954b; Reid and Martin, 1959). There is no good evidence that dietary fat has any specific effect other than that of supplying essential fatty acids. If linoleic acid is supplied at a rate of approximately 4 g per kg of dry diet or a level of 1 percent of calories, growth and skin condition are normal (Reid *et al.*, 1964). Although corn oil at a dietary level of 1 percent will permit good growth, the animals tend to have a slight dermatitis that a level of 3 percent will prevent.

Symptoms of Deficiency

Omission of a supply of unsaturated fatty acids results in a retardation of growth (Reid, 1954b). Gradually a syndrome develops that is characterized by dermatitis, poor growth of fur, loss of fur, skin ulcers, and a microcytic anemia.

BULK FORMERS

Although the guinea pig normally thrives best with roughage in its diet (Hogan and Ritchie, 1934; Crampton and Bell, 1947; Booth *et al.*, 1949; Heinicke and Elvehjem, 1955; Reid and Briggs, 1953), fairly good growth without it has been demonstrated under some conditions. The fiber content of stock diets ranges from 9 to 18 percent. With purified diets the best results have been obtained by the inclusion of 15 percent of a bulk-forming material such as gum arabic, cellophane, or cellulose. Cellophane spangles, a coarsely ground cellophane product, has been found to be effective as a bulk-forming agent in a purified diet (Reid *et al.*, 1956). However, some workers have found a pure, finely ground, wood-cellulose product to be slightly superior to either cellophane spangles or gum arabic for supplying bulk (Heinicke and Elvehjem, 1955).

MINERALS

Calcium, Phosphorus, Magnesium, and Potassium

Dietary levels of calcium, phosphorus, magnesium, and potassium must be carefully regulated for the guinea pig (Hogan and Ritchie, 1934; O'Dell *et al.*, 1957b; Maynard *et al.*, 1958). Roine *et al.* (1949) demonstrated that the rather high levels of 1.6 percent potassium and 0.34 percent magnesium in the diet are needed for maximal growth. Grace and O'Dell (1968), however, have found that with excess cations in the diet the potassium requirement is only 0.5 percent. Hogan *et al.* (1950, 1954) found that a low calcium-to-phosphorus ratio in the diet caused reduced growth, stiff joints, and deposits of calcium phosphate in the soft tissues. O'Dell *et al.* (1957b) obtained results that showed that guinea pigs fed high phosphorus diets, containing levels of magnesium and potassium commonly fed to other laboratory animals, had a marked decrease in magnesium absorption. These investigators (O'Dell *et al.*, 1956) found that the injurious effects of high phosphorus diets for the guinea pig are due in part to its inability to tolerate an acid diet: The guinea pig does not use ammonia to neutralize excess acids excreted by the kidneys.

O'Dell *et al.* (1960) found that when the guinea pig diet contained 0.9 percent calcium and 0.4 percent phosphorus the magnesium requirement was 80 mg/100 g of diet. If, however, the phosphorus was elevated to 1.7 percent, the magnesium requirement rose to 240 mg.

Manganese

Everson *et al.* (1959) studied manganese deficiency in the guinea pig and found that when this element was omitted from the maternal diet, litter size was reduced and a high percentage of the young were born dead or were delivered prematurely. All of the young born to deficient females showed ataxic symptoms at birth. A small number of defective young were maintained on the manganese-deficient diet for 3 months, and abnormal head movements and unsteadiness of gait persisted. The manganese-low diet contained under 0.002 percent.

Copper

Everson *et al.* (1967) have studied the effects of copper deficiency in the guinea pig, using a diet amply supplied with ascorbic acid but low in copper (0.5–0.7 ppm). Hemorrhages were found throughout the body. These results are of special interest in connection with a similar hemorrhagic condition that develops in the copper-deficient rat, dog, pig, sheep, and chick.

Other Minerals

Although iron, zinc, iodine, selenium, molybdenum, and chromium are presumably needed, no qualitative requirement has been demonstrated. Cobalt also is probably required for intestinal synthesis of vitamin B_{12} if the diet contains none of this vitamin.

FAT-SOLUBLE VITAMINS

Vitamin A

Bentley and Morgan (1945) found that the ingestion of 2.0 mg daily of vitamin A per kg of body weight by depleted animals resulted in the storage of detectable vitamin A in the livers. Howell *et al.* (1967) obtained satisfactory growth in young guinea pigs when 0.5 mg of vitamin A acetate was fed twice weekly. Gil *et al.* (1968), using a purified diet, found that 6.6 mg of vitamin A palmitate per kg of dry diet, or about 0.2 mg per animal daily, was necessary for optimal growth and significant liver storage. These various reports indicate a daily intake of about 0.2 mg vitamin A, or about 6 mg per kg dry diet, should be adequate.

Chevallier and Choron (1936) reported that the guinea pig had a rather low storage capacity for vitamin A and that the utilization of β-carotene was inefficient. The data of Gil *et al.* (1968) also indicate a less efficient storage of vitamin A than in the rat, but analyses of liver from guinea pigs fed a natural diet with carotene did not indicate poor utilization of the pro-vitamin.

Symptoms of Deficiency The gross effects of vitamin A deficiency in the guinea pig are cessation of growth, loss of weight, accumulation of organic debris in the bile ducts and gall bladder, clouding of the cornea, xerophthalmia, and death within a few days after the eye symptoms develop. Wolbach and Howe (1928) described the microscopic effects as a transformation of the normal epithelium of many tissues to a stratified, keratinized condition, much like the deficiency effects found in the rat. In contrast to the rat, extensive formation of keratinized epithelium occurred before the organs atrophied. The guinea pig also differs from the rat in the early and extraordinary

degree of change in the bladder and uterus in vitamin A deficiency (Kobayashi *et al.*, 1959). The deficiency causes severe retardation of growth in bones and teeth. No evidence has been found that vitamin A deficiency affects the nervous system, although lesions in the central nervous system are produced by the use of excessive amounts of the vitamin (Brusa and Testa, 1953). Excessive administration also produces effects such as metastatic calcification in the kidneys, together with parathyroid changes (Berdjis and Rinehart, 1958).

Vitamin D

The young guinea pig does not appear to require the antirachitic vitamin if the ratio of calcium to phosphorus in the diet is satisfactory. Kodicek and Murray (1943) maintained animals in good health for 3 months, with no signs of rickets on a diet low in vitamin D and with a presumably balanced salt mixture. The quantitative requirement for vitamin D in diets with unbalanced proportions of calcium to phosphorus has not been determined. A level of 0.04 mg (1600 IU) per kg of dry diet has been found to be adequate (Reid and Briggs, 1953).

Vitamin E

Shimatori *et al.* (1939) maintained guinea pigs in a healthy condition for as long as 200 days by the administration of 1.5 mg of α-tocopherol per day. The requirement during pregnancy appears to be higher than this. Farmer *et al.* (1950) found that 3 mg per day are required by primapara animals. For multipara animals this amount reduced but did not completely alleviate a tendency to hemorrhages and abortion. It seems probable, however, that an inadequate supply of vitamin K may have been partly responsible for the syndrome. As with other species of animals, the vitamin E requirement is related to the dietary content of polyunsaturated fatty acids. In the nonpregnant, growing guinea pig, a daily intake of 1.0 mg is adequate for diets containing up to 8 percent vegetable oil.

Symptoms of Deficiency Goettsch (1930) found that guinea pigs fed a certain simplified diet developed an extreme degeneration of the voluntary muscles. With the exception of the liver, which frequently showed a moderate degree of fatty infiltration, no visible lesions were found in other organs. The primary lesion in the dystrophic muscles has been described as a coagulative necrosis of the fibers, resulting in a waxy, hyaline degeneration (Goettsch and Pappenheimer, 1931). An increased content of myoglobin in the muscle of vitamin E-deficient guinea pigs has been reported (Bender *et al.*, 1959; Schottelius *et al.*, 1959). Shimatori *et al.* (1939) demonstrated that the dystrophy in the muscle of guinea pigs was due to a lack of α-tocopherol. Reproduction is also adversely affected by a lack of this vitamin.

Vitamin K

Dam *et al.* (1947) were unable to demonstrate a need for vitamin K for growth. However, Hamilton (1943) found that failure to supply the vitamin during pregnancy resulted in stillbirth or death of the young soon after birth. These effects were probably a result of hemorrhages (Mannering, 1949). A dietary level of 2 mg per kg of dry diet has proven adequate in different purified diets (Roine *et al.*, 1949; Reid and Briggs, 1953).

WATER-SOLUBLE VITAMINS

Ascorbic Acid

Mannering (1949) summarized the criteria used by various investigators for evaluating the requirement of the animal for ascorbic acid. Collins and Elvehjem (1958) reported that the ascorbic acid requirement of immature guinea pigs is 0.5 mg per 100 g body weight per day when growth is used as a criterion. Although the evidence indicates that a daily intake of 7–10 mg of ascorbic acid per kg of body weight is sufficient to prevent pathological lesions in the odontoblast layer of the incisor teeth (Crampton *et al.*, 1944; Kuether *et al.*, 1944; Pfander and Mitchell, 1952), there may be an additional requirement to protect the animal against infection. Nungester and Ames (1948) have shown that a serum level of approximately 0.4 mg per 100 ml of ascorbic acid is necessary to provide a high degree of phagocytic activity. This serum level is nearly double the level necessary to prevent lesions in the incisor teeth. To produce this degree of phagocytic activity, a 300-g guinea pig would require a daily intake of approximately 6 mg of the vitamin or approximately 150–200 mg per kg of dry diet.

Symptoms of Deficiency The gross effects produced by the absence of ascorbic acid from the diet are anorexia, retarded growth, and death. The outstanding changes to be observed are hemorrhages in almost any part of the body, a general weakness of

the tissues (especially in those with a normally high content of collagen), stiffened hind legs, beaded ribs, lowered body temperature in the late stages, and a tendency toward diarrhea. The average survival time is from 10 to 25 days, seldom exceeding 28–30 days. Lack of the vitamin causes a failure in the production of normal collagen, which results in the formation of defective connective tissue. As compared to normal collagen, the defective tissue is characterized by a lowered content of proline and hydroxyproline (Robertson, 1950, 1952; Robertson and Schwartz, 1953). Practically all tissues of the body show degenerative changes, but usually the bones and teeth are affected first. The maintenance of preformed collagen does not require ascorbic acid but present available evidence indicates that the vitamin is needed for the maintenance of rapidly formed repair collagen (Gould, 1960).

Biotin

No clear evidence has been presented indicating a dietary biotin requirement by the guinea pig.

Symptoms of Deficiency Biotin deficiency has been produced by feeding a biotin-deficient diet containing raw egg white as the source of protein (Coots *et al.*, 1959). Symptoms observed were loss of weight, alopecia, and depigmentation of the fur.

Choline

The young guinea pig requires a dietary supply of choline or its precursors: mono- or dimethylaminoethanol, plus a methyl donor. Betaine is more effective than methionine as a methyl donor. Levels of 1.0–1.5 g of choline chloride per kg of dry diet have been found sufficient to permit maximal growth in the guinea pig (Reid, 1955).

Symptoms of Deficiency Choline deficiency is characterized by severe growth retardation, muscular weakness, reduced red blood cell count, lowered hematocrit and hemoglobin values, small subcutaneous and adrenal hemorrhages, and pale kidneys (Reid, 1955). Neither severe kidney hemorrhages nor marked fatty infiltration of the liver has been observed. A chronic deficiency, which occurs when the diet contains only a small amount of choline or its equivalent, is characterized by retarded growth, anemia, and muscular weakness.

Folic Acid

It is well established that folic acid is a dietary essential for the guinea pig (Woolley and Sprince, 1945; Mannering, 1949; Woodruff *et al.*, 1953; Reid, 1954a; Reid *et al.*, 1956). Discrepancies in the findings of different investigators are, in part, a consequence of variations in the age at which the animals were placed on the experimental diets. As animals grow older, their requirements become lower.

Mannering (1949) reported that a daily intake of 100 μg of folic acid was essential for maximal growth. With a purified ration, from 3 to 6 mg per kg dry ration have ben found to be the minimal requirement for growth and the production of a normal erythrocyte picture (Reid *et al.*, 1956). The reqiurement is higher ($\geq$6 mg per kg) for maintaining a normal leukocyte count. The folic acid requirement of the guinea pig is high compared to that of most animal species.

Symptoms of Deficiency Very young animals placed on a folic acid-deficient diet display the following symptoms: retarded growth, gradual loss of appetite and activity, weakness, tendency toward diarrhea, profuse salivation in the late stages, convulsions, and death. At autopsy, a tendency toward fatty infiltration of the liver and adrenal hemorrhages have been observed (Reid *et al.*, 1956). In folic acid-deficient guinea pigs, growth may be close to normal and yet the blood picture may be definitely pathological. With a severe deficiency, both red and white blood cells are affected. The hemoglobin and hematocrit values and erythrocyte and leukocyte counts are lowered. Faulkner, Blood, and Darby (1958) reported that although deprived guinea pigs showed folic acid-deficient anemia, there was an increase in the myoglobin content of skeletal muscle that appears to be similar to that found by other workers in vitamin E deficiency (Bender *et al.*, 1959; Schottelius *et al.*, 1959).

Inositol

No definite evidence exists that the guinea pig requires a dietary source of inositol.

Niacin

The growing guinea pig requires a dietary supply of niacin (Reid, 1954a; Reid, 1961). The inclusion of 10–20 mg of niacin per kg of dry diet will meet the requirements of growing guinea pigs if the diet contains 30 percent casein or purified soybean protein.

With a 20 percent level of either of these proteins, the requirement is between 20 and 50 mg per kg of dry diet, resulting from lowered tryptophan intake.

Symptoms of Deficiency Uncomplicated niacin deficiency was produced in young guinea pigs reared on a purified diet containing 30 percent casein (Reid, 1961). As the deficiency progressed, there was retardation of growth; loss of appetite for food and water; drooling; soiled coats; diarrhea; paleness of feet, nose, and ears; and, in some cases, death. Neither oral lesions nor dermatitis was observed. Blood studies showed lowered hemoglobin and hematocrit values.

Pantothenic Acid

The young guinea pig's requirement for pantothenic acid has been found to be between 15 and 20 mg per kg of dry diet (Reid and Briggs, 1954); the requirement for the adult is apparently unknown.

Symptoms of Deficiency Riboflavin deficiency has characterized by retarded growth, anorexia, roughness of haircoat, tendency toward diarrhea, weakness, and, finally, death (Reid and Briggs, 1954). The internal symptoms of pantothenic acid deficiency include an enlargement and hyperemia of the adrenals and, in some cases, hemorrhages.

Riboflavin

Slanetz (1943) determined the riboflavin content of stock diets for the guinea pig and estimated that 3 mg per kg of diet were needed for optimal growth. The quantitative requirement of the guinea pig for riboflavin, however, has not been determined.

Symptoms of Deficiency. Riboflavin deficiency has been produced in young guinea pigs with a purified diet that lacked this vitamin (Reid, 1954a). The chief deficiency symptoms were retarded growth, rough haircoats, little loss of appetite, but no noticeable dermatitis. Using similar dietary procedures, Hara (1960) made a pathological study of the deficiency. Microscopically, corneal vascularization, atrophy of the skin, certain nerve changes (such as chromatolysis and myelin degeneration in the pons and spinal cord), and damage to the heart muscle cells were observed. The hearts showed vacuolar degeneration of the muscle cells with abnormal deposits of glycogen as well as other such prominent pathological changes as atrophy of the cells, myocardial hemorrhage, and edema.

Thiamin

The thiamin requirement of young guinea pigs was found to be 2 mg per kg dry diet (Liu *et al.*, 1967; Reid and Bieri, 1967). Both of these studies showed that the requirement in purified diets was markedly affected by the type of salt mixture used.

Symptoms of Deficiency Thiamin deficiency has been produced in the guinea pig with use of a purified diet adequate in all other known vitamins (Reid, 1954a). The average survival time of the animals on this deficient diet was 24 days. The chief symptoms of the deficiency were emaciation, tremor, and unsteady gait, with some tendency for retraction of the head in the final stage of the deficiency. At autopsy, partially digested food was present in the cecum, no fat was found around the organs, and no change was observed in the number of erythrocytes and leukocytes.

Vitamin B_6 (Pyridoxine)

The approximate requirement of the guinea pig for vitamin B_6 with a purified diet containing 30 percent protein is 2.0–3.0 mg per kg of dry diet (Reid, 1964).

Symptoms of Deficiency Two reports have been made of the production of vitamin B_6 deficiency in the guinea pig (Reid, 1954a, 1964). Animals 3–5 days old fed a diet lacking this vitamin showed anorexia, retarded growth, lessened vigor, muscular incoordination, and roughness and thinning of the haircoat. No dermatitis was observed. As the deficiency progressed, some animals had convulsions and whirled rapidly about the cage. Half of the animals succumbed relatively early, while others lived for an extended period. Both the kidneys and adrenals were enlarged and the sex organs atrophic. A hyperemic condition of the cecum, with hemorrhage, was observed in the animals that died.

Vitamin B_{12}

There is no unequivocal evidence that the growing guinea pig requires a dietary source of vitamin B_{12} (Reid, 1954a). When a diet adequate in cobalt is fed, bacterial synthesis in the digestive tract probably supplies this vitamin in sufficient amounts.

MISCELLANEOUS

Water Allowance

The daily water allowance of adult animals receiving green food in their diet is 50–100 ml. Without a

supply of green food, from 250 to 1000 ml should be provided because of the necessity for a high intake and because of considerable spillage.

Additives

Chlortetracycline has been shown to be beneficial in the maintenance of a breeding colony. O'Dell *et al.* (1957a) included it in various diets of a guinea pig colony for a period of 3 years without evidence of toxicity. It was reported to have no effect on the growth rate and caused no mortality when added to a purified diet at levels of 25–200 mg per kg. At a routine level of 25 mg per kg of dry diet, chlortetracycline hydrochloride decreased abortions and adult mortality and eliminated cervical lymphadenitis from the breeding colony.

EXAMPLES OF ADEQUATE DIETS

Stock Diets

Commercial pelleted diets formulated to suit the needs of the guinea pig are available. It is important that the materials used in the preparation of these diets should be pathogen free. These diets are made with a content of ascorbic acid that is approximately ten times the normal requirement. When precautions are taken to obtain fresh supplies of the pellets frequently, these rations support normal growth and reproduction. It is best not to store them for periods longer than 4–6 weeks, particularly when the temperature of the storage room is high. Unfortunately, no satisfactory method has yet been devised for the stabilization of ascorbic acid. An example of a satisfactory formula for a guinea pig stock ration is that formulated by the National Institutes of Health (Table 1). With allowance for considerable spillage, growing guinea pigs can be expected to require an amount of such a diet approximately equal to one twelfth of their body weight daily. After growth ceases, the intake in relation to body weight lessens. If the ascorbic acid content of this diet or that of a commercial pelleted diet becomes too low during storage, supplements of fresh kale or cabbage should be given. To obtain good reproductive performance, the feeding of greens may be necessary.

Purified Diets

Estimates of the nutritional requirements of the guinea pig have been obtained chiefly from studies with purified diets. The composition of two successful purified diets is presented in Table 2. Although the 2–5-day old guinea pig will eat a purified, powdered diet satisfactorily, some investigators (Everson *et al.*, 1959) feel that pelleting or the addition of water to the diet (B. L. O'Dell, personal communication) improves its acceptability.

SUMMARY OF NUTRIENT REQUIREMENTS

Table 3 lists, qualitatively and quantitatively, the known nutritional requirements of the guinea pig. It should be noted that where numerical values are given, those in parentheses represent tentative estimates of the minimal requirement and contain no margin of safety. The values *not* enclosed in parentheses are estimates from various adequate diets and are probably in excess of the actual requirement. Table 4 gives the daily nutrient requirement, expressed on a body weight basis, of young growing guinea pigs.

TABLE 1 Composition of the National Institutes of Health Diet for Guinea Pigs

Ingredient	Assumed Dry Matter (%)	Diet	
		Dry Basis (%)	As Fed (%)
Oats, whole, ground fine	89.7	17.64	17.70
Wheat, whole, ground fine	86.0	27.60	28.90
Alfalfa meal[a]	92.3	38.95	38.00
Soybean meal[b]	91.3	13.43	13.25
Vitamin D_2 premix (1,730,000 IU/kg)	94.0	0.10	0.10
Ascorbic acid	100.0	0.06	0.05
Sodium chloride, iodized	100.0	0.56	0.50
Limestone, ground	99.6	1.22	1.10
Dicalcium phosphate, min. of 0.2% fluorine	96.0	0.27	0.25
Delamix[c]	100.0	0.17	0.15
Total		100.00	100.00[d]

[a] Dehydrated, 17% crude protein.
[b] Dehulled, solvent extracted, containing not less than 49% protein.
[c] A trace mineral mix produced by Limestone Products Co., Newton, N. J. Contains not less than 6% Mn, 2% Fe, 0.2% Cu, 0.12% I, 0.02% Co, 26.5% Ca.
[d] Finished product at time of delivery should conform to the following calculated standards (not less than): crude protein, 18%; crude fat, 2.25%; crude fiber, 13.50%; nitrogen-free extract (NFE), 48%; ash, 8.20%; calcium, 1.20%; phosphorus, 0.40%; sodium, 0.15%; iodine, 1 ppm; carotene, 80 ppm; niacin, 40 ppm; thiamin, 4 ppm; pantothenic acid, 15 ppm; riboflavin, 6 ppm; biotin, 0.15 ppm; α-tocopherol, 40 IU/g; vitamin D, 2 IU/g; vitamin C, 450 ppm.

TABLE 2 Examples of Satisfactory Purified Diets for Guinea Pigs

Ingredient	Unit	Assumed Dry Matter (%)	Roine *et al.* (1949) Diet		Reid and Briggs (1953) Diet	
			Dry (per kg)	As Fed (per kg)	Dry (per kg)	As Fed (per kg)
Protein	g	94.	333.	300.[a]	333.	300.[b]
Cornstarch	g	—	—	—	222.	200.
Sucrose	g	100.	483.	435.	114.	103.
Glucose	g	100.	—	—	87.	78.
Gum arabic	g	96.	167.	150.	—	—
Cellophane	g	100.	—	—	17.	15.
Soybean oil	g	100.	44.	40.	—	—
Corn oil	g	100.	—	—	81.	73.
Salt mixture	g	100.	44.	40.[c]	67.	60.[d]
Magnesium oxide	g	100.	5.6	5.	5.6	5.
Potassium acetate	g	100.	27.8	25.	28.	25.
Vitamin A acetate	mg	100.	—	—	6.7	6.
β-Carotene	mg	100.	13.	12.	—	—
Vitamin D_3 (calciferol)	mg	100.	0.09	0.08	0.04	0.04
Vitamin E (α-tocopherol)	mg	100.	133.	120.	—	—
Vitamin E (α-tocopherol acetate)	mg	100.	—	—	55.	50.
Vitamin K_3 (menadione)	mg	100.	2.2	2.	2.2	2.
Ascorbic acid	mg	100.	167.	150.[e]	2222.	2000.
Biotin	mg	100.	0.4	0.4	0.67	0.6
Calcium or sodium pantothenate	mg	100.	33.	30.	44.	40.
Choline chloride	g	100.	3.3	3.	2.2	2.
Folic acid	mg	100.	3.3	3.	11.	10.
Inositol	g	100.	2.2	2.	2.2	2.
Niacin	mg	100.	111.	100.	222.	200.
p-Aminobenzoic acid	mg	100.	111.	100.	—	—
Pyridoxine hydrochloride	mg	100.	11.	10.	18.	16.
Riboflavin	mg	100.	16.	14.	18.	16.
Thiamin hydrochloride	mg	100.	11.	10.	18.	16.
Vitamin B_{12}	mg	100.	—	—	0.04	0.04

[a] To be supplied by casein.

[b] Thirty percent purified casein + 0.3% L-arginine hydrochloride, or 30% isolated soy protein + 0.5% DL-methionine, in this diet give similar growth (Reid and Mickelsen, 1963).

[c] Salt mixture IV (Hegsted *et al.*, 1941).

[d] The salt mixture of Fox and Briggs (1960) is preferred to that originally used in this diet.

[e] Ascorbic acid was fed separately at the rate of 12.5 mg per day and was not included in the diet.

TABLE 3 Amount of Nutrient Requirements per Kilogram of Dry Matter Fed

Nutrient	Unit	Diet[a] Dry	Diet[a] 90% Dry Matter
Protein (N × 6.25)	g	278.–333.	250.–300.
Calcium	g	13.	12.
Phosphorus	g	6.7	6.
Magnesium	g	3.9	3.5
Potassium	g	16.	14.
Manganese	g	0.044	0.04
Copper	g	0.0066	0.006
Iron	g	0.037	0.033
β-Carotene	mg	13.	12.
Vitamin D		Not required[b]	Not required[b]
α-Tocopherol	mg	(67.)	(60.)
Vitamin K	mg	11.	10.
Ascorbic acid	mg	(222.)	(200.)
Biotin		Not required	Not required
Choline	g	(1.7)	(1.5)
Folic acid	mg	11.(6.7)	10.(6.)
Inositol		Not required	Not required
Niacin	mg	56.(22.)	50.(20.)
Pathothenic acid	mg	(22.)	(20.)
Riboflavin	mg	18.	16.
Thiamine	mg	18.(2.2)	16.(2.)
Vitamin B_6	mg	18.(2.2–3.3)	16.(2.–3.)
Vitamin B_{12}		[c]	[c]

[a] The values that are *not* enclosed in parentheses are estimated from various adequate diets and, hence, are probably in excess of the actual requirement. The values in parentheses are tentative estimates of the minimal requirement and contain no margin of safety.

[b] Vitamin D may be required in diets with unsatisfactory calcium-to-phosphorus ratios.

[c] With adequate cobalt in the diet, bacterial synthesis in the intestinal tract probably supplies adequate vitamin B_{12}.

TABLE 4 Daily Nutrient Requirements for Growth of Guinea Pigs

Nutrient	Unit	Per kg of Body Weight per Day
Total daily dry feed	g	80.
Protein	g	20.
Calcium	g	1.
Phosphorus	g	0.5
Magnesium	g	0.28
Potassium	g	1.12
Manganese	mg	5.
Copper	mg	1.
Iron	mg	5.
β-Carotene	mg	9.6
Vitamin D		Not required[a]
α-Tocopherol	mg	4.8
Vitamin K	mg	0.8
Ascorbic acid	mg	16.
Biotin		Not required
Choline	g	0.12
Folic acid	mg	0.8
Niacin	mg	4.
Pantothenic acid	mg	1.6
Riboflavin	mg	1.3
Thiamin	mg	1.3
Vitamin B_6	mg	1.3
Vitamin $_{12}$		[b]

[a] Vitamin D may be required in diets with unsatisfactory calcium-to-phosphorus ratios.

[b] With adequate cobalt in the diet, bacterial synthesis in the intestinal track probably supplies sufficient vitamin B_{12}.

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NUTRIENT REQUIREMENTS OF THE HAMSTER

Little information is available on the quantitative requirements for individual nutrients by the golden hamster. The golden or Syrian hamster, *Mesocricetus auratus* (Granados, 1951; Whitney, 1965), is the hamster most commonly used for experimental research and, as such, is the species meant whenever reference is made to *hamster.* The somewhat larger European hamster, *Cricetus cricetus* (Whitney, 1965), and the smaller Chinese or gray hamster, *Cricetulus griseus* (Moore, 1965; Yerganian, 1958), also used experimentally, will not be considered in this review. Less is known of their nutrient requirements than those of the golden hamster. Generally, the requirements are considered to be similar to those of the laboratory rat, and hamsters will usually grow satisfactorily on diets adequate for rats. This generality, however, probably reflects our ignorance of the nutrient requirements of hamsters. Certain differences in diet adequacy for rats and hamsters have been reported (Cooperman *et al.*, 1943; Folk and Farrand, 1957; Scheid *et al.*, 1950).

ENERGY

Energy requirements of hamsters at various stages of growth, reproduction, and lactation have not been determined. Arrington *et al.* (1966) fed hamsters a semipurified diet, containing 6 percent corn oil and 5 percent cellulose, and reported an average daily food intake of about 6.7 g/day, a feed efficiency of about 5.0 g feed/g body weight gain, and a gross caloric intake of 27–29 kcal/day. Calculations were based on hamster body weights from about 40 to 100 g; 45-g hamsters ate 58 kcal/100 g body weight, and 90-g hamsters ate 28 kcal/100 g body weight. Apparently, caloric requirements are met easily from practical or semipurified diets, providing the diet is adequate in other nutrients. Information on the ability of the hamster to utilize fibrous feed materials as an energy source was not available. Presumably, hamsters are like the rat in this regard.

PROTEIN

Little information on the protein or amino acid requirements of the hamster has been published. However, from studying the purified diets that appear to be adequate, one can infer that 20–24 percent of the diet as casein will readily meet the needs of the growing hamster (Ershoff, 1956; Granados, 1951; Hamilton and Hogan, 1944; Salley and Bryson, 1957; Schweigert *et al.*, 1950). Arrington *et al.* (1966) reported that 40-g hamsters, fed a semipurified diet with casein as the protein source for 6 weeks, grew equally well at 16 percent or 20 percent dietary protein, but growth was significantly less at 12 percent or 8 percent dietary protein. Isolated soybean protein at 16 percent dietary protein supported growth equal to that produced with an equivalent amount of protein supplied as casein.

Sixty percent casein in a semipurified diet, compared to 18 or 25 percent casein, depressed growth of hamsters (Horwitz and Waisman, 1966). There is some evidence that the hamster may be able to utilize urea nitrogen (Matsumoto, 1955), but the extent to which urea may supply the protein requirement is unknown. A low protein diet (6 percent) was reported to allow for more rapid progress of experimental tuberculosis infection in hamsters than was observed with a 17 or 30 percent protein diet (Ratcliffe and Merrick, 1957).

CARBOHYDRATES

As carbohydrate sources, glucose and sucrose have been successfully included in some semipurified diets in amounts of 60–65 percent of the diet (Granados,

1951; Hamilton and Hogan, 1944; Salley and Bryson, 1957; Schweigert *et al.*, 1950), and in others as much as 72–74 percent (Christensen *et al.*, 1953; Dam and Christensen, 1952). However, improved growth and survival of hamsters has been observed when cornstarch was substituted for sucrose or when chlortetracycline at 100 mg/kg diet or alfalfa at 20 percent of the diet were used in rations containing glucose or sucrose (Ershoff, 1956). By comparison, supplying cellulose as a roughage source or replacing sucrose with dextrin was ineffective in improving growth and survival. Dam and Christensen (1961b) reported diarrhea to be a problem, particularly with high sucrose (36–74 percent) diets and to a lesser extent with high glucose (72 percent) diets. Lactose or rice starch diets generally afforded a pronounced protective effect against diarrhea.

Effects on the gastrointestinal flora may explain the above observations. Hamsters fed fat-free diets high (74 percent) in lactose or rice starch had a predominantly lactic acid flora in the colon and a pH of 5.6. This was not the case with hamsters fed the 74 percent glucose diet in which the colonic pH was 6.7 (Snog-Kjaer *et al.*, 1963). Considerable amounts of starch passed the intestinal tract and lactose caused some distention of the cecum (Dam and Christensen, 1961b). The introduction of antimicrobial agents proved inconclusive: most antibiotics caused death (Snog-Kjaer *et al.*, 1963), and one of the antibiotics reportedly induced kidney lesions (Christensen and Dam, 1961).

FAT

Christensen and Dam (1953) showed that the hamster requires a dietary source of essential fatty acids. The deficiency signs include alopecia, scaly skin, abnormal skin tightness, and a profuse secretion of cerumen (ear wax), which is probably (F. Christensen, personal communication) accompanied by a general increase in secretory activity of the fat glands of the skin. The deficiency signs were prevented by the inclusion of 10 percent lard or a linoleic acid supplement in the diet. Semipurified diets containing 3–10 percent fat as corn oil, lard, cottonseed oil, or certain other oil mixtures have been used successfully (Arrington *et al.*, 1966; Ershoff, 1956; Granados, 1951; Hamilton and Hogan, 1944; Salley and Bryson, 1957; Schweigert *et al.*, 1950).

The fatty acid composition of liver and bile phosphatides of hamsters has been shown to respond to differences in dietary lipid source (Glenn *et al.*, 1964), which is not unlike observations made with several other species. Convulsions, induced by high intakes of fat, particularly butterfat, have been reported, but the nature of the defect remains questionable. The convulsions may be related to the ether administered prior to ingestion of the high-fat test meal (Swank and Engel, 1958).

BULK FORMERS

Salley and Bryson (1957) reported that hamsters fed semipurified diets have a definite need for cellulose or some other "nonnutritive" fiber. Three to 8 percent of such material has been included in some hamster diets (Arrington *et al.*, 1966; Hamilton and Hogan, 1944; Salley and Bryson, 1957; Schweigert *et al.*, 1950).

MINERALS

Little research has been directed toward establishing the mineral requirements of hamsters. Presumably, their needs are similar to those of the rat, since satisfactory purified diets have been formulated for the hamster containing salt mixtures identical to those used in purified rat diets. In the work of Jones (1945) rickets was produced in hamsters by feeding a diet low in vitamin D and phosphorus (0.4 percent calcium and 0.02 percent phosphorus). Normal calcification was obtained and no rickets seen, even in the absence of vitamin D, when optimal amounts of calcium and phosphorus were fed (0.6 percent calcium and 0.35 percent phosphorus); the hamster is like the rat in this respect.

Phosphorus also has been implicated as an anticariogenic material for hamsters (Harris and Nizel, 1959).

Iodine deficiency has been demonstrated to cause thyroid hyperplasia in hamsters (Follis, 1959, 1964). Thyroiditis develops in the glands of iodine-deficient hamsters; in the presence of excess iodine, colloid accumulation develops.

Many of the mineral mixtures used for hamsters in the past have been notably lacking in certain required elements, such as copper, manganese, and zinc. Presumably these have been supplied by the other diet components; the possible significance of such deletions is generally unknown, with the exception of the following observations: Lack of dietary copper may cause a lighter color of the fur (F. Christensen, personal communication); copper additions to fat-supplemented lithogenic diets for hamsters decreased the incidence of gallstones but induced some liver necrosis (Christensen and Dam, 1960).

A critical review of the inadequacies of mineral mixtures used in the diets of experimental animals was presented by Williams and Briggs (1963). These investigators have developed a new mineral mixture that has been used in a semipurified diet for hamsters (Cohen *et al.*, 1967).

FAT-SOLUBLE VITAMINS

Vitamin A

Hirschi (1950) produced vitamin A deficiency in hamsters and observed the following deficiency signs: weight loss, coarsened and thinned hair coat, and xerophthalmia. In the later stages, hemorrhages developed in the genitalia and bowel. Keratinizing or squamous metaplasia in the salivary glands, respiratory mucosa, teeth, and gonads has been described in vitamin A-deficient hamsters by Salley and Bryson (1957; Salley *et al.*, 1959); control hamsters received 250 IU/week of vitamin A in cottonseed oil administered orally (Salley and Bryson, 1957). When the diet was deficient in vitamin A, the formation of gallstones increased significantly (Fortner, 1954).

Vitamin D

From the studies of Jones (1945), it is apparent that the hamster, like the rat, requires little or no vitamin D in the diet when the calcium-to-phosphorus ratio is optimal. Typical rickets does develop, however, when the diet is moderate in calcium, low in phosphorus, and deficient in vitamin D.

Vitamin E

It has been shown that the growing hamster requires vitamin E (Houchin 1942). Although the quantitative requirement for vitamin E is unknown, purified diets containing 25 mg of α-tocopherol per kg diet and small amounts of polyunsaturated lipids appear to be adequate (Hamilton and Hogan, 1944; Salley and Bryson, 1957; Schweigert *et al.*, 1950).

The signs of vitamin E deficiency are muscular weakness, purulent secretion about the eyes, and hyperirritability, followed by collapse, stupor, and death (Hamilton and Hogan, 1944; Houchin, 1942; Leicht and Gatz, 1959; West and Mason, 1958). Growth is normal until muscular weakness and dystrophy ensue. Oddly, too, the hamster is more susceptible to muscular dystrophy from vitamin E deficiency than is the rat.

Diets used to produce muscular dystrophy have usually been high in fat and have contained polyunsaturated fat sources such as cod liver oil. Lack of dietary unsaturated fatty acids in fat-free diets may explain why other investigators (Dam and Christensen, 1952) were unable to develop muscular dystrophy, despite prolonged use (2–3 months) of the vitamin-E deficient diet. Diets containing large amounts of torula yeast have been used to produce a deficiency syndrome, which responds to either vitamin E or selenium (Hopkins, 1962). Higher oxygen consumption (Houchin and Mattill, 1942), greater proteolytic activity (Koszalka *et al.*, 1961) and accelerated DNA turnover (Gerber *et al.*, 1962) of muscle are also manifested during the development of muscular dystrophy in the hamster. Therapeutic doses of 1 mg α-tocopherol have ben reported to produce recovery from vitamin E deficiency.

Vitamin K

Hamilton and Hogan (1944) obtained evidence of a need for vitamin K by the hamster. Animals fed a diet deficient in this vitamin showed depressed growth and developed small hemorrhagic areas in the muscles, the subcutaneous tissues, and the abdominal cavity, with free blood in the sinuses. The deficiency was shown to be transient, which can probably be attributed to an intestinal flora that developed that synthesized vitamin K. Granados and Dam (1950; Granados, 1951) were unable to show a requirement for this nutrient by the growing hamster. This conflicting evidence is believed to be caused by variation in the intestinal flora.

WATER-SOLUBLE VITAMINS

Ascorbic Acid

The hamster does not require a dietary source of ascorbic acid, apparently because of synthesis within the body (Clausen and Clark, 1943; Cooperman *et al.*, 1943).

Biotin

According to reports of Granados and Dam (1950) and Granados (1951) biotin is not required in the diet for growing hamsters fed semipurified diets. Cooperman *et al.* (1943), however, reported somewhat slower growth and development of a dermatitis in its absence (Rauch and Nutting, 1958). A deficiency syndrome was produced by feeding a diet con-

taining 40 percent egg white (a source of avidin) and sulfaguanidine. Reversal of the symptoms—slow growth, "kangaroo" stance, nervousness, incoordination, dry, scaly dermatitis, incrustations about the eyes, alopecia, and achromotricia—was achieved by injecting 4 μg of biotin per day.

Choline

A qualitative requirement for choline by growing hamsters has not been determined (Hamilton and Hogan, 1944), although some evidence that this nutrient is needed for successful lactation has been obtained. Danish workers (Granados, 1951; Granados and Dam, 1950) reported that a dietary source of choline is necessary for rapid growth, and Handler and Bernheim (1949) reported fatty livers in choline-deficient adult hamsters.

Folic Acid

The only information regarding folic acid was furnished by Granados (1951) and Granados and Dam (1950), who reported that this vitamin is not needed in the diet.

Inositol and p-Aminobenzoic Acid

Cooperman *et al.* (1943) observed increased growth with the addition of *p*-aminobenzoic acid and inositol to purified diets for growing hamsters, but the supplements were not tested separately to determine which was responsible. Hamilton and Hogan (1944) could find no beneficial effects on growing hamsters by the addition of either of these compounds to the diet, although they did observe many stillbirths in inositol-deficient females and concluded that inositol is essential during gestation. Granados and Dam (1950; Granados, 1951) concluded, with no qualification for gestation, that both are usually not required by the growing hamster. The only seemingly conclusive evidence was postulated by Salley and Bryson (1957), who noted that brewer's yeast, in amounts normally used in animal diets to supply B-vitamins, did not provide sufficient inositol or choline for young hamsters. In short, there is no conclusive evidence for either substance as a nutrient requirement.

Niacin

In a niacin-free diet, marked weight loss, "rough stringy" fur, alopecia, and death occurred in growing hamsters (Routh and Houchin, 1942). The signs disappeared with the daily administration of 100 μg of niacin. In later studies, Routh and Houchin did not obtain a consistent growth response to niacin (Schweigert, 1948). Similarly, several other laboratories have been unable to show a qualitative requirement for niacin by growing hamsters (Cooperman *et al.*, 1943; Granados, 1951; Granados and Dam, 1950; Hamilton and Hogan, 1944). Hamilton and Hogan (1944) obtained some evidence that niacin was required by the hamster for lactation. It appears that the hamster synthesizes niacin from tryptophan.

Pantothenic Acid

The essentiality of a dietary source of pantothenic acid by the growing hamster has been demonstrated by Routh and Houchin (1942). Deficiency signs were reported to be weight loss, red incrustation around the mouth, and death. Cohen, Amrich, and Okey (1963) reported an adverse effect of feeding cholesterol to pantothenate-deficient hamsters. Levels of dietary calcium pantothenate from about 10 mg/kg of diet (Hamilton and Hogan, 1944; Salley and Bryson, 1957) to about 40 mg/kg (Schweigert *et al.*, 1950) have been used in supposedly adequate semipurified diets.

Pyridoxine (Vitamin B_6)

A qualitative requirement for vitamin B_6 by the growing hamster has been demonstrated several times. The deficiency signs include anorexia, diminished water intake, and progressive malnutrition from low food intake. A migrating alopecia, loss of hair luster, and a mild achromotrichia were sometimes observed. Increased urinary excretion of xanthurenic acid has also been reported (Shwartzman and Strauss, 1949). Routh and Houchin (1942) were able to reverse signs and re-establish moderate growth by the daily administration of 3 μg of pyridoxine. A level of 6–12 mg of pyridoxine per kg of diet is apparently adequate for growing hamsters (Ershoff, 1956; Hamilton and Hogan, 1944; Salley and Bryson, 1957; Schweigert *et al.*, 1950).

Riboflavin

A qualitative requirement for riboflavin has been established for hamsters: Daily adminstration of 4 μg (Routh and Houchin, 1942) or inclusion of 20 mg riboflavin per kg diet (Smith and Reynolds, 1961) prevents signs of deficiency. The signs of riboflavin deficiency include variable occurrence of diarrhea,

scaly dermatitis, alopecia, arrested growth, and stupor. Smith and Reynolds (1961) further reported that deficient hamsters were not only stunted, dull, and inactive, but their haircoat lacked luster. No skin lesions have been shown to develop. A level of 6–16 mg of riboflavin per kg in purified diets is considered adequate for growing hamsters (Ershoff, 1956; Hamilton and Hogan, 1944; Salley and Bryson, 1957; Schweigert *et al.*, 1950).

Thiamin

Routh and Houchin (1942) produced polyneuritis in growing hamsters by feeding thiamin-deficient diets. The signs were reversed by the daily oral administration of 3 μg of thiamin. Semipurified diets that contain 6–10 mg of thiamin per kg have proved adequate for the growing hamster (Ershoff, 1956; Hamilton and Hogan, 1944; Salley and Bryson, 1957; Schweigert *et al.*, 1950).

Vitamin B_{12}

The hamster apparently does not require vitamin B_{12} in the diet (Granados, 1951). In a 6-week trial with young hamsters, Scheid *et al.* (1950) could not demonstrate a need for this vitamin. Under the same conditions, however, a requirement by the albino rat could be demonstrated.

Cohen *et al.* (1967) could demonstrate no outward signs or differences in growth rate or hematology due to vitamin B_{12} deficiency in hamsters; however, a metabolic deficiency was indicated by urinary excretion of methylmalonic acid and formiminoglutamic acid and by increased levels of glutathione in blood serum and liver. A dietary level of 10 μg of vitamin B_{12} per kg of diet prevented these metabolic alterations. The later investigators concluded that although there were certain differences between rats and hamsters in the metabolic defects caused by vitamin B_{12} deficiency, the hamster does require a dietary source of vitamin B_{12}.

Water

Detailed water requirements of hamsters apparently have not been determined; however, a readily available supply of good water is required. If fresh, high-moisture feedstuffs such as vegetables or fruit are used, the supply of water is less critical. With hamsters it is particularly important that the young have access to fluid other than the mothers' milk; if not, intestinal compaction or diarrhea may cause death (Whitney, 1965). Water can be supplied from inverted bottles with a suitable drinking tube or spout (Hamilton and Hogan, 1944; Whitney, 1965). The spout must be kept low so that the young have access to water. Cleanliness of water containers is important (Granados, 1951).

UNIDENTIFIED FACTORS

Several sources of unidentified factors have been reported to enhance growth of the hamster. Among these are muscle, human saliva, salivary gland extract, and possibly milk (Granados, 1961; Granados *et al.*, 1951). However, saliva did not stimulate growth of hamsters fed a commercial diet and fresh cabbage (Benoit *et al.*, 1963). An unidentified factor has also been reported present in alfalfa (Ershoff, 1956). Dried liver extract has sometimes been used to supply possible unidentified factors (Schweigert *et al.*, 1950). Adding wheat germ oil to a commercially prepared diet was reported to improve reproduction of hamsters (Oman and Magalhaes, 1957).

Danish workers were able to induce and prevent formation of gallstones in hamsters solely by dietary means (Anonymous, 1958, 1960; Christensen and Dam, 1954; Dam and Christensen, 1952). The lithogenic diets used were high (72–74 percent) in sucrose or glucose, and either low (2 percent) in fat (lard) or fat-free. Antilithogenic diets were made by incorporating yeast or soybeans (18 percent) in the lithogenic diets at the expense of carbohydrate. Once formed, cholesterol gallstones could be dissolved *in situ* by changing the lithogenic diet to a curative diet: Sucrose was replaced by yeast and rice, lard was increased from 2 percent to 10 percent, and the salt mixture was supplemented with copper (Christensen *et al.*, 1956).

A semipurified diet, the composition of which difered from the lithogenic diet in a higher level of lard (7 percent), casein (25 percent), choline hydrochloride (0.4 percent), and another salt and vitamin mixture, protected largely against gallstones. The antilithogenic activity of this diet was mainly traced to its content of 7 percent lard and the copper content of the salt mixture (Christensen and Dam, 1954, 1960).

Influences of dietary fat (Dam and Christensen, 1961a), bile acids (Dam and Christensen, 1962), and various carbohydrate sources (Dam and Christensen, 1961b) were studied in relation to incidence and type of gallstones and composition of the bile. Unsaturated fats counteracted the formation of cholesterol stones, and diets high in starch or lactose (72–74 percent) generally counteracted formation of

gallstones. Formation of cholesterol stones was nearly suppressed by 0.1 percent hyodeoxycholic acid but not by the same level of several other bile acids.

The diets with unsaturated fats, or high in rice starch, increased the ratio of bile acids to cholesterol; diets with unsaturated fats caused an increase in the lipid-soluble phosphorus–cholesterol ratio and a decrease in concentration of cholesterol in the bladder bile. In contrast to these findings, the high (72–74 percent) lactose diet, which is a good antilithogenic diet, did not change the mentioned ratios as compared with those of the antilithogenic diet (Dam *et al.*, 1965; Prange *et al.*, 1962, 1964, 1966).

The relationship of cholesterol synthesis and metabolism (Hanel *et al.*, 1954a,b; Jensen and Dam, 1966) and the influence of hormones (Dam and Christensen, 1965) with respect to alimentary production of gallstones in hamsters have also been investigated. On ectomy of the gallbladder no formation of gallstones was observed (Van der Linden *et al.*, 1959).

EXAMPLES OF ADEQUATE DIETS

In general, commercial stock diets for rats have been used successfully as colony diets for hamsters. Often they are fed with fresh vegetables or milk. Folk and Farrand (1957) found that isolated hamsters could not be maintained at normal weight levels when fed a commercial food pellet for rodents that had been stored for about 2 months at 50–70°F., although this diet produced normal growth of rats. The normal weights of hamsters could be restored by a supplement containing vitamins A, C, D, thiamin, riboflavin, niacin, and concentrated vegetable tocopherols (See Table 1 for typical body weights and growth rates.) Alternatively, fresh food pellets from the same manufacturer supported normal weights of hamsters. This indicates that vitamin destruction in commercially prepared diets may be more of a problem in raising hamsters than in raising rats. Examples of stock diets that have been reported to provide for satisfactory growth and reproduction of hamsters are shown in Table 2.

Semipurified diets have been developed that allow for normal growth of hamsters; however, they are generally less effective in supporting reproduction (Hamilton and Hogan, 1944) and have not been extensively studied in this respect. Examples of semi-

TABLE 1 Body Weights, in Grams, of Hamsters at Various Ages

	Hamilton and Hogan (1944) Colony Diet		Whitney (1965)		
Age	Males	Females	Males	Females	Avg.
Birth					2
5 days					5
10 days					10
15 days					20
21 days (weaning)	29	27			34
4 weeks	42	44			
5 weeks	55	50			
6 weeks	63	60			
7 weeks	70	69			
8 weeks (sexual maturity)	76	77	100	103	
10 weeks	85	93	108	118	
12 weeks	90	105			
18 weeks	100	115			

TABLE 2 Example Diets for Growth and Reproduction of Hamsters

Ingredient	Assumed Dry Matter in Ingredient (%)	Granados (1951) Diet		Hamilton and Hogan (1944) Diet	
		Dry[a] (%)	As Fed[a] (%)	Dry[b] (%)	As Fed[b] (%)
Wheat, grain, ground	89.0	—	—	27.6	28.
Corn, grain, ground	87.6	—	—	19.4	20.
Milk, skimmed, dried	94.3	—	—	15.6	15.
Linseed meal, solvent extracted	90.1	—	—	12.0	12.
Yeast, brewers, dried	93.0	8.0	8.	10.3	10.
Wheat, germ, meal	87.4	—	—	6.8	7.
Alfalfa meal, sun cured	90.7	1.0	1.	5.0	5.
Calcium carbonate	99.6	—	—	1.1	1.
Sodium chloride	100.0	—	—	1.1	1.
Vitamin A–D mixture[c]	100.0	—	—	1.1	1.
Sucrose	99.8	29.9	28.	—	—
Cornstarch	90.4	26.1	27.	—	—
Wheat, flour, entire kernel	88.1	7.6	8.	—	—
Corn, yellow, finely ground	89.0	7.6	8.	—	—
Casein, crude	90.7	13.6	14.	—	—
Swine, liver, dehydrated ground	92.7	3.0	3.	—	—
Salt mixture[d]	100.0	3.2	3.	—	—
Total		100.0	100.0	100.0	100.0

[a] Plus whole raw milk.
[b] Plus whole milk during lactation only.
[c] One g supplies 1,200 IU of vitamin A and 170 IU of Vitamin D.
[d] McCollum's Salt Mixture 185 [in g/100 g: Ca lactate, 35.15; Ca $(H_2PO_4)_2 \cdot H_2O$, 14.60; K_2HPO_4, 25.78; $NaH_2PO_4 \cdot H_2O$, 9.38; NaCl, 4.67; $MgSO_4$, 7.19; Fe citrate, 3.19] supplemented with 13.5 mg KI, 139 mg $CuSO_4 \cdot 5H_2O$ and 556 mg $MnSO_4 \cdot 4H_2O$/100 g.

N.B. The reader also is referred to Table B-1 in Appendix B.

TABLE 3 Examples of Semipurified Diets That Provide for Normal Growth of Young Hamsters

Ingredient	Assumed Dry Matter in Ingredient (%)	Adapted from Hamilton and Hogan (1944) Diet		Adapted from Schweigert *et al.* (1950) Diet		Adapted from Salley and Bryson (1959) Diet	
		Dry (%)	As Fed (%)	Dry (%)	As Fed (%)	Dry (%)	As Fed (%)
Glucose, monohydrate[a]	100.0[a]	61.1[a]	60.0[a]	—	—	—	—
Sucrose[a]	99.8	—	—	58.7	57.4	50.1	49.0
Vitamin supplement in glucose monohydrate or sucrose[b]	100.0	5.1	5.0	5.1	5.0	5.1	5.0
Casein, purified	90.7	18.5	20.0	22.3	24.0	22.3	24.0
Cellulose	100.0	3.1	3.0	3.1	3.0	8.2	8.0
Corn oil	100.0	—	—	5.1	5.0	—	—
Lard	100.0	7.1	7.0	—	—	—	—
Cottonseed oil	100.0	—	—	—	—	10.2	10.0
Salt mixture	100.0	4.1	4.0[c,f]	4.1	4.0[d,f]	4.1	4.0[e,f]
Liver extract[g]	92.4	—	—	1.0	1.0	—	—
Cod liver oil	100.0	—	—	0.3	0.3	—	—
Cystine	100.0	—	—	0.3	0.3	—	—
Vitamin A and D supplement	100.0	1.0	1.0[h]	—	[i]	—	[j]
Total		100.0	100.0	100.0	100.0	100.0	100.0

[a] Values expressed are for the monohydrate, not anhydrous glucose. (See *Energy*, *Carbohydrates*, and *Bulk Formers* for comments on carbohydrate source.)

[b] Percent of ingredient in vitamin supplement in glucose monohydrate or sucrose. Numbers in parentheses are mg of the vitamin provided per kg of diet as fed.

Menadione	0.060 (30)	0.012 (6)	0.060 (30)
Thiamin hydrochloride	0.016 (8)	0.012 (6)	0.016 (8)
Riboflavin	0.032 (16)	0.012 (6)	0.032 (16)
Pyridoxine hydrochloride	0.024 (12)	0.012 (6)	0.024 (12)
Calcium pantothenate	0.020 (10)	0.080 (40)	0.020 (10)
Niacin	0.100 (50)	0.040 (20)	0.100 (50)
Choline chloride	8.000 (4.000)	2.000 (1.000)	8.000 (4.000)
Folic acid	—	0.004 (2)	0.010 (5)
Biotin	—	0.0002 (0.1)	—
Vitamin B_{12}	—	0.0001 (0.05)	0.00012 (.06)
Inositol	5.000 (2.500)	2.000 (1.000)	5.000 (2.500)
p-Aminobenzoic acid	2.000 (1.000)	0.600 (300)	2.000 (1.000)
Glucose monohydrate or sucrose	84.748	95.2277	84.7379
Total	100.0	100.0	100.0

α-Tocopherol is added to the diet in the oil portion of the diet or in solution in ethyl alcohol. Dry stabilized forms of *d*-tocopheryl acetate can be mixed in the vitamin mix. All three diets contain 25 mg α-tocopherol per kg diet as fed.

[c] In parts by weight: $CaCO_3$, 125.2; $Ca_3(PO_4)_2$, 376.3; $MgCO_3$, 25.0; $MgSO_4 \cdot 7H_2O$, 32.8; NaCl, 69.0; KCl, 112.0; KH_2PO_4, 212.0; $FePO_4 \cdot 4H_2O$, 20.5; $MnSO_4 \cdot 4H_2O$, 25.5; $CuSO_4 \cdot 5H_2O$, 1.4; $Al_2(SO_4)_3$ K_2SO_4 $2H_2O$, 0.17; KI, 0.08.

[d] In parts by weight: $CaCO_3$, 600; K_2HPO_4, 645; $CaHPO_4 \cdot 2H_2O$, 150 $MgSO_4$ $7H_2O$, 204; NaCl, 335; Ferric citrate $6H_2O$, 55; KI, 1.6; $MnSO_4 \cdot 4H_2O$, 10.0; $ZnCl_2$, 0.5; $CuSO_4 \cdot 5H_2O$, 0.6 (Hegsted *et al.*, 1941).

[e] USP XIV salt mixture contains in parts by weight (all compounds USP or USP Reagent, see U.S. Pharmacopoeia, 14th Ed. p. 789, 1950): $CaCO_3$ 68.6; Ca citrate $4H_2O$, 308.3; $Ca(H_2PO_4)_2$ H_2O, 112.8; $MgCO_3$, 35.2; $MgSO_4$, 38.3; KCl, 124.7; K_2HPO_4, 218.8; NaCl, 77.1; $CuSO_4$ $5H_2O$, 0.078; ferric ammonium citrate, 15.3; $MnSO_4$ H_2O, 0.201; ammonium alum, 0.092; KI 0.040; NaF, 0.507.

[f] Williams and Briggs (1963) have criticized many of the mineral mixtures used in the past. They have developed new mineral mixtures that are more adequate and more stable in certain required elements. One has been used in a semipurified diet for hamsters at 3.5% of the diet (Cohen *et al.*, 1967). Its composition in grams of reagent grade salts to make 21 kg of total mix follows: $CaCO_3$, 4.350; $CaHPO_4$, 6.780; Na_2HPO_4, 3.906; KCl, 4.380; $MgSO_4$, 1.380; $MnSO_4$ H_2O, 92.4; ferric citrate (16.7% Fe), 90.6; $ZnCO_3$, 12.6; $CuSO_4$, 7.8; and KIO_3, 0.6.

[g] 1:20 Liver extract, Wilson Laboratories, Chicago, Ill.

[h] Vitamin A 12,000 IU/kg diet and Vitamin D 1,700 IU/kg diet were supplied as a supplement in lard by Hamilton and Hogan (1944). Dry, stabilized forms, now in use, can be included in the vitamin supplement.

[i] Vitamin A 18,000 IU/kg and Vitamin D 2,550 IU/kg diet were supplied by Schweigert *et al.* (1950) in the cod liver oil. Dry, stabilized forms, now in use, can be included in the vitamin supplement.

[j] Vitamin A 250 IU/animal weekly was administered orally by Salley and Bryson (1959) as a separate supplement in oil; Vitamin D 4,320 IU/kg diet was included also in the diet. Dry, stabilized forms, now in use, can be included in the vitamin supplement.

TABLE 4 Percentage or Amount, per kg of Diet,[a] of Nutrient Requirements

Nutrient	Growth	
	Dry	90% Dry Matter
Total protein (%)	17.8	16.0
Calcium (%)	0.67	0.60
Phosphorus (%)	0.39	0.35
Vitamin A (IU/kg)	20,000.	18,000.
Vitamin D (IU/kg)	[b]	[b]
α-Tocopherol (mg/kg)	28.	25.
Vitamin K (mg/kg)	[c]	[c]
Biotin (mg/kg)	[c]	[c]
Choline (mg/kg)	[c]	[c]
Folic acid (mg/kg)	[c]	[c]
Niacin (mg/kg)	[c]	[c]
Pantothenic acid (mg/kg)	11.	10.
Pyridoxine (mg/kg)	6.7	6.0
Riboflavin (mg/kg)	6.7	6.0
Thiamin (mg/kg)	6.7	6.0
Vitamin B_{12} (mg/kg)	0.011	0.01

[a] Most of the values represent diets that have been shown to be adequate and, hence, are probably in excess of the actual requirements.
[b] Requirement demonstrated only with unsatisfactory supply of calcium and phosphorus, about 2,000 IU/kg diet commonly used in diets.
[c] Qualitative requirement not demonstrated or in doubt; see Table 3 for amounts used in typical semipurified diets.

purified diets for growing hamsters are shown in Table 3.

Table 4 summarizes our knowledge of the levels of nutrients that apparently meet the requirements of the growing hamster. They are based primarily on levels used in successful experimental diets and, thus, may be considerably in excess of the actual requirements. Moreover, no account can be made for differences in requirements caused by variation in gastrointestinal flora, which has been postulated to explain some of the controversies on nutrient requirements existing in the literature.

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NUTRIENT REQUIREMENTS OF THE MONKEY

The use of nonhuman primates in many types of biomedical investigation has increased enormously during the past decade. The period of systematic accumulation of comparative data on nutrient requirements had largely passed prior to this new interest in nonhuman primates. Therefore, except for certain nutrients of particular current interest, most of the data on requirements are from relatively few reports. There are several difficulties in establishing nutrient requirements for primates. The members of the order Primata are extremely diverse as to size and the types of natural foods eaten. Washburn (1966) has commented on this diversity: "If we view the primates as an ancient, successful adaptation to arboreal life which led to the evolution of many forms, we can see why it is not easy to give a simple definition of the group." It is possible to overemphasize the importance of food selection by free-ranging primates as an indication of an optimum diet, particularly when the variety of selection is progressively more limited by many pressures, particularly those of man himself. The range of foods ingested by free-ranging primates is not entirely known. Nevertheless, the large variations in gut morphology between the various genera of monkeys, in the proportion of gut volume to animal size and in the intestinal contents of wild specimens (Fooden, 1964), enforce the probability of intragenera differences in optimum natural foods and, perhaps, in nutrient requirements. Ratcliffe (1966) has, however, successfully fed a wide range of primates a single diet fabricated from a variety of natural components.

Except for the rhesus monkey and chimpanzee, the data are inadequate on growth and development of nonhuman primates. The nutritional evaluation of these primates is thus very difficult. Monkeys also have a variety of infectious diseases, many of which are not well defined and which, presumably, influence nutritional status. Even in the rhesus monkey (van Wagenen and Catchpole, 1956, 1964) and chimpanzee (Gavan, 1953; Spence and Yerkes, 1937), where growth and development data are available, there are inadequate criteria of optimum nutrition. The chimpanzee, for example, becomes obese when fed a semipurified diet of high caloric density under *ad libitum* conditions (Andrus *et al.*, 1968), and weight alone is thus probably a very incomplete index of health in that species. The objectives of different investigators using primates will vary. Conceivably, the nature of the optimum diet will vary as well. Insofar as nonhuman primates are to serve as models of humans and as tools for defining a more ideal way of life for man, the nutritional objective is presumably a long and vigorous life. There are data on this subject in nonhuman primates.

Since most information about the nutrition of nonhuman primates is on the requirements of the rhesus monkey (*Macaca mulatta*), the following sections will, of necessity, emphasize that species. Pertinent comments about other species will be included in each section.

GROWTH

Several parameters of growth and development of the rhesus monkey and chimpanzee are available. Van Wagenen and Catchpole (1956) have presented the weights of a large series of male and female rhesus monkeys according to age (Figures 1 and 2). The same workers (van Wagenen and Catchpole, 1964) have data for fetal and placental weights. Pickering and Kontaxis (1961) have also presented a study of weights of rhesus monkey fetuses. Kerr and Waisman (1966b) described in detail weight changes of the rhesus monkey during the first year of life (Figure 3). Some of the most useful criteria of growth and matura-

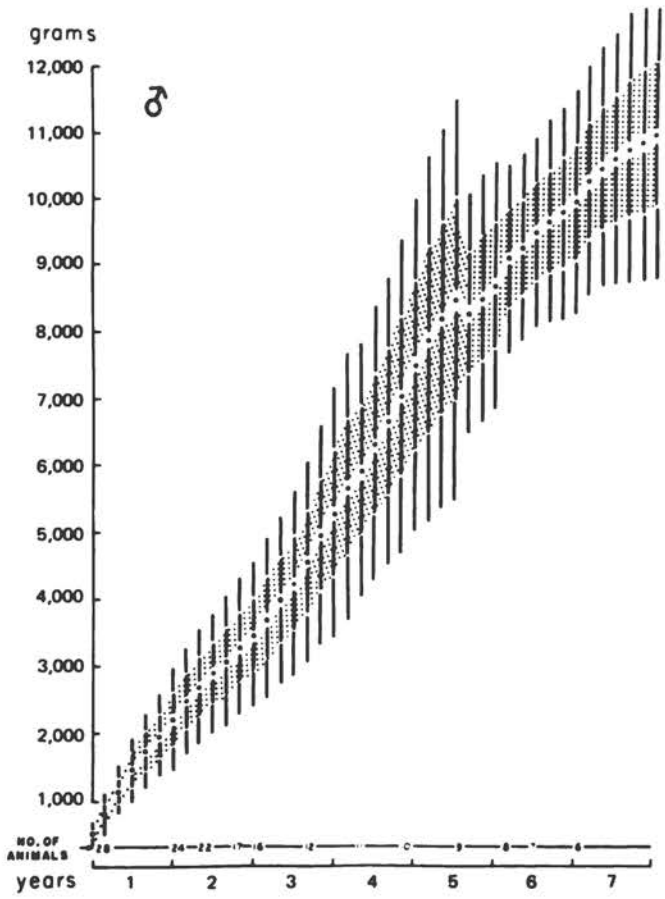

FIGURE 1 Body weight of male macaques in relation to chronological age. Means, with one (68.3 percent) and two (95.4 percent) standard deviations. (Figure and legend supplied by van Wagenen and Catchpole, 1956.)

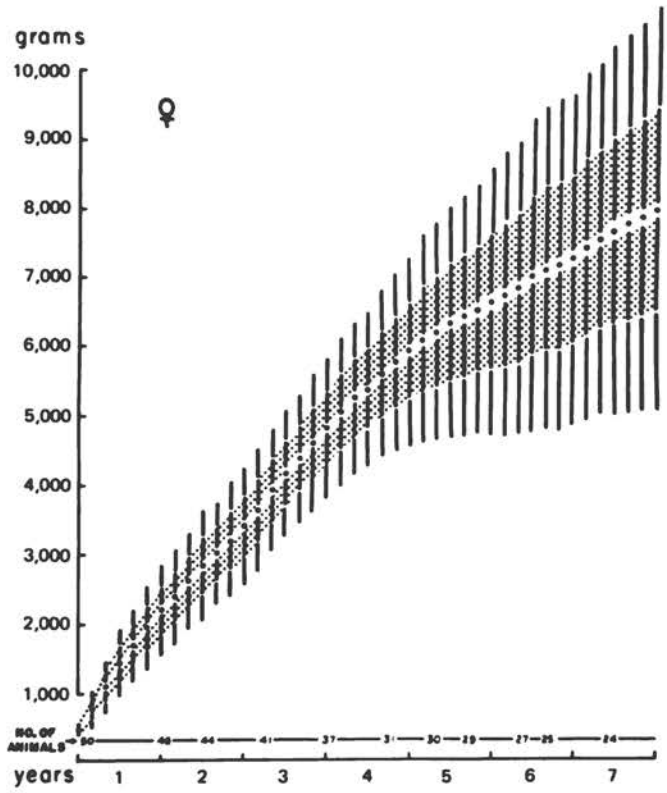

FIGURE 2 Body weight of female macaques in relation to chronological age. Included are areas showing one (68.3 percent) and two standard deviations (95.4 percent) of the mean. (Figure and legend supplied by van Wagenen and Catchpole, 1956.)

tion are emergence of teeth and appearance of centers of bone ossification. Hurme and van Wagenen (1953, 1956; van Wagenen and Hurme, 1950) have presented comprehensive data on the teeth of rhesus monkeys.

ENERGY

The question of energy requirements can be approached by considering energy expenditure or food intake. Basal metabolism has been estimated in several primates, including the rhesus monkey. The observation that basal metabolism—determined from oxygen uptake and total body calorimetry—of animals of different ages and different species can be normalized to a rather constant value when expressed in terms of surface area appears to hold for a variety of primates as well (Stahl and Malinow, 1967). Since body area is proportional to body weight to the 0.67 power (Lee and Fox, 1933), the basal metabolism per kg body weight will fall with increasing weight. Thus, mean basal metabolic rates of 650 kcal/m^2 (Bruhn, 1934) are equivalent to 49 kcal/kg for a 4-kg adult and 80 kcal/kg for a 500-g neonatal animal. These values compare (Dawes *et al.*, 1960) to observed values of oxygen uptake in anesthetized rhesus monkeys, which are equivalent to 40 kcal/kg for adults and 90 kcal/kg for infants. Bruhn (1934) have reported the basal metabolism of the macaca, mangabey, baboon, gibbon, orangutan, and chimpanzee. Similar values are available for the growing chimpanzee (Bruhn and Benedict, 1936), tree shrew (Nelson and Asling, 1962), and squirrel monkey (Malinow and Wagner, 1966). There are few, if any, good estimates of the actual increments of energy expenditure by nonhuman primates associated with work, pregnancy, and lactation. Based on comparisons with other species, a total energy requirement for the adult rhesus monkey is estimated to be 100 kcal/kg, for a neonatal animal 200 kcal/kg, for a pregnant female 125 kcal/kg, and for a lactating female 150 kcal/kg.

Another approach to energy requirements may be gained from intake figures. Kerr and Waisman (1966b)

have summarized the food intake and weights of infant rhesus monkeys during the first year of life (Figure 3). Animals maintained on Similac formulas *ad libitum* during this period gained from a mean birth weight of just under 500 g to nearly 2,500 g at 1 year. The mean *ad libitum* intake in kcal/kg of body weight/day dropped, more or less linearly, from a maximum of 264 at 60 days to 186 at 360 days. The weight gain figures were similar to those for another group of monkeys in the same laboratory that was allowed access to commercial chow during this period. The energy requirement for adolescent rhesus monkeys is probably satisfied by 100 kcal/kg/day. Deo and Ramalingaswami (1960) maintained body weight in 2.5 kg animals by supplying this level by tube feeding. Nine male monkeys in the 3.5–4.0 kg weight range were shown to consume 170 g of a commercial monkey chow *ad libitum* (Hamilton and Brobeck, 1965). This is equivalent to 650 kcal by bomb calorimetric analysis or more than 150 kcal/kg body weight. These latter animals showed marked weight gain. Robbins and Gavan (1965) found the formula of Crampton and Lloyd (1959) to predict the caloric requirement of their rhesus monkeys. This formula, kcal = 93 (wt in kg)$^{0.75}$ gives a value of 66 kcal/kg for a 4-kg animal and 50 kcal/kg for a 12-kg animal. A group of 17 rhesus monkeys with a mean body weight of 7 kg (range 4.0–12.1 kg) were maintained in mean weight equilibrium on a commercial monkey chow supplied at levels predicted by the above formula (Robbins and Gavan, 1966). Thus, a daily allowance for the adult rhesus monkey of 100 kcal/kg body weight should be generous.

PROTEIN

For a wide range of mammalian species, the minimum protein requirement is closely related to the basal metabolic rate. Hegsted (1964) has shown, from the tabulated data of Brody (1945), that endogenous urinary nitrogen is accurately predicted by the formula N (mg/day) = 146 (wt in kg)$^{0.72}$. This is equivalent to 2 mg N/basal kcal. To this minimum requirement

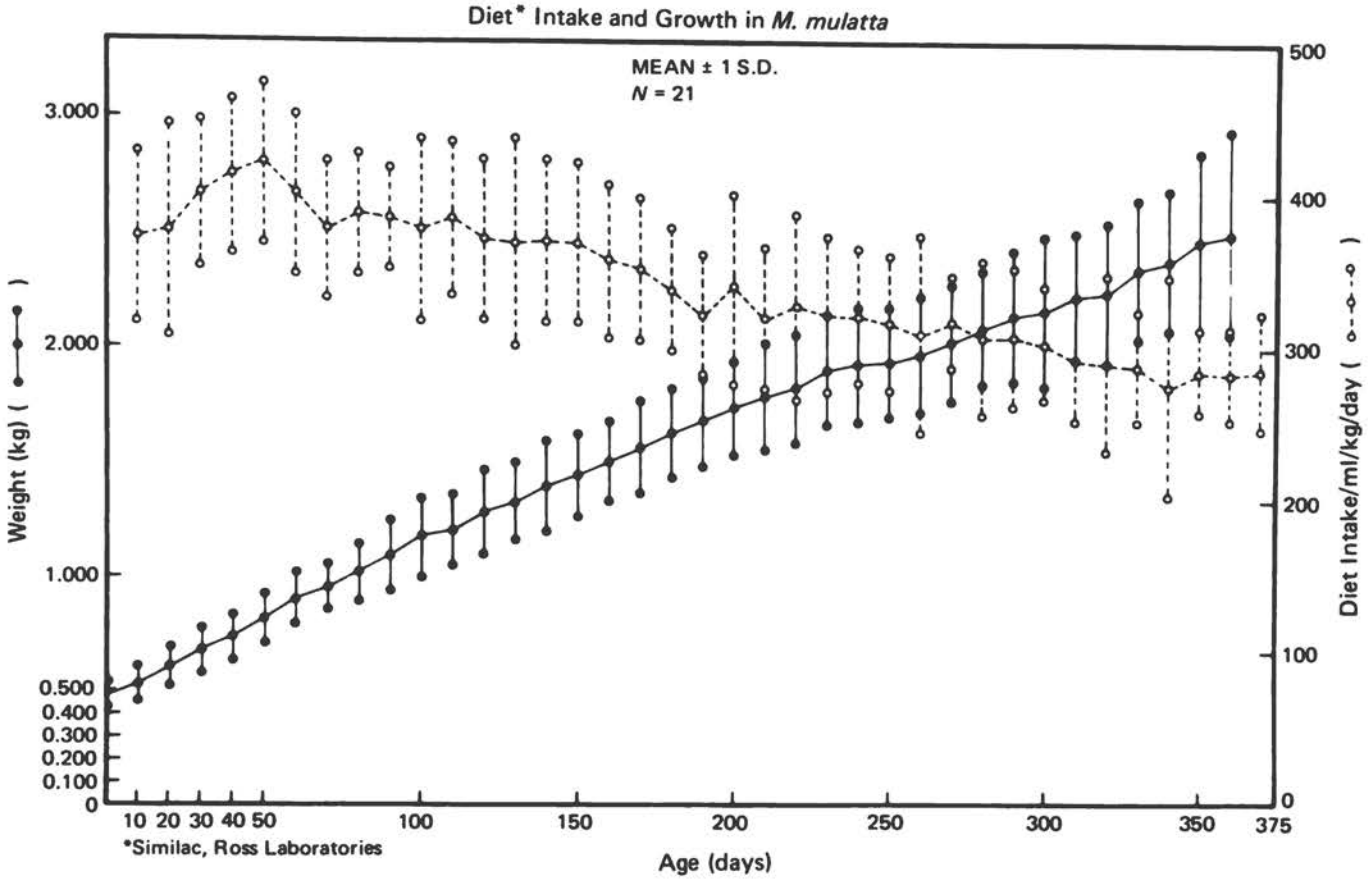

FIGURE 3 *Ad libitum* dietary intake related to growth of macaque infants during the first year of life. It is apparent that energy requirement, per unit body weight, decreases with age. (Figure and legend from Kerr and Waisman, 1966b.)

must be added allowances for growth, pregnancy, and lactation. The computed minimum protein must also consider the loss in sweat, integument, and hair, and the minimum intake must allow for true digestibility coefficient and the biological value of the protein.

Kerr and Waisman (1966b) (Figure 3) have studied weight gains and *ad libitum* intake of a commercial formula [shown to resemble the composition of rhesus monkey milk (van Wagenen *et al.*, 1941)]. In those studies, protein intake varied from 7.30 g/kg at 60 days of age (about 900 g body weight) to 5.17 g/kg at 360 days (about 2,400 g body weight). If one calculates the protein needs from the theoretical considerations discussed by Hegsted (1964) or McCance and Widdowson (1964), it is apparent that a considerable excess of protein above that theoretically required for equivalent weight gain has been supplied. For example, the Hegsted formula predicts a minimum requirement of about 1 g absorbed protein of biological value of 100 for a 1-kg monkey. The monkeys described by Kerr and Waisman were growing at about 6 g/day, resulting in a further protein requirement of about 1.2 g/day. Corrections for 80 percent digestibility and 80 percent biological value (assumed values) give a value of about 3.5 g compared to the approximately 7.0 g actually ingested. The evidence from studies of human infants indicate that substitution of an isocaloric diet containing the lower level of protein would have resulted in equivalent growth. Ordy *et al.* (1966) compared 3 levels of protein (presumably casein) at 3.5, 17, and 24 percent by weight in semipurified diets in rhesus monkeys between the thirteenth and twentieth weeks of age. The rates of weight gain on the two higher protein diets were equivalent to those seen by Kerr and Waisman (1966b), although the 13-week weights were lower in the animals of Ordy. The animals on the 3.5 percent protein diets (isocaloric) lost a small amount of weight. Plasma cholesterol, protein, and albumin levels were lower in the low protein group than in the 17 percent or 24 percent groups. Liver glycogen and alkaline phosphatase concentrations were decreased, and liver lipid levels were increased in animals fed the 3.5 percent protein diet. Based on the caloric intakes of the animals in the studies of Kerr and Waisman (Bruhn, 1934), the 3.5 percent protein diet would be equivalent to about 2.5 g protein/kg body weight.

Day and associates (1935; Langston *et al.*, 1938) observed good growth in 2- to 3-kg monkeys fed 10 g of casein per day. May *et al.* (1950) obtained good growth in 1-kg monkeys fed diets containing 3 g protein in milk formulas, consisting of as little as 1.5 percent (w/v) protein and providing approximately 9 percent of total calories as protein. In spite of the similarity of rhesus monkey milk to that of humans in other respects, rhesus monkey milk still contains 16 percent of metabolizable calories as protein (van Wagenen *et al.*, 1941). Day (1962) concluded that the quantities used by May *et al.* (1950) were adequate for the young rhesus monkey of about 1-kg body weight. It is important to note that a diet based on 3 g protein/day is probably adequate for the very young rhesus monkey only when there is a relatively low ratio of protein to calories and calories are supplied essentially *ad libitum*.

Robbins and Gavan (1966) maintained approximate nitrogen balance in a group of adult rhesus monkeys (4.0–12.1 kg) of 7 kg mean weight with an intake of 2.85 g N/day. This is a mean value of about 2.5 g of protein/kg of body weight/day. The studies of Robbins and Gavan were based on a commercial monkey diet that contained 16.4 percent protein by weight. They observed a mean digestibility coefficient for that protein of 83.4 percent. Perhaps nitrogen balance could have been maintained at a lower level of protein with equivalent or higher levels of total calories.

No definite data are available on the protein requirements of pregnancy and lactation in rhesus monkeys; extrapolation from human studies indicates an increase of 25 percent in pregnancy and 50 percent for lactation without an increase in protein-to-energy ratios. Many animals, however, require an absolute increase in protein concentration of the diet during lactation (Blaxter, 1964), and this may be true of the rhesus monkey.

There are a large number of studies of the induction of protein deficiency in monkeys, particularly of pathology of the liver and of the formed elements of blood (e.g., Bruhn and Benedict, 1936; Deo *et al.*, 1965; Follis, 1957; Ghitis *et al.*, 1963; Kerr and Waisman, 1966a; Kerr *et al.*, 1965; Mann and Andrus, 1956; Racela *et al.*, 1966; Ramalingaswami, 1964; Sood *et al.*, 1965; Waisman *et al.*, 1959; Wilgram, 1959; Wilgram *et al.*, 1958).

The deleterious effects in monkeys of excess (Kerr and Waisman, 1966a; Kerr *et al.*, 1956; Waisman *et al.*, 1959) and deficiency (Mann, 1966) of particular amino acids have been described. Excess dietary phenylalanine causes increased levels of this amino acid in the blood and urine and produces mental retardation in infant rhesus monkeys. Excess histidine causes hyperlipemia in rhesus monkeys, whereas a deficiency of total sulfur amino acids produces a similar effect on Cebus monkeys.

FAT

There is abundant evidence that the rhesus and many other species of monkeys are similar to man in responding to changes in the type of dietary fat with changes in the level of cholesterol and other lipids in the blood (Cox *et al.*, 1958; Malmros and Wigand, 1965; Portman *et al.*, 1956) and blood vessels (Portman and Andrus, 1965; Portman *et al.*, 1967; Taylor *et al.*, 1962; Wissler *et al.*, 1962). Although the rhesus monkey gets marked skin xanthomatosis and atherosclerosis of the aorta and the peripheral, coronary, and cerebral arteries when the plasma cholestorol level is elevated to a high level (Armstrong *et al.*, 1965; Mann, 1966), these changes occur only when diets similar in lipid composition to those consumed by Western man are fed. Most monkeys, including the rhesus, will thrive on diets containing very low fat levels or those containing as much as 25 percent by weight as fat. It may be necessary with extreme changes in the type or level of fat to make the changes in stages over several days or even weeks.

There are reports of a specific requirement that can be satisfied by fat that contains linoleic acid or by linoleic acid per se. Skin changes (scaliness, cracking, loss of hair, hair color changes) (Figure 4), widespread compositional changes of tissue lipids, and alterations possibly related to increased red blood cell destruction and formation (e.g., marrow hyperplasia, extramedullary erythropoiesis, blood pigment accumulation in the liver and spleen) have been observed in essential fatty acid deficiency in rhesus (Fitch *et al.*, 1961; Greenberg and Moon, 1961) and Cebus monkeys (Portman *et al.*, 1959, 1961). In the studies of Greenberg and Moon (1961), 0.5 ml of ethyl linoleate administered to young, 2-kg rhesus monkeys, chronically fed diets very low in essential fatty acids, caused a return of erythrocyte fatty acid composition toward a normal pattern: increased arachidonate and decreased eicosatrienoate levels. Cebus monkeys fed diets supplying about 300 mg of linoleate per day had erythrocyte fatty acid patterns resembling those in monkeys fed 6 g of linoleate per day, and very different from animals fed diets devoid of linoleate. Abnormal erythrocyte lipid patterns were induced by feeding diets devoid of fat or containing completely hydrogenated fat. Linoleic acid supplied at 1 percent of the total calories will thus probably prevent obvious deficiency signs. The level and type of dietary fat has an effect on the requirement of certain of the vitamins, particularly vitamin 1 (see below).

MINERALS

The optimum levels of dietary minerals are particularly difficult to determine. There are relatively few pertinent studies on primates. Harris *et al.* (1961) made a detailed study of calcium metabolism in rhesus monkeys. They used a series of monkeys with a mean body weight of 3 kg, with a diet based on the mineral formula of Hegsted *et al.* (1941) and a probable food intake equivalent to about 500 mg of Ca/day. A mean daily calcium accretion of 243 mg/day (about 80 mg/kg body weight) was calculated, and a mean urinary excretion of 32 mg/day and a fecal excretion of 215 mg/day observed. Since the fecal calcium was shown to be largely unabsorbed dietary calcium, there was an apparent absorption efficiency of about 60 percent and a net accretion of over 80 percent of the absorbed calcium. Thus, 150 mg of Ca/kg of body weight/day is apparently near a minimum requirement for young rhesus monkeys. Macdonald *et al.* (1956) calculated that rhesus monkey fetuses in the last portion of the gestational period accumulate 40 mg of Ca/kg of body weight/day, equivalent to 20 mg of absorbed Ca/day for the term fetus. Yen and Shaw (1963) studied the uptake of calcium by calcified tissues of the rhesus monkey with particular emphasis on the teeth. Vitale *et al.* (1963) have observed a magnesium deficiency syndrome in cebus monkeys fed diets low in magnesium, and they prevented the appearance of these signs with an intake of 96 mg/100 g of diet (approximately 40 mg/kg of body weight/day).

Zinc deficiency has been induced in squirrel monkeys using semipurified diets based on specially processed casein (Barney *et al.*, 1967; Macapinlac et al., 1967). It was observed that 15 ppm of added zinc supported good growth, whereas 0.5 ppm did not. A zinc requirement of 1 μg/g body weight/day was suggested.

Several salt mixtures have been used with apparent success, notably those of Hegsted (1964) and of Hawk *et al.* (1949).

FAT-SOLUBLE VITAMINS

Vitamin A

Several studies of experimental vitamin A deficiency in rhesus monkeys have appeared since the review of

Day (1944), including reports of various ocular changes (e.g., Leach and Lloyd, 1956; Ramalingaswami *et al.*, 1955; Rodger *et al.*, 1961). Ramalingaswami *et al.* (1955) described decreased plasma vitamin A levels, failure to gain weight, and decided loss of night vision in young rhesus monkeys subjected to a vitamin A-deficient diet. There was destruction of both rod and cone cells and degeneration of the retinal pigment epithelium. Degeneration of the corneal epithelium with small patches of keratinization was also manifested. The preference for the form of vitamin A aldehyde, in the aldehyde to acid oxidation reaction by liver *in vitro*, and for the rate of aldehyde oxidation was similar to that for the pig and chicken (Lakshmanan *et al.*, 1964). There are apparently no studies of minimal or optimal dietary levels of vitamin A. In the study of Ramalingaswami, control animals were protected by 1,500 IU of vitamin A administered twice weekly.

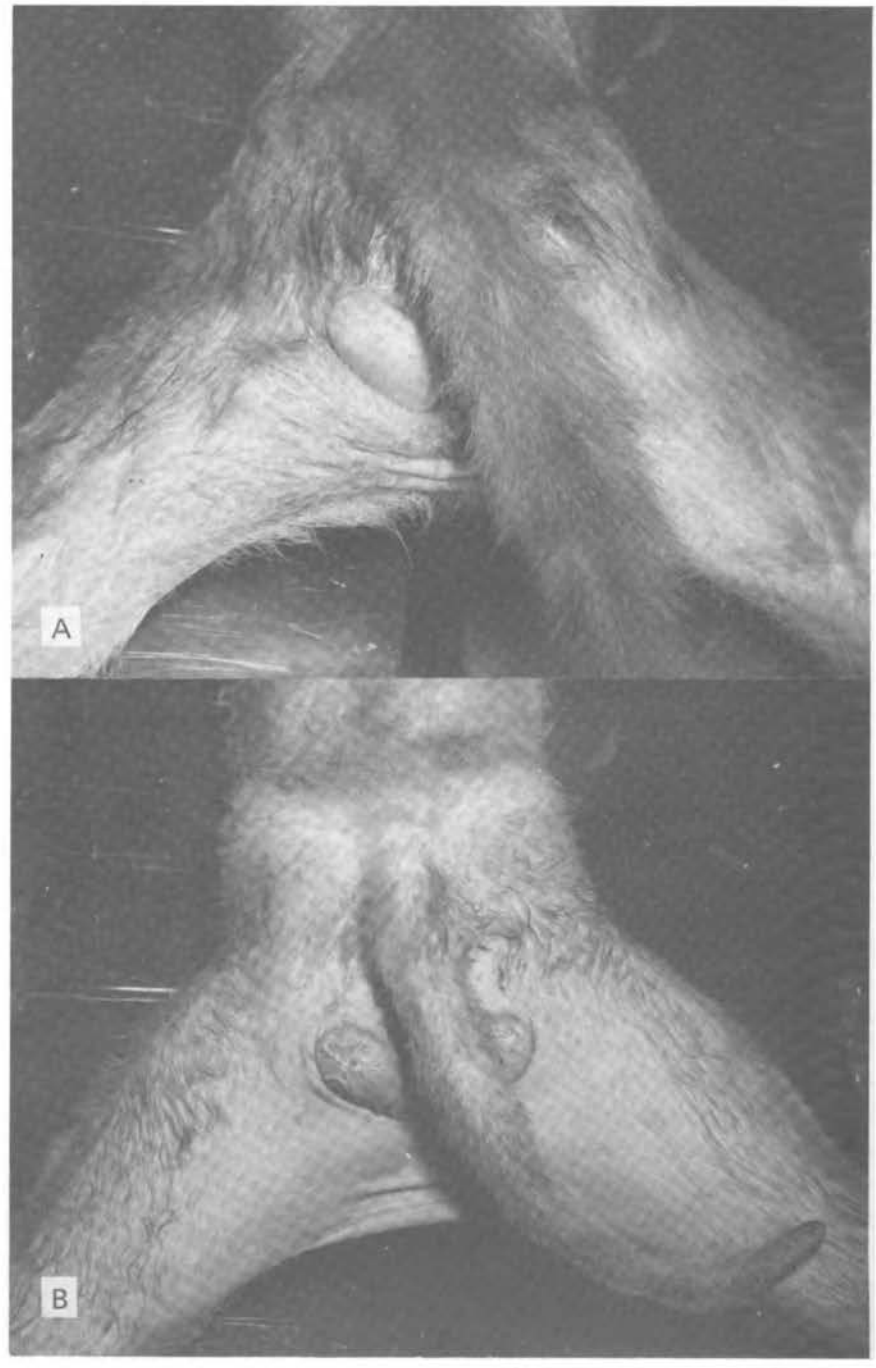

FIGURE 4 Photograph of rhesus monkey receiving a fat-containing diet (A) and a low-fat diet (B). Note the loss of hair on the monkey that was fed the low-fat diet. (From Fitch *et al.*, 1961.)

Vitamin D

A requirement for vitamin D by the rhesus monkey has been established, but minimal and optimal dietary levels are not known. Gerstenberger (1938) produced rickets in 81 young rhesus monkeys and showed that sunlight or cod liver oil would induce healing of bony lesions. Kent *et al.* (1957) accidentally produced hypervitaminosis D in a colony of 558 rhesus monkeys (2.5–10 kg) by giving them 162,000 USP units of vitamin D/day for 3 months. The findings were weight loss, anemia, elevated blood urea nitrogen and serum calcium, and increased incidence of infections. Histological findings included calcium and iron deposits with foreign body type of inflammatory reactions in the kidneys, salivary glands, and lungs. The lesions regressed after the elimination of the vitamin D excess, and they were not observed after 1 year.

There is considerable evidence that all monkeys may not be alike in their requirements for a given form and level of vitamin D or both. Rhesus monkeys have been maintained on a variety of diets, including commercial diets based on vitamin D_2 (irradiated ergosterol) without evidence of bone disease. On the other hand, many species of New World monkeys (Family Cebidae) are susceptible to demineralization and fibrous displasia of bone, which seems to respond to a change from vitamin D_2 to vitamin D_3 (irradiated 7-dehydrocholesterol). Stare *et al.* (1963) presented a description of some of these lesions in the woolly monkey (*Lagothrix lagotricha*) maintained on diets supplying 100–200 units of vitamin D_2 daily. These lesions regressed with the lower level (100–200 units) of vitamin D_3 (O. W. Portman, unpublished data). Parenteral vitamin D_2 or D_3 at 50,000 units/week induced bone recalcification, kidney calcification, and kidney failure, and it was tentatively assumed on this basis that the inadequacy of vitamin D_2 in the woolly monkey is at the absorption level (O. W. Portman, unpublished data). Hunt *et al.* (1966) have described similar lesions in *Cebus albifrons* and marmosets (*Saquinis* sp.), and Lehner *et al.* (1966, 1967) observed lesions in squirrel monkeys (*Saimiri sciurea*), which appeared during the feeding of diets containing vitamin D_2 and regressed when vitamin D_3 was substituted for vitamin D_2. The latter workers demonstrated that 10 units of vitamin D_2/g of diet were inadequate, while as little as 1.25 units of vitamin D_3/g of diet prevented bone lesions. In January 1965, the use of vitamin D_3 in lieu of vitamin D_2 was initiated in semipurified diets for New World monkeys at the Oregon Regional Primate Research Center. Prior to that time some 200 squirrel monkeys came to autopsy with a high incidence of gross or radiologically detectable bone lesions. Between January 1965 and June 1967, 305 squirrel monkeys had been autopsied and found to be apparently free of gross lesions. A particularly severe fibrous dysplasia was induced in 3 Cebus monkeys fed a diet containing vitamin D_2 and 10 percent hydrogenated coconut oil. These animals died in 5–8 months and had extremely flexible long bones (O. W. Portman, unpublished data). Cebus monkeys have, however, been maintained in apparent good health for over 5 years on diets including more unsaturated fats and vitamin D_2 (O. W. Portman, unpublished data). Hunt *et al.* (1967) have shown that vitamin D_3 increases calcium absorption in the deficient cebus monkey, whereas vitamin D_2 does not.

Vitamin E

The effects of vitamin E deficiency and the interrelationships of vitamin E requirements with the supply of other nutrients have been extensively studied in the rhesus monkey. Mason and Telford (1946, 1947) and Filer *et al.* (1949) presented the first studies of vitamin E deficiency in the rhesus monkey. Several comprehensive studies (Day and Dinning, 1956; Dinning and Day, 1957a,b) have been made of this muscular dystrophy syndrome. In addition, these workers have observed a vitamin E responsive anemia (Dinning, 1963; Porter *et al.*, 1962) and have proposed that vitamin E is a specific maturation factor for cells of the erythroid series as well as a factor influencing erythrocyte survival. Although the requirement for vitamin E depends in part on the level of unsaturated fat in the diet, Fitch and Dinning (1963) have shown that the deficiency can still be produced on a fat-free diet (Figure 5). They observed a mean requirement of 2.6 mg vitamin E/kg body weight for animals fed a diet containing 8 percent stripped lard and 3 percent cod liver oil and 0.7 mg for the fat-free-diet. These calculations, based on creatine-to-creatinine excretion ratios, are equivalent to 1 mg vitamin E for approximately 3 g of polyunsaturated fatty acids. Horwitt (1962), on the basis of the nutritional supply of vitamin E to prevent *in vitro* hemolysis of adult human erythrocytes, proposed a value of approximately 1 mg vitamin E for each 1.2 g of linoleic acid in the diet as a minimum requirement.

Vitamin K

Two studies of attempts to induce vitamin K deficiency in rhesus monkeys have been described. Metta

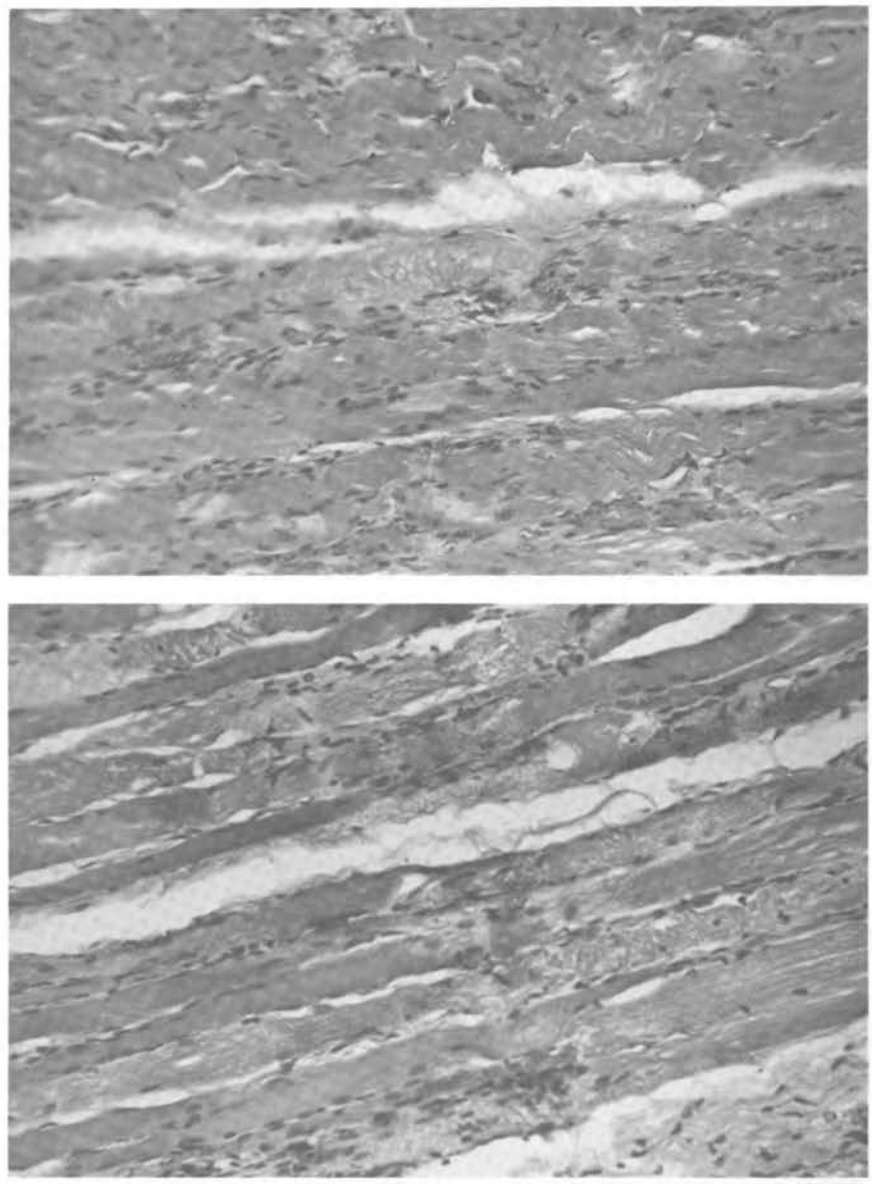

FIGURE 5 Skeletal muscle lesions in vitamin E-deficient rhesus monkeys. The upper photomicrograph is from a monkey that developed vitamin E deficiency while receiving a fat-deficient diet. The other photomicrograph is from a monkey that received the diet containing fat. Both sections illustrate degeneration of muscle fibers and increased numbers of sarcolemma nuclei. (From Fitch and Dinning, 1963.)

and Gopalan (1963) attempted to suppress the intestinal flora by feeding sugar-based synthetic diets supplemented with antibiotics. However, the diet itself supplied 4 μg vitamin K_2/kg body weight. The blood prothrombin time was not elevated. Hill *et al.* (1964), using diets based on soy protein or irradiated beef with and without antibiotics, did observe increased prothrombin times and caused a reversion to normal prothrombin times with supplements of 0.1 μg of vitamin K/kg body weight. It is not clear how successfully coprohagy was prevented in these experiments.

WATER-SOLUBLE VITAMINS

Ascorbic Acid

Shaw *et al.* (1945) produced chronic scurvy in rhesus monkeys by giving 0.25 mg of ascorbic acid/kg body weight after deficiency signs had developed on a scorbutic diet. The daily administration of 7.5 mg of ascorbic acid/kg/day completely alleviated the deficiency signs. Day (1944) estimated the requirement of ascorbic acid of 2- to 4-kg monkeys to be 2 mg/kg/day or less. The deficiency signs (Day, 1944) include hemorrhages of the gums, loose teeth, exophthalmos, muscular tenderness, subcutaneous and intramuscular hemorrhages, "rosary" swelling if the ribs, and subperiosteal hemorrhage and effusions. May *et al.* (1950, 1951; Proehl and Day, 1952) produced megaloblastic anemia in monkeys fed a milk diet. This abnormality of peripheral blood and marrow could be eliminated with ascorbic acid, folic acid, or folinic acid, but not with vitamin B_{12}. The level of ascorbic acid used was 25 mg/kg/day. May (1950) concluded that the megaloblastic anemia

produced by the low ascorbic acid milk diet actually resulted from a secondary folic acid deficiency. The concentration of liver folate in monkeys fed a milk diet with 50 mg of ascorbic acid per day was 1.42 μg/g. The concentration in monkeys with the anemia associated with the milk diet alone was 0.18 μg/g. Abt *et al.* (1962), on the basis of studies with labeled ascorbate in a 1.19-kg monkey given 25 mg of ascorbic acid/day, calculated an ascorbic acid turnover of 21 mg/day. About one half was excreted as CO_2, the remainder being excreted in the urine. Dutra de Oliviera *et al.* (1956) observed that of 50 mg/day of ascorbic acid supplied to 3- to 4.5-kg rhesus monkeys only 2–3 mg were excreted in the urine.

The specific steps in the conversion of glucuronolactone to ascorbic acid, which are absent in man and the guinea pig, have also been shown to be lacking in the rhesus monkey (Burrs, 1957; Chatterjee *et al.*, 1961). Shaw (1949) reported a requirement for ascorbic acid by the cebus monkey. Scurvy was induced in squirrel monkeys and then cured by feeding 10 mg of ascorbic acid/kg body weight/day (Macapinlac *et al.*, 1967). Elliott *et al.* (1966) have shown that L-1,4-gulonolactone can be converted to ascorbic acid by the liver of the tree shrew and slow loris. If these primates also have the capacity to reduce glucuronolactone to L-gulonolactone [the rhesus monkey apparently does not have this capacity (Chatterjee *et al.*, 1961)], they presumably do not require an exogenous source of ascorbic acid.

Biotin

Waisman *et al.* (1945) produced a chronic biotin deficiency in the rhesus monkey with a thinning of the hair and a gradual loss of hair color. The time elapsing before the appearance of hair loss in monkeys fed diets containing whole liver, liver extract, and solubilized liver could be correlated with the biotin content of the diet preparation fed. Acute deficiency was produced by feeding egg white or 3 percent succinyl sulfathiozole. In the acute disease a heavy, scaly dermatitis covered the whole body and, more conspicuously, the face. Twenty μg/day of biotin overcame the deficiency stimulation activity of egg white and sulfa drugs in 2-kg rhesus monkeys. Thus, 10 μg/kg body weight would appear to satisfy the biotin requirement.

Choline

There is little information about the choline requirement of monkeys. It could be anticipated that the level of protein, fat, and total "methyl donor" compounds would influence the requirement. Wilgram (1958, 1959) produced periportal, and in some cases centrolobular, fat deposition in cebus and rhesus monkeys by feeding a choline-deficient diet. Two cebus monkeys were fed "cirrhogenic" diets that were characterized by absence of choline, 2 percent cholesterol, and relatively low protein. There was an increased liver firmness and nodularity with increased lipid and hydroxyproline content. Microscopic examination revealed fibrous bands around the portal triads and nodular regeneration. Serum albumin was lowered, and globulins were elevated. This syndrome regressed, in part, on supplementation with dried egg yolk and whole milk powder. The role of choline, per se, in these experiments is unclear. Hoffbauer and Zaki (1965) produced increased hepatic fat in periportal areas in the parenchymal cells of the centrolobular areas of two old baboons by feeding choline-deficient diets. They were unable to induce hepatic changes in young baboons.

Folic acid

Day and associates (1935, 1940) demonstrated that folic acid deficiency in the rhesus monkey led to a macrocytic anemia and leukopenia. Cooperman *et al.* (1946) reported that 100 μg of folic acid per day were active in promoting growth, and 150 μg promoted good growth in 4-kg monkeys. Day and Totter (1947) considered the daily requirement of monkeys weighing 2–3 kg to be 80–100 μg/day. Both of these determinations are equivalent to about 40 μg/kg/day. Dutra de Oliviera *et al.* (1956) fed diets containing 200 μg of folic acid to monkeys weighing 3–4.5 kg and observed an excretion of 3–14 μg.

Niacin

Tappan *et al.* (1952) induced nicotinic acid deficiency by feeding a purified diet that contained 9 percent casein. The syndrome was characterized by weight loss and a lowered blood hemoglobin concentration. In 2- to 3-kg rhesus monkeys, 10 mg/week of nicotinic acid maintained weight, but 35 mg/week was required for optimum growth. This is equivalent to about 2 mg/kg of body weight/day. Tryptophan is evidently inefficiently converted to nicotinic acid by this species, since up to 4 g/week were required for optimum growth in the absence of nicotinic acid.

Pantothenic acid

McCall *et al.* (1946) produced pantothenic acid deficiency, which is characterized by lack of growth,

ataxia, graying and thinning of the hair, anemia, diarrhea, and cachexia. They were unable to produce complete remission of symptoms with the quantities of calcium pantothenate used (1–3 mg/day in 1.51 to 2-kg monkeys) without the addition of liver preparations. In unpublished experiments, Greenberg observed deficiency signs in four rhesus monkeys that were similar to those reported by McCall *et al.* (1946). He observed a dramatic response to 3 mg calcium pantothenate/day.

Riboflavin

Waisman (1944), Cooperman *et al.* (1945), and Greenberg and Moon (1963) have described riboflavin deficiency in the rhesus monkey. Mann *et al.* (1952) induced the deficiency in Cebus monkeys (*Cebus albifrons*), and Foy and associates (1964) studied riboflavin deficiency in the baboon. Among the signs observed in these species were leukocytopenia, anemia, hyperkeratosis, "freckled" dermatitis, neurological signs–behavioral changes, incoordination, ataxia, and blindness, and increased xanthurenic acid excretion. The minimum riboflavin requirement was thought to be 25–30 μg/kg/day for the rhesus (Cooperman *et al.*, 1945) and Cebus (Mann *et al.*, 1952) monkeys. Dutra de Oliveira *et al.* (1956) reported an excretion of about 0.08 mg of riboflavin/kg/day on an intake of about 0.25 mg/kg/day.

Thiamin

The development of a semipurified diet for rhesus monkeys by Waisman and associates (1943; Waisman and Elvehjem, 1943) was quickly followed by the demonstration of a requirement for the various B vitamins. Thiamin deficiency was produced in the rhesus monkey (Leblond and Chaulin-Serviniere, 1942; Waisman and McCall, 1944); the signs were comparable to those seen in human beriberi: weight loss, muscular weakness, loss of reflexes, convulsions, incoordination, progressive cachexia, signs of cardiac insufficiency, prostration, and death. Electrocardiographic changes and increases in the pyruvic acid level in blood were also observed. A minimum maintenance requirement for growth of 25–30 μg/kg/day was reported. Rinehart *et al.* (1948) arrived at similar minimum requirements from studies of the anemia associated with thiamin deficiency. This latter laboratory (Rinehart *et al.*, 1949) studied the nervous system pathology associated with thiamin deficiency. More recently, Dreyfus and Victor (1961, 1963) re-evaluated the material of Rinehart *et al.* in relation to the neuropathology of human Wernicke's disease. Dutra de Oliviera *et al.* (1956) observed an excretion of about 0.04 mg of thiamin/kg/day on an intake of about 0.25 mg/kg/day.

Vitamin B_6

There have been a rather large number of studies of pyridoxine deficiency in the rhesus monkey. McCall *et al.* (1946) produced pyridoxine deficiency with weight loss and anemia. Greenberg and Rinehart (1949) observed weight loss and a reduction in the blood levels of pyridoxine. Hypochromic anemia (Poppen *et al.*, 1952), diffuse arteriosclerosis (Greenberg, 1964; Greenberg *et al.*, 1958; Kuzuya, 1959, 1961; Mushett and Emerson, 1956; Rinehart and Greenberg, 1949, 1951, 1956), liver disease (fatty liver necrosis, nodular scarring, cirrhosis) (Wizgird *et al.*, 1965), oral and dental lesions (Berdjis *et al.*, 1960), neuropathology (Victor and Adams, 1956), oxaluria (Gershoff, 1964), and an apparent defect in the cysteine sulfinic acid decarboxylation activity of liver (Portman, 1962) have been reported in vitamin B_6-deficient monkeys. Greenberg and Moon (1961) were unable to detect evidence of a defect in the conversion of linoleic acid to arachidonic acid in pyridoxine deficiency. Greenberg and Peng (1965) studied the metabolism of tritium-labeled pyridoxine in the rhesus monkey.

One mg of pyridoxine/day causes a rapid remission of deficiency signs in rhesus monkeys (Greenberg and Rinehart, 1949; McCall *et al.*, 1946). Rinehart and Greenberg (1956) determined the minimum quantity of pyridoxine for maximum growth to be 51 μg/kg/day. Recently, Emerson *et al.* (1960) conducted a detailed evaluation of growth, activity, hair condition, hematology, and blood chemistry in 4-kg rhesus monkeys fed 50, 100, 500, 1,000, and 2,000 μg of pyridoxine/day, and they concluded that the 2,000-μg dose resulted in discernibly better performance than the 1,000-μg group. This study suggests an optimum pyridoxine level of 500 μg/kg body weight/day. Dutra de Oliviera and associates (1956) report that only 0.006 mg/kg/day was excreted on a daily intake of 0.25 mg/kg/day.

Vitamin B_{12}

May *et al.* (1951) reported that 15 μg of vitamin B_{12}/week provided a growth stimulus in a 2-kg monkey that had been fed a soybean formula diet. Wilson and Pitney (1955) were able to detect differences in the serum concentrations of vitamin B_{12} between normal

and nutritionally deficient monkeys. Das Gupta *et al.* (1955) made similar observations. Oxnard (1964, 1966) and Krohn *et al.* (1963) observed that the length of time rhesus monkeys were held in captivity influenced the level of vitamin B_{12} in the blood. A group of recently captured adult monkeys had concentrations of 110–680 pg/ml while a group held for a long period in captivity had concentrations of 20–70 pg/ml. Long-term captured mothers and their new-born had concentrations of less than 100 pg/ml. Recently captured mothers had a mean concentration of 122 pg/ml and their new-born had mean concentrations of 579 pg/ml. A series of prosimians (Oxnard, 1966) had high serum concentrations of vitamin B_{12}, and these were not reduced in captivity. Oxnard assumed that these changes during captivity were due to a reduction in vitamin B_{12} in the grain-based diets. He further assumed that the high levels of serum vitamin B_{12} in the prosimians might be due to the absorption of B_{12} (synthesized by bacterial flora), either directly from the gastrointestinal tract or by coprophagy. It seems unlikely, however, that the prosimians were maintained exclusively on grain-based diets. Flinn and Oxnard (1966) observed marked weight stimulating effects of 500 μg of vitamin B_{12}/week administered intramuscularly to young rhesus monkeys. Vitamin B_{12} is apparently absorbed largely from the lower ileum of the rhesus monkey (Boass and Wilson, 1963). There is evidence that the biosynthesis of DNA is the limiting step in maturation of erythrocytes in B_{12} deficiency and that the mode of action is in part via folic acid metabolism. Dzhelieva *et al.* (1965) induced increased excretion of formiminoglutamic acid (FIGLU) in rhesus monkeys by administration of antivitamin B_{12}.

WATER

Water should be offered *ad libitum*. This is rather difficult to achieve without good watering devices and vigilance that they are in working order. Feldman *et al.* (1960) studied the water intake and patterns of drinking of 20 rhesus monkeys (3.1–9.6 kg) at a temperature of 70–80° and a relative humidity of 50–65 percent. They reported a mean water intake of 1179 (575–2300) ml/m² of body surface.

EXAMPLES OF ADEQUATE DIETS

There are three general types of diets that have been used in experiments on nonhuman primates: semipurified diets, commercial pelleted rations, and formulations of natural foods. Semipurified diets are the most expensive but are obviously desirable for certain nutritional studies. Commercial pelleted rations are presumably well controlled as to certain specific compositional analyses, but the materials used may vary according to current market prices of alternative ingredients and sources. Greater control is probably obtained by a constant formulation of natural foods. This approach is laborious and best suited for a colony of substantial size. Ratcliffe (1966) has had good results feeding nonhuman primates such a formulation in the Philadelphia Zoo.

Another formulation that has been used for a substantial period of time is that of Schmidt (1955). Schmidt's colony diet is shown in Tables 1 and 2. At each feeding the boiled rice, eggs, basal mixture, and water with vitamins are mixed and fed within one half hour of preparation. The finished diet is fed twice daily to monkeys at the rate of 32 g/kg body weight/feeding. We have listed the diet of Schmidt as an illustration of the use of formulations of natural foods to feed rhesus monkeys and do not suggest that this diet is necessarily the optimum one, or necessarily in accord with all known nutrient requirements (Table 3).

TABLE 1 Composition[a] of the Basal Mixture, CHIMR, Used in the Monkey Colony Diet of Schmidt (1955)

Ingredient	Dry Matter in Ingredient (%)	Diet	
		Dry (%)	As Fed (%)
Wheat, ground	89.0	75.40	76.25
Soybean meal, solvent extracted	89.2	12.39	12.50
Milk, whole dried	96.2	4.81	4.50
Alfalfa meal, dehydrated	89.9	2.00	2.00
Sucrose	99.8	0.83	0.75
Calcium phosphate, dibasic	100	0.83	0.75
Calcium carbonate	100	1.66	1.50
Ferrous sulfate	100	0.11	0.10
Trace mineral salt	100	0.56	0.50
Yeast, brewers	100	1.39	1.25
Thiamin hydrochloride	100	0.0012	0.0011
Riboflavin	100	0.0012	0.0011
Niacin	100	0.0024	0.0022
Parvo (3% folic acid)	100	0.0151	0.0136
Vitamin B_6	100	0.0012	0.0011
d-calcium pantothenate	100	0.0025	0.0022
Vitamin E	100	0.0012	0.0011
Total		100.0	100.0

[a] Also see Tables A-1 and A-2 in Appendix A.

TABLE 2 Formula for a Colony Diet for Monkeys[a]

	Dry Matter in Ingredient (%)	Amount	
		Dry (%)	As Fed[b] (%)
CHIMR mixture	88.0	82.1	52.9
Rice, brown	88.2	14.5	9.31
Water, cooking	0	0	10.6
Salt, iodized	100	1.09	0.62
Eggs, raw	26	2.13	4.67
Ascorbic acid	100	0.167	0.093
Dry vitamin A and D_2	100	0.0065	0.0037
Vitamin B_6	100	0.0028	0.0016
Isoniazid	100	0.0055	0.0031
Water (mixing)	0	0	21.8
Total		100.0	100.0

[a] Adapted from Schmidt (1956).
[b] Feeding rate is 30 g/kg of monkey.

Table 4 lists two semipurified diets, one of which has been used with rhesus monkeys by Rinehart and Greenberg (1956) and the other for studies of several species of New World monkeys at the Harvard School of Public Health and the Oregon Regional Primate Research Center.

TABLE 3 Nutrient Requirements for the Growing 3-kg Rhesus Monkey

Nutrient	Per kg Body Weight[a]	Diet	
		Dry (per kg)	90% Dry Matter (per kg)
Energy: GE (kcal)	(70)	?	?
Protein (N × 6.25) (g)	3	189	170
Linoleic acid (g)	(0.25)	16	14
Calcium (g)	0.150	9.6	8.6
Magnesium (g)	0.040	2.6	2.3
Vitamin A (IU)	400	25,556	23,000
Vitamin D (IU)	25	1,589	1,430
Vitamin E (mg/g polyunsaturated fatty acids)	0.33–0.83	?	?
Vitamin K (μg)	0.1	6.7	6.0
Ascorbic acid (mg)	25	1,270	1,143
Biotin (mg)	0.01	0.63	0.57
Choline (mg)	Probably required	?	?
Folic acid (mg)	(0.040)	2.6	2.3
Niacin (mg)	(2)	127	114
Pantothenic acid	Required	?	?
Riboflavin (mg)	0.03	1.9	1.7
Thiamin (mg)	0.33	1.9	1.7
Vitamin B_6 (mg)	0.05–0.5	3.2–32	2.9–29
Vitamin B_{12} (mg)	0.070	4.4	4.0

[a] Values in parentheses are tentative estimates of the minimum requirement and contain no margin of safety.

There are many descriptions of the use of liquid diets for behavioral studies (Clark, 1965; Ellison and Riddle, 1961; Herndon *et al.*, 1958).

PROBLEMS REQUIRING FURTHER RESEARCH

The most obvious gaps in our knowledge of the nutrition of nonhuman primates include (a) lack of information about some of the nutrients, e.g., the mineral requirements of the rhesus monkey, one of the most widely used species in biomedical research; (b) absence of much information about the nutritional requirements of other species of nonhuman primates used in laboratory experimentation; and (c) ignorance about the effects of nutrition on more subtle parameters of health than those commonly used, i.e., growth and hematological measurements, in determining nutrient requirements.

The first deficiency requires no further comment. The need for investigation of the nutrition of the nonhuman primates other than the rhesus, which are commonly used in the laboratory, is apparent from the introduction to this chapter. The diversity of diets eaten by free-ranging primates is great. These differences may reflect the availability of food or the idiosyncrasies of the many primate species in acceptance of various forms and textures of foods as well as in optimal levels of the nutrients. Some more commonly used laboratory primates, for which nutritional requirements need to be established, include other macaques, *M. nemestrina*, *M. fuscata*, and *M. fascilularis* (*M. irus*), squirrel and Cebus monkeys, marmosets, and selected members of the genus *Cercopithecus*, e.g., *C. sabaeus*. The langurs and many of the prosimians, because of unusual dietary habits, are of special interest in comparative nutrition.

The third area requiring more research is the definition of criteria of health to be used in establishing standards of nutrition. It is difficult to select adequate and practical criteria of health. Clearly, longevity is a more valuable criterion of health than body weight. When one uses monkeys as models in psychological or cardiovascular studies or in problems of reproduction, it is important to define optimum nutrition in relation to the system under study. Recent studies of the effects of nutrition on myelination, learning, and behavior illustrate the need for new criteria of nutritional adequacy. These may include psychological testing and analysis of central nervous system composition and enzymic activity.

TABLE 4 Examples of Formulas for Satisfactory Semipurified Diets

Ingredient	Dry Matter in Ingredient (%)	Rinehart and Greenberg (1956) Diet Dry (per kg)	Rinehart and Greenberg (1956) Diet As Fed (per kg)	Rinehart and Greenberg (1956) Ration per Day[b]	Portman (New World Monkeys)[a] Diet Dry (per kg)	Portman (New World Monkeys)[a] Diet As Fed (per kg)	Portman (New World Monkeys)[a] Ration per Day[b]
Casein, vitamin-free (g)	90	165	180		231	250	
Sucrose (g)	100	743	730		625	610	
Salt mixture (g)	100	41	40[c]		41	40[d]	
Corn oil (g)	100	20	20		82	80	
Calcium stearate (g)	100	10	10		—	—	
Vitamin mix in dextrose (g)	100	21	20		21	20	
Vitamin A (IU)	100			1800			1250
Vitamin D_3 (IU)	100			180			400
Vitamin E (mg)	100			50			10
Menadione (Vitamin K_3) (mg)	100			—			4
Ascorbic acid (mg)	100			25			50
Biotin (μg)	100			10			20
Choline (mg)	100			100			500
Folic acid (μg)	100			110			100
Inositol (mg)	100			100			100
Niacin (mg)	100			5			4.9
Calcium pantothenate (mg)	100			3			3
p-Aminobenzoic acid (mg)	100			100			—
Riboflavin (mg)	100			1			1
Thiamin (mg)	100			0.5			1
Vitamin B_6 (mg)	100			1			1
Vitamin B_{12} (μg)	100			—			2

[a] Current modification of the basal semipurified diet, described in part in Portman *et al.* (1967).
[b] Supplied in addition to ingredients listed under diet.
[c] From Hawk *et al.* (1949).
[d] From Hegsted *et al.* (1941).

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NUTRIENT REQUIREMENTS OF THE LABORATORY MOUSE

Less is known about the nutritional requirements of of the mouse (*Mus musculus*) than of the rat, probably because it has been employed less extensively in nutritional research. Several investigators believe that mice resemble rats in their nutritional needs, since there is some evidence indicating that certain diets permit equally satisfactory performance with both species. On the other hand, it is obvious that considerably more research is needed to clarify certain controversial aspects of mouse nutrition.

Information pertaining to diseases, sources of various strains of mice, uses of mice, and lists of users of mice is available (Anonymous, 1958; U.S. Department of Health, Education and Welfare, 1965; Green, 1966; National Academy of Sciences, 1954, 1966; Howe and Porter, 1950; Rose and Rice, 1939; Snell, 1941).

The comments to follow include the results of research on nutrient requirements for growth, reproduction, and lactation, together with opinions based on reported or estimated nutrient contents of "suitable" mouse diets. Because of paucity of detail in many reports, it was necessary to use estimated live weights, rates of gain, and daily feed intakes to calculate reasonable approximations of nutrient requirements or intakes. There have been few, if any, attempts to segment the 2- or 3-week growth period into smaller units; hence requirements have often been stated as the amount required per day without reference to body weight or feed intake.

Basic to a study of nutrient requirements is a consideration of expected or normal rates of gain, which in turn might be related to feed intakes. Table 1 shows growth data for two strains of mice (Anonymous, 1958; Carworth Farms, Inc., 1948). These figures indicate that 21-day-old weanling mice should weigh 9–12 g and should gain 5–13 g during the next 14 days, which represents the period of most rapid postweaning growth. Strain differences are evident here with respect to growth and elsewhere in this chapter with respect to specific nutrient requirements. Falconer (1947) has presented data on preweaning growth of mice.

TABLE 1 Weights, in Grams, of Two Strains of Albino Mice from 1 to 8 Weeks Old

	Rockland RAP		Carworth Farms No. 1	
Age	Males	Females	Males	Females
1	5.0	4.5	—	—
2	7.5	7.5	6.5	6.0
3	12.0	12.0	14.5	9.0
4	15.0	14.0	22.5	13.0
5	17.5	16.5	26.5	18.5
6	20.0	19.0	28.0	20.5
7	22.5	20.5	29.0	22.0
8	24.5	22.5	—	22.5

A gain of 13–14 g in 14 days by CF No. 1 male weanlings represents the growth response obtained with several different adequate diets (J. M. Bell, unpublished data). At this growth rate, mice required 16–18 kcal of digestible energy per gram of gain. These data have been used to arrive at estimates of nutrient requirements, recognizing, however, the limitations of so doing.

ENERGY

Studies by Troelsen and Bell (1963) showed that mice consumed an average of about 3.5 g of feed daily during the 14-day period following weaning. Intake expressed as kcal based on metabolizable

energy values on a variety of diets that produced good rates of growth were about 14.5 kcal per day. The maximal level of crude fiber or cellulose that is compatible with normal or maximal growth rates depends on the nature of the fibrous material, since it may affect palatability, digestion, laxation, and intestinal biosynthesis (Bell, 1960; Dalton, 1965). Most of the more suitable diets, however, contain under 6 percent crude fiber.

PROTEIN AND AMINO ACIDS

Satisfactory protein levels in various commercial, institutional, and purified diets ranged from 20 to 30 percent (Anonymous, 1958; Bruce and Parkes, 1949; Carworth Farms, Inc., 1948; Eaton and Cabell, 1949; Fenton and Carr, 1951; Hauschildt, 1942; Kao *et al.*, 1941; Lippincott and Morris, 1942; Slanetz, 1943). Bing *et al.* (1932) investigated the protein requirements of albino mice and found growth on purified diets to approach that obtained on stock diets when 15.6 percent of the total calories came from protein. This agrees well with Korsrud's (1966) findings with whole egg powder or herring fishmeal as the protein source in semipurified diets. Weight gains approached maximum with 11.3 percent protein, or 14 percent of the total calories on egg protein diets, and with 11.9 percent protein, or 13.8 percent of total calories on fishmeal diets.

The minimal protein level in a semipurified diet, supporting satisfactory growth, reproduction, and lactation, was 13.6 percent (Goettsch, 1960).

J. M. Bell (unpublished observations), using ingredients typical of those used in stock diets (see p. 51), found 17 percent protein to be as effective for growth as 19 or 21 percent. It thus appears that the provision of about 12 percent protein of maximal digestibility and biological value is adequate for diets containing about 4.5 kcal of metabolizable energy per gram. Under more practical conditions, such as formulation of stock diets, it would seem inadvisable to provide less than 16 percent total protein, allowing for lower digestibility and lower biological value of the protein in typical diets. Hoag and Dickie (1962) found 20 percent protein to be superior to 17 percent for breeding females.

Significant heritable differences in protein requirements among strains of mice have been reported, especially relating to strains susceptible to obesity (Fenton, 1957; Fenton and Marsh, 1956).

The amino acid requirements of mice have received little attention. Bauer and Berg (1943a,b) showed that D- and L-methionine, D- and L-phenylalanine, and the L-forms of valine, leucine, isoleucine, and threonine promoted growth. Omission of arginine did not reduce the moderate growth rates being obtained. The dispensability of cystine in the presence of adequate methionine was also proven. The inability of the mouse to utilize D-histidine and D-tryptophan was reported by Celander and Berg (1953), but Harding-Gaudin (1961) found that some mice could utilize D-tryptophan. Leveille *et al.* (1961) demonstrated a requirement for 0.47 percent of sulfur-containing amino acids with diets containing 2.5 percent nitrogen (15.6 percent protein) and 0.26 percent in diets with 1.5 percent nitrogen or 9.4 percent protein equivalent. Since qualitative similarities between the rat and the mouse have been demonstrated, it is possible that the amino acid requirements of the mouse, relative to protein intakes, are similar to those of the growing rat (Maddy and Elvehjem, 1949).

FATS

Morris (1947) has found that the growth requirements for unsaturated fatty acids of the linoleic and linolenic acid series are similar in the mouse and in the rat. Laubmann (1950) found evidence that mice, especially lactating females, would exhibit a specific hunger for fats. It is not clear whether this expressed a demand for energy or for specific nutrients.

MINERALS

Very little research attention has been devoted to studying the mineral requirements. Since quantitative data are lacking, a study was made of the analytical reports and formulas of various stock diets.

Calcium and Phosphorus

Calcium varied from 0.4 percent in a purified diet (Mirone and Cerecedo, 1947; Morris and Lippincott, 1941) to 2.1 percent in a commercial ration. Phosphorus ranged from from 0.3 to 1.2 percent. In view of the greater availability of minerals in purified diets and the likelihood of subnormal growth in mice thus fed, it is possible that the calcium and phosphorus requirements of mice approach those of rats: 0.6 and 0.5 percent, respectively. Stock diets and semipurified diets containing these levels of calcium and phosphorus have been used by J. M. Bell (unpublished data) with no apparent ill-effects.

Iron

Iron was shown by Inoue (1932) to be required for both growth and reproduction. Characteristic anemia symptoms were observed in iron-deficient young mice, and birth weights and litter sizes were reduced in breeding stock. No quantitative data are available.

Manganese

Manganese contents of satisfactory mouse diets have been estimated to contain from 6 to 90 mg per kg diet, which suggests that the manganese requirement for growth may not exceed 6 mg per kg diet. Lee *et al.* (1962) showed manganese to be essential for growth of mice.

Potassium

The potassium requirement of the growing mouse has been found to be 0.2 percent of the diet (Bell and Erfle, 1958). Mice fed highly purified diets, deficient only in potassium, died within 1 week, after having exhibited outward signs of inanition. Lusterless eyes and haircoat, dry scaly tail, and general emaciation were observed in connection with severe deficiency. Partial deficiencies resulted in poor growth and a lack of "bloom."

Sodium and Chlorine

Salt (NaCl) requirements do not appear to have been studied. The levels used in various formulas ranged from 0.5 to 1.0 percent of the diet.

Zinc

Zinc is essential in mouse nutrition (Bertrand and Bhattacherjee, 1934; Day and Skidmore, 1947; Nishimura, 1953). Day and Skidmore (1947) found that an intake of about 3 mg per kg of diet resulted in deficiency symptoms, including loss of hair on shoulders and neck, emaciation, and decreased liver and kidney catalase activity.

Other Minerals

It has been suggested by Huff *et al.* (1956) that the thyroid-stressed mouse has a dietary requirement for bromine. Titanium and chromium were found to stimulate growth in mice (Schroeder *et al.*, 1963). It is probably premature to conclude that any of these three elements should be classed as nutrients.

With regard to general mineral nutrition, Slanetz (1943) stated that the mineral composition of the several stock diets that he analyzed resembled that obtained by incorporating 4 percent of Sure's (1941) salts No. 1 in a purified diet.

VITAMINS

Vitamin A

Vitamin A was shown by Wolfe and Salter (1931) to be required by the mouse. Morris (1947) estimated the daily need to be about 5 μg of β-carotene, or 0.3–0.6 μg vitamin A.

Slanetz (1943) pointed out that vitamin A requirements for all species range from 25 to 39 μg/kg body weight/day, a maximum of 200 μg per/kg of feed. He also found that various mouse diets studied ranged from 4,600 to 5,100 IU/kg of feed. Other available formulations have shown a range from 400 to 60,800 IU/kg of feed. The vitamin A requirements for pregnancy and lactation are reported to be similar to those for growth (McCarthy and Cerecedo, 1952; Morris, 1947).

The requirement therefore appears to be about 1.0–2.0 IU/day, or 250–500 IU/kg of feed. It is important to bear in mind the susceptibility of vitamin A to oxidation when formulating diets.

Vitamin A administered in doses as small as 250 IU/day during critical phases of gestation has resulted in toxicity as shown by serious reproductive disturbances and malformation of embryos (Giroud and Martinet, 1959, 1962).

Vitamin A deficiency symptoms include tremors, diarrhea, rough haircoat, eye exudates, abscesses, poor growth, rectal and vaginal hemorrhages, abortion, resorption, and permanent sterility in males.

Vitamin D

Beard and Pomerene (1920) found that mice were susceptible to the same type of rickets as rats. J. M. Bell (unpublished data) has employed a stock diet containing 150 IU/kg through many generations of albino mice (CF No. 1) with no evidence of deficiency.

Vitamin E

Bryan and Mason (1940) found that mice responded to vitamin E deficiency in a manner similar to that reported for the female rat, but these workers were unable to produce testicular injury in the male. They

reported that 350 μg of α-tocopherol daily was the minimum necessary to give normal results in the first pregnancy. In contrast, Goettsch (1942) showed that 0.5–1.0 mg of α-tocopherol given at the onset of gestation would suffice.

In life-span studies, Lee *et al.* (1962) found that vitamin E deficiency resulted in convulsions and heart failure, but that vitamin B_{12} and mineral supplementation were modifying factors.

Pappenheimer (1942) reported a muscular dystrophy and hyaline degeneration in vitamin E-deficient mice but at a lower incidence rate than was observed in rats. Cerecedo and Vinson (1944) concluded that vitamin E was related to protein metabolism; they found that muscular paralysis could be prevented either by raising the protein level in the diet or by including 20 mg of α-tocopherol/kg of diet. The possibility that selenium or sulfur-containing amino acids may have been involved in the protein level effect must now be recognized. Bruce (1950) found α-tocopherol to be helpful in reducing the mortality in litters whose dams were fed diets containing 2 percent cod liver oil.

Vitamin K

The only available report on vitamin K is that of Woolley (1945), who reported that it corrected vaginal hemorrhages and resorptions, induced by administration of *dl*-α/tocopherol quinone, an analog of vitamin K.

WATER-SOLUBLE VITAMINS

Ascorbic Acid

It has been customary for some laboratories to supply fresh milk and green vegetables to female mice during gestation and lactation (Howie and Porter, 1950; Watson, 1937). Such practices led to the early assumption that ascorbic acid was a dietary essential for mice. It now seems certain that it is not required in the diet of this species (Ball and Barnes, 1941).

Biotin

Nielsen and Black (1944) showed that mice required biotin when fed a synthetic diet adequate for growth of rats. Use of dietary sulfasuxidine accentuated the deficiency. Deficiency symptoms include alopecia, achromotrichia, and growth failure. Fenton and Cowgill (1948) and Fenton *et al.* (1950) used 20 μg/kg of diet; other than this, there is no information on which to base a biotin requirement.

Choline

Choline appears to have been recognized first as a dietary essential for the mouse by Best *et al.* (1932), who observed fatty livers in choline-deficient mice. The symptoms have been described in greater detail more recently, and these include myocardial lesions, hepatic liposis and fatty cysts, nodular parenchymal hyperplasia fibrosis, lowered conception rates in females, and low viability of young (Buckley and Hartroft, 1955; Meader and Williams, 1957; Mirone, 1954; Saucier and Demers, 1958; Williams, 1960). It seems impossible to establish a minimum requirement level for choline from the studies reported.

Folic Acid

Nielson and Black (1944) demonstrated the essential nature of folic acid for the growing mouse (Figure 1). The finding was confirmed by Weir *et al.* (1948). Cerecedo and Mirone (1947) and Cerecedo and Vinson (1944) reported that folic acid was required for reproduction. Mirone and Cerecedo (1943) reported on the value of xanthopterin in relation to lactation. Fenton *et al.* (1950) obtained satisfactory growth from mice fed semipurified diets containing 0.5 mg of folic acid/kg of diet.

FIGURE 1 Folic acid-deficient mouse. This black mouse shows graying of the hair brought about by feeding pyrimethamine, an antagonist of folic acid. (Courtesy F. M. Stout, Oregon State University.)

Inositol

Woolley (1941) described a dietary condition characterized by loss of hair, and reported that inositol was the "antialopecia" factor, because 100 mg of the purified material/kg of diet would cure the condition. Some doubt, however, has arisen over the status of inositol as a dietary essential (Cerecedo and Vinson, (1944); Martin, (1941); and Fenton *et al.*, (1950).

Niacin

No reports on either a qualitative or a quantitative niacin requirement are avaliable. Calculations and reported analyses on stock diets revealed levels ranging from 48 to 143 mg/kg of diet. Niacin levels of 50–55 mg/kg were used successfully in purified and semipurified diets (J. M. Bell, unpublished data), but this level probably exceeds the minimum requirement.

Pantothenic Acid

The deficiency symptoms in growing mice caused by lack of pantothenic acid were reported by Morris and Lippincott (1941). Loss in weight was characteristic. Other symptoms were loss of hair particularly on the ventral surface, flanks, and legs; dermatosis; partial posterior paralysis; various nerve derangements; and graying of hair in black strains.

The requirement for growth for two strains of mice is 30 μg per day (Morris and Lippincott, 1941; Sandza and Cerecedo, 1941). This level was confirmed by Fenton and Cowgill (1947b) and Fenton *et al.* (1950) with one strain of mice, but it was noted that maximum growth was not obtained with all strains at this level.

The reproduction and lactation requirements for this vitamin have not been reported, but various stock rations contain from 10 to 26 mg/kg, as compared with the growth requirement of 6.5–7.5 mg/kg of diet.

p-Aminobenzoic Acid

According to Martin (1941) and Fenton *et al.* (1950), the significance of *p*-aminobenzoic acid in mouse nutrition is still questionable. Presumbly, *p*-aminobenzoic acid functions as a folic acid precursor.

Vitamin B_6

According to Miller and Baumann (1945) and Morris (1947), mice grew satisfactorily with diets containing 1 mg of pyridoxine/kg of diet. Pyridoxamine and pyridoxal were found to be less active than pyridoxine. Deficiency symptoms include poor growth, hyperirritability, posterior paralysis, necrotic degeneration of the tail, and alopecia (Beck *et al.*, 1950).

Riboflavin

Ariboflavinosis in the mouse was described by Lippincott and Morris (1942). They reported the development of either atrophic or hyperkeratotic epidermis (but normal sebaceous glands), myelin degeneration in the spinal cord, and corneal vascularization with ultimate ulceration. Morris and Robertson (1943) found that adult mice did not survive on diets containing 0.4–0.6 μg riboflavin per gram of food and that young mice in about 9 weeks after continued growth failure. Kligler *et al.* (1944) showed that riboflavin-deficient mice had lowered resistance to *Salmonella* infection.

Riboflavin requirements for normal growth appear to be about 4 mg/kg diet (Fenton and Cowgill, 1947a,b; Wynder and Kline, 1965). Most of the formulas of commercial and other stock diets appear to provide 2–7 mg of riboflavin per kg of feed and hence are near the stated requirement. It is probable that the requirements for reproduction and lactation also are in this range, in view of the observations on stock diet performance.

Thiamin

Hauschildt (1942) established the minimum requirement of thiamin for normal growth of mice at 10 μg per day. Morris and Dubnik (1947) later found the growth requirement to be about 5–6 μg per day on a diet containing 22 percent fat. Morris (1947) and Jones *et al.* (1945) reported the deficiency symptoms to be violent convulsions, especially when the animal was held a few second by the tail; cartwheel or circular movements; brain hemorrhages; decreased food intake; poor growth; early mortality in cases of severe deficiency, silvery-streak muscle lesions; and testicular degeneration.

No studies on the specific requirements for reproduction and lactation have been reported, but Mirone and Cerecedo (1947) found that 20 mg/kg of diet were adequate. The requirements for these purposes are probably close to those for growth, since several diets proved to contain less than 20 mg/kg.. Diets used or reported by J. M. Bell (unpublished data), the *Handbook of Laboratory Animals* (National Academy of Sciences, 1954), and Rockland Farms (Anonymous, 1958) contain, by calculation from published tables

of vitamins in feedstuffs (National Academy of Sciences, 1964), about 5.5, 4.6, and 2.2–4.6 mg of thiamin, respectively, per kg of diet.

Vitamin B_{12}

Vitamin B_{12} was studied by Jaffe (1952) and reported to be required in excess of 5 μg per kg of diet for growth, and between 4 and 5 μg/kg of diet for reproduction and lactation. Lee *et al.* (1962) also found vitamin B_{12} necessary for successful gestation. Deficiency signs in young mice included retarded growth and renal atrophy.

Vitamin B_{12} was found beneficial to mice fed low-fat, high-protein diets (Bosshardt *et al.*, 1950) or fed diets containing thyroid-active material (Meites, 1952).

WATER

Mice should be provided with a readily available supply of good water. Very little specific information on requirements seems to have been published (Green, 1966), and while statements have appeared to the effect that no drinking water is needed, it is probable that the use of high-moisture foods, such as vegetables and wet mashes, led to such deductions. Restriction of water intake has been shown to result in decreased voluntary food consumption (Chew and Hinegardner, 1957). Dalton (1965) has demonstrated the effects of diet density or fiber content on water requirements, and environmental temperature is undoubtedly a factor affecting water requirements. Mice fed dry rations and housed at temperatures of 75–80°F may perish if deprived of water for a day.

EXAMPLES OF ADEQUATE DIETS

Stock Diets

Several comparisons of stock diets for mice have been made (Bruce and Parkes, 1949; Gehring, 1959; Griffin *et al.*, 1957). Four formulas that have given superior results are presented in Table 2.

In some breeding colonies (Howie and Porter, 1950; Watson, 1937) it has been regular practice to supplement the regular diet with such items as fresh lettuce, cabbage, yeast, wheat germ meal, and fish liver oil. There is reason to believe, however, that successful reproduction and lactation are possible with mice fed complete diets in dry form, preferably pelleted or cubed, and containing 20–25 percent protein (Anonymous, 1958; J. M. Bell, unpublished data; Bruce and Parkes, 1949; Eaton and Cabell, 1949; Loosli, 1945), or less (Goettsch, 1960). Benefits attributed to the use of special supplements probably reflect nutritionally inadequate stock diets, due to improper formulation, losses of nutrients during storage prior to, or subsequent to, mixing the diet, or failure to protect certain components, especially vitamin A and added fat, from oxidative destruction.

Normal variations in nutrient composition of ingredients necessitate provision of safety allowances. Hence, the formulas cited in Table 2 tend to provide higher levels of many nutrients than have been demonstrated as minimal requirements. Furthermore, different batches of feed mixed to the same formula may produce slightly different results.

Contamination of mouse diets occurs occasionally. Griffin and Thompson (1956) found *Salmonella* microorganisms in some prepared feeds and traced the problem to improperly processed meat meal. Diethylstilbestrol contamination can affect reproductive success severely and must be carefully avoided (Hadlow *et al.*, 1957). The possibility of fungal mycotoxins occurring in stock diets exists, since these compounds have given rise to problems with domestic animals.

Fortification of diets with antibiotics often has resulted in improved growth and feed conversion (J. M. Bell, unpublished data) using 10–20 mg of oxytetracycline or chlortetracycline per kg of ration. Such rations have been used successfully for breeding stock as well.

Experimental Diets

Semipurified diets have been used extensively by Fenton and Carr (1951), Fenton and Cowgill (1947a), and Morris (1947) in their studies of vitamin and protein requirements of mice; by Bell and co-workers (Bell, 1960; Bell and Erfle, 1958; Korsrud and Bell, 1967) in a variety of studies involving mice; and by others. Formulas typical of those that permitted satisfactory growth may be found in Table 3.

Table 4 summarizes the nutrient requirements of the mouse for various nutrients to the best of present knowledge.

TABLE 2 Examples of Formulas, per kg, for Satisfactory Mouse Diets

	Bell[a]		Bruce and Parkes (1949)		Gehring (1959)		Griffin *et al.* (1957)		Morris (1945)	
	Dry	90% Dry	Dry	90% Dry	Dry	90% Dry	Dry	90% Dry	Dry	90% Dry
Wheat, grain, ground (g)	444.	400.	511.	460.	588.	530.	—	—	683.	615.
Wheat, flakes (g)	—	—	—	—	—	—	55.6	50.	—	—
Wheat, germ meal (g)	—	—	—	—	—	—	94.4	85.	—	—
Corn, grain, kibbled (g)	—	—	—	—	—	—	233.	210.	—	—
Corn, flakes (g)	—	—	—	—	—	—	225.	203.	—	—
Barley, grain, ground (g)	370.	333.	—	—	166.	150.	—	—	—	—
Oats, grain, ground (g)	—	—	444.	400.	—	—	—	—	—	—
Lard, stabilized (g)	22.2	20.	—	—	—	—	—	—	—	—
Cattle, tallow (g)	—	—	—	—	—	—	44.4	40.	—	—
Corn oil (g)	—	—	—	—	—	—	—	—	64.4	58.
Alfalfa, meal, dehydrated (g)	55.6	50.	—	—	22.2	20.	16.7	15.	—	—
Milk, skimmed, dehydrated (g)	—	—	33.3	30.	55.6	50.	44.4	40.	258.	233.
Milk, whole, dehydrated (g)	—	—	—	—	55.6	50.	—	—	—	—
Cheese, meal (g)	—	—	—	—	—	—	16.7	15.	—	—
Fishmeal, herring (g)	55.5	50.	88.9	80.	22.2	20.	16.7	15.	—	—
Meat meal (g)		—	—	—	55.6	50.	200.	180.	—	—
Soybean meal, solv-extd (g)	83.8	75.	—	—	55.6	50.	94.4	85.	—	—
Liver, dehydrated (g)	—	—	—	—	—	—	11.1	10.	—	—
Yeast, brewers (g)	22.2	20.	—	—	38.9	35.	—	—	44.4	40.
Yeast, torula (g)	—	—	11.1	10.	—	—	22.2	20.	—	—
Yeast, irradiated (g)	—	—	—	—	—	—	1.1	1.	—	—
Sugarcane molasses (g)	33.3	30.	—	—	—	—	—	—	—	—
Tomato, pumace (g)	—	—	—	—	—	—	22.2	20.	—	—
Bone meal, steamed (g)	14.4	13.	—	—	33.3	30.	—	—	—	—
Dicalcium phosphate (g)	—	—	—	—	8.3	7.5	—	—	—	—
Calcium carbonate (g)	3.3	3.	—	—	—	—	—	—	—	—
Salt, iodized (g)	5.6	5.	11.1	10.	5.6	5.	2.2	2.	15.6	14.
Zinc sulfate (mg)	68.9	62.	—	—	—	—	—	—	—	—
Chlorophyllin (mg)	—	—	—	—	—	—	0.83	0.75	—	—
Vitamin A (IU)	1,667.	1,500.	—	—	—	—	1,889.	1,700.	—	—
Vitamin D (IU)	167.	150.	—	—	—	—	333.	300.	—	—
Cod liver oil (g)	—	—	11.1	10.	1.1	10.	—	—	—	—
Ferric citrate (g)	—	—	—	—	—	—	—	—	14.4	13.
Shark oil (g)	—	—	—	—	—	—	—	—	2.2	2.0
Delstrol[b] (g)	—	—	—	—	—	—	—	—	0.9	0.8

[a] Unpublished data.
[b] A fish liver source of vitamin A and D; potency not specified.

TABLE 3 Examples of Formulas, per kg Dry Matter,[a] for Satisfactory Semipurified Diets

Ingredient	Fenton[b]	Bell[c]
Casein, vitamin-free (g)	300	212
Cornstarch (g)	—	275[d]
Sucrose (g)	526	76
Glucose (cerelose) (g)	—	256
Cellulose (g)	20	55
Fat (g)	100[e]	76[f]
Salt mixture (g)	50[g]	45[h]
Cod liver oil (g)[i]	2	—
α-Tocopherol (mg)	60	—
Vitamin E (IU)	—	140
Menadione (mg)	10	200
Choline (mg)	1,500	4,000
Thiamin (mg)	5	5
Riboflavin (mg)	10	10
Niacin (mg)	10	50
Pyridoxine (mg)	5	5
Pantothenic acid (mg)	60	50
Folic acid (mg)	0.5	2.5
Biotin (mg)	0.2	1
Vitamin A, stabilized (IU)	—	5,000
Vitamin D (IU)	—	1,250
Vitamin B_{12} (μg)[j]	—	250
Inositol (mg)	—	2
Methionine, *dl*- (g)	—	1.5

[a] Purified ingredients contain some moisture, usually under 5%.
[b] Fenton and Carr (1951), Fenton and Cowgill (1947a,b; 1948), Fenton *et al.* (1950).
[c] Unpublished data.
[d] A portion of the cornstarch may be used as the carrier for the vitamin premix.
[e] Corn oil.
[f] Equal parts lard and vegetable shortening, stabilized with 1,2-dihydro-6-ethoxy-2,2,4 trimethylquinoline.
[g] Sure's salt mixture No. 2 (Sure, 1941), which is composed (g/kg) of NaCl, 173; K_2HPO_4, 333; $CaHPO_4 \cdot 2H_2O$, 98; $MgSO_4$, 51.1; $CaCO_3$, 310; ferric citrate, 28.4; KI, 0.83; $MnSO_4$, 4.13; $ZnCl_2$, 0.26; $CuSO_4$, 0.21; $Al_2(SO_4)_2K_2SO_4$, 0.21; NaF, 0.26; $CoCl_2$, 0.26; $Na_2B_4O_7$, 0.26.
[h] NaCl (iodized), 4.54; $CaHPO_4$, 77.20; $KHCO_3$, 15.33; MgO, 2.03; $MnSO_4 H_2O$, 0.34; $FeSO_4 \cdot 2H_2O$, 0.34; $CuSO_4 \cdot 5H_2O$, 0.11; and ZnO, 0.11 percent.
[i] Vitamins A and D contained in cod liver oil; potency unknown.
[j] Much of Fenton's work was done prior to the discovery of Vitamin B_{12}.

TABLE 4 Nutrient Requirements, per kg,[a] for the Mouse

	Growth		Pregnancy and Lactation	
Nutrient	Dry	90% Dry Matter	Dry	90% Dry Matter
Metabolizable energy (Mcal)	4.2	3.8	4.2	3.8
Total protein (g)	(133)	(120)[b]	—	—[c]
Calcium (g)	6.7	6	—	—
Phosphorus (g)	5.6	5	—	—
Magnesium	—	—	—	—
Manganese (mg)	22.2	20	—	—
Sodium chloride (g)	5.6	5	—	—
Iron	Required	Required	—	—
Copper	—	—	—	—
Zinc (mg)	55.6	50	—	—
Potassium (g)	(2.2)	(2)	—	—
Vitamin A (IU)	(556)	(500)	(556)	(500)
Vitamin D (IU)	167	150	—	—
α-Tocopherol (mg)	(22.2)	(20)	—	—
Vitamin K	—	—	—	—
Thiamin (mg)	(3.17)	(2.85)	5.6	5
Riboflavin (mg)	(4.4)	(4)	7.8	7
Vitamin B_6 (mg)	(1.1)	(1)	—	—
Niacin (mg)	11.1	10	—	—
Pantothenic acid (mg)	(9.4)	(8.5)	11.3	10.2
Biotin	Required	Required	—	—
Folic acid	Required	Required	—	—
Choline (g)	0.63–1.27	0.57–1.14	—	—
Vitamin B_{12} (μg)	(5.6)	(5)	0.56	0.5
Inositol	[d]	[d]	Required	Required

[a] The values in parentheses are tentative estimates of the minimum requirement and contain no margin of safety. The values that are not in parentheses are estimated from various adequate rations and hence, are probably in excess of the actual requirement.
[b] Protein of highest digestibility and biological value.
[c] —signifies no information is available on a quantitative requirement.
[d] A qualitative requirement of the nutrient is in doubt.

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NUTRIENT REQUIREMENTS OF THE LABORATORY RAT

The purpose of any attempt to describe the minimal nutrient requirement of a species is to define, in quantitative terms, the chemical nature of an optimal diet. Carlson (1943) has defined an optimal diet as

> . . . that kind and quantity of food which permits and promotes optimum growth, optimum performance of all biologic functions, optimum resistance to disease, optimum conservation of the factors of safety and powers of repair, and optimum length of life with optimum efficiency within the framework of the hereditary potentialities of the individual and the species.

Even provided the term "optimum" could be satisfactorily defined, experimental evidence is presently inadequate to approach Carlson's rigid specifications. This is true even for the rat (*Rattus rattus*), which probably has had no peer as the subject of fundamental nutritional investigations. Despite inadequate information on the physiologic processes, these are sufficient data to permit a fairly accurate estimate of the nutrient needs of the rat for growth, reproduction, and lactation.

It is important in establishing even tentative nutrient requirements to define the desirable level of performance. For the purpose of this report, it has been assumed that maximum is desirable. Treatments allowing maximal gain, or the highest level of reproduction, are considered superior. Since the rapidly gaining animal is the useful experimental subject, this philosophy appears justified. It is obvious, however, that these may not be the most desirable criteria. It has been shown (Berg, 1960; Berg and Simms, 1960, 1961; Berg *et al.*, 1963; McCay, 1947; Ross, 1961; Ross and Bras, 1965; Silberberg and Silberberg, 1955) that overconsumption of nutrients, resulting in rapid growth, may be conducive to neither the longest life-span nor the greatest freedom from organic afflictions. While qualitatively sound, the quantitative aspects of these studies are too few to be given extensive consideration in this report. There is no doubt that as data of this type accumulate, our criteria of optimal performance will change and the requirements will be modified accordingly. The need for such data has been discussed (Weeks, 1957).

The nutrient requirements are to be considered as adequate to produce desirable performance. No intentional margin of safety has been included to allow for variations in feed ingredients or abnormal animal variation.

DESIRABLE PERFORMANCES

It is impossible to describe a single level of performance that characterizes all albino rats under all conditions. The average rate of growth and mature size has increased steadily since the data were summarized by Donaldson (1924). This improvement is undoubtedly due to careful breeding and to an increased knowledge of nutrition. In addition, new strains have been developed with characteristic performance parameters (Dunn *et al.*, 1947; Palmer *et al.*, 1946).

In the absence of complete growth and reproduction data for several commonly used strains on a uniform diet, a single growth curve and a table of reproductive performance have been constructed that are believed to be realistic. It is admitted that they will not encompass all strains but, nevertheless, should prove useful as a point of reference. Fortunately, the nutrient requirements have been determined on a wide variety of strains and, therefore, in the absence of specific information to the contrary, the nutrient requirements as a percentage of the diet should apply reasonably well to all strains. It should be recognized, however, that different strains, aside from having different growth

potentials, have different metabolic characteristics that may affect their nutrient requirement (Jansen, 1962; Marshall and Hildebrand, 1963).

Growth

The growth curves for males and females are given in Figure 1. The percent of mature size, age and weight relationships, and expected daily gains have been calculated from the curve and are recorded in Table 1. The curve was determined by using the average weight of the rat at weaning time (21 days: males 45 g; females 44 g) and at 350 days of age (males 550 g; females 325 g). Points on the curve were calculated from the relationship suggested by Zucker *et al.* (1941a,b,c; Zucker, 1953; Zucker and Zucker, 1941). These workers have reported that post-weaning growth could be described by straight lines when the log of the body weight was plotted against the reciprocal of time in weeks. They further reported that the slope of the line was quite uniform for many different colonies receiving adequate diets.

In principle, this concept has been confirmed by Bertrand and Quivy (1947), Gray and Addis (1948), Harte *et al.* (1948), and Copping *et al.* (1951). A

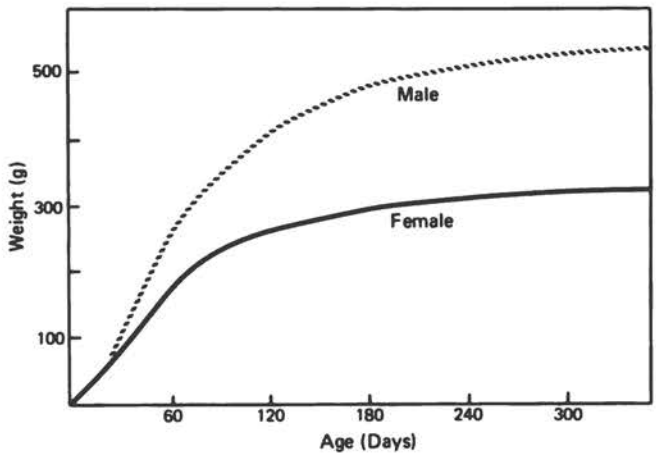

FIGURE 1 Growth curves of male and female rats. The points between 21 and 350 days were calculated in which a K value of 3.47 for males and 2.77 for females were used:

$$K = \frac{\log W_2 - log\ W_1}{1/t_1 - 1/t_2}, \text{ and where}$$

W_1 = weight at weaning (3 weeks): for males, 45 g; for females, 44 g;

W_2 = weight at 50 weeks: for males, 550 g; for females, 325 g. The values of t_1 and t_2 were the ages in weeks, or 3 and 50, respectively. The part of the curve from birth to 3 weeks was drawn free hand.

TABLE 1 Weight Gains and Nutrient Requirements Typical of Laboratory Rats[a]

		Growing Rats					Adult Rats		
		Percent of Mature Weight							
Item	Sex	10	20	30	40	70	100 Maintenance	Gestation	Lactation
Age[b] (days)	Male	23	33	42	53	108	350		
	Female	19	26	35	44	96	350		
Body weight [b] (g)	Male	55	110	165	220	385	550		
	Female	32	65	98	130	228	325		
Expected daily gain (g) from 21 days to age above	Male	5	5.4	5.7	5.5	3.9	—		
	Female	—	4.2	3.9	3.7	2.5	—	4[c]	0[c]
Ave. daily feed (g)	Male	9	15	18	21	20	19	—	—
	Female	—	10	14	15	16	13	19[d]	33[e]
Gross energy[f] (kcal/day)	Male	36	60	72	84	80	76	—	—
	Female	—	40	56	60	64	52	76[d]	132[e]
ME energy[g] (kcal/day)	Male	—	54	65	76	72	68	—	—
	Female	—	36	50	54	58	47	68	118
Net protein[h] (g/day)	Male	1.1	1.8	2.2	2.5	2.4	0.76	—	—
	Female	—	1.2	1.7	1.8	1.9	0.52	2.3	4.0

[a] For minerals and vitamins, see Table 4.
[b] See also Figure 1.
[c] Daily gain during gestation or lactation.
[d] Doe carrying litter of 8 or 9 pups.
[e] Doe and litter of 6 pups.
[f] Assuming 4 kcal/g of food.
[g] Assuming 90 percent of the gross energy is metabolizable.
[h] Net protein = net protein for maintenance + net protein for production. (see note *d* to Table 4 for explanation).

number of growth curves for the smaller strains examined by the authors also agree well with this proposal. Dunn *et al.* (1947) reported that in situations of excellent growth, the curve arched above a straight line. Mayer (1948) presented data in which the slope of the line differed significantly from that of Zucker. While this formula may not agree exactly with all conditions of rat growth, its use is warranted in estimating the growth pattern of males and females. It has the unique advantage of being mathematically defined and thus accurately reproducible. The growth curve from birth to weaning was projected on data presented by Zucker *et al.* (1941b) and Murphy and Dunn (1948). These authors discuss in detail the various phases of the growth cycle.

Published data reveal that the growth curve in Figure 1 is exceeded by the Yale (Osborne and Mendel) strain (Anderson and Smith, 1932; Dunn *et al.*, 1947; McAleese and Forbes, 1961; Mickelsen *et al.*, 1955; Pickens *et al.*, 1940), the large Sherman strain (Harte *et al.*, 1948; Spector, 1956), and the Charles River CD strain (H. L. Foster, personal communication). It is quite similar to that of the Long-Evans strain (Mills, 1955), the Sprague-Dawley strain (G. R. Dawley, personal communication; H. L. Foster, personal communication; Mayer, 1948; Ross, 1959), the Holtzmann strain (Holtzmann Rat Co., personal communication), and the rats used by Folley *et al.* (1938) and by Carlson and Hoelzel (1947). The curve exceeds growth of the Wistar strain (Dunn *et al.*, 1947; Spector, 1956), the Norway rat (Spector, 1956), those used by Deuel *et al.* (1950a) and Alfin-Slater *et al.* (1957), and much of the older growth data (Donaldson, 1924; Greenman and Duhring, 1931; Smith, 1941; Zucker *et al.*, 1941c). Figure 1 must be considered as a selective curve, involving animals suitable for growth experiments. It is almost certain that data on typical growth reported in the literature have not included runts or other undesirable animals.

The coefficient of variation of the body weights at different ages is between 10 and 15 percent from weaning to maturity (Anderson and Smith, 1932; Freudenberger, 1933; Sherman *et al.*, 1949; Smith, 1941; Zucker *et al.*, 1941c). This value suggests that experimental groups should contain approximately 10 animals, to detect successfully ($P < 0.05$) treatment differences in body weights as small as 15 percent (Cochran and Cox, 1950).

The rat is considered to be in a continual state of growth during its lifetime (Dunn *et al.*, 1947). Zucker *et al.* (1941b) observed skeletal growth at 700 days of age, while Dawson (1925) found that the epiphyseal union of some long bones did not occur until 1,000–1,270 days of age. The average life-span of the male is about 700–800 days. Females live about 10 percent longer (Carlson and Hoelzel, 1947; French *et al.*, 1953; Sherman *et al.*, 1949; Sperling *et al.*, 1955). The standard deviation of the life-span has been reported as high as 100–200 days (Shields and Mitchell, 1946), and Sherman *et al.* (1949) reported a coefficient of variation of 20 percent for this observation. These life-span data are lower than the 900 days reported by Donaldson (1924) for slower growing rats.

Reproduction

The reproduction tract of the female rat becomes functional between 40 and 60 days of age (Farris and Griffith, 1949; Goettsch, 1949; Mandl and Zuckerman, 1952; Zucker *et al.*, 1941b), when the vagina opens. Whether the age or weight (100 g) of the rat is the predominantly influencing factor remains uncertain (Asdell and Crowell, 1935; Goettsch, 1949; Mandl and Zuckerman, 1952; Zucker and Zucker, 1941). Females are usually bred initially between 80 and 100 days of age, or between 150 and 200 g (Farris and Griffith, 1949; Sherman *et al.*, 1949). At least 2 weeks are allowed after weaning a litter before rebreeding (Farris and Griffith, 1949). The most successful reproductive life is between 100 and 300 days (Farris and Griffith, 1949; Sherman *et al.*, 1949). In the Wistar Institute, females are kept for only 4 litters, although life-span production of 9–12 litters has been reported (Asdell *et al.*, 1941; Cox and Imboden, 1936).

Table 2 summarizes what can be considered satisfactory reproductive performance. It is an approximate

TABLE 2 A Satisfactory Standard of Reproductive Performance of the Laboratory Rat

Item	Value	Coefficient of Variation (%)
Fertility of mated does (%)	90	
Pups per litter	8–9	30[a]
Birth weight (g)	5.5–6	
Pups weaned (%)	90	34[b]
Weaning weight (g), 21 days		
Males	45	
Females	44	
Weight gain of the doe (g)		
During gestation	85	
During lactation	—10 to ±10	

[a] Smith (1941)
[b] Sherman *et al.* (1949).

average of the normal data of eight publications summarized by Russell (1948), a survey of eight colonies made by Smith (1941), and total of 18 additional papers reviewed by the authors (Alfin-Slater *et al.*, 1957; Dryden *et al.*, 1956, 1957; Dunn *et al.*, 1947; Farris and Griffith, 1949; Folley *et al.*, 1947; Gander and Schultze, 1955; Goettsch, 1949; Murray, 1941; Nelson and Evans, 1953, 1958; Pike *et al.*, 1954; Richardson and Brock, 1956; Schultze, 1957; Schwarz, 1958b; Sica and Cerecedo, 1948; Sure 1941a; Viswanatha and Leiner, 1956). The total gain during gestation is directly related to the number of pups in the litter, the fetuses representing 40–60 percent of the total. The data for rate of gain in Table 2 are averages of the observations of Schultze (1957), Pike *et al.* (1954), Goettsch (1949), Nelson and Evans (1953), and Murray (1941). Of this gain, approximately 60 percent is made during the last week of gestation (Murray, 1941).

Data for weight changes during lactation vary tremendously. For a healthy mother in heavy production, it seems reasonable to expect neither a large gain nor loss. The coefficients of variation have been included where data were available. Their high values suggest that approximately 65 animals are needed per treatment group to detect ($P<0.05$) treatment differences as significant as 15 percent (Cochran and Cox, 1950).

CONCEPT OF NUTRIENT REQUIREMENTS

The minimum nutrient requirements may be altered by a number of environmental, as well as dietary, factors, which cannot be considered within the province of a normal dietary standard. As interpreted in this report, the rat is housed and fed under normal laboratory conditions. Consequently, much information, presented in brief below, has been omitted in describing the requirements for the rat. The detailed reviews by Russell (1948; Nelson and Evans, 1961) on the nutrient requirements of the rat for reproduction and lactation should be consulted for a more comprehensive evaluation of the literature.

1. *Intestinal Synthesis of Nutrients* It is well established that the indigenous microflora of the gastrointestinal tract of the rat can modify the amount of dietary nutrients required. Prevention of coprophagy by the use of antibacterial agents appreciably alters this state of supplementation. This subject has been recently reviewed by Hotzel and Barnes (1966) and data obtained from animals in which coprophagy was permitted and the diets were free of added antibacterial substances.

2. *Stress* Extreme variations in temperature or those induced by the feeding of drugs and hormones have been omitted (Mitchell and Edman, 1956).

3. *Nutritionally Unbalanced Diets* Except as indicated, all requirements are based on diets containing, so far as is known, the other nutrients in the proper amounts.

4. *Depletion* Unless specified, the requirements do not apply to animals depleted of the nutrient in question.

5. *Deficiency Syndromes* In instances where classic deficiency syndromes are recorded, authoritative review articles are cited rather than a myriad of individual papers. Recently these syndromes have begun to include extensive evidence of biochemical lesions. While important in themselves, they have not been discussed except where essential to an understanding of the deficiency state.

6. *Congenital Malformations* A number of specific lesions have been described for the fetal rat from dams subjected to critical and often transient nutritional deficiencies. They are not discussed but have been comprehensively reviewed by Kalter and Warkany (1959), Hurley (1967), and Terroine (1967).

This report does not purport to include all of the papers that have contributed to our knowledge of the nutrient requirements of the rat. It is hoped that the more significant ones have not been overlooked. For further information, the reader is referred to Loosli (1945), Brown and Sturtevant (1949), McCoy (1949), Albritton (1954), Spector (1956), Cuthbertson (1957), and Nelson and Evans (1961).

ENERGY

About 75 percent of the ingredients of a normal diet are included specifically to provide calories. Restricted energy intakes will result in reduced growth and, if sufficiently severe, eventual death. Since the rat is in a state of continuous growth, realimentation results in marked recovery (McCay *et al.*, 1935, 1939; Russell, 1948). If the restriction persists as long as 300 days, however, such animals will never attain maximal size. Conversely, moderate energy restriction has been shown to increase longevity (Berg and Simms, 1960; McCay *et al.*, 1935; Ross, 1961; Silberberg and Silberberg, 1955). Retarded growth delays the onset of puberty, resulting in both cessation of estrus and

ovarian atrophy in the female and testicular degeneration in the male.

Abrupt changes in the caloric density of the diet result only in brief alterations of caloric intake, followed in a few days by an adjustment of food intake to correspond approximately with the previous level of caloric consumption (Adolph, 1947; Harte *et al.*, 1948). Sibbald *et al.* (1956, 1957a) diluted a diet with as much as 40 percent nonnutritive cellulose, and the digestible energy consumption remained unchanged, indicating that the need for calories is the predominant motivation for food consumption. Yoshida *et al.* (1957) have reported further that the daily caloric consumption of growing rats is similar when receiving diets containing from 0 to 30 percent of the diet as fat. However, deficiency states reduce food intake. Rerat and Henry (1963, 1964) altered intake by varying either protein or B-vitamin levels in growing rats and concluded that rate of growth was the primary factor affecting level of caloric consumption.

Growth

The gross energy intake of the rapidly growing rat must be adequate to cover (a) basal metabolism, which is elevated during early life (Brody, 1945); (b) activity, which likewise is more pronounced in the young (Farris and Griffith, 1949); (c) the losses during digestion and metabolism; and (d) the losses needed for growth. There are relatively few published data that have reported detailed weekly caloric intakes of rats during the early growth period. However, a useful, though not especially precise, measure was found to be 10 g of food per 100 g of body weight per day.

At present, there is no completely satisfactory mathematical expression from which to predict the voluntary energy consumption of the rapidly growing rat. Hartsook and Mitchell (1956) reported that consumption followed a quadratic form, with increasing body weight. They presented a formula for predicting feed intake of a diet containing about 4.4 kcal per gram. Hegsted and Haffenreffer (1949) have shown that the caloric intake of rats from weaning to 155 g is related to the expression $W_g^{0.882}$, where W_g is the weight in grams. Yoshida *et al.* (1957) found that the daily caloric intake of rats during the first 3 weeks after weaning was satisfactorily described by the expression 0.9 kcal $Wg^{0.87}$. Neither formulation, however, satisfactorily depicted the caloric intake of slow-growing rats as reported by Wang (1926). The formula of Hartsook and Mitchell (1956) applied to rats up to 350 g did compare favorably with the weekly caloric intake data reported by Harte *et al.* (1948) for male rats gaining at the desirable growth rate as shown in Figure 1. The formula of Yoshida *et al.* (1957) is also a good approximation for a limited growth interval (to 165 g); the food intake data of Hegsted and Haffenreffer (1949) are appreciably lower.

The standard for males shown in Table 1 is an estimate of voluntary caloric intake resulting in growth comparable to that shown in Figure 1. It is based on a data average from Harte *et al.* (1948). (Sherman strain) and Hartsook and Mitchell (1956) (strain unidentified). An examination of over 30 papers published in recent years reveals that the standard exceeds much of the intake data reported, but that comparable gains and intake have been published by Quimby (1948), Mitchell and Beadles (1952), Barnes *et al.* (1958), Sibbald *et al.* (1956), and Yoshida *et al.* (1958). When food intakes have been lower, gain has invariably been lower.

The standard for the female is much less precise, being largely dependent on the report of Harte *et al.* (1948). For growth to a weight of about 125 g the standards are essentially the same as for the male. At best, the standards for both sexes can be considered accurate to within 10–15 percent. The feed consumption data are calculated from the caloric data assuming 4.0 kcal of gross energy per gram of air-dry feed containing 5 percent fat.

To estimate the caloric requirement for rats growing at a rate slower than the standard from Table 1 is difficult. It seems clear that the intake is related, in large part, to the basal metabolism and thus more properly to a fractional power of body weight than to body weight per se (Hegsted and Haffenreffer, 1949). At present, however, published data are inadequate to calculate this requirement more accurately. In all probability, the caloric intake plotted against body weight should approximate the data in Table 1.

Maintenance

The gross energy need for maintenance encompasses the energy needed for (a) basal metabolism, (b) activity, and (c) that lost during the digestion and metabolism of the feed. Brody (1945) and Kleiber (1947) have presented convincing evidence that the basal metabolism of homeotherms is properly related to metabolic body size, i.e., $W_{kg}^{0.7}$ to $W_{kg}^{0.75}$. Because of the ease of computation, Kleiber (1947) suggested the latter figure. The activity needs of the rat have been reported to constitute a plus value of 10–22 percent above the basal metabolism (Metta and Mit-

chell, 1954; Mitchell, 1933). Brody (1945) believed that this activity component is also related to metabolic size. The energy lost in the digestion and metabolism of the diet constitutes about 10 percent of the gross energy, and the extent to which it is or is not related to metabolic size is largely academic. It seems proper, therefore, to express the maintenance energy requirement on the basis of body weight to the ¾ power or $W_{kg}^{0.75}$. The daily maintenance requirement for energy is as follows:

Gross energy	=	121 kcal/$W_{kg}^{0.75}$
Digestible energy	=	115 kcal/$W_{kg}^{0.75}$
Metabolizable energy	=	100 kcal/$W_{kg}^{0.75}$

Nasset (1957) observed that a value of 121 kcal of gross energy agreed with his experimental data. This value compares favorably with the data of McCay *et al.* (1935), Goettsch (1951), and Yoshida *et al.* (1957) for 40- to 130-g rats, as well as for heavier rats (Benditt *et al.*, 1950; Calloway and Spector, 1955; Garcia and Roderuck, 1964; Goettsch, 1951; Harstook and Mitchell, 1956; Kaunitz *et al.*, 1956, 1957, 1958; Munro and Wikramanayake, 1954; Rosenthal and Allison, 1956; Thomson and Munro, 1955). For rats between 200 and 300 g, the value obtained from the above formula, $W_{kg}^{0.75}$ is essentially the same as that obtained by using the value of 1,200–1,300 kcal/m² of body surface (Lee, 1929). At smaller weights, the latter relationship results in values that are too high and at heavier weights, too low. The proposed standard exceeds the data of Metta and Mitchell (1954), who increased the basal metabolism (70.4 kcal/$W_{kg}^{0.734}$) figure of Brody (1945) by 25 percent. It is lower than the standard of Brody (1945), who multiplied the basal metabolism by a factor of two, to obtain the maintenance requirement in terms of digestible energy.

The requirement as digestible energy assumes that the energy of an average purified diet is 95 percent digestible. The value for metabolizable energy is a compromise between data suggesting that the gross energy of a purified diet is 90 percent metabolizable (Beaton and Cheney, 1965; Metta and Mitchell, 1954; Sibbald *et al.*, 1956; Swift and Black, 1949) and the value determined by Ahrens (1967).

The data above are most applicable in the zone of thermal neutrality. The critical temperature of the rat is about 30°C (Swift and Forbes, 1939). It has been shown that the maintenance requirement declines with time on a restricted diet (Kaunitz *et al.*, 1956; Meyer *et al.*, 1956; Quimby, 1948). It appears to level off after about three to five weeks. The above requirement is probably more accurate after a short period of maintenance feeding.

Voluntary intake of calories in adult rats will usually exceed the maintenance requirement by about 25 to 30 percent.

Gestation and Lactation

The calorie requirements for gestation appear to be little above those for maintenance until about eight days before parturition (Champigny, 1963), when they increase appreciably. Restricting the total diet reduces the size and viability of the young and will induce resorption (Berg, 1965; Perisse and Salmon-Legagneur, 1960). Protein, however, appears to be more critical than energy for satisfactory reproduction (Hsueh *et al.*, 1967).

Vountary feed consumption is markedly increased during gestation. The average has been reported to be from 25 to 35 per cent (Cole and Hart, 1938; Goettsch, 1949; Murray, 1941; Slonaker, 1927). An attempt to calculate the average increase in energy consumed per pup born, over the 21-day gestation period, led to values of from 35 to 78 kcal. The daily intake figure in Table 22 is based on a requirement of 60 kcal per pup for the 21-day period in addition to the maintenance needs of the dam.

The requirement for lactation is a total of the food consumed by the dam and the litter. Normally, suckling rats will begin to consume appreciable amounts of feed after about 15 to 17 days of age. The average total feed consumed by the dam and litter is two to three times the pregestational intake (Goettsch, 1949; Hitchcock, 1927; Murray, 1941; Nelson and Evans, 1958; Slonaker, 1925, 1927). Calculations as to calories needed per pup above maintenance of the dam for a 21-day lactation period range betwen 234 and 308 kcal per pup. The figures in Table 22 are based on a requirement of 275 kcal per pup. They are somewhat lower than the consumption reported by Hsueh *et al.* (Hsueh *et al.*, 1967).

PROTEINS AND AMINO ACIDS

Growth

In establishing the protein requirements for each of the physiological functions, three factors have been considered: (a) energy concentration of the diet; (b) amino acid composition of the protein; and (c) digestibility of the protein. Since the feed intake will be related to the energy level of the diet, the protein

TABLE 3 Protein Requirements of the Rat Expressed as a Ratio of Dietary Protein (N × 6.25) to Gross Kilocalories for Diets Containing Little Fibrous Material[a]

	Protein		Protein/kcal		Dietary Protein Level[b]	
Source	True Digestibility (%)	Biological Value (%)	Growth, Gestation, or Lactation (mg protein/kcal GE)	Maintenance (mg protein/kcal GE)	Growth, Gestation, or Lactation (%)	Maintenance (%)
Ideal protein	100	100	29	10	12	4
Casein	98[c]	90[c]	33	11	13.2[d]	4.4[d]

[a] See also Tables 1 and 4.
[b] Containing 5 percent fat or about 4.0 kcal/g of diet.
[c] Figures applicable to casein properly supplemented with sulfur-containing amino acids (0.2 percent of either cystine or DL-methionine should be adequate).
[d] Dry weight basis.

requirement is most properly expressed as a protein-to-calorie ratio (Goettsch, 1948). Ideally, the minimum net protein requirement should be expressed as the amount of protein with the proper amino acid balance used per calorie of dietary net energy. Published experimental data are inadequate to derive such a relationship. It has been possible, however, to calculate from the literature—with only a minimum of assumptions—the net protein requirement as milligrams per gross kilocalorie in diets low in fibrous materials.

The net protein value* for whole egg is about 93 (Block and Mitchell, 1946). If one selects studies in which the minimum amount of egg protein, commensurate with maximum gain, has been determined, it is possible to calculate a net protein per gross calorie ratio that approaches a minimum. The following protein values, as mg/kcal of gross energy, were derived: 28, Barnes *et al.* (1946); 31, Hamilton (1939); 29, Hoagland *et al.* (1948); 25, Mitchell and Beadles (1952). Rose *et al.* (1948) used pure amino acid mixtures of about 30 mg/kcal; a diet containing casein supplemented with sulfur amino acids (Hartsook and Mitchell, 1956) calculated to 27. Goettsch (1948) determined the true digestibility and biological value of a casein–kidney bean diet and reported a ratio of 31 mg protein per kcal.

The optimal ratio of net protein per gross kcal for weanling rats has been taken as an average of these seven observations, or 29 mg per kcal. This value thus corresponds to a low-fiber diet containing 4.0 kcal/g diet and about 12 percent whole egg protein. Table 3, which records this basic requirement and expands it to diets that contain properly supplemented casein, presents data that are lower than the 49 mg protein per kcal reported by Schreiber and Elvehjem (1955) for supplemented casein and the 60 mg per kcal of Yoshida *et al.* (1957) for unsupplemented casein. Breuer *et al.* (1963) found in studies with a fiber-free diet containing 5 percent fat that a level of 14 percent casein (88 percent protein) supplemented with 0.18 percent DL-methionine supported gains nearly equal to those obtained with diets containing 20 percent casein supplemented with DL-methionine. Sibbald *et al.* (1956; 1957a,b) reported data that showed a minimum of 22 mg of apparently digested protein per kcal of apparently digested energy for most efficient nitrogen retention. This lower value agrees with the fact that the most efficient storage of nitrogen occurs at a level below that necessary for maximal gain (Barnes *et al.*, 1946; Forbes *et al.*, 1955). The data in Table 3 may be expressed in terms of metabolizable calories, assuming the diets contain 90 percent metabolizable energy (Metta and Mitchell, 1954; Swift and Black, 1949).

It is impossible, from existing data, to describe the percentage of dietary protein required for optimal growth when the diet includes a mixture of protein sources. Computation of this percentage is further complicated by the influence of (a) the proper amount of essential amino acids, (b) the proper amount of dispensable amino acids or other nonspecific nitrogen sources, (c) availability of the amino acids, and (d)

* Net protein (nitrogen) is the amount of protein (nitrogen) that is used for maintenance and production. The net protein (nitrogen) requirement for maintenance is the metabolic nitrogen + endogenous nitrogen + cutaneous nitrogen. The net protein (nitrogen) for production is the nitrogen balance (includes milk protein). The net protein (nitrogen) may be calculated as follows:

Dietary protein (nitrogen) × true protein (nitrogen) digestion coefficient × biological value (Mitchell, 1924) (B.V. is not necessarily a constant and is influenced by dietary protein or fiber).

$$\text{Net protein as \% of diet} = \frac{\text{net protein for maintenance} + \text{net protein for production} \times 100}{\text{protein intake}}$$

the amino acids that are, of necessity, carried along in foods as excess to obtain the proper level of essential amino acids. Items (c) and (d) will vary greatly, depending on the sources of proteins used. Methods for determining protein quality, such as the amino acid index technique (Oser, 1959), are useful but do not account for variations in absorbability or variations in biological value with increasing level of protein intake (Barnes *et al.*, 1946; Forbes *et al.*, 1958). If ingredients are selected to provide adequate amounts of essential amino acids, levels of 15–20 percent total protein should be sufficient (Young, 1956). In practice, stock colony diets containing 20–25 percent protein have been successful.

There is no doubt that the protein requirement declines with age after weaning (Forbes and Rao, 1959; Hartsook and Mitchell, 1956). Because of a lack of any economic incentive to change to successively cheaper diets with advancing age, the problem has not been studied extensively. Hartsook and Mitchell (1956), by use of a carcass analysis procedure, estimated that the requirements decline from about 28 percent of the diet (57 mg net protein per gross kcal) at 30 days of age to 10 percent (20 mg net protein per gross kcal) at 50 days of age. The higher value is in line with that calculated from an analysis of rat milk (Luckey *et al.*, 1954).

In the estimation of the amino acid requirements, it is necessary to give consideration to the energy concentration of the diets used (Rosenberg and Culik, 1955; Wretlind and Rose, 1950). In the early studies of Rose (1937, 1938), a diet of approximately 30 percent fat and crude sources of B-vitamins was used. In 1946, the fat level was reduced to 2–3 percent and crystalline vitamins were employed (Bowman *et al.*, 1946). The problem, introduced by altering the energy concentration of the diet, was recognized by Rose's group (Wretlind and Rose, 1950), and they devoted some time to a re-examination of the requirements. Rama Rao *et al.* (1959, 1961) studied the essential amino acid requirements of the rat by supplementing 5 percent casein in a diet containing 12 percent fat. The amino acid requirements given in Table 4 are intended for a diet containing 5 percent fat. Extrapolation of the requirements to diets of different caloric densities can probably be safely made by maintaining a constant amino acid-to-calorie ratio and allowing for variations in amino acid digestibility (Guthneck *et al.*, 1953; Kornberg and Endicott 1946; Lushbough *et al.*, 1957; Schweigert and Guthneck, 1953, 1954; Rogers and Harper, 1965).

Values for leucine, isoleucine, threonine, valine, and phenylalanine were set considering the data reported by Rose *et al.* (1949) and Rama Rao *et al.* (1959, 1961) from nutritional studies and the carcass analysis data of Williams *et al.* (1954). One third to one half of the phenylalanine requirement may be furnished as tyrosine (Rama Rao *et al.*, 1961). The value for tryptophan of 0.15 percent is intended for a diet containing adequate amounts of niacin (Forbes and Rao, 1959; Hundley, 1947; Lushbough *et al.*, 1957; Osterling and Rose, 1952; Rama Rao *et al.*, 1961; Rose *et al.*, 1949; Salman, 1954; Williams *et al.*, 1954). The histidine requirement of 0.3 percent is based on data presented by Forbes and Yohe (1955), Harper (1959), Rama Rao *et al.* (1959), and Williams *et al.* (1954). The lysine requirement is set at 0.9 percent (Calhoun *et al.*, 1960; Rama Rao, 1959, 1961; Rose *et al.*, 1949). The total sulfur–amino acid requirement is 0.6 percent, of which one third to one half may be provided by L-cystine (Hartsook and Mitchell, 1956; Rama Rao, 1961; Schweigert and Guthneck, 1954; Wretlind and Rose, 1950). The arginine level is set at 0.6 percent of the diet, based on recent reports (Hepburn and Bradley, 1964; Ranhotra and Johnson, 1965; Rogers and Harper, 1965) showing a much higher requirement than that previously reported by Rose *et al* (1949). Lower levels of arginine may be adequate in diets containing high proline and glutamic acid (Chen *et al.*, 1969).

The difference between the total nitrogen requirement and the essential amino acid nitrogen requirement should be made up with mixtures of nonessential amino acids. Breuer *et al.* (1963) found that purified diets containing amino acids in the proportions recommended in the previous issue of this publication, or the requirements stated by Rama Rao *et al.* (1959, 1961), would not support optimal growth. Studies by Hepburn and Bradley (1964), Ranhotra and Johnson (1965), and Rogers and Harper (1965) established that the diets were deficient in arginine. Breuer *et al.* (1964, 1966) and Rogers and Harper (1965) showed that asparagine was required for maximal growth. Similarly, Hepburn and Bradley (1964) and Breuer *et al.* (1964) found that glutamic acid was necessary for maximal growth, whereas Breuer *et al.* (1964) and Adkins *et al.* (1966) showed that proline was required. The responses shown to these amino acids are presumed to be due to the inability of the rat to synthesize the quantities required in very rapid growth. However, as pointed out by Breuer *et al.* (1964), rats appear to adapt to diets devoid of certain of the nonessential amino acids after a period of time, as indicated by resumption of near-maximal growth. Chen *et al.* (1969) stated that there is no "absolute requirement" for any one of the nonessential amino acids, providing the

TABLE 4 Nutrient Requirements of the Rat in Percentage or Amount per Kilogram of Diet

Nutrient	Growth		Maintenance		Gestation		Lactation	
	Dry	90% Dry Matter	Dry	90% Dry Matter	Dry	90% Dry Matter	Dry	90% Dry Matter
Energy								
GE (kcal/kg)	4,444	4,000	4,444	4,000	4,444	4,000	4,444	4,000
ME[a] (kcal/kg)	4,000	3,600	4,000	3,600	4,000	3,600	4,000	3,600
Fat (%)	5.5	5	5.6	5	5.6	5	5.6	5
Essential fatty acids[b]								
Male (%)	0.67	0.6	—	R[c]	—	—	—	—
Female (%)	0.24	0.22	—	R[c]	0.244	0.22	0.33	0.3
Net protein[d] (%)	13.3	12	4.4	4	13.3	12	13.3	12
Net amino acids								
L-arginine (%)	0.67	0.6	—	—	0.83	0.75	0.83	0.75
L-asparagine (%)	0.44	0.4	—	—	—	—	—	—
L-glutamic acid (%)	4.4	4	—	—	—	—	—	—
L-histidine (%)	0.33	0.30	0.08	0.07	0.60	0.54	0.60	0.54
L-isoleucine (%)	0.61	0.55	0.48	0.43	0.61	0.55	0.61	0.55
L-leucine (%)	0.83	0.75	0.28	0.25	0.83	0.75	0.83	0.75
L-lysine (%)	1	0.90	0.16	0.14	1.38	1.24	1.38	1.24
L-methionine (%)	0.67[e]	0.6[e]	0.26[e]	0.23[e]	0.67[e]	0.6[e]	1.1[e]	1[e]
L-phenylalanine-L-tyrosine (%)	0.89[f]	0.8[f]	0.21[g]	0.19[g]	0.89[f]	0.8[f]	0.89[g]	0.80[f]
L-proline (%)	0.44	0.4	—	—	—	—	—	—
L-threonine (%)	0.56	0.5	0.19	0.17	0.56	0.5	0.56	0.5
L-tryptophan (%)	0.17	0.15	0.08	0.07	0.22[h]	0.20[h]	0.22[h]	0.20[h]
L-valine (%)	0.67	0.6	0.34	0.31	0.67	0.6	0.67	0.6
Nonessential amino acid nitrogen (%)	0.61[i]	0.55[i]	0.47[i]	0.42[i]	0.97[i]	0.87[i]	0.93[i]	0.84[i]
Calcium (%)	0.56	0.5	—	—	0.67	0.6	0.67	0.6
Chlorine (%)	0.06	0.05	—	—	0.028	0.025	0.020	0.018
Chromium (see text)								
Copper (mg/kg)	5.6	5	—	—	?	?	?	?
Iodine (mg/kg)	0.17	0.15	—	—	0.17	0.15	0.17	0.15
Iron (mg/kg)	38.9	35	—	—	?	?	?	?
Magnesium (%)	0.04	0.04	—	—	0.056	0.05	0.056	0.05
Manganese (mg/kg)	55.6	50	—	—	56[k]	50[k]	37	33
Phosphorus (%)	0.44	0.4	—	—	0.56	0.5	0.56	0.5
Potassium (%)	0.20	0.18	—	—	0.16	0.14	0.56	0.5
Selenium (mg/kg)	0.04	0.04	—	—	?	?	?	?
Sodium (%)	0.06	0.05	—	—	0.06	0.05	0.06	0.05
Zinc[k] (mg/kg)	13.3	12	—	—	—?	?	?	?
Vitamin A[l] (mg/retinol/kg)	0.67	0.6	—	—	4	3.6	4	3.6
Vitamin D (IU/kg)	1,111	1,000	—	—	—	—	—	—
Vitamin E (α-tocopherol equivalent) (mg/kg)	39	35	—	—	33[k]	30	22	20
Vitamin k[m] (vitamin K_1 equivalent) (mg/kg)	0.06	0.05	—	—	—	—	—	—
Choline chloride (mg/kg)	833	750	—	—	<1,111	<1,000	<1,111	<1,000
Niacin[n] (mg/kg)	16.7	15	—	—	?	?	?	?
Calcium pantothenate[o] (mg/kg)	8.9	8	—	—	8.9	8	11	10
Riboflavin (mg/kg)	2.8	2.5	—	—	4.4	4	4.4	4
Thiamin hydrochloride (mg/kg)	1.39	1.25	—	—	2.8	2.5	4.4	4
Vitamin B_6 (pyridoxine hydrochloride equivalent) (mg/kg)	7.8	7	—	—	0.67	0.6	0.44	0.4
Vitamin B_{12} (mg/kg)	0.0056	0.005	—	—	0.0056	0.005	0.0056	0.005

levels of metabolically related amino acids are present in high enough quantities, e.g., the glutamic acid–proline–arginine relationship.

It is evident that specific "requirements" for the nonessential amino acids cannot be given because of the influence of the metabolic relationships between them. Therefore, the values given in Table 4 represent a pattern that has been used successfully in studies with purified diets. The value of 4 percent for glutamic acid is based on the data of Hepburn and Bradley (1964) and Breuer *et al.* (1964); that for asparagine is 0.4 percent, as found by Breuer *et al.* (1966) to be required for maximal growth. Four percent proline is the level used by Adkins *et al.* (1966). To raise the total ration to 12 percent protein equivalent (%N × 6.25), a mixture of alanine, glycine and serine should prove sufficient.

Amino acid imbalances and antagonisms can result in increased requirements for individual amino acids, an area recently reviewed by Harper (1964) and Harper and Rogers (1965). They concluded that the effect of imbalances and antagonisms on the requirement for maximum growth may be small, but the effect in diets containing suboptimal levels of protein may be considerable. They suggest that the effect of the imbalance is depression in feed intake.

Maintenance

It has been well established that endogenous nitrogen excretion is related to basal metabolism (Brody, 1945; Mitchell, 1933, 1955b). Thus, as with the maintenance energy reqiurement, the nitrogen requirement is most properly expressed as a function of metabolic body size ($W_{kg}^{0.75}$) (Brody, 1945; Kleiber, 1947). Data calculated from the literature gave values from 140 to 250 mg N/$W_{kg}^{0.75}$ (Barnes *et al.*, 1946; Goettsch, 1951; Marshall and Womack, 1954; Womack *et al.*, 1953); a median value of 200 mg N/$W_{kg}^{0.75}$ was selected. If a gross kcal requirement of 121/$W_{kg}^{0.75}$ is assumed and 200 is multiplied by 6.25, the dietary protein requirement becomes 10 mg protein per gross kcal. This figure is higher than the value (writer's calculations) obtained with whole egg diets of 8.0 of Bricker and Mitchell (1947), and of 7.2 of Kelley and Ohlson (1954) and Hartsook and Mitchell (1956). It is lower than the value of 14 derived from the data of Brody (1945). The latter arbitrarily multiplied the basal requirement of 2 mg N per basal kcal by 4. It is also lower than that used by Benditt *et al.* (1950) with an amino acid diet, essentially the same as the minimum value recommended by Mitchell (1955b), and higher than that determined for depleted rats by Nasset (1957).

The requirement is shown in Table 3 as well as Tables 1 and 4. Calculations for diets containing supplemented casein are shown in Table 3. A level of 7 percent is suggested for diets containing natural feed mixtures. This value is in agreement with those of Bricker and Mitchell (1947), when milk or soy proteins were used instead of egg protein.

The essential amino acid requirements for maintenance have been studied extensively by Wissler *et al.* (1958), Benditt *et al.* (1950), and Smith and Johnson (1967). The latter reported the requirements per unit of body surface as determined from observations on nitrogen balance and body weight maintenance. The data of Benditt *et al.* (1950) for body weight maintenance were higher and have been recalculated to metabolic body size, using Benditt's data determined on 300-g rats (mg/$W_{kg}^{0.75}$): tryptophan 20.9; histidine 20.9; phenylalanine 56.9; lysine 42.7; threonine 50.3; methionine 69.3; leucine 75.9; valine 94.9; and isoleucine 130.0. Assuming a basal energy requirement of 121 kcal/$W_{kg}^{0.75}$, these data have been incorporated into Table 4 as a percentage of the diet. The data are about 25 percent higher than those calculated by Mitchell (1955a). The tryptophan requirement agrees well with the reports of Cole and Robson (1951), and the value for methionine is almost identical with that given by Hartsook and Mitchell (1956).

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[a] Assuming 90 percent of the gross energy is metabolizable.
[b] As linoleic acid included in a diet containing 4.0 kcal gross energy per gram.
[c] Required in trace amounts
[d] Net protein = $\frac{\text{net protein for maintenance} + \text{net protein for production}}{\text{protein intake}} \times 100$. (See p. 62, for explanation.)
[e] One third to one half may be supplied by L-cystine.
[f] One third to one half may be supplied by L-tyrosine.
[g] In the presence of ample tyrosine.
[h] Amino acid requirements for gestation and lactation have not been determined except for methionine and cystine. Values listed are believed to approach the minimum.
[i] Furnished as a mixture of glycine, *l*-alanine and L-serine.
[j] Furnished as a mixture of nonessential amino acids.
[k] When rats are housed in galvanized steel cages, the dietary zinc requirement is 0.004 mg per rat per day. A value of 18 mg/kg of diet is required with isolated soybean diets.
[l] One IU vitamin A activity equivalent to 0.3 g retinol.
[m] Many rats receiving a diet devoid of vitamin K do not develop the characteristic deficiency signs, unless a sulfonamide drug is present in the diet to suppress bacterial synthesis of the vitamin in the intestinal tract.
[n] Assuming no more than 0.5 percent tryptophan in the diet.
[o] A level of 8 mg of calcium pantothenate per kg of diet was sufficient to enable the adult rat to carry out tissue acetylation reactions.

Gestation and Lactation

Protein requirements for reproduction and lactation have been reviewed by Russell (1948). She concluded that levels of 17–20 percent were adequate for diets of good protein quality. Later studies have confirmed this conclusion. Nelson and Evans (1953) reported that 5 percent protein, as unsupplemented casein, was the minimal level allowing reproduction to occur, while optimal performance occurred at 15–20 percent. Gander and Schultze (1955) reported good results for reproduction and lactation with 15–16 percent levels derived from a combination of casein, methionine, and mixed cereals. Similarly, Goettsch (1949) found 16.7 percent to be adequate for diets in which the true digestibility and biological value of the protein were 84.1 and 74.1 percent, respectively. The figure of 16.7 percent is identical to Russell's requirement for growth. Sherman *et al.* (1949) reported a diet containing 20 percent to be superior to one containing 16 percent, when the diet contained milk products, wheat, and beef muscle.

It would appear from the above that, if the data are corrected for digestibility and biological value, the net protein requirement for gestation and lactation as a percentage of the diet does not differ significantly from that for growth of weanling rats. The standard, therefore, has been set up to be identical with growth, and the data in Tables 1, 3, and 4 are so designated.

The amino acid requirements for lactation have been studied only briefly. Nelson and Evans (1958) reported that the sulfur–amino acid requirement was 1 percent of the diet, one half of which could come from cystine. Greenstein *et al.* (1957) obtained excellent results for reproduction using a water-soluble synthetic diet supplemented with about 3 percent corn oil. Weaning weights were below normal. Their amino acid mixture was not described as a minimum; since many of the values approach the growth requirement, they may be taken as "approaching" the minimum requirement for gestation and lactation and appear to be the best estimate available at present. They have been included in Table 2 with the methionine–cystine value for lactation increased to 1 percent. In the absence of specific data, the nonessential amino acid nitrogen should be supplied as a mixture of nonessential amino acids.

Symptoms of Deficiency

Protein deficiency in the growing animal results in growth reduction, anemia, hypoproteinemia, depletion of protein reserve, muscular wasting, emaciation, and, if sufficiently severe, death. In the adult, a loss of weight and body nitrogen also occurs (Cannon, 1948), and chronic deficiency may lead to edema (Alexander and Saubereish, 1957). Estrus becomes irregular and may cease, fetal resorptions occur, and the newborn are weak or dead. Similarly, reproductive capacity in the male is impaired (Goettsch, 1949; Russell, 1948). Low-protein diets also result in reduced food intake (Black *et al.*, 1950)

Removal of a single essential amino acid results in an immediate reduction in feed consumption, a situation that can return to normal within 24 hours after replacement. Prolonged deficiency leads to a syndrome typical of a protein deficiency (Cannon, 1948; Meister, 1957; Womack and Kade, 1944). A few specific signs, characteristic of a lack of one amino acid, have been reported: tryptophan, cataract and corneal vascularization, alopecia (Cannon, 1948; Meister, 1957); lysine, increase in dental caries, impaired bone calcification, blackened teeth, hunched stance, unsteady gait (Bavetta and McClure, 1957; Cannon, 1948; Harris *et al.*, 1943; Kligler and Krehl, 1952; Likins *et al.*, 1957; Meister, 1957); methionine, fatty livers (Follis, 1958). The accumulation of a porphyrin-like pigment on the nose and paws has been observed for deficiencies of tryptophan, methionine, and histidine (Cole and Robson, 1951; Forbes and Vaughan, 1954), though this condition is also observed for other deficiency states.

CARBOHYDRATE

There is no specific requirement for carbohydrate per se. Although optimal performance is possible on diets devoid of carbohydrate, certain kinds of carbohydrate have been shown to modify the response on marginal diets. In general, the complex carbohydrates, starch and dextrin, promote a higher growth rate than do the soluble mono- and disaccharides. This effect has been demonstrated on diets low in both vitamins (Ham and Scott, 1953; Hundley, 1949; Krehl *et al.*, 1946; Peterson *et al.*, 1953) and protein (Bavetta and Ershoff, 1955; Dryden *et al.*, 1956; Harper, 1959; Harper and Katayama, 1953; Harper and Spivey, 1957; Harper *et al.*, 1953; Marshall and Womack, 1954; Nino-Herrera *et al.*, 1954) and appears to be related to an intestinal microbiological population, which is more active on diets containing the complex carbohydrates.

Newly weaned rats consumed more food during their first week when diets contained dextrin in place of sucrose (Yoshida *et al.*, 1958). This results from increased water in the stomach caused by the higher

osmotic effect of the simple carbohydrates. Xylose is toxic (Booth *et al.*, 1953) and the outer layer of the potato starch granule is resistant to the rat's digestive enzymes (Jeluick *et al.*, 1952). Also, the rat has a "sweet tooth" as evidenced by its preference for a water-soluble diet containing sucrose instead of glucose (Winitz *et al.*, 1957) or a 10 percent sucrose solution instead of plain water (McCay, 1947).

The interaction of different kinds of carbohydrates, fats and proteins cannot be overlooked as exemplified by two studies (Carroll and Bright, 1967; Reussner *et al.*, 1963). A recent review of some aspects of dietary carbohydrate has been published (Hotzel and Barnes, 1966).

FAT

The addition of fat to the diet provides calories and essential fatty acids. In addition, fat is necessary for optimal utilization of the fat-soluble vitamins.

Essential Fatty Acids

Burr and Burr (1929, 1930) first demonstrated a dietary requirement for the essential fatty acids. It is now clear that arachidonic acid is the biologically important essential fatty acid (Daughaday *et al.*, 1955), although sexual maturation in the male apparently requires production of ω 6 docosapentaenoic acid (Davis and Coniglio, 1966; Kirshman and Coniglio, 1961). Arachidonic acid, found predominantly in animal tissues (Haines *et al.*, 1962; Hulanicka *et al.*, 1964), is about three times as active as linoleic acid (Deuel *et al.*, 1951; Turpeinen, 1938) and is readily synthesized, *in vivo*, from linoleic acid. Since linoleic acid is widely distributed in plant oils, it becomes the important dietary essential fatty acid.

The position of linolenic acid is less certain: It does promote growth but is inefficient in curing the skin lesions of the deficiency when fed alone. When fed in combination with linoleic acid, it is fully as effective as linoleic acid (Deuel *et al.*, 1955b). Thomasson (1953) long ago pointed out that α-linolenic acid ($\triangle$6, 9, 12-octadecatrienoic) was equal to linoleic for growth promotion and is a specific intermediary in the conversion of linoleic to arachidonic acid (Mead and Howton, 1957). Whether it cures the dermatitis of the deficiency is not clear. Holman (1958) has suggested that the term "essential fatty acid" applies only to those fatty acids that will improve growth and alleviate the skin changes. This definition thus applies only to linoleic and arachidonic acids.

The requirement for essential fatty acids is usually expressed as linoleic acid, with the greatest biological activity attributed to the *cis–cis* isomer (Privett *et al.*, 1955, 1967). It has been suggested that if the ratio of triene to tetraene is less than 0.4 in the liver, erythrocytes, and heart, the minimum requirement of linoleate has been met (Holman, 1960). Using these criteria, Pudelkewicz *et al.* (1968) report the linoleate requirement to be 1.3 percent of the calories for males and 0.5 percent of the calories for the females. Assuming a caloric density of 9 kcal/g for pure linoleic acid and a diet containing 4,000 kcal/kg, the linoleate requirement recalculates to 0.6 percent for males and 0.22 percent for females. For a summary of the requirements, see Table 4.

Reproduction is satisfactory when animals are fed at a level equivalent to that required for growth; lactation, however, requires more than 80 mg per day (Deuel *et al.*, 1954, 1955b; Mackenzie *et al.*, 1939; Quackenbush *et al.*, 1942). Optimal weaning weights have not been obtained in these studies.

It has frequently been shown that diets high in saturated fat require a higher level of linoleate for maximum performance (Aaes-Jorgensen *et al.*, 1955, 1956b; Kaunitz *et al.*, 1960; Peifer and Holman, 1959). This is not due to a direct effect of the saturated fatty acids on linoleate conversion to arachidonic acid but probably reflects the importance of linoleate in the utilization of saturated fatty acids (Mohrhaurer and Holman, 1967), justifying the requirement in relation to caloric density. Oleic acid (Lowry and Tinsley, 1966) and cholesterol (Holman and Peifer, 1960) also increase the linoleic acid requirement.

Symptoms of Deficiency

A deficiency in the growing rat (Burr and Burr, 1929, 1930) is characterized by a reduction in growth, scaly skin, a rough, thin hair coat, necrosis of the tail in the later stages, kidney damage resulting in hematuria, and eventual death. There is an increase in the basal metabolic rate, often increasing caloric intake and water consumption. Further, electrocardiographic anomalies have been reported (Caster and Ahn, 1963); females manifest irregular estrus, prolonged gestation, frequent resorptions, difficult and prolonged parturition, poor litters of low viability, and reduced lactation; and spermatogenesis is impaired in the male. In the young, skin lesions develop in from 5 to 12 weeks and become progressively worse. These symptoms become more severe as the relative humidity declines to about 40–50 percent. Growth plateaus after about 12–18 weeks (Aaes-Jorgensen and Dam, 1954a,b,c;

Aaes-Jorgensen and Holman, 1958; Aaes-Jorgensen *et al.*, 1955, 1956a,b, 1957; Barnes *et al.*, 1959b; Burr, 1942; Burr and Barnes, 1943; Deuel, 1957; Deuel *et al.*, 1950b, 1954, 1955a,b; Funch *et al.*, 1957; Holman, 1954; Kummerow *et al.*, 1952; Panos and Finerty, 1953, 1954; Panos *et al.*, 1956).

Several signs appear before the obvious gross lesions develop. Panos and Finerty (1954), Panos *et al.* (1956) and Morris *et al.* (1957) noted that the increase in basal metabolic rate occurred during the first 2 weeks. Also, Aaes-Jorgensen and Holman (1958) showed that one of the earliest and most critical measures of essential fatty acid status is the level of trienoic acid in the heart muscle; on a deficient diet, it increases markedly. This observation is useful if the diet contains no trienoic acid such as is found in cod liver oil.

The deficiency syndrome is not commonly produced in adult rats and spontaneous recovery seems to occur. Barki *et al.* (1947) produced the syndrome after a period of caloric restriction by offering a fat-free diet *ad libitum*.

Level of Fat

Fat has been long considered as an optional component of the diet, except as it supplies the essential fatty acids (EFA). *A fat deficiency does not occur* (except for the EFA deficiency) *in the sense that a specific syndrome develops.* There is, however, sufficient evidence to suggest that some fat in addition to the essential fatty acids is desirable since general performance improves. After a lifetime of study in the field, Deuel (1957) was convinced that fat should be an obligatory part of the diet.

There is good evidence to support the view that supplementary fat is desirable for growth (Deuel *et al.*, 1950b; Greenberg *et al.*, 1950, 1951; Henderson *et al.*, 1945; Lassen and Bacon, 1949; Mohrhaurer and Holman, 1967; Pearson and Panzer, 1949) and lactation (Loosli *et al.*, 1944). Females also attain sexual maturity at an earlier age on diets with added fat (Deuel *et al.*, 1947). Excellent reproduction and lactation have been maintained for 4 (Dryden *et al.*, 1957) to 46 generations (Alfin-Slater *et al.*, 1957) on diets containing 10 and 11 percent fat. Differences in reproduction and lactation are evidently small when diets range from 3 to 18 percent of fat (Richardson *et al.*, 1964). Swift and Black (1949) also reported fewer feed refusals on a 30 percent fat diet than on a 2 percent fat diet.

There also are reports in which increases in the fat content did not result in an appreciable improvement in growth (Aaes-Jorgensen and Dam, 1954a,c; Baski *et al.*, 1950; Hoagland *et al.*, 1952; Meng and Youmans, 1955; Thomasson, 1955). Furthermore, French *et al.* (1953) reported a reduced life-span on diets containing 20 percent corn oil. The same groups (French *et al.*, 1952; Swift, 1952) showed that reproduction on this same diet was also impaired.

It is probably safe to generalize that when moderate amounts of fat are added to the diet, caloric consumption is more frequently increased than depressed (Barki *et al.*, 1950; Deuel *et al.*, 1947) and that carcasses tend to contain more fat (Barki *et al.*, 1950; Dryden *et al.*, 1956; Lassen and Bacon, 1929; Scheer *et al.*, 1947b). Mickelsen *et al.* (1955) have used a diet containing 64 percent fat to obtain obese rats.

The evidence is far less conclusive for establishing the optimal level of fat. Almost any value may be derived from the literature. Barki *et al.* (1950) reported no consistent trend in growth with increasing fat level, rather the carbohydrate source was found to be important (Boutwell *et al.*, 1943; Doyden *et al.*, 1956). Peifer and Holman (1959) have suggested that the level of EFA fed may possibly account for the varying results. Many workers have reported favorable live weight gain responses with increasing fat level (Aaes-Jorgensen *et al.*, 1957; Barnes *et al.*, 1959b; Deuel *et al.*, 1947; Hoagland and Snider, 1940, 1941; Scheer *et al.*, 1947a). Scheer *et al.* (1947a) and Deuel (1955a) recommended 30 percent as the optimal level of dietary fat.

In spite of the contradictory nature of published data, a dietary standard should specify a fat level. It has frequently been implied or stated (Crampton, 1964; Goettsch, 1948; Kleiber, 1945; Mendel, 1923; Mitchell, 1955a; Wretlind and Rose, 1950) that since most nutrients are consumed and used as a function of the metabolism of energy, their level in the diet is properly related to the energy concentration of the diet. The nutrient that most profoundly affects the energy concentration of the diet is fat. Since, on an applied basis, most nutrients are expressed as a percentage of the diet, the level of energy concentration (or fat) is critical in reporting a diet composition that is minimal. Experimental diets for rats have varied widely in fat content and thus the range of energy concentrations used in practice is quantitatively significant. With existing data, it is impossible to state with certainty how much each nutrient should be changed as the caloric density (fat %) of the diet changes. The error will probably be small, however, if a constant nutrient-to-calorie ratio is maintained.

The level of fat suggested as a standard for all physiological activities is 5 percent. This figure was derived from the following sets of data: (a) That of Swift and Black (1949) showed that the greatest improvement in energy retention occurred when the fat level was increased from 2 to 5 percent, with increases in energy retention smaller when the fat level was above 5 percent. (b) The data of Deuel *et al.* (1947) exhibited the greatest reduction in number of days required to reach puberty occurring when the percentage of fat in the diet was increased between 0 and 5 percent. Again, subsequent changes with increasing levels of fat were relatively small. (c) Burns *et al.* (1951) demonstrated that 5 percent fat was a satisfactory level for the absorption of carotene and vitamin A. (d) Loosli *et al.* (1944) reported slight gain improvement of litters, when lactating mothers were fed diets containing over 5.5 percent fat. Further, many fats will provide ample essential fatty acids at this level; many studies designed to determine the nutrient requirements have contained about this level; and although increases in fat level frequently result in more rapid gains, in many instances as cited above, the carcass fat is increased. The desirability or need for this increase has not been established.

Deuel (1955b, 1957) has summarized the extensive literature on the relative value of different fats as well as their digestibilities.

MINERALS

The minimum requirements for minerals have been summarized in Tables 3 and 4. A single value is given for the growing rat, since seldom has any attempt been made to study the change in requirement with advancing age. Data for maintenance have been reported only for calcium and magnesium.

In some experiments, requirements were determined as units per day per animal. These data have been converted to units per kg of diet on the basis of an estimated feed intake of 10 g per rat per day for growth, 20 g per day for gestation, and 30 g per day for lactation.

The inherent and baffling problems of mineral interactions have been critically discussed by Davis (1960).

Chlorine

There are no accurate data suggesting a minimum requirement other than that growing rats receiving 0.28 percent chloride diets (Voris and Thacker, 1942) performed satisfactorily. Miller (1926) reported that 5 mg/day was sufficient for acceptable reproduction and lactation. This figure is listed as a possible requirement for all functions.

Symptoms of Deficiency The rat tenaciously conserves its supply of tissue chloride by drastically reducing the urinary excretion within hours of consuming a diet deficient in the element. As a result, the symptoms are less noticeable and require long periods of deprivation to develop. On a diet containing 0.02 percent chloride (Pickens *et al.*, 1940), there was a depression of appetite and a reduction in body gain of nitrogen and energy. Water consumption and heat production increased, while digestion and absorption remained normal. On a diet of 0.012 percent chloride (Greenberg and Cuthbertson, 1942), there were no outward signs except poor growth and a reduction in blood chloride and in chloride excretion. After a year on a diet of about 0.005 percent chloride, there was marked kidney pathology in addition to poor growth and poor feed efficiency (Cuthbertson and Greenberg, 1945).

Chromium

All signs of chromium insufficiency have been prevented by including 2–5 ppm trivalent chromium in the drinking water.

Symptoms of Deficiency Recent studies have suggested that under strict dietary and environmental conditions (Mertz, 1967), trivalent chromium may be a required nutrient. The deficiency was first noted when rats demonstrated a delayed glucose tolerance response (Mertz, 1967). Diets containing less than 0.17 μg/g of diet result in hyperglycemia and glycosuria similar to diabetes mellitus (Schroeder, 1966). Total mature male weight and life-span increased slightly (Schroeder *et al.*, 1963, 1967). On a 10 percent soy diet, corneal opacity develops, which can be reversed by the addition of chromium (Roginski and Mertz, 1967).

Cobalt

Cobalt, other than as a constituent of vitamin B_{12}, is apparently not required by the rat (Underwood, 1962).

Copper (See Iron)

Fluorine

Fluorine apparently is not a required element (Jenkins, 1967). Studies by Maurer and Day (1957) have shown that four generations of rats performed normally on a diet containing less than 0.007 ppm of fluorine.

Iodine

The relatively few studies that have been conducted to determine the minimum iodine requirement agree remarkably: between 100 and 200 μg/kg of diet (Halverson *et al.*, 1945; Levine *et al.*, 1933; Parker *et al.*, 1951; Remington and Remington, 1938). There appears to be no special requirement for reproduction; the studies of Parker *et al.* (1951) indicated that amounts between 100 and 225 μg/kg of diet were satisfactory. Kellerman (1934) reported that a stock diet containing 330 μg/kg of diet resulted in excellent reproduction.

Symptoms of Deficiency Iodine deficiency in the growing rat results in goiter, characterized by an enlargement of the thyroid gland (Figure 2). A prolonged deficiency in older rats results in essentially the same syndrome (Taylor and Poulson, 1956). Females deprived of iodine during pregnancy give birth to young with heavier thyroids than normal. An iodine deficiency will also inhibit reproduction (Feldman, 1960).

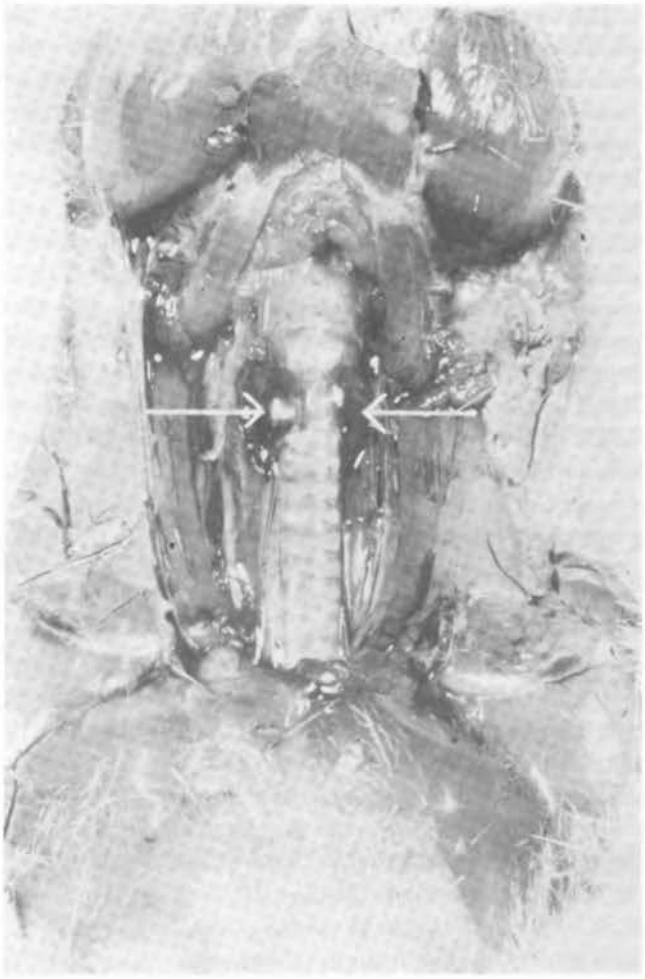

FIGURE 2 Iodine deficiency. The thyroid gland is greatly enlarged in this 9-week old rat fed a low-iodine diet containing 25 μg of iodine/kg diet. (Courtesy H. E. Parker, Purdue University.)

Iron and Copper

The iron requirement for growth and maximal hemoglobin level derived from the data of McCall *et al.* (1962a) is about 35 mg/kg of diet. This level is within the wide range of values available from the older literature.

Reports of a minimum requirement for copper have varied between 0.01 and 0.143 mg per rat per day (Hart *et al.*, 1928; Hundley, 1950; Keil and Nelson, 1931; Levine *et al.*, 1931, 1932; Mills, 1955; Mitchell and Miller, 1931; Pearson *et al.*, 1957; Rose *et al.*, 1934; Schultze *et al.*, 1934). The table of requirements (Table 4) lists an approximate average of 0.05 mg per rat per day of copper. Slightly higher amounts of copper (between 0.05 and 0.96 mg per rat per day) are needed to prevent achromotrichia. The amount essential to prevent this condition is thus about 0.1 mg per rat per day. A study by Mills (1955) suggests that the availability of copper from natural foodstuffs is superior to that supplied by pure copper ion and indicates that the studies conducted previously on minimum requirement may need re-evaluation.

No requirement values have been established for the rat for iron and copper for reproduction. Levels of 240 mg of iron per kg of diet gave satisfactory reproduction for three generations (McCall *et* al., 1962a).

Symptoms of Deficiency A deficiency of either iron or copper results in anemia of a hypochromic, microcytic type (Smith and Medlicott, 1944; Underwood, 1962). In iron deficiency, white incisor teeth, cardiomegaly, and splenomegaly develop and the cecum en-

larges (Cusack and Brown, 1965; McCall *et al.*, 1962b). Black-haired rats on a copper- or iron-deficient diet develop achromotrichia (Cusack and Brown, 1965; Henderson *et al.*, 1942; Hundley, 1950; Keil and Nelson, 1931), indicating an interrelation with pantothenic acid. (Cusack and Brown, 1965; Singer and Davis, 1950).

When whole milk supplemented with manganese was fed to rats from weaning until they gave birth, a noticeable effect on the offspring was noted unless copper or iron were added. Iron deficiency produces anemia in the dams and anemic, nonviable young; a copper deficiency did not produce anemia but the dam gave birth to severely anemic young characterized by edema and widespread subcutaneous hemorrhages (Odell *et al.*, 1961).

Magnesium

The requirement for the growing rat has been variously suggested as 60 mg/kg of diet (Medes, 1926), 50 mg/kg of diet (Tufts and Greenberg, 1938) and 200 mg/kg of diet (Kunkel and Pearson, 1948). The Kunkel and Pearson study included blood magnesium levels as a criterion and may be more reliable than methods based on other criteria. McAleese and Forbes (1961) have reported precise studies of the influence of dietary magnesium on growth of the weanling rat and the magnesium levels in bone and blood. While 100 mg/kg were adequate to support normal growth, from 350 to 425 mg of magnesium per kg of diet were necessary to maintain normal levels of blood magnesium. Thus, 400 mg of magnesium per kg of diet appears to be the requirement for the growing rat, and that for maintenance is 2 mg per kg of live weight per day or about 0.005 percent of the diet (Smith and Field, 1963).

For pregnancy and lactation, Tufts and Greenberg (1938) suggested a requirement of 500 mg/kg of diet. This is the only such study available.

Symptoms of Deficiency A deficiency of magnesium in the growing rat results in vasodilatation, hyperirritability, cardiac arrythmia, spasticity, and fatal clonic convulsion. Vasodilatation occurs after about 1 week on the diet and may disappear spontaneously within a short period of time. Convulsions occur between 21 and 30 days (Ko *et al.*, 1962; Kunkel and Pearson, 1948; McCoy, 1949; Mickelsen *et al.*, 1955). The deficiency eventually results in sudden death. Kidney calcification is a common postmortem sign (Forbes, 1964). Tufts and Greenberg (1938) reported that mothers on a deficient diet were bred successfully but did not suckle their young.

Manganese

The requirement for growth has not been adequately studied. Wachtel *et al.* (1943) reported a reasonable gain on an intake of 0.05 mg per rat per day, while Anderson and Parker (1955) showed that 0.5 mg per day was only slightly superior to 0.05 mg. Holtkamp and Hill (1950) obtained only slight improvement in growth when approximately 2 mg per rat per day was compared to 0.5 mg. On the basis of these limited data, the requirement given in Table 4 is 0.5 mg per rat per day or 50 mg/kg of diet.

The requirement for manganese during reproduction was studied by Orent and McCollum (1932), Daniels and Everson (1935), and Richardson and Hogan (1940). The values reported to give satisfactory reproduction and lactation range between 0.35 and 1.2 mg per rat per day. Table 3 shows the requirement to be 50 mg/kg diet for growth as well as gestation and 33 mg/kg diet for lactation.

Symptoms of Deficiency An inadequate level of dietary manganese results in poor growth and defective mineralization of the bone. Food consumption is reduced and early mortality results. Reproduction is impaired, characterized by testicular degeneration in the male and defective ovulation in the female. If reproduction does occur, many of the young are paralyzed and uncoordinated. Lactation is apparently not affected since manganese-deficient mothers will suckle normal young satisfactorily. Death of the young from deficient mothers apparently results from lack of viability (Underwood, 1963). Hurley *et al.* (1961) have described the skeletal abnormalities occurring in live young born to manganese-deficient dams.

Molybdenum

Molybdenum, an integral part of xanthine oxidase, a liver enzyme, might lead one to deduce that it is a required nutrient (DeRenzo *et al.*, 1953; Richert and Westerfeld, 1953). However, feeding diets containing approximately 20 μg/kg of diet (approximately 0.2 μg per rat per day), or inhibition of xanthine oxidase with sodium tungstate, resulted in no untoward signs; neither did it impair growth or reproduction (Higgins *et al.*, 1956). On the basis of present information, therefore, it is not possible to list a dietary requirement for this element.

Phosphorus (See Calcium)

Potassium

Two studies of the requirement for growth by Grunert *et al.* (1950) and Kornberg and Endicott (1946) suggest a level of 0.17–0.18 percent of the diet. The balance data of Heppel and Schmidt (1949) indicate that a level of 0.5 percent of the diet is adequate for lactation, with 0.14 percent sufficient for reproduction. Lactation experiments by Nelson and Evans (1961) have confirmed the requirement to be between 0.5 and 0.6 percent of the diet.

Symptoms of Deficiency Insufficient potassium results in a markedly reduced appetite and insignificant growth. Animals become lethargic and comatose and are often dead by 3 weeks. They have an untidy appearance, cyanotic skin, short fur-like hair, diarrhea, and distended abdomens. Postmortem examination reveals ascites and frequently hydrothorax. Pathological lesions are widespread (Kornberg and Endicott, 1946; Schrader *et al.*, 1937). In one experiment (Robbins *et al.*, 1965) a level of 0.1 percent K in the diet resulted in a symmetrical loss of hair along the back with a 50 percent reduction in hairs per follicular group.

Sodium

The sodium requirement as determined by Grunert *et al.* (1950) was 0.05 percent of the diet and was independent of the potassium intake. This level agrees with that of Miller (1923). Pregnant females on low sodium diets (0.03 percent) ate less food and showed degrees of languor and debility, which were especially pronounced during the last week of pregnancy; however, they still reproduced fairly well (Kirksey and Pike, 1962). Calculations from the older literature suggest a value of 0.13–0.5 percent of the diet (Kirksey and Pike, 1962; Miller, 1926; Nelson and Evans, 1961; Olson and St. John, 1925). Recent data by Ganguli *et al.* (1969a,b) suggest a much reduced sodium requirement for gestation and lactation of 0.05 percent of the diet.

Symptoms of Deficiency The classic sodium deficiency syndrome was described by Orent-Keiles *et al.* (1937). On a diet containing 0.002 percent sodium, growth was retarded and disturbances of the eyes were noted, including corneal lesions. Males became infertile after 2–3 months, and sexual maturity was delayed in the female. The bones were soft and most tissues were affected. Death ensued in 4–6 months. At a level of 0.007 percent sodium, Kahlenburg *et al.* (1937) noted reduced appetite, poor growth, increased heat production, and reduction in the storage of energy, fat, and protein. Digestibility was normal.

Selenium (See Vitamin E)

Sulfur

Sulfur has not generally been classified as a required nutrient except as it is an integral part of the sulfur-containing amino acids and vitamins. However, Michells and Smith (1965) have shown that dietary sulfate is readily incorporated into cartilage and will spare methionine for this purpose. It is suggested a level of 0.1 percent sulfur of the diet should be included when methionine levels are minimal. These data have support from Bernhardt and Tomarelli (1966): A mineral mix fed to meet the requirements reported in the first edition of this volume was improved by the inclusion of 0.1 percent sulfate when fed with a low (8.8 percent lactalbumin) protein diet. With adequate protein, no growth response was observed. It seems evident that the relation between sulfur and the sulfur-containing amino acids needs careful scrutiny.

Zinc

Stirn *et al.* (1935) stated that the requirement for zinc was in excess of 15 μg per rat per day. In one report (Australia CSIRO, 1952), the requirement given was in excess of 20 μg per rat per day. Hove *et al* (1937, 1938) listed the requirement as 42 μg per rat per day. If rats are housed in galvanized cages, no more than 2–4 mg/kg of diet are required (R. M. Forbes, personal communication). Rats maintained in a zinc-free environment and fed a diet based on casein or egg white require a minimum of 12 mg/kg of diet for maximum weight gain (Forbes and Yohe, 1960). The requirement is higher (18 mg/kg of diet) when isolated soybean protein is used.

Symptoms of Deficiency An inadequate intake of zinc results in marked growth retardation and eventual growth failure. It is accompanied by mild anorexia, alopecia, and a condition of hyperirritability. Gross cutaneous lesions are marked by thickening of the epidermis and loss of the hair follicles (Underwood, 1962) (Figure 3). One report (Stirn *et al.*, 1935) showed that the hair became soft and woolly and eventually light gray in color. Also, the basal

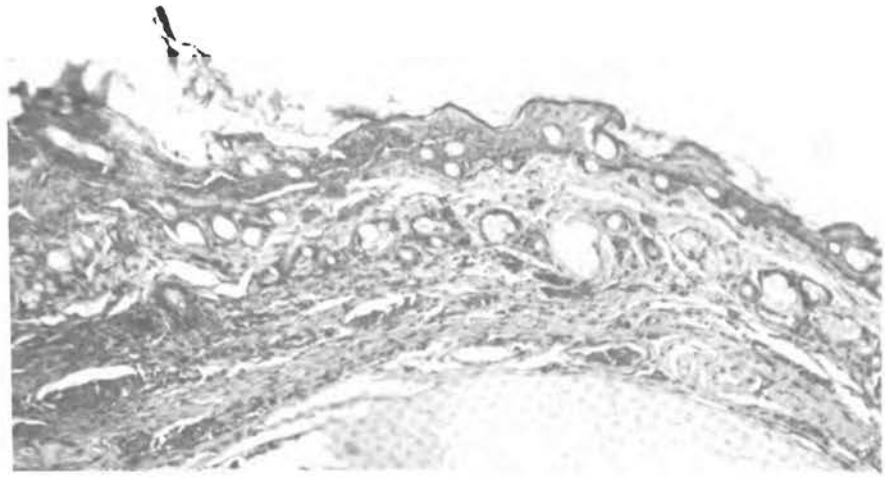

FIGURE 3 Zinc deficiency. Microsection of the epidermis showing parakeratosis of the stratum and corneum and loss of hair follicle. (W. N. Pearson, Vanderbilt University.)

metabolism may be increased (Australia CSIRO, 1952).

When less than 2 ppm of zinc is fed to females a severe disruption of the estrous cycle occurs, and no mating with normal males occurs in most cases (Hurley and Swenerton, 1966). A diet of less than 0.5 μg of zinc/g fed to growing males arrested spermatogenisis and resulted in atrophy of the germinal epithelium. Growth of the pituitary and accessory sex organs was reduced. A similar finding was made by Luecke *et al.* (1968). If atrophy of the germinal epithelium and epididymides was sufficient, the damage was not reparable with zinc supplementation (Millar *et al.*, 1958). Growth of the accessory sex organs was promoted with gonadotropins in the absence of added dietary zinc (Millar *et al.*, 1958).

WATER-SOLUBLE VITAMINS

Vitamin A

The early studies of Guilbert, Howell, and Hart (1940) demonstrated that the need for vitamin A was related to body weight rather than to energy intake. This concept appears to be logical since the vitamin is related to the maintenance of the integrity of body epithelium, which in itself is directly correlated with body mass (Mitchell, 1950). The difficulty of establishing a precise vitamin A requirement is readily envisioned by examining the following typical results that have been reported in the literature. The principal problem is one of selecting a suitable criterion of measurement: Goss and Guilbert (1939) demonstrated that 20 IU per kg of rat per day was enough to prevent the abnormal keratinization of the vaginal eipthelium. Lewis *et al.* (1942) showed that 250 IU per kg of rat per day resulted in maximal growth with a trace of stored liver vitamin A. Five hundred IU per kg yielded maximal blood levels with a moderate amount of liver storage. Paul and Paul (1946) recommended 100 IU per kg of rat per day as being optimum for maximal gain and longevity, but their data suggest that 200 IU give slightly better performance though, perhaps, insignificantly so. Sherman and Trupp (1949) produced greater longevity and slightly more live-weight gain on diets containing 12,000 IU per kg of diet, or approximately 1,200 IU per kg of body weight. In similar studies, Fraps (1947) fed β-carotene from alfalfa and observed that the numbers of litters and young, percentage born alive, and weaning weights were greater with levels up to 8 ppm of carotene. Assuming that 1 IU of vitamin A is equivalent to 0.6 μg of carotene (Rubin and De Ritter, 1954), this calculates to be 13,000 IU per kg of diet.

As a result of an evaluation of these studies, it appears that a minimal requirement for growth should be approximately 200 IU per kg of rat per day, or 2,000 IU per kg of diet. This level should give excellent gains, optimal longevity, and some traces of liver storage. For reproduction, the data of Sherman and Trupp (1949) and Fraps (1947) have been used to establish a daily requirement of 1,200 IU per kg of body weight, or 12,000 IU per kg of diet. All of the suggested levels, while minimal for certain criteria, are in excess of that required to prevent overt symptoms of deficiency.

Symptoms of Deficiency Vitamin-A deficiency is characterized by malformations of the epithelial structures and epiphyseal cartilages, followed by general growth depression. Epithelial tissues become keratinized. Disorganization of the tooth structure causes an impairment of tooth growth, a distortion of the incisor tooth, as well as a loss of the normal orange color. The retardation of skeletal growth results in compression on the brain, spinal cord, and nerve roots. There follows a herniation of the brain into the foramen magnum with consequent mechanical injury to the brain and nerve roots, which causes incoordination in about 5–6 weeks in weanling rats.

Vitamin A, as an integral part of rhodopsin, is essential for dimlight vision, and a deficiency state results in night blindness. Xerophthalmia, noted by a reddish exudate on the eyelids, develops, frequently followed by corneal opaqueness and general distortion of the shape of the eye.

In the female, failure of implantation with the production of aborted or nonviable litters is common, while testicular degeneration is characteristic in the male (McCoy, 1949; Russell, 1948; Wolbach, 1954).

Vitamin D

It is impossible to discuss vitamin D without considering calcium and phosphorus The metabolic effects of one depend largely on the others. Animal performance depends on the absolute amounts of each, as well as the relative amounts.

There are not extensive data to suggest that vitamin D is required by the rat for normal performance in the presence of adequate and balanced levels of calcium and phosphorus. Bethke *et al.* (1932) demonstrated an improvement in almost all of the criteria of calcium–phosphorus metabolism when the diet contained 300 IU of vitamin D per 100 g of food. On the other hand, Chandler and Cragle (1962) tested levels of vitamin D from 3.0 to 30,000 IU per 100 g diet over a 3-week period with no differences being observed. Recent Wisconsin studies (Becker and Hoekstra, 1966; Steenbock and Herting, 1955) have shown vitamin D to improve growth significantly when fed at a level of 1,000 IU/kg of a highly purified diet in the absence of fluorescent lights. No lower levels have been reported by these workers. The requirement is set at that level. Many diets in the literature contain from 2,000 to 12,000 IU/kg.

The requirements for calcium and phosphorus for growth and bone calcification are approximately 0.4 and 0.5 percent of the diet, with the level of phosphorus preferably below that of calcium. This level is slightly below that of Bethke *et al.* (1932), identical to that of Evans and Ali (1967), and higher than the 0.27 percent Ca and 0.21 percent P level suggested by Chandler and Cragle (1962). A calcium-to-phosphorus ratio between 1.0 and 2.0 is recommended. The tendency has been for bone calcification to increase with increases of calcium up to 0.8 percent (Lanford and Sherman, 1938), but not always (Evans and Ali, 1967; Williams *et al.*, 1957). Bone density is highly correlated with level of intake early in life (Williams *et al.*, 1957) but not later. It is clear the retention of calcium by aged rats is difficult (Hironaka *et al.*, 1960; Kane *et al.*, 1949). Adult rats transferred from adequate diets (0.16 percent) to low (0.13 percent) calcium diets will adapt to the low intake in about 6 months (Jaffe, 1956).

For reproduction, the earlier work of Cox and Imboden (1936) showed excellent performance at calcium and phosphorus levels of 0.49 percent. Lactation was improved at somewhat higher levels of calcium (Ca:P of 1.5: 1.7).

Hansard and Plumlee (1954) have determined the requirement for the maintenance of calcium status and found that it ranges from 5 to 21 mg per day, when the previous level of calcium intake ranged from 24 to 100 mg per day. At a previous intake of 40–50 mg per day (0.5–0.6 percent of the diet), a maintenance requirement of between 10 and 15 mg per day was calculated.

Symptoms of Deficiency Classical rickets, which most nearly approximates the human form, is produced on a high-calcium, low-phosphorus diet. It is denoted by an imperfect calcification of the epiphyseal plates and a significant enlargement of the carpal joint. Stiffness is also characteristic and death frequently occurs within 6 weeks (Jones, 1954; Schneider and Steenbock, 1939). A low-calcium, high-phosphorus rickets has been reported (Hansard and Plumlee, 1954; Shohl and Wolbach, 1936; Wasserman, 1960), though it does not fit the classical anatomical picture of low-phosphorus rickets. If the calcium-to-phosphorus ratio ranges between 0.6 and 2.5, and the level of each is adequate, rickets is not observed even when vitamin D is absent from the diet. Above and below these ratios, rickets will occur and vitamin D will prevent it, with the requirement becoming proportionally greater the more the ratio deviates from the above (Bethke *et al.*, 1932). On strictly deficient diets, growth is impaired (Becker and Hoekstra, 1966; Steenbock and Herting, 1955).

Boelter and Greenberg (1941, 1943) demonstrated additional lesions when the calcium-to-phosphorus

ratio was approximately 30:1 and cod liver oil was fed as a supplement. These rats showed a retardation of growth, a decrease in food consumption, an increase in basal metabolic rate, reduced activity and sensitivity, osteoporosis, and rear leg paralysis. Internal hemorrhage was frequently observed. With this abnormal diet, males failed to mate and females did not lactate properly. Guilbert and Hart (1930) demonstrated that 0.22 percent phosphorus (calcium-to-phosphorus ratio 4:1) resulted in retarded development of sexual maturity and cessation of estrus. After 110 days of age, ovulation was normal. A decrease in the calcium level eliminated these symptoms. In numerous papers reviewed by Russell (1948) and McCoy (1949), it was observed that reproduction was poor with either abnormal calcium-to-phosphorus ratios or low levels of each.

Vitamin E

The vitamin E requirement can be affected by variations in the level of dietary sulfur amino acids, selenium (Witting and Horwitt, 1946b), and fat. It is suggested that the requirement is related to the dietary fat up to the lowest level that no longer alters the tissue lipid fatty acid composition (Harmon *et al.*, 1966). The level of peroxidizability of the fatty acids ingested or stored in the tissues is a critical determinant of the requirement (Century and Horwitt, 1960). This is of special significance at low levels of linoleic acid intake (Witting and Horwitt, 1964a).

In otherwise balanced diets, the requirement, in mg per kg of diet (estimated from the original papers), is for growth, 5.0 (Witting and Horwitt, 1964b); liver necrosis prevention, 30 (Gitler *et al.*, 1957); prevention of creatinuria for 20 weeks, 10 (Witting and Horwitt, 1964b); and prevention of erythrocyte hemolysis, 35 (Gitler *et al.*, 1958; Rose and Gyorgy, 1950; Ward, 1963). Females require one third less to prevent erythrocyte hemolysis. For symptoms related to reproduction, the data summarized by Ward (1963) suggest 0.07 mg per rat per day, a value also reported by Mason (1940) as adequate to allow first litters.

The most extensive study of the vitamin E requirement was made by Evans and Emerson (1943), who found that 0.21 mg of tocopheryl acetate per rat per day was inadequate, while 0.64 mg was satisfactory to prevent all but a slight dystrophy in the third litters. This latter figure approximates a dietary level of 30 mg/kg of diet. The requirement in Table 4 is listed as 35 and 3 mg/kg of diet for growth and gestation, respectively. The level for lactation is suggested as 20 mg/kg of diet (Nelson and Evans, 1961). Diets described in the literature routinely include from 50 to 200 mg of vitamin E per kg.

A level of 40 μg of selenite selenium per kg of diet must be maintained to prevent dietary necrotic liver degeneration (Schwarz, 1958a). Maximum growth requires no more than 150 μg/kg of diet. With the vitamin E requirement satisfied, a specific requirement for selenium per se was difficult to show (Witting and Horwitt, 1964b) until recently (McCoy and Weswig, 1969).

Symptoms of Deficiency The complex and varied symptomatology associated with vitamin E deficiency has been summarized by Mason (1954). In the male there is an irreversible degeneration of the seminiferous epithelium occurring by age 40 or 50 days. The female exhibits intrauterine death and fetal resorption, a condition that is not necessarily permanent. Fetal anomalies are common. If live young are born, they may suffer from a sudden late-lactation paralysis at about 18 days postpartum. This syndrome is characterized by "clenching of the forepaws, weakness and dragging of the extremities, inability to recover posture when placed on their backs, diminution of respiration and body temperature, listlessness, and death," (Rose and Gyorgy, 1950). The skeletal muscles are dystrophic, pale, and ischemic. Those young that do not succumb may develop chronic dystrophy 5–6 months later in which waddling or an uncoordinated gait is typical.

There is often hyaline necrosis of the heart muscle, while the smooth muscles, especially those of the uterus, tend to accumulate a yellow pigment called "ceroid." The incisor teeth lose their yellow pigmentation (Mason, 1954). The kidney is affected such that those removed from deficient animals autolyze much more rapidly than do those of the controls (Emmel, 1957; Moore *et al.*, 1958). Dialuric acid will hemolyze blood from deficient animals; control blood is less affected (Rose and Gyorgy, 1950).

Dietary necrotic liver degeneration is a condition that develops at about 45–55 days post-weaning. Changes in the liver up to 1–2 days before death are only microscopic, with massive necrosis occurring just before death. The animals expire in convulsions after a period of slowed respiration. This condition has been shown to be the result of a simultaneous deficiency of both vitamin E and selenium. Either substance will prevent the condition, although vitamin E is required to prevent all necrotic changes in the liver (Witting and Horwitt, 1967). Inorganic selenium, as sodium selenite, is 500 times as active as an equal weight of vitamin E. Selenium compounds however, will not

prevent the fetal resorption syndrome (Harris *et al.*, 1958; Schwarz, 1958a; Schwarz and Folz, 1957, 1958) or the depigmentation of the incisor teeth (Alterman, 1959). Vitamin-free casein is a variable source of selenium (Bonetti and Stripe, 1962; Schwarz, 1954; Witting and Horwitt, 1964b), depending on whether it is of foreign or domestic origin. Casein from New Zealand contains from 0.1 to 0.2 ppm (Witting and Horwitt, 1967).

In addition to selenium, a number of antioxidants have been shown to either replace or spare vitamin E (Ames *et al.*, 1956; Crider *et al.*, 1961; Draper *et al.*, 1964; Gitler *et al.*, 1957; King, 1964; Moore *et al.*, 1953; Schwarz, 1958b).

Vitamin K

A single dose of 0.5 μg of vitamin K_1, or 10 μg of menadione per kg of rat, was adequate to restore normal prothrombin time within 18 hours for rats in which coprophagy was prevented (Barnes and Fiala, 1959). With diets containing casein, the requirement to maintain normal prothrombin levels was less than 50 mg/kg of diet. For a solvent-extracted soy protein diet it was increased fivefold (Matschiner and Doisy, 1965). Under germfree conditions (Gustafsson *et al.*, 1962) the requirement was 200 μg of vitamin K_1/kg of diet; with coprohagy prevented, normal rats required 10 μg (Johnson *et al.*, 1960). Menadione has about one tenth the activity of vitamin K_1 (Gustafsson *et al.*, 1962).

A level of 50 μg/100 g of diet is included in Table 4 but is undoubtedly unnecessary and excessive for most rats, even for spontaneous cases. There are no known data for gestation or lactation. Many purified diets are supplemented with 1–5 mg/kg of diet.

Symptoms of Deficiency Vitamin K deficiency reduces prothrombin level of blood, increasing clotting time. The female is more resistant to a deficiency than the male (Johnson *et al.*, 1960). Although vitamin K is synthesized by the microflora of the intestinal tract (Mickelsen, 1956), prompt deficiency can be induced by feeding sulfonamides (Almquist, 1954), diverting bile from the intestine by means of a bile fistula (Greaves, 1939), or preventing coprophagy (Barnes and Fiala, 1959; Mameesh and Johnson, 1959).

The spontaneous appearance of hypoprothrombinemia under normal feeding regimes using vitamin K-deficient diets has been reported by Greaves (1939) and Barnes and Fiala (1959), but was observed for only part of the rats even after extended periods of deprivation. Mameesh and Johnson (1959) fed a deficient diet containing an isolated soybean protein instead of casein, which reduced coprophagy in the rat, and the prothrombin levels decreased accordingly.

WATER-SOLUBLE VITAMINS

Ascorbic Acid

The rat does not require a dietary source of ascorbic acid, although it has been shown to reduce certain B-vitamin-deficiency symptoms (Daft and Schwarz, 1952; Everson *et al.*, 1954; Hotzel and Barnes, 1966) and to be involved in iron absorption (Greenberg *et al.*, 1957).

Biotin

There will be no biotin deficiency under normal feeding conditions.

Biotin is provided to the animal normally through intestinal synthesis (Barnes *et al.*, 1959a; Daft *et al.*, 1942; Skeggs and Wright, 1946). The use of raw egg white in the diet does, however, precipitate a deficiency syndrome. Nelson and Evans (1948) have suggested that 300 μg of biotin added per kg of diet containing no raw egg white or sulfonamides will improve lactation, as evidenced by about a 10 percent increase in weaning weight. This observation awaits confirmation.

A level of 1.0 mg/kg of diet is adequate to prevent symptoms on diets containing as much as 20 percent raw egg white (R. W. Luecke, personal communication).

Symptoms of Deficiency Raw egg white fed at levels as low as 5 percent of the diet (Nielson and Elvehjem, 1941), or the use of 1 percent of sulfonamides, results in a deficiency syndrome (Figure 4). The deficiency is characterized by exfoliative dermatitis, which is progressive in nature, starting at the groin. A general alopecia, "spectacle eye," and achromotrichia of colored rats occurs. Many animals have a spastic gait or a "kangaroo-like" posture. Biotin is required metabolically for gestation and probably is a factor in lactation (Gyorgy, 1954).

Choline

The requirement for choline is affected by the levels of vitamin B_{12} and folic acid (Hale and Schnefer, 1951; Mulford, 1955). The interrelations are complex

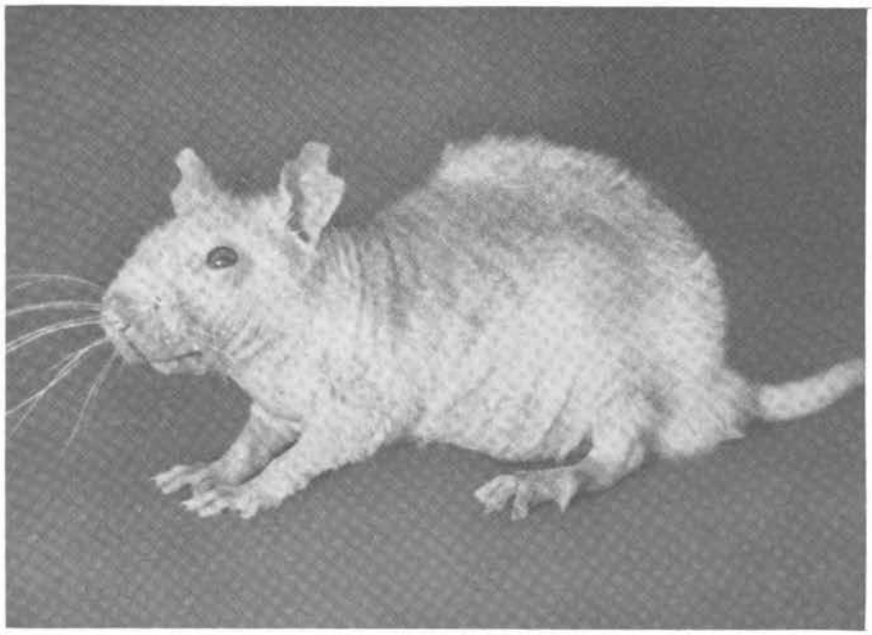

FIGURE 4 Biotin deficiency showing dermatitis, which begins around the eyes and in this individual progressed to generalized alopecia. Dietary supplementation resulted in marked improvement in 3 weeks and recovery within 3 months. (Courtesy Upjohn International Inc.)

and have been discussed in detail by Griffith and Nye (1954). The requirement also rises with an increase in dietary fat (Griffith, 1940a; Salmon, 1947). Griffith (1941) showed that diets containing over 0.8 percent methionine prevent kidney lesions in the absence of choline. Thus, a diet containing a minimum of methionine requires choline to prevent kidney damage. A significant strain difference in requirement has been reported (Copping *et al.*, 1951; Engel, 1943).

The requirement for the prevention of fatty livers is about twice that needed to prevent kidney lesions. Most of the observations place the requirement for preventing both kidney lesions and fatty livers between 5 and 10 mg of choline chloride per rat per day, or 0.5–1.0 g/kg of diet (Engel, 1942a,b; Griffith and Mulford, 1941; Griffith and Wade, 1939; Mulford and Griffith, 1942; Sure, 1940b, 1941b). It has been recommended that the requirement should be set at about 1 g/kg of diet (Brown and Sturtevant, 1949; Cuthbertson, 1957), and indeed many experimenters have routinely used this level. Because of the absence of any reported deficiency signs on these diets, it seems likely that this amount is adequate and may even be too high. The requirement has therefore been recorded in Table 4 at 750 mg/kg diet, which is identical to that suggested by Mulford and Griffith (1942) for diets containing 18 percent casein and 19 percent lard.

Sure (1940a) recommended 15 mg per rat per day for lactation. Richardson and Brock (1958) reared four generations on a purified diet containing 1.0 g/kg of diet. Their figure is certainly adequate, again, and probably excessive.

Many purified diets contain 1.0–4.0 g/kg of diet with satisfactory results.

Symptoms of Deficiency A deficiency of choline is characterized by a critical syndrome in weanling rats, which occurs 6–8 days after initiation of the study. Fatty infiltration of the liver occurs in 48 hours and reaches a maximum after 4–6 days. Marked enlargement and hemorrhagic degeneration of the kidney develop between the sixth and eighth day. The animal is noticeably sick and will succumb. The pathology includes regression of the thymus, enlargement of the spleen, and, in some cases, ocular hemorrhage (Griffith and Nye, 1954). The deficiency affects the male more rapidly and severely, indicating a sex difference in requirement (Copeland, 1944; Engel, 1942a; Griffith, 1940b). There is a marked reduction in requirement with increasing age, which is noticeable as early as 30 days of age (Barnes and Kwong, 1967). The hemorrhagic kidney is frequently not observed in the older rat, although the fatty liver is characteristic of an inadequate intake (Engel, 1942a; Griffith, 1940b; Griffith and Wade, 1939; Hale and Schnefer, 1951; Handler, 1946).

Folacin

A diet devoid of folacin does not produce a deficiency syndrome, as intestinal synthesis is adequate for growth.

There is a possibility, however, that intestinal synthesis is inadequate for the stress of lactation. Pro-

phylactic doses of 33–81 μg per female per day have resulted in increases in maternal body weight and circulating leukocytes (Nelson and Evans, 1947).

Purified diets frequently contain 0.5–4.0 mg/kg of diet.

Symptoms of Deficiency Inclusion of a sulfonamide to inhibit intestinal synthesis results in a characteristic deficiency state, which includes poor growth, leukopenia, granulocytopenia, as well as anemia. Diarrhea develops, as does rough hair coat and a porphyrin exudate around the eyes (Darke and White, 1950; Friedmann *et al.*, 1954; Mickelson, 1956; Skeggs and Wright, 1946; Stokstad, 1954). When coprophagy is prevented (Barki *et al.*, 1949), growth is improved by the inclusion of folic acid.

Inositol

Inositol is not required by the rat (Cunha, 1954; McCormick *et al.*, 1954). The rat apparently derives adequate amounts of inositol either as a result of bacterial synthesis in the intestine (Nielson and Black, 1944) or by tissue synthesis (Daughaday *et al.*, 1955). A characteristic alopecia has been reported when intestinal synthesis is reduced by sulfonamides (Nielson and Black, 1944). Many experimental diets contain between 100 and 1,000 mg/kg of diet.

Niacin

It has been demonstrated that the amino acid tryptophan is a precursor of niacin (Hundley, 1954). Therefore, the requirement for niacin is predetermined by the amount of tryptophan in the diet. The minimal tryptophan requirement is 0.15 percent of the diet when fed with an excess of niacin. It has been shown by Hundley (1947) that animals receiving diets with 15 percent casein (0.202 percent tryptophan) respond slightly to added niacin, while those receiving diets containing 20 percent casein (0.270 percent tryptophan) do not. Since it has been demonstrated that 33–40 mg of tryptophan will result in the production of 1.0 mg of niacin *in vivo* (Hankes *et al.*, 1948; Harris and Kodicek, 1950), it can be concluded that the tryptophan in excess of the requirement in the 15 percent casein diet is equivalent to 13 mg of niacin/kg of diet; while in the 20 percent casein diet the excess tryptophan is equivalent to 30 mg of niacin. The requirement, therefore, lies between 13 and 30 mg of niacin when a minimum amount of tryptophan is supplied. Table 4 lists the niacin requirement with minimal level of tryptophan at 15 mg/kg of diet. No requirement has been determined for reproduction and lactation.

Research workers frequently include from 20 to 100 mg of niacin/kg of diet.

Symptoms of Deficiency A deficiency of niacin, on low-protein or low-tryptophan diets, results in reduced growth, rough haircoat, occasionally porphyrin-caked whiskers, and alopecia. There is weight loss in the young or in adults, accompanied by anorexia. Analysis of the tissues reveals a deficiency in DPN and TPN and a reduction in the excretion of niacin and its metabolites. Death eventually occurs. There are, no doubt, difficulties with reproduction and lactation, but this problem has not received extensive study (Hundley, 1954).

Pantothenic Acid

The pantothenic acid requirement has been widely studied. Reports by Barboriak *et al.* (1957a,b) show that 8.0 mg/kg of diet (as calcium pantothenate) were adequate for growth, maintenance of acetylation reaction in the adult, and reproduction. This value is about equal to an average of results from eight earlier studies on growth and of three for reproduction. The requirement for lactation has been studied by Nelson and Evans (1961), who reported it to be 10 mg/kg of diet.

The usual levels found in purified diets range from 15 to 66 mg/kg of diet.

Symptoms of Deficiency Inadequate amounts of pantothenic acid result in poor growth and achromotrichia in rats with pigmented hair. There is a dermatitis of an exfoliative type (Figure 5). A relative humidity of less than 50 percent results in a more extensive lesion. The whiskers and hair may be stained by a porphyrin compound from the harderian glands. "Spectacle eye," spastic gait, and closure of the eyes by a sticky exudate also may occur. The animals succumb in 4–10 weeks. Frequently there is anemia and adrenal damage; reproduction is impaired with reduction in fertility and with small, undersized and slow growing litters (Barboriak *et al.*, 1957a,b; Briggs and Daft, 1954). In certain reproduction studies (Nelson and Evans, 1946), gestation was impaired when a deficient diet period was initiated on the day of mating. There were no untoward symptoms when pantothenic acid was fed from mid-gestation on. Barboriak *et al.* (1957b) demonstrated that the testes were anatomically and functionally impaired by the deficiency.

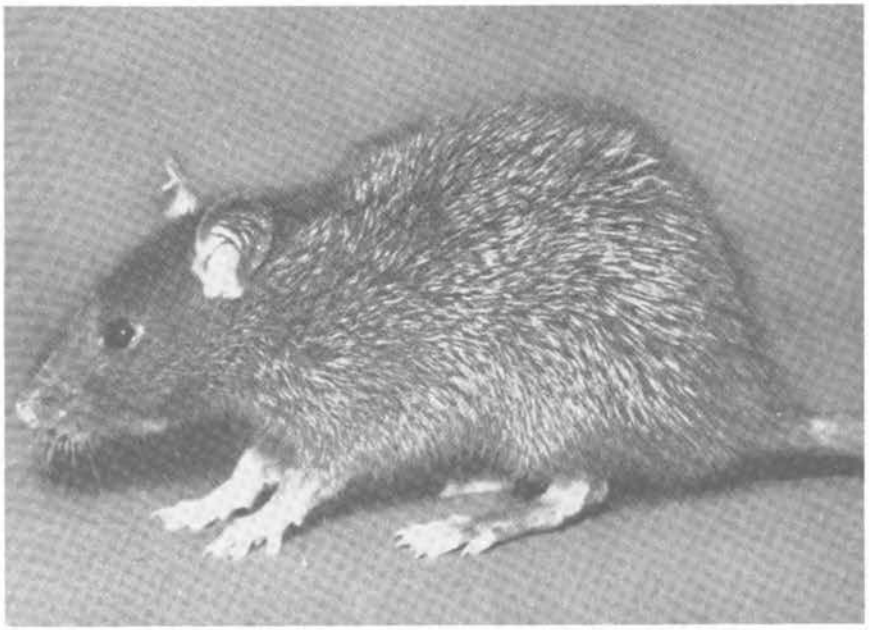

FIGURE 5 Pantothenic acid deficient black rat showing general loss of hair color (achromotrichia). Dietary supplementation with this vitamin led to restoration of much of the hair pigmentation within a month. (Courtesy Upjohn International Inc.)

Paraminobenzoic Acid

Paraminobenzoic acid deficiency has been implicated in the production of achromotrichia in black rats. However, a requirement of this vitamin has not been demonstrated under normal feeding conditions (Wright, 1954). Investigators have frequently included between 10 and 300 mg of this material per kg of diet without deleterious effect.

Riboflavin

The requirement of riboflavin for growth has been widely studied (Bessey *et al.*, 1958; Burch *et al.*, 1948; Edgar *et al.*, 1937; Fen, 1962; Mannerling *et al.*, 1941; Mills, 1948; Nieman and Jansen, 1955; Schweigert *et al.*, 1944; Sherman and Ellis, 1934; Sure, 1940a; Wagner *et al.*, 1940). The reported values range between 12 and 40 μg per rat per day, with the preponderance of data falling between 20 and 30 μg per rat per day. An approximate average figure is 25 μg per day, or 2.5 mg/kg of diet. This is slightly lower than the value suggested by Bro-Rasmussen (1958a) of 3.0 mg/kg of diet after a survey of the same literature. Since riboflavin is involved biochemically in the utilization of energy, the requirement has logically been associated with the caloric intake. Bro-Rasmussen (1958b) concluded that the requirement may be accurately expressed as 0.7–0.8 mg/1,000 kcal. If this same approach is made, using the values in Tables 1 and 4, the requirement amounts to 0.62 mg/1,000 kcal of diet.

The requirement for gestation and lactation has received less critical attention. Everson *et al.* (1948) suggested that 36–90 μg per rat per day was adequate, although Ellis *et al.* (1943) reported 100 μg to be slightly superior to 30 μg per rat per day. Barrett and Everson (1951) indicated that the need for added riboflavin was not critical until 2–3 days before parturition, when it reached a value of 75 μg per day. The only specific study on lactation reported a requirement of 120 μg per rat per day (Sure, 1940a). The level of 4.0 mg per kg of diet in Table 3 should provide these amounts.

Levels of from 4 to 22 mg/kg are found in many purified diets.

Symptoms of Deficiency A deficiency of riboflavin results in reduced growth, despite little change in appetite, and a nonspecific dermatitis, which develops slowly over the extremities. There may be some exudate around the eyes, a moderate hyperkeratosis, and alopecia (Figure 6). There is a distinct pathology of the skin. Conjunctivitis, cataract, and corneal vascularization have been reported in riboflavin deficiency, although these are not always seen. An anemia, accompanied by granulocytosis and lymphopenia, may also be seen. Anestrus has been reported to develop in the female, which is irreversible after 10 weeks, while congenital anomalies are striking for those females that do bear young. In the male, testicular atrophy occurs (Carpenter and Kodicek, 1948; Horwitt, 1954; McCoy, 1949).

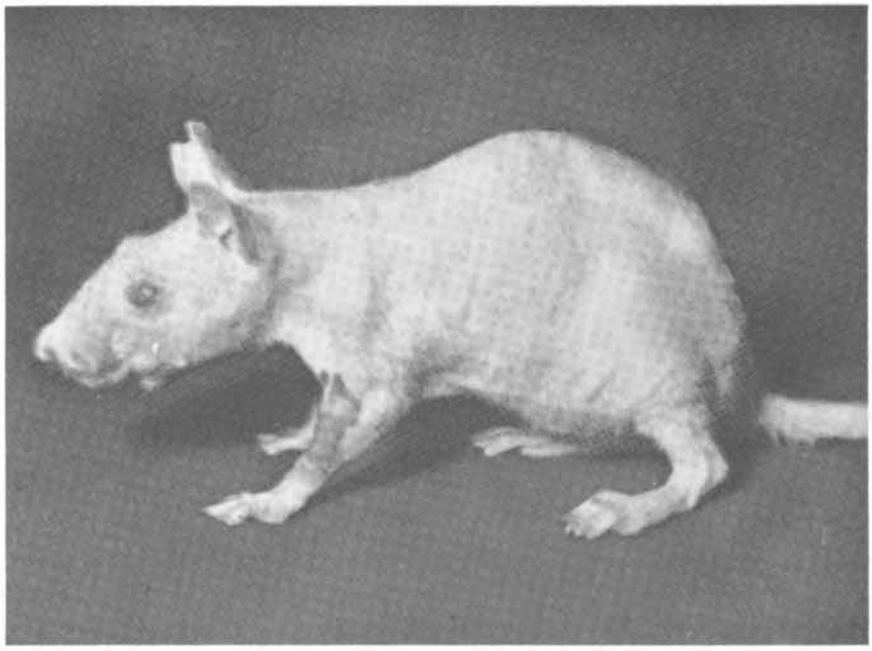

FIGURE 6 Riboflavin deficiency produced corneal keratitis and generalized dermatitis. This rat exhibited no deficiency signs after 2 months of dietary treatment with riboflavin. (Courtesy Upjohn International Inc.)

Thiamin

The minimum amount of thiamin necessary for growth depends, in part, on the age of the rat as well as the fat content of the diet. Mills *et al.* (1946) demonstrated that rats over 21 months of age required 2.0 mg/kg of diet, whereas at 2 months 1.2–1.6 mg/kg was adequate. This increased requirement in the older animal is probably due to a reduction in the efficiency of absorption (Draper, 1958). The fact that fat spares thiamin is well known (Jansen, 1954; Scott and Griffith, 1957; Yudkin, 1951). The studies of Scott and Griffith (1957) have demonstrated that with diets containing 10 percent fat, the requirement is at least 10 μg per rat per day, while with other diets of over 60 percent fat the need drops to 8 μg per day. The older literature generally substantiates their data by pointing to a value of between 0.8 and 1.6 mg of thiamin per kg of diet for diets containing less than 20 percent fat (Arnold and Elvehjem, 1938; Elvehjem, 1944; Kline *et al.*, 1945; McIntire *et al.*, 1943; Mills *et al.*, 1946; Sure, 1938; Voris *et al.*, 1942).

The median value of 1.25 mg/kg of diet has been used as the requirement in Table 4.

For reproduction, Barrett and Everson (1951) demonstrated a marked increase in requirement during the last 2–3 days of gestation, equivalent to 75 μg per rat per day. Brown and Snodgrass (1965) have recently shown that 25 mg/kg of diet or a daily estimated intake of 50 μg per rat per day supports normal gestation. Sure (1940a) reported that 120 μg per rat per day was needed for lactation. The levels suggested for gestation and lactation are 2.5 and 4.0 mg/kg of diet, respectively.

Many investigators include from 4 to 22 mg/kg of diet to ensure an ample intake.

Symptoms of Deficiency A thiamin-low diet causes marked anorexia with consequent poor growth or weight loss. Blood pyruvate is elevated and heart rate may be reduced to 50 percent. The nervous syndrome is characterized by the animal rotating its head slightly and persistently walking in a circle (Jansen, 1954) (Figure 7). In the female, the estrous cycle becomes irregular (Russell, 1948). If females are placed on a deficient diet 1–3 weeks before mating, reproduction is poor as evidenced by either subnormal birth weights, death of the young, early fetal deaths, failure of implantation, or resorption (Nelson and Evans, 1955).

Vitamin B_6

The early requirement information suggested that a value for growth was between 8 and 25 μg per rat per day of vitamin B_6 (Batchen *et al.*, 1955; Carpenter *et al.*, 1943; Clark and Lechycka, 1943; Conger and Elvehjem, 1941; El-Sadr *et al.*, 1939; Lepkovsky, 1938; Slanetz, 1943; Sure, 1940a), and a level of 12 μg was recommended in the first edition of this volume. Recently, Beaton and Cheney (1965) determined the requirement for growth on a 20 percent casein–20

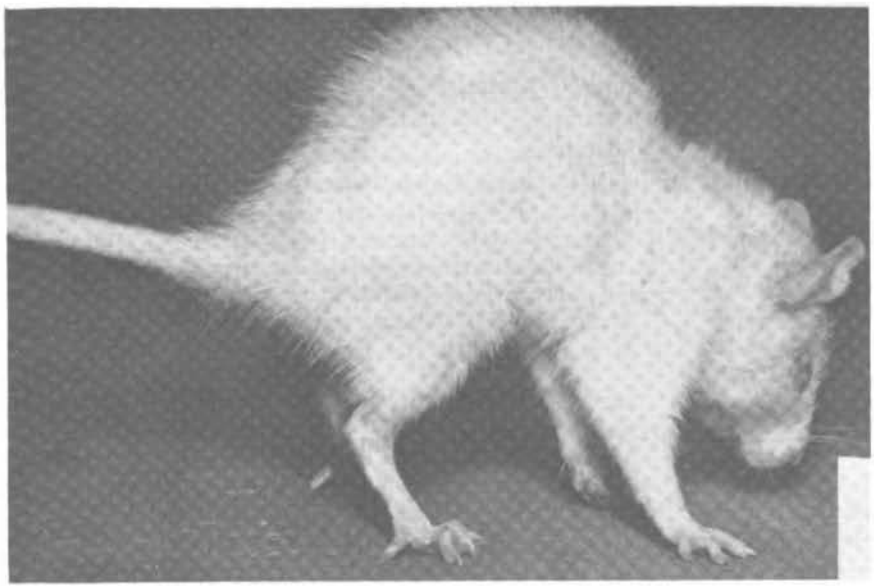

FIGURE 7 Thiamin deficiency in the rat results in spastic gait, uncoordination, and loss of balance. (Courtesy Upjohn International Inc.)

percent fat diet to be about 7.0 mg/kg of diet. This value supported maximal gain and biochemical responses but may be too high when applied to a diet lower in protein and energy. The standard on Table 4 is adjusted to be in line with this study.

Slanetz (1943) has suggested that the amount required for reproduction and lactation ranges between 12 and 20 μg per rat per day. The requirements for these functions are recorded in Table 4 as 12 μg per rat per day.

Diets containing 2 to 22 mg/kg of diet are frequently reported in the literature.

Symptoms of Deficiency Sherman (1954) summarized the extensive literature on the vitamin B_6 deficiency syndrome. It is characterized by poor weight gain, anorexia, and reduction in sexual development and behavior. The growth of the suckling animal is retarded early in life, and survival to weaning is unlikely. A symmetrical, scaly dermatitis or acrodynia is observed on the peripheral areas such as the tail, paws, mouth, nose, and ears, which eventually become red and edematous.

The vitamin is necessary for erythropoesis, and a deficiency causes a microcytosis of the erythrocytes, and anemia (Dinning and Day, 1956; Ramalingaswami and Sinclair, 1954). Convulsive seizures are observed, which include hyperexcitability and a circular running motion terminating in a tonic–clonic convulsion. Convulsions are noted in the suckling young. Less tissue fat is found as a result of an abnormal fat metabolism. The male becomes sterile, indicating (Beaton *et al.*, 1952) that the requirement for the male may exceed that for the female. Olsen and Martindale (1954) have reported that chronic vitamin B_6 deficiency results in an increase in blood pressure, and an hematuria has been observed (Agnew, 1949). Oxalturia (Gershoff and Faragella, 1959) and the formation of calcium oxalate renal stones with obstructive sequelae similar to those seen in man (Andrus *et al.*, 1960) has also been reported. Recent reports have indicated B_6 deficient rats may be deficient in insulin and growth hormone (Huber and Gershoff, 1965; Huber *et al.*, 1964).

Vitamin B_{12}

The requirement for growth and reproduction has been reported by Jaffe (1956) to be 0.5 μg/100 g of diet. This figure is lower than the data reported by Cuthbertson and Thornton (1951), Sherman *et al.* (1955), Richardson and Brock (1958). The requirement can be markedly affected by the various dietary components such as fat and protein (Erickson and Odell, 1961). Purified diets described in the literature often include between 10 and 36 μg/kg diet.

Symptoms of Deficiency Vitamin B_{12} deficiency can be induced in the young or adult rat by depleting them for an extended period of time. A deficiency is most easily produced by placing the dam on a deficient diet at the beginning of gestation. Deficient animals may then be observed at parturition or certainly by the second generation. Jaffe (1956) has indicated that deficiency of vitamin B_{12} results in a reduction of growth and reduction in liver and kidney

levels of vitamin B_{12}. There is no reduction in blood hematocrit, hemoglobin, or white or red cell counts. At parturition, deficient young are weak and smaller than normal, litter number is reduced, and mortality is high.

WATER

Adolph (1947) has demonstrated that food and water are interdependent, with water usually provided *ad libitum* to meet its requirements. A restriction in one led to a restriction in the other, but to a lesser degree. Quimby (1948) reported that full-fed rats consumed food and water at about a 1:1.9 ratio, respectively.

It has been shown that during the peak of lactation, water intake may increase fourfold (Bruce, 1950).

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APPENDIX A: COMPOSITION OF FEEDS

Table A-1 gives the composition of feeds (excluding amino acids, which are tabulated separately in Table A-2) commonly used in laboratory animal diets.* Two larger compilations are available.†

NRC NOMENCLATURE

In Tables A-1 and A-2 and in Publications 1684 and 1919, names of the feeds are based on a scheme proposed by Harris *et al.*‡ The names, called NRC names, are designed to give a qualitative description of each product, where such information is available or pertinent. A complete NRC name consists of as many as eight components, written in linear form, with components separated by commas. The components are as follows:

- Origin (or parent material)
- Species, variety, or kind
- Part eaten
- Process(es) and treatment(s) to which product has been subjected
- Stage of maturity
- Cutting or crop
- Grade or quality designations
- Classification

Feeds of the same origin (and of the same species, variety, or kind, if one of these is stated) are grouped into eight classes, each of which is designated by a number in parentheses. The numbers and the classes they designate are as follows:

(1) Dry forages or dry roughages
(2) Pasture, range plants, and feeds fed green
(3) Silages
(4) Energy feeds
(5) Protein supplements
(6) Minerals
(7) Vitamins
(8) Additives

Feeds that in the dry state contain on the average more than 18 percent of crude fiber are classified as forages and roughages. Products that contain 20 percent or more of protein are classified as protein supplements. Products with less than 20 percent of protein are classified as energy feeds. (These guidelines are approximate, and there is some overlapping.)

Abbreviations have been devised for many of the terms used in the NRC system (Table A-3). Stage-of-maturity terms are given in Table A-4.

The following tabulation shows how three feeds are described:

Components of Name	Feed No. 1	Feed No. 2	Feed No. 3
Origin (or parent material)	Cattle	Soybean	Wheat
Species, variety, or kind	—	—	—
Part eaten	milk	seed wo hulls	flour by-product
Process(es) and treatment(s) to which product has been subjected	skim dehy	solv-extd grnd	fine-sift

* These tables were prepared by E. W. Crampton and L. E. Harris. Dr. Crampton is chairman of the Subcommittee on Feed Composition, Committee on Animal Nutrition, National Research Council; Dr. Harris is a member of the Subcommittee.

† Publication 1684, *United States–Canadian Tables of Feed Composition*, lists about 400 feeds. Publication 1919, *Atlas of Nutritional Data on United States and Canadian Feeds*, lists about 6,150 feeds. Both are published by the National Academy of Sciences, Washington, D.C.

‡ Harris, L. E., J. M. Asplund, and E. W. Crampton. 1968. An international feed nomenclature and methods for summarizing and using feed data to calculate diets. Utah Agr. Exp. Sta. Bull. 479.

Components of Name	Feed No. 1	Feed No. 2	Feed No. 3
Stage of maturity	–	–	–
Cutting or crop	–	–	–
Grade or quality designations	mx 8% moisture	mx 3% fiber	mx 4% fiber
Classification	(5) (protein supplements)	(5) (protein supplements)	(4) (energy feeds)

Thus, the names of the three feeds are written as follows:

No. 1: Cattle, milk, skim dehy, mx 8% moisture, (5)
No. 2: Soybean, seed wo hulls, solv-extd grnd, mx 3% fiber, (5)
No. 3: Wheat, flour by-product, fine-sift, mx 4% fiber, (4)

The analytical data are expressed in the metric system (with the exception of the bushel weights of the cereal grains) and are shown on a dry basis. (See Table A-5 for weight–unit conversion factors and Table A-6 for weight equivalents.)

LOCATING NAMES IN THE TABLES

To locate in Tables A-1 and A-2 the NRC name of a feed, one must know its origin (the name of the parent material) and usually the variety and kind. The first word of each NRC name is the name of the parent material. For feeds of vegetable origin, the origin term is the name of the plant (e.g., alfalfa, barley, oats) not the word "plant."

A reader uncertain about the origin term that introduces an NRC name may find the term by referring to the common name of the feed in which he is interested. Common names appear alphabetically in the tables.

Names having the same origin term are arranged in a heirarchy based on whether the names include references to species, variety, or kind. Names lacking such references are arranged under the origin term as follows: first, numerically, by classes; second (within a class), alphabetically, by parts eaten.

Names that include references to species, variety, or kind are arranged under the origin term as follows: first, alphabetically, by species, variety, or kind; second (within species, variety, or kind), numerically, by classes; third (within a class), alphabetically, by parts eaten.

Many feeds have names assigned by the Association of American Feed Control Officials (AAFCO), the Canada Feed Act (CFA), or the Canada Grain Act (CGA). In addition, some feeds have regional or local names. The reader will find these names in their alphabetical place, where they are cross-referenced to th NRC names; he will also find them under the NRC names.

A 6-digit reference number is shown. The number may be used as the "numerical name" of a feed when performing linear programming with electronic computers.

The common name of the parent material is followed by the scientific name (e.g., Alfalfa. *Medicago sativa*).

TABLE A-1 Composition of Some Laboratory Animal Feeds, Excluding Amino Acids

Line Number	SCIENTIFIC NAME National Research Council Name (NAS) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Matter (%)	Dry Basis Protein (%)	Ether Extract (%)	Crude Fiber (%)	Nitrogen Free Extract (%)	Calcium (%)	Copper) (mg/kg)	Iodine (mg/kg)	Iron (%)	Magnesium (%)	Manganese (mg/kg)	Phosphorus (%)
1	ALFALFA. *Medicago sativa*													
2	–aerial part, dehy grnd, mn 15% protein, (1)	1-00-022	93.1	16.3	2.5	28.4	43.8	1.32	11.2	0.129	0.033	0.31	31.1	0.24
3	–aerial part, dehy grnd, mn 17% protein, (1)	1-00-023	93.0	19.2	3.2	26.1	41.8	1.43	10.6	0.161	0.049	0.31	31.2	0.26
4	–aerial part, dehy grnd, mn 30% protein, (1)	1-00-024	93.1	22.1	3.9	21.7	41.2	1.63	11.4	0.150	0.043	0.38	36.5	0.29
5	–aerial part, dehy grnd, mn 22% protein, (1)	1-07-851	92.9	24.2	4.0	19.9	40.8	1.59	11.9	0.215	0.048	0.36	39.8	0.30
6	–hay, s-c early blm, (1)	1-00-059	90.0	18.4	2.2	29.8	40.2	1.25	13.4	–	0.020	0.30	31.5	0.23
7	–hay, s-c, full blm, (1)	1-00-068	87.7	15.9	1.8	33.9	39.5	1.28	13.4	–	0.020	0.35	33.7	0.20
8	–hay, s-c, grnd, (1)	1-00-111	92.2	18.2	2.5	28.0	41.0	1.35	18.7	–	0.050	0.34	46.5	0.30
9	Ground alfalfa hay (AAFCO)													
10	Sun-dried alfalfa meal (AAFCO)													
11	–leaves, dehy grnd, (1)	1-00-137	92.2	22.4	3.2	21.3	41.2	1.78	11.5	–	0.039	–	39.9	0.025
12	Alfalfa leaf mean, dehy (AAFCO)													
13	ANIMAL. Scientific name not used													
14	–blood, dehy grnd, (5)	5-00-380	91.0	87.8	1.8	1.1	3.1	0.49	10.9	–	0.413	0.24	5.8	0.24
15	Blood meal (AAFCO)													
16	Blood meal (CFA)													
17	–blood, spray dehy, (5)	5-00-381	91.0	90.3	1.1	1.1	2.2	0.49	8.9	–	0.330	0.04	7.0	0.41
18	Blood flour													
19	–carcass residue, dry rendered dehy grnd,													
20	mn 55% protein mx, 4.4% phosphorus, (5)	5-00-385	93.5	57.1	10.6	2.6	2.8	8.49	10.4	–	0.047	0.29	10.2	4.31
21	Meat meal (AAFCO)													
22	Meat scrap													
23	–carcass residue w blood, dry or wet rendered													
24	dehy grnd, mx 4.4% phosphorus, (5)	5-00-386	92.0	65.0	8.8	2.2	0.7	6.46	42.1	–	–	0.17	20.8	3.44
25	Meat meal tankage													
26	Digester tankage													
27	–carcass residue w bone, dry rendered dehy													
28	grnd, mn 4.4% phosphorus, (5)	5-00-838	94.0	53.8	10.1	2.3	2.8	11.24	1.6	1.438	0.053	1.20	13.1	5.39
29	Meat and bone meal (AAFCO)													
30	Meat and bone scrap													
31	–liver, dehy grnd, (5)	5-00-389	92.6	71.8	16.3	1.4	4.0	0.54	96.4	–	0.068	–	9.5	1.35
32	Animal liver meal (AAFCO)													
33	Animal liver meal (CFA)													
34	Liver meal													
35	–bone, steamed dehy grnd, (6)	6-00-400	95.0	12.7	3.4	2.1	–	30.51	17.2	–	0.068	0.67	32.0	14.30
36	Bone meal, steamed (AAFCO)													
37	–bone charcoal, retort-charred grnd, (6)	6-00-403	90.0	9.4	–	–	–	30.11	–	–	–	0.59	–	14.14
38	Bone black (CFA)													
39	Bone char (CFA)													
40	Spent bone black													
41	–bone phosphate, precipitated dehy, mn 17%													
42	phosphorus, (6)	6-00-406	99.0	0.4	0.3	–	–	28.03	–	–	–	–	–	11.31
43	Bone phosphate (AAFCO)													
44	ANIMAL – POULTRY. Scientific name not used													
45	–fat, heat rendered, mn 90% fatty acids													
46	mx 2.5% unsaponifialbe matter													
47	mx 1% insoluble matter (4)	4-00-409	99.5	–		–	–	–	–	–	–	–	–	–
48	Animal fat (AAFCO)				–									
49	APPLES. *Malus* spp													
50	–pulp, dehy grnd, (4)	4-00-423	89.0	4.9	5.4	16.6	70.5	0.13	–	–	0.030	0.07	8.1	0.12
51	Dried apple pomace (AAFCO)													
52	BARLEY. *Hordeum vulgare*													
53	–grain, (4)	4-00-530	89.0	13.0	2.1	5.6	76.6	0.09	8.5	–	0.005	0.13	18.3	0.47
54	–grain, Pacific Coast, (4)	4-07-939	89.0	10.9	2.5	7.0	77.0	0.07	–	–	–	–	18.0	0.45
55	–malt sprouts w hulls, dehy, mn 24%													
56	protein, (5)	5-00-545	93.0	28.2	1.5	15.1	48.3	0.24	–	–	–	0.19	34.1	0.78
57	Malt sprouts (AAFCO)													
58	BEET, SUGAR. *Beta saccharifera*													
59	–molasses, mn 48% invert sugar mn 79.5%													
60	degrees brix, (4)	4-00-668	77.0	8.7	0.3	–	80.4	0.21	22.9	–	0.013	0.30	6.0	0.04
61	Beet molasses (AAFCO)													
62	Molasses (CFA)													
63	–pulp, dehy, (4)	4-00-669	91.0	10.0	0.7	20.9	64.5	0.74	13.7	–	0.033	0.30	38.5	0.11
64	Dried beet pulp (AAFCO)													
65	Dried beet pulp (CFA)													
66	BLOOD MEAL – See ANIMAL													
67	BONE – See ANIMAL													
68	BREAD. Scientific name not used.													
69	–dehy, (4)	4-07-944	95.0	11.6	1.1	0.5	84.8	0.03	–	–	–	–	–	0.10
70	BREWERS – See GRAINS													
71	BROME, SMOOTH. *Bromus inermis*													
72	–hay, s-c, (1)	1-90-947	89.7	11.7	2.4	31.8	44.9	0.36	8.6	–	0.012	0.15	58.0	0.19
73	BUTTERMILK – see CATTLE													
74	CABBAGE, *Brassica oleracea capitata*													
75	–aerial pt, fresh, (2)	2-01-046	11.7	21.8	1.9	10.3	53.8	0.51	–	–	0.008	0.17	–	0.26
76	CALCIUM PHOSPHATE, DIBASIC													
77	–commercial, (6)	6-01-080	96.0	–	–	–	–	23.12	–	–	–	–	–	18.64
78	Dicalcium phosphate (AFFCO)													
79	CARROT. *Daucus* spp													
80	–roots, fresh, (4)	4-01-145	11.9	10.1	1.6	9.2	69.0	0.42	10.9	–	0.017	0.17	31.1	0.34

(1) dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages;

Line Number	Dry Basis														
	Potassium (%)	Sodium (%)	Zinc (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folic acid (mg/kg)	Niacin (mg/kg)	Pantothenic acid (mg/kg)	Provitamin A (Carotene) (mg/kg)	Pyridoxine (mg/kg)	Riboflavin (mg/kg)	Thiamine (mg/kg)	Vitamin B_{12} (μg/kg)	Vitamin E (mg/kg)	Vitamin K (mg/kg)
1															
2	2.50	0.08	21.5	–	1665.	1.65	45.0	22.4	109.5	6.98	11.4	3.2	–	105.3	10.63
3	2.67	0.10	17.2	0.35	1632.	2.26	49.2	32.2	108.8	6.77	13.2	3.5	11.8	137.6	9.35
4	2.71	0.92	19.3	0.33	1738.	2.87	58.7	35.2	232.4	8.48	16.6	4.2	11.8	157.9	15.79
5	2.70	0.12	21.5	0.36	1994.	3.23	63.3	35.5	271.7	8.39	18.7	4.5	11.9	162.5	9.15
6	2.08	0.15	–	–	–	–	–	–	127.2	–	–	–	–	–	–
7	0.55	–	–	–	–	–	–	–	37.0	–	–	–	–	–	–
8	2.46	0.19	35.1	–	–	–	–	–	104.5	–	–	–	–	–	–
9															
10															
11	2.25	–	–	–	–	–	39.5	35.7	161.6	–	19.6	6.0	–	–	–
12															
13															
14	0.99	0.35	4.8	0.10	832.	–	34.6	1.2	–	4.84	1.6	0.2	48.5	–	–
15															
16															
17	0.45	0.36	–	–	306.	–	31.4	5.8	–	–	4.6	0.4	–	–	–
18															
19															
20	0.59	1.80	–	0.10	2091.	0.05	60.8	5.1	–	3.21	5.7	0.2	54.7	1.1	–
21															
22															
23															
24	0.61	1.82	–	–	2358.	1.63	42.6	2.6	–	–	2.6	0.5	359.4	–	–
25															
26															
27															
28	1.55	0.76	104.2	0.15	2329.	0.05	50.8	3.9	–	2.65	4.7	1.2	47.6	1.1	–
29															
30															
31	–	–	–	0.02	–	6.00	220.0	48.8	–	–	50.0	0.2	541.6	–	–
32															
33															
34															
35	–	0.48	446.9	–	–	–	4.4	2.5	–	–	0.9	0.4	–	–	–
36															
37	0.16	–	–	–	–	–	–	–	–	–	–	–	–	–	–
38															
39															
40															
41															
42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
43															
44															
45															
46															
47	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
48															
49															
50	0.49	0.14	–	–	–	–	–	–	–	–	–	–	–	–	–
51															
52															
53	0.63	0.02	17.0	0.22	1157.	0.56	64.5	7.3	–	3.26	2.2	5.7	–	6.8	–
54	0.56	0.02	16.8	0.17	1053.	0.56	49.6	8.2	–	3.26	4.8	4.5	–	40.4	–
55															
56	0.22	–	–	–	1703.	0.22	46.6	9.2	–	–	1.6	0.8	–	–	–
57															
58															
59															
60	6.19	1.52	–	–	–	–	54.8	6.0	–	–	3.1	–	–	–	–
61															
62															
63	0.23	–	0.8	–	911.	–	17.9	1.6	0.2	–	0.8	0.4	–	–	–
64															
65															
66															
67															
68															
69	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
70															
71															
72	2.67	0.63	–	–	–	–	–	–	–	–	–	–	–	–	–
73															
74															
75	2.05	0.08	–	–	–	–	–	–	–	–	–	–	–	–	–
76															
77	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
78															
79															
80	2.10	1.60	–	–	–	–	124.4	16.8	890.8	–	5.9	5.9	–	–	–

(4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-1 Composition of Some Laboratory Animal Feeds, Excluding Amino Acids

Line Number	SCIENTIFIC NAME National Research Council Name (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Matter (%)	Dry Basis Protein (%)	Ether Extract (%)	Crude Fiber (%)	Nitrogen Free Extract (%)	Calcium (%)	Copper (mg/kg)	Iodine (mg/kg)	Iron (%)	Magnesium (%)	Manganese (mg/kg)	Phosphorus (%)
81	CASEIN – see CATTLE													
82	CATTLE. *Bos* spp													
83	–whey, dehy, mn 65% lactose, (4)	4-01-182	94.0	14.7	0.9	0.0	74.1	0.93	45.9	–	0.017	0.14	0.49	0.84
84	Dried whey (AAFCO)													
85	Whey, dried													
86	–whey low lactose, dehy, mn lactose													
87	declared, (4)	4-01-186	91.0	17.3	1.4	0.2	65.4	1.70	–	–	–	–	–	1.09
88	Dried whey-product (AAFCO)													
89	Whey product, dried													
90	–buttermilk, condensed, mn 27% total solids													
91	w mn 0.055% fat mx 0.14% ash per													
92	1% solids, (5)	5-01-159	29.0	36.7	8.6	–	42.6	1.52	–	–	–	0.66	–	0.90
93	Condensed buttermilk (AAFCO)													
94	Buttermilk, concentrated													
95	Buttermilk, condensed													
96	Buttermilk, evaporated													
97	–buttermilk, dehy, feed gr mx 8% moisture													
98	mx 13% ash mn 5% fat, (5)	5-01-160	93.0	34.4	6.2	0.0	48.6	1.44	–	–	–	0.41	3.8	1.01
99	Dried buttermilk, feed grade (AAFCO)													
100	Buttermilk, dried													
101	–casein, milk acid precipitated dehy, mn													
102	80% protein, (5)	5-01-162	90.0	90.9	0.6	0.0	4.8	0.68	–	–	–	–	4.9	1.10
103	Casein (AAFCO)													
104	Casein, dried													
105	–liver, raw, (5)	5-01-166	26.0	17.3	12.3	0.0	–	0.04	–	–	–	–	–	0.88
106	Beef liver													
107	–milk, dehy, feed gr mx 8% moisture mn													
108	26% fat, (5)	5-01-167	93.7	26.9	28.2	0.2	38.9	0.95	–	–	0.018	–	0.4	0.72
109	Dried whole milk (AAFCO)													
110	Milk, whole, dried													
111	–milk, fresh, (5)	5-01-168	12.0	25.8	30.8	–	36.7	–	–	–	–	–	–	–
112	Milk, cattle, fresh													
113	–milk, skim dehy, mx 8% moisture, (5)	5-01-175	94.0	35.6	1.0	0.2	55.1	1.34	12.2	–	0.005	0.12	2.3	1.10
114	Dried skimmed milk, feed grade (AAFCO)													
115	Milk, skimmed, dried													
116	CHICKEN. *Gallus domesticus*													
117	–eggs wo shells, raw, (5)	5-08-114	26.3	49.0	43.7	–	3.4	0.19	–	–	0.008	–	–	0.76
118	CITRUS. *Citrus* spp													
119	–pulp wo fines, shredded dehy, (4)	4-01-237	90.0	7.3	5.1	14.4	66.5	1.18	6.3	–	0.018	0.18	7.6	0.13
120	Dried citrus pulp (AAFCO)													
121	Citrus pulp, dried													
122	COCONUT. *Cocos nucifera*													
123	–meats, mech-extd grnd, (5)	5-01-572	93.0	21.9	7.1	12.9	50.7	0.23	20.1	–	0.211	0.28	59.6	0.66
124	Coconut meal, mech-extd (AAFCO)													
125	Copra meal, expeller (AAFCO)													
126	Coconut meal, hydraulic													
127	Copra meal, hydraulic													
128	–meats, solv-extd grnd, (5)	5-01-573	92.0	22.9	2.0	16.1	52.5	0.18	–	–	–	–	59.8	0.66
129	Coconut meal, solv extd (AAFCO)													
130	Solv extd copra meal (AAFCO)													
131	CORN. *Zea mays*													
132	–cobs, grnd, (1)	1-02-782	90.4	2.8	0.5	35.8	59.2	0.12	7.3	–	0.023	0.07	6.2	0.04
133	Ground corn cob (AAFCO)													
134	–grits byproduct, mn 5% fat, (4)	4-02-887	90.6	11.8	7.2	5.5	72.7	0.06	16.1	–	0.007	0.26	16.1	0.58
135	Hominy feed (AAFCO)													
136	Hominy feed (CFA)													
137	–distillers grains w solubles, dehy, mn 75%													
138	original solids, (5)	5-02-843	91.0	29.7	8.8	9.3	47.5	0.38	54.9	0.05	0.022	0.38	33.0	1.04
139	Corn distillers dried grains w													
140	solubles (AAFCO)													
141	–distillers soluble, dehy, (5)	5-02-844	95.5	29.8	9.4	4.2	48.4	0.31	57.6	0.05	0.021	0.62	62.8	1.68
142	Corn distillers dried solubles (AAFCO)													
143	–germ wo solubles, wet milled solv-extd													
144	dehy grnd, (5)	5-02-898	93.0	19.4	2.2	12.9	–	0.11	–	–	–	–	17.2	0.43
145	Corn germ meal, sol extd, (wet milled)													
146	(AAFCO)													
147	–gluten, wet milled dehy, (5)	5-02-900	91.0	47.1	2.5	4.3	43.4	0.18	31.0	–	0.044	0.05	8.0	0.44
148	Corn gluten meal (AAFCO)													
149	Corn gluten meal (CFA)													
150	CORN, DENT WHITE. *Zea mays indentata*													
151	–grain, (4)	4-02-928	88.0	9.8	4.2	2.3	82.5	0.04	6.6	–	–	–	9.7	0.31
152	CORN, DENT YELLOW. *Zea mays indentata*													
153	–grain, (4)	4-02-935	86.0	10.2	4.4	2.3	81.8	0.03	4.0	–	0.003	0.17	4.8	0.31
154	CORN, FLINT. *Zea mays indurata*													
155	–grain, (4)	4-02-948	89.9	11.1	4.8	2.2	80.3	–	13.0	–	0.003	–	7.9	0.24
156	COTTON. *Gossypium* spp													
157	–seed w some hulls, mech-extd grnd, mn 41%													
158	protein mx 14% fiber mn 2% fat, (5)													
159	Cottonseed meal, 41% protein													
160	–seed w some hulls, pre-press solv-extd	5-01-617	94.0	43.6	4.6	12.8	32.4	0.17	20.7	–	0.032	0.60	22.9	1.28

(1) dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages;

Line Number	Dry Basis														
	Potassium (%)	Sodium (%)	Zinc (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folic acid (mg/kg)	Niacin (mg/kg)	Pantothenic acid (mg/kg)	Provitamin A (Carotene) (mg/kg)	Pyridoxine (mg/kg)	Riboflavin (mg/kg)	Thiamine (mg/kg)	Vitamin B_{12} (μg/kg)	Vitamin E (mg/kg)	Vitamin K (mg/kg)
81															
82															
83	1.28	0.51	–	0.42	21.	0.96	11.9	50.7	–	2.66	31.8	3.9	0.03	–	–
64															
85															
86															
87	–	–	–	–	1944.	–	65.8	75.8	–	–	61.0	–	–	–	–
88															
89															
90															
91															
92	0.79	1.07	–	–	–	–	–	–	–	–	49.3	–	–	–	–
93															
94															
95															
96															
97															
98	0.76	1.02	–	0.32	1944.	0.43	9.2	32.4	–	2.58	33.3	3.8	0.02	6.8	–
99															
100															
101															
102	–	–	–	–	232.	0.44	1.4	2.9	–	0.44	1.7	0.4	–	–	–
103															
104															
105	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
106															
107															
108	1.06	0.38	–	0.39	–	–	9.0	24.2	7.5	4.94	20.9	3.9	–	–	–
109															
110															
111	–	–	–	–	7296.	–	15.0	67.5	–	–	15.0	3.3	–	–	–
112															
113	1.78	0.53	42.6	0.35	1517.	0.66	12.2	35.8	–	4.22	21.4	3.7	44.57	9.8	–
114															
115															
116															
117	0.49	0.46	–	–	–	–	3.8	–	–	–	11.4	4.2	–	–	–
118															
119	0.69	–	16.1	–	939.	–	24.0	14.4	–	–	2.7	1.7	–	–	–
120															
121															
122															
123	1.20	0.04	–	–	989.	1.40	26.8	7.1	–	–	3.3	0.8	–	–	–
124															
125															
126															
127															
128	–	0.04	–	–	1196.	0.33	26.0	7.2	–	4.78	14.3	1.0	–	–	–
129															
130															
131															
132	0.84	–	–	–	–	–	–	–	0.7	–	–	–	–	–	–
133															
134	0.74	0.44	–	0.14	1104.	0.31	56.4	8.3	10.1	12.14	2.2	8.7	–	–	–
135															
136															
137															
138	1.10	0.05	87.9	0.33	3700.	1.10	84.6	12.1	4.0	7.10	9.9	3.8	1.60	43.4	–
139															
140															
141	2.20	0.16	104.7	0.52	6100.	1.80	125.6	23.0	0.8	13.80	23.0	7.3	7.00	59.1	–
142															
143															
144	0.22	–	–	3.22	1935.	0.22	37.7	4.4	–	–	4.4	1.1	–	93.5	–
145															
146															
147	0.03	0.11	0.24	0.16	363.	0.22	54.8	11.3	0.17	8.79	1.6	0.2	–	46.2	–
148															
149															
150															
151	–	–	26.8	0.10	–	–	17.2	4.4	0.4	–	1.5	5.1	–	–	–
152															
153	0.38	0.01	12.1	0.07	624.	0.22	26.6	5.8	4.8	8.37	1.3	4.6	–	25.6	–
154															
155	–	–	–	–	–	–	17.8	–	–	–	–	–	–	–	–
156															
157															
158	1.49	0.04	–	1.17	2957.	2.45	42.0	14.9	–	5.64	5.3	6.9	–	42.6	–
159															
160															

(4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-1 Composition of Some Laboratory Animal Feeds, Excluding Amino Acids

Line Number	SCIENTIFIC NAME National Research Council Name (NRC) American Feed Control Name (AAFCO Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Matter (%)	Dry Basis										
				Protein (%)	Ether Extract (%)	Crude Fiber (%)	Nitrogen Free Extract (%)	Calcium (%)	Copper (mg/kg)	Iodine (mg/kg)	Iron (%)	Magnesium (%)	Manganese (mg/kg)	Phosphorus (%)
161	grnd, 41% protein, (5)	5-07-872	92.5	43.6	1.5	12.7	34.5	0.17	20.7	–	0.032	0.60	22.9	1.28
162	Cottonseed meal, pre-press solv extd, 41% protein													
163														
164	–seed w some hulls, solv-extd grnd, mn													
165	41% protein mx 14% fiber mn 0.5% fat, (5)	5-01-621	91.5	44.8	2.2	13.1	33.1	0.17	21.3	–	0.033	0.61	23.5	1.31
166	Cottonseed meal, sol extd, 41% protein													
167	–seed wo hulls, pre-press solv extd grnd, mn													
168	50% protein, (5)	5-07-874	92.5	54.0	1.3	9.2	28.8	0.17	19.4	–	0.012	0.50	24.6	1.09
169	Cottonseed meal, pre-press solv-extd,													
170	50% protein													
171	CRAB. *Callinectes sapidus, Cancer* spp													
172	*Paralithodes camschatica*													
173	–processed residue, dehy grnd, mn 25%													
174	protein salt declared above 3% mx 7%, (5)	5-01-663	93.0	33.4	1.9	11.8	9.1	16.47	35.3	–	0.473	0.95	143.9	1.71
175	Crab meal (AAFCO)													
176	FISH. Scientific name not used													
177	–livers, extn unspecified dehy grnd, salt													
178	declared above 4%, (5)	5-01-968	93.0	71.5	16.8	1.1	5.8	0.54	95.8	–	0.075	–	9.5	1.34
179	Fish liver meal (CFA)													
180	–soluble, condensed, mn 30% protein, 5	5-01-969	51.0	61.6	12.7	2.0	4.1	1.20	94.5	–	0.069	0.04	23.3	1.37
181	Condensed fish solubles (AAFCO)													
182	–stickwater soluble, cooked dehy, mn													
183	60% protein, (5)	5-01-971	92.0	68.3	8.3	1.1	5.1	–	–	–	–	–	–	–
184	Dried fish solubles (AAFCO)													
185	Fish solubles, dried													
186	FISH, ALEWIFE. *Pomolobus pseudoharengus*													
187	–whole, raw, (5)	5-07-964	26.0	75.0	19.2	–	–	–	–	–	–	–	–	–
188	–whole or cuttings, cooked mech-extd													
189	dehy grnd, (5)	5-09-830	91.0	62.6	–	–	–	–	–	–	–	–	–	–
190	Fish meal, alewife													
191	FISH, ANCHOVY. *Engraulis* spp													
192	–whole or cuttings, cooked mech-extd													
193	dehy grnd, (5)	5-01-985	93.0	70.9	5.8	1.1	–	4.84	21.8	0.94	0.032	–	23.6	3.06
194	Fish meal, anchovy													
195	FISH, CARP. *Cyprinus carpio*													
196	–whole, raw, (5)	5-01-986	22.0	84.1	10.4	–	–	–	–	–	–	–	–	–
197	–whole or cuttings, cooked dehy grnd, (5)	5-09-831	90.8	74.4	–	0.8	–	–	–	–	–	–	–	–
198	Fish meal, carp													
199	FISH, CATFISH. *Ictalurus* spp													
200	–whole, raw, (5)	5-07-965	17.5	94.3	2.3	–	–	–	–	–	–	–	–	–
201	–whole or cuttings, cooked mech-extd dehy													
202	grnd, (5)	5-09-835	93.9	55.3	–	–	–	7.77	27.7	–	–	–	–	–
203	Fish meal, catfish													
204	–whole or cuttings, cooked mech-extd													
205	press cake, (5)	5-09-834	47.1	52.4	–	–	–	–	–	–	0.040	0.18	40.4	4.04
206	–whole or cuttings, cooked pasturized, (5)	5-09-833	39.9	27.8	–	–	–	–	7.5	–	0.050	1.25	15.0	2.43
207	–whole or cuttings, raw, (5)	5-09-832	42.2	27.2	–	–	–	5.57	7.1	–	0.009	0.12	10.6	2.55
208	FISH, HERRING. *Clupea harengus harengus,*													
209	*Clupea harengus pallasi*													
210	–whole or cuttings, cooked mech-extd													
211	dehy grnd, (5)	5-02-000	92.0	76.7	8.2	1.1	3.4	3.20	–	–	–	–	10.8	2.39
212	Fish meal, herring													
213	FISH, MENHADEN. *Brevoortia tyrannus*													
214	–whole or cuttings, cooked mech-extd													
215	dehy grnd, (5)	5-02-009	92.0	66.6	8.4	1.1	2.6	5.97	9.1	–	0.061	–	27.9	3.05
216	Fish meal, menhaden													
217	FISH, SALMON, *Oncorhynchus* spp,													
218	*Salmo* spp													
219	–whole or cuttings, cooked mech-extd													
220	dehy grnd, (5)	5-02-012	93.0	62.4	10.4	–	6.8	5.85	12.8	–	0.020	–	7.9	3.26
221	Fish meal, salmon													
222	FISH, SARDINE. *Clupea* spp, *Sardinops* spp													
223	–whole or cuttings, cooked mech-extd													
224	dehy grnd, (5)	5-02-015	93.0	70.4	4.6	1.1	7.0	5.27	21.7	–	0.032	0.11	23.9	2.98
225	FISH, TUNA. *Thunnus thynnus*													
226	–whole or cuttings, cooked mech-extd													
227	dehy grnd, (5)	5-02-023	87.0	65.9	7.5	1.1	3.6	6.11	–	–	–	–	–	3.53
228	Fish meal, tuna													
229	FISH, WHITE. *Gadidae* (family), *Lophiidae*													
230	(family), *Rajidae* (family)													
231	–whole or cuttings, cooked mech-extd													
232	dehy grnd, mx 4% oil, (5)	5-02-025	92.0	68.7	4.8	1.1	1.8	8.55	–	–	–	–	15.5	3.92
233	White fish meal (CFA)													
234	Fish, cod, meal													
235	Fish, cusk, meal													
236	Fish, haddock, meal													
237	Fish, hack, meal													
238	Fish, pollock, meal													
239	Fish, monkfish, meal													
240	Fish, skate, meal													

(1) dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages;

Line Number	Dry basis														
	Potassium (%)	Sodium (%)	Zinc (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folic acid (mg/kg)	Niacin (mg/kg)	Pantothenic acid (mg/kg)	Provitamin A (Carotene) (mg/kg)	Pyridoxine (mg/kg)	Riboflevin (mg/kg)	Thiamine (mg/kg)	Vitamin B_{12} (μg/mg)	Vitamin E (mg/kg)	Vitamin K (mg/kg)
161	1.49	0.04	–	–	3042.	2.45	42.0	14.9	–	–	5.3	6.9	–	–	–
162															
163															
164															
165	1.53	0.04	–	0.11	3126.	2.51	43.2	15.3	–	6.99	5.5	7.1	–	16.4	–
166															
167															
168	1.36	0.05	79.2	0.11	3568.	1.19	55.1	16.2	–	7.57	6.2	–	–	16.2	–
169															
170															
171															
172															
173															
174	0.48	0.91	–	0.07	2150.	0.12	47.3	6.1	–	7.11	6.3	0.5	455.10	–	–
175															
176															
177															
178	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
179															
180	3.43	6.00	75.1	0.39	7898.	0.43	330.8	69.4	–	23.77	28.4	10.8	8.60	–	–
181															
182															
183	–	–	–	0.29	5677.	0.48	251.2	48.8	–	26.4	8.4	–	9.60	–	–
184															
185															
186															
187	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
188															
189	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
190															
191															
192															
193	0.54	0.86	118.2	0.39	3978.	0.22	68.8	9.46	–	3.76	7.1	0.7	0.11	3.6	–
194															
195															
196	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
197	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
198															
199															
200	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
201															
202	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
203															
204															
205	0.58	0.65	121.4	–	–	–	–	–	–	–	–	–	–	–	–
206	0.45	0.50	90.2	–	–	–	–	–	–	–	–	–	–	–	–
207	0.25	0.34	67.9	–	–	–	–	–	–	–	–	–	–	–	–
208															
209															
210															
211	0.54	0.54	–	0.46	4352.	2.61	96.6	12.4	–	4.02	9.8	0.7	237.70	29.3	0.00
212															
213															
214															
215	0.76	0.33	163.0	0.28	3348.	0.22	60.8	9.6	–	–	5.2	0.8	0.11	9.8	–
216															
217															
218															
219															
220	–	–	–	–	2772.	–	24.9	6.8	–	–	5.7	0.9	–	–	–
221															
222															
223															
224	0.35	0.19	–	–	3182.	–	66.7	9.9	–	–	6.3	0.4	–	–	–
225															
226															
227	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
228															
229															
230															
231															
232	0.54	0.65	–	0.09	9692.	0.22	75.7	9.6	–	3.59	9.8	2.0	0.11	9.8	–
233															
234															
235															
236															
237															
238															
239															
240															

(4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-1 Composition of Some Laboratory Animal Feeds, Excluding Amino Acids

Line Number	SCIENTIFIC NAME National Research Council Name (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Matter (%)	Dry Basis Pro-Tein (%)	Ether Extract (%)	Crude Fiber (%)	Nitrogen Free Extract (%)	Calcium (%)	Copper (mg/kg)	Iodine (mg/kg)	Iron (%)	Magnesium (%)	Manganese (mg/kg)	Phosphorus (%)
241	FLAX. *Linum usitatissimum*													
242	–seed, mech-extd grnd, mx 0.5% acid													
243	insoluble ash, (5)	5-02-045	91.0	38.8	5.7	9.9	39.4	0.48	29.0	0.07	0.019	0.64	43.3	9.8
244	Linseed meal, mech-extd (AAFCO)													
245	Linseed meal (CFA)													
246	Linseed oil meal, expeller extracted													
247	Linseed oil meal, hydraulic extracted													
248	Linseed meal, old process													
249	–seed, solv-extd grnd, mx 0.5% acid													
250	insoluble ash, (5)	5-02-048	91.0	38.6	1.9	9.9	43.2	0.44	28.2	–	0.036	0.66	41.3	0.91
251	Linseed meal, solvent extracted (AAFCO)													
252	Solvent extracted linseed meal (CFA)													
253	Linseed oil meal, solvt-extd													
254	GRAINS. Scientific name not used													
255	–brewers grains, dehy, mx 3% dried													
256	spent hops, (5)	5-02-141	92.0	28.2	6.7	16.3	45.0	0.29	23.2	–	0.027	0.15	40.9	0.54
257	Brewers dried grains (AAFCO)													
258	Brewers dried grains (CFA)													
259	–distillers grains, dehy, (5)	5-02-144	92.5	29.2	8.2	13.8	42.5	0.05	16.2	0.05	0.014	0.08	10.8	0.40
260	LIMESTONE. Scientific name not applicable													
261	–grnd, mn 33% calcium, (6)	6-02-632	100.0	–	–	–	–	33.84	–	–	0.330	–	275.6	0.02
262	Limestone, ground (AAFCO)													
263	LINSEED – see FLAX													
264	LIVER – see ANIMAL													
265	MAIZE – see CORN													
266	MALT –see BARLEY													
267	MEAT – see ANIMAL													
268	MILK – see CATTLE													
269	MILLET. *Setaria* spp													
270	–grain, (4)	4-03-098	90.0	13.3	4.4	8.9	69.9	0.06	24.0	–	0.004	0.18	32.3	0.31
271	MOLASSES – see BEET, SUGAR, see													
272	SUGARCANE													
273	OATS. *Avena sativa*													
274	–hulls, (1)	1-03-281	93.0	6.0	2.2	29.0	56.3	0.17	5.5	–	0.011	0.09	19.9	0.20
275	Oat hulls (AAFCO)													
276	Oat hulls (CFA)													
277	–cereal byproduct, mx 4% fiber, (4)	4-03-303	91.0	17.4	6.4	4.3	69.3	0.09	6.1	–	0.042	–	48.4	0.54
278	Feeding oat meal (AAFCO)													
279	Oat middlings (CFA)													
280	–grain, (4)	4-03-309	89.0	13.2	5.1	12.4	65.7	0.11	6.6	–	0.008	0.19	42.9	0.39
281	–grain, gr 1 US mn wt 34 lb par bushel mx													
282	2% foreign material, (4)	4-03-313	91.0	13.3	5.3	13.2	64.7	0.09	–	–	–	–	41.8	0.33
283	–grain, gr 2 heavy US mn wt 36 lb per bushel													
284	mx 3% foreign material, (4)	4-03-315	89.5	13.5	4.5	10.9	67.6	–	–	–	–	–	–	–
285	Oats, grain, heavy													
286	–grain, gr 2 US mn wt 32 lb per bushel mx													
287	3% foreign material, (4)	4-03-316	89.0	12.7	4.7	12.4	66.9	0.07	–	–	–	–	–	–
288	–grain, gr 3 US mn wt 30 lb per bushel													
289	mx 4% foreign material, (4)	4-03-317	91.0	13.3	5.1	14.3	63.6	–	–	–	–	–	–	–
290	–grain, gr 4 US mn wt 27 lb per bushel													
291	mx 5% foreign material, (4)	4-03-318	91.2	13.2	4.9	16.6	60.2	–	–	–	–	–	–	–
292	Oats, grain, light													
293	–groats, (4)	4-03-331	91.0	18.4	6.4	3.3	69.5	0.08	0.4	–	–	0.98	31.4	0.47
294	Oat groats (AAFCO)													
295	Oat groats (CFA)													
296	Hulled oats (CFA)													
297	OATS, WHITE. *Avena sativa*													
298	–grain, Can 2 CW mn wt 36 lb per bushel													
299	mx 3% foreign material, (4)	4-03-378	86.5	13.2	5.2	12.0	66.1	–	–	–	–	–	–	–
300	–grain, Can 2 feed mn wt 28 lb per bushel													
301	mx 22% foreign material, (4)	4-03-379	86.5	12.7	5.1	12.0	66.8	–	–	–	–	–	–	–
302	–grain, Can 3 CW mn wt 34 lb per bushel													
303	mx 6% foreign material, (4)	4-03-380	86.5	12.7	5.3	12.1	66.5	–	–	–	–	–	–	–
304	OYSTERS. *Crassostrea* spp, *Ostrea* spp													
305	–shells, fine grnd, mn 33% calcium, (6)	6-03-481	100.0	1.0	–	–	–	38.05	–	–	0.290	0.30	133.3	0.07
306	Oyster shell flour (AAFCO)													
307	PEA. *Pisum* spp													
308	–seed, grnd, (5)	5-03-598	91.0	24.7	2.1	9.9	59.2	0.19	–	–	–	–	–	0.55
309	PEANUT. *Arachis hypogaea*													
310	–kernels, mech-extd grnd, mx 7% fiber, (5)	5-03-649	92.0	49.8	8.2	12.0	27.7	0.18	–	–	–	0.36	27.7	0.62
311	Peanut meal, mech-extd (AAFCO)													
312	Peanut meal (CFA)													
313	Peanut oil meal, expeller extd													
314	–kernels, solv-extd grnd, mx 7% fiber, (5)	5-03-650	92.0	51.5	1.3	14.1	28.2	0.22	16.7	0.07	0.029	0.04	31.5	0.71
315	Peanut meal, solv extd (AAFCO)													
316	Groundnut oil meal, solv extd													
317	Peanut oil meal, solv extd													
318	PHOSPHATE ROCK													
319	–defluorinated grnd, mx 1 part fluorine per													
320	100 part phosphorus, (6)	6-01-780	99.8	–	–	–	–	32.07	–	–	0.922	–	–	18.04

(1) dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages;

Line Number	Dry Basis														
	Potassium (%)	Sodium (%)	Zinc (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folic acid (mg/kg)	Niacin (mg/kg)	Pantothenic acid (mg/kg)	Provitamin A (Carotene) (mg/kg)	Pyridoxine (mg/kg)	Riboflavin (mg/kg)	Thiamine (mg/kg)	Vitamin B_{12} (μg/kg)	Vitamin E (mg/kg)	Vitamin K (mg/kg)
241															
242															
243	1.36	0.12	36.3	0.32	2047.	3.19	39.1	19.6	0.2	6.06	3.8	5.6	–	–	–
244															
245															
246															
247															
248															
249															
250	1.52	0.15	–	–	1346.	–	33.1	–	–	–	3.2	10.4	–	–	–
251															
252															
253															
254															
255															
256	0.09	0.28	29.9	–	1725.	0.24	47.2	9.3	–	0.72	1.6	0.8	–	–	–
257															
258															
259	0.16	0.05	54.1	0.22	1100.	1.20	45.4	7.1	8.4	4.30	3.4	2.2	0.25	–	–
260															
261	–	0.06	–	–	–	–	–	–	–	–	–	–	–	–	–
262															
263															
264															
265															
266															
267															
266															
269															
270	0.48	0.04	15.4	–	877.	–	58.4	8.2	–	–	1.8	7.3	–	–	–
271															
272															
273															
274	0.63	0.04	–	–	473.	–	10.7	3.5	–	–	4.9	–	–	–	–
275															
276															
277	0.55	0.05	483.5	0.24	1319.	0.38	30.9	25.4	–	2.42	2.0	7.7	–	26.4	–
278															
279															
280	0.42	0.07	–	0.34	1206.	0.45	17.8	14.5	–	1.35	1.8	7.0	–	6.6	–
281															
282	0.41	0.07	–	0.12	1209.	0.33	19.8	14.3	–	1.33	1.2	–	–	22.0	
283															
284	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
285															
286															
287	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
288															
289	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
290															
291	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
292															
293	0.37	–	–	–	–	–	8.9	16.2	–	1.21	1.4	7.5	–	–	–
294															
295															
296															
297															
298															
299	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
300															
301	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
302															
303	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
304															
305	0.10	0.21	–	–	–	–	–	–	–	–	–	–	–	–	–
306															
307															
306	1.13	0.04	33.0	0.20	713.	0.40	18.9	5.1	–	1.10	0.9	2.0	–	–	–
309															
310	1.25	–	–	–	1829.	–	183.7	52.4	–	–	5.8	7.9	–	–	–
311															
312															
313															
314	1.30	0.08	21.7	0.42	2174.	0.39	184.9	57.6	–	10.87	12.0	7.9	–	3.3	–
315															
316															
317															
318															
319															
320	0.09	3.96	–	–	–	–	–	–	–	–	–	–	–	–	–

(4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-1 Composition of Some Laboratory Animal Feeds, Excluding Amino Acids

Line Number	SCIENTIFIC NAME National Research Council Name (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Matter (%)	Dry Basis Protein (%)	Ether Extract (%)	Crude Fiber (%)	Nitrogen Free Extract (%)	Calcium (%)	Copper (mg/kg)	Iodine (mg/kg)	Iron (%)	Magnesium (%)	Manganese (mg/kg)	Phosphorus (%)
321	PHOSPHATE ROCK													
322	Phosphate, defluorinated (AAFCO)													
323	Defluorinated phosphate (CFA)													
324	–Rock phosphate, grnd, (6)	6-03-945	99.0	–	–	–	–	29.87	–	–	–	0.41	–	13.68
325	Rock phosphate, ground (AAFCO)													
326	POTATO. *Solanum tuberosum*													
327	–tubers, dehy grnd, (4)	4-07-850	90.3	6.5	0.6	1.6	78.1	0.08	–	–	–	–	3.2	0.22
328	Potato meal													
329	–tubers, flaked dehy, (4)	4-03-785	94.8	7.6	–	–	–	0.04	–	–	–	–	–	0.18
330	–tubers, fresh, (4)	4-03-787	24.6	9.0	0.4	2.1	84.9	–	–	–	–	–	–	–
331	POULTRY. Scientific name not used													
332	–feathers, hydrolyzed dehy grnd, mn 75% of													
333	protein digestible, (5)	5-03-795	94.0	93.0	2.6	0.6	0.0	0.21	–	–	–	–	–	0.89
334	Hydrolyzed poultry feathers (AAFCO)													
335	RICE. *Oryza sativa*													
336	–bran w germ, dry milled, mx 13% fiber													
337	$CaCO_3$ declared above 3% mn, (4)	4-03-928	91.0	14.8	16.6	12.1	44.5	0.07	14.3	–	0.021	1.04	459.1	2.00
338	Rice bran (AAFCO)													
339	–grain w hulls, (4)	4-03-939	88.0	8.9	1.9	9.9	73.9	0.10	3.3	0.05	0.008	–	20.0	0.30
340	Paddy rice													
341	Rough rice													
342	–grain w hulls, grnd, (4)	4-03-938	89.0	8.2	2.1	10.1	74.6	0.04	–	–	–	0.16	20.2	0.29
343	Grnd rough rice (AAFCO)													
344	Grnd paddy rice (AAFCO)													
345	–groats, (4)	4-03-936	88.2	9.5	1.9	1.0	86.5	0.05	3.9	–	0.003	0.10	15.1	0.28
346	Brown rice grain													
347	Rice grain wo hulls													
348	–groats, grnd, (4)	4-03-935	89.0	9.6	1.3	1.1	87.2	0.04	4.8	–	0.004	0.06	4.8	0.20
349	Grnd brown rice (AAFCO)													
350	Rice grain wo hulls, grnd													
351	–groats, polished, (4)	4-03-942	89.0	8.2	0.4	0.4	90.4	0.03	3.3	–	0.002	0.02	12.2	0.13
352	Rice, white, polished													
353	–polishings, dehy, (4)	4-03-943	90.0	13.1	14.7	3.3	60.0	0.04	12.2	0.07	0.019	0.72	–	1.58
354	Rice polishings (AAFCO)													
355	Rice polish (CFA)													
356	ROCK PHOSPHATE – see PHOSPHATE ROCK													
357	RYE. *Secale cereale*													
358	–grain, (4)	4-04-047	89.0	13.4	1.8	2.2	80.7	0.07	8.8	–	0.009	0.13	75.2	0.38
359	SAFFLOWER. *Carthamus tinctorius*													
360	–seed, solv-extd grnd, (5)	5-04-110	91.8	21.4	3.9	32.2	29.6	0.34	–	–	–	–	–	0.84
361	Solv-extd whole pressed safflower seed													
362	(AAFCO)													
363	SESAME. *Sesamum indicum*													
364	–seed, mech-extd grnd, (5)	5-04-220	93.0	51.5	5.5	5.4	27.6	2.18	–	–	–	–	51.6	1.39
365	Sesame oil meal, expeller extracted													
366	SEAWEED. *Laminariales* (order), *Fucales* (order)													
367	–entire plant, s-c grnd, (1)	1-04-190	89.4	10.7	–	8.6	–	2.05	–	–	–	7.12	–	0.20
368	SHRIMP. *Pandalus* spp, *Peneaus* spp													
369	–process residue, dehy grnd, salt declared													
370	above 3% mx 7%, (5)	5-04-226	90.0	52.7	3.2	12.2	1.7	8.17	–	–	0.010	0.60	33.4	1.77
371	Shrimp meal (AAFCO)													
372	SODIUM TRIPOLYPHOSPHATE													
373	–commercial, (6)	6-08-076	96.0	–	–	–	–	–	–	–	–	–	–	25.98
374	Sodium tripolyphosphate (AAFCO)													
375	SORGHUM, GRAIN VARIETY. *Sorghum*													
376	*vulgare*													
377	–grain, (4)	4-04-383	89.0	12.5	3.4	2.2	79.9	0.45	10.9	–	–	0.19	16.3	0.35
378	SORGHUM, MILO. *Sorghum vulgare*													
379	–grain, (4)	4-04-444	89.0	12.4	3.1	2.2	80.4	0.45	15.8	0.025	–	0.22	14.5	0.33
380	SOYBEAN. *Glycine max*													
381	–seeds, (5)	5-04-610	90.0	42.1	20.0	5.6	27.2	0.18	–	–	–	–	–	0.66
382	–seed, mech-extd grnd, mx 7% fiber, (5)	5-04-600	90.0	48.7	5.2	6.7	33.1	0.30	20.0	–	0.018	0.28	35.9	0.70
383	Soybean meal, mech-extd (AAFCO)													
384	Soybean meal, expeller extd													
385	Soybean meal, hydraulic extd													
386	Soybean oil meal, expeller extd													
387	Soybean oil meal, hydraulic extd													
388	–seed, solv-extd grnd, mx 7% fiber, (5)	5-04-604	89.0	51.5	1.0	6.7	34.3	0.36	40.8	0.146	0.013	0.30	30.9	0.75
389	Soybean meal, solv-extd (AAFCO)													
390	Soybean oil meal, solv-extd													
391	–seed wo hulls, solv-extd grnd, mx 3% fiber (5)	5-04-612	89.8	56.7	0.9	3.1	33.1	0.29	16.3	0.120	0.012	–	50.7	0.69
392	Soybean meal, dehulled, solv-extd (AAFCO)													
393	Soybean oil meal, dehulled, solv-extd													
394	SUGARCANE. *Saccharum officinarum*													
395	–molasses, dehy, (4)	4-04-695	96.0	10.7	1.0	5.2	74.8	0.43	–	–	–	–	–	–
396	Cane molasses, dried													
397	Molasses, cane, dried													
398	–molasses, mn 48% invert sugar mn 79.5													
399	degrees brix, (4)	4-04-696	75.0	4.3	0.1	–	84.8	1.19	79.5	2.100	0.025	0.47	56.3	0.11
400	Cane molasses (AAFCO)													

(1) dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages;

Line Number	Dry Basis														
	Potassium (%)	Sodium (%)	Zinc (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folic acid (mg/kg)	Niacin (mg/kg)	Pantothenic acid (mg/kg)	Provitamin A (Carotene) (mg/kg)	Pyridoxine (mg/kg)	Riboflavin (mg/kg)	Thiamine (mg/kg)	Vitamin B_{12} (μg/kg)	Vitamin E (mg/kg)	Vitamin K (mg/kg)
321															
322															
323															
324	0.60	0.03	–	–	–	–	–	–	–	–	–	–	–	–	–
325															
326															
327	2.18	–	–	–	–	–	–	–	–	–	–	–	–	–	–
328															
328	1.69	0.09	–	–	–	–	57.0	–	–	–	0.6	2.4	–	–	–
330	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
331															
332															
333	–	–	–	–	977.	–	34.2	12.2	–	–	2.4	–	–	–	–
334															
335															
336															
337	1.91	0.08	32.9	4.62	1378.	–	333.2	25.8	–	–	2.9	24.6	–	65.9	–
338															
339	0.43	0.03	2.3	0.10	1127.	0.50	38.8	10.0	–	–	1.5	3.5	–	–	–
340															
341															
342	0.38	0.07	16.9	–	899.	0.45	34.0	0.37	–	–	1.2	3.1	–	15.7	–
343															
344															
345	0.23	0.04	–	0.10	–	0.21	51.4	12.1	–	–	0.7	3.6	–	9.9	–
346															
347															
348	0.13	0.04	–	–	–	–	19.2	–	–	–	0.3	1.2	–	–	–
349															
350															
351	0.15	0.03	2.0	–	1019.	0.17	15.8	3.7	–	0.45	0.7	0.7	–	4.0	–
352															
353	1.30	0.12	29.4	0.67	1452.	0.49	590.8	64.8	–	–	2.0	21.9	–	100.0	–
354															
355															
356															
357															
358	0.51	0.02	34.3	0.07	–	0.67	1.3	7.8	–	–	1.8	4.4	–	16.8	–
359															
360	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
361															
362															
363															
364	1.29	0.04	107.5	–	1648.	–	32.3	6.9	–	13.44	4.0	3.1	–	–	–
365															
366															
367	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
368															
369															
370	–	–	–	–	6476.	–	–	–	–	–	4.4	–	–	–	–
371															
372															
373	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
374															
375															
376															
377	0.38	0.04	15.4	2.92	762.	0.22	48.4	12.5	–	5.95	1.5	4.6	–	–	–
378															
379	0.39	0.01	19.1	0.20	762.	0.27	48.0	12.8	–	4.61	1.3	4.8	–	13.5	–
380															
381	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
382	1.90	0.27	–	0.33	2970.	7.33	33.8	–	–	–	–	4.4	–	–	–
383															
384															
385															
366															
387															
368	2.21	0.38	30.3	0.36	3082.	0.79	30.1	16.3	–	8.99	3.7	7.4	2.20	3.4	–
389															
390															
391	2.25	0.1	50.1	0.36	3075.	4.01	24.1	16.1	–	8.91	3.5	2.7	2.17	3.7	–
392															
393															
394															
395	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
396															
397															
398															
399	3.17	–	30.0	–	1168.	–	45.7	51.1	–	–	4.4	1.2	–	–	–
400															

(4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-1 Composition of Some Laboratory Animal Feeds, Excluding Amino Acids

Line Number	SCIENTIFIC NAME National Research Council (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Basis Dry Matter (%)	Protein (%)	Ether Extract (%)	Crude Fiber (%)	Nitrogen Free Extract (%)	Calcium (%)	Copper (mg/kg)	Iodine (mg/kg)	Iron (%)	Magnesium (%)	Manganese (mg/kg)	Phosphorus (%)
401	SUGARCANE. *Saccharum officinarum*													
402	Molasses, cane													
403	SUNFLOWER. *Helianthus* spp													
404	–seed wo hulls, mech-extd grnd, (5)	5-04-738	93.0	44.1	8.2	14.0	26.4	0.46	–	–	–	–	24.6	1.12
405	Sunflower meal (AAFCO)													
406	Sunflower oil meal, wo hulls, expeller extd													
407	–seed wo hulls, solv-extd grnd, (5)	5-04-739	93.0	50.3	3.1	11.8	26.5	0.43	–	–	–	–	24.7	1.08
408	Sunflower meal (AAFCO)													
409	Sunflower oil meal, wo hulls, solv extd													
410	TANKAGE – see ANIMAL													
411	TIMOTHY. *Phleum pratense*													
412	–hay, s-c, early blm, (1)	1-04-882	87.7	8.7	2.6	33.2	49.3	0.60	–	–	–	–	–	0.26
413	TOMATO. *Lycopersicon esculentum*													
414	–pulp, dehy, (5)	5-05-041	92.0	23.6	14.1	31.5	–	0.30	–	–	–	–	–	0.62
415	Dried tomato pomace (AAFCO)													
416	WHALE. *Balaena glacialis, Balaenoptera* spp,													
417	*Physeter catadon*													
418	–meat, heat-rendered dehy grnd, salt													
419	declared above 3% mx 7%, (5)	5-05-160	92.0	85.8	7.4	1.1	1.4	0.30	–	–	–	–	–	0.61
420	Whale meal (AAFCO)													
421	WHEAT. *Triticum* spp													
422	–bran, dry milled, (4)	4-05-190	89.0	18.0	4.6	11.2	59.3	0.16	13.8	0.074	0.019	0.62	130.0	1.31
423	Wheat bran (AAFCO)													
424	Bran (CFA)													
425	–flour, coarse bolted, feed gr mx 2% fiber, (4)	4-05-199	89.0	17.8	3.3	3.4	73.1	0.03	5.2	–	0.002	–	50.4	0.31
426	Wheat feed flour, mx 1.5% fiber (AAFCO)													
427	Feed flour, mx 2.0% fiber (CFA)													
428	–flour byproduct, coarse sifted, mx 7% fiber, (4)	4-05-201	90.0	20.4	4.7	5.6	65.0	0.12	10.2	–	0.011	0.29	116.1	0.84
429	Wheat shorts, mx 7% fiber (AAFCO)													
430	Shorts, mx 8% fiber (CFA)													
431	–flour byproduct, fine sifted, mx 4% fiber, (4)	4-05-203	89.0	20.2	4.0	2.2	70.8	0.09	4.9	–	0.007	0.33	42.2	0.58
432	Wheat red dog, mx 4.0% fiber (AAFCO)													
433	Middlings, mx 4.5% fiber (CFA)													
434	–flour byproduct, mill run, mx 9.5% fiber, (4)	4-05-206	90.0	17.0	4.4	8.9	63.9	0.10	20.8	–	0.010	0.57	114.1	1.13
435	Wheat, mill run (AAFCO)													
436	–grain, (4)	4-05-211	89.0	14.3	1.9	3.4	78.6	0.06	8.1	–	0.008	0.18	54.8	0.40
437	–grain screenings, (4)	4-05-216	89.0	16.9	3.4	7.9	68.2	0.09	–	–	–	–	32.1	0.40
438	–grits, cracked fine screened, (4)	4-07-852	88.0	12.6	1.2	0.3	84.5	–	–	–	–	–	–	–
439	Farina													
440	Wheat endosperm													
441	–germ, grnd, mn 25% protein 7% fat, (5)	5-05-218	90.0	29.1	12.1	3.3	50.7	0.08	9.8	–	0.012	–	149.9	1.16
442	Wheat germ meal (AAFCO)													
443	–germ oil, (7)	7-05-207	100.0	–	100.0	–	–	–	–	–	–	–	–	–
444	Wheat germ oil (AAFCO)													
445	WHEAT, DURUM. *Triticum durum*													
446	–grain, (4)	4-05-224	89.5	15.0	2.2	2.5	78.3	0.17	8.6	–	0.004	–	32.1	0.45
447	–grain, Can 4 CW mn wt 56 lb per bushel													
448	mx 2.5% foreign material, (4)	4-05-225	86.5	15.7	1.9	2.6	78.0	–	–	–	–	–	–	–
449	WHEAT, HARD RED SPRING. *Triticum*													
450	*aestivum*													
451	–grain, (4)	4-05-258	86.5	16.1	2.2	3.5	76.3	0.06	12.2	–	0.006	–	71.9	0.46
452	WHEAT, HARD RED WINTER. *Triticum*													
453	*aestivum*													
454	–grain, (4)	4-05-268	89.1	14.6	1.8	3.0	78.6	0.06	5.0	–	–	0.11	36.8	0.45
455	WHEAT, RED SPRING. *Triticum aestivum*													
456	–grain, Can 4 No mn wt 56 lb per bushel													
457	mx 2.5% foreign material, (4)	4-05-282	86.5	16.3	2.0	2.8	77.2	–	–	–	-	–	–	–
458	WHEAT, SOFT. *Triticum aestivum*													
459	–grain, (4)	4-05-284	90.0	12.0	1.9	2.6	81.5	0.10	10.8	–	0.006	0.11	57.0	0.33
460	WHEAT, SOFT RED WINTER. *Triticum*													
461	*aestivum*													
462	–grain, (4)	4-05-294	89.1	12.3	1.8	2.5	81.4	0.10	11.0	–	–	0.11	42.9	0.33
463	WHEY – see CATTLE													
464	YEAST. *Saccharomyces cerevisiae*													
465	–brewers saccharomyces, dehy grnd, mn													
466	40% protein, (7)	7-05-527	93.0	48.0	1.2	3.2	40.8	0.14	35.5	–	0.011	0.25	6.1	1.54
467	Brewers dried yeast (AAFCO)													
468	–petroleum saccharomyces, dehy grnd, (7)	7-09-836	92.0	51.1	–	–	–	0.02	–	–	–	–	–	5.87
469	–primary saccharomyces, dehy, mn 40%													
470	protein, (7)	7-05-533	93.0	51.6	1.1	3.2	35.5	0.39	–	–	0.030	0.39	4.0	1.85
471	Dried yeast (AAFCO)													
472	Primary dried yeast (AAFCO)													
473	YEAST, TORULOPSIS. *Torulopsis utilis*													
474	–dehy, mn 40% protein, (7)	7-05-534	93.0	51.9	2.7	2.2	34.8	0.61	14.4	–	0.010	0.14	1.81	2.02
475	Torula dried yeast (AAFCO)													

(1) dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages;

Line Number	Dry Basis														
	Potassium (%)	Sodium (%)	Zinc (mg/kg)	Biotin (mg/kg)	Choline (mg/kg)	Folic acid (mg/kg)	Niacin (mg/kg)	Pantothenic acid (mg/kg)	Provitamin A (Carotene (mg/kg)	Pyridoxine (mg/kg)	Riboflavin (mg/kg)	Thiamine (mg/kg)	Vitamin B_{12} (μg/kg)	Vitamin E (mg/kg)	Vitamin K (mg/kg)
401															
402															
403															
404	1.16	–	–	–	–	–	–	–	–	–	–	–	–	–	–
405															
406															
407	1.08	–	–	–	3118.0	–	236.6	10.1	–	17.20	3.3	–	–	11.8	–
408															
409															
410															
411															
412	0.92	–	–	–	–	–	–	–	–	–	–	–	–	–	–
413															
414	–	–	–	–	–	–	–	–	–	–	6.7	12.9	–	–	–
415															
416															
417															
418															
419	–	–	–	–	–	–	113.8	2.8	–	–	9.1	–	–	–	–
420															
421															
422	1.39	0.07	88.7	0.54	1110.0	2.02	235.1	32.6	–	11.24	3.5	8.9	–	12.1	–
423															
424															
425	–	–	–	–	–	–	47.1	1.0	–	–	–	6.6	–	–	–
426															
427															
428	0.94	0.08	–	0.41	1031.0	1.22	105.1	19.6	–	12.22	2.2	17.6	–	33.2	–
429															
430															
431	0.67	0.74	122.3	0.42	1247.0	1.25	59.1	15.3	–	12.47	1.7	21.2	–	84.7	–
432															
433															
434	1.42	0.24	–	–	1090.0	–	124.4	14.7	–	–	2.7	16.9	–	–	–
435															
436	0.58	0.10	15.4	0.11	933.0	0.45	63.6	13.6	–	–	1.3	5.5	–	17.4	–
437	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
438	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
439															
440															
441	–	0.06	–	0.24	3344.0	2.22	52.6	12.4	–	14.44	5.7	31.0	–	147.4	–
442															
443	–	–	–	–	–	–	–	–	–	–	–	–	–	1900.0	–
444															
445															
446	–	–	–	–	–	0.44	–	–	–	–	–	7.0	–	–	–
447															
448	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
449															
450															
451	0.58	0.07	16.2	0.13	899.0	0.49	66.8	15.6	–	4.62	1.3	3.7	–	12.7	–
452															
453															
454	0.57	0.07	15.7	0.12	824.0	0.45	57.1	14.3	–	4.60	1.1	7.0	–	12.3	–
455															
456															
457	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
458															
459	0.44	0.07	15.6	0.12	876.0	0.33	66.8	14.2	–	5.33	1.3	5.3	–	12.2	–
450															
461															
462	0.44	–	–	–	874.0	0.45	64.4	12.8	–	5.16	–	5.9	–	–	–
463															
464															
465															
466	1.85	0.08	41.6	5.91	4177.0	10.43	481.2	118.1	–	46.56	37.6	98.6	–	0.0	–
467															
466	4.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–
469															
470	–	–	–	1.72	–	33.33	322.7	334.7	–	–	41.6	6.9	–	–	–
471															
472															
473															
474	–	0.01	106.7	1.20	3129.0	25.0	537.8	89.1	–	31.70	47.7	6.7	–	–	–
475															

(4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-2 Amino Acid Composition of Some Common Laboratory Animal Feeds

Line Number	SCIENTIFIC NAME National Research Council Name (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Basis Argi-nine (%)	Cys-tine (%)	Histi-dine (%)	Iso-leucine (%)	Leu-cine (%)	Ly-sine (%)	Methi-onine (%)	Phenyl-alanine (%)	Thre-onine (%)	Tryp-tophan (%)	Tyro-sine (%)	Valine (%)
1	ALFALFA. *Medicago sativa*													
2	–aerial part, dehy grnd, mn 15% protein, (1)	1-00-022	0.64	0.18	0.32	0.73	1.18	0.64	0.21	0.86	0.64	0.43	0.43	0.75
3	–aerial part, dehy grnd, mn 17% protein, (1)	1-00-023	0.75	0.34	0.43	0.75	1.40	0.86	0.21	0.86	0.86	0.43	0.54	0.97
4	–aerial part, dehy grnd, mn 20% protein, (1)	1-00-024	0.97	–	0.43	0.86	1.61	0.97	0.32	1.18	0.97	0.54	0.75	0.11
5	–aerial part, dehy grnd, mn 22% protein, (1)	1-07-851	1.08	–	0.54	0.97	1.83	1.08	0.43	1.29	1.08	0.64	0.86	1.29
6	–hay, s-c, early blm, (1)	1-00-059	–	–	–	–	–	–	–	–	–	–	–	–
7	–hay, s-c, full blm, (1)	1-00-068	–	–	–	–	–	–	–	–	–	–	–	–
8	–hay, s-c, grnd, (1)	1-00-111	–	–	–	–	–	–	–	–	–	–	–	–
9	Ground alfalfa hay (AAFCO)													
10	Sun-cured alfalfa meal (AAFCO)													
11	–leaves, dehy grnd, (1)	1-00-137	–	–	–	–	–	–	–	–	–	–	–	–
12	Alfalfa leaf meal, dehydrated (AAFCO)													
13	ANIMAL. Scientific name not used													
14	–blood, dehy grnd, (5)	5-00-380	3.85	1.53	4.62	1.10	11.32	7.58	0.99	6.70	4.07	1.21	1.98	7.14
15	Blood meal (AAFCO)													
16	Blood meal (CFA)													
17	–blood, spray dehy, (5)	5-00-381	3.63	–	5.27	1.21	11.65	9.01	1.10	6.15	3.96	1.10	2.20	7.91
18	Blood flour													
19	–carcass residue, dry rendered dehy grnd,													
20	mn 55% protein mx 4.4% phosphorus, (5)	5-00-385	3.96	0.64	1.18	2.03	3.74	4.06	0.86	2.03	1.93	0.32	0.96	2.78
21	Meat meal (AAFCO)													
22	Meat scrap													
23	–carcass residue w blood, dry or wet rendered													
24	dehy grnd, mx 4.4% phosphorus, (5)	5-00-386	3.91	0.53	2.07	2.07	5.54	4.34	0.87	2.93	2.61	0.76	1.59	4.57
25	Meat meal tankage													
26	Digester tankage													
27	–carcass residue w bone, dry rendered dehy													
28	grnd, mn 4.4% phosphorus, (5)	5-00-388	4.26	0.64	0.96	1.60	3.30	3.72	0.74	1.91	1.91	0.21	0.85	2.55
29	Meat and bone meal (AAFCO)													
30	Meat and bone scrap													
31	–liver, dehy grnd, (5)	5-00-389	4.43	0.97	1.62	3.67	5.83	5.18	1.40	3.13	2.81	0.65	1.84	4.54
32	Animal liver meal (AAFCO)													
33	Animal liver meal (CFA)													
34	Liver meal													
35	–bone, steamed dehy grnd, (6)	6-00-400	–	–	–	–	–	–	–	–	–	–	–	–
36	Bone meal, steamed (AAFCO)													
37	–bone charcoal, retort-charred grnd, (6)	6-00-403	2.00	–	0.22	0.67	0.89	1.11	0.22	0.56	0.56	–	–	0.78
38	Bone black (CFA)													
39	Bone char (CFA)													
40	Spent bone black													
41	–bone phosphate, precipitated dehy,													
42	mn 1% phosphorus, (6)	6-00-406	–	–	–	–	–	–	–	–	–	–	–	–
43	Bone phosphate (AAFCO)													
44	ANIMAL-POULTRY. Scientific name not used													
45	–fat, heat rendered, mn 90% fatty acids													
46	mx 2.5% unsaponifiable matter mx 1%													
47	insoluble matter, (4)	4-00-409	–	–	–	–	–	–	–	–	–	–	–	–
48	Animal fat (AAFCO)													
49	APPLES. *Malus* spp													
50	–pulp, dehy grnd, (4)	4-00-423	–	–	–	–	–	–	–	–	–	–	–	–
51	Dried apple pomace (AAFCO)													
52	BARLEY. *Hordeum vulgare*													
53	–grain, (4)	4-00-530	0.60	0.20	0.30	0.60	0.90	0.60	0.20	0.70	0.40	0.20	0.40	0.70
54	–grain, Pacific coast, (4)	4-07-939	0.48	0.25	0.25	0.45	0.67	0.34	0.16	0.54	–	0.15	–	0.52
55	–malt sprouts w hulls, dehy, mn 24%													
56	protein, (5)	5-00-545	–	–	–	–	–	–	–	–	–	–	–	–
57	Malt sprouts (AAFCO)													
58	BEET, SUGAR. *Beta saccharifera*													
59	–molasses, mn 48% invert sugar mn 79.5													
60	degrees brix, (4)	4-00-668	–	–	–	–	–	–	–	–	–	–	–	–
61	Beet molasses (AAFCO)													
62	Molasses (CFA)													
63	–pulp, dehy, (4)	4-00-669	0.33	–	0.22	0.33	0.66	0.66	–	0.33	0.44	0.11	0.44	0.44
64	Dried beet pulp (AAFCO)													
65	Dried beet pulp (CFA)													
66	BLOOD MEAL – see ANIMAL													
67	BONE – see ANIMAL													
68	BREAD. Scientific name not used													
69	–dehy, (4)	4-07-944	–	–	–	–	–	–	–	–	–	–	–	–
70	BREWERS – see GRAINS													
71	BROME, SMOOTH. *Bromus inermis*													
72	–hay, s-c, (1)	1-00-947	–	–	–	–	–	–	–	–	–	–	–	–
73	BUTTERMILK – see CATTLE													
74	CABBAGE. *Brassica oleracea capitata*													
75	–aerial pt, fresh, (2)	2-01-046	–	–	–	–	–	–	–	–	–	–	–	–
76	CALCIUM PHOSPHATE, DIBASIC													
77	–commercial, (6)	6-01-080	–	–	–	–	–	–	–	–	–	–	–	–
78	Dicalcium phosphate (AAFCO)													
79	CARROT. *Daucus* spp													
80	–roots, fresh, (4)	4-01-145	–	–	–	–	–	–	–	–	–	–	–	–
81	CASEIN – see CATTLE													

(1) Dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages; (4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-2 Amino Acid Composition of Some Common Laboratory Animal Feeds

Line Number	SCIENTIFIC NAME National Research Council (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Basis Argi-nine (%)	Cys-tine (%)	Histi-dine (%)	Iso-leucine (%)	Leu-cine (%)	Ly-sine (%)	Mathi-onine (%)	Phenyl-alanine (%)	Thre-onine (%)	Tryp-tophan (%)	Tyro-sine (%)	Valine (%)
82	CATTLE. *Bos* spp													
83	—whey, dehy, mn 65% lactose, (4)	4-01-182	0.43	0.32	0.21	0.96	1.49	1.17	0.21	0.43	0.85	0.21	0.32	0.74
84	Dried whey (AAFCO)													
85	Whey, dried													
86	—whey low lactose, dehy, mn lactose													
87	declared, (4)	4-01-186	0.55	0.38	–	–	–	1.43	0.24	–	–	0.24	–	–
88	Dried whey-product (AAFCO)													
89	Whey product, dried													
90	—buttermilk, condensed, mn 2% total solids w													
91	mn 0.055% fat mx 0.14% ash per 1% solids, (5)	5-01-159	–	–	–	–	–	–	–	–	–	–	–	–
92	Condensed buttermilk (AAFCO)													
93	Buttermilk, concentrated													
94	Buttermilk, condensed													
95	Buttermilk, evaporated													
96	—buttermilk, dehy, feed gr mx 8% moisture mx													
97	13% ash mn 5% fat, (5)	5-01-160	1.18	0.43	0.97	2.90	3.66	2.58	0.75	1.61	1.72	0.54	1.08	3.01
98	Dried buttermilk, feed grade (AAFCO)													
99	Buttermilk, dried													
100	—casein, milk acid precipitated dehy,													
101	mn 80% protein, (5)	5-01-162	3.78	0.33	2.78	6.33	9.56	7.78	3.00	5.11	4.22	1.11	5.22	7.56
102	Casein (AAFCO)													
103	Casein, dried													
104	—liver, raw, (5)	5-01-166	1.18	–	0.97	2.90	3.66	2.58	0.75	1.61	1.72	0.54	1.08	3.01
105	Beef liver													
106	—milk, dehy, feed gr mx 8% moisture													
107	mn 26% fat, (5)	5-01-167	0.96	–	0.75	1.39	2.67	2.35	0.64	1.39	1.07	0.43	1.39	1.81
108	Dried whole milk (AAFCO)													
109	Milk, whole, dried													
110	—milk, fresh, (5)	5-01-168	0.83	–	0.83	1.67	2.50	2.50	0.83	0.83	0.83	–	1.67	1.67
111	Milk, cattle, fresh													
112	—milk, skim dehy, mx 8% moisture, (5)	5-01-175	1.28	0.53	0.96	2.45	3.51	2.98	0.85	1.60	1.49	0.43	1.38	2.34
113	Dried skimmed milk, feed grade (AAFCO)													
114	Milk, skimmed, dried													
115	CHICKEN. *Gallus domesticus*													
116	—eggs wo shells, raw, (5)	5-08-114	–	–	–	–	–	–	–	–	–	–	–	–
117	CITRUS. *Citrus* spp													
118	—pulp wo fines, shredded dehy, (4)	4-01-237	0.22	0.12	–	–	–	0.22	0.9	–	–	0.07	–	–
119	Dried citrus pulp (AAFCO)													
120	Citrus pulp, dried													
121	COCONUT. *Cocos nucifera*													
122	—meats, mech-extd grnd, (5)	5-01-572	–	–	–	–	–	–	–	–	–	–	–	–
123	Coconut meal, mech extd (AAFCO)													
124	Copra meal, expeller (AAFCO)													
125	Coconut meal, hydraulic													
126	Copra meal, hydraulic													
127	—meats, solv-extd grnd, (5)	5-01-573	2.93	0.33	0.61	0.72	1.62	0.70	0.32	0.98	0.71	0.22	0.61	1.07
128	Coconut meal, solv extd (AAFCO)													
129	Solv extd copra meal (AAFCO)													
130	CORN. *Zea mays*													
131	—cobs, grnd, (1)	1-02-782	–	–	–	–	–	–	–	–	–	–	–	–
132	Ground corn cob (AAFCO)													
133	—grits byproduct, mn 5% fat, (4)	4-02-887	0.55	0.20	0.22	0.44	0.88	0.44	0.20	0.33	0.44	0.11	0.55	0.55
134	Hominy feed (AAFCO)													
135	Hominy feed (CFA)													
136	—distillers grains w solubles, dehy, mn 75%													
137	original solids, (5)	5-02-843	1.10	0.44	0.66	1.00	2.97	0.66	0.66	1.32	1.04	0.22	0.88	1.43
138	Corn distillers dried grains with													
139	solubles (AAFCO)													
140	—distillers soluble, dehy, (5)	5-02-844	1.20	0.42	0.66	1.31	2.76	0.99	0.52	1.36	1.08	0.31	0.99	1.46
141	Corn distillers dried solubles (AAFCO)													
142	—germ wo solubles, wet milled solv-extd													
143	dehy grnd, (5)	5-02-898	1.29	0.34	–	–	1.83	0.97	0.38	0.86	0.97	0.32	1.61	1.40
144	Corn germ meal, solvent extracted,													
145	(wet milled) (AAFCO)													
146	—gluten, wet milled dehy, (5)	5-02-900	1.54	0.66	1.10	2.53	8.35	0.88	1.10	3.19	1.54	0.22	1.10	2.42
147	Corn gluten meal (AAFCO)													
148	Corn gluten meal (CFA)													
149	CORN, DENT WHITE *Zea mays indentata*													
150	—grain, (4)	4-02-928	0.30	0.10	0.20	0.50	1.00	0.30	0.10	0.40	0.40	0.10	0.50	0.40
151	CORN, DENT YELLOW. *Zea mays indentata*													
152	—grain, (4)	4-02-935	0.58	0.10	0.23	0.47	1.28	0.23	0.20	0.58	0.47	0.12	–	0.47
153	CORN, FLINT. *Zea mays indurata*													
154	—grain, (4)	4-02-948	–	–	–	–	–	0.30	0.20	–	–	0.10	–	–
155	COTTON. *Gossypium* spp													
156	—seed w some hulls, mech-extd grnd, mn 41%													
157	protein mx 14% fiber mn 2% fat, (5)	5-01-617	4.52	0.90	1.17	1.70	2.66	1.81	0.69	2.50	1.54	0.69	0.74	2.18
158	Cottonseed meal, 41% protein													
159	—seed w some hulls, pre-press solv-extd grnd,													
160	41% protein, (5)	5-07-872	4.59	0.92	1.19	1.73	2.70	1.84	0.70	2.54	1.57	0.70	–	2.22
161	Cottonseed meal, pre-press solvent													
162	extd, 41% protein													

(1) dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages; (4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-2 Amino Acid Composition of Some Common Laboratory Animal Feeds

Line Number	SCIENTIFIC NAME National Research Council Name (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Name	Reference Number	Dry Basis Arginine (%)	Cystine (%)	Histidine (%)	Isoleucine (%)	Leucine (%)	Lysine (%)	Methionine (%)	Phenylalanine (%)	Threonine (%)	Tryptophan (%)	Tyrosine (%)	Valine (%)
163	COTTON. *Gossypium* spp													
164	–seeds w some hulls, solv-extd grnd, mn 41%													
165	protein mix 14% fiber mn 0.5% fat, (5)	5-01-621	4.64	0.93	1.20	1.75	2.73	1.86	0.71	2.57	1.58	0.71	0.77	2.24
166	Cottonseed meal, solv-extd, 41% protein													
167	–seed wo hulls, pre-press solv-extd grnd,													
168	mn 50% protein, (5)	5-07-874	5.14	1.08	1.35	2.00	3.03	2.27	0.86	2.97	1.84	0.76	0.86	2.22
169	Cottonseed meal, pre-press solv-extd,													
170	50% protein													
171	CRAB. *Callinectes sapidus, Cancer* spp													
172	*Paralithodes camschatica*													
173	–processed residue, dehy grnd, mn 25%													
174	protein salt declared above 3% mx 7%, (5)	5-01-663	1.83	–	0.54	1.29	1.72	1.50	0.54	1.29	1.08	0.32	1.29	1.61
175	Crab meal (AAFCO)													
176	FISH. Scientific name not used													
177	–livers, extn unspecified dehy grnd, salt													
178	declared above 4%, (5)	5-01-968	–	–	–	–	–	–	–	–	–	–	–	–
179	Fish liver meal (CFA)													
180	–soluble, condensed, mn 30% protein. (5)	5-01-969	4.71	3.33	4.90	3.14	4.90	5.29	1.96	2.75	2.35	1.57	0.98	3.14
181	Condensed fish solubles (AAFCO)													
182	–stickwater soluble, cooked dehy, mn													
183	60% protein, (5)	5-01-971	2.61	–	2.83	1.85	2.93	3.26	0.98	1.41	1.30	0.76	0.76	2.07
184	Dried fish solubles (AAFCO)													
185	Fish solubles, dried													
186	FISH, ALEWIFE. *Pomolobus pseudoharengus*													
187	–whole, raw, (5)	5-07-964	4.04	0.99	1.34	2.87	5.01	5.30	1.82	2.68	2.88	–	2.13	3.29
188	–whole or cuttings, cooked mech-extd													
189	dehy grnd, (5)	5-09-830	–	–	–	–	–	–	–	–	–	–	–	–
190	Fish meal, alewife													
191	FISH, ANCHOVY. *Engraulis* spp													
192	–whole or cuttings, cooked mech-extd													
193	dehy grnd, (5)	5-01-985	4.80	1.08	1.98	3.66	7.54	5.81	2.35	2.67	3.27	0.86	1.90	3.81
194	Fish meal, anchovy													
195	FISH, CARP. *Cyprinus carpio*													
196	–whole, raw, (5)	5-01-986	–	–	–	–	–	–	–	–	–	–	–	–
197	–whole or cuttings, cooked dehy grnd, (5)	5-09-831	4.64	–	1.46	2.67	4.98	5.75	1.71	2.60	2.97	–	1.99	2.87
198	Fish meal, carp													
199	FISH, CATFISH. *Ictalurus* spp													
200	–whole, raw, (5)	5-07-965	–	–	–	–	–	–	–	–	–	–	–	–
201	–whole or cuttings, cooked mech-extd dehy													
202	grnd, (5)	5-09-835	4.45	–	1.21	1.84	3.55	4.03	1.10	1.93	2.32	–	1.35	2.47
203	Fish meal, catfish													
204	–whole or cuttings, cooked mech-extd													
205	press cake, (5)	5-09-834	8.36	–	2.72	4.03	6.30	7.41	2.12	4.29	5.03	–	3.29	5.05
206	–whole or cuttings, cooked pasteurized, (5)	5-09-833	4.96	–	1.35	2.43	3.98	4.21	1.30	2.56	2.88	–	1.68	3.13
207	–whole or cuttings, raw, (5)	5-09-832	3.60	–	1.40	2.65	4.57	4.83	1.14	2.56	2.96	–	1.30	3.36
208	FISH, HERRING. *Clupea harengus harengus,*													
209	*Clupea harengus pallasi*													
210	–whole or cuttings, cooked mech-extd													
211	dehy grnd, (5)	5-02-000	4.34	1.74	1.41	3.48	5.54	7.93	2.17	2.83	2.83	0.98	2.28	3.48
212	Fish meal, herring													
213	FISH, MENHADEN. *Brevoortia tyrannus*													
214	–whole or cuttings, cooked mech-extd													
215	dehy grnd, (5)	5-02-009	4.34	1.02	1.74	4.46	5.43	5.76	1.96	2.93	3.15	0.65	1.74	3.91
216	Fish meal, menhaden													
217	FISH, SALMON. *Oncorhynchus* spp,													
218	*Salmo* spp													
219	–whole or cuttings, cooked mech-extd													
220	dehy grnd, (5)	5-02-012	5.59	0.75	–	–	–	8.17	1.72	–	–	0.54	–	–
221	Fish meal, salmon													
222	FISH, SARDINE. *Clupea* spp, *Sardinops* spp													
223	–whole or cuttings, cooked mech extd													
224	dehy grnd, (5)	5-02-015	2.90	0.86	1.94	3.55	5.06	6.34	2.15	2.80	2.80	0.54	3.23	4.41
225	FISH, TUNA. *Thunnus thynnus*													
226	–whole or cuttings, cooked mech-extd													
227	dehy grnd, (5)	5-02-023	8.03	–	–	–	–	7.11	1.95	–	–	1.03	–	2.53
228	Fish meal, tuna													
229	FISH, WHITE. Gadidae (family), Lophiidae													
230	(family) Rajidae (family)													
231	–whole or cuttings, cooked mech-extd													
232	dehy grnd, mx 4% oil, (5)	5-02-025	3.80	0.98	1.63	3.37	4.89	5.33	1.85	2.72	2.72	0.76	2.17	3.48
233	White fish meal (CFA)													
234	Fish, cod, meal													
235	Fish, cusk, meal													
236	Fish, haddock, meal													
237	Fish, hake, meal													
238	Fish, pollock, meal													
239	Fish, monkfish, meal													
240	Fish, skate, meal													
241	FLAX. *Linum usitatissimum*													
242	–seed, mech-extd grnd, mx 0.5% acid													
243	insoluble ash, (5)	5-02-045	2.52	–	0.63	1.71	1.80	1.17	0.77	1.35	1.08	–	–	1.53

(1) Dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages; (4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

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Line Number	SCIENTIFIC NAME National Research Council Name (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Basis Argi-nine (%)	Cys-tine (%)	Histi-dine (%)	Iso-leucine (%)	Leu-cine (%)	Ly-sine (%)	Methi-onine (%)	Phenyl-alanine (%)	Thre-onine (%)	Tryp-tophan (%)	Tyro-sine (%)	Valine (%)
244	Linseed meal, mech extd (AAFCO)													
245	Linseed meal (CFA)													
246	Linseed oil, exp extd													
247	FLAX. *Linum usitatissimum*													
248	Linseed oil meal, hydraulic extd													
249	Linseed meal, old process													
250	—seed, solv-extd grnd, mx 0.5% acid													
251	insoluble ash, (5)	5-02-048	–	–	–	–	–	–	–	–	–	–	–	–
252	Linseed meal, solv extd (AAFCO)													
253	Solv extd linseed meal (CFA)													
254	Linseed oil meal, solv extd													
255	GRAINS. Scientific name not used													
256	—brewers grains, dehy, mx 3% dried													
257	spent hops, (5)	5-02-141	1.41	–	0.54	1.63	2.50	0.98	0.43	1.41	0.98	0.43	1.30	1.74
258	Brewers dried grains (AAFCO)													
259	Brewers dried grains (CFA)													
260	—distillars grains, dehy, (5)	5-02-144	1.19	0.22	0.65	1.08	3.24	0.65	0.54	1.30	0.97	0.22	0.86	1.41
261	LIMESTONE. Scientific name not applicable													
262	—grnd, mn 33% calcium, (6)	6-02-632	–	–	–	–	–	–	–	–	–	–	–	–
263	Limestone, ground (AAFCO)													
264	LINSEED – see FLAX													
265	LIVER – see ANIMAL													
266	MAIZE – see CORN													
267	MALT – see BARLEY													
268	MEAT – see ANIMAL													
269	MILK – see CATTLE													
270	MILLET. *Setaria* spp													
271	—grain, (4)	4-03-098	–	–	–	–	–	–	–	–	–	–	–	–
272	MOLASSES – see BEET, SUGAR													
273	see SUGARCANE													
274	OATS. *Avena sativa*													
275	—hulls, (1)	1-03-281	0.22	0.06	0.11	0.22	0.32	0.22	0.11	0.22	0.22	0.11	0.22	0.22
276	Oat hulls (AAFCO)													
277	Oat hulls (CFA)													
278	—cereal byproduct, mx 4% fiber, (4)	4-03-303	0.77	0.26	0.33	0.60	1.10	0.11	0.22	0.71	0.53	0.22	1.00	0.82
279	Feeding oat meal (AAFCO)													
280	Oat middlings (CFA)													
281	—grain, (4)	4-03-309	0.80	0.20	0.20	0.60	1.00	0.40	0.20	0.70	0.40	0.20	0.60	0.70
282	—grain, gr 1 US mn wt 34 lb per bushel													
283	mx 2% foreign material, (4)	4-03-313	–	–	–	–	–	–	–	–	–	–	–	–
284	—grain, gr 2 heavy US mn wt 36 lb per													
285	bushel mx 3% foreign material, (4)	4-03-315	0.89	0.25	0.22	0.59	1.01	0.55	0.20	0.67	0.45	0.18	0.59	0.78
286	Oats, grain, heavy													
287	—grain, gr 2 US mn wt 32 lb per bushel													
288	mx 3% foreign material, (4)	4-03-316	–	–	–	–	–	–	–	–	–	–	–	–
289	—grain, gr 3 US mn wt 30 lb per bushel													
290	mx 4% foreign material, (4)	4-03-317	–	–	–	–	–	–	–	–	–	–	–	–
291	—grain, gr 4 US mn wt 27 lb per bushel													
292	mx 5% foregin material, (4)	4-03-318	–	–	–	–	–	–	–	–	–	–	–	–
293	Oats, grain, light													
294	—groats, (4)	4-03-331	–	–	–	–	–	–	–	–	–	–	–	–
295	Oat groats (AAFCO)													
296	Oat groats (CFA)													
297	Hulled oats (CFA)													
298	OATS, WHITE. *Avena sativa*													
299	—grain, Can 2 CW mn wt 36 lb per bushel													
300	mx 3% foreign material, (4)	4-03-378	0.58	–	0.22	0.37	0.74	0.42	0.03	0.52	0.16	–	0.17	0.59
301	—grain, Can 2 feed mn wt 28 lb per bushel													
302	mx 22% foreign material, (4)	4-03-379	0.54	–	0.17	0.25	0.68	0.31	0.12	0.46	0.32	–	0.27	0.36
303	—grain, Can 3 CW mn wt 34 lb per bushel													
304	mx 6% foreign material, (4)	4-03-380	0.59	–	0.18	0.28	0.69	0.34	0.12	0.49	0.34	–	0.27	0.42
305	OYSTERS. *Crassostrea* spp, *Ostrea* spp													
306	—shells, fine grnd, mn 33% calcium, (6)	6-03-481	–	–	–	–	–	–	–	–	–	–	–	–
307	Oyster shell flour (AAFCO)													
308	PEA. *Pisum* spp													
309	—seed, grnd, (5)	5-03-598	1.54	0.19	0.79	1.21	1.96	1.76	0.34	1.43	1.03	0.26	–	1.43
310	PEANUT. *Arachis hypogaea*													
311	—kernels, mech-extd grnd, mx 7% fiber, (5)	5-03-649	5.10	–	1.09	2.17	3.37	1.41	0.65	2.50	1.52	0.54	–	2.39
312	Peanut meal, mech extd (AAFCO)													
313	Peanut meal (CFA)													
314	Peanut oil meal, exp extd													
315	—kernels, solv-extd grnd, mx 7% fiber, (5)	5-03-650	6.41	0.65	1.30	2.17	4.02	2.50	0.43	2.93	1.63	0.54	1.96	3.04
316	Peanut meal, solv extd (AAFCO)													
317	Groundnut oil meal, solv extd													
318	Peanut oil meal, solv extd													
319	PHOSPHATE ROCK													
320	—defluorinated grnd, mx 1 part fluorine per													
321	100 part phosphorus, (6)	6-01-780	–	–	–	–	–	–	–	–	–	–	–	–
322	Phosphate, defluorinated (AAFCO)													
323	Defluorinated phosphate (CFA)													
324	—Rock phosphate, grnd, (6)	6-03-945	–	–	–	–	–	–	–	–	–	–	–	–
325	Rock phosphate, grnd (AAFCO)													

(1) Dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages; (4) energy feeds; (5) protein supplements, (6) minerals; (7) vitamins; (8) additives.

TABLE A-2 Amino Acid Composition of Some Common Laboratory Animal Feeds

Line Number	SCIENTIFIC NAME National Research Council Name (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Basis Arginine (%)	Cystine (%)	Histidine (%)	Isoleucine (%)	Leucine (%)	Lysine (%)	Methionine (%)	Phenylalanine (%)	Threonine (%)	Tryptophan (%)	Tyrosine (%)	Valine (%)
326	POTATO. *Solenum tuberosum*													
327	–tubers, dehy grnd, (4)	4-07-850	–	–	–	–	–	–	–	–	–	–	–	–
328	Potato meal													
329	POTATO. *Solenum tuberosum*													
330	–tubers, flaked dehy, (4)	4-03-785	–	–	–	–	–	–	–	–	–	–	–	–
331	–tubers, fresh, (4)	4-03-787	–	–	–	–	–	–	–	–	–	–	–	–
332	POULTRY. Scientific name not used													
333	–feathers, hydrolyzed dehy grnd, mn 75%													
334	of protein digestible, (5)	5-03-795	6.28	3.79	0.57	4.45	7.56	2.13	0.64	4.45	4.40	0.53	2.49	5.90
335	Hydrolyzed poultry feathers (AAFCO)													
336	RICE. *Oryza sativa*													
337	–bran w germ, dry milled, mx 13% fiber													
338	$CaCO_3$ declared above 3% mn, (4)	4-03-928	0.55	0.11	0.22	0.44	0.66	0.55	0.32	0.44	0.44	0.11	0.75	0.66
339	Rice bran (AAFCO)													
340	–grain w hulls, (4)	4-03-939	0.63	0.11	0.10	0.35	0.60	0.31	0.20	0.35	0.25	0.12	0.70	0.50
341	Paddy rice													
342	Rough rice													
343	–grain w hulls, grnd, (4)	4-03-938	0.60	0.11	0.10	0.30	0.60	0.30	0.19	0.30	0.20	0.11	0.67	0.57
344	Ground rough rice (AAFCO)													
345	Ground paddy rice (AAFCO)													
346	–groats, (4)	4-03-936	–	–	–	–	–	–	–	–	–	–	–	–
347	Brown rice grain													
348	Rice grain wo hulls													
349	–groats, grnd, (4)	4-03-935	–	–	–	–	–	–	–	–	–	–	–	–
350	Ground brown rice (AAFCO)													
351	Rice grain wo hulls, grnd													
352	–groats, polished, (4)	4-03-942	0.40	0.10	0.20	0.51	0.80	0.30	0.30	0.60	0.40	0.10	0.70	0.60
353	Rice, white, polished													
354	–polishings, dehy, (4)	4-03-943	0.55	0.11	0.11	0.33	0.56	0.56	0.30	0.33	0.33	0.11	0.70	0.93
355	Rice polishings (AAFCO)													
356	Rice polish (CFA)													
357	ROCK PHOSPHATE – see PHOSPHATE													
358	ROCK													
359	Rye. *Secale cereale*													
360	–grain, (4)	4-04-047	0.60	0.20	0.30	0.80	0.80	0.51	0.20	0.70	0.40	0.10	0.30	0.70
361	SAFFLOWER. *Carthamus tinctorius*													
362	–seed, solv-extd grnd, (5)	5-04-110	–	–	–	–	–	–	–	–	–	–	–	–
363	Solv extd whole pressed safflower seed													
364	(AAFCO)													
365	SESAME. *Sesamum indicum*													
366	–seed, mech-extd grnd, (5)	5-04-220	5.16	0.65	1.18	2.26	3.66	1.40	1.51	2.37	1.72	0.84	2.15	2.58
367	Sesame oil meal, exp extd													
368	SEAWEED. Laminariales (order), Fucales (order)													
369	–entire plant, s-c grnd, (1)	1-04-190	0.32	–	0.10	0.27	0.48	0.36	0.07	0.27	0.31	–	0.15	0.39
370	SHRIMP. *Pandalus* spp, *Penaeus* spp													
371	–process residue, dehy grnd, salt declared													
372	above 3% mx 7%, (5)	5-04-226	–	–	–	–	–	–	–	–	–	–	–	–
373	Shrimp meal (AAFCO)													
374	SODIUM TRIPOLYPHOSPHATE													
375	–commercial, (6)	6-08-076	–	–	–	–	–	–	–	–	–	–	–	–
376	Sodium tripolyphosphate (AAFCO)													
377	SORGHUM, GRAIN VARIETY. *Sorghum vulgare*													
378	–grain, (4)	4-04-383	0.40	0.20	0.30	0.60	1.60	0.30	–	0.51	0.30	0.10	0.40	0.60
379	SORGHUM, MILO. *Sorghum vulgare*													
380	–grain, (4)	4-04-444	0.40	0.20	0.30	0.60	1.60	0.30	0.10	0.51	0.30	0.10	0.40	0.60
381	SOYBEAN. *Glycine max*													
382	–seeds, (5)	5-04-610	–	–	–	–	–	–	–	–	–	–	–	–
383	–seed, mech-extd grnd, mx 7% fiber, (5)	5-04-600	2.89	0.67	1.22	3.11	4.00	3.00	0.89	2.33	1.89	0.67	1.56	2.44
384	Soybean meal, mech extd (AAFCO)													
385	Soybean meal, expeller extd													
386	Soybean meal, hydraulic extd													
387	Soybean oil meal, expeller extd													
388	Soybean oil meal, hydraulic extd													
389	–seed, solv-extd grnd, mx 7% fiber, (5)	5-04-604	3.60	0.75	1.24	2.80	3.82	3.26	0.67	2.47	1.91	0.67	1.57	2.70
390	Soybean meal, solv-extd (AAFCO)													
391	Soybean meal, solv extd													
392	Soybean oil meal, solv extd													
393	–seed wo hulls, solv-extd grnd, mx 3% fiber, (5)	5-04-612	4.23	0.89	1.34	2.90	4.23	3.56	0.81	3.01	2.23	0.72	2.23	3.01
394	Soybean meal, dehulled, solv extd (AAFCO)													
395	Soybean oil meal, dehulled, solv extd													
396	SUGARCANE. *Saccharum officinarum*													
397	–molasses, dehy, (4)	4-04-695	–	–	–	–	–	–	–	–	–	–	–	–
398	Cane molasses, dried													
399	Molasses, cane, dried													
400	–molasses, mn 48% invert sugar mn 79.5													
401	degrees brix, (4)	4-04-696	–	–	–	–	–	–	–	–	–	–	–	–
402	Cane molasses (AAFCO)													
403	Molasses, cane													
404	SUNFLOWER. *Helianthus* spp													
405	–seed wo hulls, mech-extd grnd, (5)	5-04-738	4.52	0.86	1.18	2.58	3.23	2.15	1.72	2.58	1.72	0.65	–	2.58
406	Sunflower meal (AAFCO)													
407	Sunflower oil meal, wo hulls, expeller extd													

(1) Dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages; (4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-2 Amino Acid Composition of Some Common Laboratory Animal Feeds

Line Number	SCIENTIFIC NAME National Research Council Name (NRC) American Feed Control Name (AAFCO) Canada Feed Act Name (CFA) Other Names	Reference Number	Dry Basis Arginine (%)	Cystine (%)	Histidine (%)	Isoleucine (%)	Leucine (%)	Lysine (%)	Methionine (%)	Phenylalanine (%)	Threonine (%)	Tryptophan (%)	Tyrosine (%)	Valine (%)
408	—seed wo hulls, solv-extd grnd, (5)	5-04-739	3.76	0.75	1.08	2.28	2.80	1.83	1.61	2.37	1.61	0.54	–	2.47
408	Sunflower meal (AAFCO)													
410	Sunflower oil meal, wo hulls, solv extd													
411	TANKAGE – see ANIMAL													
412	TIMOTHY. *Phleum pratense*													
413	—hay, s-c, early blm, (1)	1-04-882	–	–	–	–	–	–	–	–	–	–	–	–
414	TOMATO. *Lycopersicon esculentum*													
415	—pulp, dehy, (5)	5-05-041	–	–	–	–	–	–	–	–	–	–	–	–
416	Dried tomato pomace (AAFCO)													
417	WHALE. *Balaena glacialis, Balaenoptera* spp,													
418	*Physeter catadon*													
419	—meat, heat-rendered, dehy grnd, salt													
420	declared above 3% mx 7%, (5)	5-05-160	–	–	–	–	–	6.20	2.50	–	–	–	–	–
421	Whale meal (AAFCO)													
422	WHEAT. *Triticum* spp													
423	—bran, dry milled, (4)	4-05-190	1.12	0.34	0.34	0.67	1.01	0.67	0.11	0.58	0.45	0.34	0.45	0.79
424	Wheat bran (AAFCO)													
425	Bran (CFA)													
426	—flour, coarse bolted, feed gr mx 2% fiber, (4)	4-05-199	0.44	–	0.33	0.67	1.00	0.33	0.12	0.67	0.33	0.12	0.22	0.56
427	Wheat feed flour, mx 1.5% fiber (AAFCO)													
428	Feed flour, mx 2.0% fiber (CFA)													
429	—flour byproduct, coarse sifted, mx 7%													
430	fiber, (4)	4-05-201	1.07	0.22	0.36	0.79	1.35	0.79	0.20	0.79	0.56	0.22	0.45	0.87
431	Wheat shorts, mx 7% fiber (AAFCO)													
432	Shorts, mx 8% fiber (CFA)													
433	—flour byproduct, fine sifted, mx 4%													
434	fiber, (4)	4-05-203	1.11	0.22	0.44	0.78	1.33	0.67	0.11	0.56	0.56	0.22	0.56	0.89
435	Wheat red dog, mx 4.0% fiber (AAFCO)													
436	Middlings, mx 4.5% fiber (CFA)													
437	—flour byproduct, mill run, mx 9.5% fiber, (4)	4-05-206	–	–	–	–	–	–	–	–	–	–	–	–
438	Wheat mill run (AAFCO)													
438	—grain, (4)	4-05-211	0.80	0.20	0.30	0.60	1.00	0.51	0.20	0.70	0.40	0.20	0.51	0.60
440	—grain screenings, (4)	4-05-216	–	–	–	–	–	–	–	–	–	–	–	–
441	—grits, chracked fine screened, (4)	4-07-852	0.68	0.34	0.34	1.25	1.93	0.45	0.23	0.68	0.45	0.34	–	0.68
442	Farina													
443	Wheat endosperm													
444	—germ, grnd, mn 25% protein 7% fat, (5)	5-05-218	1.78	0.56	0.56	1.33	1.22	1.78	0.33	0.89	0.89	0.33	–	1.22
445	Wheat germ meal (AAFCO)													
445	—germ oil, (7)	7-05-207	–	–	–	–	–	–	–	–	–	–	–	–
447	Wheat germ oil (AAFCO)													
448	WHEAT, DURUM. *Triticum durum*													
449	—grain, (4)	4-05-224	–	–	–	–	–	–	–	–	–	–	–	–
450	—grain, Can 4 CW mn wt 56 lb per bushel													
451	mx 2.5% foreign material, (4)	4-05-225	–	–	–	–	–	–	–	–	–	–	–	–
452	WHEAT, HARD RED SPRING. *Triticum*													
453	*aestivum*													
454	—grain, (4)	4-05-258	0.83	0.20	0.20	0.80	1.10	0.40	0.20	0.90	0.40	0.20	0.90	0.80
455	WHEAT, HARD RED WINTER. *Triticum*													
456	*aestivum*													
457	—grain, (4)	4-05-268	0.79	0.28	0.34	0.79	1.01	0.51	0.22	0.79	0.47	0.20	0.67	0.67
458	WHEAT, RED SPRING. *Triticum aestivum*													
459	—grain, Can 4 No mn wt 58 lb per bushel													
460	mx 2.5% foreign material, (4)	4-05-282	–	–	–	–	–	–	–	–	–	–	–	–
461	WHEAT, SOFT. *Triticum aestivum*													
462	—grain, (4)	4-05-284	0.44	0.22	0.22	0.44	0.67	0.33	0.14	0.44	0.31	0.13	0.44	0.44
463	WHEAT, SOFT RED WINTER.													
464	*Triticum aestivum*													
465	—grain, (4)	4-05-284	0.40	0.20	0.10	–	–	0.90	–	–	–	0.30	0.40	–
466	WHEY – see CATTLE													
467	YEAST. *Saccharomyces cerevisiae*													
468	—brewers saccharomyces, dehy grnd, mn													
469	40% protein, (7)	7-05-527	2.37	0.54	1.18	2.26	3.44	3.23	0.75	1.95	2.26	0.54	1.61	2.47
470	Brewers dried yeast (AAFCO)													
471	—petroleum saccharomyces, dehy grnd, (7)	7-09-836	2.22	0.50	0.97	2.70	3.92	3.90	0.89	2.41	3.26	0.45	1.93	2.89
472	—primary saccharomyces, dehy, mn 40%													
473	protein, (7)	7-05-533	2.80	0.54	6.02	3.87	4.00	4.09	1.08	2.69	2.89	0.43	–	3.44
474	Dried yeast (AAFCO)													
475	Primary dried yeast (AAFCO)													
476	YEAST, TORULOPSIS. *Torulopsis utilis*													
477	—dehy, mn 40% protein, (7)	7-05-534	2.79	0.65	1.51	3.12	3.76	4.09	0.86	3.23	2.80	0.54	2.26	3.12
478	Torula dried yeast (AAFCO)													

(1) Dry forages and roughages; (2) pasture, range plants, and forages fed green; (3) silages; (4) energy feeds; (5) protein supplements; (6) minerals; (7) vitamins; (8) additives.

TABLE A-3 Abbreviations for Terms Used in Tables A-1 and A-2

AAFCO	Association of American Feed Control Officials
Can	Canadian
CE	Canadian Eastern
CGA	Canada Grain Act
CFA	Canada Feeds Act
cp	chemically pure
CW	Canadian Western
dehy	dehydrated
extd	extracted
extn	extraction
extn unspec	extraction unspecified
g	gram(s)
grnd	ground
ICU	International Chick Unit
IU	International Units
kcal	kilocalories
kg	kilogram(s)
lb	pound(s)
mech	mechanical
mech-extd	mechanically extracted, expeller-extracted, hydraulic-extracted, or old process
μg	microgram
mg	milligram
mm	millimeter
mn	minimum
NRC	National Research Council
ppm	parts per million
s-c	suncured
solv-extd	solvent-extracted
spp	species
US	United States
USP	United States Pharmacopeia
w	with
wo	without
wt	weight

TABLE A-4 Stage-of-Maturity Terms Used in Tables A-1 and A-2

Preferred Maturity Term	Definition	Comparable Term
Germinated	Resumption of growth by the embryo in a seed after a period of dormancy	Sprouted
Early leaf	Stage at which the plant reaches one third of its growth before blooming	Fresh new growth, very immature
Immature	Period between one and two thirds of its growth before blooming (this may include fall aftermath)	Prebud stage, young before boot, before heading out
Prebloom	Stage including the last third of growth before blooming	Bud, bud stage, budding plants, in bud, preflowering, before bloom, heading to in bloom, boot, heads just showing
Early bloom	Period between initiation of bloom up to stage at which one tenth of the plants are in bloom	Up to one tenth bloom, initial bloom, heading out, in head
Mid-bloom	Period during which one tenth to two thirds of the plants are in bloom	Bloom, flowering plants, flowering, half bloom, in bloom
Full bloom	When two thirds or more of the plants are in bloom	Three fourths to full bloom
Late bloom	When blossoms begin to dry and fall and seeds begin to form	Seed developing, 15 days after silking, before milk, early pod
Milk stage	Seeds well formed, but soft and immature	Post bloom to early seed, pod stage, early seed, in tassel, fruiting
Dough stage	Stage at which the seeds are soft and immature	Seeds dough, seed well developed, nearly mature
Mature	Stage at which the plant would normally be harvested for seed	Fruiting plants, fruiting, in seed, well matured, dough to glazing, kernels ripe
Overripe	Stage after the plant is mature, seeds are ripe and initial weathering has taken place (applies mostly to range plants)	Late seed, ripe, very mature, well matured
Dormant	Plants cured on the stem, seeds have been cast, and weathering has taken place (applies mostly to range plants)	Seeds cast, mature and weathered

TABLE A-5 Weight–Unit Conversion Factors

Units Given	Units Wanted	For Conversion Multiply by
lb	g	453.6
lb	kg	0.4536
oz	g	28.35
kg	lb	2.2046
kg	mg	1,000,000.
kg	g	1,000.
g	mg	1,000.
g	μg	1,000,000.
mg	μg	1,000.
mg/g	mg/lb	453.6
mg/kg	mg/lb	0.4536
μg/kg	μg/lb	0.4536
Mcal	kcal	1,000.
kcal/kg	kcal/lb	0.4539
kcal/lb	kcal/kg	2.2046
ppm	μg/g	1.
ppm	mg/kg	1.
ppm	mg/lb	0.4536
mg/kg	%	0.0001
ppm	%	0.0001
mg/g	%	0.1
g/kg	%	0.1

TABLE A-6 Weight Equivalents

1 lb = 453.6 g = 0.4536 kg = 16 oz
1 oz = 28.35 g
1 kg = 1,000 g = 2.2046 lb
1 g = 1,000 mg
1 mg = 1,000 μg = 0.001 g
1 μg = 0.001 mg = 0.000001 g
1 μg/g or 1 mg/kg = ppm

APPENDIX B: FORMULATING DIETS

In the previous publication, the nutrient requirements for laboratory animals—when expressed as a concentration in the diet—were given on a 90 percent dry-matter basis. When so calculated, they were often in error, because the ingredients often did not contain exactly 90 percent of dry matter. The error was serious when feeds high in moisture (e.g., milk, meat, or molasses) were used. This revision gives the requirements for each species on a 100 percent dry-matter (moisture-free) basis. Data on the composition of feeds (Tables A-1 and A-2) are expressed on a dry basis.

With these changes, diets or feed mixtures can readily be calculated on a dry basis. When this is done, an animal's nutrient requirements can be compared directly with the percentage of nutrients in the dry diet without additional calculation, and the diet can then be converted to an "as fed" basis as shown:

1. $\frac{\text{percent of ingredients in the dry diet}}{\text{percent dry matter of ingredient}} \times 100$ = parts of ingredients in the diet as fed
2. parts of ingredient in the diet as fed are totaled
3. $\frac{\text{parts of ingredient}}{\text{total parts}} \times 100$ = percent of the ingredient in the diet as fed

The method for calculating a diet on a dry basis and correcting to an as fed basis is given in Table B-1. The diet was formulated to contain 3,785 kcal/kg of metabolizable energy and 15 percent of protein on a dry basis.

The calculations to convert the diet from a dry basis to an as fed basis are shown in footnotes b and c of Table B-1. It will be noted that the final diet contains 39.4 percent corn grain, 57.9 percent skimmed milk, and 2.7 percent soybean meal.

TABLE B-1 Theoretical Example of Calculating a Diet on the Dry Basis and Correcting to an "as Fed" Basis

	Composition of Feeds			Amount in Ration ("Dry" Basis)			Amount in Ration ("as Fed" Basis)	
Ingredient	% Dry Matter "as Fed" Basis	ME[a] "Dry Basis" (kcal/kg)	% Protein "Dry" Basis	% in Ration	ME[a] (kcal/kg)	% Protein	Parts[b]	% in Ration[c]
Corn, grain	86.0	3,808	10.2	81.0	3,084	2.9	94.2	39.4
Milk, skimmed	9.6	3,910	28.5	13.3	520	8.3	138.5	57.9
Soybean meal, solvent extracted	89.0	3,174	51.5	5.7	181	3.8	6.4	2.7
Total				100.0	3,785	15.0	239.1	100.0

[a] Metabolizable energy.
[b] Calculated as follows:
$\frac{81.0}{86.0} \times 100 = 94.2$
$\frac{13.3}{9.6} \times 100 = 138.5$
$\frac{5.7}{89.0} \times 100 = 6.4.$

[c] Calculated as follows:
$\frac{94.2}{239.0} \times 100 = 39.4\%$ corn "as fed"
$\frac{138.5}{239.1} \times 100 = 57.9\%$ skimmed milk "as fed"
$\frac{6.4}{239.1} \times 100 = 2.7\%$ solvent extracted soybean meal "as fed."