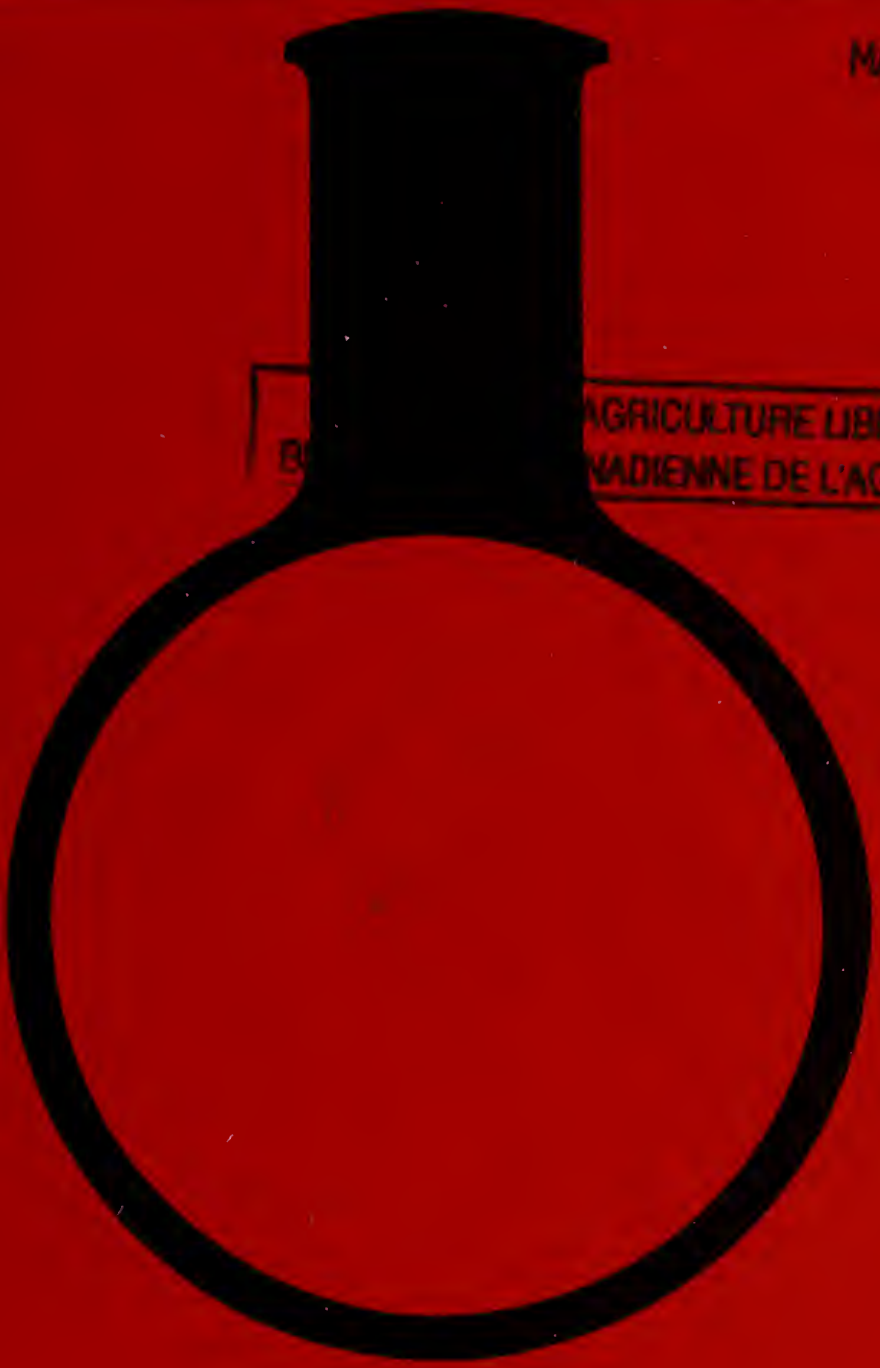
CANADA DEPARTMENT OF AGRICULTURE PUBLICATION 1238 1966



BIOLOGICAL VALUES OF PROVITAMINS A

for MAN, DOMESTIC MAMMALS, and BIRDS

A REVIEW

PUBLICATION 1238 1966

BIOLOGICAL VALUES OF PROVITAMINS A FOR MAN, DOMESTIC MAMMALS, AND BIRDS

A REVIEW

A REPORT
OF THE NATIONAL COMMITTEE ON ANIMAL NUTRITION
OF THE NATIONAL COORDINATING COMMITTEE ON
AGRICULTURAL SERVICES

CANADA DEPARTMENT OF AGRICULTURE

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ROGER DUHAMEL, F.R.S.C.
Queen's Printer and Controller of Stationery
Ottawa, Canada
1966

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FOREWORD

The National Coordinating Committee on Agricultural Services is a federal-provincial body that meets each year in Ottawa under chairmanship of the Deputy Minister, Canada Department of Agriculture, to coordinate agricultural services. It appoints committees to undertake specific projects.

The National Committee on Animal Nutrition is one of these committees. One task of the Committee is to collect, evaluate, tabulate and make available authoritative and pertinent data on nutrients required by domestic mammals and poultry, based on Canadian conditions, production and marketing requirements.

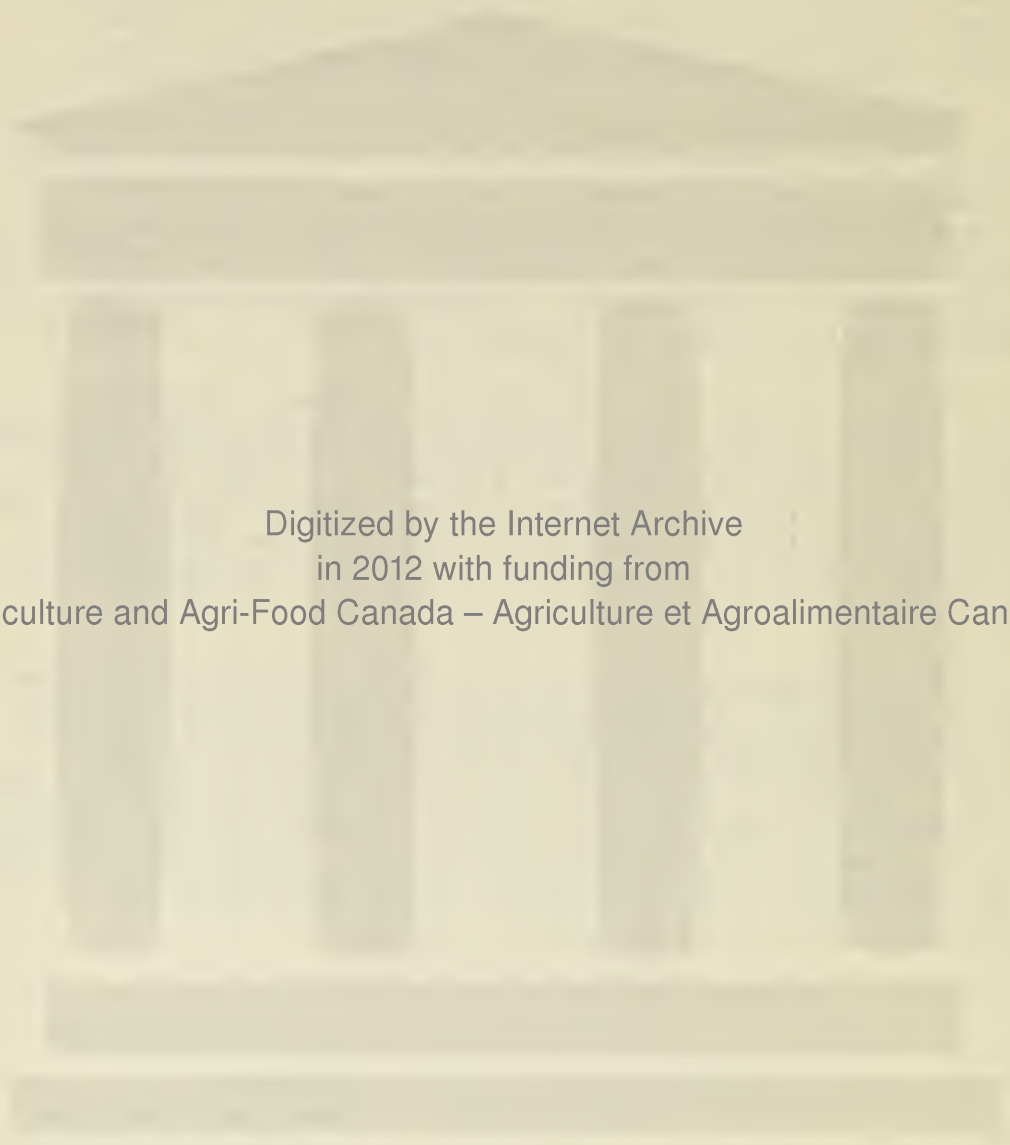
The nutritional status of vitamin A in avian and mammalian livestock has concerned nutritionists in recent years. Earlier assessments of the requirements, and of sources of provitamins A that were believed to meet these requirements, have had to be revised. A subcommittee was appointed in 1960 to review information on vitamin A requirements of domestic species and man, and biological effectiveness of carotene and other provitamins A.

This review is the subcommittee's report. Studies on cattle, sheep, horses, pigs, chickens and turkeys are discussed in detail. A review of pertinent studies with human beings is included, as well as brief summaries on research with foxes and mink, dogs and laboratory animals, including the cat, guinea pig, monkey, hamster, mouse and rat. Specific recommendations are made.

The subcommittee members are: W. E. J. Phillips, Animal Research Institute, Canada Department of Agriculture, Ottawa; E. V. Evans, Department of Nutrition, University of Guelph, Guelph; T. K. Murray and J. A. Campbell, Food and Drug Directorate, Canada Department of National Health and Welfare, Ottawa; and H. D. Branion, Department of Nutrition, University of Guelph, Guelph, Chairman. A. R. G. Emslie, Canada Department of Agriculture, Ottawa, is a member ex officio.

A. R. G. Emslie[1/]
Chairman
National Committee
on Animal Nutrition

1/ Deceased.



GENERAL CONCLUSIONS AND RECOMMENDATIONS

H. D. Branion and A. R. G. Emslie

The Committee was impressed by the needless--and heedless--confusion caused by authors reporting biological value of provitamins A in international units without giving the relationship used in converting mass units of the provitamins into "international" units.

Guilbert and Loosli pointed out that much of the confusion would be overcome if evaluation in units was discarded and requirements were expressed in weights of the pure substances. The expert committee of IUPAC recommended that "the potency of β-carotene and its preparations should be expressed not in international units of vitamin A but in international units of provitamin A." Ames urged that "all β-carotene sources, concentrates and biological requirements" be expressed "solely in terms of milligrams of all-trans-β-carotene" and suggested that a partial solution might be "to express all β-carotene levels solely on a weight basis."

Our committee recommends that, in studies on animal nutrition, quantitative measures of preformed vitamin A and of provitamins A be expressed in mass units of vitamin A acetate or alcohol and of β-carotene, respectively, and that international units be discarded.2/

Most of the committee considered that no useful purpose is served by international units and that too often international units of preformed vitamin A and of provitamin A are considered additive. In public health nutrition it may be premature to dispense with international units because of administrative problems in labeling and advertising of limits of vitamin content and of claims of vitamin preparations and vitamin content of foods; the distinction between preformed vitamin A and the provitamins should be emphasized, however.

When rations are being formulated it is necessary to equate a known mass of provitamin A with the appropriate mass of preformed vitamin. Some conditions affecting the usefulness of provitamins have been mentioned in these reviews. To be really meaningful, a "conversion factor" should be the ratio of the weight of a standard preparation of vitamin A acetate or alcohol

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2/ The committee members from the Food and Drug Directorate, Canada Department of National Health and Welfare, did not agree that this recommendation should apply to public health nutrition.

to that of a standard preparation of β-carotene that will have an equal biological effect when consumed under clearly defined conditions by a specific class of animal of stated physiological development and condition.

The literature is insufficient as yet to permit calculation of such "conversion factors" for domestic animals but shows that the "conversion factor" for poultry is different from that for mammals.

Our committee concluded that, pending acquisition of more data, and for feeding and dietary standards and nutrient allowances only, carotene from a variety of sources in typical rations should have a biological effect similar to that of the following fractions of its mass as preformed vitamin A alcohol: for cattle, sheep, horses and pigs: 1/7; for chickens and turkeys: 1/3.

This means that a biological effect similar to that of 0.3 μg of vitamin A alcohol, or of 0.344 μg of vitamin A acetate, should be obtained when cattle, sheep, horses and pigs consume 2.1 μg of β-carotene, and when chickens and turkeys consume 0.9 μg of β-carotene. Stated another way, 1 mg of carotene from various dietary sources should have a biological effect similar to that of 0.14 mg of vitamin A alcohol (476 IU of preformed vitamin A) in the rations of cattle, sheep, horses and pigs, and of 0.333 mg (1,112 IU) in the rations of chickens and turkeys.

The appropriate relationship in human dietetics appears to be similar to that for domestic livestock, i.e., 1/7, and not the 1/2 observed for the rat under very restrictive conditions.

Ratios of biological effectiveness of β-carotene to that of vitamin A for various species as recommended by different authorities are summarized in Table 1.

The following reviews reveal many serious gaps in our knowledge of vitamin A nutrition in mammals and birds, and will, it is hoped, stimulate more well-planned research.

Research is urgently required on the following:

(a) Requirements for vitamin A, and the relative efficiencies of the various isomers and provitamins in meeting these requirements, in the light of changing agricultural practices;

(b) development of convenient and reliable methods for routine determination of various isomers;

(c) assessment of the present status of vitamin A nutrition in ruminants in Canada; and

(d) basic studies on the metabolic role of the vitamin.

Table 1. Ratios of Biological Effectiveness of β-carotene and Vitamin A for Various Animals

Animal	Authority[1/]	mg β-carotene[2/] equivalent to 1 mg vitamin A alcohol	IU preformed vitamin A per mg β-carotene
Rat	International	2	1667
Mink	NAS-NRC	12	277
Fox	NAS-NRC	12	277
Dog	NAS-NRC	4 or 8	418-834
Poultry	NAS-NRC	2	1667
	NCAN	3	1112
	ARC	6	556
Horse	NAS-NRC	6-10	333-556
	NCAN	7	476
Dairy cattle	NAS-NRC	8-10	333-418
	ANRC	8	418
	NCAN	7	476
Beef cattle	NAS-NRC	8.3	400
	ANRC	8	418
	NCAN	7	476
Sheep	NAS-NRC	5.8-8.3	400-578
	ANRC	8	418
	NCAN	7	476
Swine	NAS-NRC	6.2	533
	NCAN	7	476
Man	NRC	6	556
	NAS-NRC	4	834
	BMA	6	556
	CCN	8	417
	NCAN	7	476

1/ ANRC, Animal Nutrition Research Council, N. America; ARC, Agricultural Research Council of Great Britain; BMA, British Medical Association; CCN, Canadian Council on Nutrition; MRC, Medical Research Council, Great Britain; NAS-NRC, National Academy of Sciences, National Research Council, United States; NCAN, National Committee on Animal Nutrition, Canada.

2/ On the basis of all-*trans* β-carotene.

INTRODUCTION

H. D. Branion
Department of Nutrition
University of Guelph, Guelph

This is a critical review of present information on relative potencies of isomers of vitamin A and provitamins A for various species; preferred methods of expressing relative potencies of the isomers; and gaps in the knowledge as they affect the science and practice of animal nutrition.

Vitamin A is an essential nutrient for all mammalian and avian species that have been studied. Animals that are growing rapidly, that are pregnant, or that are at a high level of production require ample supplies of the vitamin. It may be supplied in the regular diet or from other sources such as intramuscular or intraruminal injection. An adult may have body reserves sufficient to prevent detectable symptoms of physical or functional deterioration for many months, even in complete absence of dietary or other extrinsic source of the vitamin.

Vitamin A requirements of mammals and birds are met by provitamins A, the preformed vitamin or various combinations of these. Since these compounds have different potencies and their utilization is affected by a variety of conditions, a unique problem has arisen in expressing potencies and requirements. Wornick (41) stated that "in contrast to almost every other known vitamin, vitamin A activity occurs in a bewildering multiplicity of forms. Not only are there a number of active derivatives of the vitamin A molecule itself, but also a number of naturally occurring active carotenoids. As a further complication, each of these vitamin A derivatives and carotenoids can exist in a variety of isomeric forms. These innumerable forms all exhibit different biological activities. In addition, highly specialized techniques are needed for the analytical determination of each." Excellent reviews of the subject have been published (18, 26, 37).

It has been estimated that one half to two thirds of the vitamin A requirement of human adults in North America is met by provitamins A, chiefly β-carotene. Until recently, it has been normal agricultural practice to rely almost entirely on provitamins A to satisfy the requirement of adult cattle and horses, and sheep of all ages. Preformed vitamin A has commonly been supplied to calves, pigs and poultry, most often as a constituent of mixed rations or concentrates, and has been used almost exclusively for supplementation of the human diet.

The international standard of vitamin A (40) is crystalline vitamin A acetate, one international unit (IU) being defined as the biological activity of 0.344 µg of the standard. Hence, due to molecular weight, 0.300 µg of vitamin A alcohol, or 0.550 µg of vitamin A palmitate, is equivalent to 1 IU, on the basis of the all-trans isomer in each case. The biopotency of 1 mg of vitamin A alcohol is 3,333 IU; of vitamin A acetate, 2,907 IU; and of vitamin A palmitate, 1,819 IU.

Studies with young, growing rats have shown that, with small doses, 0.6 µg of pure β-carotene is biologically equivalent to 0.3 µg (1 IU) of vitamin A alcohol. Hence, the international unit for provitamin A is defined as the biological activity of 0.6 µg of the international standard preparation of β-carotene (40). Thus 1 mg of all-trans β-carotene is equal to 1,667 IU.

The WHO subcommittee on fat-soluble vitamins (40) warned: "Since two standards are available, it will now be necessary to express the vitamin A and provitamin A activity of foods or other substances in which one form only is present in terms of the respective units. It should be emphasized that when the provitamin A standard preparation is used in biological assays, the results will be a combination of two effects: (A) the provitamin A content of the material tested; (B) the availability to the animal of that content. On the other hand, if the standard preparation is used for comparison by chemical or physical methods, the provitamin A content alone will be measured. The result will be strictly valid only if no form of provitamin A other than β-carotene is present."

There is no statement in this official report of the equivalence of provitamin and vitamin A units. However, Morton (27) states: "Figures giving the provitamin A content of foodstuffs should be used with reserve and the temptation to equate or treat as additive 'provitamin units' and 'vitamin units' should be firmly resisted."

The International Union of Pure and Applied Chemistry (22) adds: "It is clear that, if the principles laid down by the 1949 Conference are to be followed, the biological activity of β-carotene and its preparations should be expressed not in vitamin A units but in provitamin A units; it does not seem correct to say, for example, that 0.6 µg of pure β-carotene is equivalent in biological potency to 0.3 µg, or 1 IU of vitamin A."

Wornick (41) concluded: "The conversion of 'carotene values' to vitamin A activity using the popular factor of 0.6 is a questionable practice."

Preformed vitamin exists in several isomeric forms, not only differing in effectiveness or biological potency but also responding differently to various assays. Sixteen cis-trans isomers of vitamin A are theoretically possible, but only six have been synthesized and are important. The biological values of these isomers as established by Ames[3/] (1), Ames et al. (3, 4) and Brown et al. (8) have been summarized by Wornick (41). The all-trans isomer of vitamin A has the highest biological activity and is the form used as the international standard, and as U.S.P. and A.N.R.C. reference standards. The biopotency of all-trans vitamin A acetate is 2,907 IU per mg and if its biopotency is taken as 100% the relative values of other isomers are: 13-cis (neo-A), 2,190 IU or 75%; 9-cis, 607 IU or 21%; 9, 13 di-cis, 688 IU or 24%; 11-cis, 690 IU or 24%; and 11, 13 di-cis, 428 IU or 15%. Gray et al. (13) fed rats various forms of vitamin A and recovered from the livers the following percentages of vitamin A esters: U.S.P. reference oil, 55.7; vitamin A caproate, 46.5; distilled ester concentrate, 44.2; vitamin A stearate, 44.3; vitamin A alcohol, 39.3; and β-carotene, 9.7.

It has been reported that the biopotency of 13-cis vitamin A is only 66% for rats and 50% for chicks (9). Fish oils contain about 35% of this isomer (35), and 19 to 26% of the 9-cis and 9, 13 di-cis isomers (8).

At present no procedure satisfactory for routine assaying of isomeric mixtures is available. The Carr-Price method (5) does not differentiate between isomers but gives the same blue color with each. The spectrophotometric procedure (39) is not applicable to isomeric mixtures since ultraviolet absorption maxima of various isomers are close to one another. Nor should the Morton-Stubbs correction be applied to all isomeric mixtures. The irrelevant ultraviolet absorption is not linear. Furthermore, the spectrophotometric assay is limited in sensitivity. Development of new procedures has been summarized (41).

Ames (2) has summarized the differences between the various estimates. The all-trans isomer assays the same with all procedures and for comparative purposes is assigned a value of 100. Corresponding values for Carr-Price, U.S.P. XVI, and biological assays for the 13-cis isomer are 100, 73 and 75; for the 9-cis isomer, 100, 77 and 25; and for natural esters in fish liver oil, 100, 85 and 70.

3/Dr. Stanley R. Ames, Research Laboratories, Distillation Products, Rochester, N. Y., is chairman of a subcommittee of the Animal Nutrition Research Council, which is considering essentially the same problem with which our committee is concerned.

Various provitamins differ in chemical structure in the terminal ring, as well as in isomeric structure. From 20 to 30 different isomers are theoretically possible for each of the major carotenoids, but cis-forms are uncommon in nature (42). Carotenoid isomers also differ in biological activity and present assay difficulties. Biopotencies of isomers of β-carotene, α-carotene, γ-carotene and cryptoxanthin are, respectively, for the all-trans: 100, 53, 42 and 57%; and for the mono-cis: 38, 13, 19 and 27%. These data were determined by rat bioassay and therefore apply only to this species. Values would be lower for other species.

Determination of carotene by the official procedure (5) and conversion of micrograms into units of vitamin A activity by a factor of 0.6 assumes that only all-trans β-carotene is present in yellow-colored material extracted from natural sources.

When preformed vitamin A is fed, it is absorbed through the intestinal wall and enters the blood stream, presumably via the lymph system. Extra vitamin A not needed for body functions is stored in the liver. β-carotene, on the other hand, must be converted into vitamin A, primarily in villi of the small intestine and, probably to some extent, in the liver. Efficiency of conversion of β-carotene and of other provitamins varies with different species.

The rat appears to be one of the more efficient utilizers or converters of carotene, whereas ruminants are less efficient. Guilbert and coworkers (14, 15, 17) considered that the minimum carotene requirement for cattle, sheep, swine, horses, rats and humans was 25 to 30 μg daily per kilogram of body weight (W_{kg}), and that of vitamin A about 4 to 6 μg. The minimum requirement for cattle, sheep, swine and horses was defined as the lowest level per unit of body weight that prevented any detectable degree of nyctalopia. The minimal level[4/] for the rat was that which just sufficed to prevent any abnormal degree of cornification detectable by vaginal smears; and for man, that which gave freedom from clinical symptoms. With all species, the minimum permitted normal growth and general well-being but little or no storage. The evidence indicated that about three times the minimum vitamin A level and five times the minimum carotene level are about the minima for reproduction and significant storage.

4/ The minimum carotene level for the rat was slightly less than that found for other species, which the authors attributed to possibly more efficient absorption from the intestinal tract.

These authors drew two pertinent conclusions: "At the biological unit level ... the ratio of efficiency of vitamin A to carotene by weight is about 3:1; at the level to meet our definition of minimum about 6:1 and at the minimum level that results in significant storage and successful reproduction about 10:1. So far as mammals are concerned it is obvious that an international unit of carotene and an international unit of vitamin A have equality only under the conditions of the biological test. Double standards for requirements must be recognized, one for carotene and one for vitamin A, and both must be considered in evaluating the status of a dietary furnishing both sources."

Guilbert and Loosli (16) concluded: "Vitamin A and carotene requirements for mammals are equal and directly proportional to body weight. The requirement for poultry is also directly related to body weight, but apparently the requirement is somewhat higher than for that of mammals. ... Vitamin A and carotene expressed in International Units on the conventional basis do not have equal biological value for either poultry or mammals. They are equivalent in the rat only at the level of dosage at which the Unit was originally defined. The higher the level of intake, the greater the divergence in efficiency of utilization of carotene compared to preformed vitamin A. Much of the confusion arising from this situation would be obviated if evaluation in units was discarded and requirements were expressed in weights of the pure substances." Their recommended allowance for mammalian species was 3,000 IU of vitamin A per 100 lb of body weight, or 5.0 mg of carotene.

Mattson (25) reviewed requirements of mammals and poultry, and Mason (24) those of human beings. Rubin and De Ritter (36) gave comparative data on requirements for vitamin A of laboratory animals, poultry, farm animals and human beings. They concluded: "Expressed on the basis of a unit of body weight, the minimum requirements for all mammalian species fall in a similar range of approximately 20 to 100 international units of vitamin A per kilogram per day. For poultry the minimum requirement for vitamin A is approximately five times greater." They also concluded: "Species such as cattle, sheep, horses and swine require five to six times as much carotene as vitamin A. Similarly high ratios have been reported for human beings."

Obviously too great reliance should not be placed on rat assays when attempting to evaluate dietary sources of provitamins A for application to other species.

It has been pointed out that conversion of β-carotene to vitamin A varies with level of intake; the higher this level, the lower the utilization and hence the less the biological

activity per unit of provitamin. Utilization of vitamin A does not appear to change with increasing levels of intake if these are not excessive.

Other dietary factors may affect utilization and conversion of carotene and vitamin A, e.g., tocopherol or vitamin E intake--since an animal deficient in vitamin E utilizes both the vitamin and the provitamin poorly. Presence of antioxidants may prevent destruction, both during storage of the ingredient, diet or ration and within the alimentary tract. Absorption may be affected by type of carrier, e.g., oily or aqueous solution, kind of feed or food, or type of fat in diet or ration. Stress also affects metabolism. All these factors generally affect utilization of carotene more than that of vitamin A.

The I.U.P.A.C. (22) pointed out in 1959 that the Ministry of Food for Great Britain, in controlling vitamin A content of margarine, passed regulations by which 0.75 μg of β-carotene may be taken as equivalent to one unit of vitamin A.

Collaborative trials (18) showed that 0.3 μg of vitamin A alcohol had a potency of one unit by rat growth assay. The 1934 standard (20) was pure β-carotene, and the unit of vitamin A was defined as "the vitamin A activity of 0.6 μg of the International Standard Preparation." Thus, it was assumed that one molecule of β-carotene yielded one molecule of vitamin A by unsymmetrical rupture of the bonds between the two β-ionone rings. If β-carotene were converted to vitamin A by symmetrical cleavage, the two compounds should have the same potency weight for weight, i.e., one molecule of β-carotene would give two molecules of vitamin A. Before this report it was considered that a molecule of β-carotene did split to give two molecules of vitamin A, whereas α- and γ-carotene, as well as cryptoxanthin, could yield only one molecule of the vitamin since they had only one β-ionone ring.

In spite of preponderance of evidence to the contrary, some reports (9, 10, 37) have been published indicating that, under certain conditions, all-trans β-carotene has a biological value approaching that of an equal weight of vitamin A. Koehn (23) showed that, in the presence of α-tocopherol, the rat can convert β-carotene quantitatively into two molecules of vitamin A. Barnett and Espoy (6) concluded that pure all-trans β-carotene has a biological activity of 2,200 to 2,500 U.S.P. units per milligram (0.4 - 0.45 μg being equivalent to one unit of vitamin A) and that it is very doubtful if carotene splits in such a manner as to give only one molecule of vitamin A.

Under favorable experimental conditions, neither preformed vitamin A nor β-carotene appears in the faeces of rats. It is assumed that the total amount ingested is absorbed, although it

is acknowledged that some of the vitamin A or carotene may be destroyed in the alimentary tract. Experiments with man and animals other than the rat have shown that some dietary carotene is excreted in the faeces. Hume and Krebs (21) reported that from 26 to 76% of carotene ingested was excreted by human adults. The term maximum effective dose was defined as that portion of the intake that was not excreted in the faeces. For vitamin A, this was assumed to be 100% of the intake. However, the subcommittee warned: "Any estimate of the minimum protective dose of carotene must be based on the maximum effective dose and not on the intake," and they recommended that, in estimating the contribution of dietary sources of carotene to vitamin A requirements of human beings, recognition be given to availability of carotene from specific dietary sources.

It is unfortunate that nutritionists, in establishing the requirements of mammals and birds, have not made greater efforts to determine maximum effective doses of provitamins A for different species and breeds. As the following reviews demonstrate, much research has been directed towards determination of minimum protective doses, requirements and allowances, but determination of the portion excreted in the faeces has been the exception.

Although the dictum of the I.U.P.A.C. (22) that the biological activity of β-carotene should be expressed in provitamin A units and not in vitamin A units has been generally accepted, the method of expressing "vitamin A requirements" in terms of β-carotene varies. Human requirements are generally given in IU of vitamin A with allowance for a greater intake if vitamin A activity is derived wholly or in part from carotene. Tables of food composition commonly give carotene content in IU of vitamin A, but carotene content of feedstuffs generally is expressed as milligrams per pound. The National Academy of Sciences, National Research Council (NAS-NRC), gives the vitamin requirement of poultry (32) in U.S.P. units of vitamin A, those of swine (31), dairy cattle (29), beef cattle (34), sheep (28), and horses (33) as either IU of vitamin A or milligrams of carotene. However, the stated equivalent value of one milligram of carotene ranges from 333 to 836 IU of vitamin A for various animal species, is 1,667 IU for poultry, and ranges from 417 to 834 IU for human beings (7, 12, 21, 30). The existence of these various "conversion factors" is a source of confusion.

A subcommittee of the Animal Nutrition Research Council (2) has advocated an efficiency ratio of 1:8 for the biopotency of vitamin A and carotene on a weight basis in the nutrition of ruminants, so that 1 mg of carotene would be equivalent to 418 IU.

Examination of available data on which the NAS-NRC committee based its various recommendations reveals that they are limited in number, and most were obtained several years ago. In intervening years breeding and management practices, resulting in increased growth rates and higher production, probably have increased and modified the requirements.

In some of the earlier studies, fish oil or natural esters were compared with the provitamin, usually considered to be β-carotene, present in feedstuffs such as alfalfa. It has been pointed out that natural esters are lower in biopotency than the all-trans isomer of vitamin A. β-carotene predominates in natural feedstuffs except yellow corn, in which cryptoxanthin is the main provitamin, but other provitamins such as α- and γ-carotene are present to a greater or less extent; their biological potency is less than that of β-carotene.

There is evidence (22) that not all solutions of the international provitamin A standard were stable. Instability of β-carotene in natural sources has been overcome appreciably by use of antioxidants, storage under nitrogen, etc. Dry, stabilized preparations of vitamin A have been available in recent years and appear to be better utilized than oily or aqueous preparations. Response is better with β-carotene in stabilized preparations than in feedstuffs.

Although the provitamins can be separated by chromatographic procedures and individual carotenes determined spectrophotometrically, common assay procedures do not separate isomers of lower biological potency from all-trans β-carotene. Isomerization of β-carotene occurs during processing and storage. Much work needs to be done on largely unsolved or unresolved problems of practical methods for measuring isomers, and establishing the biological activity of these forms for various species. A synthetic, stabilized form of all-trans β-carotene is now available for experimental purposes.

The NAS-NRC committee formerly published "recommended allowances"; these have been superseded by "nutrient requirements", it being left to practising nutritionists to decide what safety margin to provide in their formulations.

Improvements and modifications have been made in assay procedures. In 1934, for example, a factor of 1,600 was used to convert $E^{1\%}_{1cm}$ 328 mμ to IU: the factor is now 1,900.

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Addenda

In addition to the references listed above, the following contain pertinent information on vitamin A and carotene:

Isler, O., and P. Zeller. 1957. Total synthesis of carotenoids, p. 33-71. In Vitamins and hormones, Vol. 15, Academic Press Inc., New York.

Lowe, J. S., and R. A. Morton. 1956. Some aspects of vitamin A metabolism, p. 97-137. In Vitamins and hormones, Vol. 14, Academic Press Inc., New York.

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See also "Symposium on vitamin A in honor of Prof. P. Karrer," p. 291-571. In Vitamins and hormones, Vol. 18, Academic Press Inc., New York.

Relevant sections are:

CAROTENE AND VITAMIN A METABOLISM IN DOMESTIC MAMMALS

W. E. J. Phillips[5/]
Animal Research Institute, Research Branch
Canada Department of Agriculture, Ottawa

Vitamin A deficiency can and does occur in livestock in Canada. Recommended allowances for carotene or vitamin A may not be large enough for some species to provide a satisfactory safety margin to minimize the effect of individual variation, dietary or environmental stresses. Recent work suggests that the factor used to convert carotene to vitamin A may be too high in a number of cases. The increasing use of preformed vitamin A in commercial rations demonstrates that further experimental work is needed to define the conversion factor more realistically. Since biological effectiveness is significantly influenced by the form of provitamin or vitamin and by other conditions which influence their metabolism, a specific definition and terms of reference should be established for the conversion factor. It would then be possible to relate existing and future vitamin A preparations to an accepted standard. The limitations of uncritical application of a conversion factor must be stressed.

It is apparent from consideration of conversion factors for various species that exact values cannot be recommended at this time. Until more data are available it is suggested that carotene be assigned a biological value equivalent to 1/7 the weight of preformed vitamin A for cattle, sheep and pigs. For comparison, recommendations of the NAS-NRC Committee on Animal Nutrition are shown in Table 6.

Many studies not considered in this report have been made on the metabolism of vitamin A or carotene using the rat as an experimental animal. The metabolism of carotenoids is a classic example of species difference in the utilization and metabolism of a dietary constituent. It is emphasized that caution must be used in the interpretation or application of results from one species to another and between breeds of a given species. Consideration must be given to extending research in Canada to species of economic significance.

This is a summary of our knowledge on vitamin A nutrition for various mammalian species, with emphasis on information directly applicable to Canadian agriculture. It is not a general review of effects of vitamin A deficiency produced experimentally or naturally. Some factors are discussed in relation to certain species, but the reader is referred to the excellent review by Moore (60) for a detailed discussion of vitamin A metabolism.

[5/]Present address: Food and Drug Directorate, Department of National Health and Welfare, Ottawa.

Vitamin A and its provitamins are extremely important in Canadian agricultural economy. Deficiency may reduce a farmer's income through loss of livestock, lower efficiency of production or inefficient use of land. Vitamin A deficiency does occur in Canada (69): "Calves were born weak, some of which would die quickly. ... The animals were normal in all respects until the owner would suddenly notice them to be blind." These conditions are not new to the agricultural industry; in fact, it has been pointed out (5, 60) that cases of probable vitamin A deficiency are recorded in the Bible. Jeremiah (41) reported: "The wild asses did stand in high places, they snuffed the wind like dragons; their eyes did fail, because there was no grass."

The economic significance of vitamin A was shown by Ritzman et al. (79), who demonstrated that calves receiving a vitamin A - deficient diet consumed more food but gained 50% less than those receiving adequate vitamin A supplements. Protein utilization decreased about 25% owing to lack of sufficient vitamin A.

Though much has been learned about vitamin A metabolism and the requirements of livestock, there are still many gaps in our knowledge. Changing practices in Canadian agriculture may affect the requirement for, or the utilization of, vitamin A and its provitamins, so that the subject must be constantly reviewed. Knowledge gained through research is of little value, however, unless it can be compiled, summarized and converted into terms applicable by the producer.

CATTLE

Data available for calculating an absolute requirement for carotene and vitamin A in cattle are not as plentiful as one might expect. The literature contains results of many feeding trials, but the experimental designs and measurements made (or ignored) do not lend themselves to drawing unequivocal conclusions. For example, one finds results from supplemental vitamin A in rations for beef or dairy herds and no mention of carotene or vitamin A content of basal rations; similarly, effects of various levels of alfalfa, etc., have been studied without determining carotene contents. There is no literature on absolute requirements under Canadian conditions.

The estimates of requirements of vitamins reviewed are based on various criteria, such as rate of growth, survival, or other manifestations of deficiency. For vitamin A blood plasma levels or liver storage is often used. Levels of plasma vitamin A are sometimes criticized as being unrelated to liver stores but low levels of plasma vitamin A are indicative of deficiency.

Calves

At birth the calf normally has a very low level of blood plasma vitamin A and concentrations of about 9 to 10 μg per 100 ml or more must be quickly attained (1-2 days) for a favorable chance of survival (31). This may be accomplished by suitable supplementation of the calf, the dam, or both. The calf can utilize carotene; however, preformed vitamin A is much more effective.

For the postneonatal period, the most recently recommended carotene requirement (62) is 4 mg per day per 100 lb of body weight. This may be a conservative estimate. Parallel feeding trials with β-carotene and vitamin A are needed to obtain a valid conversion factor (i.e., the vitamin A activity of β-carotene). A factor of about 500 (IU of vitamin A per mg of carotene) was calculated from the limited data available. Many dietary factors affect the biological value of carotene: antioxidants (77), source of carotene (15), level of carotene in the diet (12), and quality and quantity of the main dietary components.

The time required for symptoms of vitamin A deficiency to develop depends on reserves in the body. These reserves are affected by the amount of vitamin or provitamin ingested and the amount transferred prepartum from the dam. Young animals become deficient more rapidly than older ones (87). They may exhibit the usual symptoms: rough coat, night blindness and swelling of the legs. More common in deficient calves are diarrhea with scouring, incoordination, and increased spinal fluid pressure. A less apparent change is keratinization of epithelial tissue and drying up of mucus membranes. This in turn leads to increased susceptibility to many infections of which bronchopneumonia (34) is of prime importance in calf management.

Low levels of plasma vitamin A suggest deficiency in the young calf (10, 36, 62, 84) as follows:

μg vitamin A per 100 ml plasma	Condition of calf
10 or more	Normal
7 - 8	Mild deficiency symptoms (watery eyes, cold, nasal discharge, diarrhea)
5 or less	All symptoms of advanced stage of deficiency

Various estimates of the vitamin A and provitamin A requirements of calves are shown in Table 2.

Table 2. Daily Requirements of Vitamin A and Carotene for Dairy Calves per 100 Pounds of Body Weight

Breed	Criterion	Vitamin A		Carotene	Reference
		IU	mg[1]	mg	
Holstein	Growth, liver storage	-	-	6	20
Guernsey	Growth, liver storage	-	-	10	20
Holstein	Growth only	3,000	0.90	-	49
Holstein	Growth storage	11,000	3.30	-	49
Holstein yearling	Growth	-	-	3.4	10
Dairy breeds	--	3,000	0.90	6	30
Dairy heifers	Growth	-	-	4	62
Holstein (newborn)	Prevents scouring, survival	25,000[2]	7.50	-	31
Holstein	Growth	243,400[2]	73.02	-	83

[1]/Calculated from original data based on 0.3 µg vitamin A alcohol = 1 IU.

[2]/Total IU per day.

In the period just after birth, colostrum is an important source of the vitamin. Sutton and Kaeser (99) calculated that a calf consumes about 53 mg of carotene if the colostrum feeding period is extended to 7 days (by using refrigerated colostrum). It has been suggested (52) that the newborn calf is unable to utilize carotene but later work (95) showed that it can at a rate sufficient to permit satisfactory growth and some storage. Efficiency of conversion of carotene to vitamin A, however, is not high (15). When carotene was dispersed in various carriers and fed to Guernsey calves, much of it was absorbed as such and lingered in the blood without being converted to vitamin A, although the calves were at a low plane of vitamin A nutrition. The contribution of colostrum is nevertheless significant since its ingestion caused a marked increase of carotenoids and vitamin A in the blood of the newborn calf (106). Whole milk did not supply enough vitamin A to prevent deficiency of vitamin A in the blood. Intake of carotenoids from good-quality hay was not sufficient to raise blood levels until the animal was about 6 weeks of age.

The value of feeding colostrum can be modified by management practice. Eaton et al. (19) showed that prepartum milking markedly decreases both carotenoid and vitamin A contents of colostrum and reduces blood plasma levels of carotene and vitamin A in suckling calves. Haustov (34) showed that the value of colostrum could be improved by supplementation with juice from silage, and Sokol et al. (93) recommended blending it with a complex containing α-globulin, vitamin A and antibiotics. Use of sulphonamides (80, 81) and antibiotics does not appear to have any detrimental effect on vitamin A metabolism in the calf. Application of these observations can reduce losses of newborn calves appreciably.

The diet of the dam can affect vitamin A status of the calf by modifying initial liver reserves. At birth, calves from vitamin A-supplemented cows had high plasma vitamin A levels, which increased with age, whereas no such increase was noted in calves from nonsupplemented dams (96). After 10 days vitamin A remaining in livers of calves from vitamin A-supplemented cows was higher than that in livers of calves from nonsupplemented cows. Wise et al. (105) showed that feeding one million units of vitamin A daily to dairy cows in the latter stages of gestation increased the vitamin A of the newborn calf, and should have a practical value in maintaining its postnatal health.

Growth and Fattening

Deficiency of vitamin A in mature cattle occurs in Canada. The best practical application must be made of existing information but research on vitamin A and carotene requirements must be

continued in the light of changing agricultural practices. Reassessment of recommended allowances of the vitamin and its provitamins is necessary.

In experiments, cattle deficient in vitamin A exhibit the severe classical symptoms. In the field, especially when the deficiency is mild, diagnosis is difficult even when the manifestations are many. A deficiency may produce lameness, roughening of the coat, loss of appetite followed by diarrhea, discharge from nose and eyes, convulsions, protruding eyes, edema and sometimes death. Moore (60) states that the anasarca described by Madsen and Earle (53) resulting from a deficiency is a cause for rejection of carcasses by American meat inspectors.

Table 3 shows how different are the estimates of carotene required. Guilbert (29) found 1.4 mg of carotene daily per 100 lb of body weight to be the minimum to prevent night blindness. But in practice a much higher level must be recommended. The value of 36 mg suggested by Lapsin (46) in 1957 may appear excessive. But his work and that of Perry et al. (72) show that the NAS-NRC recommendations of 1958 (62, 64) were too low for maintenance. Beef cattle that received enough carotene from yellow corn to meet their theoretical vitamin A requirements and that were also fed high levels of supplemental vitamin A gained weight more rapidly than those that were fed only the yellow corn (72). Melton et al. (58) stated that for years it has been assumed that, when sufficient carotene is available, beef cattle meet their requirements through conversion of carotene. But so many field reports have shown increased gains by fattening cattle fed supplemental vitamin A that reevaluation of carotene and vitamin A requirements of beef cattle is necessary. This need was recognized in the NAS-NRC recommendations of 1963 (67).

Table 3. Requirements of Cattle for Vitamin A or Carotene Estimated by Various Workers

Breed	Criteria	Vitamin A daily/100 lb body wt		Carotene daily/ 100 lb body wt	Reference
		IU	mg	mg	
General	Minimum[1]/ night blindness	1,100	0.33	1.4	29, 32
Jersey	Maintenance, reproduction	-	-	4.0-4.5	45
Dairy and beef	Growth, reproduction and safety factor	3,000	0.90	6	30
Guernsey Jersey Holstein	Reproduction	-	-	7.5-8.5	82
Guernsey	Reproduction	-	-	9	81
Dairy	Maintenance (dry cows)	-	-	36	46
Dairy	Lactation (yielding 40 kg milk/day)	-	-	145	46
Dairy cows	Maintenance	-	-	4.0[2]/	62
Dairy (bulls)	Maintenance	-	-	4.0[2]/	62
Beef	Normal growth, heifers and steers	1,580-2,300	0.47-0.69	4.0-5.8[3]/	67
Beef	Finishing 2-year - old cattle	1,942-2,200	0.58-0.66	4.9-5.5[3]/	67
Beef	Cows nursing calves, 3-4 months post - partum	4,200	1.26	10.6[3]/	67
Beef (bulls)	Growth and maintenance	2,100-4,050	0.64-1.2	5.4-10.3	67

[1]/Authors recommend 5 to 10 times the minimum requirement to be a practical level.

[2]/Recommended requirement, NAS-NRC (62).

[3]/Recommended requirement, NAS-NRC (67).

Reproduction

Adequate intake of vitamin A is extremely important during the stress of reproduction. It was recognized very early (57) that cattle fed diets low in carotene or vitamin A gave birth prematurely to dead, weak or blind calves. Millen and Woolam (59) and Hignet (37) have recently reviewed nutritional aspects of abnormal fetal development and female infertility in large animals in relation to vitamin A. Hart and Guilbert (33) noted that liver samples from aborted fetuses from cows reacting to the agglutination test for Brucella abortus were either negative for vitamin A or gave very low values. Infection of the placenta may restrict transfer of vitamin A to the fetus, or vitamin A deficiency may lower resistance of the placenta to infection with B. abortus. The significance of maternal transfer of vitamin A prepartum and the significance of carotene and vitamin A in colostrum has been discussed.

Severe deficiency affects reproduction in dairy bulls. If gross symptoms of deficiency occur before the breeding age, bulls fail to breed but may be induced to do so by giving vitamin A supplements (38). If bulls reach breeding age before developing symptoms, they may breed but may be too weak to mount or deliver semen. Semen obtained by artificial stimulation is fertile, though it is scanty and is high in abnormal spermatozoa. Hodgson et al. (38) concluded that the reproductive capacity of a bull should not be seriously impaired before gross symptoms of deficiency appear. On the other hand, Erb et al. (22) found that a daily supplement to bulls of 4.8 μg of vitamin A daily per pound of body weight was insufficient to prevent convulsions, night blindness, delayed sexual maturity, reduced sex drive and limited spermatogenesis. They pointed out that prepubertal damage may be permanent, especially when cystic pituitaries develop or total blindness occurs. Other workers have noted similar effects of vitamin A deficiency (11, 54). Fertility was reestablished in beef bulls by administering more than 50 μg of carotene per kg of body weight daily.

Lactation

Obviously the vitamin A requirement for lactation is higher than for maintenance. In 1958 the NAS-NRC (62) recommended that 30 mg of carotene per day be added to the maintenance requirement to allow for reproduction and showed that, when cattle are fed more vitamins A and D than they need for normal reproduction, milk volume is not increased but its vitamin content is. The committee recommended no further supplementation for lactation in dairy cattle but did for beef cattle. Kublman (44), reporting in 1942 on 30 complete lactations, had concluded that if milk secretion alone is considered and the vitamin A potency

of the milk produced disregarded the requirement of vitamin A for lactation is very little if any higher than that for reproduction.

Baker et al. (2) found that in beef cattle the hepatic stores of vitamin A decreased when 30 mg of carotene per 100 lb of body weight were fed daily during lactation. But they (1, 83) stressed that, late in gestation and in early lactation, failure to maintain vitamin A levels in the liver does not necessarily indicate inadequate carotene intake. Also, Wheeler et al. (103) in a 3-year study on range Herefords found a gradual decrease during gestation, parturition and lactation when 25 mg of carotene were fed daily per 100 lb of body weight. Evidently the NAS-NRC recommendation of 1958 (64) was too low.

Breeds

The requirement for carotene, and thus the conversion factor, may differ between breeds of livestock. Eaton et al. (19) showed that, depending on the criterion used, Holsteins converted carotene to vitamin A 1.4 to 1.8 times as efficiently as Guernseys.

In 1942 Boyer et al. (10) concluded that 18 µg of vitamin A was required per kg of body weight for Holsteins and Guernseys, or 75 to 85 µg of carotene for Holsteins and 125 to 135 µg for Guernseys. On a weight basis this would mean that the efficacy of vitamin A:carotene was between 4 to 5:1 for Holsteins and 7 to 8:1 for Guernseys.

Other Factors

Brocklesby (12) pointed out that experimental cattle kept on graded dosages of vitamin A or carotene utilize carotene less efficiently at higher levels of intake. At intakes of 60 µg per pound of body weight, carotene was equal to 1/7th the weight of vitamin A; at 180 µg, 1/13th; and at 540 µg, 1/24th. Inclusion of soybeans in the diet decreases the biological value of carotene (21, 89, 97, 98). This effect is not overcome by concomitant feeding of iodinated protein (21).

Bohman et al. (6, 7) observed that feeding extra fat with supplemental protein during the winter decreased plasma carotene and vitamin A and also liver stores of carotene and vitamin A. Level of protein in the diet may also affect vitamin A metabolism in cattle. Esh et al. (26) showed that the dietary protein level had a considerable effect on vitamin A utilization of small experimental animals.

Feed additives may also affect utilization of carotene and vitamin A. Oxytetracycline at 5 mg per pound of a feed in which alfalfa supplied the vitamin A activity increased liver stores of vitamin A in steers in two out of three experiments (17); similar effects have been noted with stilbestrol (6). Sodium bentonite, a valuable pelleting agent, was shown to adsorb carotene and vitamin A (48) and reduce their availability (47). Three percent sodium bentonite in a pelleted ration containing 25% alfalfa, however, does not affect hepatic stores of vitamin A or carotene in steers (24).

Conversion Factor

Many things influence the calculation and application of a carotene - vitamin A conversion factor. The form in which carotene or vitamin A is given is one of these, the route of administration is another. For practical purposes the oral route has the widest application. Orally administered equal doses of oil solutions of synthetic vitamin A or natural vitamin A from fish oil are not significantly different in biological value (91). Aqueous and gelatin-beadlet forms of vitamin A increase blood plasma vitamin A more than do equivalent amounts of oil solutions. In some species, carotene from dehydrated alfalfa meal is utilized less efficiently than carotene from fresh alfalfa (28). Xanthophylls and chlorophylls in the diet reduce liver storage of vitamin A from carotene (42, 43).

Guilbert and Loosli (30) suggested in 1951 that 1 mg of carotene is equivalent to 500 IU of vitamin A. In 1958 the NAS-NRC committee (62) considered that the carotene requirement for dairy cattle was about four to five times that of vitamin A. Many workers have assumed this to be on a weight basis, but the committee stated (personal communication, 1960) that their comparison was based on units of vitamin and not on weight. Thus on a weight basis the efficacy ratio was about 9:1. For beef cattle, in 1963 the NAS-NRC (67) suggested that 1 mg of β-carotene be considered equal to 400 IU of vitamin A, so that the efficacy ratio of the vitamin to carotene would be 4.2:1 on a unit basis or 8.3:1 on a weight basis.

It must be stressed that a conversion factor cannot be absolute and extreme care must be exercised in its use. Many factors that affect metabolism of carotene may be greatly different from those affecting metabolism of vitamin A.

It is also essential to consider the animal's requirement for carotene. A distinction must be made between average requirements tabulated by NAS-NRC and allowances sufficient to provide a safety margin for all animals. Individuals within a group can vary more widely than is commonly suspected: variations up to 20%

are not uncommon and up to 50% or more occur occasionally (78). The combination of individual variation and marginal allowances may account for some occurrences of vitamin A deficiency in Canada. This can be rectified by a reassessment of recommended allowances. It is then a question of economics whether to increase the allowance for all animals or to give supplements to specific animals that show deficiency symptoms.

Feed and Environment

McElroy (56) stated that good, green, well-cured hays and silages furnish ample vitamin A for cattle; over-matured hay, and hay badly weathered in the swath or burned or molded in the stack are poor sources. In 1952 vitamin A deficiency was not a common problem in Alberta feed lots but it was recommended that at least 2 or 3 lb per head daily of good, green hay be fed if other feeds available were not green. Since then the incidence of vitamin A deficiency has increased in feed lots and in breeding herds both in Canada and in the United States (12, 55, 69, 73, 78). This is probably not due to a single cause. Sound agricultural practice and management in the production, preservation and feeding of good-quality herbage is of first importance. This is not discussed here but its importance is stressed.

Several investigators consider that greater use of nitrogen-containing fertilizers has increased the nitrate content of feeds and forages to the point of interfering with carotene and vitamin A metabolism. O'Dell et al. (68) presented data from rats which indicated that nitrites played a definite role. Not only did the feeding of potassium nitrite to rats cause a more rapid depletion of vitamin A, but it induced a vitamin E deficiency with a diet normally adequate in vitamin E. Another subcommittee of this National Committee on Animal Nutrition concluded (1963) that for domestic livestock "Factual evidence does not substantiate our former concern about the effect of nitrate on vitamin A utilization. Evidently nitrate toxicity has often been incorrectly diagnosed."

Brocklesby (12) pointed out that sulphate is reduced to sulphide in the rumen and enough hydrogen sulphide may be present to interfere with the conversion of carotene to vitamin A.

The relationship of low phosphorus diets to increasing incidence of vitamin A deficiency has been considered by some. An inverse relationship was obtained with steers between plasma inorganic phosphate or dietary phosphate and plasma carotene levels (86, 100, 101). The decrease in efficiency of conversion of carotene to vitamin A is not large and hence is not considered significant in Canadian agriculture.

Environmental stress may influence the requirement of large animals for carotene or vitamin A. Page et al. (71) observed that the high summer temperatures of Arizona accelerated metabolism of vitamin A in cattle. Ershoff (23) made some preliminary studies on the survival time of vitamin A-deficient rats at low and normal environmental temperatures and concluded that vitamin A requirement was increased by prolonged exposure to cold. More recent studies (74) demonstrated that both hepatic storage and rate of metabolism in rats of orally administered vitamin A were unaffected by a low environmental temperature. Utilization of carotene, however, was greater in rats maintained at a low temperature (2 C) than at room temperature (22 C).

It is interesting to note that in a number of cases diagnosed in the field as vitamin A deficiency, cattle were found to be consuming four to six times the amount of carotene considered adequate by NAS-NRC (78). There was no essential difference in blood serum vitamin A or carotene levels of affected and apparently normal animals within the same herd. It is our opinion that here there may be competitive inhibition acting at an active site of vitamin A function, assuming that response to supplemental vitamin is indeed indicative of vitamin A deficiency. Inhibition of conversion of carotene may also result in low levels of liver or plasma vitamin A.

Chlorinated hydrocarbons will depress conversion of carotene to vitamin A (51) and some organophosphate insecticides will inhibit a number of esterases (50, 88). In cases of hyperkeratosis or X-disease described by Olafson (70) cattle exhibit symptoms similar to vitamin A deficiency. In most cases animals respond to doses of vitamin A but not of carotene. The condition develops as a result of ingestion of toxic material in lubricants (4), in petroleum products (92), or in oil-based insecticide carriers (39, 40). The causative agent in all cases was thought to be naphthalene derivatives. It is highly probable pesticides that are either chlorinated hydrocarbon or organophosphorus compounds may interfere with normal carotene or vitamin A metabolism. Up to the present little or no information is available on the effect of these compounds on vitamin requirements. An active program in this regard is currently in progress in the author's laboratory.

SHEEP

Vitamin A or its provitamins are required in the nutrition of sheep, as for other mammalian species. Under practical conditions, however, there is no serious problem since sheep can be maintained for relatively long periods on diets low in vitamin A or carotene before liver stores are depleted. Ewes grazed on green mountain feed and then fed poor-quality, low carotene

roughages, with a grain supplement to supply extra protein and total digestible nutrients during the winter, were not benefited by vitamin A supplementation (14). When range ewes were grazed on either green or dry summer range and then fed poor-quality hay during gestation and lactation, the only benefit that seemed to be derived from vitamin A was a small increase in blood levels of vitamin A in some of the ewes. Supplementation gave no consistently significant effect on percentage of lamb crop, birth weight, vigor or rate of growth of lambs. Concentration of vitamin A in blood of Merino sheep was unaffected by changes in the intake of carotene from 1 mg while grazing on dry pasture in the autumn, to 2 g per day while grazing on green pasture in the spring (76). In all sheep examined reserves of vitamin A in the liver were adequate. Bowstead (9), in a summary of sheep experiments carried out in Canada between 1919 and 1950, stated that feeding vitamin A had not proved beneficial to pregnant ewes fed nonlegume hays of average or good quality.

The classical studies in which Guilbert et al. (29) demonstrated the minimum requirement of sheep to be 800-1,200 IU vitamin A or 1.2-1.6 mg carotene daily per 100 lb of body weight have been used as the basis for the recommendations of NAS-NRC Committee on Animal Nutrition (63). Carotene requirements for late pregnancy and lactation were calculated as five times the minimum necessary to prevent nyctalopia (25 µg per kg body weight). Replacement lambs and rams require an estimated 2.5 times this minimum. For fattening lambs and for ewes during the first 15 weeks of gestation, 1.5 times the minimum is recommended. Thus the daily requirements per 100 lb of body weight are:

	Carotene mg	Vitamin A IU
Ewes - nonlactating, first 15 weeks of gestation Lambs - fattening	1.7	965
Ewes - last 6 weeks of gestation Ewes - lactation	5.8	2,316
Ewes - replacement lambs Rams - lambs and yearlings	2.8	1,158

The values represent conversion factors (IU vitamin A per mg carotene) of 568, 400, and 414 respectively or on a weight basis a potency of carotene relative to vitamin A of 1/6 to 1/8. Recent work of Myers et al. (61) suggests a reevaluation of the conversion factor. Based on levels of either plasma vitamin A or liver vitamin A, both lambs and sheep utilized carotene with diminishing efficiency as the dosage increased: for lambs this

ranged from 1/8 to 1/13 (weight basis). Diven and Erwin (16) showed that for normal sheep preformed vitamin A was 3.3 times as effective as an equal weight of β-carotene, and for vitamin A-deficient sheep it was 4.6 times as effective. This suggests that previous nutrition of the animal will influence the conversion.

Regarding recommended allowances of carotene, the work of Pierce (75) is of note. Administration of 2.5 mg of carotene per 100 lb of body weight cured night blindness but had no appreciable effect on vitamin A reserves in the liver. The recommended allowance of preformed vitamin A for reproduction appears to be adequate from the observations of Campbell et al. (13) that 4,000-6,000 IU vitamin A per head daily gave normal reproduction while subnormal reproduction was observed only with less than 2,000 IU per day. Other additives in the diet, however, may modify availability of dietary vitamin A. Erwin and Page (25) showed that soft phosphate as a source of supplemental phosphorus for sheep depressed weight gains and feed utilization. Utilization of dietary vitamin A decreased, as indicated by the magnitude of hepatic vitamin A loss in a 45-day period.

Factors influencing utilization of carotene and vitamin A, previously discussed for cattle, are in some cases applicable to sheep. Insufficient information is available to assess influence of nitrate toxicity. Sokolowski (94) fed levels of potassium nitrate up to 12.8% of the ration for periods up to 129 days without causing any visible symptoms of nitrate toxicity.

There is no evidence at the present time that vitamin A deficiency in sheep in Canada is of economic importance.

SWINE

In the Canadian swine industry vitamin A deficiency can be a real and serious problem if good management practices are not thoroughly appreciated. Whiting (104) considers a deficiency likely from his review of swine requirements and carotene content of ration ingredients, based on Canadian analyses. Deficiency symptoms are similar to those described for other species. Under natural conditions a partial posterior paralysis may develop before other symptoms are apparent. Unthriftiness, infertility or congenital malformations make this dietary nutrient extremely important to the producer.

Dietary requirements (65) for carotene recommended for swine are shown in Table 4. Daily requirements for carotene have also been calculated on the basis of 100 lb of body weight and are included in the table. Values for vitamin A allowances are not shown; however, a conversion factor of 1 mg of carotene equivalent to 533 IU vitamin A is recommended.

Table 4. Daily Requirement of Swine for Carotene (65)

	Body wt lb	Carotene per day mg	Carotene per day mg/100 lb body wt
Growing	10	1.2	12.0
	25	2.2	8.8
	50	2.4	4.8
Finishing meat type	100	4.0	4.0
	150	5.1	3.4
	200	6.0	3.0
Finishing bacon type	100	3.9	3.9
	150	4.9	3.3
	200	5.3	2.7
Breeding stock			
Gilt (bred)	300	15.0	5.0
Sow (bred)	500	18.8	3.8
Gilt (lactating)	350	27.5	7.9
Sow (lactating)	450	31.2	6.9
Boars (young)	300	15.0	5.0
Boars (adult)	500	18.8	3.8

These values appear to be adequate and provide a satisfactory safety margin. For comparative purposes the requirement as determined by other workers is shown in Table 5.

Table 5. Requirement of Swine for Vitamin A or Carotene

Criteria	Daily per 100 lb body weight			Reference
	Vitamin A		Carotene	
	IU	mg	mg	
Minimum to prevent night blindness	800-1,000	0.24-0.33	1.1-1.8	29
Maximum growth, some storage	-	-	1.13	35
Minimum for storage in baby pig	2,400	0.73	-	89

The subcommittee on Swine Nutrition, Prairie Regional Committee, National Research Council of Canada (3) assumed that 1 mg carotene = 1,000 IU and stated that "while the conversion factor may be controversial it seems advisable to take some kind of a stand on the matter." There would not appear to be any experimental basis for making this assumption. Recent work by Myers et al. (61) showed that, based on plasma vitamin A, the value of carotene relative to vitamin A alcohol ranged from 6 to 11 mg carotene, equivalent to 1 mg preformed vitamin.

Frape et al. (27) recently determined that the minimum requirement of the young pig for a stabilized source of vitamin A palmitate in a dry carrier was 800 IU per pound of feed. Normal growth occurred, however, at levels as low as 100 IU per pound of feed. For older animals Bowland (8) states that work conducted at the University of Alberta indicated that if vitamin A is fed (400 IU vitamin A per pound of feed) to growing pigs, storage is sufficient to carry them through the finishing period to market weight. From these data it is obvious that if recommended allowances are met by the producer there is little likelihood of a deficiency occurring.

The effect of nitrate consumption by swine has recently been studied. Tollett et al. (102) fed various levels of nitrates and vitamin A to growing pigs. In all cases, regardless of the amount of vitamin A, the growth of pigs receiving 3.7% nitrate was significantly depressed. It seems probable that this is not a simple question of utilization or metabolism of vitamin A and will require further study.

HORSES

Little information on the requirement of the horse for vitamin A and carotene is available on which to base an estimate of relative efficiency of carotene to vitamin A in this species. Levels of vitamin A intake required by horses were calculated by NAS-NRC (66) on the basis that "At the minimum level it requires six times as much carotene as vitamin A and ten times as much at the more liberal level for pregnancy and lactation." One milligram of carotene in the ration of horses would be equivalent to 333 to 556 IU of vitamin A (Table 6.)

Table 6. Summary of Efficacy of Carotene to Vitamin A For Various Species

(NAS-NRC Committee on Animal Nutrition (62-67))

Species	Mg β-carotene equivalent to 1 mg vitamin A	IU vitamin A equivalent to 1 mg β-carotene
Dairy cattle	8-10	333-417
Beef cattle	8.3	400
Sheep	5.8-8.3	400-578
Swine	6.25	533
Horses (growth)	6	556
(pregnancy)	10	333

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UTILIZATION OF VITAMIN A AND PROVITAMINS A BY POULTRY

E. V. Evans
Department of Nutrition
University of Guelph, Guelph

The information on efficiencies of preformed vitamin A and of carotene for poultry of various types and physiological states indicates that preformed vitamin A is more effective than carotene. Continued use of the international unit as a measure of concentrations and requirements of vitamin A and carotene is considered to be unnecessary and undesirable, and mass units (micrograms, milligrams) are recommended. Also, carotene should be considered to be one third as effective for poultry as an equal weight of vitamin A alcohol. This ratio is tentative pending completion of additional well-controlled studies. Suggestions are made for further study on factors that may affect utilization of both vitamin A and carotene.

In 1960 the NAS-NRC subcommittee (55) considered that preformed vitamin A and the provitamin β-carotene were equally effective for poultry (chickens and turkeys). They stated: "In the chick as in the rat, 0.6 micrograms of β-carotene appears to be equivalent to one U.S.P. unit of vitamin A, except when the carotene intake provides vitamin A activity greatly in excess of the requirement." The same ratio was given in earlier recommendations of this subcommittee.

In the NAS-NRC recommendations the common denominator is the U.S.P. unit and, through acceptance of conventional definitions, the subcommittee considers 0.3 μg of pure vitamin A alcohol equal in biological efficacy to 0.6 μg of β-carotene, regardless of source or carrier. Evidence on which the subcommittee based this decision is not given in detail in its publications. The following is a review of the literature.

EFFICIENCIES OF VITAMIN A AND CAROTENE

This review covers most of the readily available publications of the last 20 years, the period in which the vitamins and provitamins in feed ingredients have been determined with reasonable and increasing precision and accuracy. In the same period, control of experimental conditions has been superior to that in earlier work. As the more recent experiments in this period are probably the more reliable, the information summarized in the accompanying tables is arranged in chronological order and with dates of publication.

Published estimates of requirements for vitamin A and the provitamins and of comparative efficacy of the two forms have been based on the following criteria: growth of young poultry, liver storage of vitamin A in birds of various ages, levels of vitamin A in blood, deposition of vitamin A in eggs and, occasionally, such measures as survival time and livability. That the relative efficacies may differ with the various criteria must be recognized. Further evaluations based on the same criterion may be affected by various factors, such as: age and breed of birds; disease; concentrations of other nutrients in the ration; nutritional history of the birds, particularly depletion; level of intake of the vitamin or provitamin; source or carrier of the vitamin or provitamin, and its history, including processing and storage; antioxidants, antibiotics, bentonite or other additives or medicaments in the ration; and natural or incidental inhibitors or stimulants in ration components.

The literature reviewed does not allow definite conclusions on the effects of any of these factors. But the tables show that many of them were involved in the experiments, and a few conjectures are offered. All the efficacy ratios are expressed as international units.

Growth

Of 13 reports on growth of chicks (Table 7), sources of vitamin A were superior to those of carotene in six and equivalent in six, and alfalfa carotene was superior to fish oil vitamin A in one. Preformed vitamin A was superior to crystalline carotene in one report on turkeys, and apparently in the second (34). For bobwhite quail, cod liver oil and crystalline carotene were of equivalent efficacy.

Liver Storage

Of 13 reports on liver storage in chicks (Table 8), vitamin A was superior to carotene in ten, carotene was superior to vitamin A in two, and in one the preformed vitamin was superior, equal, or inferior to carotene, depending on the plant source.

In one of three reports with hens, carotene appeared superior to fish oil vitamin A, but in the other two preformed vitamin A was superior to carotene. Vitamin A was superior to carotene in the single report on turkey poults and the two on bobwhite quail.

Table 7. Efficacy Ratios of Vitamin A and Carotene in 13 Studies on Growth of Poultry

Date	Breed[1]	Sex[2]	Special conditions	Source[3] Vitamin A	Source[3] Carotene	Efficacy ratio[4] A/carotene	Reference
				Chicks			
1936	-	-	-	FOC	Crystalline	1.0	77
1937	WL	-	Normal and depleted	CLO	Crystalline	1.0	64
				CLO	Sun-cured alfalfa	1.0	
1939	-	-	-	FO ?	Yellow corn	1.33	78
1948	NH	M	Depleted	SA	Crystalline (stabilized)	1.0	65
				SA	Dehyd.alfalfa	1.0	
1948	BPR	MF	Depleted	FOC	Alfalfa meal	< 1.0	32
1949	NH × WL	-	-	HLO	Crystalline β	> 1.0	33
1951	BPR	-	Depleted	Ac	Crystalline β	1.0	13
	BPR	-	Depleted	CLO	Crystalline β	1.0	
1955	IR	MF	Depleted	SDA	Alfalfa meal	> 1.0	12
1955	BPR	-	-	DA	Alfalfa meal	> 1.0	24
1959	C × PR	-	Intermittent feeding	SDA	Alfalfa meal	> 1.0	28
1959	CR	MF	Depleted	SDA	Cereal grass	1.0	57
			Depleted	FO	Cereal grass	1.0	
1960	WPR	-	Coccidiosis-infected	SDA	Crystalline β & santoquin	> 3.0	16
1963	AOV	MF	Depleted	SDA or Ac	Alfalfa meal	1.0	61
				Turkey Poults			
1950	WH	-	-	Ac, CLO	Crystalline β	4.0	25
				CLO	Crystalline β	2.0	
1952	BBB	-	-	Aa or Ae	Crystalline β in oil or alfalfa meal	1.0	34
				Quail			
1952	BWQ	-	-	CLO	Crystalline	1.0	26

1/AOV, various breeds; BBB, broad-breasted bronze; BPR, Barred Plymouth Rock; BWQ, bobwhite quail; C, Cornish; CR, Columbian Rock; IR, Indian River; NH, New Hampshire; WH, White Holland; WL, White Leghorn; WPR, White Plymouth Rock.

2/M, Male; MF, mixed.

3/Aa, vitamin A alcohol in oil; Ac, vitamin A acetate in oil; Ae, vitamin A esters; CLO, cod liver oil; DA, dry vitamin A; FO, fish oil; FOC, fish oil concentrate; HLO, halibut liver oil; SA, stabilized vitamin A; SDA, stabilized dry vitamin A.

4/On the basis of international units.

Table 8. Efficacy Ratios of Vitamin A and Carotene in 19 Studies on Storage of Vitamin A in Livers of Poultry

Date	Breed[1]/	Sex[2]/	Age and special conditions	Source[3]/ Vitamin A	Source[3]/ Carotene	Efficacy ratio A/carotene	Reference
1941	BPR	-	1-8 weeks	CLO	Alfalfa meal	≤ 1.0	66
					Crystalline β in oil	≤ 1.0	
1945	RIR & RIR × LS	-	1-4 weeks	SLO	Crystalline β	> 1.0	73
1948	-	F	Breeder hens	FO	Alfalfa meal, silage, green feed	< 1.0	74
1948	BPR	MF	Depleted chicks, 2-8 weeks	FOC	Alfalfa meal	< 1.0	32
1949	NH × WL	-	Depleted chicks, 2-4 weeks	HLO	Crystalline β	≥ 1.0	33
1949	WL	F	Depleted hens	FO	Crystalline β, fresh & dehyd. alfalfa	> 1.0	22
1951	BPR	-	Depleted chicks to 8 weeks	Ac	Crystalline β	> 1.0	13
				CLO	Crystalline β	> 1.0	
1955	NH × BPR	-	Chicks normal & deutectomized	Ac	Cereal grass	> 1.0	39
1955	BPR	-	To 10 weeks	DA	Alfalfa meal	> 1.0	24
				FO	Alfalfa meal	> 1.0	
1957	WL	-	Depleted chicks to 8 weeks	FO	Fresh oats & cowpeas	< 1.0	5
				FO	Horse bean & sweet potato leaves	> 1.0	
				FO	Corn leaves	1.0	
1959	WV	-	To 31 days	SA	Alfalfa & corn	2.6	15
				FO	Alfalfa & corn	1.3	
				FO	Crystalline β	> 1.3	
1959	CR	MF	Depleted chicks	SDA	Cereal grass	> 1.0	57
				FO	Cereal grass	1.0	
1959	RW	F	Depleted hens	A	Dried or ensiled clover	> 1.0	36
1961	B	-	1-7 weeks	GB	All-*trans* in gel. bead.	3 to 4	45
1963	AOV	MF	Depleted chicks	SDA or Ac	Alfalfa meal	> 1.0 (under some conditions)	61
1963	B	MF	Depleted chicks to 7 weeks	GB	All-*trans* in gel. bead.	2.5 to 4	44
1950	WH	-	To 8 weeks	Ac	Crystalline β	> 1.0	25
				CLO	Crystalline β	> 1.0	
1948	BWQ	-	To 10 weeks	Ac, Aa or Ae	Alfalfa meal or Crystalline	2.0 to 4.0 (and higher)	56
1952	BWQ	-	To 16 weeks	CLO	Crystalline Carotene	> 1.0	26

1/B, broiler (unspecified); LS, Light Sussex; RIR, Rhode Island Red; RW, Russian White; WV, White Vantress.

2/F, female.

3/A, concentrate of vitamin A (unspecified); GB, gelatin beadlets; SLO, shark liver oil.

Levels in Blood and Eggs

Of three studies on blood vitamin A (Table 9), carotene was superior to vitamin A in one on hens and inferior in two on chicks and turkey poults. Of four reports on vitamin A in eggs, carotene gave higher levels than vitamin A in two, similar levels in one and lower levels in another.

Livability and Survival Time

In one report on livability and survival time of bobwhite quail (Table 9), vitamin A was superior to carotene.

Discussion

Some scientific papers that are often cited in discussions on vitamin A requirements of poultry are not included in the tables. Some of these (67, 71, 72, 76) gave the carotene requirements of poultry at various stages of life, but without direct comparisons with requirements of preformed vitamin A. Others (2, 23) that did compare carotene and vitamin A efficacies are omitted because losses in one or other of the vitamin sources during the experiments invalidated comparisons.

Although closely related to the main subject, neither determination of requirements nor elucidation of deficiency symptoms in poultry are reviewed here. Many excellent treatments of these two topics have already been published. Moore (51) reviewed the establishment of requirements. Deficiency symptoms were described in detail by Moore (50), Wolbach (79) and the NAS-NRC subcommittee on poultry nutrition (55).

In the results summarized, perhaps the most obvious feature is the lack of harmony. In only a few of the reports were numerical estimates of superiority given, but for liver storage vitamin A was more effective than carotene in most of the tests on poultry, on the basis of accepted units. For growth, vitamin A was used more efficiently than carotene in half of the studies and equally efficiently in all but one of the remainder. Neither the preformed vitamin nor the provitamins were in the limited number of studies on levels in blood and eggs and on livability and survival.

Although the relative efficacies may differ for different functions, vitamin A was more effectively used than carotene in more than half (60%) of the 43 studies reviewed above, and in 76% of the 25 studies reported from 1950 to the present.

The multiplicity of sources and of experimental conditions complicates the picture. As stated previously, the various factors cannot be appraised thoroughly, but comments on some are

Table 9. Efficacy Ratios of Vitamin A and Carotene in 8 Studies on Levels in Blood and Eggs and Livability and Survival Time of Poultry

Date	Breed	Sex	Age and special conditions	Source		Efficacy ratio A/carotene	Reference
				Vitamin A	Carotene		
				Levels in Blood			
1948	-	F	Breeder hens	FO	Alfalfa meal, silage, green feed	< 1.0	74
1950	WH	-	To 8 weeks	Ac	Crystalline β	> 1.0	25
1951	BPR	-	Depleted	Ac	Crystalline β	> 1.0	12
				CLO	Crystalline β	> 1.0	
				Levels in Eggs			
1943	-	-	-	SLO	Crystalline	1.0	1
				SLO	Alfalfa and carrots	1.0	
1945	RIR × LS	-	-	SLO	Crystalline β	> 1.0	73
1948	-	-	-	FO	Alfalfa meal, silage, green feed	< 1.0	74
1949	WL	-	Depleted	FO	Crystalline β, fresh and dehydrated alfalfa	< 1.0	22
				Livability and Survival Time			
1952	BWQ	-	A-free ration from 16 weeks	CLO	Crystalline Carotene	> 1.0	26

warranted. For example, depleted chicks were used in five of the eight growth studies in which carotene was superior or equivalent to vitamin A. But depleted birds were clearly used in only one of three studies on liver storage in which carotene was superior, and were used in at least seven of 12 chicken studies in which vitamin A was superior. "Depletion" is, of course, a variable and relative condition.

No differences were suggested between males and females, between breeds or between age groups in response to the two sources of A activity.

Also, carotene was inferior to vitamin A in the eight studies on liver storage in which vitamin A acetate was used in oil solution or in stabilized carriers, but was superior in the one growth study in which fish oil was used. Carotene and vitamin A acetate were equivalent for growth, however, in five of ten studies.

In this review magnitude of responses has not been related to levels of intake. It has been commonly considered (31, 43, 47, 55, 56) that relative efficiency of carotene is greater at lower than at higher levels of intake, particularly those considerably in excess of requirements; most liver storage studies fall in the latter category. In 1963, Parrish et al. (61) concluded that depleted chicks utilize equal units of vitamin A from standard preparations and carotene from alfalfa equally well but that, under certain conditions, liver storage may be somewhat higher if vitamin A is fed. Complicating effects of instability of carotene on measurements of utilization were minimized by low-temperature storage of the alfalfa and by weekly mixing of rations; other details of the experiment were also carefully controlled. In only one of five experiments involving ration levels of 400 to 1,200 IU per pound was gain or liver storage greater with vitamin A than with carotene. The authors, like others, pointed out the lack of information on level of liver storage that may be desirable or necessary. Additional work (D.B. Parrish, personal communication) confirmed earlier findings (61) and indicated that at higher levels of supplementation pre-formed vitamin A gives much larger liver stores of the vitamin than do similar levels of carotene.

The relative biological efficiencies of the two forms in practical feeding situations, involving many or all of the factors discussed above and in the section to follow, may possibly differ from those in more refined experiments with pure substances. Recently, Sibbald and Hutcheson (68, 69) reported results that tend to support the commonly accepted view that, for the chick, 0.6 μg of β-carotene is equivalent to one IU of vitamin A. These results were obtained under practically ideal

conditions in which conversion of carotene in aqueous suspension was determined after injection into the ligatured duodenum of the chick.

For practical purposes, published reports evidently do not confirm the generally accepted view that carotene and vitamin A are equally effective (in terms of international units) in poultry feeding. Rather, the evidence points to the superiority of vitamin A over carotene. The variability of experimental conditions and the fact that numerical efficiency factors were not derived in most of the studies make it difficult to select a practical efficacy ratio. However, provisional acceptance of a ratio of 1.5:1, on the IU basis, would be an improvement over the current use of the 1:1 ratio for the efficacies of vitamin A and carotene. Thus 1.5 IU (0.9 μg) of β-carotene should be considered to be equivalent to one international (or U.S.P.) unit of preformed vitamin A.5/

Factors Affecting Utilization of Vitamin A and its Provitamins

In addition to various factors that may have been operating in the studies summarized, utilization of various forms of vitamin A may be affected by many other factors and circumstances in practical feeding. Some of the reports on these influences warrant consideration here.

Age of Birds

Reports differ on the ability of young chicks to use carotene. Rubin and Bird (66) and Bolin et al. (9) reported that chicks used carotene from dehydrated alfalfa as early as the first week of life. Temperton and Dudley (73) reported similar early usage of pure β-carotene but more efficient use of vitamin

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5/ In their publication "The Nutritive Requirements of Farm Livestock, No. 1, Poultry, Summary of Recommendations," the Agricultural Research Council of Great Britian states: "By definition, the activity of β-carotene, on a weight basis, is half that of vitamin A, but its biological value in terms of vitamin A may be reduced by a variety of factors affecting the utilization of carotene in the diet. We suggest therefore that, in practice, β-carotene may have weight for weight about one-sixth of the value of vitamin A, although opinion on this matter is still divided."

A than carotene during the first 4 weeks of life. Harvey et al. (27) found that deutectomized chicks used pure carotene by the 5th day of life and probably earlier, and Laughland and Phillips (39) reported that intact and deutectomized chicks used both pure vitamin A and β-carotene from cereal grass for growth and liver storage before 2 weeks of age, the carotene giving less storage than vitamin A. Nine years earlier Mann (43) had found that undepleted chicks did not use either vitamin A or carotene for liver storage during the early weeks of life; carotene from green feed was used at about the 22nd day but preformed A from various sources was not used until birds were 35 to 42 days of age.

Disease

Though the effect of disease on vitamin A requirements and on efficiency of carotene conversion was not reviewed thoroughly, two conflicting reports were found. In 1960, Erasmus et al. (16) showed that both carotene and preformed vitamin A were used less for growth and liver storage in coccidiosis-infected than in normal birds. Much earlier, Rubin and Bird (66) reported that pullorum disease had no effect on liver vitamin A stores of young chicks.

Source of Provitamin

The extent to which β-carotene (or other carotenoids) in alfalfa, grasses or other plant sources is available for conversion to vitamin A depends first of all on the extent to which the bird can digest the particular carrier to release carotene. Digestibility is not necessarily the same for all products, or for all lots of the same kind of product. Whether or not the carotene released is in the most potent stereoisomeric form, or in the form in which it was originally in the plant, is governed by conditions of processing and storage to which the plant material has been subjected. It is well known that cis isomers of carotene are of lower biological activity than the all-trans, and the ultimate biopotency of the total carotene content of a product depends on the proportions of various stereoisomers present.

How much of the variability of the results quoted in the preceding tables can be attributed to digestibility and stereoisomerism it is impossible to say, but they may have played some part. McCaughey et al. (46) reported that dry heat treatment of alfalfa gave greater retention of carotene than did sun-curing or steam-blanching followed by forced air-drying, and lower availability of the carotene for growth and liver storage in chicks. They considered, however, that the differences could not be accounted for by change in stereoisomeric composition.

Mangelson et al. (41) found that chicks used carotene of sun-cured and dehydrated alfalfa similarly for liver storage of vitamin A. Ascarelli and Bondi (5), comparing liver storage from carotene in various fresh forages with that from vitamin A in a fish oil, found oats and cowpeas superior to the oil, corn leaves equal, and horse bean and sweet potato leaves inferior. They also reported that the results could not be explained by differences in digestibility or in stereoisomeric composition of carotene.

Natural or Added Inhibitors or Enhancers

Ascarelli and Bondi (5) found no evidence of plant components affecting storage of vitamin A from carotene sources, although Frey and Wilgus (22) believed alfalfa carried a factor enhancing utilization of carotene, for better results were obtained with alfalfa carotene than with pure carotene in oil. Sherwood and Fraps (67) found growth to be slightly but not significantly better with alfalfa leaf meal than with an oil solution of carotene. Ely (15) also reported greater liver storage in chicks with carotene from alfalfa and corn than with two dry-carrier forms. An antioxidant (BHA and BHT) was mixed with all vitamin A sources before these were incorporated into test rations, and the antioxidant may have "overstabilized" carotene in the dry carriers because chemical assays showed no losses of carotene from any of the feeds. Marusich and Bauernfeind (44) recently reported that carotene in dehydrated alfalfa was utilized as well as carotene in gelatin beadlet form, judged by liver storage. Antioxidants increased storage of carotene from alfalfa.

Mann (42) showed that ether-extractable portions of some, but not all, lots of meat meal and fish meal used in chick rations contained a factor destructive to vitamin A both in vitro and in vivo.

Utilization of vitamin A and carotene by various species is apparently affected by carotenoids and related compounds that are not active as provitamins. Moore (48) reviewed observations in this field, including those of Vavich and Kemmerer (75), who found that xanthophylls in chick rations reduced liver storage of vitamin A at high but not at low levels of carotene intake.

Ershoff et al. (18) and Ershoff and Hernandez (17), using rats reported that alfalfa and other plant materials (and other ration components to a lesser degree) contained an unknown vitamin A-enhancing factor, which in alfalfa was water-soluble. Camp et al. (12) found that choline supplements enhanced the utilization by chicks of carotene from alfalfa, but Kramke et al. (34) found no effect; the latter group did observe enhanced utilization with supplements of soybean lecithin (crude or defatted) and of soybean oil.

Naturally occurring and intentionally added antioxidants in carriers of carotene and vitamin A have received rather extensive but still insufficient study. These substances are thought to retard destruction of the vitamin and provitamins within the digestive tract and possibly within body tissues, as well as in the source stored before ingestion. These aspects are of practical importance. Indeed, some of the variability in findings presumably may have resulted from different antioxidant capacities in sources and rations, whether intentional or not. Also, variations in pro-oxidant composition undoubtedly occurred, particularly in fish oil sources. Possibly these factors explain why carotene was reported superior to fish oil vitamin A mainly before attention to antioxidants and pro-oxidants was common.

Additives of other types may also affect utilization of carotene and vitamin A, and some of these, especially antibiotics, are in common use. Burgess et al. (11) reported that penicillin in chick rations increased liver stores of vitamin A and levels of carotenoids in blood serum. Murray and Campbell (52, 53) found enhanced utilization of vitamin A in rats receiving chlortetracycline (Aureomycin). Many pelleted rations contain bentonite, a substance that adsorbs carotene and vitamin A and reduces utilization by rats of vitamin A and carotene from a purified diet (37, 38). Briggs and Spivey (10) reported that bentonite added to a synthetic diet produced vitamin A deficiency in chicks, but found no evidence for such an effect in practical chick rations. It has also been reported that "soft phosphate" interferes with utilization of carotene and vitamin A (19), presumably through adsorptive action of the clay.

Zimmerman et al. (80) found that furazolidone fed at a variety of levels had no effect on utilization of carotene by laying hens.

Quantity and Quality of Nutrients in Rations

There is considerable evidence that utilization of vitamin A and carotene by rats and other species may be affected by the level of other nutrients (fats, proteins, vitamins) in rations. Quality of proteins and fats, apparently, can also affect this utilization. Moore (49) reviewed these effects. Influence of tocopherols (29, 40) is rather widely appreciated. High and Wilson (30) found that in rats vitamin B_{12} enhanced liver and kidney storage of vitamin A from carotene more than that from preformed vitamin A. Ascarelli and Senger (6) observed improved utilization of vitamin A when rations were supplemented with soybean oil or soybean oil soapstock, but could not rule out the possibility that tocopherol content of the fats might have contributed to the improvement.

Not all of the numerous recent reports on effects of quality and quantity of protein on utilization of vitamin A are in agreement, perhaps partly because of different methods of interpretation. But relatively few of the studies have dealt with poultry. Olsen et al. (58) reported an inverse relationship between dietary protein level and storage of vitamin A in livers of 4-week-old Columbian Rock chicks, but Ascarelli and Senger (6) concluded that utilization of vitamin A by White Leghorn cockerels, judged by growth and liver storage, was not affected by variations in dietary protein within the range likely to be encountered in practice.

Among reports on effects of dietary protein on vitamin A utilization in rats, that of Esh et al. (21) suggests that vitamin A is not fully utilized when there is insufficient protein in the diet. Esh and Bhattacharya (20) found less liver storage of vitamin A in rats fed various plant proteins than in those receiving the same level of casein. Rechcigl et al. (63) reported that efficiency of utilization of vitamin A was decreased with rations containing protein of inferior quality but was unaffected by level of dietary protein.

Temperature

Interest in possible effects of environmental temperature on metabolism of carotene and vitamin A has developed in recent years. Effects reported for different species are not always in agreement. Kurnick et al. (35) reported that liver storage of vitamin A by chicks was greater during cool than in warm weather, but Ascarelli and Bartov (4) found no evidence that young chicks required more vitamin A at high temperatures. Phillips (62) found that utilization of carotene for storage as vitamin A in the liver was greater in rats kept at 2 C than at 22 C, but hepatic storage of orally administered vitamin A and rate of metabolism from storage were unaffected by these environmental temperatures.

Reliability of Methods of Analysis

A topic so far given only passing mention, but important in both investigational and practical aspects of this vitamin A-carotene problem in any species, is adequacy of the methods of analysis used for determining the two types of active substances. Closely allied to this are purity, concentration and stability of the many reference or "standard" preparations used in investigations over the years, and validity of mathematical factors used in calculations on which conclusions have been based. Undoubtedly many studies reviewed here had shortcomings of these types.

Presumably the introduction in 1949 of better-defined standards for both vitamin A and carotene enhanced the validity of later work, as have improvements in analytical techniques. However, there is little ground for complacency, particularly in analysis of mixed feeds and feed ingredients other than vitamin concentrates. The highly variable results for both carotene and vitamin A, obtained on alfalfa meal and mixed feeds in joint studies sponsored by the Association of Official Agricultural Chemists (8, 59, 60) and by the Association of American Feed Control Officials (7), and continuing attention to these methods of analysis will attest to this situation.

METHODS OF EXPRESSING POTENCIES AND REQUIREMENTS OF VITAMIN A AND THE PROVITAMINS

Recently it has been the custom, wherever possible, to express vitamin concentrations and requirements as exact weights of pure substances rather than as units of defined biological activity. But use of two "biological" units has persisted for vitamin A.

Continued use of the international unit for either preformed vitamin A or the provitamin seems to be unnecessary in view of the practicability of using mass units (micrograms or milligrams). In the first vitamin A studies, the international unit was a biological unit defined in terms of a biological response. Now, with redefinition in terms of exact weight, both for vitamin A and for carotene, international units have ceased to be strictly biological units, although they may provide a basis for equilibration of various source materials in terms of biological effect. The same result could be achieved by expressing micrograms of carotene as the equivalent weight of vitamin A, i.e., in micrograms. Recent adoption of the "niacin-equivalent" concept of tryptophan (54) is a precedent for this approach; both of these substances are conventionally measured and quoted in exact weights. The result would be the same as at present, with the same problems concerning conversion factors but without the unnecessary complication of two "international units" that have to be correlated but are not necessarily equivalent.

Interest in and need for expressing carotene content in terms of its vitamin A equivalent would be the same under such a system as at present. For either system to be reasonably satisfactory, much more information than is now available is required, and emphasis should be placed on obtaining it.

It was recommended earlier that for poultry one international unit of vitamin A be accepted provisionally as having 1.5 times the efficacy of one international unit of carotene. In units of mass this means that a vitamin A:carotene efficacy ratio

of 3:1 should be adopted provisionally for poultry; i.e., 1 μg of vitamin A should be considered biologically equivalent to 3 μg of β-carotene.

SUGGESTIONS FOR FURTHER STUDY

(a) The more closely it is desired to correlate activities of provitamins and preformed vitamin A, the greater is the accuracy required in determining the content in natural plant sources of β-carotene, of its stereoisomers and of other carotenoids. A continued study of methods of analysis is required.

(b) Confirmation of the biopotency of stereoisomeric forms will be desirable if substantial amounts are found in feed sources.

(c) Information is needed on "digestibility" by poultry of provitamins in practical sources.

(d) Careful evaluation of relative efficiencies of carotene and vitamin A for growth and for low to moderate (not excessive) liver storage of vitamin A under defined conditions of nutritive history should be undertaken. For practical purposes these conditions should be "normal" rather than "depletion", although evaluation of the possible effects of depletion is of interest. (In both c and d nutrients, especially proteins, fats and Calories, should be carefully equalized.)

(e) Extensive study is required of the effects on both vitamin A and the provitamins of various additives (antioxidants, antibiotics, pelleting agents) and of possible enhancing or inhibiting factors that may be present in ration ingredients either naturally or because of processing.

Although it is of academic interest to gain as much information as possible on all aspects of the functions and relationships of vitamin A and carotene, the emphasis on gaining information for carotene under various practical circumstances may depend on the extent to which provitamin carriers are found to be substantial and important sources of the vitamin in practical poultry feeding. Availability of new carotene sources such as fermentation products (3) that can be produced in forms incorporating antioxidants (14) may have a bearing on the situation.

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PROVITAMINS A IN HUMAN NUTRITION

T. K. Murray and J. A. Campbell
Food and Drug Directorate
Department of National Health and Welfare
Ottawa

Provitamins A are a major source of vitamin A activity in the human diet, and 900 µg of β-carotene (1,500 IU provitamin A) will satisfy the adult requirement of 0.693 µg of vitamin A alcohol (1,300 IU vitamin A). Several factors affect utilization of different sources of the provitamins, but for the purposes of public health recommendations a provitamin A unitage three times that of vitamin A appears to be sufficiently accurate. The trend towards more general use of mass units should be encouraged, but research must be directed to measurement of "digestibility" of common dietary sources of the provitamins and to the development of assay methods suitable for routine use.

Chemical analyses (18, 24) indicate that carotene provides one half to two thirds of the vitamin A activity in the diet of North Americans. It is extremely important, therefore, to know how carotene fulfills the functions of vitamin A and to know the relationship between chemical analyses and biological potency. In this report we review literature on this subject, make recommendations on the preferred method of expressing the potency of vitamin A and carotene, and point out areas in which additional research is required.

BIOLOGICAL VALUE OF CAROTENE

Information on the daily requirement of humans for vitamin A and carotene is limited. Booher, Callison and Hewston (1) found the daily intake of vitamin A (in cod liver oil and a small amount in the food) necessary for maintenance of normal dark adaptation in five human subjects ranged between 25 and 55 IU per kilogram of body weight (W_{kg}). To maintain the same state with carotene required 43 to 103 W_{kg}. Ratios of requirements in international units were remarkably constant for all subjects: to maintain normal dark adaptation carotene (in cottonseed oil) was 50 to 60% as effective as vitamin A. Wagner (20) deprived human volunteers of vitamin A until dark adaptation deteriorated and found that normal vision was restored by 2,500 IU of vitamin A or 5,000 IU of carotene. Although Moore (17) suggested that Wagner's vitamin A preparation was overevaluated and that the requirement indicated by the experiment was 1,140 rather than 2,500 IU, this has been denied (11). From results obtained with other species in which requirements were related to body weight, Guilbert, Howell and Hart (9) calculated the human requirement to be 1,400 IU of vitamin A and 2,800 IU of carotene.

The most useful study relating carotene intake to vitamin A requirement of the human adult is the Sheffield experiment (11). Volunteers were fed a diet deficient in vitamin A for 6.5 to 25 months. Doses of preformed vitamin A and of carotene were then assessed for effectiveness on the basis of protection against declining serum levels of vitamin A, deteriorating dark adaptation and high rod threshold. Absorption of small doses of vitamin A appeared to be complete and the minimum protective dose (minimum requirement) was estimated to be 1,300 IU (390 µg) of vitamin A daily. When carotene was administered, some of the dose was excreted in the feces and for this reason the authors introduced the term "maximum effective dose," meaning that portion of the total dose not excreted in the feces. On this basis a maximum effective dose of 1,500 IU of provitamin A (900 µg of β-carotene) was found to be the minimum protective dose, equivalent in biological value in the human adult to 1,300 IU of vitamin A. Thus 0.693 µg of β-carotene gave results similar to those from 0.3 µg of preformed vitamin A, a relationship remarkably close to that found in the rat, i.e., 0.6 µg of β-carotene = 0.3 µg of vitamin A.

Though all attempts at estimating minimum human requirements of vitamin A have suffered from insufficient numbers of depleted subjects, there is reasonable agreement that the requirement is near that found in the Sheffield experiment.

Of practical importance is the extent to which requirements for vitamin A can be satisfied by various sources of provitamins A. This problem is made complex by many factors that affect the utilization of carotene. Chief among these is digestibility, and Table 10 gives findings from the M.R.C. report of the Sheffield experiment on fecal excretion of carotene from different sources (11). Excretion ranged from 26% of intake for carotene administered in oil to 76% when sliced carrots were fed. In practical terms these results mean that an individual must consume 2,000 IU of carotene in oil or 6,000 IU in carrots to ensure receiving a minimum protective dose of 1,500 IU. Other studies (13-16) gave results similar to those in Table 10, but none was based on enough subjects or on enough variety of sources of carotene. There is a great need for further work in this area.

Measurement of fecal excretion of carotene is useful in comparison of various sources but may lead to erroneous conclusions because the minimum protective, as well as the maximum effective, dose of provitamins A may vary with the source. Thus it was observed in the Sheffield experiment (11) that leafy vegetables were more effective than oil solutions of β-carotene in maintaining blood levels of vitamin A, although much less carotene from the latter source appeared in the feces. Kramer

Table 10. Fecal Excretion of Dietary Carotene by Man

Source of dietary carotene	Amount excreted per unit ingested	
	Mean	Range
Carotene in oil or margarine	0.25	0.16-0.48
Carrots, homogenized	0.45	0.34-0.56
Cabbage, spinach	0.60	0.25-1.00
Carrots	0.75	0.62-0.92

and Tarjan (15) recently confirmed this result with rats. Addition of tocopherol to the diet was shown (8) to increase simultaneously fecal excretion of β-carotene and liver storage of vitamin A. Obviously part of a maximum effective dose may be degraded in the digestive tract, with loss in biological activity.

Carotenoids other than β-carotene, and isomers of β-carotene, complicate attempts to evaluate and express the contents of provitamins A. The usual practice is to separate the carotenoids during chemical assay and to make allowance for different biological values by using appropriate factors. If the factors are appropriate this should not be a major source of error. Isomers of β-carotene, however, are not usually taken into account, although their biological value may be almost 50% of that of all-*trans* β-carotene (6, 13). They may occur in raw leafy vegetables to the extent of 20% of the total β-carotene and double that amount after cooking (7). Some commercial sources of β-carotene are almost 50% *cis*-isomers (2). A simple method for estimating *cis*-isomers must be devised before proper allowance for their low biological value can be made routinely.

The foregoing shows that the contribution to vitamin A intake of dietary carotene is governed by four main factors: amount of biologically active carotenoids; *cis*-isomer content of the carotenoids; their stability in the gastrointestinal tract; and their digestibility. In practice, biologically active carotenoids can be separated and determined chemically, and correction is usually made for incomplete absorption, but the *cis*-isomer content and instability in the gastrointestinal tract are not usually taken into account. Different sources of carotene vary so greatly in digestibility (Table 10) that no single factor can be applied to individual foods. Yet it is convenient to use such a factor in evaluating all carotene sources of

vitamin A activity in mixed diets, and several such factors are in use. The Food and Nutrition Board (18) used a factor of 2 in recommending a daily intake of 3,000 IU of vitamin A or 6,000 IU of β-carotene, whereas the Canadian Council on Nutrition (5) used a factor of 4 for the same purpose. Pett (19) stated: "Since carotene is converted in the body so irregularly, at least three times the unitage must be consumed as carotene over apparent requirements for actual vitamin A." The M.R.C. report (11) recommended that separate factors be used for various classes of foods, e.g., root vegetables and leafy vegetables, but stated that a factor of 3 should be used if there was an urgent need for a single factor. Available evidence favors the last factor, but it should be emphasized that it can be properly applied only to the total carotene intake from a mixed diet.

PREFERRED METHODS OF EXPRESSING POTENCIES OF VITAMIN A AND PROVITAMINS A

The Expert Committee on Biological Standardization (10), in defining the international unit of provitamin A, recommended that vitamin A and its provitamins be expressed in their respective units. Since that time two international committees (12, 22) have also recommended that β-carotene should not be expressed as IU of vitamin A but as IU of provitamin A or in mass (weight) units. Yet in many nutrition texts, including Table of Food Values Recommended for Use in Canada (4), carotene contents of foods are given in international units of vitamin A, conversion being made on the basis that 0.6 μg of β-carotene and 1.2 μg of β-carotene are each equal to 1 IU of vitamin A. This gives the same numerical value as that given by the provitamin A unit, but the latter involves less danger of misinterpretation. Regulations under the Canadian Food and Drugs Act (3) require that carotene be expressed in international units of provitamin A when claims are made for its presence in foods and drugs.

Quantities of chemicals are generally expressed in mass units, and this principle holds for most vitamins although they are valuable almost exclusively because of their biological effect. Changing from biological to mass units presented little difficulty with the B vitamins which are not encountered in such a variety of forms of differing activity. We consider it is not fully informative, however, to describe quantities of vitamin A in mass units, because different forms of the vitamin vary so widely in biological activity. Vitamin A acetate, for example, has 1.59 times the potency of the same weight of palmitate, and *cis*-isomers of vitamin A range in biological activity from 20 to 70% of the all-*trans* form.

It is obviously necessary that potency be expressed in terms of a standard. The U.S. Pharmacopeia (20) took a step in this direction by describing vitamin A potency in terms of both U.S.P. units (equivalent to IU) and milligrams, all forms of vitamin A being expressed as the equivalent weight of vitamin A alcohol. It would be desirable to specify further that vitamin A be expressed in terms of all-*trans* vitamin A alcohol because of the lower activities of the various *cis*-isomers. All this is accomplished by use of international units, a term with which the public and clinicians are familiar. Though consumers are being educated to the meaning of statements of vitamin A activity in foods and drugs in terms of weight, it is essential that a double labeling system be used.

Retention of international units is recommended for the labeling of foods and drugs for human use for the present, but the trend toward more general use of mass units is recognized and should be encouraged when materials used and experimental conditions can be described in detail.

AREAS IN WHICH FURTHER RESEARCH IS REQUIRED

Because information on the subject is inadequate, almost any research on utilization of vitamin A and carotene by man would be of value. The following would be of particular interest:

Repetition of the Sheffield experiment with more subjects and other sources of carotene.

Measurement of "digestibility" of common sources of carotene to obtain factors appropriate for estimating vitamin A activity of individual foods.

Further study of occurrence and biological potency of *cis*-isomers of carotene.

Development of a chemical or physicochemical assay method suitable for routine use.

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UTILIZATION OF CAROTENE AND VITAMIN A BY OTHER SPECIES

H. D. Branion
Department of Nutrition
University of Guelph, Guelph

FOX AND MINK

Mattson (87) summarized the requirements of these species, and Rubin and De Ritter (124), an NAS-NRC committee (105), and Aitken (3) reviewed symptoms of deficiency and requirements for the vitamin.

Fox-- Smith (135, 136) reported that symptoms of vitamin A deficiency in the fox are nervous disturbances: trembling and cocking of the head, periods of whirling and, in some cases, coma; xerophthalmia, widespread epithelial metaplasia, demyelinization of many nerve fibers, and abortions. Bassett et al. (10) found deficiency symptoms to include head cocking, unsteadiness of gait, weaving, whirling, xerophthalmia, emaciation, coma and death. Autopsy revealed urinary calculi, gastritis, enteritis, pyelitis, and general inflammation of the urinary tract. In some cases pus was found on the feet, on jaws, and in the urinary tract. At times, the milk and adult teeth were present in the same socket, and many times the small, discolored adult incisors were chipped or broken on the upper corners.

A wide variation in vitamin A content of the liver of foxes has been observed (65, 66, 115, 116), but there has been no consistent relation to fur quality (65, 115, 116).

Coombes et al. (34) reported that foxes grew satisfactorily to maturity on an experimental ration supplying 0.1 to 0.2 µg of vitamin A per gram of wet feed. However, vitamin A levels of the liver and blood of these animals was very low. Administration of 1,500 IU of vitamin A daily as cod liver oil throughout 210 days brought the levels within the range of those of normal adults. Administration of 1,500 IU of crystalline β-carotene for 84 days caused only a small increase in vitamin A in the blood, no increase in carotene in the blood, and no liver storage of vitamin A.

Smith (135) found that the minimum vitamin A intake necessary to prevent occurrence of nervous symptoms in growing pups was between 15 and 25 IU per kilogram of body weight per day (W_{kg}/day), but 50 to 100 IU were required for liver storage. Bassett et al. (11) recommended that growing foxes be fed between 100 and 600 IU (23.2 to 138.2 µg) of vitamin A, or at least 600 IU (360 µg) of carotene per W_{kg} per day. Later, Bassett et al. (12) lowered their recommendation to 25 to 50 IU (6.2 to 12.4 µg per W_{kg} per day). This could be supplied by adding 10 to 20 IU

of vitamin A per pound of moist ration. Albritton (5) summarized the requirement of the growing fox as 0.04 to 0.08 mg of carotene daily, and of the mature fox as 0.03 mg. Spector (137) gave the requirement as 100 to 135 IU per W_{kg} daily for growth and 50 IU for maintenance.

The fox can utilize carotene as a source of vitamin A, but conversion is inefficient (11, 34). The NAS-NRC committee (105) recommended in 1953 that growing foxes be fed 100 IU of preformed vitamin or 600 IU (360 µg) of β-carotene daily per W_{kg}, i.e., 46 IU of the vitamin or 273 IU (164 µg) of β-carotene per W_{lb}. For dry feed, the vitamin A allowance was 1,720 IU per kg. By weight, the ratio of efficiency of vitamin A to carotene was 1:12.

The daily allowance of males ranged from 100 IU when weaned at 7 weeks of age to 367 IU at 19 weeks, decreasing to 257 IU at pelting at 35 weeks of age. Corresponding requirements for females were 62, 289 and 203 IU.

Helgebostad (57) reported that foxes could tolerate for 3 or 4 months as much as 40,000 IU of vitamin A per W_{kg} per day from a cod liver oil concentrate, either injected or fed. Fully grown animals tolerated 200,000 to 300,000 IU per W_{kg} per day for 6 to 8 weeks, but young animals were affected in a shorter time. Signs of hypervitaminosis were anorexia, bone changes with exostoses, decalcification and spontaneous fractures, loss of fur, exophthalmos and local hyperaesthesia of the skin.

Mink-- Warner (149) reported that kits fed a purified diet deficient in vitamin A developed night blindness, eye involvements and incoordination. Histological examination showed metaplasia of some epithelial tissues. Evans and Czarnocki (40) fed kits a purified diet containing no vitamin A supplement and observed slow growth, eye and respiratory tract infections.

Bassett (8, 9) observed signs of vitamin A deficiency--incoordination of rear quarters, diarrhea, night blindness and inferior pelts--after 58 and 84 days, respectively, on diets containing no vitamin A or 25 IU per W_{kg}. Animals getting 100 IU had no symptoms. He estimated that mink feeds containing 5% liver supply about 675 IU per animal daily, and those containing 2% lucerne green meal supply 390 to 520 IU.

Holmes et al. (66) found that the vitamin A content of livers of ranch minks varied considerably and was generally lower than in wild minks.

The NAS-NRC committee (105) tentatively suggested that the vitamin A allowance for the mink be the same as for the fox. Abernethy, quoted by Warner (149), concluded that the daily

requirement for growth of minks was between 200 and 400 IU per W_{kg} per day. At an intake of 10 IU, chronic deficiency developed, characterized by incoordination of hind quarters. Postmortem examination revealed that the cerebellum had been forced back into the foramen magnum with pressure damage to this part of the brain. There was no metaplasia. Albritton (5) concluded that growing minks require 0.09 to 0.11 mg of carotene daily and mature animals 0.08 to 0.10 mg. Spector (137) recommended 165 to 180 IU per W_{kg} for growth and 135 to 165 IU for maintenance.

Evans and Czarnocki (40) found that, at relatively high levels of feeding, carotene was 1 to 4% as effective as an equal amount (in IU) of vitamin A in promoting liver storage of the vitamin in minks. Warner (150) observed essentially no difference in liver and plasma vitamin A of minks receiving carotene at moderate levels and those on a purified diet deficient in vitamin A. But preformed vitamin A, fed at the same level, gave significant liver storage and plasma levels. Carotene probably should not be considered a provitamin A for minks.

Helgebostad (57) concluded that minks tolerated excess vitamin A to the same extent as foxes.

DOG

The vitamin A nutrition of dogs has been reviewed by Morgan (101), Michaud and Elvehjem (96, 97), Rubin and De Ritter (124) and an NAS-NRC committee (107).

Magendie (82) in 1816 reported the first evidence associating development of sore eyes in dogs with their diet. In 1921, Steenbock et al. (138) observed that dogs fed cod liver oil were normal but those deprived of "fat soluble vitamins" developed xerophthalmia. One affected dog died but xerophthalmia in two others was cured by daily administration of 20 ml of cod liver oil or by an ether extract of 30 g of saponified cod liver oil. In addition to ophthalmia, other workers reported loss of appetite, diarrhea, poor growth, respiratory infections often resulting in death (44, 46, 125, 139), bone changes and deafness (94, 95), degeneration of nerves and skin symptoms (101). Large doses of the vitamin relieved hypertension produced experimentally (70, 148). Low levels of the vitamin in blood plasma (71) and changes in the neutrophile index (36) were cited as early clinical signs of deficiency.

Herrin et al. (59) and Herrin and Nicholes (58) concluded, as a result of urea and insulin clearance tests, that a deficiency of vitamin A affected kidney function.

Livers of newly born pups were found to be deficient in vitamin A, although the maternal diet contained large amounts (22). If the milk supply of the mother was adequate, adequate stores were found in the livers of the pups after 3 months' nursing.

Frohring (44, 45) found that the minimum curative dose for vitamin A-depleted pups was 200 U.S.P. units per W_{kg} per day and the maximum curative dose 700 units. Bradfield and Smith (18) confirmed 200 U.S.P. units per W_{kg} as the daily requirement for growing pups. This permitted some liver storage: higher dietary levels did not improve growth but made the hair coat lustrous and increased liver storage appreciably. In 1940 Morgan (101) set the optimum at over 800 U.S.P. units per W_{kg} per day. Against these estimates are those of Crimm and Short (36) who concluded that dogs utilized 157 to 300 IU vitamin A per W_{kg} per week (22 to 47 IU per W_{kg} daily), and those of Michaud and Elvehjem (96, 97) who estimated the daily requirement at 20 g (67 IU) of vitamin A per W_{kg} (9 μg or 30 IU per W_{lb}). As Frohring (44-46) pointed out, rate of growth affects the amount of vitamin required.

The NAS-NRC committee (107) recommended in 1962 that puppies be fed 200 IU vitamin A per W_{kg} daily (90 IU per W_{lb}). It stated: "While older dogs may require less for metabolism, their absorption rate is much lower and provision for a similar amount in the diet might be advisable. However, the fact that adult dogs can exist for long periods without showing deficiency symptoms and have been known to raise several litters while on a deficient ration makes it appear that adults have a lower requirement." Adult dogs can be maintained on 100 IU of vitamin A per W_{kg} daily (45 IU per W_{lb}).

Evidently carotene is an effective source of vitamin A for the dog (46, 144) and Bradfield and Smith (18) found that 200 U.S.P. units of vitamin A per W_{kg} supplied by cod liver oil, carotene in oil or carrots were effective for pups. Michaud and Elvehjem (96, 97), however, reported that the weight of carotene required was two or three times that of vitamin A, and the NAS-NRC committee (107) concluded: "For dogs carotene is approximately one half as valuable as vitamin A alcohol." If this refers to IU vitamin A and provitamin A, on a weight basis the factor would be four. Albritton (5) had concluded earlier that the daily requirement of the pup was 120 μg of carotene and 59 μg for adult maintenance. Spector (137) listed the daily requirement for growth at 200 IU provitamin A per W_{kg} and for maintenance at 100 IU.

The amount of carotene in blood is slight (71). Dogs fed excessive amounts of vitamin A excrete the vitamin in urine (102). This has not been reported for other species and does not occur when carotene is the source of the vitamin.

RABBIT

Symptoms of vitamin A deficiency in rabbits include xerophthalmia (30, 83, 92, 93, 109-111, 113, 114, 119), retarded growth (113, 114), ataxia, incoordination and disturbance in equilibrium (77, 93, 114), reproductive failure (30, 31, 75, 76, 114), degeneration of nerves (92, 93, 114), sometimes blindness and deafness (93), corneal lesions and lowered ascorbic acid content of the aqueous humour (117), hydrocephalus (74, 77, 98-100), and death (117).

The requirement for vitamin A has not been estimated quantitatively. However, for growing rabbits Hogan and Hamilton (64) developed a simplified diet that contained 12,000 IU of vitamin A per kilogram of diet, and Wooley and Sebrell (155) used a purified diet that contained 6,525 U.S.P. units per kilogram of diet. Kunkel et al. (73) fed a purified diet containing an unspecified amount of vitamin A.

Mann et al. (83) and Pirie and Wood (117) cured eye lesions by oral administration of 500 to 1,000 IU of vitamin A daily. Lamming et al. (75) reported that does given 7,500 µg of vitamin A acetate weekly had an average of 6.1 living fetuses and 0.5 resorption sites at 16 to 28 days, whereas those deprived of the vitamin had 1.9 living fetuses and 3.3 resorption sites.

Mellanby (93) prevented the symptoms of vitamin A deficiency by feeding young rabbits 1 to 3 mg of carotene daily. Phillips and Bohstedt (114) found that 50 µg of carotene per W_{kg} prevented the deficiency syndrome and maintained health, but a 6- to 20-fold increase was required for a remedial dose. Pirie and Wood (117) concluded that rabbits responded better to carotene than to preformed vitamin A, judged by plasma and liver levels of the vitamin.

The NAS-NRC committee (106) made no recommendation for the vitamin A requirement of rabbits.

CAT

Gershoff et al. (47) gave anorexia as the first symptom of vitamin A deficiency in kittens. This is followed by emaciation, weakness and rigidity of the hind legs. Bronchopneumonia and lung infection are common. Squamous metaplasia occurs in a number of organs.

Albritton (5) recommended that raw food for growing cats should contain 81 µg of carotene daily, and canned food 67.2 µg. For maintenance 49.8 and 57 µg were adequate. Spector (137) gave the requirement for growth as 135 IU per W_{kg} daily, and that for maintenance as 85 IU.

Although, within a wide range, the level of dietary fat has little if any effect on absorption of vitamin A in the rat, chicken or man, in the cat rising levels of serum vitamin A follow increases in dietary fat.

For all practical purposes, carotene is not utilized by the cat as a source of vitamin A (2, 48, 120),

Gershoff et al. (47) and Da Silva et al. (38) developed purified diets containing vitamin A supplied as cod liver oil that were satisfactory for growing cats.

The NAS-NRC committee (108) in 1962 estimated from various adequate rations that 2,500 IU of vitamin A per 100 g of diet meets the nutritional requirements of growing cats. This may be higher than the actual requirement.

GUINEA PIG

Mannering (84) reviewed vitamin requirements of the guinea pig. Symptoms of vitamin A deficiency are cessation of growth, weight loss, xerophthalmia and death (16, 17, 58, 142, 152, 153). Wolbach and Howe (153) observed an atrophy of ameloblasts that led to atrophy, metaplasia and calcification of the enamel organ, followed by atrophy of odontoblasts and defective dentine formation. Earlier, they (152) reported that epithelial tissue became stratified and keratinized.

Apparently the guinea pig stores little vitamin A, even when fed diets rich in carotene (16, 25-27, 33, 133), and preformed vitamin A appears to be more than six times as effective as carotene in promoting liver storage of the vitamin. This is remarkable since the natural food of this animal does not contain preformed vitamin. However, there is wide variation between guinea pigs in their ability to convert carotene into vitamin A, and to store the vitamin (25-28).

Metabolism of vitamin A in this herbivorous animal is different from that of the rat. Clausen and McCoord (33) reported that only 6% of a massive dose of vitamin A, as haliver oil, was recovered from liver of guinea pigs in comparison with 71% from liver of rats. Mannering (84) suggested that the guinea pig may have to rely on frequent intake of the vitamin.

Chevallier and Jullien (29) reported that the basal metabolic rate of guninea pigs, without liver reserves of the vitamin, was about 20% higher than that of animals with such a reserve. Abelin (1) found that administration of thyroxine prevents transformation of carotene into vitamin A. Thyroid activity may be concerned in conversion of carotene and storage of vitamin A.

The quantitative requirement of the guinea pig for carotene or vitamin A has not been determined. Bentley and Morgan (16) found that 2 mg of carotene per W_{kg} daily, fed to depleted animals, promoted liver storage of vitamin A that was just significant. Spector (137) reported that the guinea pig required vitamin A for growth.

Roine et al. (122) obtained satisfactory growth with a purified diet containing 1.2 mg of β-carotene per 100 g of diet, and the purified diet of Reid et al. (118) contained 0.06 mg of vitamin A acetate.

The NAS-NRC committee (108) suggested in 1962 that 1.2 mg of β-carotene per 100 g of diet, or 9.6 mg per W_{kg} daily is satisfactory for the growing guinea pig. This may be higher than the actual requirement.

HAMSTER

Schweigert (127) reviewed nutritional requirements of hamsters. Hirschi (63) appears to be the only investigator to produce vitamin A deficiency in this species. He observed weight loss, coarsened and thinned haircoat, xerophthalmia, and hemorrhage in reproductive and alimentary tracts.

There is little information on the requirements for either carotene or vitamin A. Albritton (5) listed 1.1 mg of carotene daily for growth of hamsters, but Spector (137) advocated 1,800 IU per W_{kg} daily. However, synthetic diets that were satisfactory (35, 54) contained preformed vitamin A. Schweigert et al. (128) used a purified diet containing 2,000 IU of vitamin A per 100 g of diet.

The NAS-NRC committee (108) in 1962 considered that 1,300 IU of vitamin A per 100 g of diet meets the nutritional requirements of growing hamsters, although this may be higher than the actual requirement.

MONKEY

Day (39) reviewed literature on nutritional requirements of the monkey. The rhesus monkey certainly requires vitamin A but there is no information on the quantity.

In eight studies (51, 55, 60, 126, 141, 143, 145, 147) on symptoms of vitamin A deficiency in the monkey, mainly rhesus, diarrhea was the most consistent symptom but xerophthalmia, night blindness, edema, cessation of menstruation, slight increase in leucocytes, and keratinization of epithelial tissue were observed. Possibly some of these symptoms were due not solely to lack of vitamin A but also to deficiency of other vitamins.

Albritton (5) concluded that the monkey requires 36 μg of carotene daily for growth and maintenance, and Spector (137) recommended 60 IU per W_{kg} daily.

The NAS-NRC committee (108) in 1962 simply stated that vitamin A is required by the growing rhesus monkey.

MOUSE

Vitamin requirements of the mouse were reviewed by Morris (104) and nutritional requirements by others (6, 37, 41, 103, 156). Vitamin A is required (15, 52, 118, 154). Symptoms observed by these workers, by Fenton et al. (42) and by McCarthy and Cerecedo (89, 90) were xerophthalmia, characterized by a thick, colorless exudate; diarrhea, tremors and unkempt fur. Epithelium in various structures became keratinized. Reproductive function of the male mouse is very sensitive to a deficiency of vitamin A.

Morris (102) estimated the vitamin A requirement of the mouse would be met by 1 μg of β-carotene (0.1 to 0.2 μg of vitamin A) per adult mouse daily. He derived this from studies on other species by Rosenberg (123). This is minimal and does not allow for any storage. "For growth and reproduction at least three times that value for vitamin A and five times the minimum of β-carotene should be allowed." Rosenberg (123) concluded that vitamin A requirements of all mammals would be met by 25 μg of β-carotene (40 IU) or 4 μg (20 IU) of vitamin A per W_{kg}. These values are minimal for normal growth without clinical symptoms of vitamin A deficiency, and with little or no storage of the vitamin. He also advocated three times this minimum of vitamin A and five times that of β-carotene for appreciable storage and for reproduction.

McCarthy and Cerecedo (89) obtained maximum growth with a daily intake of 1 IU of vitamin A. This was adequate to maintain life over a long period, prevent deficiency symptoms, permit appreciable liver storage and allow normal reproduction of adult

males. Hence the requirement may be somewhat less than 1 IU per day. These workers fed three groups of mice 1, 2, or 3 IU of vitamin A per day, and a fourth group received 67,500 IU per kilogram of diet, or between 210 and 300 IU per mouse per day. They concluded: "Vitamin A at a level of 1 IU per day gave as good growth as was obtained at levels of 210 to 300 IU daily." Anonymous (6) also concluded that the mouse needed between 1 and 2 IU per day. Albritton (5) summarized the daily requirement of the mouse as 0.04 mg of carotene and Spector (137) as 1,000 IU per W_{kg}.

Slantez has been quoted (e.g., 108) as having concluded that common laboratory animals require 25 to 39 µg of vitamin A per W_{kg} daily, but it is evident from this paper that he meant 25 to 39 µg of β-carotene. Using the factor 0.6, this is 41 to 66 IU per W_{kg} or about 2 IU for a 40 g adult.

The NAS-NRC committee (108) also estimated that 500 IU per kg of diet or 2 IU per day should meet the requirements of growing, pregnant and lactating mice, without providing a margin of safety for growth. There is no accurate information on efficiency of carotene.

RAT

Dietary requirements of the rat were reviewed by several investigators (6, 37, 41, 81, 91, 156). Effects of vitamin A deficiency were reviewed by Wolbach (147) and McCoy (91). The NAS-NRC committee (108) stated, in part: "The syndrome of vitamin A deficiency is characterized by malformations of the epithelial structures and epiphyseal cartilages followed by growth depression. Epithelial tissues become keratinized. Disorganization of the tooth structure causes an impairment of tooth growth, a distortion of the incisor tooth, loss of the normal orange color. The retardation of skeletal growth results in a compression upon the brain, spinal cord and nerve roots, herniation of the brain into the foramen magnum with consequent mechanical injury to the brain and nerve roots, incoordination in about five to six weeks in weanling rats. Vitamin A is essential for dimlight vision, deficiency results in night blindness. Xerophthalmia is noted by a reddish exudate on the lids of the eye, opaqueness of the cornea, distortion of the shape of the eye. Failure of implantation with the production of aborted or nonviable litters is common, while testicular degeneration is characteristic in the male."

Brown and Sturtevant (20), Mattson (84) and Rubin and De Ritter (124) also reviewed vitamin A requirements of the rat. The reports indicate a wide range in estimated requirements (Table 11) because different workers used different criteria.

Table 11. Requirements of Vitamin A, Estimated by Various Workers, for the Rat

Criterion	Daily requirement, IU	Reference
Growth	3/g diet	23
	40/100 g diet	20
	25/rat	79
	$20/W_{kg}$	23
	$20/W_{kg}$	24
	$25/W_{kg}$	19
	$20\text{-}100/W_{kg}$	124
	$100/W_{kg}$	87
Growth and xerophthalmia	0.5-3/rat	13
Growth and longevity	12/g diet	129
Growth and reproduction	12/g diet	131
Growth and reproduction	$6\text{-}24/W_{kg}$	132
Growth, longevity, teeth and eyes	$100/W_{kg}$	112
Reproduction	6.6/g diet	43
Reproduction and longevity	12/g diet	130
Prevention of gross symptoms	2/rat	79
Middle ear disease	$30/W_{kg}$	24
Tooth color	3/rat	69
	$20/W_{kg}$	24
Blood level	20/rat	67
	50/rat	79
Liver storage	12/g diet	80
	6-12/g diet	23
	8-16/rat	14
	100/rat	79
	$60/W_{kg}$	123
	$80/W_{kg}$	24
	12/g diet	130

Table 11 (Cont'd)

Criterion	Daily requirement, IU	Reference
Liver storage and reproduction	55-60/W_{kg}	53
Liver storage, blood level and longevity	>20-100/W_{kg}	124
Vaginal smeal	20/W_{kg}	24
	18-33/W_{kg}	49
	18-33/W_{kg}	53

Growth was used in one third of the reports. The requirements are expressed in terms of daily intake or amount per weight of diet or per kilogram of body weight. While it can be assumed that a rat will eat 10 g of food daily and a normal, grown rat weighs 325 to 550 g, because growing rats may range in weight from 30 to 150 g the data in Table 11 have not been recalculated to a common basis for comparison.

Brown and Sturtevant (20) tentatively estimated the requirement to be 40 IU per 100 g of ration or 4 units per day for growth and concluded that oily solutions injected either subcutaneously or intramuscularly were utilized poorly if at all. This conclusion is controversial: similar results by parenteral and oral routes have been obtained (54, 71) but not always (7, 32, 50). Mattson (87) concluded that about 100 IU per W_{kg} would allow optimal growth but that the optimal level of vitamin A for longevity and reproduction be far in excess of any that have been considered previously.

Rubin and De Ritter (124) concluded that in general, if good growth and prevention of deficiency signs are the criteria, a range of 20 to 100 IU per W_{kg} per day is satisfactory but much higher levels are required for adequate blood levels, liver storage and longevity. Albritton (5) gave the daily requirement for growth and maintenance as 0.01 mg of carotene, and Spector (137) as 17 IU per W_{kg}. Anonymous (6) recommended 200 IU per day for growth, 360 IU for lactation and 240 IU for gestation.

Based on daily food intakes of 10 g during growth, 20 g during gestation and 30 g during lactation, the NAS-NRC committee (108) in 1962 listed requirements per 100 g diet during

these periods as 200, 1,200 and 1,200 IU respectively: i.e., 200 IU per W_{kg} for growth (say 20 IU per rat daily); 240 IU per rat daily during gestation and 360 IU during lactation.

The rat is most efficient in using carotene as a source of vitamin A. On the basis of rat assays, WHO (157) in 1950 defined 1 IU as equivalent to 0.6 µg of β-carotene or 0.3 µg of vitamin A alcohol. In a collaborative assay the U.S. Pharmacopeia Vitamin Advisory Board (146) reported a potency of 1,667,000 IU per gram of β-carotene, and 3,300,000 IU per gram of vitamin A alcohol. Hence 1 µg of vitamin A alcohol is equivalent in biological activity to 2 µg of β-carotene. Marusich et al. (86) found synthetic β-carotene to contain 1,730,000 IU per gram; that is, 0.58 µg of β-carotene was equivalent to 1 IU of vitamin A activity.

In 1939 Goss and Guilbert (49) concluded that the minimum dietary level of vitamin A to prevent vaginal cornification was 3.8 to 4.6 µg per W_{kg}, whereas the minimal carotene level was between 15 and 20 µg. Guilbert et al. (53) reported the optimum intake per W_{kg} to be 3.8 to 5.3 µg of vitamin A daily, compared with 15 to 20 µg of carotene. They stated: "About three times the minimum vitamin A level and five times the minimum carotene level is about the minimum for significant storage and reproduction." On the basis of their assay of U.S.P. reference oil (0.21 µg, approximating 1 IU), they concluded that at unit level the ratio of efficiency of vitamin A to carotene by weight is 3:1; at the "minimum" level, 6:1; and at the level that allows appreciable storage and successful reproduction, about 10:1. Braude et al. (19) found that 7.7 µg of vitamin A per W_{kg} was adequate for growth, but 40 µg of carotene were required. Expressing requirements in international units, Mattson (84) concluded: "When carotene is to serve as the source of the vitamin, a considerably larger amount is required."

There appears to be good evidence that utilization of β-carotene depends on conditions of the test, particularly the nature of the diet, e.g., presence of tocopherol (21, 56, 72, 140), yeast (68), antioxidants (140) and vitamin B_{12} (62, 88).

Marusich and Bauernfeind (85) reported that β-carotene fed at 10,000 IU per kg of ration provided only about one third the storage of vitamin A given by the preformed vitamin, indicating an effective ratio of 6:1 on a weight basis. At 20,000 IU per kg of ration, storage was about one fifth as good with β-carotene, a ratio of 10:1. Hence relative efficacy also depends on dosage.

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