

The first is the mammalian male reproductive organs, especially the prostate gland and the sperm (Mawson & Fischer, 1952; Hoare, Delory & Penner, 1956) and a deficiency of zinc in the diet of the male rat leads to degeneration of the reproductive organs (Millar, Fischer, Elcoate & Mawson, 1958). The second is the pancreas, and this has been traced to the α -cells. The α -cells of birds are segregated into visible brownish spots, and those of the duck have been dissected out and found to contain ten times as much zinc as the rest of the pancreatic tissue (Weitzel, Buddecke & Kraft, 1956).

Throughout this paper species differences have been highlighted, but of much greater fundamental importance are the similarities from one family of animals to another. Some species have been studied for one aspect of mineral metabolism, some for another, but the same general principles apply to all, for these ultimately depend upon the chemical properties of the mineral. The same minerals are present and they perform the same sort of functions in the bodies of grasshoppers, mealworms, oysters, trout, duck, and foxes as they do in our own.

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Requirements of different species for vitamins

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The original concept of a vitamin was an accessory food factor required in minute amount to prevent or cure certain deficiency diseases in man or animals. It is now recognized that vitamins have a more fundamental role, since many of them have been shown to be essential participants in metabolic reactions that are widespread

throughout living organisms. It would, however, be unwise to attempt any generalization regarding quantitative requirements, because the need of an organism for an exogenous source of a vitamin can be influenced by a variety of circumstances. Any micro- or macro-organism that includes a vitamin-dependent reaction in its metabolism will require an external source of that vitamin unless (a) the end-products of the reaction are supplied by some other means or (b) the organism can synthesize enough of the vitamin to meet its own needs. Either of these situations may apply to a whole group or may be peculiar to individual species or strains.

Alternative sources of end-products

Studies on the metabolic role of vitamin B₁₂ provide examples of the reduction or elimination of a need for a vitamin by provision of the end-products of the reaction in which it participates. In the nutrition of the lactobacilli, vitamin B₁₂ functions in the synthesis of the deoxyribose moiety of deoxyribonucleic acid (Wacker, Kirschfeld & Träger, 1959), hence many strains of lactobacilli can grow adequately if supplied with either deoxyribosides or vitamin B₁₂. This reaction is peculiar to the lactobacilli; some other bacteria require vitamin B₁₂ for the synthesis of methionine and, as the vitamin appears to have no other function in these organisms, methionine can entirely replace it. In animal metabolism also, vitamin B₁₂ participates in the synthesis of labile methyl groups. Thus diets rich in methylating compounds (e.g. choline, methionine) spare the need for vitamin B₁₂ although they cannot entirely replace it since it is also concerned in other metabolic pathways in higher animals.

Synthesis of vitamins

Endogenous synthesis. The ability of an organism to synthesize a vitamin will reduce or even eliminate its need for an external supply. There is greater diversity in the pathways of vitamin synthesis among micro-organisms than among animals. Certain bacteria and fungi can synthesize nicotinic acid from tryptophan, others use ornithine, and the appropriate precursor can replace all or part of the nicotinic acid needed from external sources. The protozoan, *Tetrahymena pyriformis*, has an absolute requirement for nicotinic acid or nicotinamide, which cannot be satisfied by tryptophan. Although the synthesis of nicotinic acid from tryptophan has been demonstrated in several species of animal, the amount produced is apparently not enough completely to satisfy requirements. In the presence of ample tryptophan there is still a requirement for a dietary source of nicotinic acid; if the supply of tryptophan is limited, the demand is increased.

Vitamin C is synthesized from hexose sugars by plants and most animal species. Primates and guinea-pigs are notable exceptions, in that they are unable to perform the final step in the synthetic chain, the conversion of L-gulonolactone to L-ascorbic acid, and so are entirely dependent on an outside source of the vitamin. In normal circumstances, most other animals do not require a dietary supply of vitamin C, but conditions of stress, such as exposure to infection or extremes of temperature, may

impose a greater demand than can be satisfied by endogenous synthesis, and dietary supplements are of benefit.

Exogenous synthesis. Some micro-organisms are completely dependent on an external source of vitamins which others produce in excess of their own requirements. Thus the vitamin requiring-organisms tend to flourish in close association with the vitamin producers. This tendency is of considerable ecological importance, as has been shown in a study of the influence of the vitamin B₁₂ group in regulating phytoplankton fertility in the sea (Provasoli, 1963). It may also be a determining factor in the ecology of the microflora of the alimentary tract in higher animals, and consequently in the health and well-being of the host animal.

The association between an animal and its intestinal microflora can have a marked effect on the animal's vitamin economy. It is generally agreed that ruminants can thrive without a dietary source of vitamin K or the B complex, which are produced by the action of rumen micro-organisms; comparisons between germ-free and conventional chicks and rats have given direct proof of synthesis of the vitamins B and K by micro-organisms in the gastro-intestinal tract (see review by Coates, 1968). The extent to which the host can avail itself of the products of microbial synthesis depends on the architecture of its gut. In the ruminant, where the main site of synthesis, the rumen, occupies an anterior position, the microbial products must pass through the rest of the alimentary tract, with maximal opportunity for absorption. Conversely in the chicken the greatest synthesis occurs in the caeca, and as these are situated distal to the absorptive area of the tract, the bird derives little benefit unless it has access to its droppings (Coates, Ford & Harrison, 1968). Coprophagous species such as the rat and rabbit obtain a large part of their requirements of the B complex and vitamin K by ingestion of their faeces.

A similar kind of symbiosis has been observed in certain species of insect that carry intracellular yeasts and bacteria. An external source of vitamins of the B group is indispensable for the growth of species or groups without micro-organisms, whereas those that harbour them can grow in the absence of some or all of the vitamins B. Normal larvae of two beetles (*Lasioderma serricorne* and *Sitodrepa panicea*) that contain intracellular organisms grow reasonably well on an artificial diet deficient in vitamins of the B complex, but after sterilization of the larvae supplements of thiamine, riboflavine, nicotinic acid, pyridoxine and pantothenic acid are necessary for normal development of the insects. It must be concluded, therefore, that the necessary vitamins in the normal larvae are supplied by the symbionts (Blewett & Fraenkel, 1964).

Availability of different forms of vitamins

In natural foods vitamins exist in a variety of forms which may be differently available to different species. Although a vitamin may be defined in terms of a particular chemical compound, activity may be shown by a variety of closely related substances. Vitamin B₆ occurs as pyridoxine (pyridoxol), pyridoxal, pyridoxamine and the corresponding 5'-phosphates, all of which are equally effective for mammals

and birds. Their relative activities for micro-organisms differ, thereby providing a means of differentiating between the various forms by microbiological assay.

Vitamins of the D group are formed by ultraviolet irradiation of the corresponding provitamins, which are all sterols with a $\Delta_{5,7}$ double bond in the B ring of the steroid nucleus. The two most important are vitamin D₂ (ergocalciferol), formed by irradiation of the vegetable sterol, ergosterol, and vitamin D₃ (cholecalciferol), formed from the animal sterol, 7-dehydrocholesterol. The two forms have generally been considered equally effective for mammals, but there is recent evidence that the squirrel monkey utilizes ergocalciferol much less efficiently than cholecalciferol. The same is true for birds; the ratio of activity for the chick of vitamin D₃ to D₂ is about 35:1 (McChesney, 1943). The reason for these differences has not been explained but may become clear when the biochemical mechanism of action of vitamin D is finally resolved.

Vitamins frequently occur in nature bound to larger molecules, in which form they are not readily able to penetrate cell membranes. Thus higher animals, with their more elaborate digestive systems, are generally better able than micro-organisms to utilize bound forms of vitamins. For instance, pantothenic acid occurs most frequently as its nucleotide, coenzyme A, and also in other combined forms such as pantetheine (*N*-(pantothenyl)- β -amino-ethanethiol). Animals respond equally as well to coenzyme A and pantetheine as to the free vitamin, but micro-organisms are not all able to use the combined forms. There are exceptions to the general rule. The bound form of nicotinic acid that occurs in maize and other cereals is poorly utilized by man, pig, rat and chick and similarly by *Lactobacillus arabinosus* and *Lb. caseii*. In contrast, riboflavine, which occurs widely in the prosthetic groups of the flavo-proteins, appears to be equally available in all its forms to micro- and macro-organisms. Bound forms of vitamin B₁₂ follow the general pattern in being unavailable to micro-organisms, but their availability to higher animals is complicated by the special mechanism necessary for the transport of vitamin B₁₂ across the intestinal mucosa. Absorption of physiological amounts of vitamin B₁₂ demands the presence of intrinsic factor, a binding substance that is produced in the upper part of the alimentary tract and which is to some extent species-specific. Although vitamin B₁₂ bound to pig intrinsic factor is available to man, it is only poorly utilized by the rat and chick; rat intrinsic factor renders the vitamin less available to the chick. Furthermore, the bound form in which vitamin B₁₂ is present in sow's milk is poorly utilized by the rat and chick but is presumably adequately absorbed by the baby pig (Coates, Gregory, Harrison, Henry, Holdsworth & Kon, 1955).

In the nutrition of micro-organisms the intact vitamin required by higher animals may sometimes be replaced by part of the molecule, which is used either as a starting point for the synthesis of the whole vitamin (e.g. some yeasts synthesize thiamine from its thiazole and some from its pyrimidine moiety) or *per se* in place of the intact vitamin (e.g. the thiazole moiety can be used instead of thiamine by some flagellates). Among the vitamin B₁₂-requiring bacteria, some can use Factor B, the cobamide portion of the molecule with no nucleotide; others require the presence of a nucleotide, but it may contain an adenine instead of a benzimidazole derivative.

For most protozoa and all higher animals the presence of a nucleotide containing benzimidazole is essential for vitamin activity.

These differences in availability of the naturally occurring vitamins are of considerable practical importance in the applications of microbiological assays to the measurement of vitamins in foods. Where bound forms are present, erroneously low values may be obtained unless the vitamin is first released by, for instance, treatment with enzymes; conversely, assay organisms that respond to fractions of a molecule may grossly overestimate the activity for animals or man.

Effects of vitamin deficiency

In micro-organisms a lack of any essential vitamin is usually manifested by a failure of growth and cell division. In higher animals the signs of deficiency are more varied, according to the dominant physiological function in which the vitamin is concerned. There is a broad similarity between species in the effects of deprivation of a particular vitamin. Thus rickets in the young and osteomalacia in the adult are invariable consequences of a lack of vitamin D; deficiency of vitamin B₆ has been observed to result in blood dyscrasias in rats, mice, cats, dogs, pigs, monkeys and man, and also in convulsions in several species. Some individual signs of deficiency are typical of only one or a few species, possibly because certain peculiarities of metabolism, behaviour or body conformation predispose towards trauma of a characteristic type. The xerophthalmia characteristic of the vitamin-A-deprived rat is a particular manifestation of the general need for vitamin A in maintaining the integrity of mucous membranes, but is rarely seen in that form in other species; the neurological defects observed in animals deprived of riboflavine take the form of 'curled toe paralysis' in chicks but appear as 'stiff legs' in the young pig. There are a few outstanding inter-species differences in the effects of deficiency that cannot readily be explained in the light of present knowledge. Muscular dystrophy has been observed in at least twenty species resulting from a lack of vitamin E, but other distinct signs are characteristic of only one or a few. A dietary deficiency of vitamin E in the rat and chicken leads to reproductive failure in the male and female, but in cattle, goats and sheep there is little evidence that the deficiency interferes with reproduction (see review by Blaxter, 1962). Encephalomalacia and exudative diathesis are peculiar to the chicken and turkey (see review by Scott, 1962); liver degeneration has been observed in the rat and pig, and steatitis (a yellow pigmentation of the fat) in the rat, pig and mink but not in cattle. The picture is further complicated by the fact that some of the signs of deficiency (e.g. encephalomalacia in chicks, muscular dystrophy in cattle) can be prevented or cured by administration of antioxidants, and others (e.g. exudative diathesis in chicks, muscular dystrophy in chicks and cattle but not reproductive failure in rats) by dietary supplements of selenites. Comparative studies have revealed the complexity of the role of vitamin E in animal metabolism and must clearly be continued until the mechanism of action of the vitamin, and its interrelationship with other antioxidants and with selenium, are fully resolved.

Another notable inter-species difference exists between man and animals in the

effects of deficiency of vitamin B₁₂. In animals, lack of vitamin B₁₂ is characterized by a failure of growth in the young, but the adult shows little effect of deprivation except in the reproductive performance of the female; severe deficiency in birds results in poor hatchability of the eggs, and in mammals retarded foetal development, immature birth weights and a high incidence of malformed infants has been observed. In man the classic sign of deficiency is Addisonian pernicious anaemia, a megaloblastic anaemia accompanied in severe deficiency by neurological complications, particularly a demyelination of the spinal chord. The corresponding syndrome has never been produced in any species of experimental animal, although a demyelinating disease in monkeys that was partially relieved by crude liver extract (Hamerton, 1942) may have resulted from dietary lack of vitamin B₁₂. Vitamin B₁₂ is required in very small amounts, is stored in the tissue and is passed on from dam to offspring. Lack of evidence of reproductive failure in women may simply indicate that severe deficiency of vitamin B₁₂ is never encountered in human beings during the child-bearing years, but the failure to produce anaemia in experimental animals even after long periods of deprivation is strong evidence for a difference in the mechanism of blood formation between man and other animals.

Individual variability

This discussion has so far been concerned with differences between species or classes of organism in requirements for vitamins, but variability within species must also be considered. Evidence with mice, rats and chickens indicates that substantial strain differences exist in requirement for nutrients; differences between individuals may be even greater. After a careful study of the effects of vitamin A deficiency in several strains of rat, Williams & Pelton (1966) concluded that the large inter-individual differences made it impossible to establish the vitamin A requirements of any particular strain, and even less feasible to determine the needs of experimental rats in general. Attempts to assess the vitamin requirements of different classes of livestock or of man can, at best, yield only approximate values applicable to an average individual under average conditions. Quantitative comparisons between species must be accepted with even greater reservation.

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