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THE ROLE OF MICRONUTRIENTS IN THE BIOSYNTHESIS
OF THE CRUSTACEAN EXOSKELETON

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ABSTRACT

Exoskeleton biosynthesis is reviewed from the standpoint of identifying possible nutrient demands of this process in crustaceans; specifically examined are the possible roles of selected micronutrients - vitamins C, A, D, E, and K. The functions of these in vertebrate systems are discussed as well as the possibility that they function in crustacean exoskeleton biosynthesis and molting.

Key words: Nutrition, Crustacea, exoskeleton, ascorbic acid, vitamin C, fat-soluble vitamins, vitamins A, D, E, K.

INTRODUCTION

The rigid body-covering of crustaceans provides them not only with a name but also an integument and skeleton. Although this common characteristic of all arthropods has been studied extensively (for review see Richards 1951; Dennell 1960; Travis 1960a; Hackman 1971; and Nelville 1975), one aspect not well examined is the relationship between the exoskeleton and specific nutrients in the diet. A primary reason for this omission has been the lack of the required tools, i.e., reliable crustacean test diets. As suitable diets are being developed, a review of the available information on crustacean exoskeleton biosynthesis and promising research directions is appropriate. Specifically of interest, along with the major nutrient classes of proteins, carbohydrates, and lipids, are vitamin C, and vitamin D, as well as the other fat-soluble vitamins A, E, and K.

The examination of this particular group of micronutrients reflects the tendency of comparative animal nutritionists to view the nutritional physiology of a relatively small taxonomic group of animals, the white rat and its vertebrate relatives, as representative of the norm. While historically vitamin C and the fat-soluble vitamins were sometimes considered to be required only by vertebrates, the more usual approach has been to also include these vitamins in invertebrate test diets. This assumption will be examined, with regard to the synthesis of the crustacean integument, in an attempt to identify possible differences in nutritional physiology between crustaceans and vertebrates.

GENERAL STRUCTURE OF THE INTEGUMENT

The crustacean integument (see Figure 1) is made up of two basic parts: a single layer of epidermal cells, and the cuticle, a non-living portion that has been secreted externally by the epidermis. This cuticle is composed principally of layered chitin impregnated with protein that has been variously modified with lipids and mineral salts, depending on species, body location and depth. This range in composition of the cuticle layers has lead to a confusing assortment of classification schemes and terms, the most common of which are summarized by Aiken (1980). The following discussion will use the terminology originated by Richards (1951) i.e. epicuticle, exocuticle, endocuticle, and membranous layer.

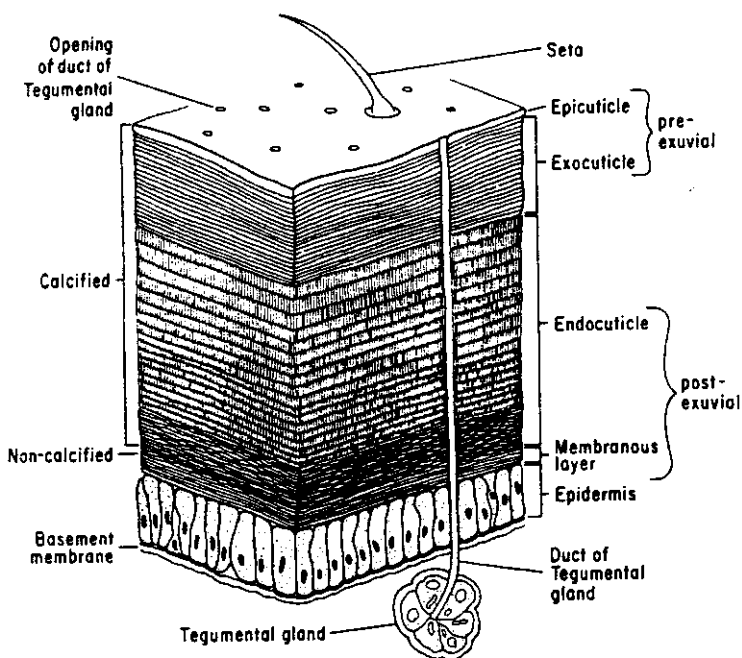


Figure 1. The crustacean exoskeleton in cross section (adapted from Aiken 1980).

In most crustaceans, the outer most component of the integument, the epicuticle, consists of two layers: a lipid outer layer and a lipoprotein inner layer. Some calcium is found in the epicuticle, typically in scattered aggregates (Digby 1968; Hegdahl et al. 1977b). Permeability of the cuticle seems related to the composition and presence or absence of this epicuticle (Yonge 1936; Mary and Krishnan 1974). The epicuticle may also protect the underlying chitinous regions from bacterial attack (Fisher et al. 1978).

The bulk of the cuticle is often referred to as the procuticle and has three zones: the exocuticle, endocuticle, and membranous layer. The exocuticle is composed of laminar sheets of chitin fibrils enveloped in a matrix of protein that may be sclerotized or tanned. The exocuticle is formed prior to ecdysis, but calcification of this layer does not occur until later. Following ecdysis, the exocuticle is calcified rapidly and serves as the only cuticle until the endocuticle and the membranous layer are formed medially. Like the exocuticle, the endocuticle consists of chitin and protein laid down in laminae parallel to the surface. As laminae are formed, calcite crystals are deposited generally parallel to the chitin microfibrils (Hegdahl et al. 1977a). The membranous layer is a similar chitin-protein layered complex but is not calcified.

MINERALS

With the exception of the membranous layer, the cuticle of most crustaceans is heavily mineralized, primarily with calcium carbonate with minor amounts of magnesium, phosphorus, and sulfur. The amount of calcium (up to 99% of the total inorganic component of the exoskeleton) can vary tremendously depending on species, location on the body, and stage of the molt cycle (for extensive reviews, see Richards 1951; Travis 1960a, 1963, 1965; Dall 1965a, b; Greenaway 1974a, b, c; Welinder 1974, 1975a, b; Huner et al. 1976, 1979a, b).

Compared with other nutrient groups, mineral requirements of crustaceans have received little attention. Early work with shrimp (for review see New 1976) indicated a need for dietary calcium and phosphorus for growth. A ratio close to 1:1, calcium to phosphorus, was considered appropriate, with the total amount of dietary minerals being as high as 19.5%. Gallagher and co-workers (1978) suggested a ratio of 1:2 as optimum for juvenile American lobsters. Sedgwick (1980) found that the addition of a 7.0% mineral mix promoted growth if a vitamin mix was included in the diet but that minerals were toxic in the absence of vitamins.

Passano (1960) suggested dietary calcium would not be a requirement for marine crustaceans because it is so abundant in sea water. Recently Deshimaru and Yone (1978) reported growth of the prawn, Penaeus japonicus, was not inhibited by a lack of supplemental calcium, although additions of phosphorus (2.0%), potassium (1.0%), and trace metals (0.2%) improved growth. Deshimaru et al. (1978) later demonstrated the prawn could obtain adequate calcium from the surrounding sea water in the absence of a dietary source. Fresh water, of course, has a lower calcium content but should still provide adequate amounts in most cases (Rawson 1939). The work by Mills and Lake (1976) and Mills et al. (1976) suggests that exceptionally low calcium concentrations in fresh water can be compensated for by reduced exoskeleton mineralization, although there must be a minimum requirement (Greenaway 1974c). Thus, while the information available is still limited and much of the early work on crustacean mineral requirements may have been flawed by the lack of adequate test diets, it would appear that crustaceans meet most, if not all, of their demands for calcium from the surrounding aqueous medium, particularly in the marine environment. Crustacean requirements for other minerals, such as phosphorus, that are not readily available from the water, probably have to be satisfied by dietary intake.

VITAMIN D

Vitamin D, typically included in crustacean diets (see Table I), and is of interest because of its recognized importance in vertebrate bone mineralization and its possible analogous role in the mineralization of the crustacean exoskeleton. It is important to note, however, that in higher vertebrates vitamin D is often considered more as a hormone than a vitamin. Rather than serving as a catalyst for enzymatic activity, vitamin D regulates cell function by interacting directly with the genome. It is, therefore, appropriate to briefly review the current model for steroid hormonal action.

Table I. Vitamin Requirements of the Chick and Dietary Vitamin Levels for Trout and Crustaceans (mg or [IU]/100 g dry diet)

	Vitamin				
	C	A	D	E	K
Chick ^a	NR ^b	150 [IU]	20 [IU]	1 [IU]	0.05
Trout ^c	10	200 [IU]	100 [IU] ^d	3	8
Shrimp ^e	25	200 [IU]	12.5 [IU]	1.25	0.12
Shrimp ^f	2,000	9.6 (β -carotene)	1.2	20	4
Shrimp ^g	875	3.5 (β -carotene)	0.5	35	3.5
Crab ^h	200	1.2 (β -carotene)	0.6	20	4
Lobster ⁱ	500	65,000 [IU]	32,500 [IU]	100	-

^aNational Research Council 1977. ^bNR - not required. ^cNational Research Council 1973. ^dBarnett et al. 1979. ^eDeshimaru and Shigeno 1972. ^fKanazawa et al. 1977. ^gSedgewick 1980. ^hPoniat and Adelung 1980. ⁱD'Abramo et al. 1981.

In vertebrates, steroid hormones are synthesized in endocrine tissues, released into the blood stream, and transported bound to specific proteins. Upon reaching the target tissues, the hormones are concentrated within the cells by the action of intracellular receptor proteins that selectively bind the hormone. The hormones then regulate the functions of the target cells by affecting gene expression and the subsequent synthesis of proteins. Regulation of a hormonal system can be complex, involving feedback inhibition of precursor synthesis and of the active metabolite as well as interactions with other hormones. Vitamin D metabolism and function in higher vertebrates clearly fits this steroid hormone model. The only significant difference is that the precursor molecule cannot be synthesized *de novo*.

Although not every effect of vitamin D is explained through hormonal action, the steroid model has been useful in examining vitamin D's role in higher vertebrates. According to Deluca (1979) providing there is an adequate exposure to sunlight or UV irradiation, the epidermis of mammals can synthesize enough cholecalciferol from dietary

sources of 7-dehydrocholesterol that dietary input is unnecessary. In mammals, cholecalciferol, either from the diet or from epidermal synthesis, is rapidly cleared from the blood by the liver, which hydroxylates it to form 25-hydroxycholecalciferol. This step is self-regulating by a negative feedback process. The 25-hydroxycholecalciferol is transported by a specific serum protein to the kidney, where it is further hydroxylated to form 1, 25-dihydroxycholecalciferol. Although the transport mechanism for 1, 25-dihydroxycholecalciferol has yet to be identified, it is assumed also to include a specific transport protein. The active metabolite, 1, 25-dihydroxycholecalciferol, is concentrated by and acts at the site of various target tissues, principally the kidney, gut, and bone, by altering protein synthesis so as to affect the calcium flux of the cells. Although the molecular mechanism is not always completely understood, the result is an increase or decrease in calcium movement in each of the target tissues so as to maintain physiologically normal calcium levels in the blood. In bone, for example, the metabolite's action mobilizes calcium into the circulatory system when blood calcium levels are low. In maintaining blood calcium levels, 1, 25-dihydroxycholecalciferol also interacts with two other hormones, the parathyroid hormone and calcitonin. These two hormones also act directly in regulating the production of the active metabolite in the kidney; the parathyroid hormone promotes the production of, 25-dihydroxycholecalciferol while calcitonin inhibits the production.

Although limited evidence indicates a vitamin D dietary requirement in crustaceans (Conklin 1980; Kanazawa this volume), there is no real indication that crustaceans regulate serum calcium levels in an analogous fashion to that found in vertebrates. Injections of mammalian parathyroid extracts stimulate the movement of minerals from the crustacean exoskeleton to the hemolymph (McWhinnie et al. 1969). However, under more normal physiological conditions, studies with crustaceans indicate hemolymph calcium concentrations are not maintained through use of the shell calcium. For example, no movement of shell calcium was found in the lobster Homarus americanus, after hemolymph calcium levels were halved (Hayes et al. 1962).

Although the mineralized cuticle serves admirably as both a skeleton and a protective integument, its rigid structure does present problems with respect to the animals growth. Arthropods, of course, have resolved this difficulty by molting and the most dramatic movement of calcium from the crustacean exoskeleton occurs in preparation for this process. Both Travis (1955b) and Dall (1965a) have suggested that the resorptive processes occurring in preparation for molting appear to be primarily for the conservation of organic constituents, not calcium. However, the existence in many crustaceans of various mineral storage mechanisms, such as gastroliths, seems to indicate regulation and thus a possible regulatory role for vitamin D. Gastroliths are paired mineralized structures formed between the epidermal and cuticular lining of the stomach and have the typical cuticular structure (Travis 1963). In species with gastroliths, minerals are deposited in these structures as the exoskeletal mineral content is decreased during premolt (Travis 1960b; McWhinnie 1962). At ecdysis, the gastroliths are shed with the cuticular lining into the stomach, and upon digestion the stores become available to the animal.

Several problems arise in attempts to assign gastrolith and mineral regulation to a vitamin D system. First, gastroliths are not found in all crustaceans, and their presence is not restricted to species inhabiting a specific environment with respect to calcium availability. Thus, gastroliths appear superfluous particularly for marine species, which presumably can meet their calcium requirements from the surrounding medium. Second, most evidence indicates the molt and the formation of the gastroliths are both regulated by an ecdysteroid hormone, probably 10-hydroxyecdysone (Kleinholz and Keller 1979). To further postulate the involvement of another hormonal regulatory agent such as vitamin D appears unnecessary and thus unattractive. Third, while the involvement of a parathyroid-like hormone in gastrolith calcium mobilization was postulated by McWhinnie and co-workers (1969), no further evidence has been reported. It should also be noted that McWhinnie and co-workers, however, used parathyroid gland extracts and not the purified hormone. Finally, gastrolith stores contain only a small portion both of the calcium that is resorbed from the integument before molting (McWhinnie 1962) and of the calcium required following the molt (Travis, 1960b). The calcium stored in the gastroliths is probably advantageous only in reducing the time necessary to establish minimum rigidity for consumption of the old exoskeleton and for beginning feeding. The gastroliths, then, are considered highly specialized adaptations, and their presence does not indicate calcium conservation in a closely regulated hormonal system.

The actual mechanism by which calcium is transported to and from the exoskeleton has always intrigued crustacean biologists. Simkiss (1976), in reviewing the mineralization of organic matrices in invertebrates, points out the inadequacy of existing models and the complexities involving both the intracellular and extracellular phases of this process. Although the recent model proposed by Roer (1980) does not include the mechanism of calcium deposition in the organic matrix of the exoskeleton, it is attractive in its simplicity with regard to the two-way transport required at different stages of the molt cycle. Roer's model suggests the exoskeleton epidermis is continually actively transporting calcium out of the cells and that it is the morphology of the epidermal cells that dictates the direction of the net calcium movement. As the model presumes the epidermal cells are connected by intercellular junctions that restrict any paracellular calcium movement, molt-related changes in the morphology of these cells results in the transport of calcium. During postmolt the squamous cells of the epidermis elaborate numerous protoplasmic projections that extend through the pore canals of the cuticle. This comparatively large surface area on the external side of the epidermis results in a net calcium movement to the exoskeleton. At the onset of premolt, the cell extensions are lost and the cells become more columnar in appearance when the old cuticle and its pore canals are separated from the epidermis. These changes reverse the surface ratio on the two sides of the intercellular junctions, thus reversing the direction of net calcium movement. Although structural changes occur in the intestinal epithelium of higher vertebrates in response to vitamin D (Bikle et al. 1981), there is no evidence of vitamin D's involvement in the changes in the structure of crustacean epidermis. As stated earlier, these molt related cellular changes in crustaceans are presently assumed to be regulated by the molting hormone, 20-hydroxyecdysone.

VITAMIN A

Morphological changes in epithelial cells of higher vertebrates are known to occur in response to vitamin A. Vitamin A profoundly affects cell division and differentiation of epithelial cells. Deprivation of this vitamin, therefore, leads first to retardation and ultimately to cessation of growth. As with vitamin D, vitamin A in higher vertebrates appears to act primarily as a hormone rather than as an enzymatic co-factor (for review see Ganguly et al. 1980). In arthropods no clear evidence demonstrates an analogous role for vitamin A in growth (see Dadd this volume).

Based on work done with insects (for references see Dadd 1977a), vitamin A probably has a role in the crustacean visual cycle (Fisher and Kon 1959) that is the same as that in the vertebrate visual cycle. An important difference between vertebrates and invertebrates, however, is in the vitamin's distribution. In crustaceans, vitamin A is concentrated in the eye (Fisher and Kon 1959), but in higher vertebrates, "the amount of the vitamin involved in the visual cycle is only a very small fraction of the total amount of the vitamin in the rest of the animal body" (Ganguly et al. 1980).

PROTEINS

The chemical composition of the cuticle is predominated by chitin and protein, the relative amounts and ratios of which vary considerably among species. The amount of protein present appears inversely related to the degree of calcification (Travis 1960a). Amino acid analysis has revealed different protein compositions of the cuticle depending on species, region of the cuticle, and solvent extractions (Travis et al. 1967; Sameshima et al. 1973; Hackman 1974; Welinder 1974, 1975a). Although the information available on cuticle protein composition is still limited, it does not appear likely that modifications in the amino acid dietary ratios will be needed in order to satisfy a unique amino acid requirement for cuticle formation.

VITAMIN K

One amino acid absent from the crustacean exoskeleton is γ -carboxyglutamic acid (King 1978). This amino acid, which was discovered originally in the vertebrate blood clotting factor prothrombin, appears to be an important component of bone proteins and is thought to have a role in calcium binding. Work with vertebrate systems indicates that the formation of γ -carboxyglutamic acid is vitamin K dependent (Stenflo and Suttie 1977). In a limited survey of mineralized tissues in vertebrates and invertebrates, King (1978) found γ -carboxyglutamic acid only in the vertebrates. The apparent absence of this amino acid in invertebrates and the fact that vertebrate plasma coagulation factors do not affect arthropod hemolymph coagulation (Durliat and Vranckx 1981) suggests these vitamin K dependent activities do not play a role in the physiology of crustaceans.

VITAMIN C

Penaeid shrimp have been found to have a vitamin C, or ascorbic acid, requirement for growth (Kitabayaski et al. 1971b; Deshimaru and Kuroki 1976; Guary et al. 1976; Lightner et al. 1977). Lack of ascorbic acid in the diet of Penaeus californiensis and P. stylirostris results in a high incidence of "black death," a deficiency disease of shrimp characterized by reduced growth rates, poor feed conversion ratios, decreased resistance to stress and reduced rates of wound repair (Lightner et al. 1979). In vertebrates, an ascorbic acid deficiency results in impaired collagen formation (Barnes and Kodicek 1972), and results reported for crustaceans by Lightner and co-workers (1979) parallel the effect found in vertebrates. In ascorbic acid-deficient shrimp, Lightner et al. (1979) recorded a decrease in the amount of hydroxyproline, which was assumed to reflect a decrease in the collagen content. Collagen, or collagen-like proteins, depending on the definition used (Adam 1978), are present in crustaceans and may be an important element of integument structure and function, particularly in the specialized case of wound repair.

Ascorbic acid may also be important in other aspects of the crustacean exoskeleton formation and function. In vertebrates, ascorbic acid is involved with alkaline phosphatase activity; for example, catfish grown on diets without ascorbic acid supplementation were found to have low serum alkaline phosphatase activity (Wilson and Poe 1973). Gould and Shwachman (1942) found that morphological changes in guinea pig osteoblasts, as a result of an ascorbic acid deficiency, were correlated with decreased alkaline phosphatase levels. Although similar studies have yet to be done on crustaceans, Travis (1955a) found extensive changes in both the morphology and presumed alkaline phosphatase activity in epidermal cells of the spiny lobster just preceding ecdysis.

Ascorbic acid may be involved in the sclerotization or tanning of the crustacean epicuticle. The insect cuticle, which is not particularly calcified, is hardened primarily by tanning (Nelville 1975), a cross-linking of cuticle proteins by quinones. The quinones are formed through the oxidation of various phenols; a reaction catalyzed by the enzyme tyrosinase. Dadd (1960) first suggested that ascorbic acid had a role in the regulation of the enzymes involved in insect sclerotization, and recent work by Navon (1978) with the Egyptian cotton leafworm clearly supports Dadd's speculations. An ascorbic acid deficiency in this insect leads to the premature hardening of the cuticle, causing impaired ecdysis and mouth-part function and leading to death by starvation. Navon's studies and other studies with insects (Chippendale 1975; Kramer et al. 1978) indicate that various derivatives of vitamin C and related compounds, while not as effective as ascorbic acid, do have a hierarchy of effectiveness similar to that found in vertebrates. In crustaceans, sclerotization (see Yamaoka and Sheer 1971) of the epicuticle also is catalyzed by tyrosinase (Stevenson and Schneider 1962) and most likely is also affected by ascorbic acid.

Another possible vitamin C function was suggested by Guary and co-workers (1975), who found that the ascorbic acid levels of juvenile shrimp Palaemon serratus diminished immediately before and after molting, when the animals were not feeding. These decreases were suggested to be the result of vitamin C metabolism during the synthesis

of chitin. Although no direct evidence supports this suggestion, Guary and co-workers cite evidence of Antonowicy and Kodicek (1968) that vitamin C has an essential role in the analogous vertebrate synthesis of mucopolysaccharides.

CARBOHYDRATES

Based on a relationship between the crustacean molt cycle and glycogen levels of the hepatopancreas and epidermis, researchers have postulated that the precursor of chitin is glucose derived from stored glycogen (Passano 1960; Travis 1960a, 1963). Stevenson and Hettick (1980) have suggested the pathway shown in Fig. 2 for the synthesis of chitin; identification of enzymes for each of these steps has yet to be demonstrated in individual species, therefore this pathway represents a composite picture.

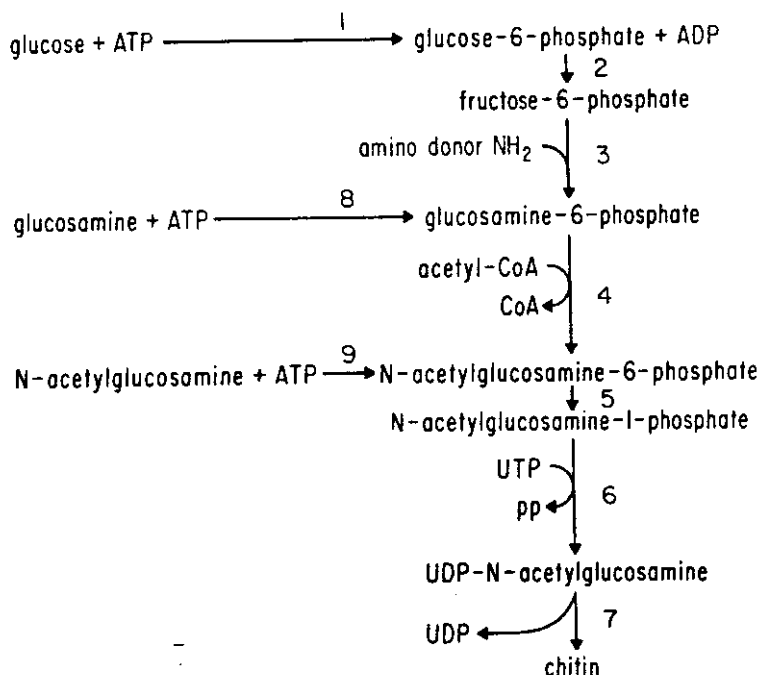


Figure 2. Suggested metabolic pathway for the synthesis of chitin in arthropods (adapted from Pahlic and Stevenson 1978; Stevenson and Hettick 1980). Individual enzymatic steps 1-6 have been demonstrated in the locust (Candy and Kilby 1962); 1,3,5,6,7, 8 and 9 in the crawfish (Lang 1971; Speck et al. 1972; Gwinn and Stevenson 1973b; Pahlic and Stevenson 1978), and step 7 in brine shrimp and a crab (Carey 1965; Hohnke 1971).

This scheme also provides a pathway for the direct utilization of intermediate products, glucosamine and N-acetylglucosamine, arising from the breakdown of the old cuticle at molt. The findings of Hornung and Stevenson (1971) and Gwinn and Stevenson (1973a) compared the incorporation of N-acetylglucosamine with glucose in chitin synthesis support this purposed recycling. Gwinn and Stevenson (1973a) indicated that over half of the postmolt chitin in crayfish consisted of recycled components of the former cuticle. The utilization of the chitin breakdown product, N-acetylglucosamine, may partly explain the benefits of including shrimp by-product meals in crustacean diets (New 1976). A stimulatory effect on growth also has been noted in shrimp with inclusion of glucosamine in the diet (Kitabayashi et al. 1971a), though glucosamine is not an indispensable nutrient (Deshimaru and Kuroki 1976) as indicated by the above pathway.

LIPIDS

Lipids have a central role in the biology of crustaceans, not only as energy sources but also as structural elements. Although information on the lipid composition of the crustacean exoskeleton is not extensive, it is known that the lipid content is low (approximately 1% or less) (Allen 1971; Ando et al. 1977; van den Horst et al. 1973). Cuticle lipids, as mentioned earlier, are thought to help control water permeability and perhaps help protect the chitinous regions from bacterial attack.

Cholesterol and polyunsaturated fatty acids are critical structural elements of cell membranes and are thus important for elaboration of the crustacean epidermis. In this respect, it is surprising that arthropods seem to be generally incapable of synthesizing cholesterol or polyunsaturated fatty acids (Dadd 1977a, b). Sterols, especially cholesterol, seem essential for membrane structure throughout the animal kingdom (Nes 1974). In crustaceans, the extensive morphological changes in the epidermal cells associated with molting must require cholesterol for membrane synthesis. Indeed, Guary and Kanazawa (1973) found that after being injected with labelled cholesterol, the epidermis of Penaeus japonicus had a high radioactive count.

Recently, several groups of investigators have shown phospholipids are a necessary crustacean dietary factor (Kanazawa et al. 1979; Conklin et al. 1980), and a phospholipid involvement in exoskeleton formation has been noted in juvenile lobsters (Homarus americanus) (Conklin et al. 1980). In lobsters a lack of dietary phospholipids commonly resulted in death due to the animal's inability to extricate itself at molt from the old exuvium. Also, lobsters fed phospholipid-deficient diets had abnormal calcium deposits embedded on the inner surface of their shed exoskeleton (Bowser and Rosemark 1981). Although the mechanisms of these exoskeletal abnormalities remain unknown, recent work has led to several possibilities. Phospholipids may be directly involved in membrane formation and there is also the possibility that they affect other nutrient levels.

One of the roles phospholipids apparently play in crustaceans is in the transport of fat-soluble nutrients through the hemolymph. D'Abramo et al. (1982) has shown that a dietary phospholipid deficiency in homarid lobsters reduces cholesterol transport in the hemolymph. Since crustaceans cannot synthesize this steroid, the drop in hemolymph

cholesterol could either affect epidermal membrane synthesis directly or indirectly by limiting the synthesis of the molting hormone for which cholesterol is a precursor. The phospholipid transport system may also carry fat-soluble vitamins. A further complication is the possibility that phospholipids in the pelletized diet reduce the rapid leaching of water-soluble vitamins and thus increase the available level of these vitamins in the diet (Castell personal communication).

The effectiveness of phospholipids in preventing molt related deaths is dependent on its specific constituents (D'Abramo et al. 1981). For the lobster, lecithin is the most effective phospholipid, but both lecithins and cephalins promoted growth in the prawn Penaeus japonicus (Kanazawa et al. 1979). In both animals, however, the effectiveness of the phospholipids seems also to depend on the constituent fatty acids, in particular the presence of polyunsaturated fatty acids.

VITAMIN E

The importance of polyunsaturated fatty acids in the diet of crustaceans is well established and reflects these animals' limited biosynthetic abilities (see Castell this volume). In vertebrates the amount of dietary vitamin E required is related to the amount of polyunsaturated fatty acids present (Harris and Embree 1963). Indeed the primary function of vitamin E in animals is thought to be prevention of the oxidation of fatty acids located within cellular membranes (Dam 1962). Crustaceans, with their apparent dependence on polyunsaturated fatty acids, undoubtedly will be found also to require vitamin E. Other possible biological functions such as co-factor interaction in enzymatic activities have yet to be clearly demonstrated in vertebrates or crustaceans.

CONCLUSIONS

With the limited data presently available, the critical nutrient requirements for crustacean exoskeleton biosynthesis remain unknown. Enough evidence suggest, however, that attempts to use the well-known nutritional requirements of higher vertebrates as a direct model for understanding invertebrate nutrient physiology will be misleading. A better approach will be to look closely at the specific role that each nutrient has in the physiology of these animals.

The apparent evolution of a hormonal function for some of the fat-soluble vitamins such as vitamin D and perhaps vitamin A may reflect highly specialized adaptations unique to higher vertebrates. Dacke (1979) has argued that the calcium-regulating system of higher vertebrates, which involves vitamin D and other hormones, is associated with the specific needs of bony terrestrial animals to compensate for the lack of a surrounding calcium-rich medium. Limited evidence suggests that utilization of vitamin K by vertebrates may have a similar association with calcium. Unfortunately, our knowledge of the nutritional physiology of some of the lower vertebrates, such as fish, that remain in a calcium-rich environment is still rudimentary. An interesting recent paper by George and co-workers (1981) demonstrated a vitamin D requirement for trout but found no involvement of vitamin D with the skeletal system. The researchers did, however, demonstrate

changes in the cellular morphology of muscle cells, which they speculate are related to intracellular calcium movement.

For other nutrients such as vitamin C and vitamin E that have more general cellular functions in vertebrates, the use of comparative models may be helpful. Although some evidence suggests the involvement of vitamin C in crustacean exoskeleton synthesis, whether the majority of crustaceans require the vitamin in their diet or can synthesize it, is unknown.

Although caution is stressed in the interpretation of comparative nutritional data between widely divergent groups, this should not be taken to the extreme. Most vitamins are involved in a variety of ways in the nutritional physiology of animals, even though some functions are more critical than others, depending on the species. Thus, a similar set of nutrient requirements ultimately may be demonstrated, even though divergent animal groups may evolve unique systems for the utilization of these nutrients.

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LITERATURE CITED

- Adam, E. 1978. Invertebrate collagens. *Science* 202:591-598.
- Aiken, D. E. 1980. Molting and growth. Pages 91-163 in J. S. Cobb and B. F. Phillips (eds.) "The Biology and Management of Lobsters", Vol. 1. Academic Press, New York.
- Allen, W. V. 1971. Amino acid and fatty acid composition of tissues of the Dungeness crab (*Cancer magister*). *Journal of the Fisheries Research Board of Canada* 28:1191-1195.
- Ando, T., A. Kanazawa, S. Teshima, J. Patrois, and H. J. Ceccaldi. 1977. Variation in the lipids of tissues during the molting cycle of prawn. *Bulletin of the Japanese Society of Scientific Fisheries* 43:1445-1450.
- Antonowicz, I., and E. Kodicek. 1968. The effect of scurvy on glycosaminoglycans of granulated tissue and costal cartilage. *Biochemistry Journal* 110:609-616.
- Barnes, M. J., and E. Kodicek. 1972. Biological hydroxylations and ascorbic acid with special regard to collagen metabolism. Pages 1-43 in R. S. Harris, P. L. Munson, E. Diczfaesy and J. Glover (eds.) "Vitamins and Hormones", Vol. 30. Academic Press, New York.

- Barnett, B. J., C. Y. Cho, and S. J. Slinger. 1979. The essentiality of cholecalciferol in the diets of rainbow trout (Salmo gairdneri). Comparative Biochemistry and Physiology 63A:291-297.
- Bikle, D. D., R. L. Morrissey, D. T. Zolock, and H. Rasmussen. 1981. The intestinal response to vitamin D. Review of Physiology, Biochemistry and Pharmacology 89:64-142.
- Bowser, P. R., and R. Rosemark. 1981. Mortalities of cultured lobsters, Homarus, associated with molt death syndrome. Aquaculture 23:11-18.
- Candy, D. J., and B. A. Kilby. 1962. Studies on chitin synthesis in the desert locust. Journal of Experimental Biology 39:129-140.
- Carey, F. G. 1965. Chitin synthesis in vitro by crustacean enzymes. Comparative Biochemistry and Physiology 16:155-158.
- Chippendale, G. M. 1975. Ascorbic acid: An essential nutrient for a plant-feeding insect, Diatraea grandiosella. Journal of Nutrition 105:499-507.
- Conklin, D. E. 1980. Nutrition. Pages 277-300 in J. S. Cobb and B. F. Phillips (eds.) "The Biology and Management of Lobsters", Vol. 1. Academic Press, New York, p. 277-300.
- Conklin, D. E., L. R. D'Abramo, C. E. Bordner, and N. A. Baum. 1980. A successful purified diet for the culture of juvenile lobsters: the effect of lecithin. Aquaculture 21:243-249.
- D'Abramo, L. R., C. E. Bordner, D. E. Conklin, and N. A. Baum. 1981. Essentiality of dietary phosphatidylcholine for the survival of juvenile lobsters. Journal of Nutrition 111:425-431.
- D'Abramo, L. R., C. E. Bordner, and D. E. Conklin. Relationship between dietary phosphatidylcholine and serum cholesterol in the lobster Homarus sp. Marine Biology 67:231-235.
- Dacke, C. G. 1979. "Calcium Regulation in Submammalian Vertebrates". Academic Press, New York, p 222.
- Dadd, R. H. 1960. Some effects of dietary ascorbic acid on locusts. Proceedings of the Royal Society of London 153:128-143.
- Dadd, R. H. 1977a. Qualitative requirements and utilization of nutrients: insects. Pages 305-346 in M. Rechcigal, Jr. (ed.) "CRC Handbook Series on Nutrition and Food", Section D, Vol. I. CRC Press, Cleveland, Ohio.
- Dadd, R. H. 1977b. Qualitative requirements and utilization of nutrients: arthropods other than insects. Pages 347-352 in M. Rechcigal, Jr. (ed.) "CRC Handbook Series in Nutrition and Food", Section D, Vol. I. CRC Press, Cleveland, Ohio.
- Dall, W. 1965a. Studies on the physiology of a shrimp, Metapenaeus sp. (Crustacea: Decapoda: Penaeidae). III. Composition and structure of the integument. Australian Journal of Marine and Freshwater Research 16:13-23.
- Dall, W. 1965b. Studies on the physiology of a shrimp. Metapenaeus sp. Australian Journal of Marine Freshwater Research 16:181-203.

- Dam, H. 1962. Interrelations between vitamin E and polyunsaturated fatty acids in animals. Pages 527-558 in R. S. Harris and I. G. Wool (eds.) "Vitamins and Hormones", Vol. 20. Academic Press, New York.
- Deluca, H. F. 1979. Vitamin D. Monographs on Endocrinology, Vol. 13. Springer-Verlag, Berlin, 80 pp.
- Dennell, R. 1960. Integument and exoskeleton. Pages 449-472 in T. H. Waterman (ed.) "The Physiology of Crustacea", Vol. I - Metabolism and Growth. Academic Press, New York.
- Deshimaru, O., and K. Shigeno. 1972. Introduction to the artificial diet for prawn Penaeus japonicus. Aquaculture 1:115-133.
- Deshimaru, O., and K. Kuroki. 1976. Studies on a purified diet for prawn. VII. Adequate dietary levels of ascorbic acid and inositol. Bulletin of the Japanese Society of Scientific Fisheries 42:571-576.
- Deshimaru, O., and Y. Yone. 1978. Requirement of prawn for dietary minerals. Bulletin of the Japanese Society of Scientific Fisheries 44:907-910.
- Deshimaru, O., K. Kuroki, S. Sakamoto, and Y. Yone. 1978. Absorption of labelled calcium-⁴⁵Ca by prawn from sea water. Bulletin of the Japanese Society of Scientific Fisheries. 44:975-977.
- Digby, P. S. B. 1968. Mobility and crystalline form of the line in the cuticle of the shore crab, Carcinus maenas. Journal of Zoology 154:273-286.
- Durliat, M., and R. Vranckx. 1981. Action of various anticoagulants on hemolymphs of lobsters and spiny lobsters. The Biological Bulletin, Marine Biological Laboratory, Woods Hole. 160:55-68.
- Fisher, W. S., E. H. Nilson, J. F. Steenbergen, and D. V. Lightner. 1978. Microbial diseases of cultured lobsters: a review. Aquaculture 14:115-140.
- Fisher, L. R., and S.K. Kon. 1959. Vitamin A in invertebrates. Biological Reviews 34:1-36.
- Gallagher, M. L., W. D. Brown, D. E. Conklin, and M. Sifri. 1978. Effects of varying calcium/phosphorus ratios in diets fed to juvenile lobsters (Homarus americanus). Comparative Biochemistry and Physiology 60A:467-471.
- Ganguly, J., M. R. S. Rao, S. K. Murthy, and K. Sarada. 1980. Systemic mode of action of vitamin A. Vitamins and Hormones, 38:1-54.
- George, J. C., B. J. Barnett, C. Y. Cho, and S. J. Slinger. 1981. Vitamin D3 and muscle function in rainbow trout. Cytobios 31:7-18.
- Gould, B. S., and H. Shwachman. 1942. Bone and tissue phosphatase in experimental scurvy and studies on the source of serum phosphatase. American Journal of Physiology 135:485-494.
- Greenaway, P. 1974a. Total body calcium and haemolymph calcium concentrations in the crayfish Austropotamobius pallipes (Lereboullet). Journal of Experimental Biology 61:19-26.

- Greenaway, P. 1974b. Calcium balance at the premoult stage of the freshwater crayfish Austropotamobius pallipes (Lereboullet). Journal of Experimental Biology 61:27-34.
- Greenaway, P. 1974c. Calcium balance at the postmoult stage of the freshwater crayfish Austropotamobius pallipes (Lereboullet). Journal Experimental Biology 61:35-45.
- Guary, J. C., and A. Kanazawa. 1973. Distribution and fate of exogenous cholesterol during the molting cycle of the prawn Penaeus japonicus Bate. Comparative Biochemistry and Physiology 46A:5-10.
- Guary, M. M., H. J. Ceccaldi, and A. Kanazawa. 1975. Variations du taux d'acide ascorbique au cours du developpement de l'ovaire et du cycle d'intermue chez Palaemon serratus (Crustacea: Decapoda). Marine Biology 32:349-355.
- Guary, M., A. Kanazawa, N. Tanaka, and H. J. Ceccaldi. 1976. Nutritional requirements of prawn - VI, requirement for ascorbic acid. Memoirs Faculty of Fisheries, Kagoshima University 25:53-57.
- Gwinn, J. F., and J. R. Stevenson. 1973a. Role of acetylglucosamine in chitin synthesis in crayfish. I. Correlation of ¹⁴C-acetylglucosamine incorporation with stages of the molting cycle. Comparative Biochemistry and Physiology 45B:769-776.
- Gwinn, J. F., and J. R. Stevenson. 1973b. Role of acetylglucosamine in chitin synthesis in crayfish. II. Enzymes in the epidermis for incorporation of acetylglucosamine into UDP-acetylglucosamine. Comparative Biochemistry and Physiology 45B:777-785.
- Hackman, R. H. 1971. The integument of Arthropoda. Pages 1-62 in M. Florkin and B. T. Schneer (eds.) "Chemical Zoology", Vol. VI - Arthropoda, Part B. Academic Press, New York.
- Hackman, R. H. 1974. The soluble cuticular proteins from three arthropod species: Scylla serrata (Decapoda: Portunidae), Boophilus microphus (Acarina: Ixodae) and Agrianome spinicollis (Coleoptera: Cerambycidae). Comparative Biochemistry and Physiology 49B:457-464.
- Harris, P., and Embree, N. 1963. Quantitative consideration of the effects of polyunsaturated fatty acid content of the diet upon the requirement for vitamin E. American Journal of Clinical Nutrition 13:385-392.
- Hayes, D. K., L. Singer, and W. D. Armstrong. 1962. Calcium homeostatic mechanisms and uptake of radioisotopes in the lobster. American Journal of Physiology 202:383-386.
- Hegdahl, T., J. Silness, and F. Gustausen. 1977a. The structure and mineralization of the carapace of the crab (Cancer pagurus L.). 1. The endocuticle. Zoological Scripta 6:89-99.
- Hegdahl, T., F. Gustausen, and J. Silness. 1977b. The structure and mineralization of the carapace of the crab (Cancer pagurus L.). 3. The epicuticle. Zoological Scripta 6:215-220.

- Hohnke, L. A. 1971. Enzymes of chitin metabolism in the decapod, Hemigrapsus nudus. Comparative Biochemistry and Physiology 40B:757-779.
- Hornung, D. E., and J. R. Stevenson. 1971. Changes in the rate of chitin synthesis during the crayfish molting cycle. Comparative Biochemistry and Physiology 40B:341-346.
- Huner, J. V., J. G. Kowalczyk, and J. W. Avault, Jr. 1976. Calcium and magnesium levels in the intermolt (C₄) carapaces of three species of freshwater crayfish (Cambaridae: Crustacea). Comparative Biochemistry and Physiology 55A:183-185.
- Huner, J. V., L. B. Colvin, and B. L. Reid. 1979a. Postmolt mineralization of the exoskeleton of juvenile California brown shrimp, Penaeus californiensis (Decapoda: Penaeidae). Comparative Biochemistry and Physiology 62A:889-893.
- Huner, J. V., L. B. Colvin, and B. L. Reid. 1979b. Whole body calcium, magnesium and phosphorus levels of the California brown shrimp, Penaeus californiensis (Decapoda: Penaeidae) as functions of molt stage. Comparative Biochemistry and Physiology 64A:33-36.
- Kanazawa, A., S. Teshima, and S. Tokiwa. 1977. Nutritional requirements of prawn - VII Effect of dietary lipids on growth. Bulletin of the Japanese Society of Scientific Fisheries 43:849-856.
- Kanazawa, A., S. Teshima, S. Tokiwa, M. Endo, and F. A. A. Razeq. 1979. Effects of short-necked clam phospholipids on the growth of the prawn. Bulletin of the Japanese Society of Scientific Fisheries 45:961-965.
- King, K., Jr. 1978. Distribution of γ -carboxyglutamic acid in calcified tissues. Biochimica et Biophysica Acta 542:542-546.
- Kitabayashi, K., H. Kurata, K. Shudo, K. Nakamura, and S. Ishikawa. 1971a. Studies on formula feed for kuruma prawn-I. On the relationship among glucosamine, phosphorus, and calcium. Bulletin of the Tokai Regional Fisheries Research Laboratory; 65:91-140.
- Kitabayashi, K., K. Shudo, K. Nakamura, and S. Ishikawa. 1971b. Studies on formula feed for kuruma prawn-II. On the utilization values of glucose. Bulletin of the Tokai Regional Fisheries Research Laboratory 65:109-118.
- Kleinholz, L. H., and R. Keller. 1979. Endocrine regulation in crustacea. Pages 159-213 in E. J. W. Barrington (ed.) "Hormones and Evolution", Vol. I. Academic Press, New York.
- Kramer, K. J., L. H. Hendricks, Y. T. Liang, and P. A. Seib. 1978. Effect of ascorbic acid and related compounds on the tobacco hornworm, Manduca sexta Johannson (Lepidoptera: Sphingidae). Journal of Agricultural and Food Chemistry 26:874-878.
- Lang, R. 1971. Chitinsynthese bei dem Flusskrebs. Orconectes limosus: Aklurtat der phosphoglucosaminisomerase and einbau von glucose-U-¹⁴C in chitin. Zeitschrift fur Vergleichende Physiologie 73:305-316.

- Lightner, D. V., L. B. Colvin, C. Brand, and D. A. Danald. 1977. Black death, a disease syndrome of penaeid shrimp related to a dietary deficiency of ascorbic acid. *Proceedings of the World Mariculture Society* 8:611-618.
- Lightner, D. V., B. Hunter, P. C. Magarelli, Jr., and L. B. Colvin. 1979. Ascorbic acid: Nutritional requirement and role in wound repair in penaeid shrimp. *Proceedings of the World Mariculture Society* 10:513-528.
- Mary, R. F., and G. Krishnan. 1974. On the nature and role of protein constituents of the cuticle of crustaceans in relation to permeability of the cuticle. *Marine Biology* 25:299-309.
- McWhinnie, M. A. 1961. Gastrolith growth and calcium shifts in the freshwater crayfish, Oronectes virilis. *Comparative Biochemistry and Physiology* 7:1-14.
- McWhinnie, M. A., M. O. Cahoon, and R. Johanneck. 1969. Hormonal effects on calcium metabolism in crustacea. *American Zoologist* 9:841-855.
- Mills, B. J., and P. S. Lake. 1976. The amount and distribution of calcium in the exoskeleton of the intermolt crayfish Parastacoides tasmanicus (Erichson) and Astacopsis fluviatilis (Gray). *Comparative Biochemistry and Physiology* 53A:355-360.
- Mills, B. J., P. Suter, and P. S. Lake. 1976. The amount and distribution of calcium in the exoskeleton of intermolt crayfish of the genera Engaeus and Geocharax. *Australian Journal of Marine and Freshwater Research* 27:517-523.
- National Research Council - National Academy of Sciences. 1973. Nutrient requirements of trout, salmon, and catfish. *Nutritional Requirements of Domestic Animals*, No. 11. 57 pp.
- National Research Council - National Academy of Sciences. 1977. Nutrient requirements of poultry. *Nutritional Requirements of Domestic Animals*, No. 1. 62 pp.
- Navon, A. 1978. Effects of dietary ascorbic acid on larvae of the Egyptian cotton leafworm, Spodoptera littoralis. *Journal of Insect Physiology* 24:39-44.
- Nelville, A. C. 1975. "Biology of the Arthropod Cuticle", Springer-Verlag, New York. 448 pp.
- Nes, W. R. 1974. Role of sterols in membranes. *Lipids* 13:742-753.
- New, M. B. 1976. A review of dietary studies with shrimp and prawns. *Aquaculture* 9:101-144.
- Pahlic, M., and J. R. Stevenson. 1978. Glucosamine-6-phosphate synthesis in the crayfish epidermis. *Comparative Biochemistry and Physiology* 60:281-285.
- Passano, L. M. 1960. Molting and its control. Pages 473-536 in T. Waterman (ed.) "The Physiology of Crustacea", Vol. I. Academic Press, New York.

- Ponat, A., and D. Adelung. 1980. Studies to establish an optimal diet for Carcinus maenas. II. Protein and lipid requirements. Marine Biology 60:115-122.
- Rawson, D. S. 1939. Some physical and chemical factors in the metabolism of lakes. Pages 9-26 in F. R. Moulton (ed.) "Problems in Lake Biology", American Association for the Advancement of Science, Publication No. 10, Lancaster, Penn.
- Richards, A. G. 1951. "The Integument of Arthropods" University of Minnesota Press. Minneapolis, Minnesota. 411 pp.
- Roer, R. D. 1980. Mechanisms of resorption and deposition of calcium in the carapace of the crab Carcinus maenas. Journal of Experimental Biology 88:205-218.
- Sameshima, M., T. Katayama, and O. Deshimaru. 1973. Constituents of the amino acids composed of the protein in the exoskeletons of the crustacea. Memoirs Faculty of Fisheries, Kagoshima University 22:33-37.
- Sedgwick, R. W. 1980. The requirements of Penaeus merguensis for vitamin and mineral supplements in diets based on freeze-dried Mytilus edulis meal. Aquaculture 19:127-137.
- Simkiss, K. 1976. Cellular aspects of calcification. Pages 1-31 in N. Watabe and K. M. Wilber (eds.) "The Mechanisms of Mineralization in the Invertebrates and Plants". University of South Carolina Press, Columbia, South Carolina.
- Speck, U., K. Urich, and U. Herz-Hubner. 1972. Nacheveis einer regulation der glucoaminbildung bei dem flusskrebs Orconectes linosus zur zeit der hautung. Zeitschrift fur Vergleichende Physiologie 76:341-346.
- Stenflo, J., and J. W. Suttie. 1977. Vitamin K-dependent formation of γ -carboxyglutamic acid. Annual Review of Biochemistry 46:157-172.
- Stevenson, J. R., and R. P. Schneider. 1962. Tyrosinase activity of organs containing tegumental glands in the crayfish. Journal of Experimental Zoology 150:17-25.
- Stevenson, J. R., and B. P. Hettick. 1980. Metabolism of chitin precursors by crayfish tissues during chitin synthesis. Journal of Experimental Zoology 214:37-48.
- Travis, D. F. 1955a. The molting cycle of the spiny lobster Panulirus argus Latreille. II. Pre-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues. The Biological Bulletin, Marine Biological Laboratory, Woods Hole 108:88-112.
- Travis, D. F. 1955b. The molting cycle of the spiny lobster, Panulirus argus - III. Physiological changes which occur in the blood and urine during the normal molt cycle. The Biological Bulletin, Marine Biological Laboratory, Woods Hole 109:484-503.
- Travis, D. F. 1960a. Matrix and mineral deposition in skeletal structures of the decapod Crustacea. Pages 57-116 in R. F. Sognaes (ed.) Publication of the American Association for the Advancement of Science Vol. 64, Washington.

- Travis, D. F. 1960b. The deposition of skeletal structures in the Crustacea. I. The histology of the gastrolith disc, skeletal tissue complex and the gastrolith in the crayfish, Orconectes (Cambarus) virilis Hagen. The Biological Bulletin, Marine Biological Laboratory, Woods Hole 118:137-149.
- Travis, D. F. 1963. Structural features of mineralization from tissue to macromolecular levels of organization in decapod Crustacea. Annual of the New York Academy of Science 109:177-245.
- Travis, D. F. 1965. The deposition of skeletal structures in the Crustacea. 5. The histomorphological and histochemical changes associated with the development and calcification of the brachial exoskeleton in the crayfish, Orconectes virilis Hagen. Acta Histochemica 20:193-222.
- Travis, D. F., D. J. Francois, L. C. Bonar, and M. J. Glimcher. 1967. Comparative studies on the organic matrices of invertebrate mineralized tissues. Journal of Ultrastructure Research 18:519-550.
- van der Horst, D. J., R. C. H. M. Oudejans, A. G. Plug, and I. van der Sluis. 1973. Fatty acids of the female horseshoe crab Xiphosura (Limulus) polyphemus. Marine Biology 20:291-296.
- Welinder, B. S. 1974. The crustacean cuticle I. Studies on the composition of the cuticle. Comparative Biochemistry and Physiology 47A:779-787.
- Welinder, B. S. 1975a. The crustacean cuticle II. Depositions of organic and inorganic material in the cuticle of Astacus fluviatilis in the period after molting. Comparative Biochemistry and Physiology 51B:409-416.
- Welinder, B. S. 1975b. The crustacean cuticle III. Composition of the individual layers in Cancer pagurus cuticle. Comparative Biochemistry and Physiology 52A:659-663.
- Wilson, R. P. and W. E. Poe. 1973. Impaired collagen formation in the scorbutic channel catfish. Journal of Nutrition 103:1359-1364.
- Yamaoka, L. H., and B. T. Scheer. 1971. Chemistry of growth and development in crustaceans. Pages 321-341 in M. Florkin and B. T. Scheer (eds.) "Chemical Zoology", Vol. V. Academic Press, New York.
- Yonge, C. M. 1936. On the nature and permeability of chitin. II. The permeability of the uncalcified chitin lining the foregut of Homarus. Proceedings of the Royal Society of London 120:15-41.

QUESTIONS AND ANSWERS - CONKLIN

FARMANFARMIAN (Rutgers University): For what it might be worth let me give you a little information on work in my laboratory with Macrobrachium and dietary supplementation with calcium. One's first thoughts observing a crustacean voraciously consuming its exuvia is that there might be a calcium deficiency. To examine this possibility calcium supplements of 1, 2, and 3% were added to a repelletized commercial marine ratio we have used. In no case, over an approximately 14 weeks growth trial, was growth stimulated significantly and there was no change in the appetite for the exuvia.

CONKLIN: Undoubtedly, the various minerals deposited in the exoskeleton matrix are important elements of this structure and, because of its abundance, calcium is typically the one examined. Most of the reported work on crustaceans seems to support your results; a dietary source of calcium is not necessary for crustaceans. Presumably, the requirement for calcium is being met by uptake from the water. I do feel, however, that the focus on calcium has obscured the necessity of examining the dietary need for other important minerals such as phosphorus and sulfur.

