Table II. Frequencies and assignments for hydroxyapatite, fluorapatite and fluorchlorapatites

Apatites					Assignment
$Ca_{10}(PO_4)_6(OH)_2$ Frequency cm^{-1}	$Ca_{10}(PO_4)_6F_2$	$Ca_{10}(PO_4)_6F_{0.554}Cl_{1.446}$	$Ca_{10.4}(PO_4)_6F_{1.449}Cl_{0.579}$	$Ca_{10}(PO_4)_6F_{2-x}Cl_x$ ($x = \pm 0.06$)	
365 m	325 w	319 w	340 w	335 m	ν_2 PO_4 ?
425 vw	438 w	428 w	430 w	420 vw	ν_3 1050–ν_4 605 = 445
475 w	478 w	472 m	475 m	440 vw 472 m	ν_3 1050–ν_4 605 = 445 ν_3 1050–ν_4 572 = 478
572 s 605 s 635 s	565 s 580 s 605 s	555 s 570 s 605 s	565 s 575 s 602 s	565 s 578 s 605 s	ν_4 PO_4
965 m	968 w	962 w	962 w	962 w	ν_1 PO_4
1050 s 1095 s	1025 s 1050 s 1096 s	1032 s 1045 s 1085 s	1045 s 1090 s 1095 s	1005 s 1055 s 1092 s	ν_3 PO_4
3578 m	–	–	–	–	OH

363 cm^{-1} should also thus become IR-active. The triple degeneracies of ν_3 and ν_4 are both lost. This loss of degeneracy causes the band at about 1090 cm^{-1}, which could also arise due to interactions between the 6 phosphate ions in the apatite unit cell. However, the band at about 962 cm^{-1}, which is IR-inactive in the undistorted ion, arises from the lowering of the symmetry.

2 bands which appear consistently in the spectra of fluorapatite and fluorchlorapatites absorb in the region 420–480 cm^{-1}. They are assigned as the difference tones, as the numerical values of their frequencies are close in magnitude to the difference between ν_3 and ν_4. STUTMAN et al.[2] also observed 1 band in this region and they excluded it from a ν_4 designation[6].

Zusammenfassung. Es wird eine Zuordnung der IR-Absorptionsbanden zu den Tetraederschwingungen des PO_4-Tetraeders in Apatit gegeben.

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Astaxanthin in the Blue Oceanic Barnacle *Lepas fascicularis*[1]

Specimens of the blue, oceanic, stalked barnacle *Lepas fascicularis* are encountered occasionally during spring cruises, or more rarely when swept shoreward by strong prevailing winds. This pelagic, hermaphroditic crustacean adheres, through its stalk, to such objects as floating feathers, bits of tar, cork, wood or other flotsam, and relatively frequently to the small oceanic siphonophore *Velella lata*, whose blue color it closely matches. After the barnacle's own float has grown sufficiently large and air-laden to afford independent support, the animal relinquishes its adherence to a foreign object.

Inspection of the materials consumed by *L. fascicularis* reveals that the animal feeds extensively upon microcrustaceans such as copepods. Earlier studies of the carrying host, *V. lata*, had shown that these animals also feed upon small pelagic crustaceans, floating marine eggs, larvae and microscopic detritus, and that the blue pigment of this colonial coelenterate involves astaxanthin chromoprotein complexes[2]. And indeed BALL reported, in 1944, that the blue eggs of *L. fascicularis* involved protein conjugated with a xanthophyllic carotenoid suspected of being astaxanthin[3].

In April 1966 a blue *Lepas* was taken from one of a *Velella* population which had been wind-transported toward shore off the Scripps Institution. Since this species of barnacle is of such chromatic interest, but so relatively rare, it was decided to complete such carotenoid analyses as might be feasible, in order to outline some main facts about its body pigmentation (e.g. whether pigmentary or Tyndall-blue) until, in a later spring, additional specimens should make possible a more searching investigation.

[1] These researches are among those supported by Research Grant No. GB-2312 to the senior author from the National Science Foundation. We are indebted also to Dr. W. A. NEWMAN for his help and information concerning the biology of this barnacle.

[2] D. L. FOX and F. T. HAXO, XVth Int. Congr. Zool., Sect. III, No. 2 (1958).

[3] E. G. BALL, J. biol. Chem. *152*, 627 (1944).

Because the specimen was wanted by a colleague for anatomical examination and identification, its tissues were not dissected for separate analysis on this first occasion. The whole animal (wet weight = 1.84 g) was stored in a small volume of ethanol under refrigeration until all carotenoid pigment had been leached from its body. On immersion in the alcohol, the body, stalk and appendages turned from blue to pink in color; the internal tissues gradually underwent the same color change, while the white, bubble-filled float remained essentially unchanged in appearance.

When the barnacle, now a pale straw-color, conferred no more color to alcohol, the extracted pigments were transferred from the orange-yellow ethanol solution into hexane. Herein they exhibited a single plateau or very shallow, saddle-like maximum extending from 454–474 nm, at a density reflecting an original concentration of about 1.2 mg of carotenoid/100 g wet tissue (astaxanthin units). The partition ratio of this crude extract between hexane and 95% methanol was 19:81, suggesting a preponderance of unesterified xanthophyllic carotenoids.

After exposure to ethanolic NaOH at 60–70 °C, there remained, in addition to major amounts of a red soap, a persistent neutral fraction, which exhibited maxima at 473, 446 and ~ 423, and a minimum at 465 nm, thus suggesting the presence of β-carotene and/or derivatives thereof. The partition ratio of 33:67, found for this neutral residue, suggested that a dihydroxy β-carotene (e.g. zeaxanthin, with a known partition ratio of 11:89) might be accompanied by minor proportions of β-carotene, which is completely epiphasic, or possibly by one or another of the monohydroxy β-carotenes, which are 82–86% epiphasic[4]. This neutral fraction amounted to 24.4% of the total carotenoid, while the remaining 75% was astaxanthin, recovered from the red Na-salt as free astacene, and exhibiting a single, smooth maximum at 493 nm in pyridine.

The blue body-color of *L. fascicularis* is therefore concluded to be that of a conjugated astaxanthin chromoprotein, as in its own eggs[3], in numerous other crustaceans[5], and in the siphonophore *Velella*[2]. The barnacle exhibits its blue pigmentation irrespective of whether it has anchored itself to *Velella* or to inanimate flotsam.

With the availability of a more favorable supply of this unusual *Lepas* species, gathered during a spring cruise, it should be possible to explore for the potential selective fractionation of carotenoids among its tissues, and to determine, for example, the kinds and relative concentrations of neutral carotenoid fractions in the somatic and gonadal tissues.

Zusammenfassung. Die blaue Farbe der ozeanischen Cirripedien, *Lepas fascicularis*, wird als Chromoproteid mit Astaxanthin als Carotinoidkomponente identifiziert.

D. L. Fox and G. F. Crozier

Department of Marine Biology, Scripps Institution of Oceanography, University of California, San Diego, La Jolla (California, USA), August 23, 1966.

[4] F. J. Petracek and L. Zechmeister, Analyt. Chem. *28*, 1484 (1956).

[5] D. L. Fox, *Animal Biochromes* (Cambridge University Press, 1953), p. 117.

Sodium Intake in Goldfish

Freshwater fish constantly lose sodium in their feces, in their urine, and by diffusion through surface tissues. These sodium losses are corrected in 2 ways: through the absorption of sodium ions from the surrounding water by the gills and through the ingestion of sodium-containing foods. The control of the absorption of sodium by the gills has been studied extensively (see Black, 1957, for a comprehensive bibliography and a review of the field[1]). To our knowledge, no study has been conducted on regulation of sodium ingestion in these animals.

Materials and animals. The 12 goldfish used in these experiments were common pool goldfish (*Carassius auratus*) between 4 and 6 inches long. They were purchased locally. The pellets used in the experiments consisted of a gelatine-albumin-starch-agar base. In addition, various salt mixtures were added to all but the salt-free pellets. The standard pellets contained Hegsted salts[2]. The sodium pellets contained sodium chloride. The potassium pellets contained potassium chloride and the sodium-free salt pellets contained a commercial sodium-free salt mixture. A small amount of corn-oil and of a glucose vitamin mixture was added to all pellets used in experiment 2.

Method. In the experiments presented here, regulation of sodium intake in the goldfish was specifically studied by measuring changes in the preference of the goldfish for sodium following sodium depletion. A simultaneous preference measure (the relative amount eaten of 1 food when 2 or more foods are available) was used, rather than a single intake measure (the total amount of food eaten when only 1 food is available). It was necessary to use the simultaneous preference measure because of the large variations in day to day intake of many goldfish[3] and because of the possibility that sodium deprivation might cause anorexia in the fish. The simultaneous preference measure is relatively insensitive to these variations, while the single intake measure is rendered invalid by them.

The basic procedure used was the following: (1) the fish were maintained in tap water with standard pellets available for 1 h/day; (2) the fish were pre-tested by making available equal numbers of sodium pellets and control pellets (either salt-free pellets or potassium pellets); (3) the fish were subjected to a sodium stress by maintaining them in distilled demineralized water for 5 days and making pellets containing a sodium-free salt mixture available for 1 h each day; (4) the fish were tested by making available equal numbers of sodium pellets and control pellets. In Experiment 1, salt-free

[1] V. S. Black, *Excretion and Osmoregulation*, in *The Physiology of Fishes* (M. E. Brown; Academic Press Inc.) *1*, 163 (1964).

[2] D. M. Hegsted, R. C. Mills, C. A. Elvehjem, and E. B. Hart, J. biol. Chem. *138*, 459 (1941).

[3] P. N. Rozin and J. Mayer, Am. J. Physiol. *201*, 968 (1961).