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).

pellets were used as control pellets. In experiment 2, potassium pellets were used as control pellets.

Results. In experiment 1, 5 out of the 6 fish completing the experiment increased their preference for the sodium pellets as a result of deprivation (Figure 1). Before deprivation, 4 fish showed a stronger preference for the salt-free pellets than for the sodium pellets, 1 fish showed a stronger preference for the sodium pellets, and 1 fish ate an equal number of both types. After deprivation, 5 fish showed a stronger preference for the sodium pellets than for the salt-free pellets and only 1 showed a stronger preference for the salt-free pellets. In experiment 2, all 6 fish increased their preference for the sodium pellets as a result of deprivation (Figure 2). Before deprivation, 3 fish showed a stronger preference for the sodium pellets than for the potassium pellets, and 3 showed a stronger preference for the potassium pellets than for the sodium pellets. After deprivation, all 6 fish showed a stronger preference for the sodium pellets than for the potassium pellets.

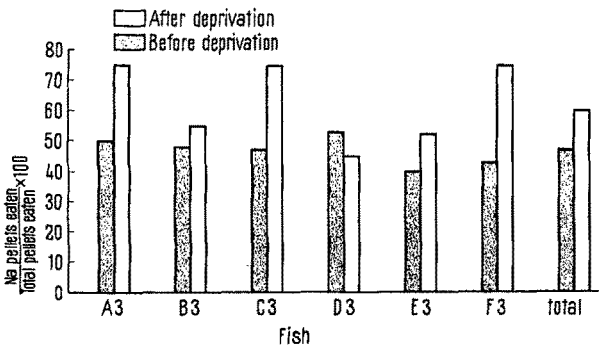

Fig. 1. Relative preference shown for sodium pellets before and after deprivation using salt-free control pellets.

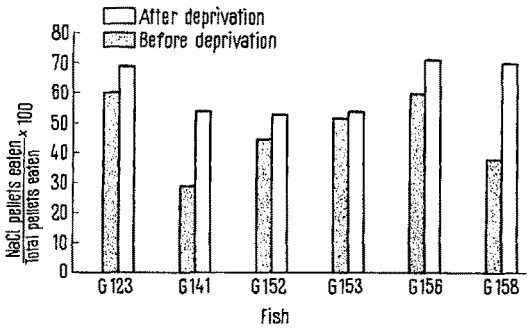

Fig. 2. Relative preference shown for sodium pellets before and after deprivation using potassium control pellets.

Conclusion. The results from experiment 1 show that goldfish increase their preference for salt-containing foods when they are kept in water with a low sodium content. The results of experiment 2 suggest that the sodium ion in the salt-containing foods is responsible for this increased preference[4].

Résumé. Les poissons rouges sentent le manque de sodium et, lorsqu'ils sont placés dans de l'eau contenant très peu de sodium, leur préférence pour les aliments contenant du sel est accrue.

S. A. COPANS and J. MAYER

The Department of Nutrition, Harvard School of Public Health, Boston (Massachusetts 02115, USA),
July 29, 1966.

[4] This work was supported in part by a grant-in-aid from the National Institute of Neurological Diseases and Blindness (NB-01941), National Institutes of Health, U.S. Public Health Service, Bethesda, Maryland, and the Fund for Research and Teaching of the Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts.

Exotoxin in Malaria Infection (*Plasmodium berghei*)

Recent investigations have shown [1,2] that large amounts of plasmodium proteins are necessary to induce protective immunity against *Plasmodium berghei*. About 5 g/kg body weight of the antigen must be injected in the course of 28 days in order to provide 80% of mice of the Swiss strain with an established immunity. This dose is much higher than the average amount of antigen needed for immunization against soluble heterologous proteins. From this we concluded that only a small part of the plasmodium acts as the specific antigen which produces the protective immunity. But it was not known whether an exotoxin, perhaps stored in the parasite or in the host cell, was responsible for the mechanisms of immunity. As the assumed exotoxin should appear in the serum after the rupture of the host cell during schizogony, the following investigations were carried out.

Methods. 5 groups (I–V) of 25 Swiss mice each were injected with different amounts of serum obtained from mice with high parasitemia (12th day post infection with *Pl. berghei*). A total of 9 injections (3 each week) were given i.p. without any adjuvants to each animal: (group I) 0.005 ml per shot (total 1.5 mg serum protein), (II) 0.01 ml (3 mg), (III) 0.1 ml (30 mg), (IV) 0.3 ml (90 mg), and (V) 0.6 ml (180 mg). 1 day after the last injection the mice were infected with about $5 \cdot 10^6$ parasites (*Pl. berghei*). 100 untreated animals kept under equal conditions (standard diet, water, room temperature) served as controls. For blood smears see Table.

Results. Surprisingly, all immunized mice developed daily-increasing parasitemia, just like the untreated animals. And like the control group, of which no animals survived the 25th day post infection, all mice of groups IV and V died at the same time. However, 2 animals (8%) of group I, 11 (44%) of group II, and 1 (4%) of group III survived this critical time. Though developing

[1] C. JERUSALEM, IInd Int. Conf. Protozool., London, 1965, p. 208.
[2] C. JERUSALEM, IX. Int. Congr. Mikrobiol., Moskau, 1966, p. 571.