Some Observations on Pigments of the Pacific Sand Dollars, *Dendraster excentricus* and *Dentraster laevis*[1]

Two of the commoner flat echinoid species of the Pacific coast are the purple sand dollar, *Dendraster excentricus*, and its light tan or buff-coloured, smooth-surfaced relative, *D. laevis*, which possesses fewer, shorter, slenderer spines and which inhabits deeper, colder water (CLARK[2]). The animals live on sandy floors, often burying or half-burying themselves therein. They are typical feeders on sandy detritus.

Collections of each species were made by the diving instructor, Mr. CONRAD LIMBAUGH, on July 20–21, 1954, *Dendraster excentricus* being taken from a depth of about 6 fathoms beneath the surface in the New Valley of Scripps Submarine Canyon, and *D. laevis* from depths of 8–11 fathoms, on the floor of the La Jolla Submarine Canyon.

In addition to the relatively minor morphological differences between the two species, the obvious differences in their colours and in their respective habitats, there is a striking biochemical contrast which led to this investigation. When preserved in dilute formaldehyde solution, each species readily yielded its own characteristic pigment, the purple animals conferring a deep port-wine purple, and the buff ones, a conspicuous green colour to the aqueous solution, while the whole external surfaces of the latter animals changed from light buff to deep green in colour. A similar discharge of pigment occurs in distilled water.

An earlier report briefly mentioned the presence of echinochrome in the ectoderm, endoderm, intestine, ovary, spermary and egg cases of *Dendraster excentricus* (FOX and SCHEER[3]). In the present study, two or more such fractions were recognized, and will be discussed below.

Dendraster laevis. This species often exhibits small green patches or scars when first collected. The effect may be replicated quickly by rubbing or scraping the animal's surface to rupture the ectoderm, which at once turns green, while the pigment is readily liberated into sea water. The whole internal organs yield the green pigment into distilled water, just as does the whole intact animal.

In the leaching of the pigment by distilled water, the prevailing pH (5) is not the controlling factor, since sea water adjusted to the same pH value leached no pigment even after an overnight period; the animals remained alive. The pH had risen, however, after a few hours, to 5·8.

Sea water containing 10% (v/v) of concentrated formaldehyde produces the colour change to green only very slowly, and without evident leaching.

The green pigment is not soluble in ether or in chloroform; it loses its colour at pH values below about 3 (dilute acetic acid), turns green at about pH 5; above pH 10·0 it is precipitated in murky green flocs. The maxima at pH 5 are at 395, 430, (510), and 620 mμ, and at pH 10, 395, 455 and 660 mμ (Fig. 1).

The addition of very small amounts of hydrosulphite produces no obvious effects; somewhat more of the salt causes a discharge of the colour, due to pH effects, since some of the colour may be restored, not by shaking in air but by neutralizing with bicarbonate.

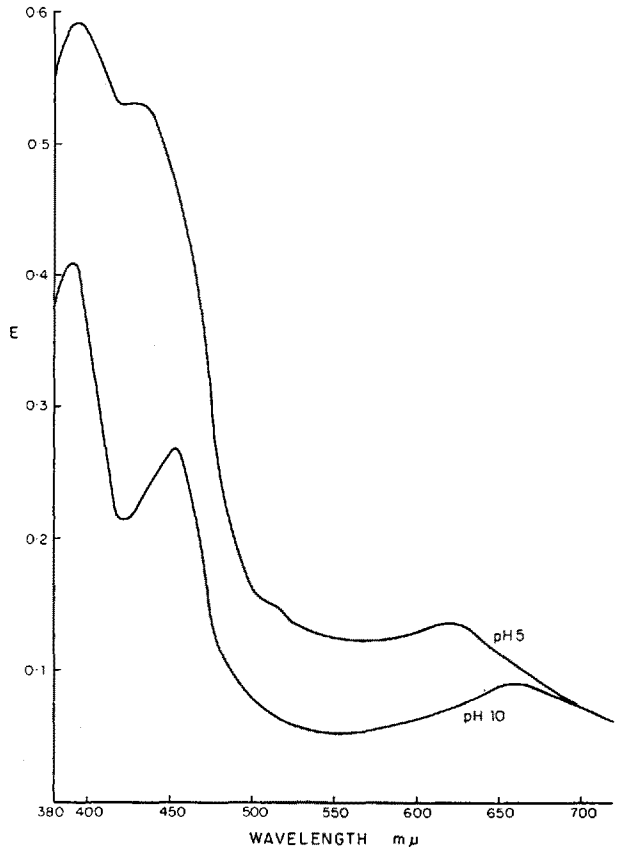

Fig. 1.–The absorption spectrum of the water-soluble pigments from *Dendraster laevis*. (*A*) at pH 5·0; (*B*) at pH 10·0.

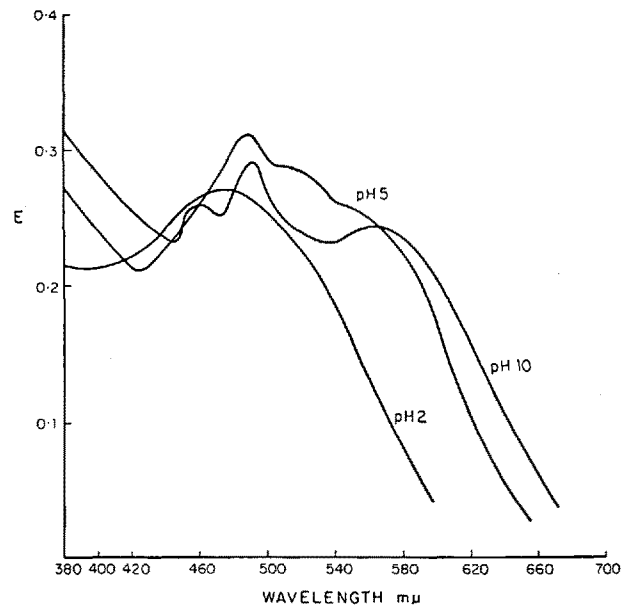

Fig. 2.–The absorption spectrum of the water-soluble pigments from *Dendraster excentricus*. (*A*) at pH 2; (*B*) at pH 5; (*C*) at pH 10.

[1] Contribution from the Scripps Institution of Oceanography, University of California, New Series No 773.

[2] H. L. CLARK, Allan Hancock Found. Publ., First Series Allan Hancock Pacific Expedition *8*, 225 (1948).

[3] D. L. FOX and B. T. SCHEER, Biol. Bull. Woods Hole *80*, 441 (1941).

When the shell of *D. laevis* is treated with HCl and ethyl ether (GOODWIN and SRISUKH[1]) no pigment passes into the ether as the skeleton dissolves, indicating the absence of any echinochrome-like naphthoquinone pigments.

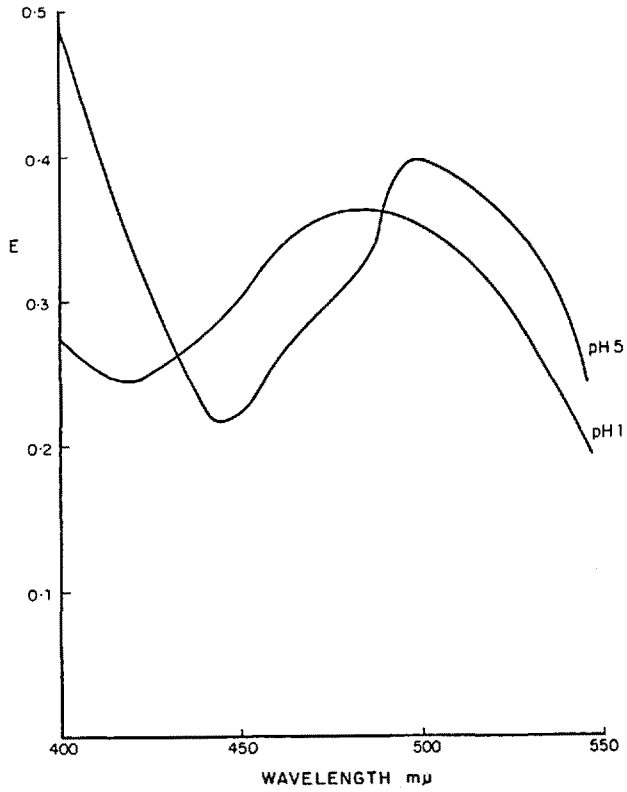

Fig. 3.–The absorption spectra of the least adsorbed pigment fraction from the water-soluble purple pigments of *Dendraster excentricus*. (i) at pH 1; (ii) at pH 5.

Dendraster excentricus. The water-soluble purple pigment of this species turns orange-red at pH 1 and is precipitated as a brownish mass at pH 10. Absorption maxima of aqueous systems at different pH values are shown in figure 2.

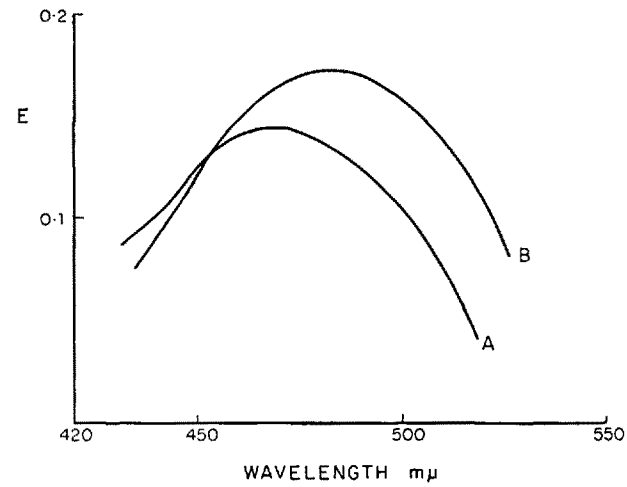

Fig. 4.–The absorption spectra in ether of the two main "echinochrome-like" pigments from *Dendraster excentricus*. (*A*) the less strongly adsorbed pigment; (*B*) the strongly adsorbed pigment.

[1] T. W. GOODWIN and S. SRISUKH, Biochem. J. *47*, 69 (1950).

The pigment passed uninterruptedly through a celite column, but was adsorbed by alumina, from which 10% HCl removed an orange-purple fraction (*A*) whilst a firmly bound purple component (*B*) remained at the top of the column.

The colour changes in fraction *A*, according to prevailing pH (Fig. 3), suggest that the firmly adsorbed purple fraction *B*, not yet isolated in pure form, may be mainly responsible for the prominent peak at 480 mμ, observed in the crude extracts (Fig.2). These pigments are not extractable with ether, but are reversibly reducible with hydrosulphite, which suggests a quinonoid character.

Treating the shells of *D. excentricus* with HCl and ether yields an orange ethereal extract, indicating the presence of echinochrome-like pigments (cf. FOX and SCHEER[1]). Chromatography on $CaCO_3$ yields two main fractions, although there are small traces of others as well. Fraction *A*, eluted with ether, exhibits a rather broad absorption maximum at 470 mμ; and *B*, eluted with ethanol, a maximum of similar shape at 485 mμ (Fig. 4). Both pigments are extractable from etheral solution with soda, and both are reversibly reducible with hydrosulphite. They do not appear to be any of the quinonoid pigments already described by LEDERER[2].

Thus the two species of *Dendraster* are in contrast through their pigments, as summarized in the table. The state of the water-soluble pigments in the tissues has not yet been investigated. The green pigment of *D. laevis* is normally present in its colourless form.

A Comparison of the Pigments of *Dendraster excentricus* and *D. laevis*

Pigment	*D. laevis*	*D. excentricus*
Water-soluble	Green; colourless below pH 5·0	Two pigments, purple above pH 5·0, orange-purple below pH 5·0
Ether-soluble	absent	Two echinochrome-like pigments present

Nutritional or metabolic differences between the two species, as emphasized by these marked pigmentary contrasts, would be well worth detailed study.

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Zusammenfassung

Dendraster laevis bildet ein grünes, wasserlösliches Pigment (farblos bei pH unter 5) und kein Echinochrom. *Dendraster excentricus* dagegen erzeugt zwei purpurrote, wasserlösliche und zwei echinochromähnliche Pigmente.

[1] D. L. FOX and B. T. SCHEER, Biol. Bull. Woods Hole *80*, 441 (1941).

[2] E. LEDERER, Biochem. Biophys. Acta *9*, 92 (1952).