cessively high corticoid concentrations—as at times of very acute stress—the regulation of the corticotrophin discharge may become relatively independent of circulating cortical hormone(s).

Summary. In the rat large doses of desoxycorticosterone acetate do not prevent the decrease in the concentration of adrenal ascorbic acid which accompanies exposure to cold. It is suggested that during severe stress, corticotrophin discharge is conditioned by factors other than mere lack of corticoids.

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Adenochrome-Like Pigment of the *Polyzoa bugula neritina* (L).

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Bugula neritina (L), a *Polyzoa* (Bryozoa) of the order *Cheilostomata,* presents a dark purple pigment not commonly found in other animals of the same order.[1] As far as we know no study of the pigments of Polyzoa has yet been reported.[2,3] Our specimens were collected in the bay of Rio de Janeiro near the Hydrobiological Station of the Institute.

The pigment of *Bugula* appears morphologically in the granular corpuscles of the distal part of the *zooecium* (Fig. 1) and is easily extracted with distilled water. The extract is wine-red in color and shows a Tyndall effect and a pH of 6.8.

The physical and chemical properties investigated showed that the pigment closely resembles the adenochrome isolated by Fox and Updegraff from the branchial hearts of the *Octopus*.[4] The absorption spectrum, however, exhibits a different maximum (545 mμ as compared with 505 mμ for the adenochrome).

Fox and Updegraff suggested that adenochrome is probably a waste product, as shown by its localization in the excretory tissues of the *Octopus* and the apparent storage of most of its nitrogen in the amide state, releasable as free ammonia in the presence of KOH.[4] In *Bugula* the pigment is accumulated in the distal part of the *zooecium* and around the *brown body* which is known to be an excretory waste mass. However, until now we have been unable to establish any relation between the pigment and the excretory function of *Bugula*. Other water-soluble pigments behaving as indicators of pH have been reported by Crozier in two sponges and a nudibranch but unfortunately no detailed chemical and spectroscopical data were published.[5]

Isolation and properties of the pigment. Animals from a colony (about 30 g) were washed with filtered sea water to free them of associated organisms, then weighed and extracted with boiling distilled water for 5 minutes. The residue was reextracted with boiling water until the solution was only slightly yellow. The extract was filtered and washed with petroleum ether (b.p.40-60°). The aqueous phase was made alkaline (pH 11.0) with KOH and the flocculent purple pigment was separated by centrifugation. The precipitate was dissolved in 10% acetic acid, then diluted with a few ml of

[1] Marcus, E., *Bol. Fac. Fil., São Paulo,* 1937, **1**, 67.

[2] Lederer, E., *Biol. Rev.,* 1940, **15**, 273.

[3] Fox, D. L., *Ann. Rev. Bioch.,* 1947, **16**, 443.

[4] Fox, D. L., and Updegraff, D. M., *Arch. Biochem.,* 1942-43, **1**, 339.

[5] Crozier, W. J., *J. Biol. Chem.,* 1918, **35**, 455.

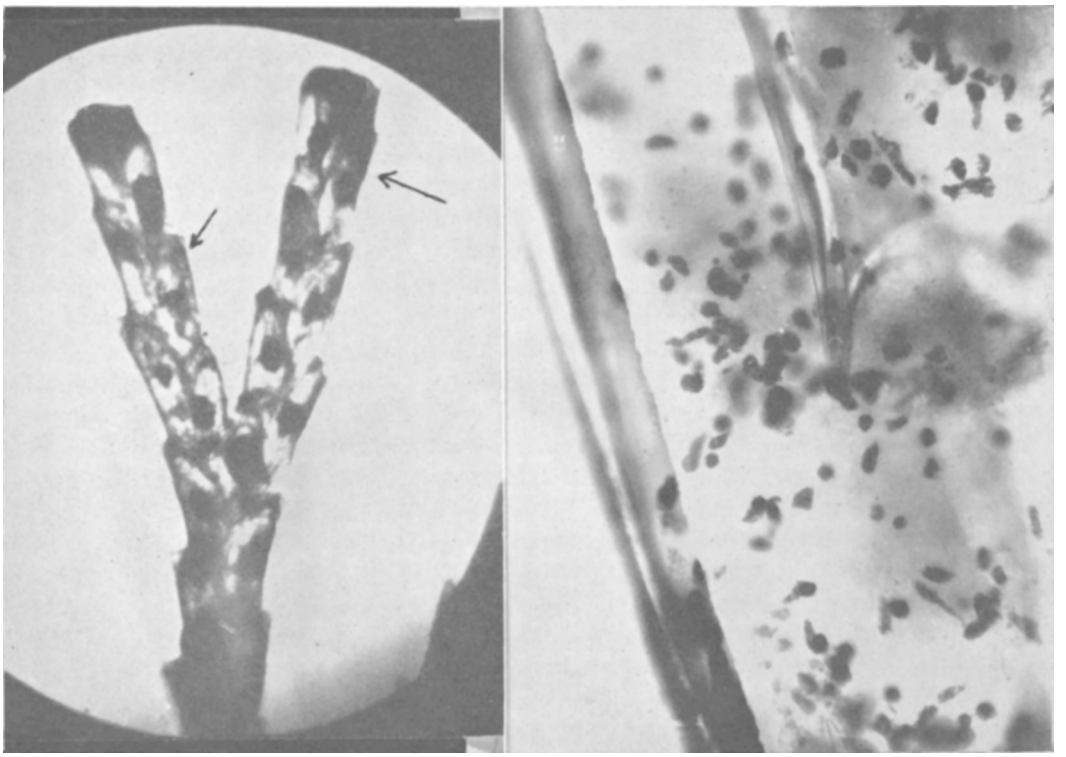

FIG. 1.
Left: Fresh preparation showing the distal distribution of the pigment in the *zooecium* (arrows). × 50. Right: Microphotography of the pigmented corpuscles. × 180.

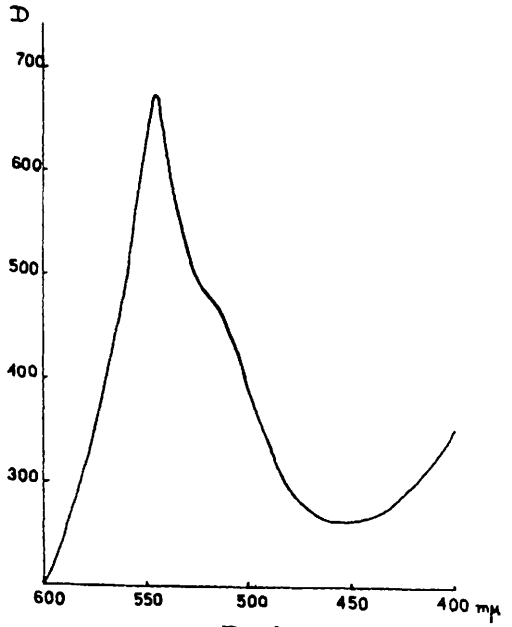

FIG. 2.
Absorption spectrum of the pigment in water at pH 6.8. Ordinate: optical density. Abscissa: wavelength in mμ.

water and reprecipitated with 4 N KOH. After centrifugation the precipitate was washed 3 times with 95% ethanol, once with ether and finally dried at 37°C. From 30 g of washed animals 21 mg of dry pigment were isolated. The powder had no melting point. Large variations in the pigment content were observed among individual colonies, the darker being richer than those presenting a pink color.

The absorption spectrum was determined with a Beckman spectrophotometer and the absorption curve showed a sharp maximum at 545 mμ, the concentration being 0.6 g/1000 and the pH 6.8. Fig. 2 shows a typical curve.

Since the color and the stability of the solutions of the pigment depend on the pH, titrations with alkali and acid were made to determine the behaviour of the solution at different H-ion concentrations. A water solution containing 4 mg of the pigment in 10 ml of solution was titrated with 0.2 N NaOH or

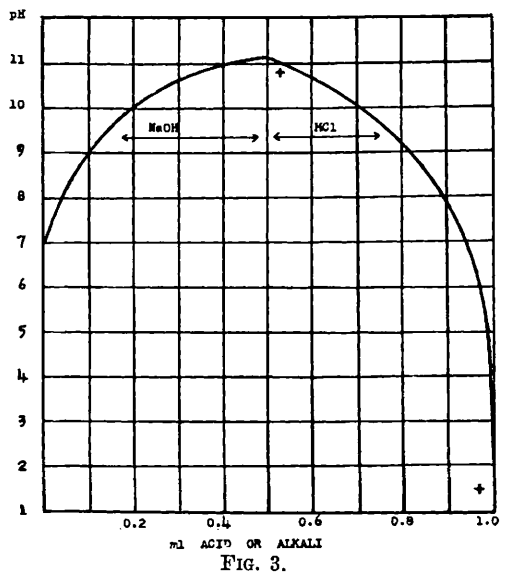

FIG. 3.

Titration curve of the pigment dissolved in water. Ordinate: pH units. Abscissa: ml of standard alkali or acid (0.2 N).

HCl solutions and the pH determined at intervals with a Macbeth pH-meter equipped with glass electrodes. At pH 11.1 all the pigment was precipitated and back titration to pH 9.2 with standard acid redissolved it; it reprecipitated at pH 2.0. A titration curve is shown in Fig. 3 and the precipitation zones are indicated with a cross. Inspection of this curve reveals the marked buffering capacity of the dissolved pigment. The changes of color with pH are recorded in Table I.

The pigment is soluble in water, very soluble in alkaline solutions, but insoluble in the common organic solvents. Addition of acetone to the aqueous extract (10 volumes of acetone to 1 volume of the extract) precipitates the pigment. The dried precipitate is easily dissolved in alkali or acid. When water solutions of the pigment are treated with concentrated KOH or NaOH small bubbles develop which react with Nessler reagent. Fox and Updegraff reported a similar reaction with adenochrome.

TABLE I.

pH	Color
2.4	blue-purple
3.0	purple
5.0	red-wine
6.8	red-wine
8.0	red
9.5	purple
11.0	purple-blue

The pigment of *Bugula* is adsorbed on MgO, $Ca(OH)^2$ and $CaCO$.[3] It was possible to liberate it from the $CaCO_3$ with 10% acetic acid solution. Strong adsorption of the pigment was also obtained on Brockmann's aluminum hydroxide, but all of the solvents tried failed to extract it from the adsorbate. Zinc dust in KOH and sodium hydrosulfite produced rapid decolorization. $KMnO_4$ and H_2O_2 also discharged the color, which partially returned after the addition of sodium hydrosulfite. Biuret and xanthoproteic reactions were negative. The nihydrin test was positive. The murexide test for purines and the Salkowski test for indole were negative. Boiling water failed to precipitate the pigment even when acidified with acetic acid. Reduced sulfur was absent and the Liebermann test for phenol was negative.

Summary. A water soluble pigment was isolated from the Polyzoa *Bugula neritina* (L) and its chemical and physical properties were determined. A close resemblance was verified between this pigment and the adenochrome obtained from the branchial hearts of the *Octopus* by Fox and Updegraff.

We wish to express our thanks to Dr. W. Cruz of the Instituto Oswaldo Cruz for some spectrophotometric determinations and to Prof. P. Drach, Roscoff Marine Laboratory, for his interest during the progress of this work.