Reports

Occurrence of Titanium, Vanadium, Chromium, and Sulfuric Acid in the Ascidian Eudistoma ritteri

Abstract. The body tissues of the colonial ascidian *Eudistoma ritteri* are shown by quantitative spectrographic analysis to contain up to the following amounts of related metals (parts per million of dry weight): titanium, 1512; vanadium, 471; and chromium, 144. Body tissues and tunic fluid show a *p*H of 1.5 due to the presence of sulfuric acid.

Half a century ago Henze demonstrated the presence of relatively high concentrations of vanadium in phlebobranch ascidians and found this to occur in certain blood cells in association with high concentrations of sulfuric acid (*1*). Since that time numerous workers have confirmed the presence of vanadium in blood cells or other tissues of ascidians and have noted that relatively high concentrations appear to be restricted to certain members of the ascidian order Phlebobranchiata (*2, 3*).

The more recent discovery of 475 ppm of vanadium in *Euherdmania claviformis* (*4*) and the reports of positive tests for vanadocytes in *E. claviformis* and *Pycnoclavella stanleyi* (*5*) are of particular interest, for they constitute the first indications of comparably large concentrations of vanadium, and of the presence of vanadocytes, in any members of the ascidian order Aplousobranchiata. It seemed desirable that other species among the less specialized aplousobranchs should also be examined for vanadium content. Further, the reports of quantities of manganese in several ascidians (*6, 7*), the discovery that individuals of *Molgula manhattensis* may concentrate either vanadium or niobium (*8*), and the finding that *Pyura stolonifera* accumulates iron (*9*), suggested that other related metals might also be present in ascidians.

Eudistoma ritteri Van Name, 1945, represents a relatively unspecialized aplousobranch ascidian common along central California shores. Initial tests for vanadium in *E. ritteri* included microscopic examination of the blood for vanadocytes and treatment of the blood cells and other tissues with osmic acid and neutral red by the diagnostic tests of Webb (*2*) and George (*10*). The blood shows an abundance of morular "green-cell" corpuscles, which, however, fail to exhibit the characteristic staining reactions of vanadocytes; they neither take up neutral red nor darken in the presence of osmic acid. However, these tests proved positive when applied to other body tissues. The entire epidermis concentrates neutral red heavily; the whole body blackens in the presence of osmic acid. The fluid of the tunic has a *p*H of 1.5 as shown by a glass-electrode *p*H meter and indicator papers specific for low *p*H. When $BaCl_2$ is added to the tunic fluid, a heavy precipitate of $BaSO_4$ is formed, indicating the presence of sulfuric acid.

Chemical analyses of the tissues for vanadium were carried out with the phosphotungstic acid method described by Sandell (*11*). The bodies of approximately 1500 to 2000 zooids were removed from living colonies, inspected to insure that digestive tracts were empty of food and feces, dried at about 110°C, weighed, ashed at 600°C, and tested. The phosphotungstic acid method yielded rather erratic results, suggesting the presence of one or more elements, such as titanium, which are known to interfere with the test for vanadium in this method.

Samples of zooids, dried and ashed as before, were subjected to quantitative spectrographic analysis (*12*). The results, shown in Table 1, indicate that titanium is present in quantities of up to 1512 ppm, vanadium in amounts of up to 471 ppm, and chromium in amounts of up to 144 ppm in dried, whole, unpreserved zooids. The vanadium concentration in the lower abdomen is greater than that in the zooid body as a whole. Zooids preserved in 10 percent neutral formalin for 4 years show roughly similar quantitative relationships between the three metals, but the concentration of each is drastically reduced. Spectrographic analysis of salt formed by evaporating sea water collected from the immediate environment of *E. ritteri* indicates that these three metals are below the level of detection.

The concentrations of vanadium reported here are similar to those obtained in *Euherdmania* (*4*). Titanium has previously been reported from ascidian tissues (*6*) only in trace amounts (1.7 ppm dry weight). Chromium has not been reported to be concentrated by ascidians according to my present knowledge.

The metals now known to be accumulated in relatively large amounts by some ascidians show the following arrangement relative to one another in the periodic table:

Ti V Cr Mn Fe
Nb

All belong to the "related metals" class and have a similar number of electrons in the outermost and the next-to-the-outermost shells. Manganese has been reported by I. Noddack and W. Noddack (*6*) to be concentrated by *Ciona intestinalis* at 120 ppm of the dry weight, and Vinogradov (*7*) reports "large quantities in *Didemnum albidum* and *Microcosmus glacialis* which have $CaCO_3$ spicules."

The function of vanadium in ascidians remains uncertain. However, the chemical similarities among at least some of the substances listed suggests the possibility that these metals may play more or less interchangeable physiological roles. The findings of Carlisle (*8*) further suggest the possibility that small genetic or physiological differences, even within a single species, may favor the concentration of one or another of these elements. The presence of relatively great amounts of vanadium in both *Euherdmania* and *Eudistoma* indicates that, in this feature, these genera are more closely allied to the ascidians of the order Phlebobranchiata than to the aplousobranchs with which

Table 1. Quantities of titanium, vanadium, and chromium in spectrographically analyzed samples of *Eudistoma ritteri* zooids removed from the tunic (parts per million of dry weight).

Unpreserved zooids: Whole	Unpreserved zooids: Abdomens only	Whole preserved zooids	Sea water salt
	Titanium		
1361–1512	1110	529	0
	Vanadium		
370–471	660	18	0
	Chromium		
72–144	100	2	0

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

they are generally placed. This provides further evidence to support the contention of Berrill (*13*) and others that the polycitorid aplousobranchs (*Eudistoma* and close allies) have clear affinities with the diazonid phlebobranch ascidians. Further studies of the distribution of metals in ascidians are continuing (*14*).

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12. The quantitative spectrography was done by the American Spectrographic Laboratories, Inc., 557 Minna St., San Francisco, Calif.
13. N. J. Berrill, *Phil. Trans. Roy. Soc. London Ser. B* **226**, 43 (1936); *J. Morphol.* **81**, 269 (1947).
14. This report is a portion of a thesis submitted in partial fulfillment of the requirements for the Ph.D. degree at Stanford University. Thanks are due to D. P. Abbott of the Hopkins Marine Station for advice and encouragement during the work.
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Wave-Guide Modes in Retinal Receptors

Abstract. Retinal receptor wave-guide modal patterns have been photographed. The inner and outer segments of the rod and cone receptors of albino rats, rhesus macaque monkeys, and human beings have been studied. The wave-guide modal pattern is believed to be the normal form of energy transfer in these parts of the retinal receptors in these species.

Since 1933, when Stiles and Crawford (*1*) discovered the directional sensitivity of the retina, increased interest has been directed toward the optical properties of the retinal receptors. In the past few years, with the rapid development of fiber optics, the retinal receptors have been viewed with interest because they are, in fact, a functioning and highly efficient fiber bundle. That is, the resolution capacity of the retina is essentially limited in the fovea by the diameter of the individual receptors (*2*).

Inasmuch as the diameters of the components of the receptors begin to approach the wavelength of light, it has been assumed that the inner segment, ellipsoid, and outer segment of the retinal receptors act as dielectric wave guides (*3*). If this assumption is accepted, it then becomes logical to look for, identify, and determine the role played by wave-guide modal patterns in the retinal receptors. At this time, wave-guide modes have been seen (*4*), they are being identified under various conditions, and first thoughts have been given to the possible roles they play in the visual mechanism (*5*).

Electromagnetic waves are guided in a wave-guide. The modal pattern shown in Fig. 1 is the result of interactions which have occurred in the receptor acting as a wave guide. A wave-guide modal pattern may be defined as a distribution of field which propagates down a wave guide with a well-defined phase velocity. These patterns result in a nonuniform distribution of energy in the receptor.

While the apparatus needed for visualization of these modal patterns is rather simple in principle, the accomplishment of the goal presents serious problems. In essence, one creates a schematic eye with a piece of retina placed in a special chamber (in normal saline) and oriented as it might be *in situ*. An intense source of light is imaged by a lens upon the retinal tissue. Since a modal pattern may change when the wavelength, the angle of incidence of the luminous energy, or both the wavelength and angle of incidence are varied, it is useful to control the wavelength composition of the light and to insert a diaphragm (analogous to the iris of the eye) to limit the angular subtense of the incident cone of light. A microscope is situated above the flat retinal preparation, and hence one observes the light which has passed through the receptors. The limiting resolution of the microscope, the maintenance of receptor orientation, the elimination of vibration effects during time exposures, the graininess of the film, and the need for rapid dissection of the specimen present the major problems to the investigator.

Similar wave-guide modal patterns have been observed by focusing upon the outer segments of the receptor of albino rat, rhesus macaque monkey, and human retinas. All receptors studied exhibited the mode form of energy transfer. An example of such a pattern recorded from a cone receptor located in the macula area of a monkey is shown in Fig. 1.

In some receptors the perceived modal pattern changes when wavelength is varied. The patterns also often change when the focal plane within the receptor is varied. In any given mosaic

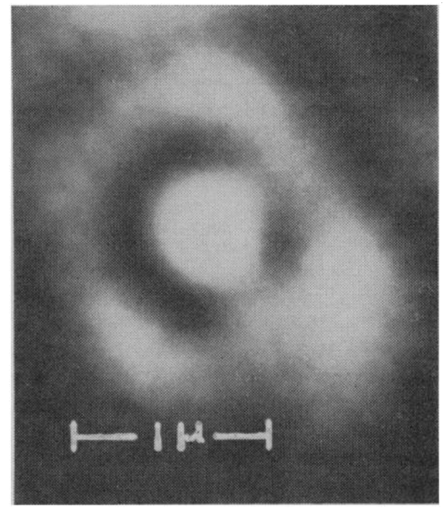

Fig. 1. A wave-guide modal pattern recorded from the outer segment of a cone in the macula of a monkey.

of outer segments a dominant modal pattern is seen. Some departures from the dominant pattern are due to differences in diameters, cross-sectional shape, and orientation. In all three species, when the outer segments of the receptor were viewed, only a small number of different patterns were seen. When the pattern changed as wavelength was varied, only one or two changes occurred within the range of 450 μ to 600 mμ. Rods and cones may be readily differentiated in monkey and human retinas. In some receptors modal patterns vary in the intensity of their excitation, their transmissivity as a function of wavelength, or both. The directionality of the retinal receptors is readily observed.

The role(s) played by wave-guide modes in vision may be many fold. The differential transmissivity of modal patterns as a function of wavelength obviously influences the result. The modal patterns themselves give rise to regional nonuniformities in energy concentration in the outer segments of the receptor. It is important to know, particularly in receptors responsible for color vision, whether there are corresponding nonuniformities in pigment concentration (and how many pigments are involved), or in potential to evoke a response. It also becomes necessary to explore further electro-physiological and psychophysical correlates.

At this time it can be said that the wave-guide mode form of transmission is probably the normal form of energy transfer in these species, and that the response of a given receptor may be modified by the presence of the mode form of transmission (*6*).

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