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ON THE POSSIBLE USE OF THE FOULING ASCIDIAN *CIONA INTESTINALIS* AS A SOURCE OF VANADIUM, CELLULOSE AND OTHER PRODUCTS

SUR LA POSSIBILITE D'UTILISER LA SALISSURE BIOLOGIQUE, L'ASCIDIE *CIONA INTESTINALIS,* COMME SOURCE DE VANADIUM, DE CELLULOSE ET AUTRES PRODUITS

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ABSTRACT

Raft tests carried out in the Haifa and Kishon harbours showed at times a heavy settlement of the tunicate *Ciona intestinalis.* Preliminary analyses have been made of the vanadium content of the blood, and isolation of the cellulose from the mantles has been carried out. The figures obtained confirm the data available from the literature as to the high content of these products in ascidians in general and in *Ciona intestinalis* in particular. The possible uses of these products are discussed.

RÉSUMÉ

Au cours d'essais sur radeaux entrepris dans les ports d'Haïfa et Kishon, de denses colonies de tuniciers *Ciona intestinalis* sont apparues de temps à autre. Des analyses préliminaires ont été faites sur le vanadium contenu dans le sang et la séparation de la cellulose à partir des manteaux a été effectuée. Les chiffres obtenus confirment les données théoriques connues sur la grande quantité de ces produits trouvés chez les ascidiens en général et chez *Ciona intestinalis* en particulier. Les utilisations possibles de ces produits sont discutées.

I. *Introduction*

Attention has been drawn to the mass occurrence of the ascidian *Ciona intestinalis* on experimental panels for marine anti-fouling paints in Haifa harbour (Komarovsky and Schwarz, 1957). In these experiments, steel panels coated with anti-fouling paints, which did not contain a sufficiently high Cu_2O content to prevent fouling, became covered with a thick layer of *Ciona intestinalis* after about six weeks of immersion, during the early spring of 1951.

Consecutive series of experiments with anti-fouling paints showed that the main season of settlement of this organism on submerged surfaces is from early spring to early summer. The

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occurrence of this organism, both on experimental panels and on the shaded areas of ships' hulls, indicated a preference of this species for lower light intensities.

On the basis of this information, the authors undertook a series of preliminary field tests, specifically designed to offer a suitable surface of attachment for this particular species — one of the most common tunicates occurring along the Mediterranean coast of Israel. Several reasons for the choice of this particular tunicate as the object of the present study were found:

1. The blood of this organism is rich in vanadium — the total vanadium content may reach over 1 % of the ash weight per specimen.
2. The mantle of this ascidian is composed mainly of a high grade animal cellulose. Moreover, the size of individual specimens occurring along this coast is among the largest recorded in literature, reaching in many cases about 25 cm. in length. Therefore, an attempt to extract both the vanadium and the cellulose appeared to be of possible interest.

This investigation has been made possible through a grant kindly offered by the Ford Foundation, upon the recommendation of its scientific adviser in Israel, Prof. E. Bergman. The processing of biological data has been carried out at the Sea Fisheries Research Station, Haifa, while the field work and field observations were performed at the Haifa and Kishon harbours with the kind assistance of Mr. J. Boxer of the Haifa Port Authority, to whom the authors are greatly indebted.

II. *Review of literature*

1. *Cellulose*

For many years, the sessile tunicates have been known as one of the very few sources of cellulose in the animal kingdom.

Schmidt (1848) recognized the skeletal material of the tunicate mantles to be " animal cellulose ". Berthelot (1858) called this material " tunicin ". In 1893, Winterstein described the purification of tunicin followed by its cellulosic characterization through chemical degradation. Since then, considerable work has been done concerning the structure of tunicin. Thorough treatments of the subject may be found in Grignard's Traité de Chimie Organique. Two quotations follow (Vol. VIII 2, p. 699).

« La cellulose de Tunicates se distingue cependant des celluloses végétales par une plus grande résistance aux agents chimiques. Ainsi Berthelot avait reconnu qu'elle n'est pas carbonisée par la fluorure de bore qui attaque cependant immédiatement le papier-filtre ou le coton. Sa nitration ne peut être effectuée qu'après qu'elle a été réduite en poudre très fine. Une fois séchée, elle ne reprend, au sein de l'eau, que très lentement de l'eau d'hydration, contrairement à la cellulose de coton ».

« La cellulose animale parait donc renfermer le même motif de structure que la cellulose végétale, mais en diffère soit par son degré de condensation qui serait plus élevé, soit par son état physique qui correspondrait à une plus grande régularité dans l'arrangement des groupes glucose constitutifs et assurerait une plus grande imperméabilité aux agents chimiques ».

Panby (1952) made the following observations:

" In tunicates, the cellulose occurs as tough homogeneous tissue, rather than as freely fibrous cell walls. Except for this fact, no distinct difference has been found between plant and animal cellulose. "

" Tunicin was ultrasonically dispersed (5 samples of tunicin and 2 samples of plant cellulose) and showed similar structure by electron microscope to the plant cellulose. Thin filaments were formed (micelle strings) of about 100-140 Å in diameter in tunicin, 85-90 Å in cotton, 70-80 Å in wood. Micelle strings or animal cellulose are more resistant towards alkali swelling than cotton... wood has least resistance. Hydrolysis with hot, dilute mineral acids affects tunicin strings much less than plant cellulose. Degree of polymerization of nitrated tunicin (method of limiting viscosity) is high (minimum = 4,000)."

"It is thus far impossible to decide whether differences be purely in micelle dimensions or whether better lattice order within the strings as well."[1]

2. *Vanadium*

Vanadium is known to be a major metallic constituent in the blood of many species of ascidians. A comprehensive account of the matter may be found in Vinogradov (1953). A number of pertinent statements from this book will be quoted or paraphrased.

p. 421. The following table lists some of the trace elements found in *Ciona intestinalis* (Noddoch 1939):

% of dry weight

Ti	0.00017	Ni	0.0016	Sn	0.00035
V	0.062	Cu	0.0013	Pb	0.00011
Mo	0.00008	Zn	0.033	As	0.0003
Mn	0.012	Cd	0.00006	Sb	0.00001
Fe	0.025	Bi	0.00002	Ca	0.00002
Co	0.00023	Ce	0.00004		

p. 422. Henze (1911) discovered vanadium in the blood pigments of tunicates. So, for example, the corpuscles of *Phallusia mammelata* contain 18.5 % V. The blood in the tunicates is acid due to the presence of free sulfuric acid in the cells.

p. 424. "Webb (1939) found that the mantle of *Ascidia mammelata* contained 0.61 mg.V, the intestines 1.10 mg. and the blood 3.75 mg., the latter thus containing 65% of the V."

p. 427. "The Ascidiae which are the richest in V are warm water species, while typical Arctic species are poorer as a rule. This phenomenon is also observed in cosmopolitan forms. The ash of *Ciona intestinalis* from Roscoff contains 1.5 % whereas the same species from the Gulf of Kola contains but 0.05 %. *Ciona intestinalis* from the North never attains the size observed in specimens from warmer seas. Note that among warm water forms of ascidians there are species which do not concentrate V."

"In sea water, vanadium is present in very small amounts and cannot be detected even in many litres (probably there is about n x 10 - 7 %)...[2] The only source, then, could be in the silts of the seas. Apparently, a local enrichment of silts by vanadium from dead Ascidiae takes place.[3] Note that vanadium occurs in oils in the form of vanadium porphyrin complexes."

Considerable elucidation of the structure of the vanadium complex in the blood of the ascidians was achieved through the work of Boeri et al (1950) and of Bielig and Bayer et al. (1954).

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[1] For further information, see E. Ott and H. M. Spurlin: Cellulose and Cellulose Derivatives, 2nd Edition, Part I (1954) p. 25.

[2] Tests run by the senior author indicate the concentration of V in sea water to be approximately 1/litre (i.e. 0.001 ppm or 1 × 10-7 %).

[3] Some authors (cf. Webb 1939) appear to challenge this view and claim it possible that the organisms concentrate the V from aqueous solution.

Concerning the role of the respiratory metals, Fulton (1921) makes the following remarks: "... The absence of metals in some respiratory pigments appears to indicate that the function of oxygenation is not dependent on the presence of the metal, but that the presence of the metal acting as a catalyst increased the capacity for ready oxygenation and reduction."

3. *Other constituents*

Since the tunicates are protochordates — i.e. precursors of the vertebrates — it is to be expected that their metabolism utilizes and produces various biologically active substances which might be of interest to man. Some work has been done on the isolation of bio-chemicals from the body of *Ciona intestinalis*. These include glycine, taurine, choline and spermine (Ackermann, 1953). Considerable work has also been done on the physiology of the tunicates (Day, Hecht).

II. *Biological methods and observations*

The material for this investigation was collected over a period of years both from experimental panels for antifouling paints tested on rafts, and from a variety of sailing craft where the tunicates were found hanging from the underside of the hulls. The specification for the types of rafts and steel experimental panels used are given in a previous report (Komarovsky and Schwarz, 1957).

Specimens of *Ciona intestinalis* collected both from the panels of the raft tests and from the boat hulls, served as material for the laboratory experiments.

All the specimens taken for laboratory work were preserved in a 4 % formaldehyde solution.

The population of *Ciona intestinalis* occurring on submerged surfaces along the coast, shewed striking differences from year to year. In 1951, a very heavy growth of this organism took place on experimental panels attached to the starboard of a stationary ship in Haifa harbour. Since that year, growth tests on similar rafts have been conducted, and as before, the main development of *Ciona intestinalis* took place during the spring and early summer of each year. However, subsequent years have exhibited much lighter growths than in 1951. Nevertheless in a series of raft tests carried out in the Kishon Harbour, adjoining the main Haifa Harbour — in 1960 — a considerable growth of this tunicate was recorded on the panels during May and June.

III. *Chemical methods and results*

1. Methods

As a point of practical interest, it should be noted that the organisms may be worked up simultaneously for their cellulose (tunicin) content and for their vanadium. This is because the mantle, which contains all of the cellulose, may be easily removed from the rest of the structure which contains most of the vanadium (cf. Webb, op. cit.).

a. *Cellulose* (tunicin)

The tunicin was obtained in a pure state by Winterstein's method as described by Grignard (op. cit.) p. 699.

The tunic was removed from the residue by cutting it at the base and slipping out the entrails. Boiling with water and treatment with cold 1 % HCl, followed by washing and drying, served to remove encrustations and some of the gelatinous layer. The mantle was then boiled for one hour with 1 % KOH, washed and treated with hot H_2SO_4 (2 %) for one hour. After a final water wash, the tunic was in the form of a tough transparent film.

When the film was dried with alcohol, and then with ether, it appeared as a semi-opaque fibrous membrane which showed little tendency to absorb water.

b. *Vanadium*

A variety of decomposition techniques have been tried out. The final determinations have been run on a semi-quantitative basis by rapid paper chromatographic techniques. A description of these techniques, in respect both to vanadium analysis in general, as well as to its analysis in tunicates will be published by the senior author of this paper.

2. RESULTS

a. *Cellulose*

Little work has so far been carried out by the authors on possible special uses for the tunicin. However, the following observations may be noted concerning the possible yields and the special properties of the mantles.

(1) The peculiar behaviour of the tunicin concerning its hydrophylic character while still wet, and its hydrophobic character when once dried, have been noted in the literature (Grignard, op. cit.). This behaviour forms the basis of some speculation concerning possible uses of the material.

(2) The weight of the pure dry tunicin from a single specimen measuring roughly 10 cm. in length and 1-2 cm. in diameter (i.e. area abt. 40 cm_2) was 2-3 mg. Simple calculations will show the approximate thickness of such a film to be 5×10^{-5} cm. = 5000 Å.

(3) During the growth experiments of 1951, the organisms were found very closely packed on the test plates. Considering the diameter to be 1-2 cm., an area of 1 m_2 of plate would contain roughly between 2,500-10,000 specimens (about 140 kg. wet weight by actual measurement).

We found the cellulose of one organism to weigh 2-3 mg.; therefore, the total weight over 1 m^2, would range between 5-30 g. In other words (considering one growth season per year) a heavy yield might run between 50 and 300 kg. per hectare. Note however that the organisms grow in the shade, so that 1 hectare of water area could be made to support many hectares of growing plates.

b. *Vanadium*

The analytical results so far obtained by us on the vanadium content of the tunicates are not uniform. They range from approximately 0.04 % of ash weight to about 0.7 %.

The reasons for this divergence of results have not been as yet clarified, but they may be due to a number of causes:

(1) Faulty decomposition techniques — leading to partial volatilization of V.
(2) Variation in V content of various batches of tunicates.
(3) Variable V content among individuals of the same batch.

A thorough investigation is being carried out on this problem.

The sea water from an uncontaminated area has been tested by us for vanadium, and found to contain approximately 0.001 ppm.[4]

[4] Some analyses of the V content of holothurians from the Red Sea (off Eilath) have also been made and positive results have been obtained.

Concerning the possible yield of V per unit area, very little can be said until much more data have been gathered, and until the analytical details have been cleared up. On the basis of calculations similar to those made for the cellulose, possible yields might run between 5-30 kg. V/hectare.

IV. *Possible uses*

1. *Cellulose*

a. The membranes (purified tunics) themselves might have unique properties, enabling them to compete successfully with membranes made of cellophane and other materials. Some suggestions for possible uses are listed:

(1) In dialysis — i.e. for separating molecules of various sizes in solution, due to the " sieve size " of the membrane.

i) for drug purification: e.g. procaine alkaloids

ii) for clinical use (artificial kidneys, etc.)

iii) in sugar manufacture (separation of molasses from sucrose)

iv) for separation of azeotropes (Hagerbaumer, 1955)

v) for molecular weight determinations (using centrifugation techniques, etc.)

(2) For use in electronics industry for purposes where very fine separating films are necessary.

b. The individual tunics may possibly be formed into larger sheets, by such methods as partial solution in Schweizer's reagent, pressing of sheets, then washing free of solvent.

Possible uses include:

(1) For currency paper of very high chemical resistance.

(2) For parachute and/or balloon fabrics.

c. The membranes might be disintegrated into fibres which could then be used in the manufacture of highly resistant filter paper and currency paper.

Possible methods of fibre formation include:

(1) rupture of membrane structure in supersonic fields.

(2) rupture by rapid (explosive) dehydration.

d. Derived cellulosic materials of higher than average molecular weight should be considered.

2. *Vanadium*

a. Some thought has been given to the extraction of the vanasium in the form of a porphyrin complex as it occurs in the blood. Specific uses for this type of compound might be found, especially in the field of pharmacology. In the 19th century, considerable use was made of vanadium as a therapeutic agent (The Encyclopedia Americana, 1955).

b. Consideration might be given to the possibilities of preparation of high-purity vanadium salts or metal. It is doubtful whether the tunicates could be made to compete with vanadium bearing ores for normal supplies of the metal. However, for special grades of V, this source might be competitive.

3. *Other constituents*

Should a non-destructive procedure (see 2.a.) for the extraction of vanadium be adopted, thought might be given to the possibilities of working up the tunicate residues for important biochemicals.

V. *Discussion*

The data presented in this report are a first indication of possible uses of V from the blood, and of animal cellulose from the mantle of the tunicate *Ciona intestinalis*. The figures given for these products in regard to both concentrations per organism as well as weights per area are — in the view of the authors — highly stimulating for further work along the lines outlined above. Nevertheless, both the figures obtained by the authors as well as those found in the literature concerning the abundance of these products, are to be viewed in the light of natural fluctuations in populations from year to year. These fluctuations proved to be considerable at the localities along the coast of Israel where tests were run.

Whereas in 1951 the heaviest settlement of *Ciona intestinalis* took place on our experimental panels in record time (this was found to be the exception rather than the rule), subsequent years (until the present one) showed much less growth. A suggestion is made that this may be the result not only of purely climatic factors. The absence or presence of a variety of minor factors, either environmental or of an interspecific nature might also play important roles. The way is therefore open for new assumptions that may open new vistas of research in the future.

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