MUCUS HYPERSECRETION IN A NORMALLY ISOLATED NON-INNERVATED CELL (1)

by

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Résumé

La cellule basale ciliée à mucus des urnes libres du liquide coelomique du Ver marin *Sipunculus nudus* a été étudiée, à l'état vivant au microscope à contraste de phase (Phase III). La structure et l'activité sécrétoire des urnes, sans coloration et en coloration vitale, ont été étudiées dans les conditions normales et expérimentales. Le débit sécrétoire normal a été évalué à un micron par minute environ. Le taux en était modifié pour peu de temps par l'introduction de diverses sortes de corps étrangers et de bactéries mortes dans le liquide. L'inoculation massive de bactéries vivantes produisait une hypersécrétion et un taux sécrétoire dix fois au moins plus élevé pendant plus de trois heures.

Introduction

Prodigious amounts of mucus are sometines produced by epithelial cells in animal mucous membranes. The "urn cell" in the coelomic fluid of the marine worm *Sipunculus nudus* has this capacity. The urn's ciliated-secretory base cell secretes a mucous tail onto which débris becomes stuck as the cell swims through the fluid; the amount and quality of the mucus are affected by physiological changes within the worm (Cantacuzène, 1928) (Bang and Bang, 1962). During attempts to induce reproducible changes in the secretory mechanism, we have observed structural details in living urns under highpower phase microscopy, watched the process of secretion, measured the rate of secretory output, and elicited marked hypersecretion for a period of several hours.

Materials and methods

Procedures of collection and maintenance of worms have been described previously (Bang and Bang, 1962). For microscopic studies, 2 or 3 drops of freshly drawn blood were put on a clean slide and covered lightly with a coverslip, so that the coverslip remained just barely floating. Experimental fluids were introduced directly into the worm, or into freshly drawn blood in a test tube, or under the coverslip. Preparations were examined in a Zeiss binocular phase microscope.

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Introduced materials.

Seawater solutions of India ink 1:100, and carmine powder 1:1000, were filtered before use. Filtered seawater solutions of neutral red and of methylene blue at dilutions of 1:100 to 1:4000 were prepared; the most concentrated solutions usually killed urns quickly, while the most dilute gave indecisive staining. All dilutions retarded or arrested the output of the stainable component in normally secreting urns, but for long periods of observation, neutral red at 1:1000 and methylene blue at 1:2000 were least toxic. Hypersecreting urns tolerated concentrations of 1:100 neutral red and 1:500 methylene blue for several hours. A commercial preparation of 8. p. 100 pilocarpine in normal saline was used.

Secretory rates were measured by means of a micrometer inserted into the ocular; urns had to be in perfect profile, securely attached at one end and entirely free at the other, so that a focal point on the free end could be chosen, and its rate of progress along the micrometer units followed with a stopwatch. Secretory rates are based on the estimated size of the linear units in the Phase III system, and are therefore only approximate.

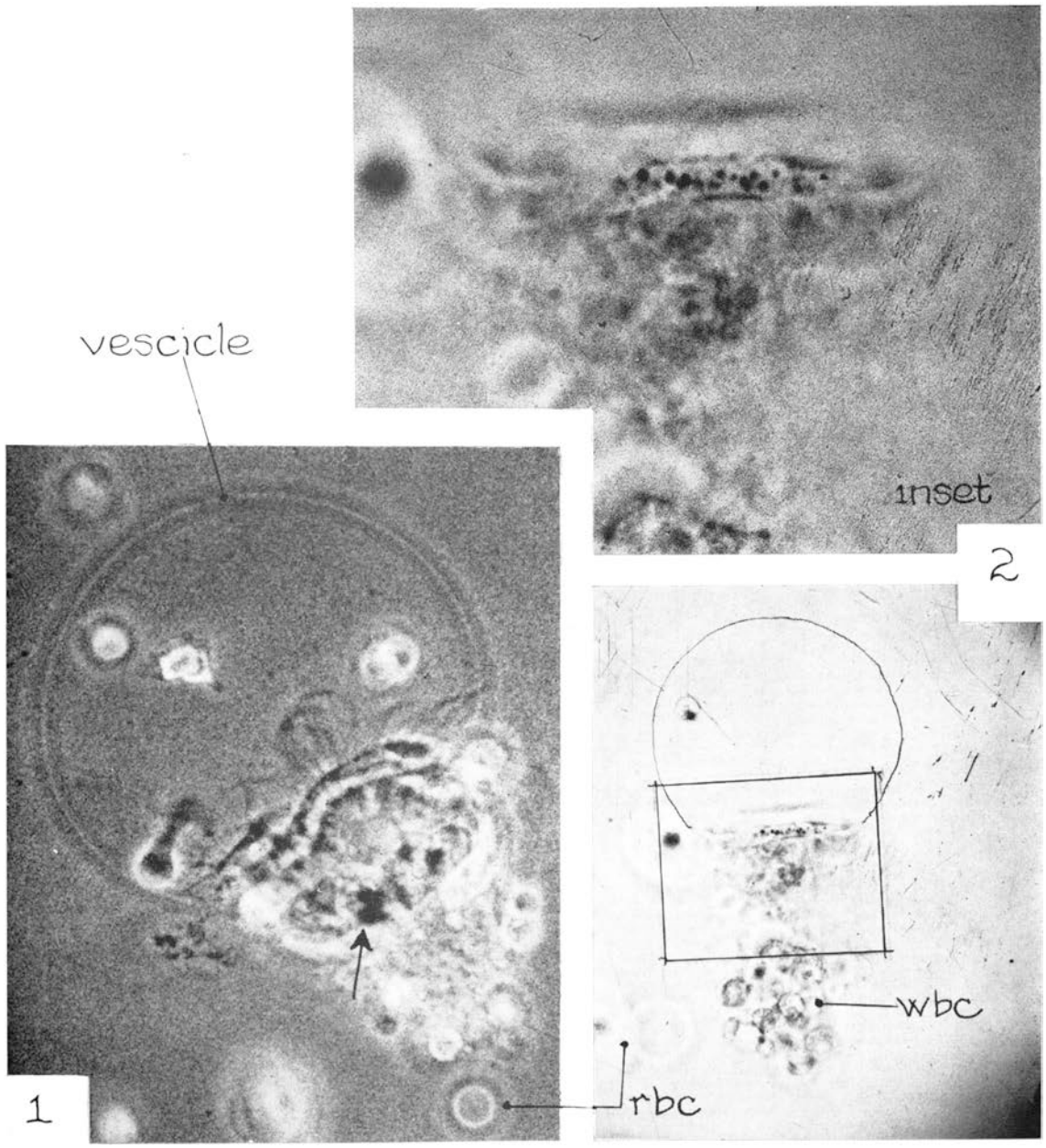
Bacterial inocula.

Killed bacteria were a *Vibrio* sp. *limulus* (Bang, 1955). Inocula were prepared by adding 3.5 ml of a cloudy suspension of the Vibrio to 1 cc of seawater. The live bacteria used for massive inocula were obtained from Mr. William Barker, who had maintained a cloudy broth suspension of bacteria found in the blood of a *Carcinus maenas* crab parasitized by *Sacculina carcini,* collected near Roscoff.

The normal urn

Sipunculus nudus (Hyman, 1959) and the origin (Selenski, 1922), activities, and functions of the cells in its coelomic fluid are well known. Free urns originate as elevations of connective tissue at the site of ciliated cells in the peritoneal lining; these vacuolate and pinch off, carrying with them the ciliated cell. The vacuolated cell becomes the urn vescicle, the ciliated cell, the secretory base cell. Cultures of free urns may be prepared by withdrawing coelomic fluid with a sterile syringe into sterile test tubes and stoppering them with cotton plugs. Urns remain alive in the original fluid for as long as three weeks. They survive also for a least five days in vaseline-sealed tubes in which they become immobile but resume motility when unsealed.

When observing an urn profile under Phase III the entire field is occupied by the central portion of the base and the upper part of the secretory tail. Photography is severely limited by refraction, depth, and constant motion; the photographs in Plate 1 are of vitally stained cultured (thus relatively quiescent) urns. The drawings are based on sketches of dozens of unstained and vitally stained urns.



B.G. Bang and F.B. Bang PLATE I

1. — Semiprofile of cultured urn, showing neutral red staining of the paranuclear secretions in the throat area (arrow). Note the immobilized cilia, stuck on the slip cover, and cell debris caught in the mucous tail.

2. — Profile of cultured urn stained with methylene blue, focused on secretory droplets in the ciliated region. The vescicle has been outlined in ink. Inset shows enlargement of secretory-ciliated area.
rbc = living red blood cells wbc = dead white blood cells trapped in the mucous tail, some of which have partially stained with methylene blue.

Photographs reproduced from color slides.

In phase microscopy, the entire urn is nearly transparent. The saucer-shaped rim of the base cell is a ciliated-secretory structure supporting about 1000 cilia set at regular intervals roughly ten rows

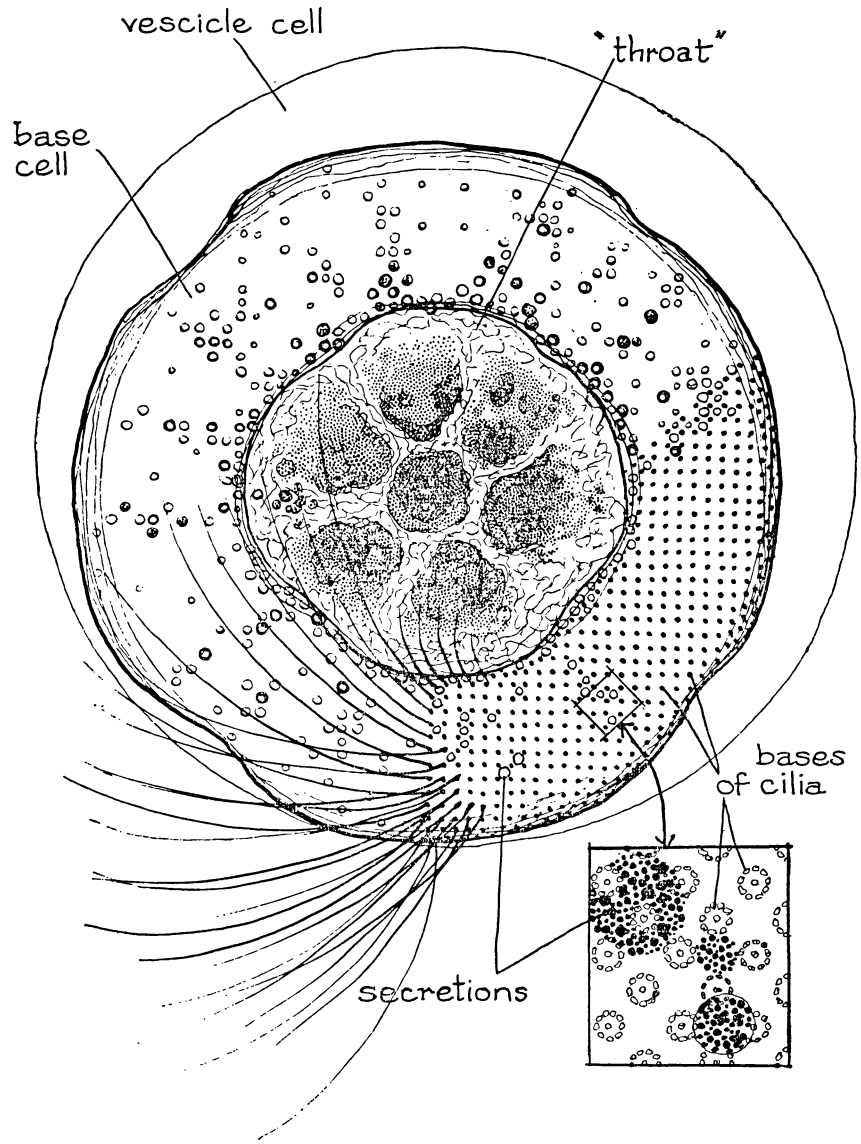


FIG. 1

Interpretation of possible structure of base cell suggested by observation of vitally stained living urns. Inset shows details of droplets containing granules which stain rich purple with toluidine blue, and fuschia PAS, in 1.5 μ sections through the urn-base ciliated area.

deep and one hundred circumferentially (Fig. 1). Secretory droplets evidently form between the cilia (Fig. 2, inset, and Fig. 1, inset) and must form and discharge continuously since they are never all simul-

taneously stained. More brilliantly staining secretions collect in the central throat in foci which open into a series of delicate sheaths. Secretions proceed distally within the sheaths at about one micron per minute, and break through the ends of the sheath membranes to form the secretory tail. Vital stains lose most or all of their color at the moment of breakthrough.

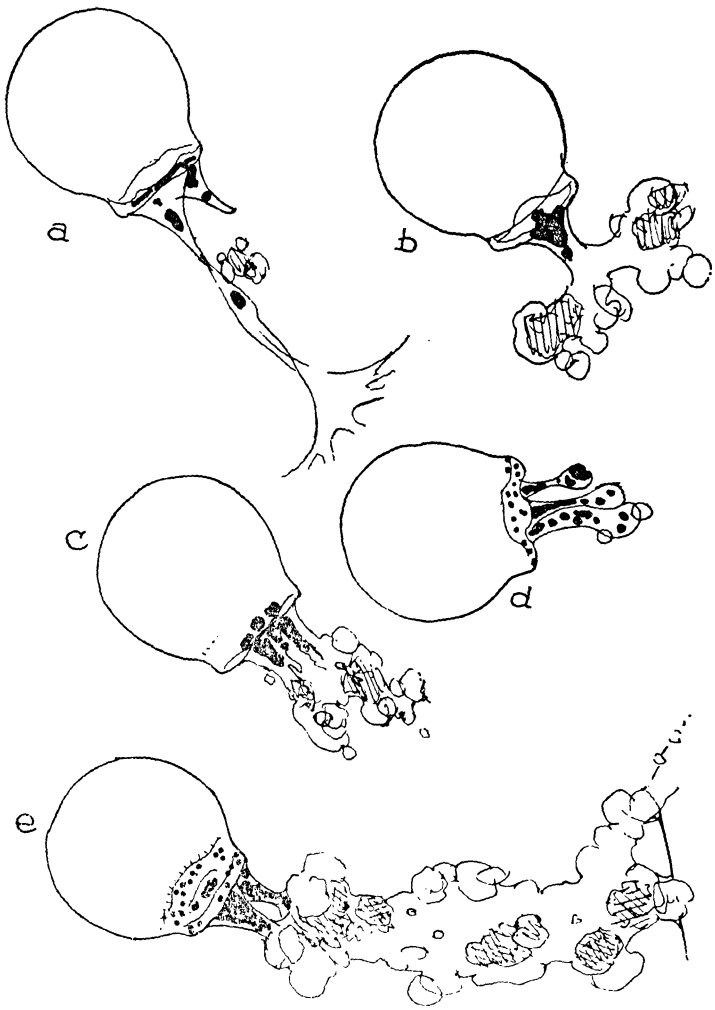


FIG. 2

Types of vital staining most commonly seen in fresh preparations of coelomic fluid. - a - d, normal worms. - e, traumatized worms. Granular tail secretions are laden with dead-cell débris, some of which shows various degrees of vital staining, minimized in the drawings.

Experimental alteration of secretion

The blood cells in the fluid of normal worms are distributed in a quite orderly and homogenous way, and most of the urns have small symmetrical tails (Fig. 2, a, b, c, d). Traumatized worms, however,

show many degrees of clumping of the large cells (urns, giant urns, and innominate vescicles), which adhere to one another principally by massive secretions of mucus. Swimming urns in such preparations have long, untidy tails (Fig. 2e). Several kinds of foreign materials were introduced into the blood of normal worms *in vitro* to determine whether some of these effects could be reproduced. Into three parts

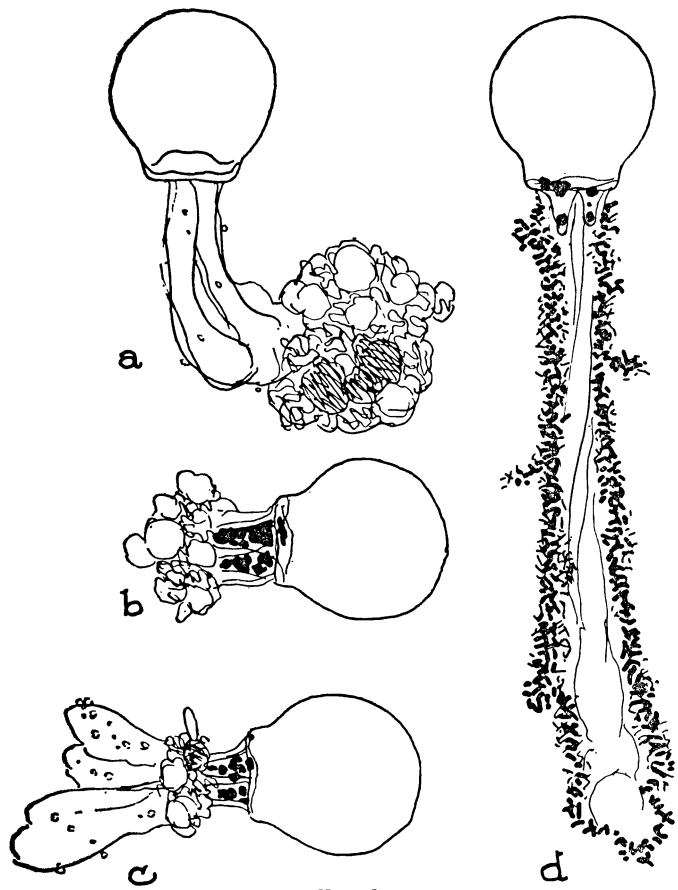


FIG. 3

Types of urn tails secreted following infection with small inocula of live bacteria (a, b, c) and massive inocula of live bacteria (d). a : an unstained urn with original débris-laden tail still attached to hypersecreted tail. - b: vitally stained urn with normal tail. - c: vitally stained urn with forked hypersecretion showing below normal original tail. - d: typical "bacterial tail" showing adherent bacteria and agglutinated rafts of bacteria stuck on the clear, agranular, outpouring hypersecretion.

of whole blood freshly drawn from normal worms added one part each of:

1. normal seawater
2. 1:100 India ink in seawater
3. 1:1000 carmine powder in seawater
4. 8 p. 100 pilocarpine solution in normal saline
5. a solution of killed marine bacteria in seawater.

In general, each of these caused an immediate brief acceleration in the rate of secretion (about twice the normal rate for 1½ minutes), and no further effect on the secretory rate. Particulate matter continued to adhere to the secretory tails until the fluid was cleared of particles.

Next, slime which had formed at the waterline of some of the worm tanks was scraped off and prepared as a thick suspension. 3.5 ml of this suspension was added to 1 cm^3 of seawater and injected into normal worms, or one part of the seawater preparation was added to three parts of normal whole blood *in vitro*. These slime preparations, which contained living bacteria and numerous other organisms, caused hypersecretion at rate up to 10 times normal in about one third of the urns, both *in vivo* and *in vitro*. The effects were reproducible, but individual urns showed quite varying responses (Fig. 3, a, b, c).

Experimental hypersecretion

In vivo injection of 1 cm^3 of a cloudy broth suspension of live marine bacteria caused intensive hypersecretion in all visible urns. Within a few minutes after injection, rates from ten times to over thirty times normal were sustained for well over an hour. The addition of one part of the cloudy suspension to three parts of whole blood *in vitro* produced the same effect. In both cases, hypersecretion continued at an equal rate in unstained preparations and in preparations to which neutral red (1:100) or methylene blue (1:500) had been added after hypersecretion had begun. Most of the urns in a given preparation were still secreting at about ten times the normal rate after three hours; by four hours those still viable were discharging at about five times the normal rate, and by five hours secretory rates were normal although the secretion was abnormal in appearance.

Hypersecreting urns had several distinguishing characteristics. In stained preparations, the stainable component appeared in the throat and appendages in the form of small discrete stained masses apparently suspended in a clear medium (Fig. 3b), and this obtained throughout the period of observation. The droplets in the ciliated region, which ordinarily show as paler but distinctly stained structures, failed to show staining at any time during the hypersecretion process. The rapidly outpouring secretion was entirely colorless and failed to stain at any time with either of the vital dyes at any strength. This secretion seemed to pour from the base cell much as melted Vaseline would pour from a tube under constant pressure; it preserved a regular width, and appeared to consist of several blended streams. Swarms of living bacteria and rafts of agglutinated bacteria stuck to each tail along its entire length. Urns with such "bacterial tails" were immediately recognizable because of their excessive length —up to seven or eight times the length of the urn vescicle—their lack of color, and the curious shimmering caused by the agitating bacteria. Occasional urns retained the normal débris-laden secretion, which was pushed distally when hypersecretion began, but which remained attached (Fig. 3a). Results of the *in vivo* experiments, in

which the various materials were introduced into the coelomic cavity, are summarized in Table 1.

TABLE I

Matetials added to blood	# of expts	Secretion rate, units per minute (#/per expt)				
		.5–1.5	1.5–2.5	5–10	10–15	over 15
normal blood	30	30				
seawater	2		2			
india ink	4		4			
pilocarpine	5	1	4			
dead vibrio	8	2	6			
tank scum	7	3	1	3		
controls	5	5				
live bacteria	11			2	4	5
controls	11	11				

Discussion

Urns are reminiscent of goblet cells in several ways. They develop from undifferentiated epithelial cells, have no connection with the central nervous system (Florey, 1932), do not respond to pilocarpine, respond immediately to bacterial infection by hypersecretion, and can simultaneously manufacture and discharge mucus (Florey, 1930). We do not know whether there is urn cell hyperplasia during infection and recovery, or whether fixed urns are released to become freeswimming under these conditions.

The significance of the course of vital staining will not be clear until the fine structure of the cell is revealed by electron microscopy. Since we know that the base-cell nucleus is in the throat (Bang and Bang, 1962), the brilliant staining of the paranuclear area suggests Golgi activity in this region just as in other mucous cells, but the mechanism of droplet formation between the cilia is not understood.

As a potential model for biochemical studies of mucus secretion the urn seems particularly promising. Recent studies (Tappan and Zalar, 1963) have shown that differences in the mechanisms of response to the mediation of acetylcholine in solitary cells and in autonomically innervated cells may be less striking at the molecular level than has been thought. We know of no other normally freeliving cell capable of sustained synthesis of great volumes of mucus both *in vivo* and *in vitro* for several hours.

Summary

The mucous-ciliated base cell of the freeswimming urns in the coelomic fluid of the marine worm *Sipunculus nudus* has been studied vitally in the Phase III microscope. The structure and secretory activity of unstained and vitally stained urns have been studied under normal and experimental conditions. The normal secretory output has been observed to be about one micron per minute. The rate was briefly altered by the introduction of several kinds of foreign materials and dead bacteria into the fluid. Introduction of large inocula of live bacteria caused hypersecretion at a rate of at least ten times normal for over three hours.

Zusammenfassung

Die bewimperte, basale Schleimhautzelle der freisch°immenden Urnen der Coelomflüssigkeit des marinen Wurmes *Sipunculus nudus* ist mit Hilfe des Phasenkontrastmikroskopes III lebend studiert worden. Die Struktur und die sekretorische Aktivität ungefärbter Urnen ist unter normalen und experimentellen Bedingungen studiert worden. Das normale Sekretionsquantum ist ungefähr ein Mikron pro Minute. Dieses Quantum wird für kurze Zeit geändert durch die Einführung verschiedener Sorten von Fremdkörpern und abgestorbener Bakterien in die Coelomflüssigkeit. Die Einführung grösserer Mengen lebender Bakterien bewirkt eine Hypersekretion die während mehr als drei Stunden mindestens zehn mal so stark ist als die normale Sekretion.

REFERENCES

BANG, F.B., 1955. — A bacterial disease of *Limulus polyphemus*. *Bull. John Hopkins Hosp.*, 98, *pp.* 325-351.

BANG, F.B. and BANG, B.G., 1962. — Studies on Sipunculid blood: immunologic properties of coelomic fluid and morphology of "urn cells". *Cah. Biol. Mar.*, III, *pp.* 363-374.

CANTACUZÈNE, J., 1928. — Recherches sur les réactions d'immunité chez les Invertébrés. *Arch. Roum. Pathol. Exp. et Microb.*, I, *pp.* 7-80.

FLOREY, H., 1930. — Experimental inflammation of the colon. *Brit. J. Exp. Pathol.*, 13, *pp.* 349-359.

FLOREY, H., CARLETON, H.M. and WELLS, A.Q., 1932. — Mucus secretion in the trachea. *Brit. J. Exp. Pathol.*, 13, *pp.* 269-284.

HYMAN, L.H., 1959. — "The Invertebrates", 5. *McGraw-Hill, Inc.*

SELENSKI, W., 1922. — Untersuchengen uber die sorgenanten Urnen der Sipunculidien. *Ztschr. f. Wiss. Zool.*, 90, *pp.* 536-595.

TAPPAN, V. and ZALAR, V., 1963. — The pathophysiology of bronchial mucus. *Ann. N.Y. Acad. Sc.*, 106, *pp.* 722-745.