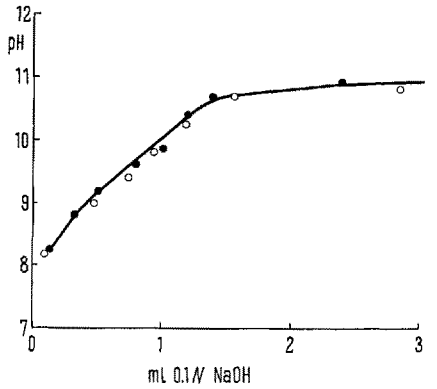
reference cell; a peak was obtained having a $\lambda_{max}$ at 360 nm. In similar experiments, with slightly less concentrated solutions, we have obtained a small peak with a $\lambda_{max}$ at 346 nm (see Table II); however, when the experiments were repeated with an equal ionic strength solution of sodium chloride instead of magnesium chloride, the same small peak having a $\lambda_{max}$ at 346 nm was obtained, which is further evidence that no complex formation is taking place between novobiocin and $Mg^{++}$ ions.

Table II. Difference spectra

| Sample solution<br>Concentration of novobiocin sodium | Reference solution<br>Concentration of novobiocin sodium | $\lambda_{max}$ |
|---|---|---|
| $8 \cdot 10^{-4} M$ [a] | absent (water) | 308 |
| $5 \cdot 10^{-4} M$ | $1 \cdot 10^{-4} M$ | 323 |
| $1 \cdot 10^{-3} M$ | $6 \cdot 10^{-4} M$ | 333 |
| $1 \cdot 10^{-2} M$ | $9.6 \cdot 10^{-3} M$ | 351 |
| $5 \cdot 10^{-3} M + 5 \cdot 10^{-3} M$ $MgCl_2$ | $5 \cdot 10^{-3} M$ | 346 |
| $5 \cdot 10^{-3} M + 1.5 \cdot 10^{-2} M$ NaCl | $5 \cdot 10^{-3} M$ | 346 |

[a] 1 mm cells used here; 1 cm cells in the other experiments.




Potentiometric titration curves for 20 ml of a solution of a mixture $5 \cdot 10^{-3} M$ novobiocin sodium and $5 \cdot 10^{-3} M$ magnesium chloride (●); summation curve (see text) (o).

In addition, potentiometric titration curves have been recorded, using 0.1 $N$ NaOH as titrant, for 20 ml solutions of (a) $5 \cdot 10^{-3} M$ novobiocin sodium, (b) $5 \cdot 10^{-3} M$ magnesium chloride, and (c) a mixture of $5 \cdot 10^{-3} M$ novobiocin sodium and $5 \cdot 10^{-3} M$ magnesium chloride. It was found that on summation of the titration curves (a) and (b), an identical curve was obtained to that obtained with the mixture (c) (Figure). This finding confirms the results recently reported by NIEBERGALL et al.[3].

Our results are not intended to throw light on the exact mode of action of novobiocin. Whether the primary effect of the antibiotic is on the bacterial cytoplasmic membrane[4] or on deoxyribonucleic acid synthesis[5] is not yet known. However, the results presented here demonstrate clearly that novobiocin and $Mg^{++}$ do not form a complex; it is thus extremely unlikely that novobiocin acts by inducing an intracellular deficiency of $Mg^{++}$.

*Résumé.* La novobiocine et les ions de magnésium ne forment pas un «complexe». De ce fait, il est très peu probable qu'elle occasionne une déficience des ions de magnésium dans la cellule bactérienne.

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## Fluorescence of Blood Cells of the Tunicates *Phallusia mamillata* and *Ciona intestinalis*[1]

The ascidiaceae *Phallusia mamillata* Cuvier (family Ascidiidae) and *Ciona intestinalis* L. (family Cionidae) possess different types of blood cells[2–6]. Most of these cells are vanadocytes, that contain in electronmicroscopically dense areas (vanadophores[5]) vanadium of low valency (reduction of osmic acid) and an acid (indicator reaction with methyl red), first observed with *Ph. mamillata*[7] and later with *C. intestinalis*[8]. The presence of disulfatovanadium(III) acid and of a yellow acid reductone-like substance, belonging to the hemovanadin system, has been proved in the hemolysate of the vanadocytes of *Ph. mamillata*[9]. A second cell type of both species are vacuolized compartment cells, that in the case of *Ph. mamillata* have been considered as direct precursors of the vanadocytes[4]. Further blood cells, which have also

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been described, are not considered here in connection with the following observations and investigations: (1) type of fluorescence in blood cells of both species of ascidiaceae; (2) localization of the fluorescent material, and (3) histospectrographic measurement of the emitted fluorescence.

*Methods.* Blood samples of *Ph. mamillata* (vein puncture[9]) and *C. intestinalis* (heart puncture[10]) were centrifuged (2 min at 100 *g*). The sediment was transferred with adhering plasma to a glass slide, covered with a cover slip and examined under a fluorescence microscope (Leitz Ortholux), to which a Zeiss spectrograph of high luminosity was connected. The spectrograms were recorded on panchromatic plates (Ferrania Superex) and evaluated with a microphotometer (Leeds and Northray, Philadelphia)[11]. Osmic acid was used as 1% solution, methyl red as saturated solution of the sodium salt in sea water.

*Results.* In *Ph. mamillata* only the compartment cells, which do not give the reaction with osmic acid and methyl red, show granules with a brilliant blue fluorescence, while the vanadocytes lack any fluorescence (Figure 1a). The fluorescent material is resistant to UV-irradiation. The fluorescence spectrum (Figure 2) shows besides the Hg-lines (5461 and 4359 Å) 2 maxima at about 5200 and 4600 Å.

In *Ciona intestinalis* only the vanadocytes, characterized by a faint brown colour with $OsO_4$ and a red one with methyl red, show a yellow fluorescence (Figure 1b) with maxima at about 5900, 5200, and 4600 Å (Figure 2). By UV-irradiation for about 10 min the yellow fluorescence is transformed into a blue one, similar but not as brilliant as that of the *Phallusia* cells. This transformation is characterized by the disappearance of the maximum at 5900 Å and the accentuation of the maxima at 5200 and 4600 Å. The same transformation takes place within a few seconds in blood samples which had been stored for some hours, even at 4 °C. Some of the vanadocytes of *Ciona* already show the blue fluorescence instead of the yellow one in the freshly drawn blood.

*Discussion.* It is concluded that the stable blue fluorescent substance in the compartment cells of *Phallusia* is similar to that which, in the vanadocytes of *Ciona*, arises from the yellow fluorescent component. The fact that neither fluorescent component is observed in *Phallusia* vanadocytes may be explained by the quenching effect of highly concentrated vanadium (V-content 15 times that in *Ciona* vanadocytes[10]). Futhermore the finding that the test cells, present in the oocytes of *Ciona*, show a yellow fluorescence changing to blue[12], and are stained like the vanadocytes with osmic acid and with methyl red, may support the hypothesis, that the test cells[13,14], like the blood cells, are derived from the mesenchym.

The work on the chemical structure of the fluorescent material up to the present has shown that it is not identical with the yellow organic compound isolated from the hemovanadin system.

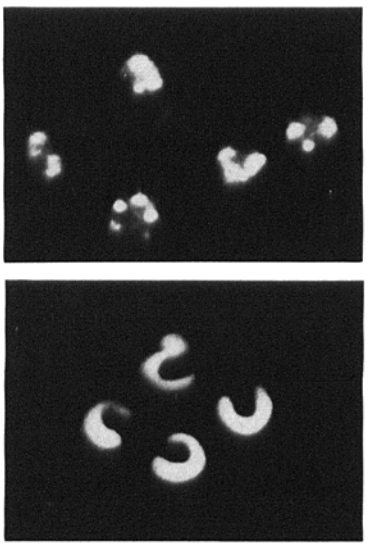


Fig. 1. Blood cells under Wood light (1 cm = 10 $\mu$). (a) *Phallusia mamillata*, blue fluorescent compartment cells; (b) *Ciona intestinalis*, yellow fluorescent vanadocytes.

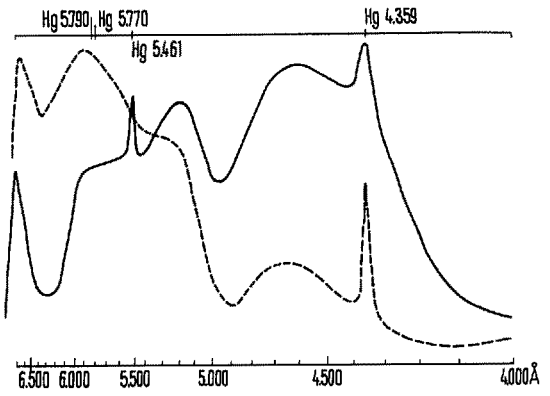


Fig. 2. Fluorescence spectra (Wood light). —— (a) blue fluorescent cells of *Phallusia mamillata*; ---- (b) yellow fluorescent cells of *Ciona intestinalis*.

*Zusammenfassung.* Von den Blutzellen der *Phallusia mamillata* Cuvier zeigen nur die stark vakuolisierten (compartment cells) im UV-Licht eine stabile blaue Fluoreszenz ($\lambda_{max}$ = 5200 und 4600 Å). Sie ist in den Zellgranula lokalisiert. Bei den Blutzellen von *Ciona intestinalis* L. tritt dagegen eine gelbe Fluoreszenz ($\lambda_{max}$ = 5900, 5200 und 4600 Å) auf, die nur von den Vanadocyten ausgeht. Diese Fluoreszenz, welche auch von den Testazellen der Oocyten gegeben wird, geht innerhalb einiger Minuten in die blaue Fluoreszenz über.

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