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The Dynamics of Antibiotic Activity of nemertine Tissues and Its Significance in the System of Protective Mechanisms of the Organism

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Abstract

Obviously, nemertean tissues normally have antibiotic properties, intensifyed by injury and in contact with bacteria. The highest antibiotic activity was found in mature nemertines, especially in females during the reproductive season. During winter, the antibiotic activity of tissue of the females in the damage focus is significantly reduced compared with the antibiotic activity of tissues during the reproductive period. In males, seasonal fluctuations of the antibiotic activity are negligible.Therefore, the antibiotic activity of the tissue regularly increases in the area of the wound track not only due to bacterial infection or the introduction of a foreign body, ensuring tissue sterility and preventing, thus, the development of necrosis, but also due to the physiological state of animals, particularly those related to reproductive cycles.

Keywords: *Lineus ruber*, antibiotic activity, test-microbs, humoral factors, *Sarcina lutea, Micrococcus lysodeicticus*, spore-forming*Bacillus subtilis*.

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1. Introduction

The participation of humoral factors in the development of specific immunity in worms has been shown in many investigations [1--18]. Bactericidal and bacteriostatic substances were detected in the blood of the perivisceral fluid of the earthworm *Lumbricuster-restris*[9]. Earthworm cavity fluid also contains antifungal substances [9,]. In the tissues of the nemertins were identified bactericidal and bacteriostatic substances [19--30]. In the epithelium of nemertines there are well-developed mucous cells, and in the underlying connective tissue (cutis) there are multicellular packages of glands containing acid sulfated mucopolysaccharides and antibiotic substances. Antibiotic activity of nemertine tissues *Lineus ruber* increases in the area of the wound canal not only due to bacterial infection or the introduction of a foreign body, providing sterility of tissues and thus preventing the development of necrosis, but also in connection with the physiological state of animals, especially associated with reproductive cycles.

2. Methods and Equipment

The introduction of a sterile cotton thread into the tissues of adult nemerteans was carried out during different seasons of the year in order to study the dynamics of antimicrobial activity of tissues. *Sarcinalutea* and *Micrococcus lysodeicticus*, which are sensitive to the animalantibiotics, as well as spore-forming *Bacillus subtilis* were used as the test organisms.The threads were removed from the nemerteans after 5, 10 minutes, after 1, 3, 6, 12, 24, 48 hours and placed in Petri dish with plain agar, seeded by superficial or interior way with the daily culture of bacteria at the rate of 1 billion cells per 100 ml of agar (according to the bacterial turbidity Standard). Sterile threads, which have not been inserted into the tissue served as a control.

3. Results

In 5 minutes after stay of cotton thread in the tissues of nemerteans and placing it on the surface of agar with seeded *S. lutea* a slight inhibition of the growth of *Sarcina* colonies was observed near the threads fibers. In no case the clear zones of growth were observed (Figure 1). After 1 - and 3-hours staying of fibers in the tissues of nemerteans the zones of negative growth with a width of 2 to 3.3 mm have appeared (Figure 2).

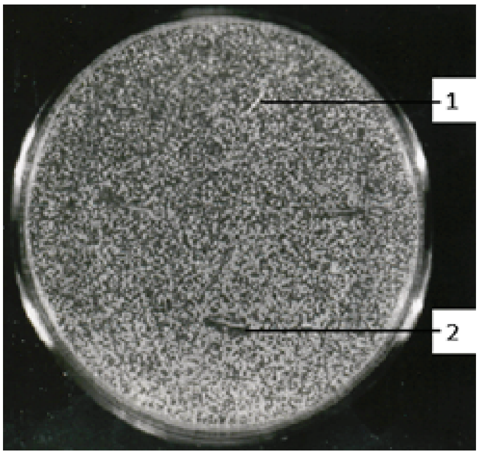

Figure 1: The effect of antibiotic substances adsorbed on the threads, which were in the tissues of nemerteans for five minutes on the daily culture of *S. lutea*. Plain agar.1 - the absence of sterile zones, 2 - slight inhibition of bacterial growth.

In case of removing of thread fibers within 6 hours after the introduction into the tissues of animals and putting them on a Petri dish, there were distinct zones of negative growth around them, with sizes of 3-5 mm in diameter (Figure 3). Around the fibers, remained in the body of animals for 12 hours, negative growth zone were revealed with

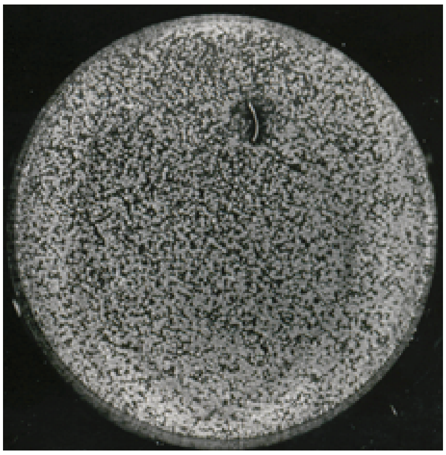

Figure 2: The formation of the zone of negative growth of *S. lutea* around the thread extracted after 1 hour from nemerteans tissue.

a width of 5.6-6 mm. The threads that were in the damage focus for 24 hours, cause the formation of the broad areas of negative growth 7 mm. in width (Figure 4).

The dynamics of changes in antibiotic activity in the damage focus, when using the test-microbe *S. lutea,* largely coincides with the dynamics of changes in antibiotic activity using *M. lysodeicticus*. However, the width of the negative growth zones using *M. lysodeicticus* as a test microbe was slightly smaller. Around the thread fibers extracted from the tissue after 5, 10 minutes and after 1 hour, there was a slight inhibition of growth of *M. lysodeicticus*.

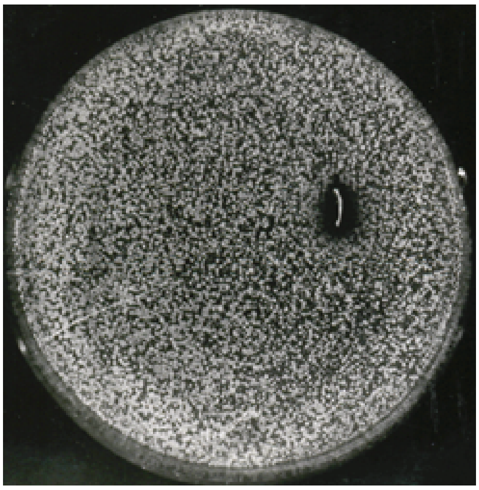

Figure 3: The zone of negative growth of *S. lutea* around the thread extracted from the nemerteans body in 6 hours after introduction.

Figure 4: The zone of negative growth of *S. lutea* around the thread extracted from the nemerteans body in 24 hours after introduction.

4. Discussion

Clear zone of negative microbial growth of 1.5-2 mm in width were detected around the fibers extracted from the tissues of nemerteans after 6-12 hours. The width of the negative growth zones of *M. lysodeicticus* was increased and reached 2.5-4 mm around the fibers remained in the tissues of animals during the day.In some experiments on the dynamics of antibiotic activity in the damage focus *B. subtilis* was used as the test microbe. Around the thread fibers extracted from the nemerteans tissues after 5 minutes, 1, 3 and 6 hours, there was a slight inhibition of growth of *B. subtilis*. Zones of negative microbial growth of *B. subtilis* of 1.5-2 mm in width were detected around the fibers remaining in the tissues of the nemerteans within 24-36 hours.

5. Conclusion

The results of these experiments denote the regular intensification of antibiotic activity in the damage focus, starting from 3 to 48 hours after the start of the experiment. However, in one series of experiments with 5-10-day culture of *Sarcinalutea* it was found that the tissues of some nemerteansexhibited antibiotic behavior at the very early stages after the injury. The zone of negative growth were obtained around the thread fibers, extracted from the body of nemerteans in 10 minutes and 1 hour after the start of the experiment.Obviously, nemerteans tissues normally have antibiotic properties, intensifying by injury and in contact with bacteria. The highest antibiotic activity was found in mature nemerteans, especially in females during the reproductive season. During winter, the antibiotic activity of tissue of the females in the damage focus is significantly reduced compared with the antibiotic activity of tissues during the reproductive period. In males, seasonal fluctuations of the antibiotic activity are negligible.Therefore, the antibiotic activity of the tissue regularly increases in the area of the wound track not only due to bacterial infection or the introduction of a foreign body, ensuring tissue sterility and preventing, thus, the development of necrosis, but also due to the physiological state of animals, particularly those related to reproductive cycles.The strengthening of the tissue antibiotic activity of females during the period, approaching to eggs spawning, is also associated with the fact that the capsule formed around the eggs by the secretions of cutaneous glands, has also antibiotic properties, providing a sterile environment in which the eggs develop and in which at first time young nemerteans live.

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Conflict of Interest

The author has no conflict of interest to declare.

References

[1] Ali, A.E., Arakawa, O., Noguchi, T., Miyazawa, K., Shida, Y., Hashimoto, K. (1990). Tetrodotoxin and related substances in a ribbon worm *Cephalothrix linearis* (Nemertean). *Toxicon*, vol 28., pp. 1083--1093.

[2] Asakawa, M., Toyoshima, T., Shida, Y., Noguchi, T., Miyazawa, K. (2000). Paralytic toxins in a ribbon worm *Cephalothrix species* (Nemertean) adherent to cultured oysters in Hiroshima Bay, Hiroshima Prefecture, Japan. *Toxicon*, vol. 38, pp. 763--773.

[3] Asakawa, M., Ito, K., Kajihara, H. (2013). Highly toxic ribbon worm *Cephalothrix simula* containing tetrodotoxin in Hiroshima Bay, Hiroshima Prefecture, Japan. *Toxins*, vol. 5, pp. 376--395.

[4] Bang, F. B. (1967). Serological responses among invertebrates other than insects.*Federat. Proc.,* vol. 26, № 6, pp. 1680--1684.

[5] Beckers, P., Bartolomaeus, T., von Döhren, J. (2015). Observations and experiments on the biology and life history of *Riseriellus occultus* (Heteronemertea: Lineidae) *Zool. Sci.*, vol. 32 pp. 531--546.

[6] Beleneva, I., Magarlamov, T.Y., Kukhlevsky, A. (2014). Characterization, identification, and screening for tetrodotoxin production by bacteria associated with the ribbon worm (Nemertea) *Cephalotrix simula* (Ivata, 1952) *Microbiology,* vol. 83, pp. 220--226.

[7] Blunt, J.W., Copp, B.R., Keyzers, R.A., Munro, M.H.G., Prinsep, M.R. (2014). Marine natural products. *Nat. Prod. Rep.* vol. 31, No. 2, pp. 160-258.

[8] Butala, M., Sega, D., Tomc, B., Podlesek, Z., Kem, W.R., Kupper, F.C., Turk, T. (2015). Recombinant expression and predicted structure of parborlysin, a cytolytic protein from the Antarctic heteronemertine *Parborlasia corrugatus*. *Toxicon*, vol. 108, pp. 32--37.

[9] Hemalatha, K., Madhumitha, G., Roopan, S. M. (2013). Indole as a core anti-inflammatory agent- a mini review. *Chem. Sci. Rev. Lett.*, vol. 2, pp. 287-292.

[10] Cooper, E.L. (1968). Transplantation immunity in annelids. 1. Reactions of xenografts exchanged between *Lumbricusterrestris* and *Eiseniafoetida*. *Transplantation,* vol. 6, pp. 322--337.

[11] Cushing, J. E., Neely, J. L (1969). Comparative immunology of sipunculidcoelomic. *Journal*. *Invertebrate Pathology,* vol. 14, pp. 4--12.

[12] Jacobsson, E., Andersson, H.S., Strand, M., Peigneur, S., Eriksson, C., Lodén, H., Shariatgorji, M., Andren, P.E., Lebbe, E., Rosengren, K.J., Tytgat, J., Göransson, U. (2018). Peptide ion channel toxins from the bootlace worm, the longest animal on Earth. *Sci. Rep.* vol. 8 p. 4596.

[13] Kajihara, H., Sun, S.C., Chernyshev, A.V., Chen, H.X., Ito, K., Asakawa, M., Maslakova, S.A., Norenburg, J.L., Strand, M., Sundberg, P., et al. (2013). Taxonomic identity of a tetrodotoxin-accumulating ribbon-worm *Cephalothrix simula* (Nemertea: Palaeonemertea): A species artificially introduced from the Pacific to Europe. *Zool. Sci.*, vol. 30, pp. 985--997.

[14] Kem, W.R. (2001). Nemertine Neurotoxins. In: *Neurotoxicology Handbook: Natural Toxins of Animal.* Humana Press: Totowa, NJ; pp 573-594.

[15] Kem, W. R. (1958). Structure and activity of Nemertine Toxins. *Integr. Comp. Biol.,* vol. **25**, pp. 99--111.

[16] Kem, W., Soti, F., Wildeboer, K., LeFrancois, S., MacDougall, K., Wei, D.-Q., Chou, K.-C., Arias, H.R. (2006). The nemertine toxin anabaseine and its derivative DMXBA (GTS-21): Chemical and pharmacological properties. *Mar. Drugs*, vol. 4, pp. 255--273.

[17] Kitajima, M., Takayama, H. (2016). Monoterpenoid bisindole alkaloids. T*he Alkaloids: Chemistry and Biology.* vol. 76, pp. 259-310.

[18] Kwon, Y.S., Min, S.K., Yeon, S.J., Hwang, J.H., Hong, J., Shin, H.S. (2017). Assessment of neuronal cell-based cytotoxicity of neurotoxins from an estuarine nemertean in the Han River Estuary. *J. Microbiol. Biotechnol*., vol. 27, pp. 725--730.

[19] Lalit, K., Shashi, B., Kamal, J. (2012). The diverse pharmacological importance of indole derivatives: a review. *Int. J. Res. Pharm. Sci.* vol. 2, pp. 23-33.

[20] Magarlamov, T.Y., Beleneva, I.A., Chernyshev, A.V., Kuhlevsky, A.D. (2014). Tetrodotoxin-producing *Bacillus* sp. from the ribbon worm (Nemertea) *Cephalothrix simula* (Iwata, 1952). *Toxicon*, vol. 85, pp. 46--51.

[21] Magarlamov, T.Y., Shokur, O.A., Chernyshev, A.V. (2016). Distribution of tetrodotoxin in the ribbon worm *Lineus alborostratus* (Takakura, 1898)(Nemertea): Immunoelectron and immunofluorescence studies. *Toxicon,* vol. 112, pp. 29--34.

[22] Miyazawa, K., Higashiyama, M., Ito, K., Noguchi, T., Arakawa, O., Shida, Y., Hashimoto, K. (1988). Tetrodotoxin in two species of ribbon worm (Nemertini), *Lineus fuscoviridis* and *Tubulanus punctatus*. *Toxicon,* vol. 26, pp. 867--874.

[23] Netz, N., Opatz, T. (2015). Marine indole alkaloids. *Mar. Drugs*, vol. 13, pp. 4814-4914.

[24] Ponomarenko, L.P., Makarieva, T.N., Stonik, V.A., Dmitrenok, A.S., Dmitrenok, P.S. (1995). Sterol composition of Linneus torquatus (Nemertini) Anopla *Comp. Biochem. Physiol.* vol. 11, N. 4, p. 575-577.

[25] Rangel, M., Falkenberg, M. (2015). An overview of the marine natural products in clinical trials and on the market. *J. Coast. Life Med.,* vol. 3, pp. 421--428.

[26] Rubiolo, J.A., Ternon, E., Lopez-Alonso, H., Thomas, O.P., Vega, F.V., Vieytes, M.R., Botana, L.M. (2013). Crambescidin-816 acts as a fungicidal with more potency than crambescidin-800 and -830, inducing cell cycle arrest, increased cell size and apoptosis in Saccharomyces cerevisiae II *Mar. Drugs.* vol. 11, No. 11, pp. 4419-4434.

[27] Rubiolo, J.A., Lopez-Alonso, H., Roel, M., Vieytes, M.R., Thomas, O., Ternon, E., Vega, F.V., Botana, L. M. (2014). Mechanism of cytotoxic action of crambescidin-816 on human liver-derived tumour cells. *Br. J. Pharmacol*. vol. 171, No. 7, p. 1668-1675

[28] Strand, M., Hedström, M., Seth, H., McEvoy, E.G., Jacobsson, E., Goransson, U., Andersson, H.S., Sundberg, P. (2016). The bacterial (*Vibrio alginolyticus*) production of tetrodotoxin in the ribbon worm *Lineus longissimus*---Just a false positive? *Mar. Drugs.* vol. 14, p. 63.

[29] Turner, A., Fenwick, D., Powell, A., Dhanji-Rapkova, M., Ford, C., Hatfield, R., Santos, A., Martinez-Urtaza, J., Bean, T., Baker-Austin, C. (2018). New Invasive Nemertean

Species (*Cephalothrix simula*) in England with high levels of tetrodotoxin and a microbiome linked to toxin metabolism. *Mar. Drugs,* vol. 16, pp. 452.

[30] Vlasenko, A., Velansky, P., Chernyshev, A., Kuznetsov, V., Magarlamov, T.Y. (2018). Tetrodotoxin and its analogues profile in nemertean species from the Sea of Japan. *Toxicon,* vol. 156, pp. 48--51.

[31] Voogt, P.A. (1973). Biosynthesis and composition sterols in nemertean Cerebratus marginatus. *Arch. Int. Physiol. Biochem.*, vol. 81, pp. 871-880.