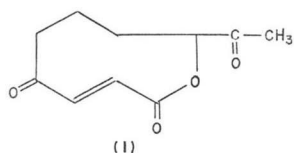


Communications to the editor

THE CYTOTOXIC EFFECTS OF A NEW ANTIBIOTIC VERMICULINE

Sir:

In our previous paper the isolation of a new antibiotic vermiculine from culture broth of *Penicillium vermiculatum* DANGEARD as well as the physico-chemical and antiprotozoal properties of the substance were described.¹⁾ The



substance consists of a nine-membered lactone (I) and its structure was published by SEDMERA *et al.*²⁾

The molecule of vermiculine resembles pyrenophorol³⁾ and pyrenophorin⁴⁾, which showed an inhibitory effect on EHRlich ascites carcinoma cells. Of two other fungal metabolites having a macrolide structure, cytochalasin B was shown to influence the growth of AH 130, MSL and EAC tumor as well as NOVIKOFF hepatoma, through inhibition of cytokinase activity.⁵⁾ The incorporation of ³²P into nucleic acids of EAC cells and especially the synthesis of proteins in HeLa cells was suppressed when the macrolide antibiotic, cyanenin, was added to the reaction mixture.⁶⁾

The possible cancerostatic effect of vermiculine, using three types of ascites tumors, EHRlich carcinoma (EAC), lymphadenoma L-5178 and sarcoma 37, was studied. The cells of the tumors were partially adapted and selected for *in vitro* growth.^{7,8)} Cells, cultivated 24 hours at 37°C in primary suspended culture, were separated and hydrolyzed for 20 minutes by addition of HClO₄ at 90°C. The concentration of total nucleic acids in the hydrolyzates was estimated spectrophotometrically.⁹⁾ The ED₅₀, *i.e.* the concentration of the test substance which decreased

nucleic acids by 50% from that of the basal medium control was determined. The effect of vermiculine on decreasing the amount of nucleic acids in the cells of the three tumors used are shown in Fig. 1. The ED₅₀ was EAC 1.7 μg/ml; L-5178 0.5 μg/ml; S-37 1.7 μg/ml, respectively. The inhibition of incorporation of ¹⁴C-labelled precursors, *l*-valine, adenine, thymidine and uridine into cold TCA-insoluble fractions of EAC cells by vermiculine was also studied in order to determine its effect on protein and nucleic acid synthesis as well as synthesis of RNA and DNA.¹⁰⁾ The substance at all concentrations tested reduced the synthesis of proteins and nucleic acids.

Fig. 1. The effects of vermiculine on *in vitro* level of nucleic acids in tumor cells.

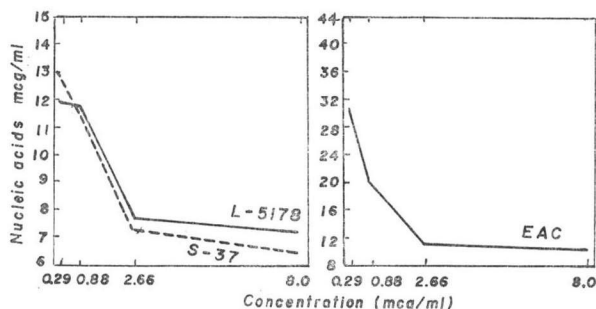
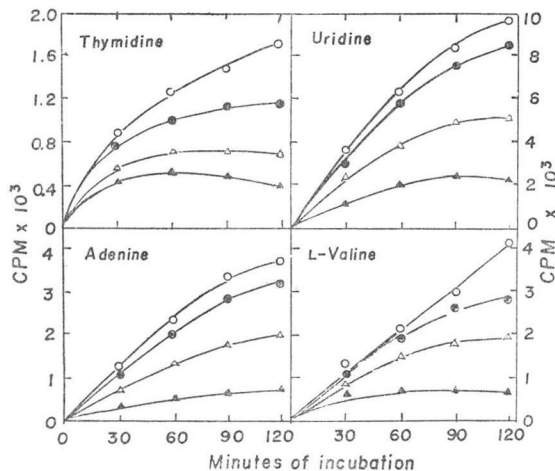


Fig. 2. The dynamic of incorporation of ¹⁴C-labelled precursors into the EAC cells in the presence of vermiculine (0, 6.25, 12.5 and 25 μg/ml). Precursors used: adenine-8-¹⁴C, *l*-valine-¹⁴C(U), thymidine-2-¹⁴C and uridine-¹⁴C(U).



At the higher concentration the antibiotic inhibited the incorporation of DNA precursors to a greater extent than that of RNA precursors (Fig. 2).

The cytotoxic effect of vermiculine was evaluated using HeLa cells. The modified method of OYAMA and EAGLE¹¹⁾ was used for estimating the growth of cells. The ED₅₀ for vermiculine was found to be 1.8 µg/ml.

Vermiculine is a cytotoxic antibiotic, which in concentrations from 0.5 to 1.7 µg/ml inhibited the growth of EAC, Lymphadenoma L-5178 and sarcoma 37 cells.

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References

- 1) FUSKA, J.; P. NEMEC & I. KUHR: Vermiculine, a new antiprotozoal antibiotic from *Penicillium vermiculatum*. J. Antibiotics 25: 208~211, 1972
- 2) SEDMERA, P.; J. VOKOUN, M. PODOJIL, Z. VANĚK, J. FUSKA, P. NEMEC & I. KUHR: The structure of vermiculine, a new lactone from *Penicillium vermiculatum*. Tetrahedron Letters 1973: 1347~1348, 1973
- 3) KIS, Z.; P. FURGER & H. P. SIGG: Über die Isolierung von Pyrenophorol. Experientia 25: 123~124, 1969
- 4) ISHIBASHI, K.: Studies on antibiotics from *Helminthosporium* sp. fungi. II. Pyrenophorin, a new antibiotic produced by *Pyrenophora avenae* (*Helminthosporium avenae*). J. Agr. Chem. Soc. Japan 35: 257~262, 1961 (Cit. Agr. Biol. Chem. 25: A24, 1961)
- 5) ESTENSEN, R. D.: Cytochalasin B. I. Effect on cytokinesis of NOVIKOFF hepatoma cells. Proc. Soc. Exp. Biol. Med. 136: 1256~1260, 1971
- 6) BETINA, V.: Effects of the macrolide antibiotic cyanein on HeLa cells growth and metabolism. Neoplasma 16: 23~32, 1969
- 7) MAKUKHO, L. V.; L. P. IVANITSKAYA, L. YA. VOLKOVA, T. P. PREOBRAZHENSAYA & L. P. TEREKHOVA: The screening of antitumor antibiotics inhibiting synthesis of nucleic acids in cancer cells. Antibiotics (Russian) 17: 117~121, 1972
- 8) IVANITSKAYA, L. P. & L. V. MAKUKHO: Primary suspended culture of tumor cells of animal ascites carcinoma as a model for screening antitumor substances. Voprosy Onkologii 19: 67~72, 1973
- 9) SPIRIN, A. S.: Spectrophotometric determination of total nucleic acids. Biochemistry (Russian) 23: 656~661, 1958
- 10) FUSKA, J.; M. MIKO, P. NEMEC & L. DROBNICA: Screening of the cytotoxic action of fungus filtrates on EHRlich's ascites carcinoma, utilizing ¹⁴C-labelled precursors. Neoplasma 18: 631~636, 1971
- 11) OYAMA, V. I. & H. EAGLE: Measurement of cell growth in tissue culture with a phenol reagent (FOLIN-CIocalTEAU). Proc. Soc. Exp. Biol. Med. 91: 305~307, 1956