Inhibition of Protein and DNA Syntheses in Ehrlich Ascites Tumour by Nivalenol, a Toxic Principle of *Fusarium nivale*-Growing Rice

In the summer of 1954, 25 young men of Tokyo took *Fusarium* sp.-growing rice to suffer from nausea, vomiting and drowsiness[1,2]. The similar poisonings due to so-called 'scabby grains' occurred at the other districts at that time. TSUNODA et al.[1] isolated a toxic fungus *Fusarium* sp. F2 from actually damaged wheat, and URAGUCHI[3] made toxicological examinations on this fungus. Mice which ate the mouldy grains went off feed, lost their movements and sometimes died. The toxic principle, which was fractionated from charcoal-absorbable materials of the fungus filtrate, killed mice at the s.c. dose of 70 μg/10 g, though the chemical characteristics of this isolate were left undisclosed because of the reduced toxicity of the fungus.

Recently, several tens of *Fusarium* species were collected from damaged wheat and re-examined for toxicity. Based on the lethal action of the cultured filtrate of each strain, *F. nivale* Fn 2 was selected as the standard strain for the isolation of toxic agent(s)[4], and Nivalenol was isolated from the charcoal-absorbable materials of the *F. nivale*-growing rice[5,6]. Pathological examinations proved that Nivalenol gave necrosis and degeneration of the intestine, bone marrow, lymph nodes, thymus and testis of mice, indicating that actively-dividing cells of these tissues were injured[5,6]. Biochemical investigations revealed that the toxin inhibited poly U-directed synthesis of polypeptide in reticulocyte ribosome[7,8].

Based on these biological findings on the toxin, the authors investigated effects of the toxin on the syntheses of protein and nucleic acids in Ehrlich tumour cells, and the results obtained indicated that Nivalenol inhibited the syntheses of protein and DNA of the cells.

Ehrlich ascites tumour cells, strain 4N, were maintained in ddS-male mouse and harvested 8–10 days after i.p. inoculation. After washing the cells with 0.9% NaCl solution until contaminated reticulocytes were removed, the packed cells were diluted with an equal volume of the saline and stored in an ice-cold bath. For the uptake of radioactive precursors into maclomolecules of the cells, 0.2 ml of the cell suspension was mixed with 0.6 ml of Locke-Ringer's solution and preincubated with shaking at 37 °C for 15 min in the presence or absence of the toxin. At the end of the preincubation, 0.1 μC of 1-C^{14}-leucine, 2-C^{14}-uracil or 2-C^{14}-thymidine was added to the cell suspension, making a total volume of 1.0 ml, to continue the incubation. After 40 min of incubation, the solution was mixed with an equal volume of 10% PCA to fractionate acid-insoluble precipitate into protein, RNA and DNA according to the method of REICH et al.[9].

As for the polymerization reaction of DNA in vitro, the supernatant of osmotically-ruptured tumour cells was prepared according to the method of SMELLIE et al.[10], and a standard assay system contained the following components (in μmoles unless otherwise specified); *Tris* buffer (pH 7.6) 5, KCl 15, $MgCl_2$ 1, EDTA-K 0.1, 2-mercaptoethanol 1.5, dXTP 0.05, DNA 50 μg and 0.1 μC H^3-TTP. Total volume was 0.25 ml. After 30 min of the incubation at 37 °C, the reaction was stopped by the addition of 1.0 ml of cold PCA, and the acid-insoluble materials were filtrated on a disc paper of Whatman 3MM to count the radioactivity with a scintillation counter.

When the cells were preincubated for 15 min in the presence of the toxin, as shown in Table I, Nivalenol at the concentration of 1–10 μg/ml inhibited markedly the uptakes of C^{14}-leucine and C^{14}-thymidine without affecting significantly the uptake of C^{14}-uracil. Experiments on the time course indicated that the toxin at the concentration of 5 μg/ml inhibited the uptakes of C^{14}-thymidine and C^{14}-leucine with and without a lag-phase, respectively, as shown in Figure 1. The inhibitory effect of the toxin on the uptake of labelled amino acid was also observed with C^{14}-lysine, C^{14}-proline and C^{14}-phenylalanine, as shown in Table II. However, the inhibitory effect of Nivalenol on the uptake of C^{14}-thymidine was not significant when the toxin was added to the reaction mixture at 0 time or after 15 min of the incubation, as shown in Figure 2.

Table I. Effects of Nivalenol on the syntheses of protein, DNA and RNA in tumour cells

Nivalenol (μg/ml)	Incorporation (cpm)		
	1-C^{14}-leucine into protein	2-C^{14}-thymidine into DNA	2-C^{14}-uracil into RNA
0	4179 (100)[a]	753 (100)	1296 (100)
1.0	3924 (94)	622 (83)	1176 (90)
2.5	3505 (84)	575 (76)	–
5.0	2793 (67)	481 (64)	1087 (84)
7.5	2119 (51)	438 (47)	–
10.0	1383 (33)	319 (42)	923 (71)

[a] Numbers in parentheses indicate the % activities of the control.

Table II. Effects of Nivalenol on the uptake of various kinds of C^{14}-labelled amino acids into protein in tumour cells

Nivalenol (μg/ml)	Incorporation (cpm)			
	C^{14}-leucine	C^{14}-lysine	C^{14}-proline	C^{14}-phenylalanine
0	552 (100)[a]	968 (100)	244 (100)	1546 (100)
2.5	269 (49)	610 (63)	65 (27)	1123 (73)
7.5	94 (17)	247 (25)	36 (15)	332 (21)

[a] Numbers in parentheses indicate the % activities of the control.

[1] H. TSUNODA, O. TSURUTA, S. MATSUNAMI and S. ISHII, Proc. Fd Res. Inst. (Minist. Agr. Forest. Japan) *12*, 26 (1957).
[2] K. URAGUCHI, T. TATSUNO, F. SAKAI, M. TSUKIOKA, Y. SAKAI, O. YONEMITSU, H. ITO, M. MIYAKE, M. SAITO, M. ENOMOTO, T. SHIKATA and T. ISHIKO, Jap. J. exp. Med. *31*, 1 (1961).
[3] K. URAGUCHI, Shokuhin-Eisai Kenkyu *8*, 25 (1958).
[4] H. TSUNODA, N. TOYAZAKI, N. MOROOKA, N. NAKANO, H. YOSHIYAMA, K. OKUBO and M. ISODA, Proc. Fd Res. Inst. (Minist. Agr. Forest. Japan) *23*, 89 (1968).
[5] T. TATSUNO, Y. UENO, I. UENO, Y. MORITA, M. HOSOYA, M. SAITO, M. ENOMOTO, K. OKUBO, N. MOROOKA, N. NAKANO and H. TSUNODA, Folia pharmac. Jap. *64*, 12 (1967).
[6] T. TATSUNO, M. SAITO, M. ENOMOTO and H. TSUNODA, Nature, in press.
[7] Y. UENO, M. HOSOYA and T. TATSUNO, J. Jap. Biochem. Soc. *39*, 708 (1967).
[8] Y. UENO, M. HOSOYA, I. UENO, Y. MORITA and T. TATSUNO, J. Biochem., Tokyo, in press.
[9] E. REICH, R. M. FRANKLIN, A. J. SHATKIN and F. L. TATUM, Proc. natn. Acad. Sci. USA *48*, 1238 (1962).
[10] R. M. S. SMELLIE, H. M. KEIR and T. N. DAVIDSON, Biochim. biophys. Acta *35*, 389 (1959).

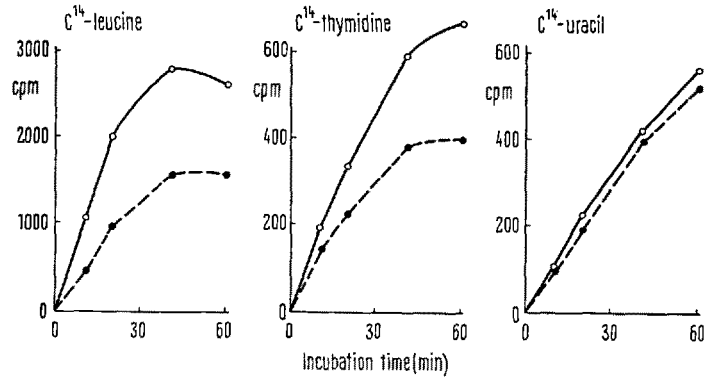

Fig. 1. Time courses of the incorporation of labelled precursors in tumour cells. o——o control, ●-----● Nivalenol (5 μg/ml).

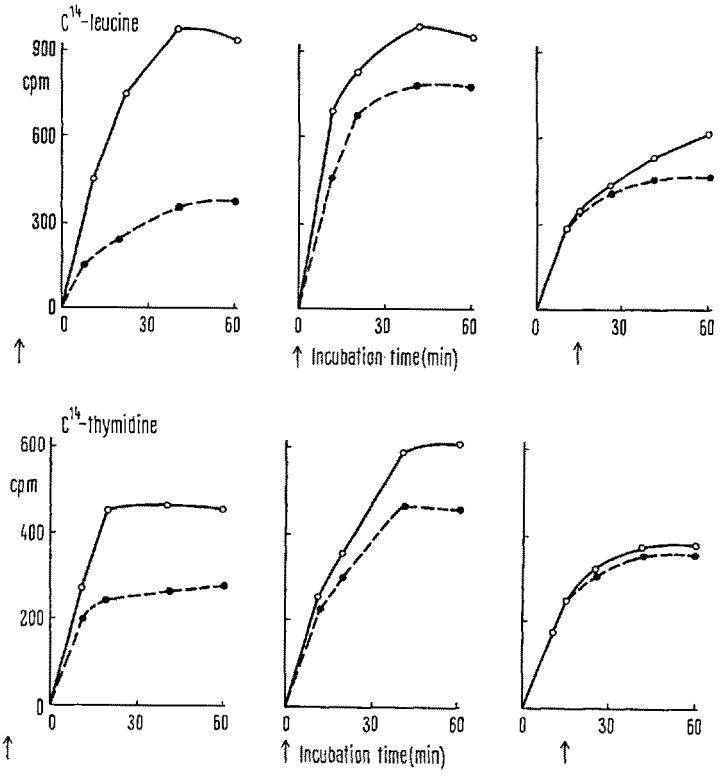

Fig. 2. Effects of the addition time of Nivalenol on the uptakes of C^{14}-leucine and C^{14}-thymidine. o——o control, ●-----● Nivalenol (5 μg/ml).

Table III. Effects of Nivalenol on DNA polymerase of tumour cells

Nivalenol (μg/ml)	Incorporation (cpm/mg protein)		
	Exp. 1	Exp. 2	Exp. 3
0	749	562	660
1	–	547	670
5	674	550	–
10	–	–	629
50	738	–	–

Further experiments conducted with DNA polymerase of the cells revealed that Nivalenol at the concentration of 1–50 μg/ml did not affect the incorporation of H^3-TTP into DNA in vitro, as shown in Table III.

Summing up the above experiments, it is concluded that Nivalenol, the toxic agent of *F. nivale*-growing rice, interferes with the syntheses of protein and DNA of Ehrlich ascites tumour cells, and the observed inhibition of protein synthesis is presumably caused by inactivation of the ribosome system of the cells as in the case of reticulocyte[7,8]. The inhibition of DNA synthesis in vivo is assumed to be caused by an inhibition of synthesis of enzymes required in DNA synthesis, as in case of cyclohexamide[11,12].

Résumé. La Nivalénole, principe toxique, synthétisé par le *Fusarium nivale*, inhibe les synthèses de la protéine et du DNA dans la cellule de la tumeur Ehrlich.

Y. Ueno and K. Fukushima

Microbial Chemistry, Faculty of Pharmaceutical Sciences, Science University of Tokyo, Ichigaya, Tokyo (Japan), 10 June 1968.

[11] M. R. Siegel and H. D. Sisler, Biochem. biophys. Acta *87*, 70 (1964).

[12] This investigation was partly aided by the Cancer Research Grant from the Ministry of Welfare. Nivalenol was kindly supplied by T. Tatsuno, the Institute of Physical and Chemical, Research, Tokyo.