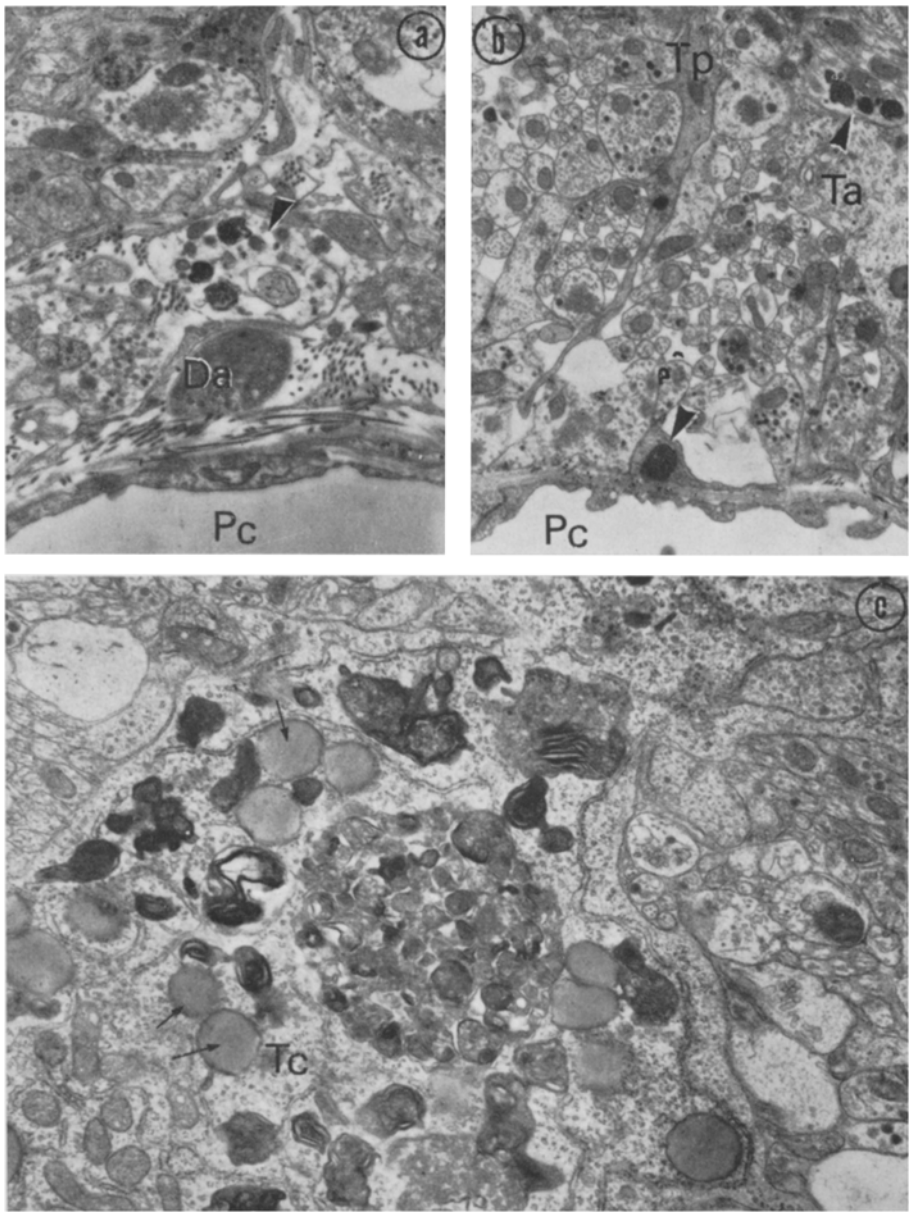

Fig. 3. a) and b) Axon cytolysome formation in early degenerating terminals (arrowhead). 1 degenerating axon (Da) with recognizable dense core vesicles is in the perivascular space. Ta, tanycyte cell body; Tp, tanycyte process; Pc, portal capillary. ×12,600. c) Many late degenerating fragments inside a tanycyte 48 h after GTG injection. Large lipid droplets (arrows) are strikingly increased in number. Tc, tanycyte cell body. ×18,980.

New Antilymphoma L-Asparaginase from *Fusarium* Species

Antitumour and immunosuppressive activities of L-asparaginases are summarized in recent reviews[1–3]. In spite of the wide distribution of L-asparaginases among microorganisms[4–9], the antitumour activity is limited to L-asparaginases from bacteria of Enterobacteriaceae[10–13], *Mycobacterium tuberculosis*[14] and *Aspergillus terreus*[15]. Among them, the enzymes from bacteria of Enterobacteriaceae are now being used in patients with leukaemia, but a wide range of toxic effects on various organs have been observed[16]. Hence we have looked for antitumour asparaginases from different microorganisms.

After screening a large number of microorganisms[7], we noticed that L-asparaginases of *Fusarium* species and ascomycetous fungi having a *Fusarium* asexual state suppressed the growth of Gardner lymphosarcoma and were devoid of L-glutaminase and endotoxin, which are inseparable from the L-asparaginase activity of enterobacteria and may be responsible for the cytotoxic effect[17,18].

The fungi were grown on agar slants and small pieces of grown mat were inoculated into 500 ml of DP-medium in 2 l flasks. DP-medium contained per liter: dextrin, 30 g; Pharmamedia (Traders Mill Co., Fort Worth, Texas), 40 g; NaCl, 5 g; KH_2PO_4, 5g; K_2HPO_4, 1.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; pH 6.2. Flasks were incubated at 28 °C for 3 days and the contents of 2 flasks were trans-

ferred to 30 l of DP-medium in a 50 l stainless-steel fermentor, and incubated for 5 days at 28 °C with stirring and aeration (30 l per min). The activity of L-asparaginase in the culture filtrate of *Fusarium oxysporum* IFO 9331 was 2.5 IU/ml.

Assay of L-asparaginase was carried out using *Tris*-HCl buffer (pH 7.2) according to the method described by ROBERTS et al.[19] with some modifications.

L-Asparaginase preparation of *Fusarium oxysporum* IFO 9331 was obtained from the culture filtrate by acetone precipitation, Sephadex G-75 gel-filtration, DEAE- cellulose chromatography and isoelectric focusing. The purification was 500-1,000 fold and the final preparation contained 127 IU of L-asparaginase per mg of protein.

L-Asparaginase preparations from other fungi were prepared from the culture filtrates by acetone precipitation, Sephadex G-75 gel-filtration (IFO 9660), or ammonium sulfate precipitation to remove precipitable impurity proteins (IFO 5421 and 9661) and lyophilization. The preparations from *Fusarium oxysporum* IFO 9660, *Fusarium roseum* IFO 5421, *Fusarium solani* IFO 5893 and *Hypomyces solani* IFO 9661 contained 15 IU, 0.35 IU, 1.8 IU and 0.57 IU of L-aparaginase per mg of powder, respectively.

L-Asparaginases thus obtained were similar to each other in their properties. They deamidated L-asparagine selectively. The deamidation rate of D-asparagine was approximately 1% of that of L-asparagine. Neither L- nor D-glutamine was deamidated. *Km* values for L-asparagine of the enzymes from *Fusarium oxysporum* IFO 9331 and 9660 were 4.3×10^{-5} and 8.3×10^{-5} *M* respectively. The deamidation of L-asparagine was competitively inhibited by D-asparagine, *Ki* values being 4.3×10^{-4} and 5.0×10^{-4} *M* for the enzymes from IFO 9331 and 9660, respectively. Optimum pH for the reaction was 7.2 for these enzymes.

Antitumour activities of the L-asparaginase preparations are summarized in the Table. All the L-asparaginase preparations, as well as *Escherichia coli* L-asparaginase, strongly suppressed the growth of 6C3HED Gardner lymphosarcoma in C3H mice. It should be pointed out that, by the treatment with the fungal L-asparaginases, the volume of ascites once increased, and then resulted sometimes in the complete regression of tumour. On the other hand, there was little initial increase of ascitic volume by the treatment of *E. coli* L-asparaginase.

Clinically, allergic reactions were reported as the side effects of *E. coli* L-asparaginase in patients[16]. During

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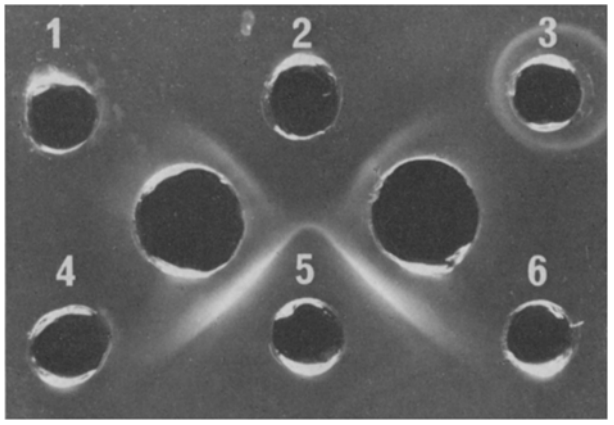
Antitumour activity of L-asparaginases of *Fusarium* and its related species[a]

Experiment No.	Treatment	Dose IU/mouse/day × 5	No. of mice	Body wt. change (day 1–5)	Survival time (day)
I	*Escherichia coli*	2.5	5	−2.5	25 — — — —[b]
	(Worthington Biochemicals)	5.0	5	−2.8	21 23 — — —
	Escherichia coli	2.5	5	−3.0	— — — — —
	(Kyowa, crystalline)	5.0	5	−3.2	16 17 21 — —
	Fusarium oxysporum	2.5	5	0	13 16 — — —
	IFO 9331	5.0	5	−0.4	— — — — —
	Control		5	+2.4	12 13 13 17 17
II	*Fusarium oxysporum*	2.5	5	0	16 16 17 — —
	IFO 9660	5.0	5	+0.2	— — — — —
	Control		5	+1.6	14 14 14 15 15
III	*Fusarium roseum* IFO 5421	5.0	3	0	12 29 —
	Control		4	0	12 13 13 13
IV	*Fusarium solani* IFO 5893	5.0	5	−1.4	14 14 14 15 16
	Control		5	+1.2	10 10 11 11 12
V	*Hypomyces solani* IFO 9661	5.0	5	−0.6	19 — — — —
	Control		5	+0.6	11 11 11 11 11

[a] 10 million of 6C3HED lymphosarcoma cells were transplanted i.p. and drugs were injected i.p. for 5 consecutive days from 24 h after tumor implantation. [b] —, The tumour-transplanted animals survived more than 30 days after the tumour transplantation.

the treatment of acute myelocytic leukaemia, precipitating and complement-binding antibodies were demonstrated[20]. We investigated the cross-reaction between *E. coli* L-asparaginase preparation and L-asparaginases obtained from *Fusarium* species on agar-immuno-diffusion. Anti-*E. coli* L-asparaginase antiserum was raised in rabbits by multiple injection of *E. coli* L-asparaginase (Worthington Biochem. Co.) mixed in Freund's complete adjuvant. As seen in the Figure, *Fusarium* and *Hypomyces* L-asparaginase preparations gave no identical precipitation lines with *E. coli* L-asparaginase. Thus, L-asparaginases from *Fusarium* and *Hypomyces* were antigenically different from *E. coli* L-asparaginase.



Immunoprecipitation of several kinds of L-asparaginases against anti-*E. coli* L-asparaginase antiserum. The antiserum was raised in rabbits by injecting i.v., 2 ml of L-asparaginase from *E. coli* (Worthington, 200 IU/6 mg/2 ml) in physiological saline solution twice 3 days apart. 1 and 3 weeks later, rabbits were boosted s.c. with the same amount of the L-asparaginase emulsified in Freund's complete adjuvant (Difco). The sera were collected 1 week after the last injection. Micro-gel diffusion was carried out on microslides using 1% agar (Difco, Noble) in veronal buffer, 0.06 *M*, pH 8.6. Central wells: 50 µl of anti-*E. coli* L-asparaginase rabbits sera. Peripheral wells: 10 µl of 0.2% of *E. coli* L-asparaginase and 10 µl of 2% of other L-asparaginase preparations: 1. *Fusarium solani*, IPFO 5893; 2. *Escherichia coli* (Kyowa, crystalline); 3. *Fusarium oxysporum*, IFO 9660; 4. *Fusarium oxysporum*, IFO 9331; 5. *Escherichia coli* Worthington; 6. *Hypomyces solani*, IFO 9661.

It may be important to supply various L-asparaginases which differ in the antigenic properties to each other, so as to avoid the problems arising from antigen-antibody reactions such as neutralization of the enzyme activity or anaphylactic shock.

SCHEETZ et al.[21] have pointed out the inability of L-asparaginase from mycelia of *Fusarium tricinctum* to suppress Gardner lymphosarcoma; therefore, we should like to examine the properties and antilymphoma activities of intracellular L-asparaginases of *Fusarium* species studied in the present report.

Résumé. Les champignons de *Fusarium* et ceux qui ont le type fusarium dans leur état asexues sécrétent l'asparaginase. Celle-ci n'a pas l'activité de la glutaminase et elle arrête le developpement de la leucémie chez les souris.

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Equivalence of Continuous Infusion and Single Injection of ^{3}H-Thymidine for Analysis of Intravascular Kinetics of Neutrophilic Granulocytes in the Rat

A quantitative description of the turnover of neutrophilic granulocytes requires the measurement of the mean intravascular lifespan of these cells. In dogs[1] and in man[2], autotransfusion studies after in-vitro labelling with ^{32}P-diisopropylfluorophosphate and subsequent scintillation counting of isolated leukocyte samples have been used to determine this parameter. The method is, however, not applicable to small laboratory animals. A new experimental approach to this problem was therefore developed using continuous infusion of ^{3}H-thymidine and autoradiography to determine the replacement of unlabelled peripheral blood granulocytes by labelled granulocytes coming from the bone marrow. Results for the rat, as well as a discussion of the principles of the method, were published previously[3,4]. Experiments reported here demonstrate that continuous infusion of ^{3}H-thymidine in this experimental system can be replaced by a single injection of the radioactive precursor, resulting in a considerable simplification of the technical procedure.

Materials and methods. ♂ Wistar AF-Han rats (250–350 g) were used in the experiments. For a period of 120 h the animals received a continuous infusion of either ^{3}H-thymidine in 0.9% saline (3 µCi/g body wt. per day) or 0.9% saline following a single i.v. injection of ^{3}H-thymidine (2 µCi/g body wt.). Blood samples were obtained at 12-h-intervals by repeated punctures of the tail artery[5]. The percentage of labelled blood granulocytes was determined by autoradiography of leukocyte-enriched blood smears. Details of the methods employed have been published[4].

Results. The percentage of labelled blood granulocytes obtained at various times after starting a continuous infusion or after giving a single i.v. injection of ^{3}H-thymidine is shown in Figure 1. It can be seen that the replacement of unlabelled blood granulocytes by labelled granulocytes from the marrow is the same for both schedules of

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