trainee by a training grant from the National Institutes of Health. We thank Dr Ron Baker for making *E. coli* T_3A-2 sensitive to S13 and Dr Ethel Tessman for criticisms.

ANNE S. VANDERBILT
IRWIN TESSMAN*

Department of Biological Sciences,
Purdue University,
Lafayette, Indiana.

*Present address: Department of Molecular Biology and Biochemistry, University of California, Irvine, California.

Received May 11, 1970.

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Specific Inhibition of Influenza Replication by α-Amanitin

α-AMANITIN, a bicyclic polypeptide from the toadstool *Amanita phalloides*[1], is known specifically to inhibit the DNA-dependent RNA polymerase of mammalian cells by interaction with the enzyme rather than with the DNA template[2,3]. No influence of the toxin on the replication of a number of DNA or RNA-containing viruses has been found so far (ref. 4 and personal communication from L. Philipson). Actinomycin, which reacts with the DNA template and so impairs the DNA-dependent RNA polymerase reaction, depresses the multiplication of influenza viruses but does not interfere with the replication of most of the other RNA-containing viruses[5–7]. Because the mode of action of this antibiotic on the synthesis of influenza viruses is still obscure, we extended our studies to the action of α-amanitin on the multiplication of this virus group.

As a model system for influenza virus the fowl plague virus (FPV) strain "Rostock" and, as a control, a parainfluenza virus (Newcastle disease virus, NDV) were propagated in chick fibroblasts. The plaque test and the determinations of viral neuraminidase, haemagglutinin and the inner component (RNP-antigen) were performed according to standard methods. Viral plus and minus strand RNA were determined by the hybridization technique[8]. Viral RNA polymerase activity was assayed in a cytoplasmic extract prepared 5 h after infection[9].

FPV multiplication is inhibited completely by 50 μg/ml. of α-amanitin, whereas NDV multiplication is not affected (Table 1). FPV replication is even more efficiently blocked when the inhibitor is added to the cultures 2–3 h before infection. This holds true not only for the infectivity, but also for the amount of haemagglutinin, neuraminidase and RNP-antigen (Table 2).

Table 1. DOSE EFFECT OF α-AMANITIN ON THE YIELD OF FPV AND NDV

α-Amanitin (μg/ml.)	HA units		P.f.u. $\times 10^{-5}$	
	FPV	NDV	FPV	NDV
0	256	32	110	140
1	224	32	110	250
10	32	32	14	150
25	4	32	8	—
50	< 2	32	2	—
100	< 2	32	1·9	150

The toxin was added immediately after infection. The cell-associated virus-specific activities were determined 8 h after infection after breaking the cell by freezing and thawing three times.

Table 2. EFFECT OF α-AMANITIN ON THE SYNTHESIS OF VIRUS-SPECIFIC RNA *in vivo*, AND ON THE PRODUCTION OF OTHER VIRAL COMPONENTS

	No α-amanitin	25 μg/ml. α-amanitin added 2·5 h before infection
Radioactivity (d.p.m.) in:		
Total RNA	71,500	34,000
Plus strand	5,700	1,700
Minus strand	4,300	320
Cell-associated parameter:		
HA units	128	1
P.f.u. $\times 10^{-5}$	33	1·6
RNP-antigen titre	64	4
Neuraminidase units	520	38

To each of three FPV-infected cell cultures 100 μCi of ^{3}H-uridine was added 2·5 h after infection. When α-amanitin was investigated, 200 μCi ^{3}H-uridine was investigated. Total RNA was extracted by sodium dodecyl sulphate plus phenol 3·5 h after infection. Viral plus and minus strand RNA was determined by hybridization[9].

Up to 50 μg/ml. of α-amanitin had no influence on the incorporation of ^{3}H-uridine or ^{3}H-leucine into the corresponding macromolecules of uninfected cells, when the isotopes and the toxin were administered simultaneously. If the pulse was given 3 h after the addition of α-amanitin there was also no significant effect. Six hours after addition of the inhibitor, however, the uptake and phosphorylation of ^{3}H-uridine and RNA and protein synthesis reached only about 50 per cent of the values for untreated cells.

The results in Table 2 demonstrate that a substantial amount of viral plus strand RNA is still being synthesized in conditions when the plaque test indicates a twenty-fold inhibition of FPV multiplication. The production of viral minus strand RNA, however, is barely measurable. A similar result has been obtained recently using mithramycin as an inhibitor of RNA synthesis[10]. FPV-specific RNA polymerase, which synthesizes chiefly minus strand RNA[11] *in vitro*, was not inhibited by 180 μg/ml. α-amanitin. There is no measurable inactivation of FPV by incubating the virus for 16 h with 60 μg/ml. α-amanitin at 4° C.

The results indicate that α-amanitin inhibits specifically influenza virus replication without interfering significantly with the overall synthesis of RNA and protein in the host cell. The delayed effect on these activities might be rather a non-specific toxic alteration of the metabolism in our cell system, which is essentially different from the systems used by others[2,3]. At this time the multiplication cycle of FPV is in any case completed, so that it is not very probable that α-amanitin inhibits influenza virus multiplication by interfering with cellular RNA synthesis as has been discussed for the action of actinomycin[5,7]. Because much viral RNA is still being synthesized in the presence of α-amanitin, it could be that this inhibitor also affects some step of the translation of viral RNA.

We thank Professor Th. Wieland of the Max-Planck-Institut für Medizinische Forschung, Heidelberg, for a gift of α-amanitin, and Miss H. Krombach and Miss B. Piontek for technical assistance. The work was supported by the Deutsche Forschungsgemeinschaft.

R. ROTT
C. SCHOLTISSEK

Institut für Virologie,
Justus Liebig-Universität,
Giessen.

Received February 20, 1970.

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