It is also conceivable that the groups with adult social experience were sexually exhausted due to socially induced autosexual or homosexual activities. This possibility cannot be entirely discounted since the latency to spermatophore production in non-copulating group-reared males (Group IV) was significantly higher (Mann-Whitney U $p < 0.04$) than that in the group deprived after adulthood was attained (Group III). However, there was very little difference between the group deprived as nymphs (Group II) and the group deprived in the adult stage (Group III) in this regard. Thus sexual exhaustion does not entirely account for the low performance of all males with social experience as adults.

An alternate explanation to the aforementioned possibilities is that social deprivation increases excitability with reference to both sexual behavior and aggression. Furthermore, such excitability may have a demonstrable physiological basis. Social deprivation has been shown, in aphids, to lead to hyperactivity of the corpora allata[8], which have important physiological functions. The c. allatal hormone stimulates male accessory gland activity and influences behavior in a number of insects[9]. Although these specific mechanisms may not operate in house crickets, their physiology may be modified by social deprivation in a somewhat similar manner. It is proposed that this modification of the physiology involves a lowering of threshold to sexual excitability in singly-reared male house crickets.

In summary, it is concluded that visual and tactual social deprivation from the egg stage enhanced the likelihood of copulation in 2- or 3-day adult male *Acheta domestica* when reared at 34 ± 1 °C.

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Enhanced Inhibition of RNA Synthesis by Amanitins in in vitro Cultured Cells

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Summary. The inhibition of RNA synthesis by α-amanitin on in vitro cultured cells is very slow. The action of various analogues of the toxin was tested and some of them proved more effective. Moreover pretreatment of cell cultures with DEAE-dextran greatly enhanced the effect of β-amanitin.

α-Amanitin is a toxic polypeptide of *Amanita phalloides*. It is a useful tool in biology and biochemistry for its ability to inhibit RNA-polymerase B selectively by binding to the enzyme[2–4].

α-Amanitin brings about a very early inhibition of RNA synthesis in liver and kidney when administered to animals[5,6], but it acts very slowly on in vitro cultured cells[7]. This slow action is probably due to a scarce penetration. This hypothesis receives support from 2 observations. Different types of in vitro cultured cells display different sensitivity to α-amanitin, while RNA synthesis in isolated nuclei is inhibited by equal doses of the drug[8]. Furthermore, the toxicity of amanitin for cultured macrophages increases several times after conjugation of the toxin to albumin which allows amanitin to enter by pinocytosis[9].

A rapid inhibition of the amanitin-sensitive RNA-polymerase B of cultured cells would be required, especially in studies on replication of DNA viruses, in order to try to discriminate between viral and host cell transcription. With the aim at obtaining this effect, we performed two series of experiments on cell cultures. In the first series we examined the effect of a number of naturally occurring and chemically modified amanitins, all known to inhibit RNA-polymerase B in vitro[10–12], in order to compare their transcription-blocking activity with that of α-amanitin. In the second series of experiments we tried to increase amanitin penetration by treating cells with DEAE-dextran, a polycation which is known for its enhancing effect on penetration of several substances into cultured cells. We also tested the effect of the polyenic antifungal antibiotic amphotericin B, which had been reported to facilitate the penetration of α-amanitin[13].

Materials and methods. HEp-2 and BHK cell monolayers were grown for 36 h in 35 mm plastic Petri dishes in Eagle's minimum essential medium (MEM) supplemented with 10% foetal bovine serum. Amanitins were added to the medium at the concentration of 10 or 30 μg/ml, as indicated in the Tables I and II.

To perform pretreatment with DEAE-dextran, monolayers were washed twice with phosphate buffered saline lacking Ca^{++} and Mg^{++} (PBS A), kept in contact with 2 ml/dish of a solution containing 500 μg/ml DEAE-dextran (m.w. 2×10^6, Pharmacia) and 1 mg/ml glucose in PBS A for 15 min at 37 °C, washed 3 times with PBS A and refed with medium. Amphotericin B (Squibb) was dissolved in dimethylsulphoxide (10 mg/ml) and further diluted with the medium.

RNA synthesis was measured by pulse-labelling the monolayers with (^{3}H) uridine (29 Ci/m*M*; the Radiochemical Center, Amersham) at the concentration of 1 μCi/ml of medium in the presence of a 20-fold excess of cold thymidine. After a 30 min pulse, monolayers were

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Table I. Effect of various amanitins on RNA synthesis of HEp-2 cells

	Concentration (μg/ml)	Time of contact (h)	RNA synthesis (cpm/mg protein)
Control	0	6	44602 ± 2244
α-Amanitin	10	6	38844 ± 1873 (13)
β-Amanitin	10	6	41108 ± 684 (8)
Amanin	10	6	39070 ± 670 (12)
Amanitin-tiophenyl ester	10	6	35235 ± 1368 (21)
α-Amanitin-azotrifluoracetic	10	6	37990 ± 837[a] (15)
O-Methyl-γ-amanitin	10	6	30910 ± 1200[a] (31)
Control	0	2	46125 ± 2675
O-Methyl-γ-amanitin	10	2	32008 ± 1628[a] (31)
O-Methyl-γ-amanitin	30	2	29670 ± 2542[a] (36)
O-Methyl-γ-amanitin	50	2	27090 ± 2477[a] (41)

α-Amanitin, β-amanitin and amanin are naturally occurring amanitins[3]. Amanitin-tiophenylester[16], α-amanitin-azo-trifluoroacetic[11] and *O*-methyl-γ-amanitin[12] were prepared in the laboratory of Professor TH. WIELAND. Results are presented as means of 4 values ± SE, with Student's *t*-test for significance of difference between means. [a] $P < 0.05$ in comparison with the control. Percentage of inhibition in parentheses.

Table II. Effect of pretreatment with DEAE-dextran and glucose (Dex) on RNA synthesis of HEp-2 cells treated with α- or β-amanitin

Treatment	Concentration of amanitin (μg/ml)	RNA synthesis (cpm/mg protein)		
		1 h[a]	2 h[a]	12 h[a]
None	0	41137 ± 1336 ($n = 3$)	42569 ± 569 ($n = 8$)	37054 ± 3995 ($n = 2$)
α-Amanitin	30		40448 ± 2431 (5) ($n = 4$)	
β-Amanitin	10		40866 ± 2785 (4) ($n = 4$)	
β-Amanitin	30	41548 ± 2280 ($n = 4$)	37748 ± 3454 (11) ($n = 4$)	
Dex	0	45521 ± 2364 ($n = 4$)	45514 ± 3541 ($n = 4$)	41764 ± 1848 ($n = 2$)
Dex + α-amanitin	30		15342 ± 1104[b] (64) ($n = 4$)	
Dex + β-amanitin	10		17954 ± 2247[b] (58) ($n = 2$)	
Dex + β-amanitin	30	22488 ± 2469[b] (45) ($n = 4$)	13234 ± 946[b] (69) ($n = 7$)	5634 ± 994[b] (8) ($n = 2$)

[a] Time of contact with amanitin (h). Results are presented as means ± SE, with Student's *t*-test for significance of difference between means. [b] $P < 0.02$. n = number of specimens. In parentheses, percentage of inhibition with respect to control cultures.

washed twice with 0.01 *M Tris* buffered saline, pH 7.5, harvested in the same buffer, precipitated with cold 10% $HClO_4$, filtered through glass fibre discs (Whatman GF/C) and the radioactivity was measured in a Packard Tri-carb liquid scintillation spectrometer. Proteins were determined according to the method of LOWRY et al.[14]. Cells viability was measured by a trypan blue exclusion test[15].

Results and discussion. A comparative study of the levels of inhibition of RNA synthesis in HEp-2 cells (Table I) shows that some amanitin derivatives are more effective than α-amanitin. A significant reduction was obtained with the chemically modified compounds α-amanitin-azo-trifluoracetic[11] and *O*-methyl-γ-amanitin[12]; this last analogue gave the strongest reduction.

To accelerate the effect of amanitins, we treated cell cultures with Amphothericin B or DEAE-dextran. Amphothericin B alone, at the concentration of 2 μg/ml, caused a 70% inhibition of HEp-2 cells RNA synthesis after a 6 h contact (only 10% was due to the solvent dimethylsulphoxide); this inhibition did not increase appreciably when α-amanitin was added to cell cultures together with Amphothericin B.

In the experiments with DEAE-dextran, we used a concentration of 500 μg/ml, according to BARBANTI-BRODANO et al.[17], since lower concentrations were found to be much less effective (data not shown). Pretreatment with this dose for 30 min proved slightly toxic in our conditions, as revealed by RNA synthetic activity, viability test and morphological examination. We found that this toxicity was totally abolished by shortening the time of pretreatment to 15 min and by adding 1 mg/ml glucose (Table II). Table II also shows that subsequent to the treatment with DEAE-dextran β-amanitin, which has a free carboxylic group[3] is more active than the amido-substituted analogue α-amanitin.

A very similar effect was obtained also in another cell line. Pretreatment of BHK[21] cell monolayers with DEAE-dextran led, upon addition of 30 μg/ml β-amanitin, to a 66% inhibition of total RNA synthesis within 2 h.

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