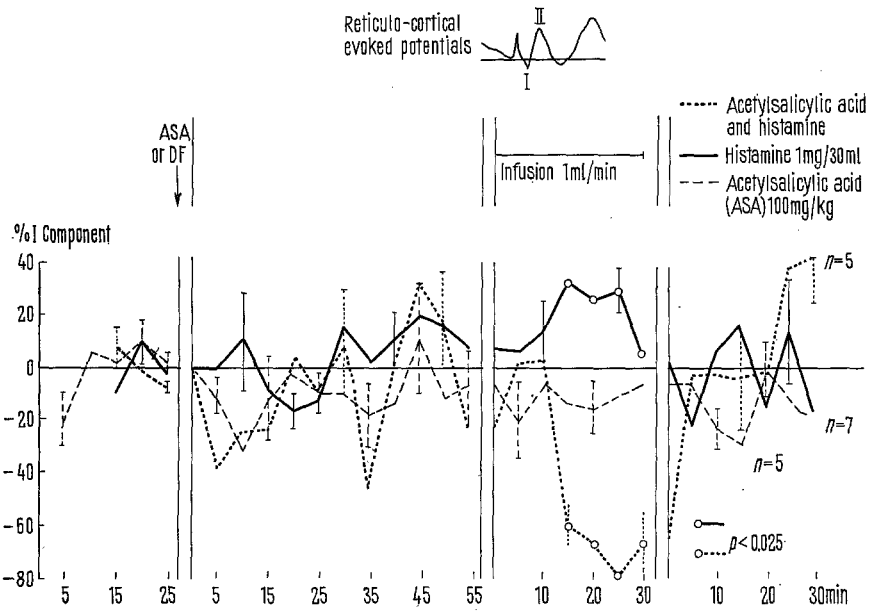

Fig. 2. The reticulo-cortical evoked potentials, enhanced by i.v. histamine (——), are markedly *depressed by peroral analgesia* with acetyl salicylic acid (. . . . .). Infusion of this analgesic alone does not alter the previous evoked cortical activities.

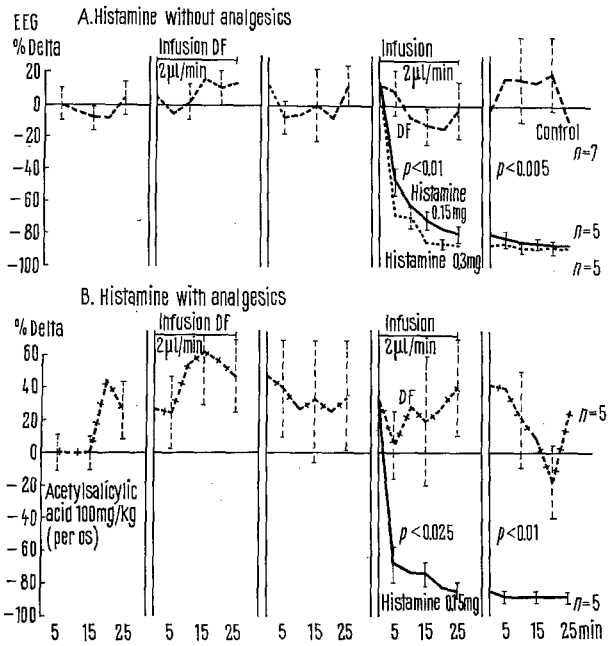

Fig. 3. The *waking effect* of intraventricular histamine (decreased delta activities in A) is *not abolished by analgesia* with acetyl salicylic acid per os in B. It is not due to reflex stimulation (pain), but to direct stimulation of activating brain centres.

di-HCl in 0.05 ml tyrode-like fluid of adjusted osmolarity during 30 min into the third ventricle of the rabbit.

Here again histamine induced a *marked EEG arousal reaction* with decreased delta activities. This waking effect is not of peripheral reflex origin; it is not due to painful afferents since it is not abolished by analgesia with acetyl salicyclic acid (Figure 3). *The activating effect of intraventricular histamine has a central origin*, due to direct stimulation of the surrounding reticulo-thalamic activating systems.

In *conclusion*, the waking effect of *intravenous histamine* (which does not pass the blood-brain barrier) must be explained reflexly by visceral afferents stimulating chiefly the reticular activating system and, to some extent also, the thalamo-cortical and hippocampal activating mechanisms. Part of the afferents are nociceptive pain inducing fibres, since their waking action is markedly reduced by analgesia.

The waking effect of *intraventricular histamine* infused into the third ventricle is central, and not attributable to pain, since it is not suppressed by analgesia[7].

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Effect of Dimethyl Sulfoxide on RNA Synthesis in S-180 Tumor Cells

Dimethyl sulfoxide (DMSO) has been found to affect a number of biological processes, both in vitro and in vivo. Concentrations of DMSO greater than 10% have been shown to inhibit protein synthesis in bone marrow cells in vitro[1]. This inhibition was found to be readily reversible by removal of the drug. NIEUWEBOER (in [2]) found that incorporation of leucine into the 10,000 *g* supernatant of rat liver homogenate was stimulated by 5–10% DMSO, but was inhibited at higher concentrations. ARCHER et al.[3] reported that DMSO caused dilatio

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of the endoplasmic reticulum and diminution of ribosomes in rat fibroblasts. The drug has also been shown to inhibit cell proliferation in in vitro cultures of fibroblasts and bacteria[4,5]. In related studies, DMSO was found to reversibly inhibit amino acid transport[6] and DNA synthesis[7] in tumor cells, and also to inhibit thyroidal transport and organification of iodine[8]. In addition, it has been reported to be teratogenic in hamsters[9] and to increase fetal resorption in rats[10].

The mechanism of DMSO inhibition of certain biosynthetic and physiological processes remains obscure. The aprotic nature of the compound may facilitate reversible binding to macromolecules (probably by hydogen bonding) and may hinder substrate-active site interactions, however, it should be noted that the activity of certain enzymes may be increased by DMSO[11,12]. The present report describes the reversible inhibition of RNA synthesis by DMSO.

Sarcoma-180 ascites tumor cells (a gift of Dr. Titus C. Evans, University of Iowa) were maintained in male Ha/ICR mice (Schmidt) by weekly i.p. injection of 5×10^6 cells. Incorporation of tritiated uridine (^{3}HUR), specific activity 20 Ci/mM, was used to quantitate RNA synthesis. 1 ml of packed tumor cells was incubated in 20 ml of Tyrode's solution containing 5μCi ^{3}HUR and 0, 5, 10 or 15% DMSO, in a water bath shaker at 37 °C. After various incubation times, an aliquot of cell suspension was removed and spun in a refrigerated centrifuge for 1 min. 5% trichloracetic acid (TCA) was added and the samples were stored in an ice bath for 1 h to remove unincorporated ^{3}HUR. Two additional washes with iced 5% TCA ensued. The samples were weighed and solubilized in Soluene (Packard Inst. Co.). An aliquot of the digest was placed in a counting vial and 10 ml of scintillator (containing 5 g PPO and 0.2 g POPOP/l toluene) was added. The samples were counted in a liquid scintillation spectrometer (Packard) utilizing an Absolute Activity Analyzer (Packard) for quench correction. The data was expressed as disintegrations per min (dpm)/g wet weight of packed cells. Additional experiments included provision of a second 5 μCi ^{3}HUR after 10 min incubation, and incubation in 15% DMSO for 8 min, washing the cells 2 times with Tyrode's, and resuming the incubation in fresh medium without DMSO.

The effect of incubation in various concentrations of DMSO on RNA synthesis is shown in Figure 1. Each point represents the mean of from 3–5 experiments and the uncertainties are the standard errors of the mean. To determine if the decreased slope of the control curve after about 10 min incubation was due to depletion of precursor in the medium, a second 5 μCi ^{3}HUR was added at this time (dotted line). The original slope of the control curve was re-established. The inhibitory action of various concentrations of DMSO on RNA synthesis is apparent. Incubation with 15% DMSO reduced the incorporation rate to about 7% of control levels.

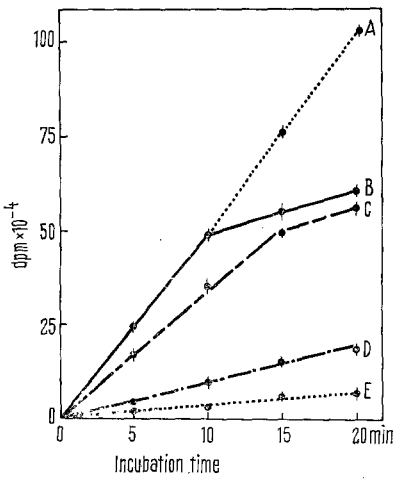

Fig. 1. Uridine uptake as a function of incubation time. Curve (A) Control, additional ^{3}HUR added at 10 min (B) Control, no additional ^{3}HUR, (C) 5%, (D) 10%, and (E) 15% DMSO.

The inhibitory action of the drug was found to be readily reversible (Figure 2). After incubation in 15% DMSO for 8 min, and subsequent removal of the drug by washing the cells in fresh Tyrode's (arrows), RNA synthesis proceeded at a slightly higher rate than in the control cells.

It was considered salient to compare the relative inhibitory action of DMSO on RNA and DNA[7] synthesis (Table). The data in all cases were taken from the linear

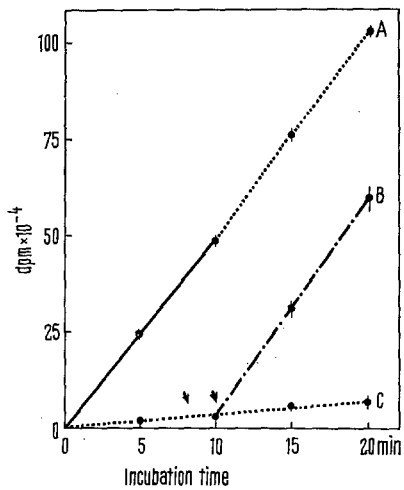

Fig. 2. Reversibility of inhibition. (A) Control (from Figure 1). (B) Incubated in 15% DMSO for 8 min, washed (arrows), incubated in fresh medium. (C) Incubated in 15% DMSO.

Nucleic acid synthesis (% control)

Nucleic acid	DMSO concentration: 0%	5%	10%	15%	15%, washed
RNA	100	71	19.5	6.9	122
DNA[a]	100	64	43	7.6	160

[a] From HAGEMANN and EVANS[7].

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portion of the synthesis curves (RNA: first 10 min incubation, DNA: 15 min). Although RNA synthesis was significantly more depressed in 10% DMSO than was DNA ($p < 0.001$), the inhibition of nucleic acid synthesis, in general, did not appear to be selective. It is of interest to note that, in both cases, removal of the drug by washing resulted in a significant overshoot in synthetic activity.

An etiologic relationship between the results presented herein, and previous studies on RNA-dependent processes has not been established. Where the inhibitory effect of DMSO is acute, it is doubtful if RNA mediation plays an important role. Conversely, these data may relate to the mechanism of DMSO action on cell proliferation, such as the reversible inhibition of fibroblast growth in culture[4], its effect on fetal development in vivo[9,10], and its ultrastructural effects on ribosomes[3].

Zusammenfassung. Es hat sich gezeigt, dass Dimethyl-Sulfoxid (DMSO) die RNA-Synthese in Sarkom 180 Tumorzellen in vitro hemmt. In 15% DMSO betrug die Syntheserate nur 7% der Kontrolle. Diese Hemmung war leicht reversibel durch einfaches Entfernen des Wirkstoffes durch Auswaschen der Zellen mit Tyrodes-Lösung. Nach Entfernung des DMSO wurde eine leicht erhöhte Aktivität der Synthese beobachtet. Die Angaben wurden mit der Kinetik der Hemmung von DNA-Synthese verglichen und ihre möglichen Beziehungen zu anderen Wirkungen von DMSO beschrieben.

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Antitumor Activity of an Aqueous Extract of *Amanita phalloides* Fr.

It was previously shown, by electron microscopic examination, that the earliest evidence of a structural damage caused by non-lethal doses of the total extract of *Amanita phalloides* Fr. occurs in the nucleus and nucleolus, and that it is in these structures that the changes begin to regress; the signs of damage in the cytoplasm and its regression occur after nuclear changes[1,2]. Since these lesions seem to indicate that the *Amanita phalloides* extract interferes with the metabolism of the nucleic acids, experiments were performed to determine if such an extract might have an antitumour activity in rats.

Materials and methods. Wistar strain albino rats of both sexes, weighing about 200 g were used. Yoshida ascites tumour AH 130 was inoculated i.p. (0.3 ml of a liquid containing about 15×10^3 tumour cells in 1 mm^3). An aqueous extract of fresh mushrooms was prepared. The material previously lyophilized was diluted with saline just before use. This solution was injected i.p. (1.5 mg/200 g of body wt.). Preliminary controls had shown that this dose was not lethal for rats. In control animals the same amount of saline was given.

The action of pure α-amanitine (0.5 mg/200 g i.p.) and of an extract enriched in phalloidine (30% of phalloidine) were also tested. These substances were kindly supplied by Prof. TH. WIELAND from Heidelberg. The ascites tumour cells were also examined after incubation in vitro at 37 °C respectively with total mushroom extract, pure α-amanitine and phalloidine extract.

Experimental. 1st Group. 7 rats in which the ascites tumour cells had been inoculated 2 days before, were injected with *Amanita phalloides* extract. All these animals died 24 h later.

2nd Group. 19 rats were inoculated with ascites tumour cells. In 10 of these animals the aqueous extract of *Amanita phalloides* was injected at the same time. The 9 control animals died in 2 weeks with ascites. None of the treated animals died, nor did they develop ascites.

3rd Group. Reduced quantities of the *Amanita phalloides* extract were given in different groups of 3 rats each. The inhibitory activity of the extract during the transplantation of the tumour was present with doses of 0.75, 0.5 and 0.3 mg. When 0.15 mg of *Amanita phalloides* extract was given, an ascites developed, but 2 days later than the controls.

4th Group. 9 rats, in which the *Amanita phalloides* extract inhibited the growth of the tumour, were inoculated again after 10–15 days with the same dose of tumour cells. The animals did not develop ascites.

5th Group. When the tumour cells were inoculated with pure α-amanitine, the rats showed a cutaneous toxic reaction and all developed ascites and died. The inoculation of an extract enriched in phalloidine in 12 rats inhibited the growth of the tumour in 4 animals. The other 8 rats died with ascites.

6th Group. Experiments in vitro: (a) Controls. After 24 h of incubation at 37 °C most of the tumour cells were still well preserved. (b) Incubation with *Amanita phalloides* extract. After 1 h of incubation, phenomena of nuclear picnosis and initial alterations of the cytoplasm were already present. After 6 h the destruction of the tumour cells was very pronounced and it was complete after 24 h. At this time the normal cells (plasmacells, mesothelial cells, etc.) were still preserved. (c) Incubation with pure α-amanitine. No differences were noted between the cells of this series and the control ones after 1 h, 6 h and 24 h. (d) Incubation with an extract enriched in phalloidine. After 24 h numerous tumour cells were still present and well preserved. However, some cells showed initial signs of nuclear and cytoplasmatic damage. It was observed that only a few cells were completely destroyed.

Discussion. These experiments show that an aqueous extract of *Amanita phalloides* at certain doses inhibits the implantation of the ascites tumour cells; whereas it has no antitumoral activity when given after the development of the tumor. The rats in which the *Amanita phalloides* extract has inhibited the tumour do not develop the tumour even when a second inoculation is performed. This seems attributable to a phenomenon of active immunization, similar to what happens with other experimental techniques (cryolization[3] or irradiation with betatron[4]). The antitumoral activity does not seem linked

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