mycin' syrup (Pfizer Italiana, S. p. A., Italy) was used for oral administration in doses containing 75 mg oxytetracycline activity per kg rabbit weight.

Results and discussion. The figure summarizes the various feeding experiments. Flies of series 1, first fed on an i.p.-injected rabbit, were affected within a few hours. Of the 103 flies (4 replicates), 11 were moribund 3–5 h after engorgement, and 70 flies were dead within 48 h. A less drastic effect was observed in series 2 (55 flies), treated on the fifth day of their lives; only 2 died on the day of treatment. In older flies, the reaction of the drug was much more delayed and moderate. In series 3 (85 flies in 3 replicates), the rise in the rate of mortality that followed treatment on the 15th day was slow, reaching the total of 49 dead flies 17 days later. The mortality in all three series was much higher than that of controls (95, 3 replicates, $p < 0.005$ calculated for day 5 of series 1, day 7 series 2 and day 17 after treatment for series 3). Because of lower absorption and partial excretion of the drug following oral administration, flies of series 4, fed one day after eclosion, reacted less incisively than series 1 (96 in 4 replicates; 46 dead by day 10, $p < 0.005$). Pupae, progeny of the fed flies, were collected and were found to be of low vitality. Only 17% of 71 lived for more than a single week. (No normal control pupae were collected for comparison.) Flies moribund following treatment and untreated flies were examined histologically. Most of the mycetome cells in treated flies were full of dead, elongate and dark brown, bacterioids, in sharp contrast to the blue staining (in Mayer's haemalum) of all the bacterioids in control flies. The affected mycetomes were markedly vacuolated and in different stages of disintegration. The borders of the gut were disrupted both towards the lumen and the haemocel. Brown bacterioids were also dispersed in the haemocel. These histological observations indicate that death of the flies was apparently effected by destruction of the mycetome system, although a direct toxic effect of the tetracycline cannot be excluded.

It has been suggested that sulfaquinoxaline, which impairs the fecundity of tsetse flies, could be considered for control of tsetse flies in the wild[8,9]. The results of laboratory experiments presented here show that the tetracyclines should also be viewed as potential tools for this control. The drug could be added to the fodder or drinking water of domestic animals. It is nonpoisonous and should, in the right dosage, kill tsetse flies selectively and quickly.

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Effect of L-amino-ethyl-cysteine, a sulfur analogue of L-lysine, on virus multiplication in mammalian cell cultures[1]

A. Scioscia-Santoro*, D. Cavallini*, Anna M. Degener, R. Perez-Bercoff and G. Rita

Institute of Biological Chemistry and Institute of Virology, University of Rome, viale di Ponte Tuburtina 28, I–Roma (Italy), 12 August 1976*

Summary. L-amino-ethyl-cysteine clearly inhibits replication of Mengovirus; another RNA-virus (vesicular stomatitis virus) is completely insensitive. Protein synthesis is not impaired, but no active viral RNA-polymerase is detected.

In previous studies, we have looked for the action of a 4-sulfur analogue of L-lysine (AEC) on the metabolism of mammalian cell cultures[2]. In this paper we report the effects of AEC on the replication cycle of Mengovirus.

Materials and methods. L929 cells were grown at 37 °C in Eagle's minimal essential medium (MEM) containing 8% of calf serum (CS). Mengovirus and Vesicular Stomatitis Virus (VSV) were replicated on L929 cells. Titers of stocks viruses: Mengovirus, 3.8×10^9 PFU/ml; VSV, 2.7×10^9 PFU/ml. [^{3}H]-Uridine (specific activity 27 Ci/mmole) and [^{14}C]-protein hydrolysate (specific activity 50 mCi/matom) were obtained from the Radiochemical Centre, Amersham. Actinomycin D was purchased from Sigma. S-(β-aminoethyl)-L-cysteine HCl was synthesized through the condensation of L-cysteine and β-bromoethylamine by the method of Cavallini et al.[3]

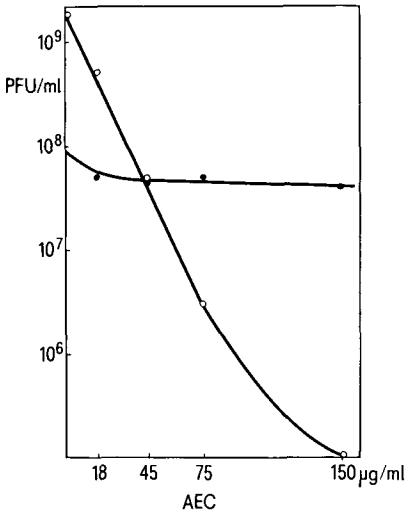

Fig. 1. Replication of Mengovirus (○) and VSV (●) in presence of AEC.

Results. 1. Dose-response curve. Barely confluent monolayer of L cells in 60 mm plastic dishes were treated with different concentrations of AEC 24 h before infection. After removal of the media, cultures were infected with Mengovirus (30 PFU/cell). After incubation for 1 h at 37 °C, the monolayers were carefully washed 3 times with PBS. 5 ml of fresh medium containing the appropriate amount of AEC were added to each plastic dishes and incubation at 37 °C was continued for 24 h. The cultures were frozen and thawed 4 times to release the intracellular virus, and after low-speed centrifugation the virus in the clarified supernatants was titrated by a plaque assay.

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Figure 1 shows that at 18 μg/ml (a concentration which has no effect on the host cell)[2], there is a 75% reduction in the virus yield. Moreover, a linear relationship between the concentration of AEC and the reduction of the virus production is found. To rule out the possibility that this reduction was caused by a toxic effect on the host cell, the same experiment was repeated using VSV (20 PFU/cell). The virus was titrated following the procedures given above and the results are shown in the same figure 1. It is clear that AEC has no effect on VSV replication and that concentrations completely harmless to VSV resulted in an almost complete inhibition of Mengovirus replication.

2. Reversibility and time-course of Mengovirus sensitivity to AEC. The following experiment was devised to determine whether or not the action of AEC was confined to a particular period during the replication cycle of Mengovirus. For these experiments we used a higher dose, because in the short life-time of virus this dose was ineffective on the cellular macromolecular synthesis[2]. L-cells were infected with Mengovirus (10 PFU/cell) and adsorption was allowed to proceed at 37 °C during 1 h. The cells then were carefully washed 3 times with PBS and the cultures replenished with 5 ml of fresh prewarmed MEM. At different times thereafter 300 μg/ml of AEC were added and incubation proceeded for a total of 10 h (the end of the adsorption period being time 0). The virus yield was determined by plaque formation. No differences were observed in the effect of AEC added immediately before incubation or as late as 6 h after the end of the adsorption period; in every case the inhibition was greater than 90% of the untreated controls. To look for the reversibility of this effect, Mengovirus-infected L-cells were incubated with fresh medium, and AEC (300 μg/ml) was added and removed after 2 h, the 'pulse-period' beginning at 0.2 and 4 h after the end of the adsorption. Once again, no difference was observed in the inhibitory effect of AEC, which in all cases was greater than 90%. These results indicate that, on one hand, the action of AEC is irreversible and an exposure of the infected cultures (as short as 2 h) is sufficient to prevent Mengovirus multiplication while, on the other hand, they suggest that the target of AEC is either a very late step in the replication cycle of Mengovirus or, alternatively, that the compound can indiscriminately interfere with virus-directed synthesis.

3. AEC and virus-directed synthesis. In view of these findings, it was of interest to analyze the effect of AEC on the virus-induced protein, RNA and enzyme synthesis. a) L-cells from confluent monolayers were detached with trypsin, suspended in MEM (Spinner modified) containing 4 μg/ml of Actinomycin D and incubated at 37 °C for 1 h with stirring. Cells collected by low-speed centrifugation were infected in a small volume with Mengovirus (30 PFU/cell), and virus adsorption was allowed to proceed for 1 h at 0 °C. Warmed medium was added to the cells in order to have a suspension containing 5×10^6 cells/ml. [^{14}C]-labelled protein hydrolysate was added to 0.6 μCi/ml. The infected cell suspension was divided into 2 portions and to one 300 μg/ml of AEC was added. During incubation at 37 °C under constant agitation, aliquots were removed at different time intervals and precipitated with an equal volume of ice-cold 10% TCA. After 20 min in ice, the precipitates were collected by centrifugation, suspended in 0.1 M NaOH, heated at 60 °C for 30 min, cooled, precipitated with 5% TCA and collected on GF/C filters. This procedure was followed to eliminate the spurious acid-precipitable radioactivity due to amino-acyl-charged tRNAs. As shown in figure 2b, AEC has almost no effect on the virus-directed protein synthesis. Of course, this type of experiment gives no indication of the *quality* and the processing of the proteins synthesized in the presence of the analogue, and cannot discriminate between biologically active or altered, functionally unactive, proteins. b) Since the synthesis of viral RNA is a virus-directed event, requiring at the very least the synthesis of an active, viral-coded RNA-replicase, an analysis of the pattern of viral RNA-synthesis can be more useful to understand the mechanism of the action of AEC. For this purpose, L-cells from confluent monolayers were trypsinized and suspended, cell-directed RNA-synthesis was inhibited with Actinomycin D and cells were infected as described above. The cells were diluted to 6×10^6 cells/ml with prewarmed media, and [^{3}H]-uridine was added to a final concentration of 10 μCi/ml. To one half of the suspension AEC was added to a final concentration of 300 μg/ml. Incubation proceeded at 37 °C under constant stirring and at different time intervals the amount of TCA precipitable radioactivity was determined. Figure 2a shows that in the presence of AEC, Mengovirus-directed RNA-synthesis proceeds as in control for the first $2^1/_2$–3 h, that is, during the logarithmic phase of RNA-synthesis. Then a dramatic change in the time course of synthesis is evident. Thus, while the synthesis of viral RNA in the control switches on to the linear phase, the synthesis in the presence of AEC stops.

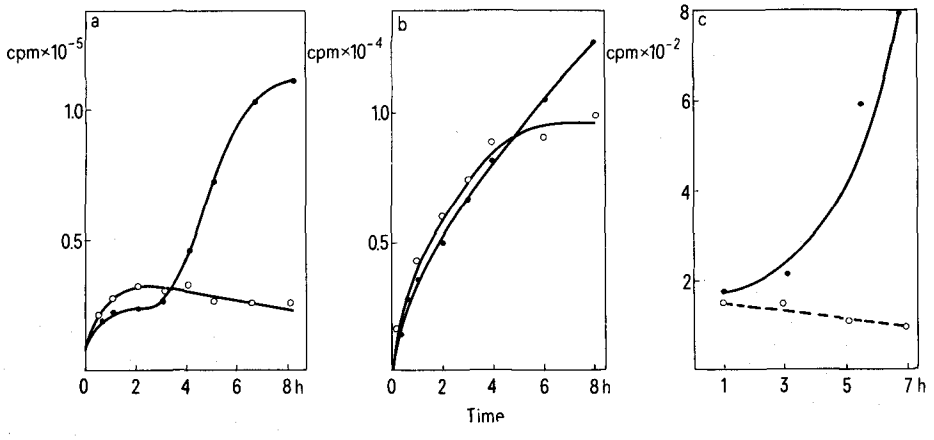

Fig. 2. *a* Mengovirus directed RNA-synthesis in infected cells in growth medium (●) and in growth medium plus 300 μg AEC/ml (○); *b* Mengovirus directed protein synthesis in infected cells in growth medium (●) and in growth medium plus 300 μg AEC/ml (○); *c* Activity of Mengovirus RNA-polymerase contained in infected cells in growth medium (●) and in growth medium plus 300 μg AEC/ml (○).

c) Synthesis of the virus-induced RNA-replicase. L-cells, treated and infected as described above in section b, were collected at 2-h-intervals, and the amount of viral RNA-polymerase in the post-mitochondrial supernatant was extracted and the activity was tested according to Baltimore and Franklin[4-6], using as a marker [^{14}C]-UTP. Figure 2c shows that, in the presence of AEC, the activity of viral RNA-polymerase contained in Mengovirus-infected cells is not detectable.

Discussion. The results presented in this paper clearly show that the amino-acid analogue can efficiently block the replication of Mengovirus. At the same concentration AEC has no effect on the multiplication of another (although rather different) RNA-virus, replicating in the same host cell. This inhibitory effect is not due merely to a toxic effect on the host cell (see figure 1). 2 other considerations give additional support to this conclusion: a) a drastic reduction in virus yield is obtained when AEC is added to the incubation mixture just after infection, a time too short to produce a detectable effect on the host cell multiplication[2]; b) the lowest concentration of AEC tested (18 µg/ml) resulted in a reduction of 75% of the virus yield, whereas a 10-fold greater amount of AEC, continuously present in the culture medium for 48 h, has no effect on the cell's growth[2]. Viral protein synthesis seems to be less quantitatively impaired by AEC (see figure 2b); but no *active* viral RNA polymerase was detected in the cytoplasm of Mengovirus-infected AEC-treated cells (figure 2c). This result and the pattern of the viral RNA-synthesis in the presence of AEC strongly suggest that the virus-coded polymerase responsible for the *late* synthesis of virus RNA might be the target of this lysine analogue. This point is of great interest because the mechanism responsible for the change in the rate of virus RNA-synthesis from the logarithmic to the linear phase is at present unknown. Analysis of the RNA-structures and the polarity of the chains synthesized in the presence of the compound may help to clarify the issue. Work along these lines is in progress.

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Water, a powerful attractant for the gravid females of Plodia interpunctella and Cadra cautella

Y. S. Chow, D. F. Yen and S. H. Lin

Institute of Zoology, Academia Sinica, Taipei (Taiwan), and Division of Life Sciences, National Science Council, Taipei (Taiwan), 27 September 1976

Summary. In the sex pheromone controlling experiments, not only large number of the males of the almond moth and Indian meal moth, but also great number of ovipositional females were caught. The increased catch of the fertilized females was due to the presence of detergent in the water.

The almond moth Cadra cautella and the Indian meal moth Plodia interpunctella are serious grain pests in storage house in Taiwan. Their biology and physiological ecology have been reported[1-6]. Although modern controlling techniques, such as sterile male release[7], sex pheromone[8] and juvenile hormone [9,10] have been studied, no practical way of controlling the moths is known. In our sex pheromone attraction experiments, when the female sex pheromones were used in combination with water solvent and soap powder, a great number of males and fertilized females of these 2 species[11] were caught. It is hoped that if we can eliminate large numbers of the females by using soap water in combination with sex pheromone, eventually these 2 species could be controlled. In the granary at Hwa-Shan, a large grain storage house at Taipei City, the populations of these 2 species are quite stable throughout the whole year. In 1974, we confirmed that when the synthetic sex pheromones cis-9-tetradecenyl acetate and cis-9,trans-12-tetradecadienyl acetate were mixed in a polyethylene cap at a ratio of 1:1 to 1:2, the mixture could attract a large number of the males of both species[12]. First, we used sticky paper fastened within a tube pheromone trap to immobilize the attracted insects. However, these traps became soon useless because the air of the storage house contains much dust which decreases the holding power of the sticky paper. Recently, Yushima and Tamaki[13] in Japan have marketed a plastic pheromone trap which was originally designed for controlling bigger moths, such as Spodoptera litura and S. littoralis in the vegetable field. In the box-shaped trap, they used detergent water for killing insects. We also succeeded in using this kind of trap to catch a large quantity of Spodoptera male moths in field experiments[14]. In order to improve our traps in the storage house, we put several detergent (main ingredient is alkyl benzene sulfonate) water traps[15] in the storage house to see if they

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