precipitation; fecal calcium was measured, after dry ashing, by the same method. Plasma phosphate was determined by the method of Fiske and Subbarrow [*J. Biol. Chem.* **66**, 375 (1925)]. During the two 5-day calcium balance periods urine and stools were collected, the latter being marked by carmine red, and the calcium intake was accurately determined.

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Adenovirus Multiplication: Inhibition by Methisazone

Abstract. *Methisazone (5 to 40 μM) inhibited the multiplication of types 3, 7, 9, 11, 14, 16, 17, 21, and 28 adenovirus; SV15 (a simian adenovirus) was also inhibited. A study of adenovirus 11 under single-cycle conditions showed that multiplication of the virus was completely inhibited by 30 μM methisazone when addition of the compound was delayed until 13 hours after infection. A survey showed that the structure-activity relations of the action of methisazone against adenoviruses and pox viruses are similar.*

Methisazone (1-methylisatin 3-thiosemicarbazone) will inhibit the multiplication of vaccinia (*1*) and smallpox (*2*) viruses in mice and is effective in the prophylaxis of smallpox (*3*) and alastrim (*4*) in man, as well as in the treatment of eczema vaccinatum (*5*) and vaccinia gangrenosa (*6*). The compound had no apparent effect in mice that had been infected intracerebrally with 15 other viruses, mostly arboviruses (*1*), and it was concluded that the spectrum of antiviral activity of methisazone was extremely limited. In this report we show that methisazone is highly active against certain types of adenovirus and that its spectrum of activity is therefore much wider than had been suspected.

Methisazone was dissolved in the minimum amount of dimethylformamide and added to 900 ml of distilled water; this suspension was autoclaved at 1.7 atm for 10 minutes to effect solution of the compound. To the 900 ml of solution 100 ml of tenfold concentrated Eagle growth medium was then added, which gave a final concentration of 40 μM methisazone. Further dilutions, down to 5 μM methisazone, were prepared in Eagle medium.

In a preliminary study of toxicity, monolayers of HeLa cells in bijou bottles were incubated with 40 μM methisazone for 2 hours; the compound was then washed off, and the cells were infected with adenovirus 11 and incubated for 72 hours. Cell cultures that had not been exposed to methisazone were infected similarly. Cells were disrupted by three cycles of freezing and thawing, and the amount of virus in the supernatant fluid was determined by hemagglutinin titration with patas monkey red cells on perspex plates for 1 hour at 37°C.

In comparison with the controls, there was no depression in the titer of virus from cells exposed to methisazone and we concluded that exposure to 40 μM methisazone did not affect the ability of cells to support multiplication of adenovirus 11. Uninfected HeLa cells were also incubated with 40 μM methisazone for 48 hours, and at the end of this time they appeared normal; when subcultured they grew normally and could be maintained in further passage in the usual way. We concluded that 40 μM methisazone was not toxic for HeLa cells and that this would be a suitable concentration for use in studies of antiviral effect.

Monolayers of HeLa cells, in bijou bottles, 12-ml screw-capped tubes, or in 50-ml prescription bottles, were infected with adenovirus 11 in a multiplicity of around four TCD_{50}'s (tissue culture doses 50 percent infective) per cell after the cultures had been at 4°C or at room temperature for 1 hour to permit absorption to take place. Resid-

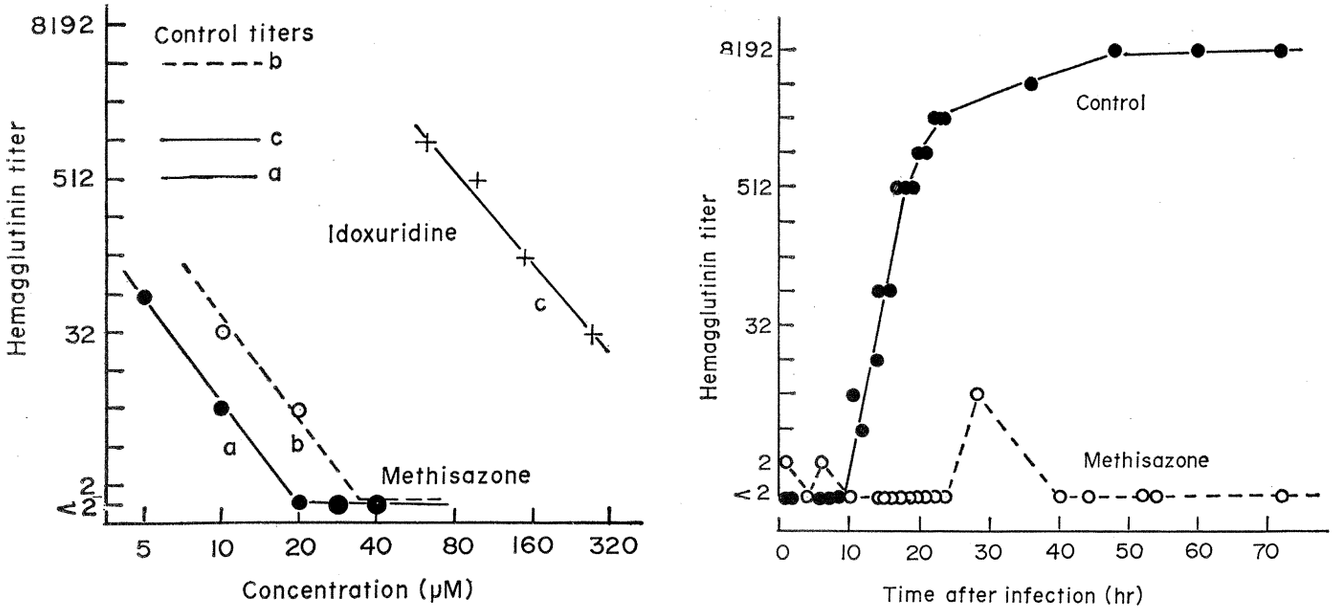

Fig. 1 (left). Dose-response lines of antiviral effect of methisazone (*a*, hemagglutinin production; *b*, infectivity and idoxuridine *c*, hemagglutinin production). Upper lines indicate level of titers in control cultures that did not contain antiviral compounds. Fig. 2 (right). Inhibition by methisazone of the production of hemagglutinin by adenovirus 11 in HeLa cells during a single growth cycle.

ual virus was removed by washing the cultures three times with phosphate-buffered saline or Eagle medium. Medium containing from 5 to 40 μM methisazone was added to respective groups of cultures, and a control group was set up in medium without methisazone. Cultures were incubated for 42 hours and were then subjected to three cycles of freezing and thawing. The amount of virus in the supernatant fluids was determined by titrating the hemagglutinin with patas monkey red cells in perspex plates. The titer of hemagglutinin from the cultures without methisazone was between 512 and 1024. In the presence of 5 μM methisazone this was reduced to 64; a further reduction occurred at 10 and 20 μM, and in the presence of 30 to 40 μM methisazone the formation of hemagglutinin was completely inhibited.

The dose-response curve derived from this experiment is shown in Fig. 1a, which shows that complete inhibition of hemagglutinin production is obtained with 30 μM methisazone. A similar experiment was carried out with 5-iodo-2′-deoxyuridine (idoxuridine) (Fig. 1c), which has been found to inhibit multiplication of adenovirus 5 (*7*). This compound also suppressed formation of hemagglutinin but was much less active than methisazone, since complete inhibition was not obtained even with a 200 μM concentration.

The main hemagglutinin in most types of adenovirus is attached to the virus particle (*8*), and therefore these results imply that methisazone also inhibits the production of infective virus. In order to establish this beyond doubt, the content of infective virus in the cultures was determined by serial titration in HeLa cells, the end point being read after 4 days. The dose-response line for infectivity thus obtained lay parallel to the dose-response line for hemagglutination but two to three $\log_2$ units higher (Fig. 1b). The infectivity titer of control cultures was 4096, and the production of infective virus was completely inhibited in cultures treated with 30 and 40 μM methisazone. Inhibition of hemagglutinin production was observed in similar experiments with adenovirus types 3 and 9, and also types 7, 14, 16, 17, 21, and 28. The hemagglutinin of types 9 and 17 was titrated with rat red blood cells. In types 7 and 14 the hemagglutinin is produced as the result of infection but is separate from the infective particle (*8*). In a similar experiment in primary patas monkey kidney cells the hemagglutinin of SV15, a simian adenovirus, was inhibited to a similar extent.

The effect of methisazone on the growth curve under single-cycle conditions was investigated. Parallel series of HeLa cell cultures were infected with adenovirus 11. After residual virus had been washed off, the cultures were incubated with normal medium and with medium containing 30 μM methisazone, respectively. At intervals up to 72 hours, two tubes were taken from each series and pooled, and the content of hemagglutinin was titrated in twofold dilutions with patas monkey red cells; results are shown in Fig. 2. In the absence of methisazone hemagglutinin appeared after 10 hours; the titer then rose logarithmically and attained a maximum after 48 hours. In the presence of methisazone no formation of hemagglutinin occurred over a period of 72 hours except for a small amount found in the tubes sampled after 28 hours. Formation of hemagglutinin was thus inhibited over a period extending beyond the normal growth cycle. In a similar experiment tubes were sampled at intervals and the content of infective virus was determined by titration in HeLa cells. Results obtained were similar to those of Fig. 2 and showed that methisazone also inhibited the formation of infective virus.

An attempt was made to determine the time during the growth cycle of the virus at which methisazone acts. Replicate cultures of HeLa cells were infected with adenovirus 11 under single-cycle conditions, and at increasing intervals after infection the medium was removed from two cultures and replaced with medium containing 30 μM methisazone. Additions of the compound were made at hourly intervals up to 24 hours. Cultures were incubated further for a period of 18 hours, and the hemagglutinin content of all cultures was determined. Production of hemagglutinin was completely inhibited when the addition of methisazone was delayed until 13 hours after infection. Increasing amounts of hemagglutinin appeared when addition of the compound was delayed beyond this time. It therefore appears that methisazone acts at a late stage in the cycle, when infective virus is just about to appear.

A preliminary study of the structure-activity relations of antiviral activity of methisazone against adenoviruses showed that this compound followed the same pattern that it does with pox viruses. Thus, activity was abolished by replacing sulfur with oxygen in the side-chain (isatin 3-semicarbazone) and by substituting two alkyl groups in the 4′-position [isatin 3-(4′,4′-dibutyl) thiosemicarbazone]; it was reduced to one-half by removal of the 1-methyl group (isatin 3-thiosemicarbazone) and reduced still further by substitution in the 5-position (5-methylisatin 3-thiosemicarbazone).

Successful use of methisazone in pox virus infections of man makes it reasonable to expect that this compound may find some application in the prevention and treatment of adenovirus infections of man.

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Airflow Control by Auditory Feedback: Respiratory Mechanics and Wind Instruments

Abstract. *The auditory signal provided by a soprano recorder in a breathing circuit can help human subjects to regulate inspiratory and expiratory airflow rates at constant preset levels. This method of airflow control is useful in studies of the static and dynamic mechanical properties of the lungs and may have additional applications in human respiration physiology.*

Production of a tone of constant pitch and loudness on a soprano or descant recorder requires a low and constant driving pressure (1 to 7 cm H_2O for tones of increasing pitch) and a low and constant airflow rate (0.05 to 0.11 liter/sec for tones of increasing pitch). The auditory signal provided by