ential development of dendritic spines and their interconnections occurs in response to the localized blood flow and biochemical changes associated with the afferent input; (iv) that the patterning of these interconnections lays the foundation for later behavioral performance. This mechanism may thus provide the neuroanatomical framework underlying the complex of phenomena generally referred to as the influence of early experience on development and behavior.

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15. We have since completed another series of experiments in which the spines on the apical as well as basilar, oblique, and terminal dendrite branches were increased by extra stimulation in 9- and 13-day-old animals.
16. It is also conceivable that sensory stimulation increased the number of spines staining because of local chemical changes associated with increased neuronal activity.
17. In the adult animal sensory stimulation will increase regional blood flow in the receiving and relaying pathways of the central nervous system; see S. Feitelberg and H. Lampl, *Arch. Exp. Pathol. Pharmakol. (Naunyn-Schmiedebergs)* **177**, 725 (1935); H. Serota and R. Gerard, *J. Neurophysiol.* **1**, 115 (1938); L. Sokoloff, in *Regional Neurochemistry*, S. Kety and I. Elks, Eds. (Pergamon, Long Island City, 1961), p. 107.
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Rhodanine: A Selective Inhibitor of the Multiplication of Echovirus 12

Abstract. *A search for compounds which have previously unrecognized antiviral activity led to the discovery that rhodanine inhibits the multiplication of echovirus 12 and also the development of virus-induced morphologic changes. Eighteen derivatives and analogs of rhodanine were synthesized and tested against echovirus 12. These compounds were considerably less active than rhodanine or were inactive, and some of them were more toxic to the host cells than rhodanine.*

It is timely to find previously unknown antiviral activities of organic compounds so that new effective inhibitors can be synthesized through guidance of inhibition-structure relationships, as exemplified by the knowledge on the structural requirements for selective inhibitors of enteroviruses by 2-(α-hydroxybenzyl)-benzimidazole and related compounds (*1*).

We have found that rhodanine (2-thio-4-oxothiazolidine) is a selective inhibitor of the multiplication of echovirus type 12. Rhodanine and its derivatives have been tested in diverse biological systems (*2*). 5-(β-Carboxyethyl)-rhodanine has been used as an insecticide for plants (*3*). 3-Methyl-5-(4′-nitrophenylazo)-rhodanine cured dogs which were parasitized by *Ancylostoma caninum* and *Uncinaria stenocephala* (*4*). Tuberculostatic and other bacteriostatic activities in vitro were reported for 3-amino-5-(4′-hydroxy-3′-methoxybenzylidene)-rhodanine (*5*) and 3-arylrhodanines (*6*) and 3,5-dialkylrhodanine (*7*). Soil fungi have been controlled by 3-(2′-cyanoallyl)-rhodanine (*8*). *Aspergillus niger, Botrytis cinerea, Penicillum italium*, and *Rhizopus nigricans* have been controlled by 3-aryl- (*9*) and 5-propylidenerhodanines (*10*). Rhodanine and 5-methylrhodanine have been reported to show antithyroid activity (*11*). Electrical and drug-induced spasms have been responsive to 3-ethyl- and 3-phenylrhodanine (*12*). 5-(α-Furanyl)-rhodanine has been studied for activity against Ehrlich, Yoshida, and sarcoma 180 ascites tumor cells (*13*).

In addition to these diverse biological activities, minimum antiviral activity against Columbia SK virus in mice for piperonylidenerhodanine has been reported by Cutting and Furst (*14*). Consequently, we decided to include rhodanine and its derivatives in our studies on viruses.

The antiviral spectrum of rhodanine was determined in tube cultures of primary kidney cells from the rhesus monkey. The cultures were maintained in 2 ml of Eagle's minimum essential medium which contained different concentrations of rhodanine. About 100 tissue culture infective doses ($TCID_{50}$) of virus were inoculated in 0.1 ml amounts into groups of tubes with and without rhodanine. Virus multiplication was determined by recording the development of virus-induced cytopathic effects (CPE). Inoculated cultures were maintained at maximum for 9 days, depending on the virus used. Details of experimental procedure have been described (*15*).

A virus was considered susceptible to rhodanine if, on any day of the observation period, there was more than 90 percent reduction of virus-induced cell damage in the presence of 150 μg of rhodanine per milliliter as compared to the control cultures. If there was less than 50 percent reduction of CPE at this concentration of rhodanine, a virus was classified rhodanine-insusceptible. Between these extremes, a group of slightly susceptible viruses was established.

Rhodanine at a concentration of 150 μg/ml is nontoxic to monkey kidney cells as judged by microscopic examination; a concentration of 200 μg/ml sometimes causes granulation of cells, but only after exposure for 4 days or more.

The spectrum of the virus inhibitory activity of rhodanine was determined. Only the echovirus 12 was particularly susceptible. Many other picornaviruses, reovirus 3, vesicular stomatitis virus, and three representatives of DNA-containing viruses—adenovirus 7, herpes simplex, and vaccinia virus—were insusceptible. Since herpes simplex virus

did not multiply in primary cells of the monkey kidney, the experiments were carried out in BS-C-1 cells, which is a continuous cell line derived from kidney tissue of *Cercopithecus aethiops* (*16*). It had been ascertained previously that echovirus 12 was inhibited by rhodanine also in BS-C-1 cells.

Coxsackievirus A9, echovirus 7, the prototype strain of echovirus 9, and an influenza virus B strain were slightly susceptible to rhodanine. However, echovirus 12 was far more susceptible to rhodanine than any of these viruses, since development of CPE was significantly inhibited even at concentrations of rhodanine as low as 12.5 μg/ml.

It was of interest to determine whether echovirus 12 strains other than the prototype strain Travis were susceptible to rhodanine. Accordingly, six field strains of echovirus 12 (supplied by the Communicable Disease Center, Atlanta, Georgia) were tested, and five were susceptible. The sixth, apparently insusceptible, was found on closer examination to be a mixture of echovirus 12 and another virus, presumably an enterovirus. As reported (*17*), virus-selective inhibitors do help in recognizing and separating mixtures of viruses.

These results demonstrate that the antiviral activity of rhodanine is essentially restricted to a single virus type. This limitation of the inhibition of rhodanine is in contrast to that of such inhibitors as isatin-β-thiosemicarbazone, 2-α-hydroxybenzylbenzimidazole, and guanidine, which affect large numbers of virus types within a virus group (*18*).

Rhodanine is virus-selective in two respects: (i) only echovirus 12 is markedly inhibited; (ii) concentrations of rhodanine which block echovirus 12 multiplication are not toxic for host cells, as determined by their morphologic appearance.

The lack of toxicity of rhodanine on the uninfected host cell was further substantiated. The synthesis of RNA by monkey kidney cells, as measured by the conversion of uridine-^{3}H to a trichloroacetic acid (TCA)-insoluble form (*19*), and by the cells' protein biosynthesis from L-leucine-^{3}H (*19*), remained essentially unaffected by rhodanine up to a concentration of 200 μg/ml.

To establish that rhodanine does not directly inactivate extracellular virus, but inhibits virus multiplication, is shown by the following experiments. Echovirus 12 was kept at 37°C in Eagle's minimum essential medium, both in the presence and absence of rhodanine (300 μg/ml). After 22 hours, the residual virus infectivity was determined, and the rhodanine was diluted to ineffective concentrations. The culture maintained in the presence of rhodanine showed a titer of 1.4×10^8 plaque-forming units (PFU) and the control showed 1.0×10^8 units. Rhodanine has no effect on extracellular virus.

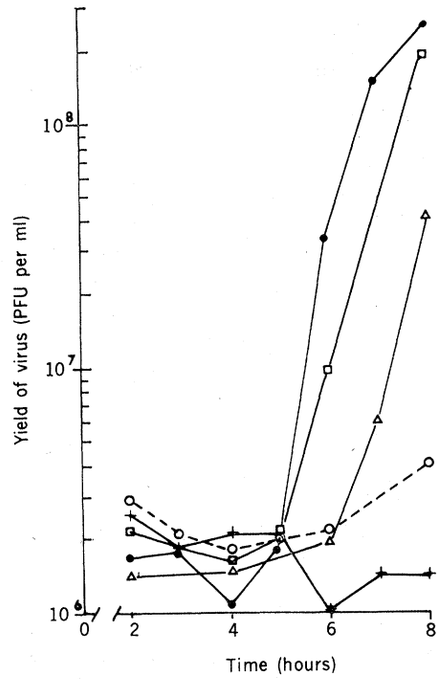


Fig. 1. Single cycle growth curve with echovirus 12 in the presence or absence of rhodanine. ●, Control; □, 1.9 μg/ml; △, 5.5 μg/ml; ○, 17 μg/ml; and +, 50 μg/ml.

A single-cycle experiment was then conducted by introducing the compound after adsorption of the virus by the cells. The total yield of virus was determined at intervals after inoculation. Replicate plates, each with about 2×10^6 kidney cells from the rhesus monkey, were exposed to 10 PFU of echovirus 12 per cell for 30 minutes at room temperature. The inoculum was then removed and 2.5-ml quantities of Eagle's minimum essential medium containing increasing concentrations of rhodanine were added to the plates. The cultures were then incubated at 37°C. Two plates were used per variable. At intervals, groups of cultures were frozen at −20°C until they were assayed by the plaque technique.

The results of a single-cycle experiment are shown in Fig. 1. During the latent period, the amount of recovered infective virus was very similar in treated and untreated cultures. The virus in the control cultures then increased rapidly; there was no increase in cultures treated with 50 μg/ml or higher concentrations of rhodanine. The curves obtained with 100 and 200 μg of rhodanine per milliliter were omitted from Fig. 1, since they showed no difference from the curve for 50 μg of rhodanine per milliliter. At 17 μg/ml, rhodanine reduced the final yield of virus to about 1 percent; at 5.5 μg/ml, the yield was about 10 percent that of the control cultures. These results indicate that rhodanine inhibits some step in the reproductive sequence of echovirus 12 which occurs after virus adsorption has taken place. Thus, rhodanine inhibits echovirus 12 multiplication.

The virus inhibitory activity of rhodanine is not confined to one cell type, such as the primary kidney cells of the monkey; it is also demonstrable in BS-C-1 cells. In addition, the virus-inhibitory activity of rhodanine was also shown in Vero cells; the latter is a continuous kidney cell line of the green monkey (*20*) in W1-38 cells from human fetal tissue (*21*); inhibitory activity was also observed in another diploid cell strain from human fetal lung tissue.

The development of viral CPE was used as an indicator of virus multiplication. Since only small inocula were used, we could not decide whether cells actually infected with echovirus 12 were protected by rhodanine from viral CPE. These very few cells could not have been detected in the culture. This decision, however, was obtained by exposing cultures to large amounts of virus (40 PFU per cell) in a single-cycle experiment and then recording viral CPE as the percentage of cells affected when maximum virus titers had been reached 8 hours after virus inoculation. At this time, about 50 percent of the cells in untreated cultures exhibit viral CPE. Even at high concentrations of virus, it was not possible in this system to infect all cells in the cultures. In all treated cultures, in which no virus multiplication was detectable (200 to 25 μg per milliliter of rhodanine), no viral CPE was observed. At 12.5 μg/ml, a concentration at which virus multiplication was inhibited by 95 percent, less than 10 percent of the cells in the cultures showed viral CPE. Rhodanine not only inhibits echo virus 12 multiplication but also inhibits development of virus-induced morphologic changes.

Previous work (*22*) has led to the hypothesis that picornavirus-induced morphologic changes depend on the synthesis of viral capsid protein. The present data support the interpretation that echovirus 12 capsid protein is not

biosynthesized in the presence of rhodanine.

After the discovery that rhodanine (**1**) has inhibitory activity against echovirus 12, certain derivatives and analogs of rhodanine were synthesized and tested. These compounds were synthesized by described procedures. Each compound was purified by recrystallization and chromatography so that the antiviral testing would be conducted on pure samples. The physical constants on these compounds were in agreement with recorded values. Eighteen derivatives and analogs were selected for the initial comparison with rhodanine against echovirus 12.

To examine the effect of substitution on the nitrogen atom, the four rhodanines (*2–5*) were tested. The *N*-alkyl derivative, 3-methylrhodanine (**2**) (*23*), showed no antiviral activity but did exhibit significant toxicity toward the host monkey kidney cells. Three functionally substituted rhodanines, 3-aminorhodanine (**3**) (*24*), rhodanine-*N*-acetic acid (**4**) (*25*), and its ethyl ester (**5**) (*25*) were without activity against the echovirus 12; the ethyl ester **5** exhibited considerable toxicity.

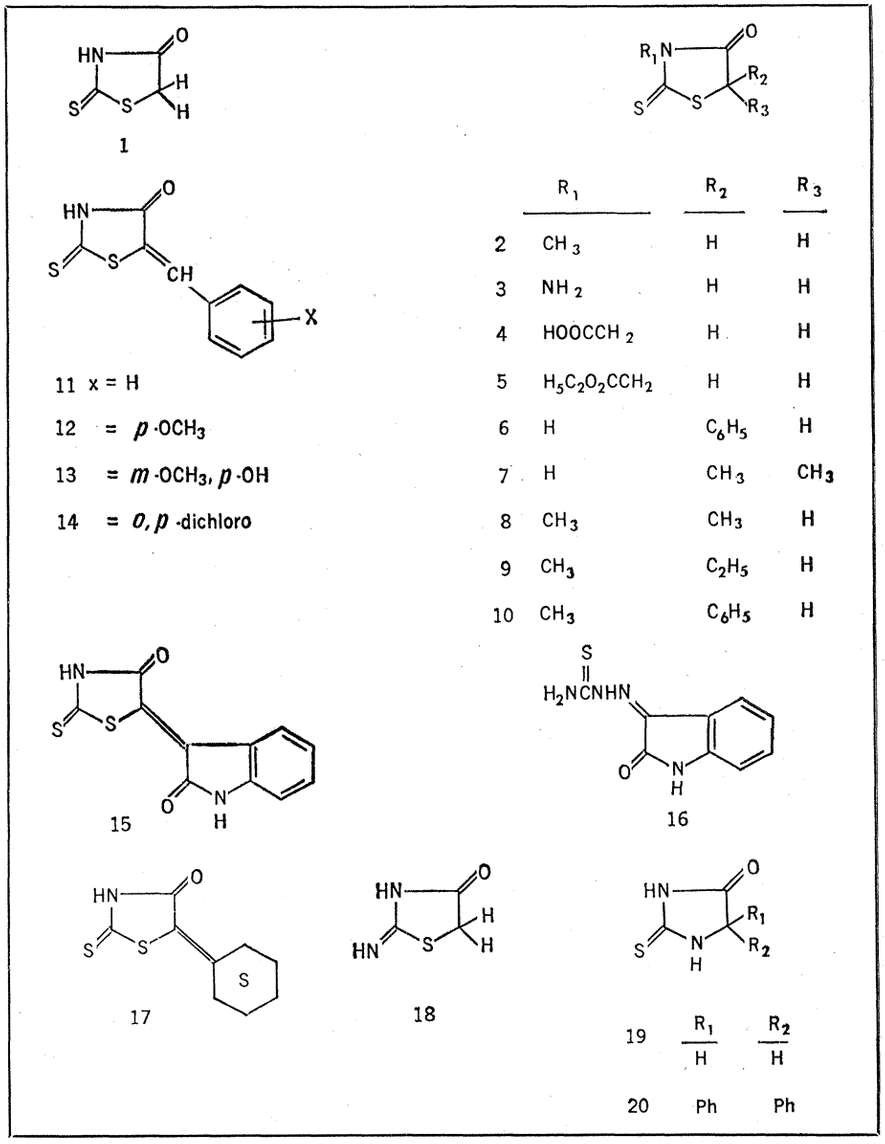
In contrast to the four nitrogen-substituted derivatives, two 5-substituted rhodanines, 5-phenylrodanine (**6**) (*26*), and 5,5-dimethylrhodanine (**7**) (*27*), were not toxic at the concentrations tested. Both **6** and **7** exhibited very slight inhibition at nontoxic concentrations.

Rhodanine derivatives possessing substituents in both the 3- and 5-positions, 3,5-dimethylrhodanine (**8**) (*28*), 3-methyl-5-ethylrhodanine (**9**) (*28*), and 3-methyl-5-phenylrhodanine (**10**) (*28*) exhibited pronounced toxicity, again possibly due to the absence of a proton on the nitrogen atom, which may be needed for proper bonding.

Five 5-arylidene derivatives were tested; 5-(4′-methoxybenzylidene)-rhodanine (**12**) (*29*) appeared to be slightly inhibitory; but 5-benzylidene-rhodanine (**11**) (*30*) was inactive. 5-(3′-Methoxy-4′-hydroxybenzylidene)-rhodanine (**13**) (*31*) and 5-(2′,4′-dichlorobenzylidene)-rhodanine (**14**) (*32*) were inactive and exhibited considerable toxicity. A particularly interesting compound is the condensation product of isatin with rhodanine (**15**) (*33*) in which the rhodanine moiety replaces the β-thiosemicarbazone moiety of isatin-β-thiosemicarbazone (**16**) which is a potent antiviral agent (*34*). However, compound (**15**) exhibited no antiviral activity against echovirus 12. A single 5-alkylidene derivative of rhodanine, 5-cyclohexylidene-rhodanine (**17**) (*35*), appeared slightly inhibitory.

Replacement of the exocyclic sulfur atom of rhodanine with an imino group, pseudothiohydantoin (**18**) (*36*), resulted in the loss of antiviral activity. The isomeric compounds, thiohydantoin (**19**) (*37*) and 5,5-diphenylthiohydantoin (**20**) (*38*) were inactive.

These derivatives and analogs **2** to **19** were considerably less active than rhodanine or were inactive against echovirus 12, and some of these compounds were more toxic than rhodanine to the host cells. However, a number of other structural variations remain to be tested for elucidation of structure-activity relations on a broad basis.



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"Antidotal Thirst": A Response to Intoxication

Abstract. *Albino rats increased their intake of water soon after they were given a load of 0.12 molar lithium chloride in the stomach. Alterations in blood volume and tonicity could not account for the magnitude of the thirst observed, which served to facilitate the renal excretion of the toxic lithium ions.*

Thirst occurs as an occasional adverse side effect in manic-depressive patients under treatment with lithium salt. While the effects of lithium on blood tonicity have not been reported, alterations in the blood volume of these patients cannot account for the occurrence of thirst (*1*). Apparently this thirst is not necessarily secondary to the diuretic effects of the toxic lithium ions (*2*). We investigated the possible causes of drinking that follows the administration of single, nonlethal doses of lithium chloride. The results suggest that a unique homeostatic mechanism may be involved in the increased drinking.

Male Holtzman rats (350 to 450 g) were housed in individual cages at 21° ± 0.5°C, on a 12-hour light-dark cycle (lights on 06:00 to 18:00). The rats were given free access to Teklad rat chow and tap water before testing.

Immediately after the tests were completed, each rat was put under ether anesthesia and a terminal blood sample was drawn from the dorsal aorta. Cumulative urine samples were collected every 8 hours throughout the experiments.

All tests began at midnight when the rats were placed in individual metabolism cages without food or water. Eight hours later, 29 rats received a 10-ml dose of 0.12*M* LiCl by stomach tube (catheter). After an additional 8-hour period of deprivation, blood and urine samples were taken from 11 animals. Seven of the 18 remaining animals were offered water to drink for 8 hours before the test was terminated, and five animals were returned to their home cages with food and water freely available for 4 days. The six remaining animals given LiCl were killed after a 16-hour period of food and water deprivation in the metabolism cages.

After an initial 8-hour period of deprivation, 23 other rats were given 10 ml of 0.12*M* NaCl by stomach tube (catheter). These rats were then deprived for an additional 8 hours in the

Table 1. The effects of a 10-ml stomach intubation of 0.12*M* LiCl or 0.12*M* NaCl on the physical parameters shown (mean ± S.D.). The vertical listing of the groups corresponds to the order in which they are described in the text. In all cases the food and water or water only are freely available when given; D, deprived.

Treatment	Rats (No.)	Water intake (ml)	Urine		Serum		Hematocrit (% by volume)
			Volume (ml)	Li^+ (total meq)	Osmolality (milliosmol/kg)	Li^+ (meq/liter)	
			LiCl groups				
8-hour D	11		7.9 ± 3.6	0.358 ± .120	285.8 ± 3.0	1.63 ± .24	51.5 ± 2.1
8-hour D + 8-hour water	7	41.9 ± 12.4	40.4 ± 10.1	0.245 ± .077	290.0 ± 4.6	1.17 ± .33	50.0 ± 2.2
8-hour D + 4 days food and water	5	52.8 ± 1.8					
16-hour D	6		4.6 ± .7	0.120 ± .014	285.2 ± 2.7	1.18 ± .18	50.0 ± 1.4
			NaCl groups				
8-hour D	5		5.7 ± .7		290.4 ± 4.9		47.2 ± 2.8
8-hour D + 8-hour water	3	6.0 ± 3.0	8.8 ± 2.3		289.0 ± 4.0		44.0 ± 1.0
8-hour D + 4 days food and water	7	43.4 ± 3.8					
32-hour D	4		8.2 ± 1.1		292.0 ± 4.1		49.0 ± 1.1
32-hour D + 8-hour water	4	17.8 ± 5.7	7.0 ± 3.5		290.3 ± 1.7		50.3 ± .5