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ANTIVIRAL ACTIVITY OF OXIDIZED POLYAMINES AND ALDEHYDES*

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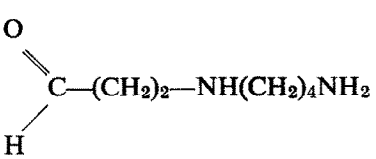
Abstract—Amine oxidase was isolated from lamb serum, purified 50-fold by chromatographic techniques, and used in the preparation of the aminoaldehydes of spermine and spermidine. Crude spermine aldehyde inactivated a number of animal viruses, but failed to affect virus hemagglutination. Chromatographic fractionation of the aldehyde of spermidine or spermine indicated that fractions containing peak amine aldehyde concentrations produced maximal inactivation of vesicular stomatitis virus. Inactivation of vesicular stomatitis virus by column-purified oxidized spermidine (a monoaldehyde) or spermine (a dialdehyde) was a function of the concentration of aldehydic groups. Viral inactivation produced by the amine aldehydes proceeds in a linear-first-order reaction with respect to time.

Studies to determine structure-antiviral activity relationships were conducted with a number of aliphatic and aromatic aldehydes. These results suggest that the aldehyde moiety is the primary determinant of antiviral activity.

SPERMIDINE and spermine are naturally occurring aliphatic polyamines found in a wide variety of microbial, plant and animal tissues.[1] The polyamine concentration appears to be closely associated, in as yet an undefined manner, with cellular synthetic activity.[2] These amines have been shown to stabilize ribosomes,[3] alter enzyme activity,[4–6] and protect helical DNA[7] and RNA[8] against thermal denaturation. Interaction of the polyamines with polynucleotides and ribosomes is reflected in the stimulation of RNA and DNA synthesis[9] and amino acid incorporation.[10]

Spermidine and spermine are oxidatively deaminated by an enzyme present in the sera of cattle and other ruminating animals.[11,12] The oxidation products of spermidine (a) and spermine (b) have the following structures:[13,14]

(a) 

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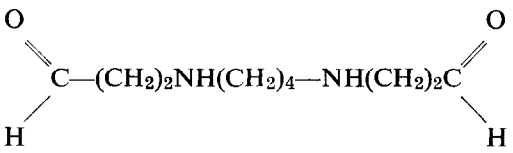
(b) 

Initially it was observed that oxidized spermine is toxic to mycobacteria.[15] Further studies have since indicated that oxidized polyamines can also inactivate other bacteria,[16] mammalian cells,[17] spermotozoa,[16] and bacterial, plant and animal viruses.[18,20]

In the present paper the effects of oxidized polyamines on several animal viruses are noted, and data are presented which indicate that the viral inactivation appears to be primarily related to the aldehyde moiety of these compounds.

MATERIALS AND METHODS

Enzyme purification. All operations were carried out at 4°. Ammonium sulfate (Mann, enzyme grade) was added to 100 to 200 ml of lamb serum (Microbiological Associates) to 45 per cent of saturation; the mixture was centrifuged at 35,000 *g* for 15 min. Ammonium sulfate was added to the supernatant fraction to 55 per cent of saturation and, after 1 hr, centrifuged. The precipitate was resuspended in a minimum volume of 0·10 M sodium phosphate buffer, pH 7·0, and its enzymic activity was determined spectrophotometrically, using benzylamine as the substrate.[21] Protein was estimated by measuring absorption at 280 mμ.[22] The specific activity of the resuspended precipitate was 7 to 9, relative to about 1 for the crude serum.[21]

The enzyme was then applied to a column of Sephadex G-150 (Pharmacia) equilibrated with 0·01 M sodium phosphate buffer, pH 7·0, having a volume approximately twenty times that of the enzyme solution and a height to diameter ratio of 15:1. The enzymic activity was markedly retarded on the column. Those fractions having a specific activity of 25 or greater were pooled. The pH was adjusted to 5·8 with 0·5 N HCl and applied to a column of phosphorylated cellulose (Bio-Rad Lab.), equilibrated with 0·01 M sodium phosphate buffer, pH 5·8. The cellulose column volume was approximately equal to that of the solution to be applied, with a height to diameter ratio of 15:1. The enzyme was not absorbed; the specific activity was 50 to 60. The overall enzymic yield was 20 per cent of the starting activity.

Although further enzyme purification was not routinely practised, the enzyme can be purified further by a factor of 2 to 3 by adsorption on benzyldiethylaminoethyl cellulose.[23] The enzyme, in 0·01 M phosphate buffer, pH 7·4, was applied to the cellulose column equilibrated with the same buffer, and eluted with two column volumes of 0·20 M NaCl in the phosphate buffer.

The enzyme was finally concentrated by adding ammonium sulfate to 60 per cent of saturation; the precipitate was resuspended in a minimum volume of 0·03 M phosphate buffer, pH 7·4.

Preparation of oxidized polyamines. Twenty-five μmoles of spermidine or spermine (Calbiochemical) were incubated in a 50-ml Erlenmeyer flask with 400 spectrophotometric units[21] of purified amine oxidase, adjusted to a final volume of 8·0 ml with 0·1 M Tris, pH 7·4, at 34°. The enzymic reaction, generally complete in 2 hr, was followed by assaying for aldehydes,[24,25] spermidine,[26] and spermine.[27] The incubation mixture was fractioned immediately or stored at −20°. Isotopically labelled radioactive

spermidine (aminopropyl) tetramethylene-1,4-^{14}C-diamine · 3 HCl and spermine [bis-(aminopropyl)-tetramethylene-1,4-^{14}C-diamine] · 4 HCl were obtained from New England Nuclear Corp. Radioactivity was determined by liquid scintillation counting using Bray's solution.[28]

A volume, generally 4 ml, of the enzymic reaction mixture was diluted with 2 vols. of water, adjusted to pH 5·5 to 5·8 with 0·5 N HCl, and applied to a column of phosphorylated cellulose having a volume of 3 to 4 ml and equilibrated with 0·01 M phosphate buffer, pH 5·8. Several volumes of buffer and water were applied, followed by 20 ml of 0·05 N HCl, and finally the oxidation product was eluted with 0·20 N HCl. All column fractions were analyzed for aldehydes, spermidine and spermine, lyophilized and then diluted to the desired aldehyde concentration with phosphate-buffered saline (PBS), pH 7·2,[29] containing 500 units of penicillin G and 100 μg of streptomycin per ml.

Preparation of other aldehydes. The glutaraldehyde (Fischer) was a 50% solution of biological grade, redistilled before use and appropriately diluted. The orthophthaldehyde (Mann Lab.), phenylacetaldehyde, benzylamine, and propionaldehyde (Eastman Organic), and all other compounds (Aldrich) were diluted immediately before use. The compounds available as the acetal (see Table 3) were converted to the corresponding aldehyde by mixing with an excess of an aqueous suspension of Dowex 50 (X-4) in the H^+ form.

Cell cultures. Porcine kidney (PK(15)), monkey kidney (LLC-MK_2) and canine kidney (MDCK) were propagated in 75-mm^2 plastic tissue culture flasks using reinforced Eagle's medium[30] containing 10% fetal bovine serum. The fetal bovine serum had no amine oxidase activity when evaluated with benzylamine as the substrate.

Primary cultures of sheep choroid plexus (SCP) cells were prepared by the trypsinization of choroid plexuses removed from exsanguinated Suffolk and Hampshire sheep by the method previously described.[31]

Virus. The Indiana strain of vesicular stomatitis virus (VSV) was passaged once in baby hamster kidney cells (BHK 21-F) and twice in PK(15) cells. Stock VSV containing $4{\cdot}3 \times 10^7$ PFU/ml was stored at $-70°$ until used.

Type 2 neuroattenuated P712, Ch, 2ab strain of poliovirus containing $2{\cdot}7 \times 10^9$ PFU/ml was prepared by serial passage in rhesus monkey kidney or HeLa cell cultures.

Tenth passage of visna virus grown in SCP cells ($6{\cdot}3 \times 10^7$ $TCID_{50}$/ml) was used; the initial passage was made from visna virus K485 obtained from Drs. H. Thormar and P. A. Pálsson, Institute for Experimental Pathology, University of Iceland.

ECHO 7 virus was prepared by serial passage in monkey kidney cells. Influenza A virus (NWS strain) in chorioallantoic fluid was prepared from infected embryonated eggs.

Five virus preparations were used in experiments involving hemagglutination: (1) ECHO 7 virus, (2) NWS strain of influenza A virus, (3) VSV grown in PK(15) cells and concentrated 100-fold by centrifugation at 54,500 *g* for 1 hr, (4) measles virus antigen (Microbiological Assos.), (5) pneumonia virus of mice (PVM) passaged in baby hamster kidney (BHK 21/13) cells.[32]

Conditions of inactivation. Five-tenths ml of virus and 0·5 ml of the oxidized polyamine solution, previously diluted with PBS to 8×10^{-4}M, were mixed in glass test tubes, and incubated in a water bath at 37° for 3 hr with intermittent shaking.

In early experiments using crude oxidized spermine, the mixture was first centrifuged at 60,000 *g* for 2 hr in a Spinco SW 65 swinging bucket rotor to remove excess polyamine; pelleted virus was then resuspended in reinforced Eagle's medium containing 0·5% BSA and assayed for infectivity and hemagglutination. Subsequent experiments indicated that the antiviral activity of the uncentrifuged virus–aminealdehyde system was identical to the centrifuged system. This indicated that the excess aldehyde did not alter the cell cultures sufficiently to effect the virus assay. Therefore, the centrifugation step was omitted in subsequent experiments.

Kinetic experiments involving different incubation periods were performed in replicate tubes containing identical virus-oxidized polyamine mixtures.

Infectivity assay. Infective VSV was quantitated by plaque assay on monolayers of PK (15) or LLC-MK_2 cells. Plaque assays of poliovirus, ECHO 7 and influenza virus were performed in HeLa, LLC-MK_2 and MDCK cells respectively. Confluent monolayers were prepared in 60-mm plastic tissue culture dishes, washed twice with PBS and inoculated with 0·5 ml of virus dilution prepared in reinforced Eagle's medium containing 0·5% BSA.* Each virus dilution was inoculated onto three plates. After an adsorption period of 2 hr at 37°, the inoculum was removed, and the cell sheet was overlaid with 5·0 ml 0·95% agar (Noble Difco) in reinforced Eagle's medium containing 4% heat-inactivated (56° for 30 min) fetal calf serum in the assay of VSV, poliovirus 2 and ECHO 7, and with 5·0 ml 0·85% "Ionagar" No. 2 (Colab Lab., Inc., Chicago Heights, Ill.), in reinforced Eagle's medium containing 10% inactivated fetal calf serum and 0·02% DEAE-dextran (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) in the assay of influenza virus.† The plates were then incubated at 36° in a humidified atmosphere of 5% CO_2 in air. After 2 or 3 days, a second overlay consisting of 0·005% neutral red and 0·95% Noble agar in the aforementioned reinforced Eagle's medium was added and the plates were incubated further at 36°. Plaques were counted on the third or fourth day after inoculation.

Visna virus was assayed by inoculating confluent SCP cell monolayers and inspecting them for cytopathic effects after 14 days, by the method previously described.[31]

Hemagglutination titrations. Hemagglutination titrations were performed in glass tubes as described by Choppin *et al.*[33]

Suspension of 0·5% human 0 erythrocytes in PBS and 0·25% BSA were used in hemagglutination assays of the NWS strain of influenza virus and ECHO 7 virus.

The hemagglutination of PVM and measles antigen were tested using 0·8% mouse and 1·0% green African monkey respectively. Hemagglutination titration of concentrated VSV was performed by adding 0·25% goose erythrocytes prepared in phosphate buffer, pH 5·8, to the virus preparation diluted in 0·5% BSA-borate saline buffer, pH 9, by the method described by Halonen *et al.*[34] Results were read after 2 hr incubation at 25° or 37°

RESULTS

Effect of oxidized spermine on viral infectivity and hemagglutination. As indicated in Table 1, crude oxidized spermine at a final concentration of 4×10^{-4}M inactivated the 5 RNA-containing viruses tested to different degrees. The concentration of the oxidized spermine was based on the determination of the aldehyde concentration,

*The BSA had no detectable amine oxidase activity, using benzylamine as a substrate.

†Dr. R. Krug, Sloane-Kettering Institute for Cancer Research, personal communication.

and, assuming that the aldehyde formed was *N*, *N'*-bis-(2 formylethyl)-butane-1, 4 diamine. The most pronounced reduction in infectivity was observed with VSV, influenza and visna viruses; poliovirus and ECHO 7 were least affected.

Incubation with similar concentration of oxidized spermine, however, failed to change the hemagglutination titer of the 5 viruses tested. The hemagglutination titer is an indicator of virus-cell surface interaction; thus it would appear that the virus surface was not altered.

TABLE 1. INACTIVATION OF ANIMAL VIRUSES BY OXIDIZED SPERMINE

	Infective virus*		
	Oxidized spermine	Control	Log_{10} virus inactivated
Vesicular stomatitis	$3{\cdot}3 \times 10^4$	$5{\cdot}9 \times 10^7$	3·3
Influenza	$\leqq 5{\cdot}0$	$2{\cdot}3 \times 10^6$	$> 5{\cdot}7$
Visna	$3{\cdot}6 \times 10^2$	$6{\cdot}3 \times 10^7$	5·3
Poliovirus	$3{\cdot}9 \times 10^7$	$2{\cdot}0 \times 10^8$	0·7
ECHO 7	$2{\cdot}5 \times 10^6$	$1{\cdot}5 \times 10^7$	0·8

*Present after incubation with oxidized spermine or spermine control 4×10^{-4}M at 37° for 3 hr. Infectivity expressed as PFU per milliliter (vesicular stomatitis, influenza, poliovirus and ECHO 7) and $TCID_{50}$ per milliliter (visna virus).

Since VSV demonstrated pronounced sensitivity to oxidized spermine and could be easily assayed by the plaque technique, it was chosen for use in subsequent experiments.

Identification of oxidized polyamines. In a study of the partially purified amine oxidase and putrescine as the substrate, and the assay conditions comparable to that described for the oxidation of the polyamines, no aldehyde formation was detected. When the same enzyme preparation having 65 units of activity was incubated with 4·0 μmoles of spermidine for 3 hr, the analysis of the incubation media indicated 3·8 μmoles equivalent of aldehyde functional groups. The fluorometric analysis for spermidine showed 0·05 μmoles of spermidine remained.

In a comparable experiment 4·0 μmoles of spermine was used as the substrate and the enzymic reaction mixture was analyzed; 7·7 μmoles equivalent of aldehyde functional groups were found. The molar absorbancy obtained in the colorimetric (*N*-methyl-2-benzothiazolone hydrozone hydrochloride) test for aldehydes was twice that obtained for oxidized spermidine. Analysis for spermidine indicated, on the basis of comparison to an inactivated enzyme control incubation mixture, that 0·06 μmoles of spermidine was formed. This would appear to confirm a previous report of a small quantity of spermidine formation by serum amine oxidases.[13] The assay of amines by means of 2, 4-dinitrofluorobenzene, reading at 380 mμ where the absorbencies of the reaction products of primary and secondary amines are about equal, indicated 8·1 μmoles equivalent of amine groups. The 8·1 μmoles of amine groups are presumed to be because of the secondary amine groups of spermine. In another comparable incubation experiment, to which a tracer quantity of ^{14}C spermine [bis-(aminopropyl)-tetramethylene-1,4^{14}C-diamine]·4 HCl was added, followed by chromatographic fractionation of the reaction mixture, 91 per cent (51,000 cpm) was eluted with the

aldehyde component by 0·20 N HCl. The chromatographic elution pattern of the amineraldehyde indicated that the compound possessed a minimum of a net-plus two charge.[26] The above observations are consistent with other previous findings, in which beef serum was used as the enzyme source, that spermidine is oxidized to a monoaldehyde whereas spermine is oxidized to a dialdehyde, *N*, *N'*-bis (2 formylethyl)-butane-1,4-diamine.[13,14]

In another experiment a comparison was made of the antiviral activity of the crude enzymic reaction mixture to that of an aliquot subjected to cellulose column fractionation. To simplify biological testing, column fractions were analyzed for aldehydes, combined as indicated, lyophilized, resuspended in the minimum volume of PBS required for analysis (2 ml), and re-analyzed for aldehydes. To permit a direct comparison to the crude reaction mixture, the column fraction containing the major portion of the aminoaldehydes was further diluted to the same concentration of aldehydes originally present in the unfractioned preparation. As shown in Table 2, the spermine aldehyde was eluted with 0·20 N HCl; this fraction had the same antiviral activity as the unfractioned preparation. Approximately 90 per cent of the amineraldehydes applied to the column were recovered in the main fractions (7 and 8); after lyophilization and resuspension in PBS, only 70 per cent of the aldehydes were accounted for.

In a similar column fractionation experiment the enzymic oxidation products of spermidine were purified and tested for ability to inactivate VSV. In this experiment 93 per cent of the amineraldehydes were eluted with 0·20 N HCl; after lyophilization and resuspension in PBS, 75 per cent of the aldehydes were accounted for. The antiviral

TABLE 2. INACTIVATION OF VESICULAR STOMATITIS VIRUS BY COLUMN FRACTIONED OXIDIZED SPERMINE

Column fraction no.	Applied to column	Fractions combined	Aldehyde concn. (M)	Log_{10} virus inactivated
1	Incubation mixture	1, 2	7×10^{-5}	0·07
2	Buffer, H_2O			
3	0·05 N HCl	3, 4	6×10^{-5}	0·20
4	0·05 N HCl			
5	0·05 N HCl	5, 6	9×10^{-5}	0·50
6	0·05 N HCl			
7	0·20 N HCl	7, 8	4×10^{-4}*	3·37
8	0·20 N HCl			
9	0·30 N HCl	9, 10	9×10^{-5}	0·80
10	0·30 N HCl			
Control-spermine (4×10^{-4}M)			0	
Unfractioned-spermine aldehyde			4×10^{-4}	3·40

*Concentration adjusted by dilution to correspond to controls.

activity was associated with the main aldehyde containing fraction. The apparent loss of aldehydes, as a result of lyophilization, could not be explained as simply because of mechanical losses, and may be because of instability of the aldehydes.[13]

Relationship between viral inactivation and aldehyde concentration. Since preliminary experiments using column-fractionated oxidized polyamines indicated that, on a molar basis, twice as much oxidized spermidine was required to produce the same

inactivation as that obtained with oxidized spermine, further studies were done to relate antiviral activity with aldehyde concentration.

VSV was exposed to column-purified oxidized spermine and spermidine at various molar concentrations of aldehydic groups and the degree of viral inactivation determined. As indicated in Fig. 1, the compound's effectiveness was a function of the concentration of the aldehyde moiety.

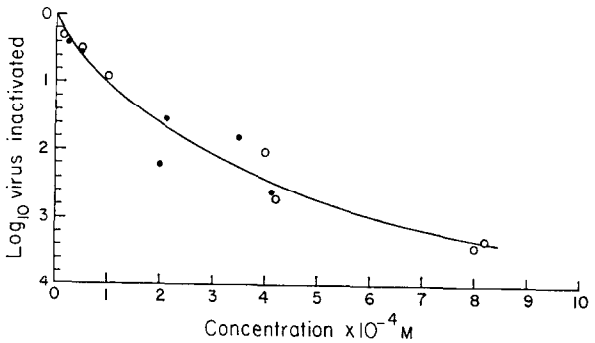


FIG. 1. Relationship between aldehyde concentration of column-purified oxidized spermine (○) and spermidine (●) and the inactivation of vesicular stomatitis virus. Virus and oxidized polyamine preparations varying in aldehyde concentration were incubated for 3 hr at 37° and the mixture assayed for surviving infective virus.

Rate of VSV inactivation by oxidized polyamines. To determine the rate of VSV inactivation, replicate virus samples were incubated with column-purified oxidized spermine or spermidine at 37°. At intervals, mixtures were assayed for remaining

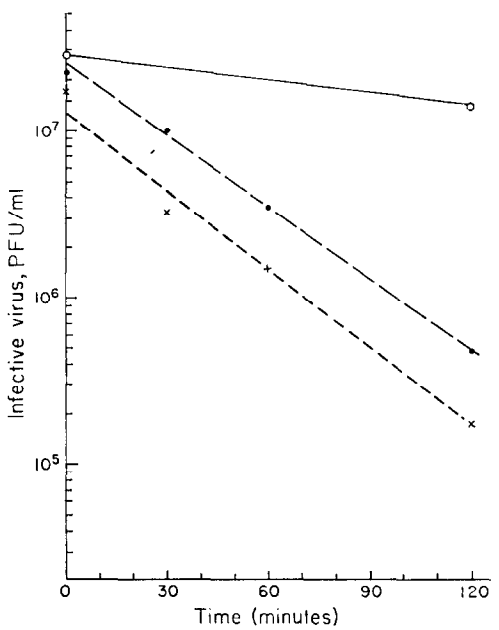


FIG. 2. Rate of inactivation of vesicular stomatitis virus by oxidized spermine 4×10^{-4}M(× — — ×), oxidized spermidine (● — — ●) 5×10^{-4}M, and control unreacted spermine, 4×10^{-4}M (○——○), at 37°C.

infective virus. As shown in Fig. 2, inactivation of VSV by both oxidized polyamines proceeded linearly in the manner of a first-order reaction.

Antiviral activity of synthetic aldehydes. Since the above results indicated that the aldehyde moiety of the oxidized polyamines appeared to be the primary determinant of the inactivation of VSV, experiments were conducted to determine the relative effectiveness of simple aliphatic and aromatic aldehydes in inactivating VSV. The compounds were evaluated at a final concentration of 4×10^{-4}M, the concentration of amine aldehydes previously found to inactivate 3 to 5 $\log_{10}$ of VSV. The results of these experiments are shown in Table 3. The data appear to indicate that an amine

TABLE 3. EFFECT OF ALDEHYDES ON THE INFECTIVITY OF VESICULAR STOMATITIS VIRUS

Aldehydes	Log_{10} VSV inactivated
Monoaldehydes	
Formaldehyde	0·4
Acetaldehyde*	0
Aminoacetaldehyde	0·2
Phenylacetaldehyde	0
Propionaldehyde	0
Valeraldehyde	0·2
Hexaldehyde	0·2
Heptaldehyde	0·1
Octylaldehyde	1·0 to 4
Unsaturated monoaldehydes	
2-Propenal (acrolein)*	1·6
trans-2-Hexanal	1·7
3, 7-Dimethyl-2,6-octadienal	3·7
Dialdehydes	
Malondialdehyde*	3·8
Glutaraldehyde	5·2
Orthophthaldehyde	5·1
Isophthaldehyde	1·0
Terephthaldehyde	0·5

(All compounds were evaluated at 4×10^{-4}M at 37° for 3 hr.)
*Prepared from corresponding acetal.

constituent in the aldehyde molecule is not essential for antiviral activity. The saturated monoaldehydes had little or no antiviral activity with the exception of octylaldehyde which showed variable moderate activity in comparison to the aminealdehydes. The unsaturated monoaldehydes were intermediate in their effectiveness, the long-chain polyunsaturated citral (3,7-dimethyl-2,6-octadienal) was as effective as the amino-aldehydes. The aliphatic dialdehydes were as effective antiviral agents as the amine-aldehydes. The study of the isomers of phthaldehyde indicates that the antiviral activity is a function of the relative positions of the aldehyde groups.

DISCUSSION

In previous studies of enzymically oxidized polyamines, beef plasma was used as the source of enzyme;[15,18] in the present studies the enzyme was isolated from lamb serum and purified 50- to 60-fold by simple and rapid chromatographic techniques. The enzyme's properties appeared to be similar to those previously reported with the exception of its molecular weight. Gel filtration studies of the lamb serum enzyme, relative to proteins of known molecular weight, indicated a molecular weight of

approximately 100,000. Studies of the beef plasma oxidase had previously indicated a molecular weight of 255,000 ± 20,000 on the basis of sedimentation velocity measurements.[35]

The animal RNA viruses used in the present study varied in their susceptibility to oxidized spermine. This is in keeping with an earlier report that oxidized spermine inactivated two arboviruses (Sindbis and West Nile) to a greater degree than Newcastle disease virus or vaccina virus.[20] The susceptibility of a given animal virus may be related to the ease with which the compound can penetrate the viral envelope or capsid in a manner similar to that postulated for bacteriophages and oxidized polyamines.[36]

The agglutination of red blood cells by viruses requires interaction between combining sites on the virus surface and erythrocyte receptors. As virus hemagglutination remains unaffected after treatment with oxidized spermine, the compound apparently does not affect virus attachment. A similar conclusion was reached after study of the adsorption of T5 bacteriophage inactivated by oxidized spermine.[36,37]

The mechanism by which the amine aldehydes inactivate animal viruses is not known. Earlier reports suggested that the terminal carbonyls of the oxidized polyamines are of primary importance in determining toxic activity to cells and microorganisms in mammalian cell cultures[17] and bacteriophages.[36,38] The dialdehyde of spermine is a more effective inhibitor than the monoaldehyde of spermidine and reduction of oxidized spermine to the corresponding alcohol destroys the bacteriocidal effect.[39] Studies of the interaction of oxidized spermine with bacterial DNA indicate two types of bonds are involved; electrostatic binding by secondary amino groups and nonelectrostatic binding by the two carbonyl groups. The aminoaldehydes, oxidized spermidine and monofunctional reagents did not induce cross-links in DNA.[40] Most recently, these investigators concluded that the amino groups of the DNA bases are responsible for the irreversible binding of oxidized spermine.[41]

Conflicting results have been reported in comparing the effectiveness of the oxidized polyamines with aldehydes not containing primary or secondary amino groups. The unsaturated monoaldehyde, acrolein, was as toxic to mammalian cells as oxidized spermidine.[17] On the other hand, butyraldehyde and glyoxal at high concentrations did not affect the growth of *S. aureus*[39] and a number of aldehydes (butyraldehyde, amino-butyraldehyde, phenylacetaldehyde, imidazoleacetaldehyde or indolacetaldehyde) failed to inactivate T5 bacteriophage at concentrations far exceeding those at which oxidized spermine proved effective.[35] The latter finding led the authors to conclude that both the two terminal carbonyl groups and the secondary amines were required for phagocidal activity.

The mechanism by which non-amine containing aldehydes inactivate VSV is not suggested by these experiments. However, the bifunctional aldehydes are known to interact with DNA *in vitro* and *in vivo*; attempts to locate the binding site indicated that the aldehydes are preferentially associated with the guanine and cytidine moiety of DNA core.[42] A similar interaction would be possible for this RNA containing virus. A variety of reactions are also possible with the viral protein. It is known that glutaraldehyde forms Schiff bases with lysine amino groups and recent studies of commercial glutaraldehyde indicate that the reagent breaks down to form unsaturated aldehydes, which also give stable Michael-type adducts with the amino groups of lysine.[43]

The present study suggests that the determinant of antiviral activity for VSV is the aldehydic group, as shown by : (1) peak antiviral activity appeared in column fractions

containing the highest aldehyde concentration; (2) the degree of virus inactivation was a function of the aldehyde concentration; (3) a simple monoaldehyde and several dialdehydes can be as effective antiviral agents as either oxidized spermine or oxidized spermidine.

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