and 12, where the alkyl group is either ethyl or propyl. However, when the methionine residue is substituted with an alkylcysteinamide residue the biological activity is drastically reduced. It is apparent, in accord also with previous observations[2,4], that whereas methioninamide cannot be replaced by other naturally occurring amino acids, it may be substituted, even with advantage, by synthetic non-natural sulphur-containing amino acids.

(2) The terminal amide group is not essential for biological activity: methionine nitrile (compounds No. 14 and 15) can replace the methioninamide residue with limited loss of activity.

(3) The all D-enantiomer of a highly active hexapeptide[5] is devoid of activity and does not antagonize either the L-enantiomer or eledoisin[6]. On the contrary, the presence of a single D amino acid can have influence on the biological activity[3] (cf. No. 16) provided the C-terminal pentapeptide fragment is the same as in eledoisin.

(4) N-ε-acylation of the highly active hexapeptide No. 18 does not appreciably alter the overall activity: in one case (No. 19) a 3-fold increase was observed.

Riassunto. Vengono descritte le proprietà di una serie di peptidi sintetici affini per struttura ed attività all'eledoisina.

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[6] After this work was completed we learnt of a similar study by Prof. SCHRÖDER et al.[7]: our results point to the same conclusions.
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Inhibition of the Infective Activity of Bacteriophage f_2 by Spermine

It was shown recently that spermine antagonizes the inhibition of reproduction of phage f_2 caused by streptomycin (SCHINDLER[1]), in spite of that it exerts a high inhibitory activity itself. REITER observed that spermine at a concentration of 1 mg/ml (approximately 3 m*M*) inhibits adsorption and penetration of phages T 1, T 3, T 7, and PLT 22. T even phages were not inhibited (REITER[2]). FERROLUZZI-AMES and AMES[3] report that the reproduction of T 4r$^+$ phage is about 95% inhibited by spermine at 20 m*M* concentration. Phage P 22, even though it adsorbs normally to *Salmonella typhimurium*, does not develop in the presence of spermine (AMES and DUBIN[4]). Spermine (1 mg/ml) stimulates injection of streptococcus-P9 phage DNA into the host cell (BROCK and WOOLEY[5]).

Spermine, as well as other polyamines, interact with nucleic acids. Spermine interacts with DNA, changing its thermal denaturation profile (MAHLER and MEHROTRA[6], MANDEL[7], TABOR[8]). It protects DNA against hydrodynamic shear, strengthening its molecule longitudinally (KAISER, TABOR, and TABOR[9]). Interactions with RNA of low molecular weight were described. Spermine becomes bound to polyuridylic acid (HUANG and FELSENFELD[10]) and s-RNA (CANTONI[11]). It is capable of blocking the messenger activity of polyuridylic acid in cell-free protein synthesizing systems (OCHOA and WEINSTEIN[12]). MITRA and KAESBERG[13] have shown that spermine brings about a compact tertiary structure in turnip yellow mosaic virus RNA.

In view of these facts, a brief study was undertaken into the effect of spermine on phage f_2 reproduction. Throughout these experiments, experimental procedures were used as described in detail elsewhere (SCHINDLER[1]). Spermine hydrochloride dissolved in distilled water was used.

First, the inhibitory effect of spermine on phage f_2 reproduction in *E. coli* K 13 Hfr was demonstrated: Bacteria growing in exponential phase in broth ($4 \cdot 10^7$ cells/ml) were infected with f_2 (0.3 phage particles per bacterium) with simultaneous addition of spermine. After 5 min, infective centres were titrated in a control flask without spermine. After 90 min, chloroform was added to all flasks and phage was titrated. In another experiment infected cells were centrifuged 5 min after infection and sedimented cells were resuspended in warm broth without spermine. Average yield of phage particles was calculated by dividing phage titre by infective centre titre. Table 1 shows that spermine inhibits phage development in concentrations ranging from 50–200 μg/ml (approximately $1.5 \cdot 10^{-4} M$ to $6 \cdot 10^{-4} M$). Although spermine inactivates about 30% of phage T 2 (MORA and YOUNG[14]), the inhibition of f_2 is not due to the interaction of spermine with

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free phage f_2 particles. After incubation of $1 \cdot 10^8$ plaque-forming units per ml in 0.5% peptone water with spermine at concentration 0.5, 10, 100, and 200 µg/ml respectively, at 37°C for 60 min, no drop in titre was observed.

Spermine does not inhibit the adsorption of phage f_2 particles significantly (Table II).

Table I. Inhibitory effect of spermine on the reproduction of bacteriophage f_2

Spermine µg/ml	Exposure min	Phage yield pfu/ml[a]	Relative yield
0	–	426	1.00
50	5	218	0.51
50	90	332	0.78
100	5	257	0.61
100	90	271	0.64
200	5	174	0.41

[a] Plaque-forming units per ml.

Table II. Adsorption of f_2 to *E. coli* K 13 in the presence of spermine

Spermine µg/ml	% of adsorbed pfu[a]	Relative value
0	96	1.00
0.5	81	0.84
10	84	0.88
100	78	0.81
200	82	0.85

[a] Plaque-forming units.

Table III. Development of phage f_2 in spheroplasts of *E. coli* K 13 infected by infectious RNA and subsequently exposed to spermine

Spermine µg/ml	Titre pfu/ml[a]	Relative titre
0	$1.6 \cdot 10^3$	1.00
5	$0.6 \cdot 10^3$	0.37
10	$0.6 \cdot 10^3$	0.37
100	$0.5 \cdot 10^3$	0.30

[a] Plaque-forming units per ml.

Table IV. Inactivation of infectious f_2 RNA by spermine

Spermine µg/ml	Infective centres per ml	Relative value
0	$1.1 \cdot 10^2$	1.00
5	$0.9 \cdot 10^2$	0.82
10	$0.4 \cdot 10^2$	0.36
100	$0.2 \cdot 10^2$	0.18

The effect of spermine on the phage development in spheroplasts infected with infectious f_2 RNA was investigated next. Infectious RNA was isolated by phenol extraction omitting ethanol precipitation (GIERER and SCHRAMM[15]). It contained 10^4 infectious units/ml. 0.2 ml of spheroplasts, prepared according to GUTHRIE and SINSHEIMER[16], was mixed with 0.2 ml of RNA. After 5 min at 37°C, when about 90% of spheroplasts (as against 15 min incubation) were infected, spermine was added. In control tubes, infected centres were assayed. After 90 min infected spheroplasts were osmotically shocked and freeze-thawed three times in acetone-dry ice mixture. Liberated phage was subsequently titrated. Microscopic control shows disruption of spheroplasts. The observed uniform drop in phage development independent of spermine concentration (Table III) suggests that the inhibitory effect perhaps occurs at a site which is blocked already at a low concentration of spermine.

In order to study the effect of spermine on the biological properties of phage RNA, 0.1 ml of RNA was mixed with 0.1 ml of spermine solution and incubated for 60 min at 37°C. Then 0.2 ml of spheroplasts was added and the infectivity of RNA assayed by scoring infective centres after 15 min incubation. Results are shown in Table IV. The infective activity of RNA is inhibited. The degree of inactivation depends on the concentration of spermine.

From these experiments it can be concluded that spermine, at concentrations from 0.5 µg/ml to 200 µg/ml, inhibits the development of bacteriophage f_2. It was shown that this inhibition is not caused by interaction with phage particles prior to their contact with the cell, and that spermine does not significantly inhibit adsorption to the host cell. Due to the susceptibility of phage RNA to spermine, it is probable that the interaction with phage RNA plays a most important role in the inhibitory activity of spermine. Spermine interacts directly with cytoplasmic membrane (TABOR[17], GROSSOWITZ and ARIEL[18]) and affects its permeability (NEČINOVÁ, VEREŠ, and BURGER[19]). The interaction with f_2 RNA could take place at this site at the moment of RNA release from the capsid. Spermine also apparently interacts with the intracellular development of f_2 in infected spheroplasts. No definitive explanation of the mechanism can be suggested, because spermine could perhaps interfere with RNA penetration into the spheroplast and/or with proper phage synthesis[20].

Résumé. La spermine a un effet inhibiteur sur la reproduction du phage f_2. Cette inhibition dépend de la concentration de la spermine. Ne diminuant pas le taux du phage libre et n'ayant pas d'effet sur l'adsorption, la spermine réduit l'activité de l'ARN infectieuse et la production du phage dans les sphéroplastes infectés.

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