does not cause swelling but a cytopathic effect, as slight cytoplasmic coartation, can be observed.

α-staphylotoxin-treated cells show a precocious and progressive cytoplasmic vacuolation (Figure 3). In these cells the mitochondria are not induced to swell but a mitochondrial fragmentation is often observable. In the control cultures any cytopathic effect or mitochondrial change occur.

Discussion. The effect of diphtheria toxin is comparable to that previously obtained in this laboratory[1-3].

O-streptolysin and α-staphylotoxin do not induce mitochondrial changes at these concentrations, but cause a cytopathic effect. This finding eliminates the doubt that the negative results concerning the mitochondria are due to a lack of penetration of these toxins into the cell. The cytopathic effect induced by these toxins is comparable to that described elsewhere[5-7]. Tetanus toxin does not induce any morphological change in the mitochondria but the lack of a cytopathic effect[7] does not allow the conclusion that the toxin penetrates into the cells. On the other hand, a mitochondrial swelling in the liver cells of tetanus toxin treated rats is described[4], while in mice treated with tritiated tetanus toxin the uptake of labelled toxin by the neurones is only occasionally reported[8].

In our experiments, nevertheless, the cells were entirely surrounded by the solution containing tetanus toxin, and it is probable that it reached the mitochondria in the cytoplasm and that tetanus toxin was then deprived of swelling properties in vivo[9].

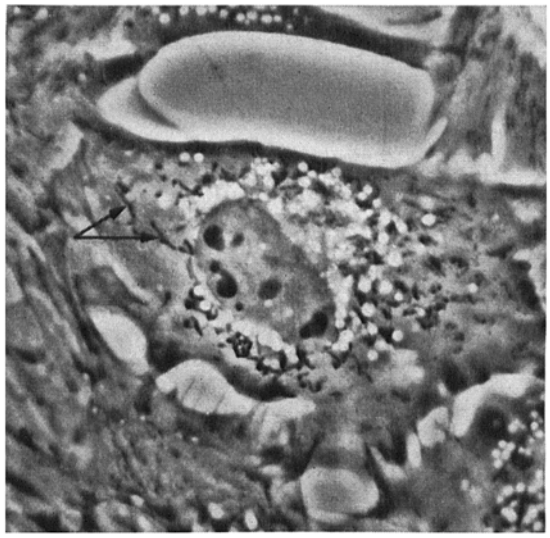


Fig. 3. α-Staphylotoxin treated cell (2,5 MLD/ml for 5 min). Note the cytoplasmic vacuolation and rod-like mitochondria (arrows). Phase-contrast microscope. About × 1000.

Riassunto. In precedenti lavori è stato studiato il rigonfiamento causato dalla tossina difterica nei mitocondri di cellule coltivate in vitro. Si è fatto un confronto fra l'azione della tossina difterica e quella di altre tossine batteriche come la O-streptolisina, la α-stafilotossina e la tossina tetanica sui mitocondri di cellule di cuore di embrione di pollo coltivate in vitro. Solo la tossina difterica ha manifestato capacità rigonfianti mentre le altre tossine, alle dosi usate e con questo tipo di cellule, sono apparse sprovviste di questa proprietà.

F. Paradisi[10]

Clinic of Infectious Diseases of the University, Napoli (Italy), 10 July 1967.

[5] G. Penso and G. Vicari, Rc. Ist. sup. Sanità *20*, 1109 (1957).
[6] G. Penso, P. Merucci and G. Vicari, Rc. Ist. sup. Sanità *22*, 1075 (1959).
[7] I. Gabliks and M. Solotorovsky, J. Immun. *88*, 505 (1962).
[8] A. A. Fedinec, Fedn Proc. Fedn Am. socs exp. Biol. *21*, 230 (1962).
[9] Acknowledgment: I wish to express my gratitude to Dr. Puccini of the Eli Lilly Co. and to Prof. De Barbieri of the Istituto Sieroterapico Milanese for the generous supply of bacterial toxins.
[10] Present address: Clinica delle Malattie Infettive, Università di Napoli, Ospedale Gesù e Maria, Via Cotugno 1, Napoli (Italy).

Structure Activity Relationships of Compounds able to Suppress the Antipolio Action of Guanidine

Previous work from our laboratories and from others has demonstrated that the influence of guanidine on poliovirus replication is antagonized by a number of compounds; 2 methyl-donors, methionine and choline, being the most active ones (Lwoff and Lwoff[1]; Loddo and Schivo[2]).

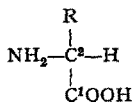
As a working hypothesis, Lwoff has suggested that guanidine would interfere with an essential methylation of a viral structure[3]. However, since it was found that the guanidine effect is antagonized by ethionine, by 2 demethylated methionine analogs, homocysteine and α-aminobutyric acid, and by the methyl-free choline analog ethanolamine[4], the methylating hypothesis was no longer tenable.

A structure-activity relationship of compounds able to suppress guanidine effect is attempted in this paper, in order to shed some light on the mechanism by which guanidine inhibits virus replication.

The techniques used were previously described in detail[5]. In Table I are listed, in decreasing order of potency, the amino acids found active against guanidine. In Table II the inactive ones are listed. As it appears from

[1] A. Lwoff and M. Lwoff, C. r. hebd. Séanc. Acad. Sci., Paris *259*, 949 (1964).
[2] B. Loddo and M. L. Schivo, Boll. Soc. ital. Biol. sper. *41*, 960 (1965).
[3] A. Lwoff, Biochem. J. *96*, 289 (1965).
[4] G. L. Gessa, B. Loddo, M. L. Schivo and A. Tagliamonte, Boll. Soc. ital. Biol. sper. *42*, 813 (1966).
[5] B. Loddo, G. L. Gessa, M. L. Schivo, A. Spanedda, G. Brotzu and W. Ferrari, Virology *28*, 707 (1966).

these Tables the basic structure essential for an antiguanidine action is the following:



that is: (a) amino acids must be L-isomers; the D-forms are inactive; (b) they must be α-aminated; (c) the H linked to C_2 cannot be substituted; (d) $-NH_2$ should not be included in a ring structure; (e) R must be constituted by at least 1 methyl group; it must contain neither basic nor acid groups. The presence of an OH group decreases or abolishes the antiguanidine property. The presence of an -SH radical allows an antiguanidine effect to occur when located in C 4 (homocysteine); on the contrary, it abolishes the antiguanidine effect when it is linked to carbon 3 (cysteine). Moreover, the antiguanidine effect is roughly proportional to the length of the C chain. Finally the antiguanidine effect is not restricted to natural amino acids: α-aminobutyric acid and ethionine are highly active.

Although the data presented in Table III are too scanty for a definite conclusion, the fact that L-α-alanilol and α-aminobutanol are highly active while L-β-alanilol is inactive, indicates that a structure similar to that present in the active amino acids is also present in the active amino alcohols.

Subsequently, we have compared the pattern of the antiguanidine action of the most active representative of the 2 groups of compounds: i.e. methionine and leucine, among amino acids; choline and ethanolamine among amino alcohols. Figure 1 shows that the slope of the lines expressing the dose activity relationships for amino acids is different from that for amino alcohols.

In fact, amino acids such as methionine and leucine possess a wider range in their antiguanidine effectiveness than amino alcohols, as choline and ethanolamine: i.e. amino acids maintain the ability to suppress the action of guanidine up to a guanidine concentration of $1.3 \times 10^{-3} M$, while amino alcohols (at the same molar concentrations) are able to antagonize only up to a guanidine concentration of $3.3 \times 10^{-4} M$. However, against a lower concentration of guanidine, amino alcohols hold their antiguanidine action to a much greater extent (Figure 2).

Table I. Suppression by amino acids of the inhibitory action of guanidine on the multiplication of polio 1 virus (BRUNHENDERS)

Amino acids	Lowest molar concentration capable of suppressing the inhibition of guanidine HCl $3 \times 10^{-4} M$ at 12 h[a]
L-methionine	3.2×10^{-5}
L-leucine	3.4×10^{-5}
L-homocysteine	3×10^{-4}
L-α-aminobutyric acid	4×10^{-4}
L-valine	3×10^{-4}
L-isoleucine	8×10^{-4}
L-ethionine	10^{-3}
L-phenylalanine	10^{-3}
L-tyrosine	3×10^{-3}
L-α-alanine	5×10^{-3}

[a] I.e. the concentration able to increase by at least 2 logs the plaque forming units (pfu) of cultures treated with guanidine.

The mechanism of action of guanidine antagonists is not clear. Several facts, however, suggest that antiguanidine compounds interact directly with the guanidine molecule. The action of guanidine can be suppressed indiscriminately by any of the several active compounds; i.e. they are in this effect interchangeable. The antiguanidines are able to suppress also the conditioning effect of guanidine for the growth of guanidine-dependent poliovirus[5].

Table II. Amino acids unable, at the maximum concentration tolerated by the cells, to antagonize guanidine HCl inhibition of poliovirus 1 multiplication (BRUNHENDERS strain)

Amino acids	Concentration used (μg/ml)
D-isomers:	
D-methionine	2000
D-leucine	2000
β- and γ-aminated:	
L-β-amino-butyric acid	1000
L-γ-amino-butyric acid	1000
α-methylated:	
L-α-methyl-tyrosine	1000
L-α-amino-isobutyric acid	2000
NH_2 cyclized:	
L-proline	2000
Orotic acid	1000
Glycocholbetaine	1000
R = H:	
Glycine	1000
Basic groups in R:	
L-lysine	1000
L-arginine	300
L-canavanine	20
L-asparagine	1000
L-Histidine	300
L-Tryptophane	200
Acid groups in R:	
L-glutamic acid	1000
L-aspartic acid	1000
L-serine	1000
L-homoserine	1000
-SH group in C_3:	
L-cysteine	300

Table III. Suppression by amino-alcohols of the inhibitory action of guanidine on the multiplication of polio virus 1 (BRUNHENDERS)

Amino alcohols	Lowest molar concentration capable of totally suppressing the inhibition of guanidine HCl ($3 \times 10^{-4} M$) at 12 h
Choline HCl	4×10^{-6}
Ethanolamine	4×10^{-6}
L-α-alaninol	1.5×10^{-5}
DL-α-amino-butanol	1.2×10^{-5}
β-alaninol	10^{-3}

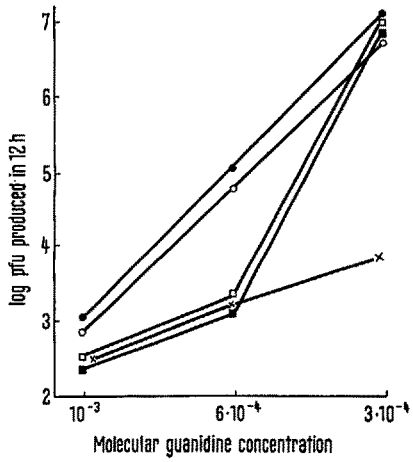


Fig. 1. Pattern of the antiguanidine effect of different compounds. Challenging a constant dose of antagonist against increasing guanidine concentrations. x——x guanidine; ●——● guanidine + L-methionine; o——o guanidine + L-leucine; □——□ guanidine + choline; ■——■ guanidine + ethanolamine. The concentration of the antagonists was $3.3 \times 10^{-4} M$. pfu, plaque forming units.

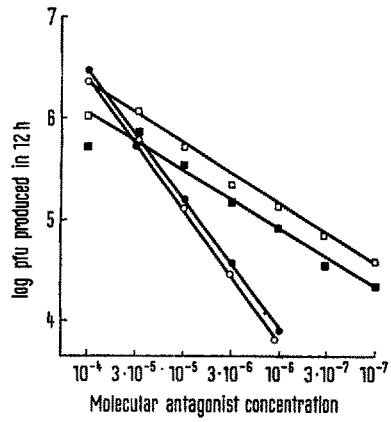


Fig. 2. Pattern of the antiguanidine effect of different compounds. Challenging increasing concentrations of each antagonist against a constant dose of guanidine. ●——● guanidine + L-methionine; o——o guanidine + L-leucine; □——□ guanidine + choline; ■——■ guanidine + ethanolamine. The guanidine concentration was $3.3 \times 10^{-4} M$. pfu, plague forming units.

Conversely, it is possible that guanidine might exert its antipolio action by reacting with the same structures present in an amino acid or in an amino alcohol essential for poliovirus growth (during replicase synthesis) but not for culture cells.

Consequently, a potential antipolio agent might be found among some analogs of those amino acids which most actively antagonize guanidine. It is very suggestive that a valine analog, D-penicillamine, inhibits very effectively and selectively polio virus growth[6,7].

Riassunto. L'inibizione da parte della guanidina sulla crescita del virus polio può essere antagonizzata da amino-acidi e amino-alcoli. Viene definita la struttura responsabile dell'effetto antiguanidinico negli amino-acidi attivi. Una struttura simile è presente anche negli amino-alcoli attivi. I risultati ottenuti suggeriscono che i composti antiguanidinici interagiscono con la molecola guanidinica sottraendola dai recettori connessi con l'effetto antivirale.

B. Loddo, G. L. Gessa,
A. Tagliamonte and W. Ferrari

Institutes of Pharmacology, University of Cagliari and Modena and Institutes of Hygiene, Virology and Microbiology, University of Cagliari (Italy),
24 April 1967.

[6] G. L. Gessa, B. Loddo, G. Brotzu, M. L. Schivo, A. Tagliamonte, A. Spanedda, G. Bo and W. Ferrari, Virology *30*, 618 (1966).

[7] This work was supported by the Consiglio Nazionale delle Ricerche, Roma.

The Influence of Chlorothiazide and Tolbutamide upon Intestinal Serotonin Levels in the Sprague-Dawley Rat

Rats pretreated with sulfamerazine and several antibiotics develop increased serotonin levels in some areas of the gastrointestinal tract[1-3]. The mechanism(s) of this increase is unknown, but it has been suggested that lumenal sterilization is responsible[2,3]. There are 2 possible mechanisms by which lumenal organisms could alter tissue serotonin levels. Firstly, amines may undergo an enterohepatic circulation with subsequent destruction by intestinal bacteria[4]; and secondly, bacterial utilization of dietary amino acids may reduce the availability of these amine precursors for tissue decarboxylase. However, it seems unlikely that the elevated serotonin levels observed following sulfamerazine are due primarily to an antibacterial effect because, the addition of sulfasuxidine to the diet leads to a greater recovery of intestinal serotonin in tryptophan deficient rats compared to controls[5]. Furthermore, serotonin levels following sulfamerazine are not uniformly elevated in those bowel areas normally inhabited by micro-organisms[1], and in man, urinary tyramine and tryptamine have been shown to be of tissue rather than of bacterial origin[4]. In order to study the possibility that the increased mucosal serotonin levels following sulfamerazine are due to a non-specific effect

[1] J. H. Thompson and L. B. Campbell, Nature *212*, 850 (1966).
[2] R. S. Stacey and T. J. Sullivan, J. Physiol. *137*, 63P (1957).
[3] T. J. Sullivan, Br. J. Pharmac. *16*, 90 (1961).
[4] V. L. DeQuattro and A. Sjoerdsma, Clinica chim. Acta *16*, 227 (1967).
[5] E. M. Gal and P. A. Drewes, Proc. Soc. exp. Biol. Med. *110*, 368 (1962).