NEW LIMITATION CHANGE

TO
Approved for public release, distribution unlimited

FROM
Distribution authorized to U.S. Gov't. agencies and their contractors; Critical Technology; NOV 1965. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Releases Branch, Frederick, MD 21701.

AUTHORITY
Fort Detrick/SMUFD ltr dtd 14 Feb 1972

THIS PAGE IS UNCLASSIFIED
TRANSLATION NO. 1565

DATE: 29 NOVEMBER 1965

DDC AVAILABILITY NOTICE

Reproduction of this publication in whole or in part is prohibited. However, DDC is authorized to reproduce the publication for United States Government purposes.

STATEMENT #2 UNCLASSIFIED

This document is subject to special export controls and each transmittal to foreign government or foreign nationals may be made only with prior approval of

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

with Tech Release Sec 720
THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.
NEUTRALIZATION BY VARIOUS METABOLITES OF THE
INHIBITORY EFFECT OF GUANIDINE ON THE
DEVELOPMENT OF POLIOVIRUS

[Following is a translation of a report by
A. Lwoff and M. Lwoff of the Microbial Phy-
siology department of the Pasteur Institute,
(presented by J. Trefouel) in the French-
language journal Comptes Rendus de l'Academie
des Sciences, Paris (Reports of the Academy
1964, pages 948-952.]

The development of type I poliovirus in
Earle's medium is totally inhibited by 3 ×
10⁻⁴M guanidine. Choline, methionine and
valine neutralize this effect the mechanism
of the action of these antiguanidines is dis-
cussed below.

Guanidine-requiring strains of poliovirus do not de-
velop in the absence of guanidine which can be replaced by
substance possessing this radical⁴. In cells infected with
guanidine⁵-requiring poliovirus, the viral RNA-replicase does
not appear in the absence of guanidine, which is thought to
contribute to the achievement of a tertiary or quarternary
structure of the enzyme⁶.

The original strains of poliovirus are inhibi-
ted by
guanidine, which prevents the formation of the viral RNA-
replicase⁷. If cellular metabolites intervene in this in-
formation, as they do in the structure of phosphorylase or
in glutamic acid dehydrogenase, the blockage of viral devel-
optment by guanidine is perhaps due to an inhibitor-effector
competition. We have tried to find evidence for substances
capable of diminishing the inhibitory effect of guanidine.
KB cells cultivated in lactalbumin medium enriched with autolysate of yeast and serum were washed, infected with poliovirus of type I (original strain LSc 2 ab of Sabin), and after elimination of non-adsorbed virus, were put in suspension in nutritive media containing extract of bovine embryo at a final concentration of 2%. The kinetics of viral development during the course of one full cycle was established by counting the cell units forming plaques.

The inhibitory action of guanidine varies according to the type of medium used. A concentration of $3 \times 10^{-4}$M guanidine blocks development in Earle's medium (yield 0.1 to 0.01% of the control). In medium consisting of lactalbumin + hydrolysate of yeast, the inhibition is much less marked and above all more irregular. The addition to Earle's medium of yeast autolysate at a concentration of 0.3 to 0.6% permits a respective yield of [illegible]. Finally, in modified Eagle's medium, called "4EM" (concentration of vitamins and amino acids four times that of the original mixture), the yield is 100%; there is no inhibition in this case.

We then studied the effects of the constituents of Eagle's medium on the development of poliovirus in Earle's medium with added guanidine, the concentration of the various substances being the same as in the "4EM" medium. The results are shown in the figure below and the table gives the percentage yield as compared to the controls.

<table>
<thead>
<tr>
<th>Guanidine $3 \times 10^{-4}$M</th>
<th>0.1 - 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>$+$ L(+)-valine $1.5 \times 10^{-3}$M</td>
<td>11</td>
</tr>
<tr>
<td>$+$ L(-)-methionine $4 \times 10^{-4}$M</td>
<td>21</td>
</tr>
<tr>
<td>$+$ choline $3 \times 10^{-5}$M</td>
<td>30</td>
</tr>
</tbody>
</table>

The L(-)-leucine at $1.5 \times 10^{-3}$ showed a feebler activity (Rdt 1%). Similarly, L(+)-alanine at $4 \times 10^{-3}$ gives feebler activity (Rdt 4%), but only in the presence of the vitamins in Eagle's medium. The other constituents of the medium (group B vitamins, hypoxanthine, amino acids other than those already mentioned) are devoid of antiguanidine activity under the conditions of these experiments. However, "4EM" medium, while antiguanidine in its full complement, is a mixture of inhibitory and anti-inhibitory substances. It appeared necessary thus to examine the effects of the constituents at varying concentrations to exclude the possibility of inhibitory action caused only by the isolated
Kinetics of the poliovirus development at 36° in Earle's medium + embroyic extract.

Abscissas: Hours after infection; ordinates: units forming plaques per an illiliter, in log-
arithmetic coordinates. O = control; g = guanidine, 3x10^{-4}M; chol-cholmic 3.3x10^{-5}M; meth-
L(-) methionine 4x10^{-4}M; val = L(t)- valine 1.5x10^{-3}M.

components. Note that methionine, which is antiguanidine in Earle's medium at a concentration of 4 x 10^{-4}M, complete-
ly inhibits poliovirus development at a concentration of 2 x 10^{-3}M.

Thus certain constituents of cell culture media dimin-
ish the inhibitory action of guanidine, and the problem of
determining the mechanism of this effect is one which quite
naturally comes to mind. Two hypotheses among others can be
envisaged. The first is that antiguanidine substances com-
bine with the guanidine and form a less active complex. KB
cells can, for example, methylate the guanidine in the pre-
sence of an excess of choline or methionine. This supposi-
tion is not in contradiction with the fact that guanidine is
not metabolized by the animal organism under normal condi-
tions. The second hypothesis is that antiguanidines repre-
sent either effectors or substances intervening in the syn-
thesis of effectors of viral development. These effectors

- 3 -
play the same role for the susceptible strain as does guanidine for the guanidine-requiring strain. Current experiments should permit us to choose between the two hypotheses. It was known that one substance favored the development of poliovirus: semi-carbazide. This compound augments the rate of development in Earle's medium, doubling time 9 min. instead of 14, and also the yield of units forming plaques. It does not have, as shown in the table below, an ability to neutralize the inhibitory effect of guanidine, nor does it affect the antiguanidine action of choline. The yields are shown below (the numbers of represent the percentage yield as compared to the control):

<table>
<thead>
<tr>
<th>Substance</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semi-carbazide 2.10^{-2}M</td>
<td>333</td>
</tr>
<tr>
<td>Guanidine 3.10^{-4}M</td>
<td>0.03</td>
</tr>
<tr>
<td>Semi-carbazide + guanidine</td>
<td>0.07</td>
</tr>
<tr>
<td>Choline 3.3,10^{-4}M</td>
<td>72</td>
</tr>
<tr>
<td>Choline + guanidine</td>
<td>8</td>
</tr>
<tr>
<td>Semi-carbazide + choline</td>
<td>445</td>
</tr>
<tr>
<td>Semi-carbazide + choline + guanidine</td>
<td>110</td>
</tr>
</tbody>
</table>

Whatever the mechanism may be of the action of choline, methionine and valine, it does not remain unless normal cellular metabolites neutralize the inhibitory effect of guanidine on the sensitive strain's development. It was previously known that normal cellular metabolites, such as arginine, glycine, creatine and glutamine assure the development of the guanidine-requiring strain. It was also known that normal cellular metabolites, such as arginine and glycine, inhibit the development of the guanidine-sensitive strain of poliovirus, while they are without effect on the guanidine-resistant strain. Thus a number of essential metabolites influence the development of poliovirus in one way or another. Their action depends, of course, on the genetic composition of the virus, on the intracellular concentration of the metabolites concerned. This is probably a function of the nature and physiological state of the cells, of the constituents of the medium, the modifications of the medium provoked by cellular metabolism, that is to say, particular parts of the metabolism, of the cell concentration, the duration of the experiment and finally the perturbations of cellular metabolism caused by the viral infection itself. Any analysis of the results must take into consideration the multiplicity of factors and their possible interaction.

Finally, it is necessary not to forget, during the course of chemo-therapy assays in vitro and in vivo, that
essential metabolites can diminish or accentuate the effect of certain antiviral agents.

Presented at the meeting on 15 July 1964. This study was supported by aid from the "National Foundation" of the United States and the general Delegation for scientific and technical Research.

Notes


