TESTING OF CHEMICALS FOR INHIBITION OF THE KILLER ACTION OF PARAMECIUM AURELIA*

BY MARY WILLIAMSON, WINIFRED JACOBSON, AND C. CHESTER STOCK

(From the Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York, New York)

(Received for publication, January 24, 1952)

Nearly 100 chemicals, chiefly purines and pyrimidines and a few antibiotics, have been tested for their ability to interfere with the killing action of *Paramecia* and to produce sensitives from killer animals, presumably through inactivation of kappa. Kappa, a cytoplasmic factor in certain stocks of *Paramecium aurelia*, dependent upon, but not initiated by, the dominant allele of the killer gene, is known to be involved in the production of the antibiotic paramecin (1-3). Sensitive stocks of *Paramecia* with the recessive killer gene, which therefore cannot maintain kappa, and those killer animals depleted of kappa are affected by paramecin and ultimately die in its presence (1).

The advantages of using Protozoa as test animals in testing essential nutrients and their analogues for effects on sensitivity, locomotion, growth, reproduction, and survival have been emphasized previously (4, 5). *Paramecia* with kappa, an independently multiplying cytoplasmic entity containing desoxyribonucleic acid (6, 7), appeared to offer a system for demonstrating possible selective interference with nucleic acid metabolism.

Inactivation of kappa by high temperature (8), x-rays (9), nitrogen mustards (10), streptomycin (11), and Chloromycetin (12) has been reported previously. This paper reports a survey of compounds for possible ability to decrease the content of active kappa in *Paramecia* as detected by decreased killing ability. Observation of the detrimental effects of 2,6-diaminopurine on the killing ability (13) encouraged inclusion in this study of many compounds which might be expected to influence nucleic acid metabolism.

Materials and Methods

The test organism was *P. aurelia*, variety 4, stock 51 killer, mating type VII (51.7K), cultured in Sonneborn’s baked lettuce infusion inoculated

* This work was supported from a grant by the Charles F. Kettering Foundation to the Wellcome Research Laboratories and by a grant to the Sloan-Kettering Institute from the American Cancer Society. We are greatly indebted to Dr. George B. Brown, Sloan-Kettering Institute, and to Miss G. B. Elion and Dr. George H. Hitchings, Wellcome Research Laboratories, for helpful discussions in addition to provision of many of the compounds used in the study.
with *Aerobacter aerogenes* (11). Semi-starved *Paramecia* grown at two fissions per day (6) for 3 or 4 days prior to exposure to the test chemicals were found to be less susceptible to their toxicity than were well fed animals dividing at the maximum rate of five to six fissions per day. The salt solution described by Taylor and van Wagtendonk for *Colpoda duodenaria* (14) was used in making all dilutions.

The test materials were dissolved in the salt solution at a concentration of 2000 γ per ml. and the pH adjusted to 6.4 to 7.0. Salt solution and culture fluid were used to dilute the solutions to 1500, 1000, 500, 200, and 100 γ per ml. The culture fluid throughout the tests constituted one-fourth the total volume. The tests were run in triplicate by adding ten to twenty killer animals contained in a micro drop (0.01 ml.) to 0.2 ml. of each dilution of the test chemical. If the concentrations of the chemical were toxic, the experiments were repeated with lower levels of 100, 50, 25, 10, and 5 γ per ml.

After incubation at 27° for 24 hours with the chemicals, the animals were observed for changes in locomotion, general morphology, growth, and reproduction. A check on pH was made in each test with brom thymol blue to determine that none of the observed effects were due to appreciable changes in pH. Accordingly, single animals from each of the three highest tolerated levels of test chemical were isolated into fresh culture medium and allowed to reproduce at the maximum rate for 48 hours, i.e. eight to ten fissions at 27°. The resultant clones, the progeny of the single isolations (15), were examined for changes mentioned above.

Clones from treated animals were often a fission or two behind control clones. It was necessary to isolate from the three highest tolerated levels of the chemicals because sometimes animals surviving the highest level failed to reproduce or produced weak, non-viable clones. After these observations, the clones were subjected to the "killer-sensitive" test (11, 16) to detect changes in killing ability effected by the chemical on the parent *Paramecia*.

The killer-sensitive test was conducted as follows: The clones to be tested were divided into three parts: to the first part (A) were added known killer animals, to the second part (B) were added known sensitive animals, and to the third part (C) were added a few drops of culture fluid for the control. After incubation for 24 hours at 27° the killer-sensitive tests were read. If killing action were found in (A), part or all of the clone being tested had become sensitive due to decrease in effective kappa as a result of exposure of the parent to the chemical. Killing in (B) alone would indicate that the chemical had no detectable effect on the original animals. If killing were observed in (A), (B), and (C), the control, the clone contained both killers and sensitives and the compound was presumed to have
M. WILLIAMSON, W. JACOBSON, AND C. C. STOCK

some effect on kappa. The progeny of the untreated controls were tested in like manner to insure that chemical treatment alone, and not other conditions of the test, had altered paramecin production. These control animals remained good killers in every series of tests.

EXPERIMENTAL

It was found that the first compound tested, 2,6-diaminopurine, SK1395, has the ability to inhibit the killing action of 51.7K Paramecia (13). Further studies revealed that the effect could be obtained in 24 hour exposures to SK1395 in concentrations from 100 to 1000 γ per ml. The animals are killed at 1500 γ per ml. and abnormal and weak ones are observed after exposure to 1000 γ per ml. Quantitative, more detailed studies of the effects of SK1395 will be presented elsewhere. When the striking effect of SK1395 was observed, the studies were extended to numerous purines and pyrimidines. Also included were various antibiotics and miscellaneous compounds. The results of the tests of these materials, presented in Table I, emphasize the high degree of specificity of the action of 2,6-diaminopurine. The lack of effect of 7-amino-1-α-triazolo(d)pyrimidine illustrates this. Another observation of interest but of obscure significance is the marked difference in toxicity to the Paramecia of the D and L forms of 9-α-arabofuranosyladenines, 25 γ per ml. compared to 1500 or more.

The inhibition of killer ability with streptomycin confirmed previous findings (11). It is active from 50 to 1000 γ per ml., while dihydrostreptomycin is active from 25 to 1500 γ per ml. A report of the activity of Chloromycetin (12) appeared at the time of our independent observations of its effect on the lethal action of killers at concentrations of 500 and 1000 γ per ml. Terramycin inhibits the killing ability at 100 to 200 γ per ml., whereas the sample of Aureomycin was active at 10 to 25 γ per ml.

In view of the fact that A. aerogenes provided an essential food for the Paramecia, it was necessary to determine whether 2,6-diaminopurine and the antibiotics were affecting the bacteria and in this manner were indirectly influencing the lethal properties of the killers. Experiments were carried out, therefore, to determine the influence of SK1395 and the antibiotics tested separately on the bacteria under conditions similar to those employed in the test for effects on kappa. Sterile culture medium containing 500 γ of SK1395 per ml. was inoculated with 0.1 ml. of a 24 hour culture of A. aerogenes. The culture was incubated for 24 hours at 27° and a heavy growth produced. Similar cultures containing the antibiotics individually at the highest levels not toxic to Paramecia also produced heavy growths of the bacterium. Colony counts of serial dilutions of the

Table I
Compounds Tested for Ability to Decrease Killing Action of *P. aurelia*, Variety 4, Stock 51.7 Killers

<table>
<thead>
<tr>
<th>Compound*</th>
<th>Highest level tolerated†</th>
<th>Effect on <em>Paramecium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>2,6-Diaminopurine</td>
<td>1000</td>
<td>Inhibits killing action from 100-1000 γ per ml.; produces weak and abnormal forms at 1000 γ per ml.</td>
</tr>
<tr>
<td>Adenine sulfate</td>
<td>500</td>
<td>May stimulate growth</td>
</tr>
<tr>
<td>Adenosine</td>
<td>T.</td>
<td>None observed</td>
</tr>
<tr>
<td>9-α-D-Arabofuranosidyladenine</td>
<td>25</td>
<td>May stimulate growth</td>
</tr>
<tr>
<td>9-α-L-Arabofuranosidyladenine</td>
<td>T.</td>
<td>None observed</td>
</tr>
<tr>
<td>Adenyl acid (yeast, α and β)</td>
<td>“ “</td>
<td>May stimulate growth</td>
</tr>
<tr>
<td>“ “ (muscle, 5-PO₄)</td>
<td>“ “</td>
<td>None observed</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>1000</td>
<td>Inhibits killing action 50-1500 γ per ml.</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>500</td>
<td>Inhibits killing action 25-1500 γ per ml.</td>
</tr>
<tr>
<td>Dihydrostreptomycin</td>
<td>25</td>
<td>Inhibits killing action 10-25 γ per ml.</td>
</tr>
<tr>
<td>Aureomycin</td>
<td>500</td>
<td>Inhibits killing action 200-500 γ per ml.</td>
</tr>
<tr>
<td>Chloromycetin</td>
<td>T.</td>
<td>May slow growth slightly</td>
</tr>
<tr>
<td>Neomycin</td>
<td>200</td>
<td>Inhibits killing action 100-200 γ per ml.</td>
</tr>
</tbody>
</table>

* The following additional compounds at the levels indicated in parentheses (in micrograms per ml.) have shown no effect on the killing action: 2,6-diaminopurines substituted as follows, 7-methyl (T.); 9-p-nitrophenyl (T.); 6-dimethylamino (200); 8-methyl, chloro, thio, methylthio, amino, and iodo all at (T.) and p-chlorophenyl at 500; other purines, 6-methylaminopurine (1000); 2,8-dichloroadenine (T.); 2,8-dichloro-9-α-L-arabofuranosidyladenine (T.); 2-chloroadenosine (T.); 2,8-dichloroadenosine (T.); 2-chloro-9-β-D-glucopyranosidoadenine (T.); 2,6,8-trichloropurine (T.); 2-thiol-6-aminopurine (T.); guanylic acid (T.); 2-amino purine HCl (500); isoguanine sulfate (T.); isoguanine riboside (crotonoside) (T.); 1-v-triazoloprimidines; 7-amino (1000); 5-amino-7-hydroxy (T.); 5,7-diamino (T.); 8,4-diaminopyrimidines, 5-chloroaacetamido (100); 5,6-dimethyl (100); 5-p-hydroxyphenoxo (500); 5-methyl (1000); 6-phenyl (50); 2-amino-6,8-dimethylpyrimidines, 4-p-carboxyanilino (T.); 4-p-hydroxyanilino (200); 4-p-chloroanilino (5); other pyrimidines, 2-hydroxy-4,6-dimethyl (T.); 2,4,6-trihydroxy (barbituric acid) (T.); 2,4,5-trihydroxy (iso-barbituric acid) (T.); 2,4,6-trichloro (5, toxic); 2,4-dihydroxy-6-methyl (T.); 2-amino-4-methyl-6-sulfanilyl (500); 2-chloro-4-dimethylamino-6-methyl (castrix) (1000); 2-amino-4-hydroxy-5,6-dimethyl (500); 2-thiothymine (T.); 2-thiol-4-amiu (thiocytosine) (T.); 5-bromo-isocytosine (T.); cytidyllic acid (500); 2-amino-6-methyl-4-pyrimidone (T.); substituted uracils, 5(dimethylaminocetamino) (T.); 5-(β-phenylethylaminoacetamino) (T.); 5-formylamino (T.); 5-iodo
TABLE I—Concluded
(T.); 5-amino (T.); 5-bromo (T.); 5-nitro (T.); 5-(glucosidamino)uracil; p-amino-
benzoylglutamic acid (T.); 5-bromosacetamido (200); thiouracil (T.); miscellaneous,
ribose nucleic acid (T.); desoxyribose nucleic acid (calf thymus) (T.); folic acid
(T.); 4-aminofolic acid (T.); 4-amino-\textit{N}^{10}\text{-methyl}folic acid (T.); \textit{other pteridines},
2,4-diamino-7-hydroxy-6-carboxylic acid (500); 2,4,6,7-tetrahydroxy (1000); 2-sulfa-
nilamido-4,6,7-trihydroxy (T.); 2-sulfanilamido-4-hydroxy-6,7-diphenyl (500). For
the above compounds we are indebted to the Calco Chemical and Lederle Labora-
tories Divisions of the American Cyanamid Company, Chas. Päzer and Company,
National Research Council Chemical-Biological Coordination Center, Merck and
Company, Inc., Parke, Davis and Company, The Squibb Institute for Medical Re-
search, Southern Research Institute, Dr. C. K. Cain, McNeil Laboratories, Inc.,
Dr. G. H. Hitchings and Miss G. B. Elion, Wellcome Research Laboratories, Dr. G.
B. Brown and associates, Sloan-Kettering Institute for Cancer Research.
† Highest level tolerated or 1500 μ; highest level tested (T.).

cultures indicated no significant effect of SK1395, nor of the antibiotics
in the concentrations employed, in decreasing the number of organisms
below that of a normal culture. Any possible qualitative influence upon
the capacity of the organisms to serve successfully as an essential food
for the \textit{Paramecia} remains to be explored.

The marked disturbance of the killer action by 2,6-diaminopurine made
it of interest to test a few natural substances for ability to block the effect.
With the exception of one detail, the blocking experiments were conducted
in the same manner as those already described for testing the ability of
chemicals to interfere with the killer action. In the blocking experiments
the test material was added simultaneously with the 2,6-diaminopurine.
The test materials were tried in concentrations of 500, 200, 100, 50, 25, 10,
and 5 μ per ml. against 500 μ per ml. of SK1395. Controls were main-
tained by (1) exposing animals to these levels of test materials in the
absence of SK1395, (2) exposing them to SK1395 in the absence of other
test materials, and (3) retaining them in the salt solution-culture fluid
mixture in the proportion used as a solvent for test materials. After
incubation for 24 hours, isolated animals were allowed to undergo maximum
fissions for 48 hours, and the killer-sensitive tests subsequently run. A
number of materials were found to block the action of SK1395. These
are presented in Table II. Controls exposed under type (1) conditions
did not show a decrease in killer action. Indeed, it was thought their
killing power had been enhanced following exposure to adenine and its
derivatives. Controls under type (2) conditions showed a characteristic
decrease in killing ability with some sensitives produced, while type (3)
controls maintained average killing ability.

The inhibitory action of 2,6-diaminopurine and its blocking by adenine,
adenosine, and the adenylic acids are quite consistent with earlier obser-
vations in certain biological systems. SK1395 has been found to have inhibitory effects on certain bacteria (17, 18), on vaccinia virus in chick embryonic tissue culture (19), in tissue cultures of mammalian cells (20, 21), and to be effective against a mouse leukemia (22). Lactobacillus casei grown in a medium containing folic acid, but not adenine, is inhibited by 2,6-diaminopurine. The inhibitions from low concentrations of 2,6-diaminopurine are blocked by either guanine or adenine, while only the latter was effective against larger amounts of the inhibitor (18). Biese- sele et al. (20, 21) have reported damage to tissue cultures of mouse sarcomas by 2,6-diaminopurine with concentrations that show very slight or no damage to cultures of embryonic mouse skin or heart cells. Added adenine blocked the damaging actions of the diaminopurine.

**TABLE II**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Approximate molar ratio of compound to 2,6-diaminopurine for blocking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine</td>
<td>1:100</td>
</tr>
<tr>
<td>Adenosine</td>
<td>1:30</td>
</tr>
<tr>
<td>Adenylic acid, yeast</td>
<td>1:5 or 10</td>
</tr>
<tr>
<td>&quot; &quot; muscle</td>
<td>1:15 or 25</td>
</tr>
<tr>
<td>Guanylic acid</td>
<td>No effect at 2:1</td>
</tr>
<tr>
<td>Desoxyribonucleic acid</td>
<td>200 γ per 500 γ*</td>
</tr>
<tr>
<td>Ribonucleic acid</td>
<td>1000 &quot; &quot; 500 &quot;</td>
</tr>
</tbody>
</table>

*Weight ratios.

The blocking of the biological effects of diaminopurine by nucleic acid derivatives other than adenine varies considerably from system to system. Thus in the present experiments the adenylic acids and adenosine were found effective, although appreciably less active than adenine on a molar basis, whereas guanylic acid is inactive. The action of ribonucleic acid and desoxyribonucleic acid could possibly be due to their polynucleotide adenine content or to the presence of small amounts of impurities, although the quantities effective do not suggest the latter. In the experiments of Bieseles et al. (21) with normal and malignant rodent tissues only free adenine was effective, while in the experiments with vaccinia virus-chick embryonic tissue (19) both adenylic and guanylic acids were active. In view of the conversion of 2,6-diaminopurine to nucleic acid guanine in the rat (23, 24) and its ready utilization for both pentose nucleic acid adenine and guanine in L. casei (25) under conditions permitting growth, it is not possible to formulate a single mechanism for its inhibitory effects. Dimin-
ished killer action of Paramecia appears to be due to a decrease in effective kappa. The failure of 7-amino-1-ν-triazolopyrimidine, an antimetabolite of adenine (26), to affect the killer activity raises a question of whether 2,6-diaminopurine can be acting to prevent the utilization of adenine to form new kappa. Unpublished data indicate no decreased effectiveness of paramecin in the presence of 2,6-diaminopurine and, further, that the amount of kappa detectable by staining procedures is considerably reduced after exposure of the Paramecia to 2,6-diaminopurine. Possibly this may result from the formation of kappa with an abnormal purine composition and a resultant increased breakdown of kappa.

SUMMARY

Among nearly seventy purines and pyrimidines tested, 2,6-diaminopurine uniquely exhibits the ability to interfere with the killer action of variety 4, stock 51 killer, mating type VII, Paramecium aurelia. The action of 2,6-diaminopurine is blocked by adenine, adenosine, and adenylic acid. Several antibiotics also affect the killer action.

BIBLIOGRAPHY

6. Preer, J. R., Jr., Genetics, 33, 349 (1948).
TESTING OF CHEMICALS FOR INHIBITION OF THE KILLER ACTION OF PARAMECIUM AURELIA
Mary Williamson, Winifred Jacobson and C. Chester Stock


Access the most updated version of this article at http://www.jbc.org/content/197/2/763.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/197/2/763.citation.full.html#ref-list-1