Ethidium bromide (3,8-diamino-5-ethyl-6-phenyl phenanthridinium bromide) has been shown to inhibit completely the incorporation of preformed purines into the nucleic acids of Ehrlich ascites carcinoma cells, but the drug does not appreciably inhibit the incorporation of radioactive glycine into nucleic acids or proteins (4). These studies have been interpreted in terms of disruption of the normal relationship between intracellular pools of purine ribonucleotides synthesized from endogenously supplied purines or from purine ribonucleotides synthesized endogenously, although the detailed mechanism remains obscure. A combination of ethidium bromide and azaserine, an agent which inhibits purine biosynthesis de novo (7), prolonged the survival time of mice given implants of Ehrlich ascites carcinoma by 400 per cent, and 50 per cent of the mice so treated were free of tumor at 50 days (5). Ethidium bromide has been shown not to be metabolized by mouse tissues and tumors and hence is itself the active inhibitor (6).

A large number of phenanthridinium derivatives have been synthesized and tested for their antitrypanosomal (1), antibacterial (8), and antiviral (9) activity. In the present study, ten phenanthridinium compounds in addition to ethidium bromide have been tested for their effects on the incorporation of adenine into nucleic acids, and of glycine into proteins and nucleic acids in Ehrlich ascites carcinoma cells in vitro. It was hoped to establish the relationship between chemical structure and inhibition of these reactions and to attempt to predict the antitumor activity of these compounds (in combination with azaserine) on the basis of these in vitro tests.

**MATERIALS AND METHODS**

The phenanthridinium compounds used in this study were gifts of Dr. M. R. Gurd, Boots Pure Drug Co., Ltd., Nottingham, England. They are the following (together with their trivial names or code numbers): 3,8-diamino-5-methyl-6-phenylphenanthridinium bromide (dimidium); 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide (ethidium); 3,8-diamino-6-phenyl-5-propylphenanthridinium bromide (14927); 5-allyl-3,8-diamino-6-phenylphenanthridinium bromide (1446); 3,8-diamino-6-ethyl-5-methylphenanthridinium bromide (92610); 3,8-diamino-6-p-aminophenyl-5-methylphenanthridinium bromide (1887); 3,8-diamino-6-(4-dimethylaminostyryl)-phenanthridine 5,4′-dimethoxynaphthalate (929289); 3,8-diamino-6-decyloxy-5-ethyl-5,6-dihydro-6-phenylphenanthridine (8516); 3-amino-8-(6-amino-6-methyl-4-pyrimidinylamino)-6-p-aminophenylphenanthridine 5,1′-dibromide.
(prothidium, 2801); 2-(2-amino-1,6-dimethyl-4-
pyrimidinylamino)-6-methylphenanthridinium di-
methosulphate (2988). Adenine-8-C\(^{14}\) of specific
activity 0.62 mc/mmole was purchased from Iso-
tope Specialties, Inc., and glycine-2-C\(^{14}\), 1.2 mc/
m mole, from Volk Radiochemicals, Inc. All com-
 pounds were dissolved in 0.154 M sodium chloride
for use.

Female ICR Swiss mice, 20–25 gm., were given
intraperitoneal inoculations of approximately
1 \( \times \) 10\(^6\) Ehrlich ascites tumor cells (hypotetra-
ploid line) and used 5–6 days thereafter. Tumor
cells were removed by capillary pipette after laparotomy, and the ascitic fluid was removed by centrifu-
gation. Without further washing, 24 mg.
wet weight of cells were added to 2 ml. of calcium-
free Krebs-Ringer phosphate medium, pH 7.4,
containing 5.5 mM glucose, in 10 ml. Erlen-
meyer flasks. The flasks were incubated, with
shaking, at 37\(^{\circ}\) C. in an air atmosphere. When
glycine-C\(^{14}\) was the precursor used, 1 mm gluta-
tamine was added, as it has been shown to be limit-
ing for de novo purine synthesis in these cells (4).
Glutamine had no effect on the incorporation of
the other precursors tested.

The uptake of isotope into tumor cells was
measured by the membrane filtration technic (4).
After a 5-minute equilibration period the radioac-
tive precursor under study, with or without drug,
was added. At frequent intervals thereafter dupli-
cate 0.1-ml. samples were removed and inactivated
with 0.4 M perchloric acid. This suspension
was then filtered through Schleicher and Schuell
coarse membrane filters, the acid-insoluble frac-
tion being retained on the membrane. This was at-
tached to a planchette with rubber cement, air-
dried, and its radioactivity was measured. For
glycine, which is incorporated into both nucleic
acids and proteins, one set of 0.1-ml aliquots was
treated as described above and was a measure of
the total isotope incorporation into the acid-in-
soluble fraction. Another set of aliquots was heated
with 0.4 M perchloric acid at 100\(^{\circ}\) C. for 30 minutes
to hydrolyze the nucleic acids. The protein was
then collected on membrane filters, and its radio-
activity was measured. The radioactivity in the
nucleic acids was calculated by difference. The
average deviation between duplicate samples was
approximately 6 per cent. Each experiment has
been repeated twice, and the results reported here
are from a representative experiment. Radioactiv-
ity measurements were made in a windowless gas-
flow counter and were made to less than 10 per
cent error.

RESULTS

The effects of ethidium bromide and ten struc-
turally related compounds on the incorporation of
radioactive adenine into the perchloric acid-in-
soluble fraction of Ehrlich ascites carcinoma cells
are shown in Charts 1–3. Because ethidium brom-
ide had been shown to inhibit this process com-
pletely within 15 minutes at a concentration of
2.5 \( \times \) \( 10^{-4} \) M (4), each new compound was tested at
three concentrations—2.5, 1.25, and 0.625 \( \times \)
\( 10^{-4} \) M—in order to determine potency relative to
that of ethidium bromide. Because inhibition of
adenine incorporation by ethidium bromide is not
linear from the beginning of the incubation but ex-
hibits a definite delay before the onset of maxi-
mum inhibition (4), measurements were made at
intervals during the course of the 1-hour incuba-
tion to determine lag periods. The effect of each
compound on the incorporation of radioactive
glucose into nucleic acids and into proteins was
tested at a concentration of 2.5 \( \times \) \( 10^{-4} \) M, and the
results have also been incorporated into Charts
1–3.

For convenience in presenting these data, the
drugs have been divided into three groups on the
basis of chemical structure, and each group is pre-
 sented in a separate chart. Group I contains ethid-
ium bromide and three very closely related homo-
logs, whereas Group II consists of more diverse
variants. Group III contains two phenanthridin-
um nuclei linked to a 1,6-dimethyl-4-amino-
pyrimidinium salt, which is a component of an-
other active trypanoside, antrycide (2). For
purposes of comparison, the activity of each com-
pound against Trypanosoma congolense in mice\(^{1}\) has
also been indicated on these charts.

Only compound 2988 inhibited completely the
incorporation of both adenine and of glycine into
macromolecules, whereas the other compounds
studied had only slight effects on glycine incorpo-
ration either into proteins or nucleic acids.

Ethidium bromide was the most rapid inhibitor
of adenine incorporation into nucleic acids, but
compounds 1427, 1390, 2516, and dimidium all
achieved complete inhibition, although after
longer delays than required by ethidium bromide.
Compounds 2989, 1887, and 1446 were almost as
active, and would probably have achieved com-
plete inhibition had the period of observation been
extended. Prothidium and compound 2610 inhibi-
ted only slightly.

\(^{1}\) These data of Drs. T. I. Watkins and G. Woolfe were com-
municated by Dr. M. R. Gurd.
DISCUSSION

These results have indicated that a wide variety of phenanthridinium derivatives is able to inhibit almost specifically the incorporation of adenine into nucleic acids of Ehrlich ascites cells in vitro. This type of biochemical block is believed to be unique for this class of compounds. These compounds were originally investigated for their trypanosomal activity (1), but it may be seen that there is apparently no correlation between their trypanocidal activity and their effects on adenine incorporation.

Because so many of this relatively small group of compounds had inhibitory activity, few definite conclusions concerning the relationship between chemical structure and biochemical activity in this system can be made. Compound 2610, which lacks a 6-phenyl group, is the least effective inhibitor of adenine incorporation into nucleic acids. Neither the 3,8-diamo grouping nor quaternization of the phenanthridine nitrogen appears to be necessary for this biochemical activity.

Although ethidium bromide had little if any carcinostatic potency by itself, in combination

![Chart 1](chart1.png)

**Chart 1.**—Effects of various phenanthridinium compounds on the incorporation of adenine and of glycine into macromolecules.

Tumor cells, 24 mg. wet weight, were incubated in 2 ml. of calcium-free Krebs-Ringer phosphate medium, pH 7.4, at 38° C in air with 5.5 X 10^-3 M glucose and either 1.8 X 10^-4 M adenine-8-C14 or 2 X 10^-4 M glycine-8-C14. Drug concentrations: 0 (---●---); 2.5 X 10^-4 M (---○---); 1.25 X 10^-4 M (---×---); 0.625 X 10^-4 M (Δ---Δ). "Anti-T. congolense" indicates the activity of each compound against Trypanosoma congolense in mice.
with azaserine a potent tumor-inhibitory effect was reported (5). This has been explained on the basis of the complete deprivation of the supply of purine nucleotides for nucleic acid synthesis induced by these two drugs, one of which inhibits their synthesis de novo, and the other, the utilization of nucleotides made from exogenously supplied purines for these processes. In addition to ethidium bromide, its 5-methyl, propyl, and allyl analogs (dimidium, 1427, and 1446), all possess antitumor activity in combination with azaserine in several ascites tumors. Whether compound 9988 has a specific effect on macromolecule synthesis or just a nonspecific cytotoxicity is not yet known. It is obviously different in its effects from

\[ \text{ANTI} \text{ CONGOLENSE} \]

\[ \text{GLYCINE-PROTEIN} \]

\[ \text{COUNTS PER MINUTE} \]

\[ \text{MINUTES} \]

\[ \text{CHART 2.——See legend for Chart 1} \]

G. A. LePage, personal communication.
the other compounds and should be tested for its carcinostatic efficacy. The results of this study are tentatively assumed to support the promise of antitumor activity by the other compounds also, although this is yet to be tested.

Ethidium bromide has been shown not to be metabolized by mouse tissues and tumor cells, and the active inhibitor is therefore ethidium bromide itself. The same is probably true of most of the other compounds included in this study. However, comparison with antrycide (9) suggests that prothidium and 2988, which are linked with the pyrimidinium derivative, may be metabolized. Differences in activity among this group of compounds may be associated with differences in permeation into the cells or degree of association with critical intracellular biochemical components.

Although the mechanism of action of these compounds is not known, the present study suggests that all except compound 2988 may act similarly.

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REFERENCES

Inhibition of Purine Metabolism in Ehrlich Ascites Carcinoma Cells by Phenanthridinium Compounds Related to Ethidium Bromide

J. Frank Henderson

Cancer Res 1963;23:491-495.

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