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 percent, and 50 percent 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 antityrpanosomal (1), antibacterial (8), and antiviral (3) 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-phenyl phenanthridinium 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 (929289); 3,8-diamino-6-decyloxy-5-ethyl-5,6-dihydro-6-phenylphenanthridine (92516); 3-amino-8-(2-amino-6-methyl-4-pyrimidinylamino)-6-p-aminophenylphenanthridine 5,1'-dibromide
RESULTS

The effects of ethidium bromide and ten structurally related compounds on the incorporation of radioactive adenine into the perchloric acid-insoluble fraction of Ehrlich ascites carcinoma cells are shown in Charts 1–3. Because ethidium bromide had been shown to inhibit this process completely within 15 minutes at a concentration of $2.5 \times 10^{-4} \text{ M}$ (4), each new compound was tested at three concentrations—2.5, 1.25, and $0.625 \times 10^{-4} \text{ M}$—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 exhibits a definite delay before the onset of maximum inhibition (4), measurements were made at intervals during the course of the 1-hour incubation to determine lag periods. The effect of each compound on the incorporation of radioactive glycine into nucleic acids and into proteins was tested at a concentration of $2.5 \times 10^{-4} \text{ M}$, 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 presented in a separate chart. Group I contains ethidium bromide and three very closely related homologs, whereas Group II consists of more diverse variants. Group III contains two phenanthridinium nuclei linked to a 1,6-dimethyl-4-amino-pyrimidinium salt, which is a component of another active trypanoside, antrycide (2). For purposes of comparison, the activity of each compound against Trypanosoma congolense in mice 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 incorporation 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 2289, 1887, and 1446 were almost as active, and would probably have achieved complete inhibition had the period of observation been extended. Prothidium and compound 2810 inhibited only slightly.

1 These data of Drs. T. L. Watkins and G. Woolfe were communicated 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-diamino 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

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**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 × 10^{-5} M glucose and either 1.8 × 10^{-4} M adenine-8-C^14 or 2 × 10^{-3} M glycine-2-C^14. Drug concentrations: 0 (■—■); 2.5 × 10^{-4} M (O——O) 1.25 × 10^{-4} M (X——X); 6.25 × 10^{-5} M (△——△). "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 (dimidiu.m, 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 the actions of...
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 pro-

thidium 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.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. H. George Mandel, in whose laboratory this work was done, and to Drs. M. R. Gurd, T. I. Watkins, G. Woolfe, and D. A. Peak, of the Boots Pure Drug Co., Ltd., for assistance and permission to use unpublished data.

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