of apparent chaos, as for the single species systems discussed in detail above. The behavior of the system of Eq. 5 in these various regimes is illustrated in Fig. 2.

Equations 1 and 2 are two of the simplest nonlinear (density dependent) difference equations that can be written down. Their rich dynamical structure, and in particular the regime of apparent chaos wherein cycles of essentially arbitrary period are possible, is a fact of considerable mathematical and ecological interest, which deserves to be more widely appreciated. Without an understanding of the range of behavior latent in such deterministic difference equations, one could be hard put to make sense of computer simulations or time-series analyses in these models.

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Hycanthone Analogs: Dissociation of Mutagenic Effects from Antischistosomal Effects

Abstract. N-*Oxidation at the diethylamino group of hycanthone, of lucanthone, and of two chlorobenzothiopyranoindazoles resulted in a marked reduction in mutagenic activity, while antischistosomal activity was retained or even enhanced. Introduction of chlorine into the 8-position of benzothiopyranoindazoles reduced acute toxicity but had no effect on chemotherapeutic potency. These dissociations of biological activities indicate that safer antischistosomal compounds of this class can be developed.*

The geographical distribution and the nature of human schistosomiasis require special care in the selection of chemotherapeutic agents for the treatment of this infection. More than 200 million human subjects are infected with schistosomes and the incidence is on the increase. Even a low frequency of delayed serious complications, produced by mutagenic, teratogenic, and carcinogenic actions of a drug, can involve a large absolute number of individuals. Populations infected with schistosomes are not protected by national drug laws or regulatory agencies. Moreover, in an undetermined number, and possibly the majority, of subjects infected with *Schistosoma hematobium*, overt clinical and pathological manifestations disappear in adulthood (*1*). This must be taken into account when considering a drug for the mass treatment of children whose life expectancies are longer and whose reproductive potentials are greater than those of adults. As was stated by Rubidge *et al.* (*2*), "urinary tract bilharziasis is a relatively mild disease in South Africa and serious sequelae are rare. Hence, therapy must be safe."

It is estimated that during the past 6 years, in Brazil, Africa, and the Middle East, at least 700,000 human subjects infected with *S. hematobium* and *S. mansoni* have been treated with the antischistosomal thioxanthenone derivative hycanthone (the drug is ineffective in infections produced by *S. japonicum* prevalent in mainland China and the Philippines) (*3*). Reports from a variety of laboratories have indicated that

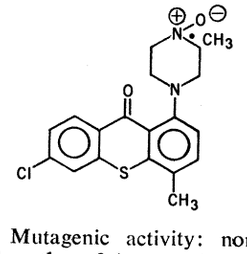


Fig. 1. Mutagenic activity: none detectable (less than 0.1 percent as active as hycanthone). Antischistosomal activity: intramuscular, 0.4; oral, 0.3.

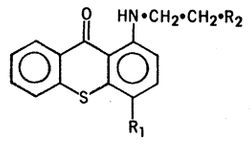
hycanthone is mutagenic (*4*) and teratogenic (*5*), and that it induces prophage (*6*), mitotic crossing-over (*7*), cytogenic changes (*8*), and malignant transformations (*9*); hycanthone is carcinogenic in mice infected with *S. mansoni* (*10*). As pointed out by Firminger (*11*), a report (*12*) which seemingly did not support the last observation was based on such a small number of animals that no significant negative results could have been obtained. Since a number of compounds chemically related to hycanthone exhibit antischistosomal activity, the question arose whether structural alterations can bring about a dissociation of undesirable toxicological properties from chemotherapeutic activity. Data summarized below indicate that this is the case.

A chloroindazole analog (IA-4, structure in Table 1) of hycanthone has the same antischistosomal activity in mice as hycanthone (*13*), while its acute toxicity and its hepatoxicity are lower (*13*, *14*). Compound IA-4 failed to induce demonstrable malignant transformations in cells infected with Rauscher virus (*9*). Its mutagenic activity was found to be lower in *Salmonella* (*15*), bacteriophage T4 (*15*), and mouse lymphoblasts (*16*); no mutagenic effects were detected in yeast (*17*); no cytogenetic effects were detected in rat bone marrow cells (*18*). Furthermore, in contrast to hycanthone and to a number of chemical carcinogens, IA-4 failed to induce breaks in rat liver DNA (*19*). Another indazole analog (IA-3) had lower antischistosomal activity; but since there is decreased acute toxicity, the chemotherapeutic index of IA-3 approximately equals that of IA-4 (*13*).

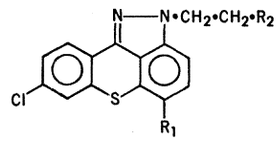
We found that chloro substitution in position 8 produced a marked decrease in the acute toxicity of the indazole analogs for mice. For example, the median intramuscular lethal dose (LD_{50}) of IA-3 and of IA-4 was more than seven times higher than that of the corresponding deschloro derivatives.

In further studies of the effect of structural modifications on antischistosomal activity and on mutagenicity, *N*-oxides of active thioxanthenones and benzothiopyranoindazoles were prepared. The parent bases were oxidized with *m*-chloroperbenzoic acid in dichloromethane solution, and after chromatography (Al_2O_3) the *N*-oxides so obtained were converted to their water-soluble methanesulfonate salts. *N*-Oxidation at the diethylaminoethyl group consistently resulted in a marked reduction in mutagenicity for *Salmonella*

Table 1. Mutagenic and antischistosomal activities in mice of hycanthone, hycanthone analogs, and their *N*-oxides; IM, intramuscular.



A



B

Compound	Ring system	R_1	R_2	Mutagenic activity (colonies per plate)	Relative antischistosomal activity (*21*)	
					IM	Oral
Hycanthone	A	CH_2OH	$N:(C_2H_5)_2$	1262	1.0	0.5
Hycanthone *N*-oxide	A	CH_2OH	$\overset{\oplus}{N}:(C_2H_5)_2$ $\lvert$ $O^{\ominus}$	56	0.3	0.25
Lucanthone	A	CH_3	$N:(C_2H_5)_2$	70	0.1	0.2
Lucanthone *N*-oxide	A	CH_3	$\overset{\oplus}{N}:(C_2H_5)_2$ $\lvert$ $O^{\ominus}$	22	0.5	0.4
IA-4	B	CH_2OH	$N:(C_2H_5)_2$	100	1.0	1.5
IA-4 *N*-oxide	B	CH_2OH	$\overset{\oplus}{N}:(C_2H_5)_2$ $\lvert$ $O^{\ominus}$	18	1.0	1.2
IA-3	B	CH_3	$N:(C_2H_5)_2$	70	0.6	0.7
IA-3 *N*-oxide	B	CH_3	$\overset{\oplus}{N}:(C_2H_5)_2$ $\lvert$ $O^{\ominus}$	5	2.0	2.0

strain TA1538 (*hisD3052 uvrB△ rfa*). This was assayed for drug-induced reversions essentially by the method of Ames *et al.* (*20*), with rat liver microsomes. Data given in Table 1 are the average numbers of drug-induced mutations per plate above spontaneous mutation background (17 colonies per plate). Values represent the averages of four or more plates, each containing 0.5 μmole.

Antischistosomal activity in mice (*21*) of the *N*-oxides of compounds containing the hydroxymethyl group was either reduced (hycanthone *N*-oxide as compared to hycanthone) (Table 1) or remained unchanged (IA-4 *N*-oxide as compared to IA-4) (Table 1). On the contrary, antischistosomal activities of lucanthone *N*-oxide and of IA-3 *N*-oxide were greater than those of lucanthone and of IA-3, respectively. The schistosomicidal activity of IA-3 *N*-oxide exceeded that of hycanthone, regardless of whether the compound was administered intramuscularly or orally (Table 1). In addition, IA-3 *N*-oxide exhibited less than 1 percent of the mutagenicity of hycanthone. Furthermore, the *N*-oxide (Fig. 1) of a 6-chloro-*N*-methylpiperazinylthioxanthenone, which had itself shown lower intrinsic mutagenicity (*22*), exhibited no detectable mutagenic activity while it retained, albeit low, antischistosomal activity. The lower mutagenic activities of the *N*-oxides could be ascribed, in part, to lower penetration, but growth inhibition studies indicate that this is not the major factor with lucanthone *N*-oxide, IA-3 *N*-oxide, and the *N*-oxide of the piperazinyl derivative (Fig. 1).

Our observations indicate that the antischistosomal effects of hycanthone are brought about by mechanisms different from those producing mutagenic and some acute toxic effects. Hence, structural modifications of the hycanthone molecule provide opportunities to reduce or eliminate certain acute and potentially serious long-term toxic host effects while maintaining or, in some instances, increasing schistosomicidal activity.

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