11. To our knowledge the records obtained by F. Zettler and M. Järvilehto from *Calliphora* [*Z. Vergl. Physiol.* **68**, 202 (1970)] are the only previously published intracellular records of laminar responses of the higher Diptera that are accompanied by electrode marking experiments. Their records show the waveforms of the laminar and retinular responses to be quite similar. Their subsequent investigations, however, seemed to indicate that these were recorded from the processes of the retinula cells [M. Järvilehto and F. Zettler, *ibid.* **69**, 134 (1970)]. Recently the same group also reported on a hyperpolarizing monopolar neuron in the lamina of *Calliphora* [H. Autrum, F. Zettler, M. Järvilehto, *ibid.* **70**, 414 (1970)].
12. We thank Drs. J. Grossfield, L. Pinto, and T. H. Goldsmith for advice and criticism of the manuscript. Supported in part by NSF grant GB-24666.

18 December 1970

Hycanthone Resistance: Development in Schistosoma mansoni

Abstract. *Following the administration of relatively high doses of the antischistosomal drug hycanthone to mice and hamsters infected with* Schistosoma mansoni, *a number of the worms survived. After a period of 6 to 12 months these parasites resumed production of viable eggs that gave rise to schistosomes that proved resistant to hycanthone and to two other related antischistosomal compounds. This drug resistance has remained stable for three subsequent generations of worms.*

The activity of the thioxanthone derivative hycanthone against *Schistosoma mansoni* in experimental animals and man has been reported (*1–5*). It has been stated that a single intramuscular injection of 50 mg per kilogram of body weight to mice infected with *S. mansoni* results in the elimination of over 91 percent of the worms and that only one-fourth of this dose is required to destroy 98 percent of these parasites in hamsters (*2, 3*).

In an attempt to explore the mode of the antischistosomal action of hycanthone, it was noted that its onset of action is very slow. After the intramuscular administration of 60 mg of hycanthone per kilogram to mice infected with a Puerto Rican strain of *S. mansoni,* a complete shift of the worms from the mesenteric veins to the liver was observed only after 9 to 10 days. After 3 weeks most of the parasites recovered in the liver remained motionless when placed in 75 percent horse serum, but many of them exhibited motor activity when they were incubated in the same medium containing 5-hydroxytryptamine ($5 \times 10^{-5} M$) (*6*). These surviving worms were damaged functionally and morphologically. For example, alterations in the reproductive system of the females were similar to those observed after administration of other antischistosomal compounds (*7, 8*), the glycogen stores of the males were greatly reduced, both sexes appeared stunted, and their wet weight was decreased. Four to 12 months after the administration of the drug, live worms were found in 257 out of a total of 268 mice. Most of the schistosomes were located in the mesenteric veins. While after 4 months the reproductive system of most females gave the appearance of being damaged, a progressive recovery occurred thereafter, and after 12 months few, if any, abnormalities were noted. This coincided with the reappearance of live eggs in the liver of the host. The miracidia hatching from these eggs were infective to the intermediate host of *S. mansoni*, the snail *Biomphalaria glabrata.* After a period of 5 to 6 weeks the snails that had been infected with these miracidia were shedding cercariae that, in turn, were infective to mice and gave rise to adult schistosomes resistant to hycanthone. Intramuscular administration of hycanthone (80 mg/kg) to the host produced no hepatic shift, no damage to the female reproductive system, no weight loss, and no glycogen depletion in the progeny of the worms that had recovered from the effects of the drug. Administrations of the same dose, repeated four times at intervals of one or several days, were equally ineffective. Miracidia hatched from eggs produced by these hycanthone-resistant worms (F_1) gave rise to a second generation (F_2) of hycanthone-resistant schistosomes. This resistance has remained stable for two subsequent generations (F_3 and F_4).

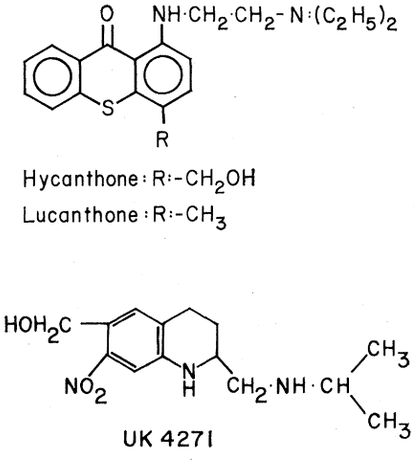

Fig. 1. The structures of hycanthone, lucanthone, and UK4271 (an aminoalkyl tetrahydroquinoline).

The appearance of this type of drug resistance was observed without exception in 163 mice infected with *S. mansoni* originating from eggs deposited in the livers of nine out of nine schistosome-infected mice to which a single dose of hycanthone (30 or 60 mg/kg) had been administered 6 to 12 months previously. This was not limited to the mouse host. A similar pattern was observed in hamsters infected with *S. mansoni.* The treatment of these animals with hycanthone (16 mg/kg, single intramuscular dose) resulted at first in a complete hepatic shift, but 8 months thereafter paired worms (approximately 10 to 20 percent of the number found before treatment) had reestablished themselves in the mesenteric veins. The miracidia hatched from the eggs of these worms gave rise, in hamsters, to schistosomes that were resistant to hycanthone. Intramuscular administration to these hamsters of doses of hycanthone as high as 80 mg/kg had no effect on the worms. This resistance has remained stable in the hamster host for two subsequent generations (F_2 and F_3).

Hycanthone-resistant schistosomes exhibited cross-resistance to two chemically related antischistosomal compounds, lucanthone (*9*) and an aminoalkyl tetrahydroquinoline (UK4271) (*10*) (Fig. 1). An alkylaminoalkyl group in the side chain is a structural feature common to these three compounds. By contrast, hycanthone-resistant schistosomes proved as susceptible to the effects of a nitrovinylfuran (*11, 12*) as hycanthone-susceptible worms.

It remains to be determined whether, in geographic areas where schistosomiasis is endemic, eggs excreted by human subjects previously treated with hycanthone give rise to strains of schistosomes resistant to this drug. Furthermore, the dose of hycanthone used in humans is 5 to 20 times lower than those employed in this study for hamsters and mice, respectively. Yet

in these animal hosts a significant number of schistosomes escaped destruction. Six to 12 months were required until production of viable eggs was resumed. Therefore, it can be expected that live eggs are absent from the stools of human subjects for a similar period (*4*, *5*), regardless of whether or not all the parasites have been eliminated.

It appears unlikely that the development of hycanthone-resistant schistosomes can be accounted for by the presence of two preexistent populations of worms, one drug-susceptible and one drug-resistant. Such a hypothesis is not consistent with the hepatic shift and other changes involving *all* the worms after the initial treatment. In addition, many months are required for functional and morphological recovery. By contrast, the progeny of these worms proved completely unaffected by this drug. Conceivably a preexistent resistant population might be affected by the products of the dying susceptible schistosomes rather than by the drug itself. This appears unlikely because, when mice were infected with a mixture of cercariae of a susceptible strain and a resistant strain, only a proportion of worms equal to that of the susceptible strain was affected by administration of hycanthone.

Possibly, hycanthone-resistant oocytes are induced or selected by this drug. Since hycanthone has been found to be a potent mutagen (*13*), administration of this compound might contribute to an increase in the drug-resistant oocyte population.

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9. When these observations were communicated to Dr. C. R. Lambert, he informed us of unpublished observations made in 1962 which indicate the development of lucanthone-resistance in the progeny of a Liberian strain of *Schistosoma mansoni* that had survived treatment of the host with lucanthone. Since the results given in this report were obtained with a Puerto Rican strain, it appears that resistance to thioxanthones can occur in at least two geographic strains of *S. mansoni*. To our knowledge, no other type of antischistosomal compound has been found to produce drug-resistant strains of schistosomes.
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14. Supported by grants from NIH and from the Rockefeller Foundation. The furoate and methane sulfate salts of hycanthone were kindly supplied by Dr. Sidney Archer and UK4271 by Dr. R. Foster.

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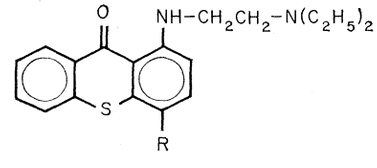
Hycanthone: A Frameshift Mutagen

Abstract. *Rapid spot-test screening of antischistosomal agents reveals that hycanthone is a potent frameshift mutagen while the closely related compound, miracil D, is nonmutagenic in* Salmonella. *Both hycanthone and miracil D are frameshift mutagens for T4 bacteriophage during growth in* Escherichia coli.

Over 1000 mutations in the histidine operon of *Salmonella* have been classified as to type (missense or nonsense base substitution, frameshift, extended deletion) on the basis of reversion analyses and other genetic and biochemical tests (*1*). From among the spontaneously revertible strains we have selected 13 mutants which appear suitable for use in rapid spot-test screening of new compounds for mutagenic activity (Table 1). Each strain produces a low number (about one to ten) non-histidine-requiring colonies when approximately 2×10^8 bacteria are spread evenly on the surface of a petri dish containing enriched minimal medium (*2*). At the plating density utilized, the enrichment in this medium allows most amino acid–requiring mutants to undergo roughly four cell divisions before growth ceases (*3*). Thus we have chosen strains that mutate spontaneously but do so at low frequencies (less than 10^{-8}). The strains also have been chosen for their diversified, yet clear-cut, responses to mutagens in spot tests [see (*4*) and (*5*) for descriptions of spot tests]. Different sensitive tester strains have been described earlier by Ames (*6*). The plating medium allows ready discernment of revertant colonies on an accumulated background population of mutant bacteria exceeding 10^9 per plate. On experimental plates the medium also allows detection of mutations that are delayed in expression and mutations that are induced only in growing bacteria during DNA replication. Further points on methodology and the usefulness and validity of tests of this type are discussed by Ames (*6*).

Conversations with Rogers and Bueding concerning some of their data (*7*) led us to screen by the spot-test procedures a series of compounds potentially useful in the treatment of schistosomiasis. Among these are HC (hycanthone monomethanesulfonate and hycanthone furoate) and MD (miracil D; also known as lucanthone); HC and MD are closely related compounds (*8*) with planar ring structures (Fig. 1) which may allow intercalation into DNA [see (*9*)]. Both hycanthone derivatives behaved similarly in our mutation tests and are therefore both identified as HC although they were tested individually; the furoate is less toxic to bacteria than is the monomethanesulfonate.

In initial tests, crystals of the compounds were applied with sterile toothpicks near one edge of the petri dish; later, tests were performed with the relevant compounds dissolved in distilled water at 10 mg/ml. All manipulations were performed under subdued light and all incubations were carried out in the dark to avoid any possible photodynamic effects. Table 1 shows that HC is effective in eliciting reversions of several frameshift mutations but is inactive in reverting mutations involving base substitutions. Figure 2 shows a test with *hisA3043*. Miracil D is inactive as a mutagen in our tests with *Salmonella*. Tests on additional representative frameshift mutants show



R = –CH_2OH (Hycanthone) or –CH_3 (Miracil D)

Fig. 1. Structures of hycanthone and of miracil D.