ments in which the drug apparently inhibited the movement of exogenous NE into storage sites. In control rats whose cardiac NE stores were previously labeled by $H^3$-NE (50 μc/kg, intravenous injection, 24 hours previously), the intravenous injection of *l*-NE (2 mg/kg) reduced the heart $H^3$-NE by 60 percent (Table 1; typical of three experiments). In rats previously treated with desipramine, the *l*-NE displaced only 20 percent of the $H^3$-NE, though, as shown above, the neuronal accumulation of *l*-NE was virtually unchanged. After a larger dose of *l*-NE (10 mg/kg, intramuscularly) about 90 percent of heart $H^3$-NE was displaced in control rats compared to 45 percent in animals treated with desipramine (*20*). These results indicate that the exogenous *l*-NE taken up by adrenergic neurons displaced a considerable fraction of the endogenous amine from storage sites; previous treatment with desipramine appears to have interfered with this displacement.

In conclusion, our results add to accumulating evidence that desipramine, and perhaps other tricyclic antidepressants, exert an intraneuronal action. Admittedly, the action of desipramine at the neuronal membrane can account for the blockade of NE uptake after administration of tracer doses of the catecholamine. However, when a large dose of *l*-NE is injected, desipramine does not block neuronal uptake, yet it apparently prevents the exogenous amine from freely mixing with endogenous NE stores labeled with $H^3$-NE. Since the storage vesicles contain most of the endogenous neurohormone (*21*), we favor the view that desipramine may act also on storage vesicles to prevent *l*-NE and other amines from displacing endogenous NE. Whether or not this intraneuronal action of desipramine bears any relationship to the pharmacological mechanism by which the drug produces its characteristic clinical effects remains unanswered.

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22. Dr. Stefano [the Consejo Nacional Investigaciones Cientificas Technicas, Republica Argentina] was supported as visiting associate by NHI; Dr. Kurzepa was an international fellow from the National Institute of Mother and Child, Warsaw, Poland. He is now deceased. Desipramine HCl was supplied by Dr. F. H. Clarke. Phenoxybenzamine was a gift from Dr. Glenn E. Ullyot.

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## Resistance to Metronidazole by Trichomonas foetus in Hamsters Infected Intravaginally

Abstract. *Induced resistance to the antitrichomonad agent metronidazole administered to hamsters infected intravaginally with* Trichomonas foetus *and treated with subcurative levels of metronidazole was demonstrated in vitro and in vivo.*

Metronidazole [1-(β-hydroxyethyl)-2-methyl-5-nitroimidazole] is effective against trichomoniasis, but it has been suggested that resistance to it could become clinically significant (*1*). Several workers have shown that resistance to metronidazole by *Trichomonas vaginalis* can be induced in vitro (*2*). There is apparently only one reference to the characteristics in vivo of these resistant organisms (*3*). We have no report on the induction of resistance in vivo.

Hamsters (*Mesocricetus auratus*, young virgin females) were infected intravaginally with *Trichomonas foetus* and subsequently treated orally with suboptimum doses of metronidazole (50 mg/kg; 12 doses over a 3-week period). The trichomonas organism was then isolated and compared with the parent strain for sensitivity to metronidazole. The assay system employed was a twofold tube dilution assay in Diamond's medium (10 per-

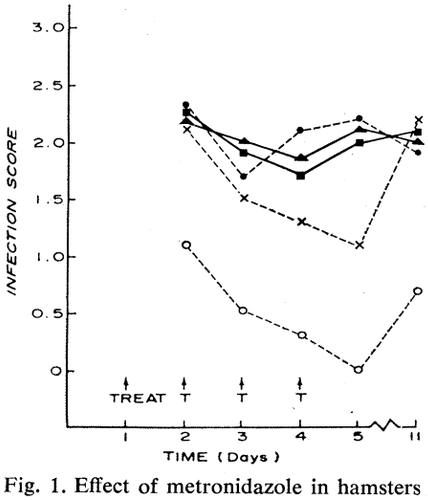


Fig. 1. Effect of metronidazole in hamsters infected intravaginally with a metronidazole-sensitive or a metronidazole-resistant strain of *Trichomonas foetus*. ▲—— Infection control, resistant strain; ■—— metronidazole (200 mg/kg), resistant strain; ● - - - - infection control, sensitive strain; × - - - - metronidazole (50 mg/kg), sensitive strain; and ○ - - - - metronidazole (100 mg/kg), sensitive strain.

cent rabbit serum; *p*H 7.0; without agar), with incubation for 24 hours at 37°C. The end point of activity was complete inhibition of growth. After isolation of metronidazole-resistant organisms, experiments were done in hamsters to compare the efficacy of metronidazole against the metronidazole-sensitive and -resistant strains.

Hamsters were infected with 24-hour cultures of vaginal washings from donor animals infected with either the sensitive or the resistant strain of *T. foetus*. After 1 week a vaginal smear was taken to confirm infection. Groups of ten animals each were then treated orally with up to 200 mg of metronidazole per kilogram of body weight, daily for four successive days. A group serving as infection controls was treated with the drug diluent, 0.5 percent gum tragacanth.

Twenty-four hours after each treatment, a vaginal washing was taken and placed in Diamond's medium containing 100 units of penicillin G and 100 μg of streptomycin per milliliter. The sample was then incubated for 24 hours at 37°C, examined, and scored for trichomonads present. An additional sample was taken 1 week after the last treatment. The degree of infection, designated as the infection score, was determined by assigning a value of 0, 1, 2, or 3 to each culture examined, 0 indicating no detectable organisms and 3 indicating more than 100 trichomonads per microscopic (× 10) field.

The isolate from the animals treated with the aforesaid suboptimum doses of metronidazole was 8 to 16 times more resistant than the parent strain. The minimum inhibitory concentration of metronidazole for the parent strain ranged from 0.0975 to 0.195 μg/ml, whereas that for organisms isolated from the metronidazole-treated animals ranged from 1.56 to 3.12 μg/ml. There was no further increase in resistance to metronidazole in infected animals in which treatment was continued for a period of about 3 months.

After four oral treatments with metronidazole, hamsters infected with the metronidazole-sensitive strains of *T. foetus* had a significant reduction in parasites, even at 50 mg/kg per day (Fig. 1). One week after the last treatment, an exacerbation of the infection was observed. Animals infected with the metronidazole-resistant strain of *T. foetus* and treated with metronidazole, even at 200 mg/kg daily for 4 days, showed no change in parasite numbers during the test period.

Resistance to antiprotozoal agents is an important problem in animal and human therapy. Although metronidazole-resistance apparently is not a widespread clinical problem (*4*), its presence may be more common than believed. Most clinicians do not isolate organisms and test for resistance in cases of therapeutic failure. Furthermore, epidemiological problems cloud the search for resistant organisms in recurrent cases, as the long-term follow-up required is of little value if the treated individual cannot be isolated (*5*). Chloroquine-resistance in human malaria first became evident after the drug had been used for many years. Similarly, metronidazole-resistance could prove to be a significant clinical entity.

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13 January 1969

## DDT: Sublethal Effects on Brook Trout Nervous System

Abstract. *When brook trout are exposed for 24 hours to sublethal doses of DDT, the cold-blocking temperature for a simple reflex, which shows lability related to thermal history, is altered in a way suggesting that DDT is affecting the thermal acclimation mechanism. Sublethal dosage of DDT also prevents the establishment of a visual conditioned avoidance response.*

Fish show behavioral changes after exposure to sublethal concentrations of pesticides (*1, 2*) that may act on either peripheral or central (or both) nervous structures. One receptor system (the lateral line) is markedly affected by sublethal concentrations of DDT (*3*). Although there is little supporting evidence, the central nervous system (CNS) nevertheless seems the most likely site for the pesticide-sensitive region responsible for changes in complex behavior.

Two different behavioral responses of brook trout *Salvelinus fontinalis* to sublethal exposure to DDT implicate the CNS as the target site. The first is represented by changes in the low temperature (cold-block temperature) which is just sufficient to extinguish the propeller tail reflex (*4*). The spinal cord is the site for this cold blockage (*5*). The second response involves visual conditioning of an avoidance response that is formed in the optic tectum (*6*).

The fish ranged from 6 months to 2 years old. They were fed DDT-free beef liver once daily. To minimize the amount of detritus present, the fish were not fed for 3 days prior to and during the period of treatment. All DDT exposures were for 24 hours and were carried out in 6 liters of continuously-aerated water at the acclimation temperature in glass jars, one fish per jar (*7*). The DDT was always added to the water in 0.3 ml of acetone. Control fish were treated the same as were experimental ones, except that no DDT was dissolved in the acetone. The fish were tested in clean water, and, unless otherwise specified, each experiment

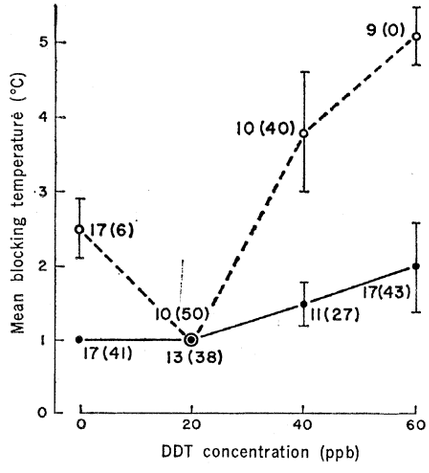


Fig. 1. The effect of acclimation temperature and exposure to DDT on the cold-block temperature of the propeller tail reflex in the brook trout. The numbers refer to the total number of fish tested and (in parentheses) the percentage which failed to block down to the lowest temperature obtainable, 1°C ± S.E. also shown. Solid line, fish acclimated at 9°C; broken line, fish acclimated at 18°C.