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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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

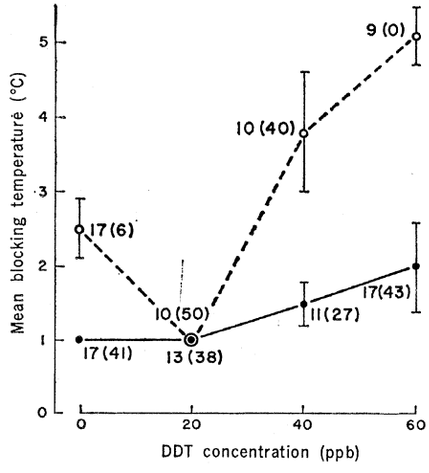

Fig. 1. The effect of acclimation temperature and exposure to DDT on the cold-block temperature of the propeller tail reflex in the brook trout. The numbers refer to the total number of fish tested and (in parentheses) the percentage which failed to block down to the lowest temperature obtainable, 1°C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

began immediately after the 24-hour exposure to DDT.

Cold-block temperature was determined as described (*5*), except that our stimulus was a 10 msec train of square wave pulses (1 msec and approximately 10 volts each at a rate of 400 per second).

As expected, for control fish acclimated at 18.0°C, the cold-block temperature was significantly higher than for the ones acclimated at 9.0°C (Fig. 1). Treatment with DDT also altered the cold-block temperature. The response of DDT-treated fish acclimated at 9.0°C was not quite the same as that of those acclimated at 18.0°C (Fig. 1). The difference, however, may be more apparent than real. The lowest temperature obtainable with our apparatus was 1°C and not only did many fish acclimated at 9°C fail to block, but also, for those fish that did block, the blocking temperature was about 1°C. If much lower temperatures had been possible, the response of fish acclimated at 9°C might have differed.

To say that DDT can alter the cold-block temperature just as can thermal acclimation, may be more than a convenient analogy. Sublethal concentrations of DDT, like thermal acclimation, shift the selected temperature of brook trout (and other salmonids) (*2*). Low doses lower the selected temperature; higher doses raise it in a pattern very similar to that shown in Fig. 1 for the cold-block temperatures of fish acclimated at 18°C. The DDT may be interfering somehow with the thermal acclimation mechanism. This would be consistent with our hypothesis that DDT acts upon central nervous structures because changes in selected temperature are thought to be controlled by the CNS (*8*).

The effect of DDT on a nervous function more complex than the propeller tail reflex was investigated by exposing trout acclimated at 9°C to 20 parts per billion of DDT and then comparing their ability to learn a simple conditioned avoidance response with the ability of untreated fish.

Brook trout have an individual preference for either the lighted or darkened side of a two-chambered aquarium. We trained our trout to avoid the side of their preference. The nonpreferred lighting was the conditioning stimulus; electric shock was the unconditioned stimulus. Fish were considered to be conditioned when they showed eight consecutive proper avoidances. The apparatus and method of training were similar to that used by Roots and Prosser (*5*).

Table 1. The response of fish during the establishment of the conditioned avoidance response. The "avoidance" response is the one used for the training criterion. The fish tested at various times after DDT exposure were all different fish and had not previously been tested.

Day	Fish tested (No.)	Average trials* (No.)	Misses† (%)	Escapes‡ (%)	Avoidances§ (%)
		Untreated			
	12	30.3	13.0	52.0	35.0
		DDT-treated			
1	6	>25	94.0	6.0	0.0
4	6	>28.3	52.0	45.1	2.9
7	6	>29.5	43.0	49.2	7.8

* Trials per fish until trained to avoid preferred side. † Failures to leave the initially preferred side of a two-chambered aquarium during the electric shock period. Misses typically occurred early in the training session. ‡ Successful exit during the electric shock period. § Departure after lighting change but before the electric shock.

Although untreated naive fish took only about 30 trials to become conditioned, not one of the DDT-treated naive fish became conditioned (Table 1). Training was discontinued after approximately 25 trials because by this time, after almost 6 minutes of intermittent electric shock, the fish had become refractory and were sitting on the bottom, often at an angle, failing to exhibit any overt response to the shock. The duration of the DDT effect was investigated by testing the response of naive fish 4 and 7 days after DDT exposure. These fish showed some improvement in performance. Fewer misses and more escapes and avoidances were observed. However, the improvement was at best slight, for it did not appear that the fish would ever show the required eight consecutive avoidances. Even after 7 days, no fish showed even two consecutive avoidances.

Evidently, DDT treatment reduces the ability of fish to form an association between the connecting doorway and escape from shock. There was no apparent impairment in either the swimming or visual abilities of the fish.

The effect of DDT treatment on the retention of the conditioned avoidance response was determined by comparing the number of trials initially required to attain full conditioning with the number required for the same fish when tested 24 hours later. Six fish served as controls and six as experimentals. The second performance of control fish was enhanced by the previous training, the difference between the initial 32.7 ± 3.04 (S.E.) trials and the subsequent 11.2 ± 1.20 trials being significant ($P < .005$). The fish exposed to DDT required 27.8 ± 1.80 trials before treatment and 24.2 ± 1.80 trials after treatment. The difference is not significant ($P > 0.5$). It is as if the DDT treatment had converted the previously-trained fish into naive ones. Yet, clearly something has been retained by these fish, for if they had not had the training session prior to the DDT exposure, then, as shown in Table 1, they would not have been able to learn at all.

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