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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

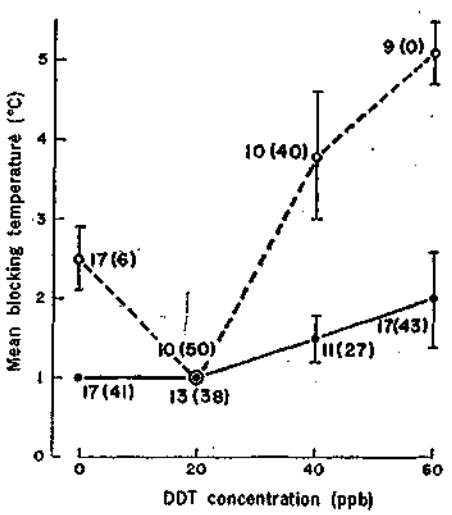

Fig. 1. The effect of acclimation temperature and exposure to DDT on the cold-block temperature of the propeller tail reflex in the brook trout. The numbers refer to the total number of fish tested and (in parentheses) the percentage which failed to block down to the lowest temperature obtainable, 1°C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

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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Circadian Rhythm of Serotonin in the Pineal Body of Immunosympathectomized Immature Rats

Abstract. *In the pineal body of the immature rat the circadian rhythm of serotonin persists when sympathetic innervation is abolished by the administration of nerve growth factor antiserum. This rhythm is regulated by a mechanism that does not involve the sympathetic innervation and is, therefore, fundamentally different from that in the adult.*

In the past 10 years major advances in our knowledge of pineal physiology have been established. Compounds within the pineal body have been identified and their levels measured. Many of them have been shown to have a circadian rhythm that is dependent upon environmental lighting mediated through the retina and the sympathetic nervous system (*1*).

Serotonin is one of these compounds. In the rat pineal it exists in high titers (*2*) and has a circadian rhythm in which levels are lowest 4 hours after the onset of darkness and highest 6 to 8 hours after the onset of light (*3*). Sympathetic postganglionic fibers from the superior cervical ganglion (*4*) regulate the rhythm. After bilateral superior cervical ganglionectomy or severance of preganglionic fibers the cycling of serotonin in the pineal body is abolished (*5*, *6*). Although this role of the sympathetics has been confirmed in the adult rat, the following observations suggest that pineal serotonin may not be regulated through sympathetic innervation in the immature animal.

As early as 6 days postpartum the circadian rhythm of serotonin is present (*7*), but at this age there is a sparsity of intrapineal sympathetic nerve fibers (*8*). To learn the significance of this apparently inadequate innervation we undertook the study, described in this report, of serotonin levels in totally denervated pineals in young rats. Because of stress and mortality after superior cervical ganglionectomy, we chose to denervate the pineal by immunosympathectomy (*9*).

Holtzman rats of both sexes were given bovine nerve growth factor antiserum (NGFA) (*10*) within 6 hours after birth and again 24 hours later. Experimental and control animals were maintained together in a controlled environment. The temperature was 19° ± 1°C, and fluorescent lights were kept on from 5:00 a.m. to 7:00 p.m., that is, a cycle of 14 hours of light and 10 hours of darkness.

Animals were decapitated at either 8 or 20 days of age—one group at 1:00 p.m. and another at 11:00 p.m. Pineals were dissected quickly, weighed on saline-moistened filter paper on a Roller Smith torsion balance, homogenized in groups of two in 0.5 ml of 0.1*N* HCl and 0.5 percent ascorbic acid, and refrigerated. Within 24 hours serotonin was assayed on a Farrand spectrofluorometer (model No. 104244B) according to the method of Quay (*11*). Since fluorescence microscopy reveals the extent of sympathetic nerve suppression (*12*), a sample of pineal bodies from each group of treated animals was examined by this method. In addition, stretch preparations of the iris of all the 20-day-old animals used in the experiment were examined by fluorescence microscopy. In normal control pineals (from animals 8 and 20 days old), the sympathetic innervation was present and similar to that described elsewhere (*8*).

In animals injected with NGFA (600 unit/g) there was no nerve fluorescence in the iris preparations, and although denervation of the pineal was almost complete, occasionally a few scattered fibers remained. When the dosage was increased to 1100 unit/g, pineal nerve fluorescence was abolished.

Prolonging the light period into the normal dark period prevents the nocturnal fall in pineal serotonin in both adult and immature animals (*6*, *7*). To clarify the role of sympathetics in the immature rat, it was important to determine whether prolonged lighting would modify the amount of serotonin in denervated pineals. Thus on the day the animals were killed a third group was given four additional hours of lighting, that is lights remained on until 11:00 p.m., the time they were killed.

Results in Table 1 show that at both 8 and 20 days of age there is a circadian rhythm in rat pineal serotonin and that after immunosympathectomy the rhythm persists unchanged. Thus its regulation must be through a mechanism fundamentally different from that in the adult.

In the adult, central nervous system stimuli that regulate serotonin levels reach the pineal via sympathetic fibers (*5*). Since rhythm persists in the young animal after denervation, it seems reasonable to speculate that in the immature rat the rhythm is intrinsic to the pineal itself. However, the origin and nature of its regulator remains unknown. An endocrine influence in immature rats has not been studied, but in the adult, removal of endocrine organs does not modify pineal serotonin rhythm (*6*).

The results of Table 1 also show that in sympathectomized animals additional lighting did not elevate serotonin levels above the nocturnal low. From the first observation—that serotonin rhythm persists in the absence of sympathetics—one might presume that innervation has no regulatory function on serotonin metabolism in the young rat. However, since additional lighting prevents the nocturnal fall in serotonin in intact

Table 1. Serotonin levels in the pineals of normal and immunosympathectomized young rats. Each group contained 10 or 12 animals. There is no significant difference between pineal weights of groups in each of the four treatment categories. N.S., not significant.

Treatment	Pineal weight (mg)	Serotonin (ng/pineal)	Significance of difference	
			Groups	Level
		Rats 8 days old		
None				
Day*	0.470 ± 0.035	30.5 ± 2.20	Day:night	.001
Night†	.502 ± .022	13.3 ± 1.60	Day:night-lighted	.02
Night-lighted	.410 ± .013	23.1 ± 1.50	Night:night-lighted	.01
NGFA (1100 unit g^{-1} day^{-1})				
Day	.473 ± .015	29.6 ± 1.63	Day:night	.001
Night	.527 ± .030	10.0 ± 2.33	Day:night-lighted	.001
Night-lighted	.483 ± .017	14.5 ± 2.78	Night:night-lighted	N.S.
		Rats 20 days old		
None				
Day	0.586 ± 0.039	54.5 ± 4.59	Day:night	.001
Night	.632 ± .037	13.5 ± 4.12		
NGFA (600 unit g^{-1} day^{-1})				
Day	.549 ± .031	61.0 ± 3.52	Day:night	.001
Night	.550 ± .034	19.0 ± 1.81		

* Day, 1:00 p.m. † Night, 11 p.m.