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LABORATORY STUDIES OF THE EFFECT OF U-5897 ON THE REPRODUCTION OF WILD MALE RATS

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LABORATORY STUDIES OF THE EFFECT OF U-5897 ON THE REPRODUCTION OF WILD MALE RATS

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Abstract: The effects of U-5897 (3-chloro-1,2-propanediol), a male chemosterilant that acts by inducing epididymal lesions, were tested in the laboratory on ricefield rats (*Rattus argentiventer*), Polynesian rats (*R. exulans*), black rats (*R. rattus*), Norway rats (*R. norvegicus*), and laboratory rats (Long-Evans strain). For each species, seven treatment groups of five males each were given single oral doses ranging from 0–300 mg/kg. No lesions were induced in ricefield and Polynesian rats at any dose level. Some Norway and laboratory rats developed lesions at doses of 50 mg/kg, and all developed them at 100 mg/kg and above. Black rats developed lesions at 100 mg/kg, and higher, but the response was incomplete; at least one male at each dose level failed to develop lesions. Some Norway, laboratory, and Polynesian rats died from doses of 200 mg/kg and higher.

The concept of controlling wildlife populations by interfering with one or more of the reproductive processes was discussed by Davis (1961), and attempts to apply the concept have been reported by several workers (Balser 1964, Linhart and Enders 1964, Linhart et al. 1968, Howard and Marsh 1969). Because reproductive inhibitors have advantages over traditional control chemicals in both safety and specificity, there is considerable interest in finding effective ones for use against species causing damage.

One possible compound for rats is U-5897, a chlorohydrin. It was first shown to have a reversible antifertility effect in male monkeys, (*Macaca mulatta*), guinea pigs (*Cavia cobaya*), and white rats (Kirton et al. 1970, Ericsson and Youngdale 1970). Subsequent studies revealed that male white rats are permanently sterilized when given single oral doses of 45 mg/kg, approximately five times the minimum dose that produces reversible antifertility (Ericsson 1970). In both instances, the compound is a post-testicular artifertility agent that apparently does not affect libido (Ericsson 1970, Ericsson and Baker 1970). Sterility induced in white rats is due to the formation of a lesion in the caput epididymidis that completely blocks passage of sperm to the exterior. Recently Ericsson and Connor (1969) reported that Norway rats also develop the epididymal lesion when treated by gavage or via drinking water.

The unique properties of this compound suggested it might be a useful male sterilant for rat species causing agricultural damage. Accordingly, we tested its effects on Polynesian, black, ricefield, and Norway rats, and on laboratory rats for comparison.

METHOD

The Polynesian and black rats used were trapped in Hawaii, and the ricefield rats on Mindano Peninsula in the Philippines; they were shipped separately to this laboratory by air freight. Norway rats were trapped locally, and laboratory rats (Long-Evans strain) were purchased.[1] The Polynesian, black, and ricefield rats were isolated for 2 weeks before assignment to treatment groups, and the Norway and laboratory rats for 1 week. All animals were maintained in a routine manner in individual cages with unlimited access to water and a commercial rat ration. Room temperature was con-

[1] Simonsen Laboratories, Gilroy, California.

Table 1. Effects on the reproductive tract, and mortality produced in male rats by single oral doses of U-5897.

Treatment (mg/kg of U-5897)	Number of Animals			Results With Survivors at Necropsy		
					Organ Weights (grams ± S.D.)	
	Dosed	Died	Bilateral Lesions	Body Weight (grams ± S.D.)	Testes[b]	Epididymides[b]
				Ricefield rats		
All	24	0	0	204.1 ± 7.9	5.252 ± .293	1.996 ± .348
				Polynesian rats		
Control	5	0	0	73.8 ± 9.4	1.528 ± .128	.609 ± .310
100	5	0	0	91.0 ± 7.5	1.760 ± .308	.743 ± .111
150	5	0	0	74.2 ± 14.0	1.663 ± .344	.624 ± .216
200	5	3	0	70.0 ± 12.0	1.552 ± .401	.550 ± .274
250	5	5	—	—	—	—
300	5	5	—	—	—	—
				Black rats		
Control	5	0	0	208.2 ± 20.9	3.246 ± .918	1.114 ± .570
100	5	0	2[a]	213.0 ± 16.4	3.648 ± .731	1.556 ± .522
150	5	0	1	212.8 ± 21.2	3.628 ± .528	1.355 ± .294
200	5	0	2	209.8 ± 5.9	2.689 ± .796	1.124 ± .475
250	5	0	3[a]	213.4 ± 45.9	2.839 ± .826	1.719 ± .302
300	5	0	4	212.2 ± 15.9	1.835 ± .242	1.063 ± .553
				Norway rats		
Control	5	0	0	334.6 ± 45.8	3.038 ± .280	1.007 ± .179
50	5	0	2[a]	318.2 ± 27.2	3.048 ± .306	.943 ± .225
100	5	0	5	329.6 ± 29.8	4.168 ± 1.057	.928 ± .205
150	5	0	5	322.8 ± 32.3	3.922 ± 1.138	.848 ± .167
200	5	2	3	287.3 ± 31.6	3.950 ± 1.809	.655 ± .175
250	5	2	3	271.6 ± 76.1	3.478 ± 2.252	.614 ± .260
300	5	5	0	—	—	—
				Laboratory rats		
Control	5	0	0	360.6 ± 16.8	2.959 ± .222	.964 ± .067
50	5	0	1	376.8 ± 16.1	2.854 ± .177	1.198 ± .134
100	5	0	5	330.4 ± 13.2	2.637 ± .617	.853 ± .056
150	5	1	4	304.5 ± 42.4	2.588 ± .922	.674 ± .098
200	5	4	1	191.0 —	1.699 —	.508 —
250	5	5	—	—	—	—
300	5	5	—	—	—	—

[a] Lesion was unilateral for one rat.
[b] Mean of totals for left and right sides.

trolled to 76 F (± 4.0 F), and lights were preset to provide 14 and 10 hours of light-dark daily. The day before treatment, the food in each animal's cage was removed at 4:00 PM, and dosing began at 8:00 AM the following morning.

For dosing, U-5897 was diluted with distilled water to provide stock solutions of 1, 2, 3, and 4 percent. Although aqueous solutions are reported to be chemically and physiologically stable at room temperature (Ericsson 1970), solutions more than 4 weeks old were never used. Males of each species were dosed at six different levels: 0, 100, 150, 200, 250, and 300 mg/kg of body weight. Body weight was measured to the nearest gram. A seventh treatment level, 50 mg/kg, was added for the Norway and laboratory rats. The chemical was administered as single oral doses by gavage

Fig. 1. The testis and epididymis of a laboratory rat showing the lesion (arrow) that develops following treatment with U-5897.

with a 3-inch, 18-gauge, blunt-tipped needle attached to a 2.5 cc syringe and measured to the nearest 0.1 cc. The standard volume delivered was 1.0 ml (± 0.2 ml) for Polynesian rats and 2.0 ml (± 0.2 ml) for the other species. The proper dose was obtained by using the appropriate stock solution. Control males were dosed with distilled water.

Ten days after treatment, each male was killed by an overdose of ether. After body weight was recorded, the testes and epididymides were removed, trimmed of excess fat, weighed individually, and examined for epididymal lesions or other effects of the treatment. When a lesion was found, a smear was made of the material extruded from the epididymis just distal to the lesion to determine how completely the epididymal tubules were blocked. The cauda epididymides of each male were also checked for the presence, approximate concentration, and motility of sperm, to ensure that all treated males were sexually active at the time of treatment. Juvenile males do not respond to this compound (Ericsson, personal communication), and it is quite possible that sexually inactive adults would react differently from sexually active ones.

To detect any changes not seen in gross examination, the testes and epididymides from each male were fixed in Bouin's solution, embedded in paraffin, cut at 7 μ, stained with hematoxylin-eosin, and examined microscopically. Whenever possible, epididymides with lesions were sectioned at the site of the lesion and at sites both proximal and distal to it.

RESULTS

U-5897 had only two primary effects in these tests. Some treated rats died within 72 hours of treatment, and some survivors developed epididymal lesions. The lesions were usually bilateral; most were located in the caput epididymidis, but occasionally the corpus or cauda epididymidis were involved. In general, the number of animals with lesions, the number killed, and the rapidity of death were proportional to the dosage. In spite of these general patterns, however, the various species responded quite differently. The data are summarized in Table 1.

Ricefield rats were unaffected by the treatment; none either died or developed epididymal lesions. This was true of all ricefield rats treated, though 18 (representing all six treatment groups) of the original 42 were eliminated from the study at necropsy because they were sexually inactive.

No lesions developed in Polynesian rats, but the treatment killed three of five dosed at 200 mg/kg and all dosed at 250 and 300 mg/kg. In contrast, no black rats were killed, and at least one male in each treatment group developed lesions. However, though the number of affected black rats

Fig. 2. Photomicrographs of testicular and epididymal cross-sections obtained from black rats treated with U-5897 (Magnif. 98×). (A) Cauda enididymidis showing the lesion (L) blocking the movement of sperm (S) to more distal areas (D) of the epididymis. (B) Edematous seminiferous tubules (E) caused by a lesion. (C) Seminiferous tubules showing the degenerative changes that occur in the germinal epithelium after the lesion-causing edema has subsided. (D) Normal seminiferous tubules from a control male.

increased with the dosage, even 300 mg/kg did not sterilize all five rats treated. In two rats, one dosed at 100 mg/kg and one at 250 mg/kg, the lesion was only unilateral and was located in the corpus epididymidis.

The response of Norway and laboratory rats was essentially similar. Some Norway rats died at 200 and 250 mg/kg, and all died at 300 mg/kg; one laboratory rat died at 150 mg/kg, and 14 of 15 died at 200 mg/kg and higher. In both, all survivors dosed at 100 mg/kg and higher developed bilateral lesions. Two Norway and one laboratory rat dosed at 50 mg/kg were also affected, but in one of the Norway rats the lesion was only unilateral.

Analysis of variance of body, testis, and epididymal weights for each type of rat showed no significant differences among treatment groups (including controls), with two exceptions: testis weight in black and laboratory rats. The *lsd* method of multiple comparison (Steel and Torrie 1960) showed that the testes of black rats in the 300-mg/kg group weighed significantly less ($P < 0.025$) than those of the other treat-

ment groups for the species, and that the testes of laboratory rats in the 50-mg/kg group weighed significantly more ($P < 0.05$). This latter difference was probably due to the testing schedule. The 50-mg/kg group was dosed 2 weeks later than the other groups and the rats had grown in the interval.

The epididymal lesion and its effect were always visible macroscopically (Fig. 1). There was invariably a color change in the epididymis just distal to the lesion; instead of the characteristic creamy color usually associated with sexually active males, the epididymis appeared more like those seen in juvenile males. Smears from this area had few sperm, whereas smears from the epididymis proximal to the lesion showed high sperm concentrations (Fig. 2A). Apparently, the lesion caused fluid to accumulate in the testis, and the resulting pressure caused degeneration of the seminiferous tubules. At necropsy 10 days after treatment, the testes from treated males were occasionally still edematous (Fig. 2B), but most showed degeneration of the tubules (Fig. 2C). For comparison, a cross-section from the testis of a control black rat is shown (Fig. 2D). If the 10-day interval between dosing and necropsy was increased, tubular degeneration would have been greater and one would expect the testes of affected animals to weigh significantly less than those of the controls, as they did in black rats dosed at 300 mg/kg.

The lesion produced by U-5897 affects reproduction in two ways. The main effect is the prevention of the passage of sperm to the exterior during ejaculation. The secondary effect is a reduction in the number of normally functioning seminiferous tubules. However, degeneration of the germinal epithelium is not complete, and some normal tubules are known to persist in the testes of affected animals as long as 1 year after treatment with U-5897 (Ericsson 1970).

DISCUSSION

The variable response between rat species illustrates the danger of extrapolating laboratory data from one species to another on the basis of studies with limited dosages. U-5897 had no effect on ricefield rats; it killed Polynesian, Norway, and laboratory rats; it sterilized Norway and laboratory rats; and it partially sterilized black rats. As a male chemosterilant for rodent control, U-5897 thus appears to offer promise for Norway rats at about 100 mg/kg, but little for Polynesian rats at any level. Only additional studies will show whether doses higher than 300 mg/kg will reliably sterilize black and ricefield rats without causing mortality.

In practical terms, the difference in response to U-5897 means that when combinations of species are present in an area (as often happens in agricultural damage situations), no single dose will provide blanket control. On the other hand, it could be possible to tailor the treatment rate so that one species was affected and not another; this kind of specificity is sometimes very desirable in control programs.

It is also possible that some of the differences observed between species resulted from the manner in which the rats reacted to dosing. The trauma associated with stomach-tubing may vary from one species to another, and hence the compound could be metabolized differently. To test this possibility, a study is underway at this laboratory to compare the effects of U-5897 on rats dosed by stomach-tubing and by adding the chemical to the food supply. The latter phase of the study will be designed to provide information on the acceptability of the chemical.

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