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Rhodamine B: A Systemic Fluorescent Marker for Studying Mountain Beavers (*Aplodontia rufa*) and Other Animals

Abstract

Mountain beavers (*Aplodontia rufa*) consuming 15 to 45 mg/kg of Rhodamine B dye developed fluorescent bands in claws within 2 to 4 days and in hair within 5 to 7 days. These bands were invisible in ambient light but appeared orange under ultraviolet light in both live and dead animals. The 1- to 4-mm bands persisted in claws for 6 weeks and in hair for 17 to 28 weeks in free-roaming animals. Bands of dye were also deposited in hair and claws of pocket gophers (*Thomomys mazama*) and in feathers of domestic chickens (*Gallus* sp.) when these animals ingested Rhodamine B dye. The long lasting systemic marking effect should increase the versatility of Rhodamine B in studying mammals and birds.

Introduction

Rhodamine B is an industrial and analytical dye that has been widely used as a marker and tracer in animal studies. This dye is compatible with many organic and inorganic chemicals (Evans and Griffith 1973) and is available from most chemical suppliers. Wildlife uses of Rhodamine B have been mainly for short term marking of the gut, feces, urine, and blood through consumption of dyed bait and through external application of dye solutions for staining hair and feathers. Wadkins (1948), New (1958), Kozlik *et al.* (1959), Gast (1963), Evans and Griffith (1973), and Bruggers and Bortoli (1979) reported on Rhodamine B for studying movement and dispersal of animals; Evans and Griffith (1973), Batcheler and Bell (1974), and Lindsey *et al.* (1979) evaluated its use in animal damage control programs.

During research to evaluate and develop methods for controlling conifer damage caused by mountain beavers (*Aplodontia rufa*), I noted that Rhodamine B, when taken orally by mountain beavers, marked the gall bladder, gut, feces, urine, and oral and urogenital openings and was systemically deposited in the claws and hair. Although systemic action of Rhodamine B dye in animals was noted by Evans and Griffith (1973: 76-77) and by Ellenton and Johnston (1975: 60-67), systemic marking in the claws and hair was not described. This discovery led to concurrent studies on mountain beavers reported herein and on coyotes (*Canis latrans*, Johns and Pan 1981) to study the potential of Rhodamine B as a systemic marker.

Methods and Materials

Commercial Rhodamine B dye (Matheson Coleman and Bell, Manufacturing Chemists, Norwood, Ohio*) was administered in laboratory-held and free-roaming adult and subadult mountain beavers by gavage or by ingestion of fresh apple baits. Laboratory

*Reference to manufacturers or trade names does not imply endorsement by the Federal Government.

held mountain beavers were trapped near Olympia, Washington, held at least seven days, treated, and examined daily until dye marking appeared, then every two weeks thereafter. Field animals were live trapped, weighed, and then treated by gavage or fed apple baits and released at their capture site; they were recaptured and examined at one week and then monthly thereafter.

Test groups of one to five mountain beavers were treated about every two months throughout the year. Twelve caged mountain beavers (5 M, 7 F) were treated by gavage with dosage levels of 5, 15, 25, and 35 mg/kg of body wt.; five field animals (2 M, 3 F) received 30 mg/kg doses. All animals were given single doses of 1.0 to 3.0 ml of a Rhodamine B-water solution; quantities of Rhodamine B in solution were based on individual animal weights to provide consistent mg/kg dosage levels. Ten caged (6 M, 4 F) and four field mountain beavers (1 M, 3 F) were fed apple baits. Thirteen mountain beavers received single treatments, and one animal received two treatments spaced 2 months apart. Baits were prepared by applying Rhodamine B to the surface of apple chunks in concentrations ranging from 0.1 to 0.34 percent (by weight of total bait).

All animals were examined for fluorescent marking in the dark with an ultraviolet (UV) lamp. Gastrointestinal tracts of animals kill-trapped or necropsied during testing were also examined. I used long-wave (366 nm) UV lamps for all examinations.

Blood plasma from four caged mountain beavers treated by gavage with 30 mg/kg doses of Rhodamine B and two untreated control mountain beavers were examined with a Turner Model 111 fluorometer at the Denver Wildlife Research Center. Blood samples ranging from 3 to 4 ml/animal were collected at 1 day, 7 days, and 28 days post treatment. I anesthetized the animals and obtained samples by cardiopuncture using vacutainer tubes containing sodium heparin. The plasma, separated by centrifuging, was initially frozen and then thawed and diluted to 4 cc volume with deionized water for examination.

To evaluate systemic marking on other animals, I treated five adult pocket gophers (*Thomomys mazama*) captured near Olympia, Washington, and six 3- to 5-week-old white, domestic chickens (*Gallus* sp.) by gavage with Rhodamine B. All pocket gophers were treated in September at a 30 mg/kg level, and the chickens (two per treatment level) were treated in May with 5, 15, and 30 mg/kg. Pre- and post-treatment procedures were similar to laboratory-held mountain beavers.

Results

Mountain Beavers

Rhodamine B or a metabolite was internally deposited by systemic action in claws and hair of mountain beavers treated by gavage or by consumption of dyed apple. These bands (Figs. 1 and 2) were invisible in ambient light but fluoresced orange under UV light in both live and dead animals. Claws and hair were marked in all mountain beavers treated by gavage with doses of 15 to 35 mg/kg; the markings of the 5 mg/kg dose were not consistent. Rhodamine B was observed in bands 1- to 4-mm long within 2 to 4 days in claws and in bands 1- to 2-mm long within 5 to 7 days in whiskers and guard hairs. In hair, banding was most evident in the face whiskers and tactile hairs on the forelegs. The dye was present in roots of pulled whiskers within 16 to 40 hours post ingestion; without pulling the hair, the bands were not visible until Day 5.



Figure 1. Fluorescent marking in claws of a mountain beaver treated by gavage with Rhodamine B.

Single bands appeared from single treatments and double bands from two treatments spaced 2 months apart. As growth occurred, the bands moved outward and eventually disappeared as hair and claws were worn, broken, or shed.

Mountain beavers readily accepted apple baits coated with Rhodamine B. Consumption of Rhodamine B ranged from 13 to 45 mg/kg. Hair and claws of all mountain beavers were positively marked except for one animal that ingested 13 mg/kg dye. Oral and urogenital areas and front feet of all animals eating dyed apple were visibly stained red for up to 2 weeks. Feces and urine were visibly stained red for 1 to 3 days. Internally, the dye marked the stomach, intestines, and gall bladder. In some animals, the dye diffused through the gall bladder and stained adjacent internal tissue. No adverse physical or behavioral effects from consuming Rhodamine B were observed in laboratory animals.

Duration of banding in caged animals was 19 to 26 weeks in the hair and 13 to 21 weeks in the claws. Duration of hair marking in field animals was 17 to 28 weeks, but the claw marking lasted only 6 weeks because claws were worn away more quickly in free-roaming than in caged animals.

Rhodamine B was detected in blood plasma at 1 day and 7 days but not at 28 days

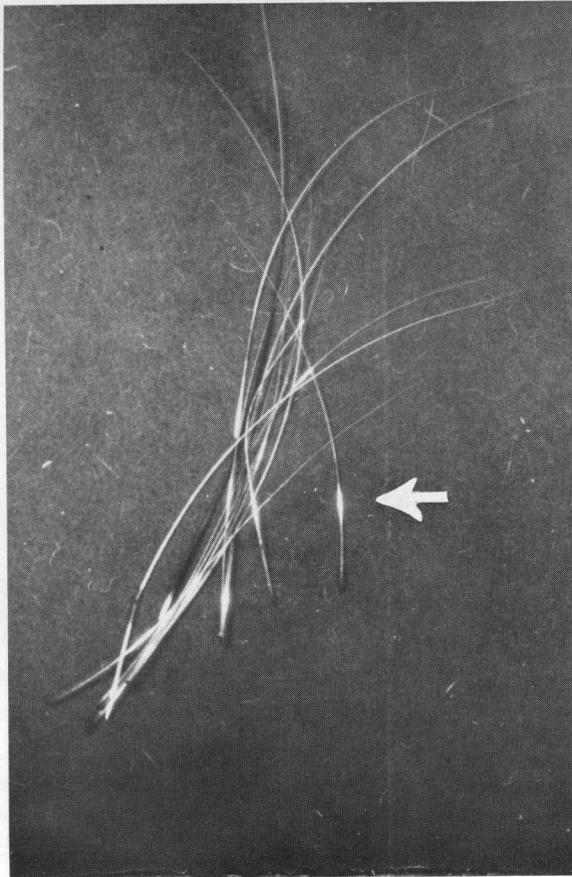


Figure 2. Fluorescent markings in face whiskers removed from a mountain beaver treated by gavage with Rhodamine B.

post ingestion. Rhodamine B was not present in control plasma. Quantitative measurements of Rhodamine B in plasma were not made, but fluorometer data indicate that greatest concentrations of dye were in the 1-day plasma samples.

Pocket Gophers and Chickens

I observed fluorescent bands similar to those described for mountain beavers in whiskers and claws of the five pocket gophers dosed at 30 mg/kg. Bands in whiskers appeared 7 to 15 days post treatment and remained 5 to 6 weeks. Bands in claws appeared 2 to 7 days post treatment and remained 10 weeks. No bands were seen in body hairs.

Domestic chickens developed fluorescent bands in feathers within 24 hours after receiving Rhodamine B. Bands were about 3 mm long and extended the width of the feathers (Fig. 3). Banding was most evident in the primary and secondary wing feathers, but the dye was deposited in all feathers. Banding lasted 1 to 2 weeks in chickens receiving 5 mg/kg Rhodamine B and 15 to 26 weeks in those receiving 15 and 30 mg/kg. The beaks and toenails were not marked.

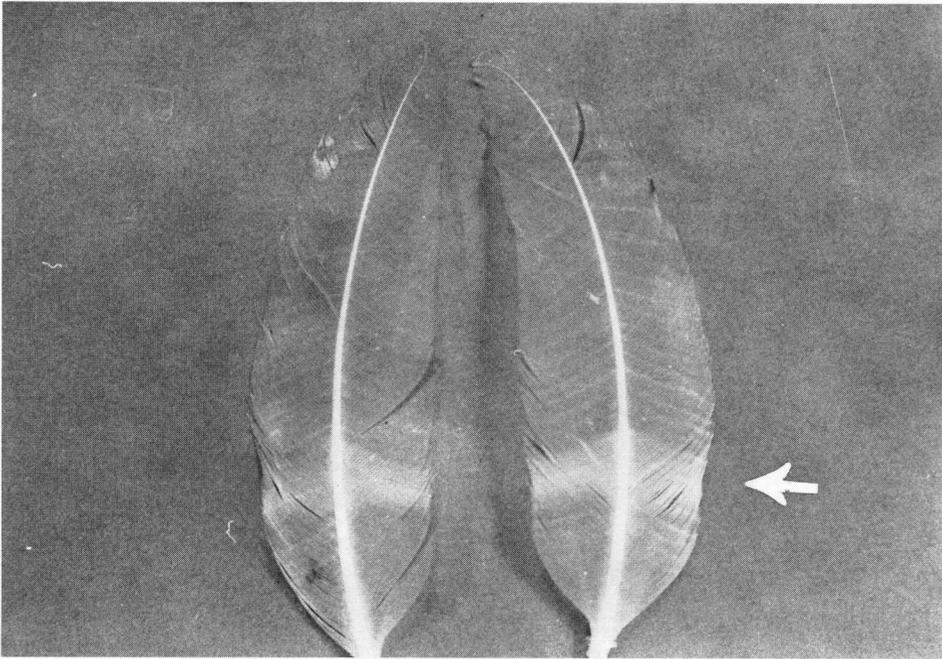


Figure 3. Fluorescent marking in primary feathers of a white domestic chicken treated by gavage with Rhodamine B.

Discussion

Fluorescent banding of claws, hair, and feathers with Rhodamine B expands its already wide application as a marker in animal studies. Because fluorescent bands are transported outward in claws and hair, the rate of growth should reveal when Rhodamine B was ingested. Furthermore, scanning for Rhodamine B does not require necropsy; thus, animals can be retained for further studies. With the use of portable UV lamps, field-trapped animals can be examined and immediately released, reducing stress from capturing and handling. Also, because fluorescent bands are invisible in ambient light, systemic marking should not affect the social structure of birds as noted by Evans and Griffith (1973) when they used an external dye to mark pheasants (*Phasianus colchicus*).

Use of Rhodamine B as a systemic marker may be limited to certain periods of the year in some animals because of the growth characteristics of the hair, claws, and feathers. Johns and Pan (1981) reported that fluorescent banding occurred only in actively growing hair. With animals such as mountain beavers, hair and claws grow continuously, so systemic marking should be effective throughout the year.

The internal "gut" and systemic "hair and claw" marking qualities of Rhodamine B, along with radio telemetry, have allowed us to evaluate methods for controlling mountain beavers and to estimate their exposure to experimental pesticides. Long-term marking gives greater latitude than short-term gut and fecal markers in conducting control and hazard studies. For example, pre- and post-control activity indices or other data can be obtained with less concern that the marker will disappear before animals can be captured for examination.

Rhodamine B, because of its extensive industrial uses, is being investigated for

evidence of carcinogenicity (International Agency for the Research on Cancer 1978, Tomatis *et al.* 1978). Nestmann *et al.* (1979), reported that commercial Rhodamine B dye, but not purified dye, possessed mutagenic activity in *Salmonella* in short term screening tests. Nestmann *et al.* (1979), however, further stated that chemicals showing mutagenic responses in these tests were not necessarily carcinogenic to mammals. Most importantly, Rhodamine B is exempt from legal tolerance requirements in or on raw agricultural commodities (Federal Register 1974). Secondly, no adverse physiological effects from using Rhodamine B for marking wildlife have been reported.

Studies on coyotes (Johns and Pan 1981), plus these on mountain beavers, pocket gophers, and domestic chickens, indicate that systemic hair/claw/feather marking with Rhodamine B is not species specific and should have application to other mammals and birds.

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