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B. E. Johns[1] *and H. P. Pan*[1]

Analytical Techniques for Fluorescent Chemicals Used as Systemic or External Wildlife Markers

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ABSTRACT: New approaches to wildlife marking with fluorescent chemicals were examined using the analytical techniques of fluorometry, chromatography, and tissue examination under ultraviolet light. A miniaturized, portable, and economical chromatography system was utilized. Quinacrine dihydrochloride was used as a marker and was found to be detectable at 50 ppb in 1 ml of blood. Rhodamine B was demonstrated to be a long-lasting marker of coyote (*Canis latrans*) claws and hair when the chemical was ingested orally and systemically distributed. The use of the analytical methods presented, in combination with fluorescent chemicals, will provide researchers and managers with additional ways to mark wildlife. The possibility of the systemic marking of hair with a variety of chemicals offers an expanded future for wildlife marking.

KEY WORDS: fluorescence, marker, blood, claw, hair, bait, quinacrine, rhodamine B, rat, starling, coyote, vertebrate pest control

The purpose of our research was to examine new ideas in wildlife marking that could result in development of markers and techniques that would advance the field of wildlife marking.

Fluorescent materials have been used as wildlife markers for many years [*1-5*].[2] Their uses include bait identification, direct application to the exterior of the animal, and internal marking of the digestive tract and excretory products. The marking of bones and teeth has been accomplished by ingestion of an oral antibiotic or selected fluorescent dyes [*6-8*]. Hair has also

[1]Physiologist and research chemist, respectively, Denver Wildlife Research Center, U.S. Fish and Wildlife Service, Denver, Colo. 80225.

[2]The italic numbers in brackets refer to the list of references appended to this paper.

received considerable attention as a deposition site for numerous compounds and promises to be a significant diagnostic tissue [*9, 10*].

Our research has demonstrated three analytical procedures for fluorescent marker detection. In addition, we have further developed the use of systemic distribution of fluorescent chemicals as a means of wildlife marking. The analytical procedures include fluorometry, chromatography, and direct observation of systemically marked claws and hair under ultraviolet illumination. The fluorometric technique is valuable for the detection of fluorescent materials in very small quantities. Paper or thin layer chromatography, even though it is less sensitive than fluorometry, requires only inexpensive equipment and provides the availability of identification of a marking compound. Furthermore, the chromatographic system we developed has the advantage of being miniaturized. The direct observation of systemically marked claws and hair is a very simple procedure because it requires only a long-wave ultraviolet light source and a darkened area. One chemical that has a prolonged affinity for blood and another that marks claws and hair by distribution through the circulatory system were used as markers in this experiment.

Procedures

Fluorometric and Chromatographic Analysis of Blood

In search of new wildlife markers, we looked for chemicals that are persistent and detectable in small blood samples and have an acceptable safety margin. Quinacrine dihydrochloride, an antimalarial drug used during World War II, met these criteria. In humans, quinacrine is readily absorbed in the digestive tract and slowly disposed of by the body. It is detectable in the urine 2 months after discontinuance of therapeutic use and it has a high affinity for leukocytes in addition to being bound to plasma protein [*11*].

1. Two stock solutions of different concentrations were prepared with quinacrine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.). One, 100 ppm in 0.1 *N* hydrochloric acid (HCl), was used to prepare standard rat blood samples of known quantities of the chemical, and the other, 5000 ppm in deionized water, was used to dose the test animals by gavage.

2. The 100-ppm quinacrine stock solution was diluted with 0.1 *N* HCl for the preparation of albino rat (*Rattus norvegicus*) blood samples with known quantities of quinacrine. To 0.95 ml of blood, which contained 10 percent 0.1 *M* sodium oxalate to prevent clotting, was added 0.05 ml of the diluted quinacrine stock solution. The control blood contained 0.05 ml of 0.1 *N* HCl. These preparations resulted in 1-ml blood samples that contained, respectively, 0, 10, and 50 ppb quinacrine.

3. Four albino rats, 325 to 390 g, maintained on a commercial laboratory rat food, were dosed with quinacrine dihydrochloride at 0, 0.1, 1, and 10 mg/kg body weight. The gavage volume was 0.2 ml per 100 g. The rats were

killed with chloroform 3 h after treatment, and blood samples were drawn from the left renal vein with a needle and syringe. The syringe contained 0.2 ml of 0.1 *M* sodium oxalate to prevent clotting. The total volume of each blood sample drawn plus anticoagulant was 2.0 ml.

4. Three starlings (*Sturnus vulgaris*), 95 to 102 g, were not fed for 2 h and then dosed by gavage with quinacrine dihydrochloride at 0, 1, and 10 mg/kg body weight. All other procedures and equipment were the same as used for the albino rats except that the bird blood samples were drawn from the beaker that collected blood from the decapitated bird.

5. One millilitre of each blood sample was processed with the procedure for the double extraction of quinacrine as described by Brodie et al [12]. For fluorometric analysis, the extracts were transferred to 12 by 75 mm cuvettes and read in a Turner® Model 111 fluorometer. The primary filter (Turner® 47B + 2A) isolated the activating energy at the 436-nm line, and the secondary filter (Turner 2A-12) transmitted the fluorescent light at 510 nm. The illumination source was an F4T4/BL General Electric ultraviolet mercury arc lamp with major wavelength emission at 360 nm and all longer mercury emission lines.

6. A miniaturized chromatography system was used for analysis of the extracts of blood from dosed rats and starlings. The system is a modification of the concepts presented by Rockland and Dunn [13] and Pan [14]. Whatman No. 1 filter paper or Eastman silica gel chromatogram thin-layer sheets were cut into 10 by 100-mm strips. One millilitre of methyl alcohol was placed in the bottom of 20 by 150-mm screw top tubes. Fifty microlitres of each blood sample extract was spotted repeatedly on a strip. After that the strip was placed in a tube and the lid screwed on for development. A battery-powered long-wave (360-nm) ultraviolet light was used to detect the fluorescent spots on the developed strips.

Systemic Marking of Coyotes with Orally Dosed Rhodamine B

G. D. Lindsey[2] had noticed that the hair of small rodents orally dosed with rhodamine B fluoresced in bands that appeared to persist. Personal communication with him led us to consider dosing coyotes (*Canis latrans*) with the dye.

All coyotes used were adults maintained on dry dog food and housed in outdoor concrete-floored wire pens. Table 1 shows the date they were dosed, the number of coyotes dosed, sex distribution, dose, and methods of administration. The rhodamine B was obtained from Eastman Kodak Co., Rochester, N.Y. A battery-powered long-wave (360-nm) ultraviolet light was used for fluorescent detection under a dark cloth or in a darkened room.

[1]Reference to trade names does not imply endorsement of commercial products by the federal government or any of its agencies.

[2]Wildlife biologist, U. S. Fish and Wildlife Service, Forestry Sciences Laboratory, Olympia, Wash. 98502.

TABLE 1—*Oral dosing of coyotes with rhodamine B.*

Date	Sex	Dose, mg/kg body weight	Method of Administration[a]
28 Aug. 1979	1 M, 1 F	30	gelatin capsule[b]
	1 M, 1 F	0	...
16 Oct. 1979	1 M, 1 F	5	tallow-mixture[c]
	1 M, 1 F	5	tallow-tablet[d]
	1 M, 1 F	5	horse meat[e]
	1 M	15	horse meat
	1 F	15	tallow-mixture
	1 M	0	tallow
8 Jan. 1980	3 M, 1 F	30	lamb meat[f]
5 Feb. 1980	1 M	30	horse meat
	1 F	15	horse meat

[a]All coyotes fed tallow or meat baits were prebaited for at least 1 day with a similar bait.
[b]A gelatin capsule containing the dose was administered behind the tongue with a balling gun.
[c]A mixture of beef tallow and rhodamine B to form a 13-g bait.
[d]Beef tallow with rhodamine B tablet in the center to form a 13-g bait.
[e]A horse meat and rhodamine B mixture to form a 13-g bait.
[f]A lamb meat and rhodamine B mixture to form a 26-g bait (two coyotes were used) or 114 g of lamb meat mixed with rhodamine B dissolved in a small amount of water (two coyotes were used).

Results and Discussion

Fluorometric and Chromatographic Analysis of Blood

The fluorometric examination of the extract of 1 ml of rat blood that contained a known amount of the marker demonstrated that quinacrine dihydrochloride was detectable at a concentration of 50 ppb but not 10 ppb. Blood from rats dosed at 1 and 10 mg/kg body weight was positive for quinacrine. However, the 0.1 mg/kg blood was negative. The starling blood was positive at 10 mg/kg and negative at 1 mg/kg. Silica gel chromatographic strips gave the best results. Both rat and starling blood samples from the 10-mg/kg doses provided positive chromatograph readings for quinacrine with spots having migration distance (Rf) values of 0.30 on silica gel strips when methanol was the solvent. The blood of animals that received lower doses was negative.

The use of these two analytical techniques offers the wildlife researcher methods that are not burdened with rare and costly equipment. Fluorometers are available in many laboratories. The chromatographic equipment described is small and portable enough to be taken to the field and operated by hand without electricity except for that provided by the batteries in the ultraviolet lamp. The extraction procedure is simple and is done at ambient temperature by hand shaking the samples in small disposable tubes. Our research with quinacrine dihydrochloride is in the initial phase and we intend

to develop it as a marker that will be field operational. The analytical techniques that we used with this fluorescent compound should be applicable to other fluorescent chemicals that may be useful as wildlife markers.

Systemic Marking of Coyotes with Orally Dosed Rhodamine B

All observations were made on coyotes that were restrained or sedated. The different sizes and types of bait used had no apparent effect upon bait consumption or success of marking except in one instance when one of the two animals that were offered tallow baits containing tablets of rhodamine B ate the tallow but left the tablet on the pen floor. This type of reaction has been observed in other studies at our coyote facility even when the tablet was a cellulose placebo. Since we saw no rejection of any other rhodamine B baits, we believe that the rejection was caused by the hardness of the tablet in the bait, not the chemical.

Figures 1 and 2 illustrate the two tissues that provided the best marking of coyotes—claws and hair. All coyotes dosed at 15 or 30 mg/kg body weight had marked claws. At these two doses, any growing hairs were marked. The large guard hairs were good examples. The 5-mg/kg dose did not make a visible mark on claws or hair. The fluorescent bands on the claws varied in width from 1 to 4 mm. The growing hair was marked with a band that was a fraction of a millimetre wide at its greatest intensity and then spread in either direction until it faded out. Both claws and growing hair were marked 24 h after dosing. The mark on a claw could be seen at this time by pushing back the skin at the base of the claw. To examine the hair at the 24-h interval the hair was pulled out and the base examined. As both claws and hair grew, the band was no longer hidden as at the 24-h interval and was detectable in place on the coyote.

The red-orange fluorescent bands in claws and hair appeared to result from the incorporation of the circulating rhodamine B, or its metabolite, into the tissue structure. Preliminary research had not confirmed that the bands contained rhodamine B. The bands were firmly attached to the tissue and the mark apparently would last as long as the claw or hair was on the coyote. The claws and hair stayed marked for at least 175 days on a coyote with no signs of deterioration. The necessity for the hair to be growing in order to receive the marker may appear to be a drawback. However, we found that even though the general body hair of a coyote stopped growing when the fur became prime, the hair over the toes and claws was still actively growing and was marked when the animal was dosed. It should also be noted that the coyotes might have nonsystemic markings of various intensities on the hair of the muzzle, anal, and urinary areas. However, these did not last, and the marking on the muzzle might not have been due to the ingestion of the marker.

Blood plasmas from the marked coyotes were examined fluorometrically,

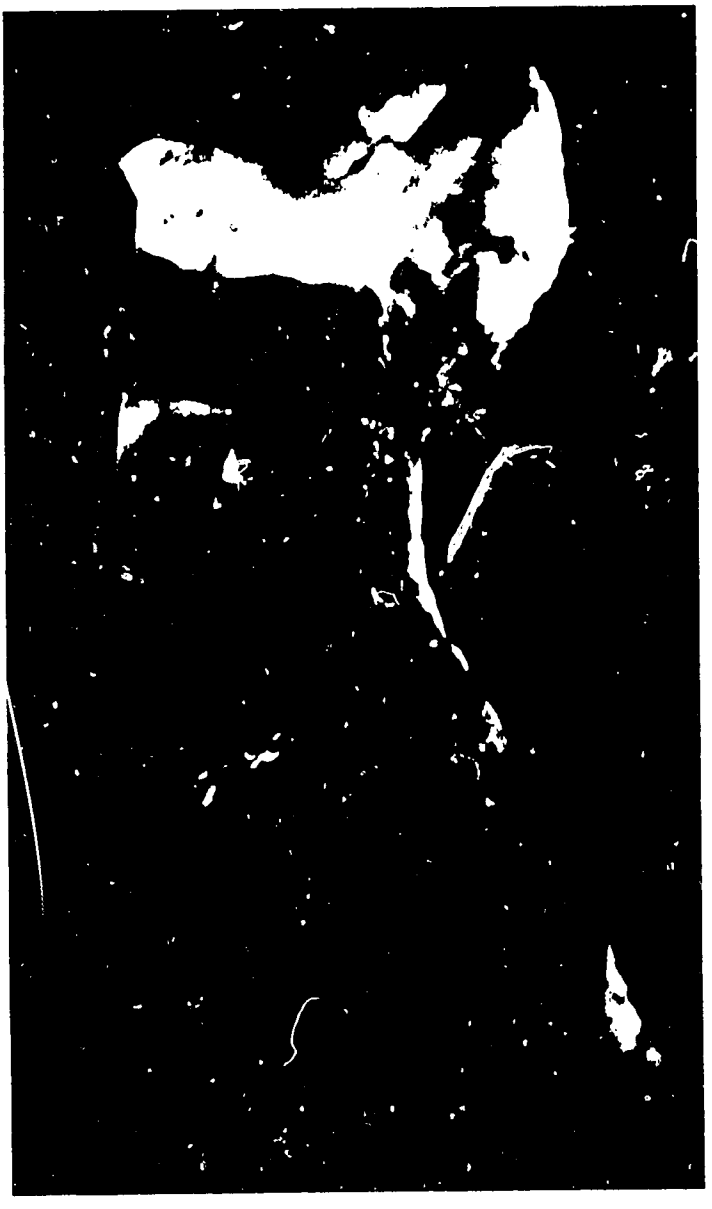

FIG. 1—*Coyote claw with a fluorescent band 30 days after an oral dose of 30 mg/kg rhodamine B. (The claw length is 19 mm.)*

and hair samples from the same coyotes were extracted in various solvents and the resultant samples read on the fluorometer. The plasmas provided positive readings for only a few days after dosing, and the hair extracts, although positive, never provided a degree of sensitivity greater than direct visual observation of the hair under ultraviolet light.

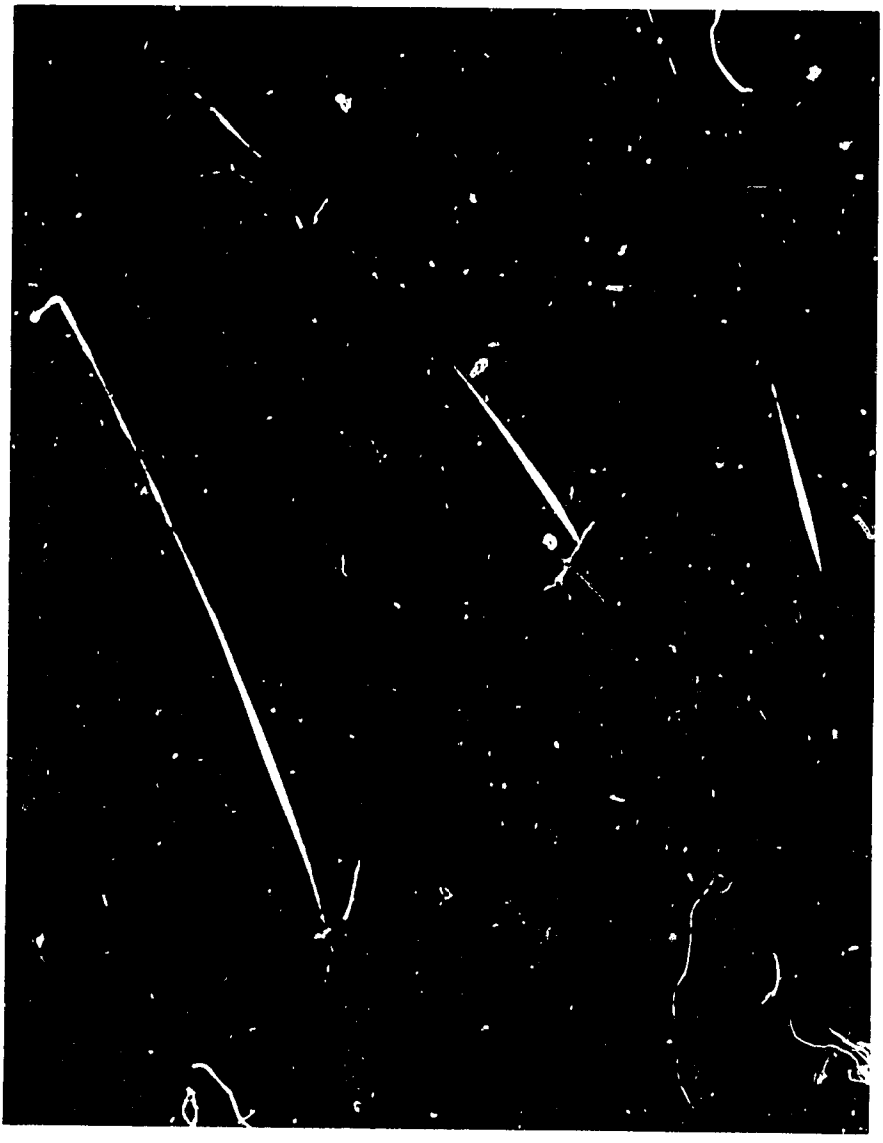

FIG. 2—*Coyote guard hairs with a fluorescent band 14 days after an oral dose with 30 mg/kg rhodamine B.*

Conclusions

We believe that this work with new marking techniques can be expanded into useful methods for field application. Both researchers and managers should consider the use of fluorometry, chromatography, and systemic claw and hair marking as a means that is available for marking wildlife. The

systemic marking of hair may extend beyond the use of fluorescent compounds and develop into a very useful marking technique with nonfluorescent chemicals. We know that there is the potential for a whole host of compounds to be systemically incorporated into hair. Our laboratory is working on methods of identifying organic compounds in hair, and we are hopeful that economical techniques will be developed to allow detection of very small quantities of chemicals that have been systemically incorporated into hair.

Acknowledgment

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