

ELIMINATION OF TRITIATED THYMIDINE FROM BIRDS

Esmeralda Party,* Anat Barnea,*[†] Ilona Linins,*[‡] and Edward L. Gershey*

Abstract—The kinetics with which tritiated thymidine is eliminated from birds and the concentration of radioactivity remaining in the carcass after 350–430 h were measured. Birds injected with tritiated thymidine eliminated 98% of the radioactivity injected within 28 h. Ninety-eight percent was identified in the excreta, and less than 2% of the radioactivity injected remained in the birds 28 h after injection.

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INTRODUCTION

RADIOACTIVE LABELING of DNA with tritiated thymidine is a practical and frequently used technique for studying biochemical processes (e.g., nucleotide metabolism or DNA synthesis), molecular events (e.g., cell cycle control), and more integrated biological phenomena (e.g., development and animal behavior). Because of its long physical half-life and wide distribution in the body of all organisms, tritiated thymidine is commonly used in animal experimentation. However, when studies are conducted *in vivo*, the fate of the radiolabeled material and its potential environmental impacts are necessary for regulatory, management, and disposal purposes.

In this case, we were interested in studying seasonal neurogenesis and neuronal replacement in a population of wild free-ranging black-capped chickadees (*Parus atricapillus*). The study required the capture of wild birds at different times of the year and their treatment with tritiated thymidine, which is incorporated into newly formed DNA and in this way acts as a cell birth marker (Goldman and Nottebohm 1983). In order to study seasonal patterns of neurogenesis in the wild and to avoid the effects produced by the stress of captive life, it was desirable to release the birds as quickly as possible after their treatment with tritiated thymidine. The same treated birds were recaptured after six weeks and sacrificed for brain analysis. The six week survival period allowed

enough time for the neurons forming at injection time to migrate to their final destination and go through final anatomical differentiation (Alvarez-Buylla and Nottebohm 1988).

For these experiments, and for others, it was necessary to understand the kinetics with which tritiated thymidine is eliminated from the animal and the amount and distribution of the incorporated material. The practical consequences of answering these questions were requisite to establishing whether the study could be conducted at all and under what conditions.

MATERIALS AND METHODS

Three zebra finches (*Taeniopygia guttata*) were injected intramuscularly with 185 kBq of tritiated thymidine.[§] Each bird was placed in a sealed cylindrical acrylic container with a volume of 13.5 L. The container had a removable bottom and two ports. Fresh air was drawn through one port, and container air was removed for sampling through the other port using a sampling pump at a rate of 0.3–1.1 L min⁻¹. Each bird was kept in the acrylic cage with water and feed for 26–30 h after the injection of the tritiated thymidine and then transferred to a standard wire bird cage. Birds had constant free access to water and feed. At each collection time, all feces and seeds were gathered and placed in separate 20 mL scintillation vials. A small amount (0.5–1.0 mL) of water was added to rinse the cage bottom and the liquid was collected with a Pasteur pipette into a 20 mL scintillation vial. The cage bottom was then wiped thoroughly with several cotton swabs dampened with water which were then placed in 7 mL scintillation vials.

One milliliter of water and 15 mL of scintillation cocktail[¶] was added to each feces and seed vial and vortexed. Fifteen milliliters of scintillation cocktail was added to the liquid collected from the bottom of the cage and vortexed. Two milliliters of scintillation cocktail were added to each cotton swab vial. Each vial was counted for 10 min in the tritium setting (0–18.6 keV) of a beta liquid scintillation counter.[¶] An aliquot of tritiated

* The Rockefeller University, 1230 York Avenue, New York, NY 10021; [†] Present address: Life Sciences, The Open University of Israel, Max Rowe Educational Center, 16 Klausner Street, P.O.B. 39328, Ramat Aviv, Tel Aviv 61392, Israel; [‡] Present address: Columbia University, Department of Environmental Health and Safety, 500 W 120 Street, New York, NY 10027.

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[§] Product number NET027Z, DuPont NEN Research Products, 549 Albany Street, Boston, MA 02118.

[¶] Ready Safe, Beckman, 2500 Harbor Boulevard, Fullerton, CA 92634.

[¶] Packard 2000, Packard Instrument Company, One State Street, Meriden, CT 06450.

water[#] or toluene^{**} calibrated reference standard (115,000 dpm and 172,000 dpm, respectively) was added to several vials of each category as an internal standard. These vials were recounted in the beta liquid scintillation counter for 1 min to calculate counting efficiency.

Air was filtered continuously during the first 26–28 h through two silica^{††} and two charcoal^{‡‡} sampling tubes placed in series at a flow rate of 0.3–1.1 L min⁻¹. The tubes were changed at 3.75–4.5 h, 21.25–23 h and 26.25–28.5 h after injection. The contents of each sampling tube were emptied into a 7 mL scintillation vial, 6 mL of scintillation cocktail was added to each vial, and the vials were counted for 10 min in the tritium setting (0–18.6 keV) of a beta liquid scintillation counter.

The birds were sacrificed 15–18 d post-injection, and organs and blood were collected and digested with a tissue solubilizer^{§§} according to manufacturer's recommendation. A weight of tissue solubilizer 2.5 times the weight of the brain tissue or egg, and five times that of liver, heart, or breast tissue, was added to each type of tissue and samples were incubated for 24 h at 37°C. A solution of 1:2 tissue solubilizer:ethanol equivalent to 2.5 times the volume of the blood sample was added and the blood incubated at 60°C for 1 h. The blood mixture was then treated with 30% hydrogen peroxide in a volume equivalent to 2.5 times the original volume of blood collected and incubated at 60°C for 1 h. Fifteen milliliters of scintillation cocktail was added to each vial and the vials were counted in the tritium setting (0–18.6 keV) of a beta scintillation counter for 10 min each. Subsequently, a known amount of tritiated water or toluene calibrated standard was added to each vial and recounted for 1 min in a beta scintillation to calculate counting efficiency.

The radioactivity from the feces, seeds, cage rinses, and cotton swab wipes were summed for each collection point to calculate the value for total excreted radioactivity. The radioactivity from the silica and charcoal tubes for a given time point was summed to calculate the radioactivity in the air. Finally, the radioactivity from the body parts was summed and the total radioactivity was adjusted for the weight of the bird in order to calculate the radioactivity in tissue.

RESULTS

Counting efficiencies varied 10-fold, from 5 to about 50%, depending on the composition of the sample. The feces, with their high uric acid content, showed the

[#] Product number NES003, DuPont NEN Research Products, 549 Albany Street, Boston, MA 02118.

^{**} Product number NES004, DuPont NEN Research Products, 549 Albany Street, Boston, MA 02118.

^{††} Product number 226–10, SKC Inc., 334 Valley View Road, Eighty Four, PA 15330.

^{‡‡} Product number 226–01, SKC Inc., 334 Valley View Road, Eighty Four, PA 15330.

^{§§} Protosol, DuPont NEN Research Products, 549 Albany Street, Boston, MA 02118.

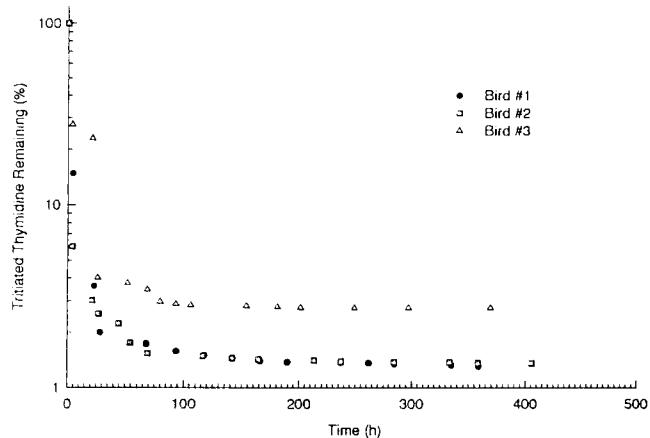


Fig. 1. Tritiated thymidine elimination rate from birds' bodies. Radioactivity was eliminated from birds' bodies very quickly, 98% in 28 h.

Table 1. Distribution of radioactivity in bird tissue 15–18 d post injection of tritiated thymidine.

Tissue	kBq g ⁻¹
Blood	6.03
Liver	4.07
Heart	3.63
Breast	1.18
Brain	0.59
Egg	0.15

greatest degree of quench and lowest efficiency of counting.

The radioactivity was rapidly eliminated from the bodies of the birds, primarily by excretion. After 28 h, less than 2% of the initial radioactivity remained in the bodies of the birds (Fig. 1); the remaining radioactivity was bound to tissues or circulating in the blood.

The radioactivity in the air was monitored continuously for the first 26–28 h; the air in the container changed at a rate of 1.6–4.7 air changes h⁻¹. Only a very small fraction of the injected radioactivity was found in the air (0.06%) during the first 28 h of the experiment.

Immediately following injection, the birds had an estimated average tissue concentration of 0.014 MBq g⁻¹. At the time of sacrifice (15–18 d post injection) their average tissue concentration was 2.14 kBq g⁻¹. The distribution varied with the tissue, with blood showing a higher concentration of radioactivity than the other tissues sampled (Table 1). Since the concentration in the heart and liver were much higher than brain and breast muscle, one could assume tissue differences reflected their infusion with blood.

CONCLUSION

The results of these experiments indicate that 98% of the total radioactivity injected was eliminated by excretion within the first 28 h, and a negligible amount

(0.06%) was airborne. Less than 2% of the initial radioactivity remained in the bird 28 h after the initial injection of tritiated thymidine, mainly in the blood. This remaining amount decreased very slowly thereafter. The concentration of tritium in the tissue dropped by approximately 100-fold 15–18 d after the injection. This indicates that the researcher may design a protocol that will result in a carcass that contains 1.85 kBq g^{-1} (U.S. NRC 1991) or less at the time of sacrifice. By controlling the initial amount of radioactivity and not sacrificing the animal within the first 28 h, the resulting carcass may qualify for disposal without regard to its radioactivity. By holding the bird captive before release into the environment for the initial 28 h after injection, the environmental impact would be as low as reasonably achievable (ALARA).

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