# Excretion of Radiocarbon of $^{14}C$-Labeled 16-alpha,17-alpha-Dihydroxyprogesterone Acetophenide (DHPA) by Beef Heifers

T. S. Rumsey[1] and E. C. Schreiber[2]

This study investigated the excretion patterns of radiocarbon from beef heifers administered the progestogen DHPA and whether methoxychlor affected these patterns. Four heifers were given the estrus synchronization treatment consisting of feeding 120 mg. of unlabeled DHPA daily for nine consecutive days and an intramuscular injection of 5.0 mg. of estradiol valerate on the second day of DHPA treatment. Capsules of labeled DHPA were administered orally to two heifers each on the second and ninth days and one animal from each group received methoxychlor (112 mg. per kg. body weight, daily) during the nine-day treatment period. All of the label was recovered in the feces (97.4 to 97.9%) and urine (2.1 to 2.6%), and the average biological half-lives were 22.2 and 21.6 hours, respectively, for feces and urine. The administration of DHPA-4-$^{14}C$ on the second or ninth synchronization days of feeding methoxychlor had little effect on the fecal and urinary excretion patterns.

Wiltbank *et al.* (1967) and Lantz *et al.* (1968) have used DHPA in combination with estradiol valerate to synchronize estrus in beef cattle. Metabolic residues of this material, however, remain in rats, mice, rabbits, and guinea pigs for an extended length of time. Data are not available on the rate of elimination of DHPA or its metabolites from ruminants. In addition, commonly used pesticides such as DDT and methoxychlor affect the hormonal systems of rats, mice, and swine (Smally and Earl, 1966; Tullner, 1961; Ware and Good, 1967). The effect of these pesticides appears to be estrogenic in nature, but the mechanism and their overall effects on the normal system are not known.

The purposes of this study were to determine the $^{14}C$-excretion patterns after dosing heifers with DHPA-4-$^{14}C$ and the effect of methoxychlor [1,1,1-trichloro-2,2-bis(*p*-methoxyphenyl)ethane] on these excretion patterns.

## METHODS AND MATERIALS

**Animals.** Five Angus heifers, which averaged 250 kg. in body weight, served as experimental animals. Approximately four months prior to and during the study, the heifers were individually fed twice daily a diet consisting of 45% alfalfa hay, 45% timothy hay, 8% cottonseed meal, 1% trace mineralized salt, and 1% bonemeal. The hays were ground through a 10-mm. screen, the diet was pelleted (10-mm. die), and the heifers received a daily amount equal to 2.0% of their body weight. The heifers were visually observed to exhibit regular estrual cycles before the start of the study, but were not checked for heat synchronization or ovulation during the study.

**Chemical.** The labeled and unlabeled 16-$\alpha$,17-$\alpha$, dihydroxyprogesterone acetophenide and the estradiol valerate were supplied by E. R. Squibb and Sons, Inc., New Brunswick, N. J. The carbon-14 label was located at the four position of the progestogen molecule.

**Analytical.** The system described by Robbins and Bakke (1967) and as adapted to beef cattle by Rumsey (1969) was used to quantitatively collect urine, feces, and respired $^{14}CO_2$. Urine was continuously collected via bladder catheters and thymol was added to the urine collection vessels to prevent microbial action. Total urine and feces were measured once daily and representative samples were frozen until analyzed for carbon-14 activity. During each collection period, samples of muscle and fat tissue were surgically removed from the flank area through a 3-cm. skin incision using local anesthesia. The samples were frozen until analyzed for carbon-14 activity. The sampling procedure had no apparent adverse affects on the heifers.

The procedure for trapping and counting respiratory $^{14}CO_2$ was the same as described by Robbins and Bakke (1967). Feces, urine, muscle tissue, and fat tissue were prepared and counted in duplicate as outlined by Schreiber (1968). This method consisted of digesting the sample with hyamine hydroxide 10-X (Packard Instrument Co., Inc., Downers Grove, Ill.) and decoloring the digest with 30% peroxide. This preparation was neutralized with concentrated HCl and counted in a mixture of toluene, absolute ethanol, and liquifluor (T. M. Pilot Chemicals, Inc., Watertown, Mass.). Counting vials served as the vessels in which the samples were prepared, and three 10-minute counts were obtained for each sample and averaged.

Recoveries of $^{14}C$-activity using this method were determined in our laboratory by adding known amounts of uniformly labeled glucose-$^{14}C$ to cold samples and are shown in Table I. The recoveries for urine, muscle, and fat were above 90%. The recoveries for feces were lower but consistent for the three concentrations tested. On the basis of these data, the method was used to quantitate the activity in samples from this study.

**Trial 1.** This trial was a preliminary test to determine the amount of activity as DHPA-4-$^{14}C$ to administer to each animal, the type of excretion pattern to expect, and the length of the collection period needed to accurately describe the excretion pattern during trial 2. One heifer was used in a 17-day collection study. The heifer was placed in the collection system and prepared for collection on the day prior to the study. While in the system the heifer was bucket-fed twice daily and water was available at all times except during respiratory collections. Respiratory samples were collected for a 1-hour period daily (9:00 to 10:00 a.m.), during the collection study and for eight consecutive hours following the administration of DHPA-4-$^{14}C$. Total collections of feces and urine were obtained daily.

The heifer was administered 38.5 mg. of DHPA-4-$^{14}C$ (102 microcuries) on the fourth day of the collection study. The labeled DHPA was dissolved in benzene (55 mg. of solute per milliliter of solution with a specific activity of 2.64 microcuries per milligram). Seven-tenths milliliter of the benzene solution was added to 500 mg. of lactose in a 000

[1] Animal Husbandry Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Md. 20705

[2] Biopharmaceutical Research, Squibb Institute for Medical Research, New Brunswick, N. J. 08903

**Table I. Recoveries of Activity from $^{14}C$-Labeled Glucose Added to Feces, Urine, Muscle, and Fat Samples**

| Microcuries Added per Milliliter[b] | Per Cent Recovery[a] | | | |
|---|---|---|---|---|
| | Feces | Urine | Muscle | Fat |
| 0.05 | 67.8 | 90.7 | ...[c] | ... |
| 0.025 | 67.9 | 95.1 | ... | ... |
| 0.0125 | 64.6 | 92.5 | 99.0 | 100.4 |

[a] Recoveries are an average of duplicate samples and three 10-minute counts per sample.
[b] For feces, muscle, and fat, $^{14}C$-labeled glucose was added to the aqueous homogenates of the samples.
[c] Recoveries at the two higher levels of activity were not determined for muscle and fat samples.

**Table II. Activity Recovered in Feces, Urine, Respired $CO_2$, Muscle, and Fat of a Beef Heifer Treated with 102 Microcuries of DHPA-4-$^{14}C$**

| | Sample[a] | | | | |
|---|---|---|---|---|---|
| | Microcurie/Day | | | Millimicrocurie/Gram | |
| Day | Feces | Urine | $CO_2$ | Muscle | Fat |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1 | 10.48 | 0.34 | 0.00 | 0.00 | 0.20 |
| 2 | 53.60 | 1.18 | 0.00 | | |
| 3 | 23.89 | 0.38 | 0.00 | 0.00 | 0.66 |
| 4 | 8.67 | 0.16 | 0.00 | | |
| 5 | 4.21 | 0.09 | 0.00 | | |
| 6 | 1.44 | 0.04 | 0.00 | | |
| 7 | 1.29 | 0.01 | 0.00 | 0.04 | 0.15 |
| 8-14 | 0.00+ | 0.00 | 0.00 | 1.20 | 0.12 |
| Total from 1-14 days | 103.58 | 2.20 | 0.00 | | |
| % of total | 97.9 | 2.1 | 0.0 | | |

[a] Values for feces, urine, muscle, and fat samples were prepared in duplicate and all samples were counted three times at 10 minutes each.

gelatin capsule and dried under reduced pressure and room temperature to a constant weight. This capsule was placed in a number 10 gelatin capsule and orally administered to the heifer with the aid of a balling gun.

The results of days 1, 2, and 3 were averaged to establish the biological background. Samples from days 4 through 10 were analyzed individually, and samples from days 11 through 17 were analyzed as a composite. Muscle and fat samples from the flank were obtained on days, 2, 4, 6, 10, and 16 of the collection study.

**Trial 2.** Four heifers were placed in metabolism crates for a 24-day collection study. One crate contained the respiratory collection system. During the 24-day period, the heifers were fed and collections were made the same as in trial 1. Days 1, 2, and 3 of the collection period were used to establish a biological background. On days 4 through 12 all heifers were treated daily with 120 mg. of cold DHPA per animal and on day 5 the heifers received 5 mg. of estradiol valerate. The cold DHPA was placed in sesame oil (14.7 grams per liter) and added to the morning feed and the estradiol valerate was injected intramuscularly.

Two of the heifers received DHPA-4-$^{14}C$ (approximately 100 microcuries per animal) on day 5 of the collection study (5D animals), and the remaining two heifers received the same amount on day 12 (12D animals). The labeled DHPA was prepared and administered in capsule form the same as in trial 1. Within the 5D and 12D groups, one heifer received 112.0 mg. per kg. body weight of methoxychlor added to the feed daily on days 4 through 12.

Samples of muscle and fat tissues were obtained from the 5D animals on days 3, 6, 11, 15, 20, and 24 during the collection study and from the 12D animals on days 10, 12, 15, 20, and 24.

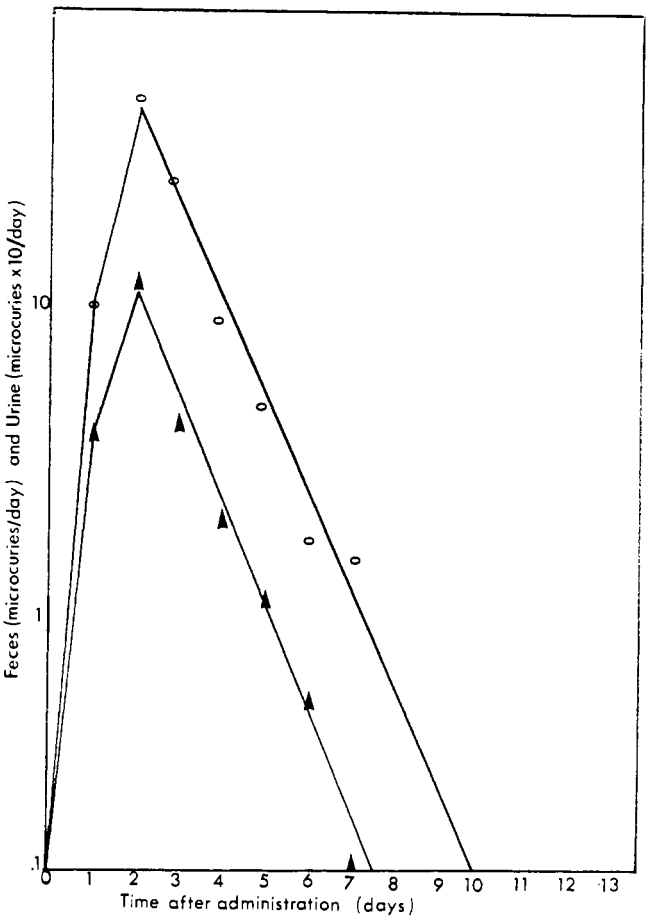


**Figure 1. Fecal and urinary excretion of DNPA-4-$^{14}C$ in the preliminary study**

Least squares excretion curves and biological half-lives of $^{14}C$-activity were: Feces —○—○—, log $Y = 2.48 - 0.38(X)$, $b½ = 19.6$ hr.; urine —△—△—, log $Y = 1.76 - 0.37(X)$, $b½ = 19.8$ hr.

## RESULTS AND DISCUSSION

**Trial 1.** The total microcuries of activity measured in the feces, urine, respired $CO_2$, muscle, and fat are presented in Table II. The heifer was administered 102 microcuries of DHPA-4-$^{14}C$, which was completely recovered (103.7%) within seven days post-administration. None of the activity appeared in the respired $CO_2$, 2.1% appeared in the urine, and 97.9% was measured in the feces. The carbon-14 excreted in the feces probably does not represent unabsorbed DHPA but is radioactivity which entered the gastrointestinal tract via the biliary pathway. This observation is based upon the high degree of parallelism seen in the excretion patterns of radiocarbon in urine and feces (Figure 1). If the carbon-14 in the feces was present as the result of poor absorption rather than excretion, a biphasic, initially nonlinear, excretion pattern would be expected. The first slope of this curve would be substantially steeper than the second. This difference in slope would come about because in the early portion of the excretion pattern the elimination of unabsorbed material would be superimposed upon DHPA or metabolites which are being excreted by a process that appears to follow first-order kinetics. Once fecal elimination of unabsorbed drug was no longer taking place, the second portion of the curve would appear as a less steep, more linear curve. In view of the similarity of the half times of the fecal and urinary elimination curves (19.6 and 19.8 hours, respectively, after day 1, Figure 1), it seems reasonable to conclude that the drug is well absorbed following oral administration. A very small

**Table III. Activity Recovered in Feces and Urine of Beef Heifers Administered DHPA-4-$^{14}$C on Second and Ninth Days during Nine-Day Estrus Synchronization Treatment with DHPA[a]**

| | Second Day | | Ninth Day | | |
|---|---|---|---|---|---|
| | 360[b] | 378 | 346[b] | 379 | Average |
| Activity recovered in: | | | | | |
| Feces, μCi | 89.64[c] | 106.07 | 98.93 | 99.77 | 98.60 |
| Urine, μCi | 2.28 | 2.72 | 2.20 | 2.68 | 2.47 |
| Total activity recovered, μCi | 91.92 | 108.79 | 101.13 | 102.45 | 101.14 |
| Total activity administered, μCi | 100.20 | 100.20 | 101.90 | 101.90 | 101.05 |
| Per cent recovered: | | | | | |
| Feces | 97.51 | 97.50 | 97.82 | 97.38 | 97.55 |
| Urine | 2.49 | 2.50 | 2.18 | 2.62 | 2.45 |
| Total | 91.73 | 108.57 | 99.24 | 100.53 | 100.02 |

[a] No activity was recovered as respiratory $^{14}CO_2$ and the amount of activity measured in muscle and fat tissue was not significantly greater than background.
[b] Heifers 360 and 346 received 1000 mg./kg. body weight of methoxychlor during the 9-day estrus synchronization treatment.
[c] Average of three 10-minute counts and duplicate samples.

**Table IV. Least Squares Excretion Curves and Biological Half-Lives of Activity in Feces and Urine of Beef Heifers Administered DHPA-4-$^{14}$C[a]**

| Treatment | Least Squares Regression Equation | Biological Half-Life, Hours |
|---|---|---|
| Feces | | |
| Second day | $\log Y = 2.54 - 0.40(X)$ | 18.9 |
| Ninth day | $\log Y = 2.27 - 0.29(X)$ | 25.4 |
| − Methoxychlor | $\log Y = 2.31 - 0.31(X)$ | 24.2 |
| + Methoxychlor | $\log Y = 2.51 - 0.38(X)$ | 20.2 |
| Overall average | $\log Y = 2.41 - 0.35(X)$ | 22.2 |
| Urine | | |
| Second day | $\log Y = 1.75 - 0.40(X)$ | 19.1 |
| Ninth day | $\log Y = 1.63 - 0.32(X)$ | 24.0 |
| − Methoxychlor | $\log Y = 1.92 - 0.43(X)$ | 17.7 |
| + Methoxychlor | $\log Y = 1.46 - 0.29(X)$ | 25.5 |
| Overall average | $\log Y = 1.69 - 0.36(X)$ | 21.6 |

[a] DHPA-4-$^{14}$C was administered on either the second or ninth days of the recommended estrus synchronization treatment and the pesticide methoxychlor was fed to half of the heifers during the treatment period.

amount of activity was measured in the muscle and fat tissues. However, it is unlikely that this activity represents a true residue, since the actual counts per minute in the samples were not significantly greater than background, and 100% of the administered dose was recovered in the feces and urine over a short period of time. During days 1 through 7 post-administration, the changes in activity in the feces and urine were highly significant ($P < 0.005$). The greatest quantities were excreted on day 2.

The daily excretions of activity in the feces and urine were plotted on a log scale *vs.* time in days, and least squares regression curves were fit to the points for days 2, 3, 4, and 5 after administration (Figure 1). Days 6 and 7 were not used to estimate the regression equation because the amount of activity measured on these days was small and therefore less accurate, leading to an inaccurate estimate of the regression slope. The slopes of the regression curves were similar for both feces and urine. The regression equation for feces was $\log Y = 2.48 - 0.38\ X$ with a biological half-life for radiocarbon in the feces of 19.6 hours. For urine, the equation was $\log Y = 1.76 - 0.37\ X$ with a biological half-life of 19.8 hours.

The activity in the composite samples for the last week of the collection was unmeasurable for both feces and urine. This would indicate that none of the activity administered as DHPA-4-$^{14}$C is associated with a long biological half-life. On the basis of the data from this trial, it appears that approximately 100 microcuries of activity as labeled DHPA-4-$^{14}$C was sufficient to measure the excretion pattern of the label and this pattern in both the feces and urine was linear with a comparatively short half-life in both the feces and urine.

**Trial 2.** The same excretion patterns were found with the four heifers in trial 2 as were found in trial 1. None of the activity was measured in the respired $CO_2$, the amount of activity in the muscle and fat tissues was not significantly greater than background, and most of the activity was obtained in the feces and urine during the first seven days post-administration. A slight amount of activity was measured in the urine up to 20 days post-administration; this was not significantly greater than background and could have resulted from residual activity in the urine collection system.

A summary of the total excretion of carbon-14 activity in the urine and feces is shown in Table III for the individual animals. On the average, 100.02% of the activity was recovered in the feces and urine. The urine accounted for 2.45% (ranging from 2.18 to 2.262) and the feces accounted for 97.55% (ranging from 97.38 to 97.82). These values are very similar to those obtained in trial 1. The low recovery (91.92%) for animal 360 and the high recovery (108.79%) for animal 378 could have resulted from an error in preparing the capsules of labeled DHPA. A total of 200.4 μCi was prepared for both animals but by mistake was probably not divided into two equal doses of 100.2 μCi.

The activity recovered in the feces and urine was analyzed statistically and regression equations were fit to the data as in trial 1. These equations and the biological half-lives for $^{14}$C-activity are shown in Table IV. When the data for all the heifers were averaged, the regression slopes and the biological half-lives were similar for both feces and urine and similar to those shown in Figure 1 for the first trial.

When DHPA-4-$^{14}$C was administered on the second day of the normal treatment for estrus synchronization, the excretion rates in both the feces and urine were somewhat greater compared to the excretion of activity from animals treated on the last (ninth) day; however, this difference was not significant. The effect of feeding methoxychlor upon the excretion of $^{14}$C-activity was not significant for the feces but approached significance ($P < 0.10$) for the urine. Methoxychlor appeared to delay the excretion of activity in the urine but the mechanism of this effect is not known. It is doubtful that these differences are of practical significance, since the level of methoxychlor was much higher than that encountered under normal management, and only an insignificant amount of the activity remained in any of the heifers beyond 10 days.

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