Distribution, Elimination, and Residue Studies in the Cow with the Synthetic Prostaglandin Estrumate

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After intramuscular administration of radiolabeled Estrumate (cloprostenol; ICI 80,996) to cows, the levels of radioactivity in blood, milk, and urine were monitored. Residual concentrations in tissues were determined at 0.5, 24, 48, and 72 h after dosing, using four cows at each time point. Less than 0.75% of the dose was eliminated in milk, and this was largely contained in the samples at 4 h. Blood levels reached peak values in the range 0.18 to 0.86 ng/mL within the first hour after dosing and had fallen to undetectable levels by 24 h. Elimination was about equally divided between urinary and fecal routes; approximately half the dose was recovered in urine within 16 h. Significant levels of radioactivity in tissues were largely confined to samples taken almost immediately after dosing, and detectable persistence of residues in the edible tissues did not occur. The status of the estrous cycle at the time of dosing appeared to have no effect on any of the parameters monitored.

Estrumate (Trademark, the property of Imperial Chemical Industries Limited) is a synthetic prostaglandin which has been shown to cause luteal regression at low doses by the intramuscular route (Cooper, 1974; Cooper and Furr, 1974). The use of Estrumate for the synchronization of estrous in cattle is of commercial benefit, particularly when combined with the use of artificial insemination (Cooper and Furr, 1974; Cooper and Rowson, 1975). A single, intramuscular dose of Estrumate has been shown to be a highly effective treatment of certain "infertile" conditions in the cow (Jackson and Cooper, 1976). These conditions include chronic endometritis, subestrous, the removal of fetal mummies, and the termination of normal but unwanted pregnancies.

Having regard to the potent biological activity of the compound, this study was designed to measure the elimination of ICI 80,996 and derived material in milk and clearance from edible tissues.

In a preliminary study using two cows, it was shown that fecal elimination during a 72-h collection period accounted for 39–43% of the dose. The corresponding urinary figures (0–15-h collection only) were 55 and 52%. Thus, the overall recoveries were 94 and 95%, respectively. Between 65 and 75% of the total fecal elimination occurred in the 12–24-h period.

MATERIALS AND METHODS

The material used was labeled with ^{14}C in the C(15) position (Figure 1) using the highest feasible specific activity, which was 120 μCi/mg and which represented ca. 80% isotopic abundance for the C(15) position (White, 1977).

Sixteen Friesian dairy cows, having a nominal weight of 500 kg and yielding around 16 L of milk/day, were pretreated with prostaglandin $F_{2\alpha}$ so as to cause half the cows to be in the luteal phase of the estrous cycle and half in the follicular phase at the time of dosing with [^{14}C]ICI 80,996.

All the cows were dosed by intramuscular injection in the rump with 5 mL of a citrate buffer formulation containing 500 μg ICI 80,996 and 60 μCi radioactivity. The last milking prior to dosing occurred about 14 h earlier, in the previous evening. After dosing, milk was collected for up to 3 days, urine for up to 24 h (by means of urethral catheterization), and blood was sampled for up to 24 h. Feces were not collected. Milk results exclude those from cow 13, which was found to be suffering from mastitis.

The cows were divided into four equal groups, each intended to contain a pair in the luteal phase and a pair in the follicular phase. The groups were slaughtered at 0.5, 24, 48, and 72 h after dosing. An additional untreated cow was slaughtered to provide control tissues. Samples of the following tissues were removed: muscle, liver, kidney, subcutaneous and abdominal fat, skin, heart, adrenals, ovaries, uterus, the injection site, and blood. Bile was also sampled from some cows. The injection site was defined as a cylinder of tissue having a diameter and length equal to twice the length (5 cm) of the needle used.

Milk was counted in "Instagel" (10 mL + 10 mL of milk) using nine replicate blanks prepared from predose milk from the same cow. Six replicate aliquots for counting were prepared from each sample, no later than 24 h after sample collection, and were stored at 4 °C until counting facilities became available. All the samples were counted for 100 min/10^4 counts on the same Intertechnique SL30 counter operating at 10 °C. Correction of cpm to dpm was by internal standardization, using 50 μL of [^{14}C]-*n*-hexadecane containing 10000 dpm, and "spiked" samples were counted to 10^4 accumulated counts.

Urine and bile samples were prepared in triplicate and were counted in a phosphor system containing 0.5% PPO + 0.05% dimethyl POPOP in Triton X-100:toluene (1:2), using a mixture of 10 mL of phosphor + 1 mL of sample or 10 mL of phosphor + 0.5 mL of sample + 0.5 mL of water. An Intertechnique SL30 counter operating at 10 °C was used, with a count limit of 10 min/10^4 counts. Counting efficiencies were computed from the external standard ratio using a quench correction curve described by a third-order polynomial.

Whole blood (0.5 mL) and tissue samples (70–400 mg) were combusted using the Kalberer-Rutschmann modification of the Schöniger oxygen flask technique. For these samples, three replicates were used, together with at least nine blank replicates. The absorbent used was 15 mL of 12% ethanolamine in methanol, and after combustion and rotation of the flasks (20 min), 10 mL of this absorbent + 10 mL of 0.8% butyl-PBD scintillation phosphor in toluene was counted using Packard 2002 room temperature counters. Three different counters were employed for these samples, but all samples of a given tissue were counted on the same counter (count limit was 100 min/10^4 counts). Correction of cpm to dpm was by internal

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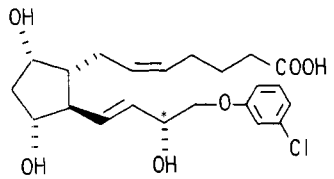

Figure 1. Structure of ICI 80,996. Asterisk denotes ^{14}C labeling position.

Table I. Cumulative Urinary Excretion (% Dose) of Total ^{14}C

Collection period, h	No. of cows	Mean urinary excretion ± SE
0-0.5[a]	4	8.2 ± 1.9
0-1	12	8.2 ± 1.8
1-2	12	19.3 ± 3.5
2-3	12	30.8 ± 3.3
3-4	12	38.3 ± 3.2
4-8	12	47.3 ± 2.2
8-12	12	50.4 ± 1.7
12-16	12	52.5 ± 1.4
16-24[b]	8	53.4 ± 1.4

[a] Cows 1-4 only; 0-16 h results are from cows 5-16. [b] Cows 5-12 only.

standardization as described for milk, using a 1-min count limit.

The limits of detection for the results for the individual cows were based on the single-sided confidence limit ($p = 0.01$) for the mean of the replicate assays, using a variance derived from blank tissue data (Case et al., 1977). Tissue values which differed from zero by less than this limit were defined as not detectable. These assay detection limits varied with the tissues but were in the range 0.05 to 0.20 ng/g. The mean tissue values (average of values from *all* four cows for the given sample time) were not considered to be significant unless they exceeded the assay detection limits (given in Table II).

RESULTS

It should be noted that all the concentrations of ^{14}C-labeled material described below have been expressed as the radiochemically equivalent levels of ICI 80,996 (free acid) for convenience of comparison, and this should not be taken to imply that the material measured was solely of unchanged ICI 80,996.

Excretion of ^{14}C material in milk represented a minor route of elimination, since the highest amount recovered in milk by 24 h was 0.74% of the dose. In all cases, well over half (and in many cases over 90%) of this material had been eliminated by 4 h after dosing. This rapid elimination is depicted in Figure 2, which shows a fall in milk concentrations having a mean $t_{1/2}$ value of 5.4 h. The highest concentration seen after 24 h (i.e., in the 24–36-h samples) was 0.0067 ng/mL, i.e., 6.7×10^{-6} ppm.

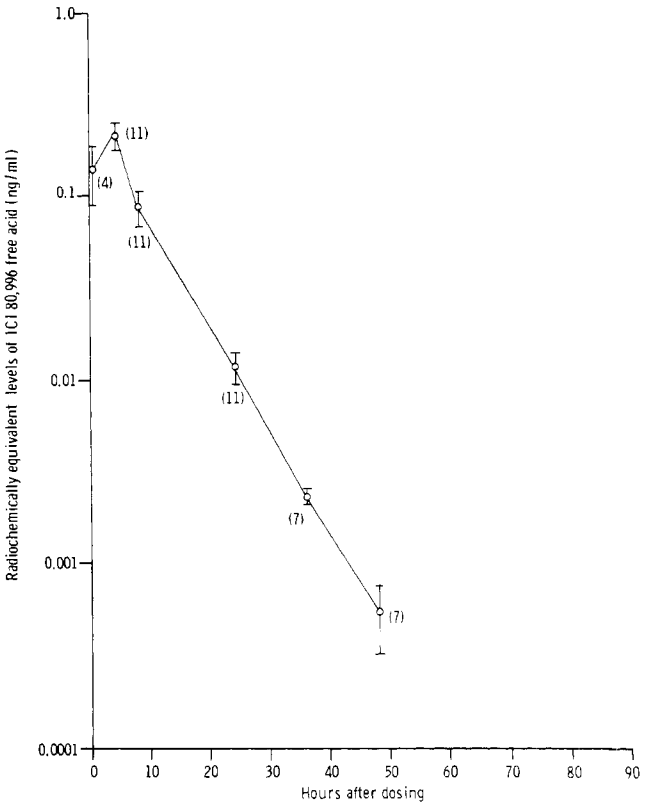

Figure 2. Mean levels of total ^{14}C in milk. Vertical bars represent standard error of mean values (values of n are indicated in parentheses).

As shown in Table I, elimination of radioactivity in urine was largely complete by 16 h after dosing and the mean recovery up to this time was 52.5 ± 4.8% (SD). Elimination in urine was rapid, as shown in Figure 3, and the elimination rate declined with a mean $t_{1/2}$ value of 2.8 h.

Peak blood levels occurred between 15 min and 1 h after dosing and varied between 0.18 and 0.86 ng/mL (Figure 4). The subsequent rapid decline in levels paralleled that for urine and was described by a mean $t_{1/2}$ value of 3 h (taken over the 4–12-h period).

At 0.5 h after dosing, residues were observed in all tissues sampled except the fats, three out of four muscle samples,

Table II. Individual Tissue Residue Levels of Total ^{14}C (ng/g)[a]

Tissue	Cow no. (0.5 h sample time) 1[b]	2[b]	3	4	Mean ± SE[c]	Limits of detection
Muscle	0.123	ND	ND	ND	ND	0.090
Liver	7.23	5.46	8.38	7.92	7.25 ± 0.641	0.020
Kidney	26.5	13.6	19.2	17.0	19.1 ± 2.73	0.094
Subcut. fat	ND[d]	ND	ND	ND	ND	0.200
Abdom. fat	ND	ND	ND	ND	ND	0.200
Adrenals	0.387	0.237	0.254	0.332	0.303 ± 0.035	0.173
Uterus	0.851	0.600	1.01	0.650	0.778 ± 0.095	0.048
Ovaries	1.86	1.06	0.729	0.627	1.07 ± 0.279	0.127
Heart	0.827	0.457	0.686	0.481	0.613 ± 0.088	0.090
Skin	0.463	ND	0.152	0.120	0.192 ± 0.094	0.105
Inj. site	212	171	129	135	162 ± 19.1	0.053
Blood (ng/mL)	0.863	0.500	0.699	0.547	0.652 ± 0.082	0.020
Bile (ng/mL)	49.7	19.8	55.5	24.7	37.4 ± 8.90	

[a] Expressed as the radiochemically equivalent level of ICI 80,996 free acid. [b] These cows were in the luteal phase; others were in the follicular phase. [c] Mean and standard error on *all* four values obtained. [d] ND = below detection limits for assay.

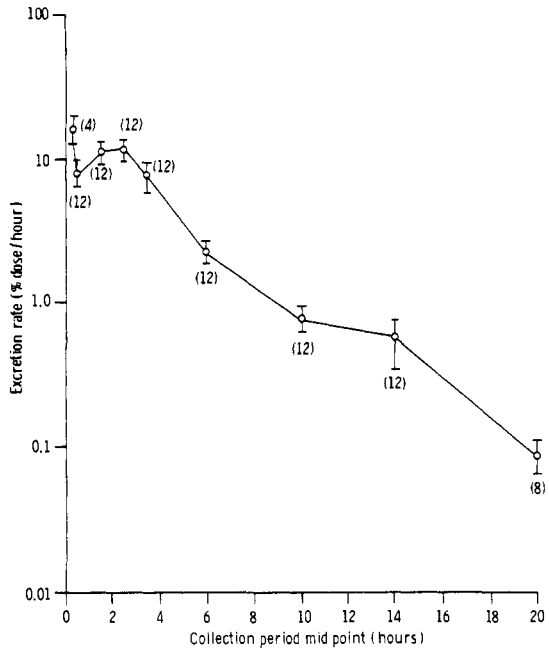

Figure 3. Mean urinary excretion rate of total ^{14}C. Vertical bars represent standard error of mean values (values of *n* are indicated in parentheses).

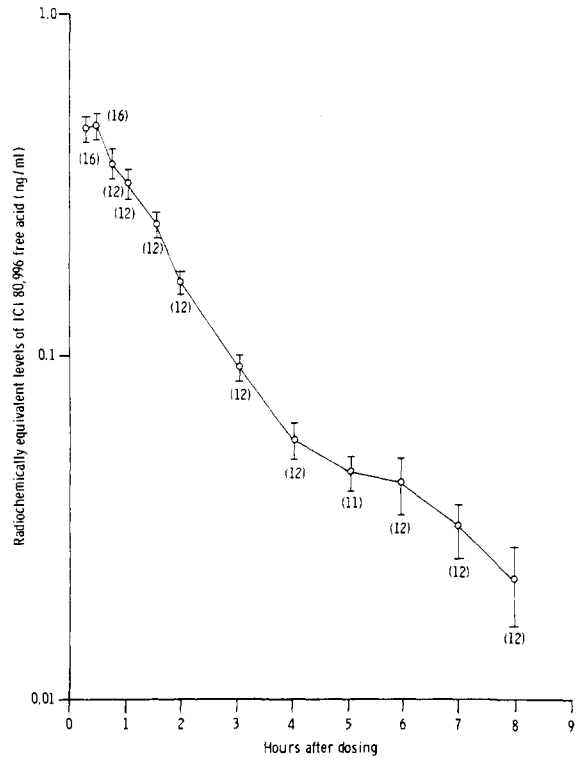

Figure 4. Mean levels of total ^{14}C in whole blood. Vertical bars represent standard error of mean values (values of *n* are indicated in parentheses).

and one skin sample (Table II). By 24 h, measurable levels were contained only by liver (0.027 to 0.057 ng/g), kidney (up to 0.17 ng/g), and some skin and injection sites (up to 0.61 ng/g and 0.95 ng/g, respectively). By 48 h, detectable levels of radioactivity occurred in only three samples: one kidney (0.095 ng/g), one uterus (0.054 ng/g), and one injection site (0.060 ng/g). At 72 h, detectable levels occurred only in one uterus (0.053 ng/g) and in all injection sites (0.16 to 0.38 ng/g).

Of the samples examined, liver, bile, kidney, and the injection sites, taken at 0.5 h, were the only ones to contain

Table III. Individual Tissue Residue Levels of Total ^{14}C (ng/g)[a]

Cow no.	24-h sample time 5[c]	6[c]	7[c]	8[c]	Mean ± SE[b]
Liver	0.027	0.057	0.045	ND	0.036 ± 0.010
Kidney	ND[d]	0.127	0.169	0.119	0.123 ± 0.019
Skin	0.611	0.253	ND	ND	0.205 ± 0.155
Inj. site	ND	0.484	0.553	0.950	0.493 ± 0.198
Bile	NA[e]	NA	0.271	0.086	0.179 ± 0.093
Other tissues	ND	ND	ND	ND	ND

Cow no.	48-h sample time 9[c]	10[c]	11	12	Mean ± SE[b]
Kidney	0.095	ND	ND	ND	ND
Uterus	ND	ND	ND	0.054	ND
Inj. site	ND	ND	0.060	ND	ND
Bile	NA	NA	NA	NA	NA
Other tissues	ND	ND	ND	ND	ND

Cow no.	72-h sample time 13[c]	14	15[c]	16	Mean ± SE[b]
Uterus	ND	0.053	ND	ND	ND
Inj. site	0.163	0.180	0.263	0.384	0.248 ± 0.050
Bile	NA	NA	NA	NA	NA
Other tissues	ND	ND	ND	ND	ND

[a] Expressed as the radiochemically equivalent level of ICI 80,996 free acid. [b] Mean and standard error on *all* four values obtained. [c] These cows were in the luteal phase; others were in the follicular phase. [d] ND = not detected (for limits see Table II). [e] NA = not assayed.

relatively high levels of ^{14}C material. The injection sites at this time contained 40.0 ± 3.4% (SD) of the dosed radioactivity. In general, significant levels were largely confined to samples taken at 0.5 h after dosing (Table III). No evidence was seen which would indicate dependence of any of the parameters studied on the stage of the estrous cycle at the time of dosing.

DISCUSSION

Preliminary studies in rodents indicated that the ^{14}C label in the C(15) position was metabolically stable and that biliary excretion was a major route of elimination (Bourne, unpublished data). This has now been confirmed by the data reported for the cow. Preliminary results of metabolism studies in this species show that ICI 80,996 is excreted in urine partly unchanged and partly as its tetranor acid, formed by β-oxidation of the aliphatic carboxylic acid side chain. Further transformation products of this tetranor acid are also eliminated in urine (Bourne, unpublished data).

Intramuscular administration of ICI 80,996 is followed by very rapid clearance from the site of injection, giving rise to maximum tissue levels at about 30 min after dosing. Neither the drug nor its metabolites accumulate in fat, and only very low levels, of the order of 1 ng/g, are found in nonexcretory tissues, such as the uterus, ovaries, and heart, at 30 min.

ICI 80,996 and its metabolites are excreted very rapidly, approximately equal amounts being eliminated via the kidney and in bile. Less than 0.75% of the dose is eliminated in the milk and detectable persistence of residues in the edible tissues of the cow does not occur.

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LITERATURE CITED

Case, D. E., Barnfield, D. J., Reeves, P. R., "Liquid Scintillation Counting", Vol. 4, Heyden & Son Ltd., London, 1977, pp 243–251, Chapter 19.
Cooper, M. J., Furr, B. J., *Vet. Rec.* **94**, 161 (1974).
Cooper, M. J., *Vet. Rec.* **95**, 200 (1974).
Cooper, M. J., Rowson, L. E. A., *Ann. Biol. Anim. Biochim. Biophys.* **15**, 427 (1975).
Jackson, P. J., Cooper, M. J., *Int. Congr. Dis. Cattle* **2**, 903 (1976).
White, D. F., *J. Label. Compd.* **13**, 23–40 (1977).

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Volatile Components of Fermented Egg, an Animal Attractant and Repellent

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Since field tests have indicated that the volatile components of a fermented egg product (FEP) are attractive to coyotes and repellent to deer, it seems possible that a dual-purpose synthetic mixture could be prepared from the important compounds in this material. Both properties are potentially useful for controlling animal damage to agricultural and forest products. The quantities of 13 volatile fatty acids and eight amines were measured by gas–liquid chromatography. To identify other important compounds (alcohols, alkyl aromatics, esters, ketones, terpenes, and organosulfur compounds), we collected the headspace volatiles and analyzed them by combination capillary–column gas chromatography and mass spectrometry. The volatile constituents of FEP parallel those found in fermented food products. Also, some of the same volatile fatty acids and amines are found in anal gland secretions of canids. Thus, both food associated and chemical signaling aspects may be involved in coyote and deer responses.

A fermented egg product (FEP) recently patented as a bait for synanthropic flies (Mulla and Hwang, 1974) has been shown to be both repellent to deer and attractive to coyotes. The development of a material for either of these purposes has been a major research objective for many years.

The feeding activity of deer has become an increasingly important problem in agricultural crops, residential shrubbery, tree plantations, and reforestation areas. Reforestation areas are of special concern in the Pacific Northwest where protection of timber resources is particularly important. During the past two decades, reforestation efforts have been seriously hindered because deer and elk browse Douglas fir seedlings. If these seedlings could be protected, timber regeneration would be substantially accelerated. The approach toward finding methods to protect these resources from large game animals has generally been that of searching for nontoxic repellents which, when applied to the plants, will prevent browsing.

The coyote is currently one of the more frequent topics in discussions of animal damage control. Many stockmen are convinced that control of coyotes is necessary to remain in business, while others believe that claimed livestock losses to coyotes are exaggerated. Unfortunately, this dilemma cannot be resolved because not enough information is available on the effects of coyotes in natural biological systems, or their population distributions. Attractants can be used as a tool for gathering this information. For example, FEP was used from 1971 to 1975 to obtain indices of coyote population throughout the Western states (Linhart and Knowlton, 1975).

The dual potential of decomposed proteinaceous matter was recognized when a putrified fish formulation that had been used as a coyote lure was found to be an effective deer repellent (Campbell and Bullard, 1972). It was the best of 225 candidates (selected from over 4000 candidate chemicals in a screening program) that were evaluated in a standardized test for deer repellency (Dodge et al., 1967). Later, FEP was tested because it had some of the same properties. It also was effective in the deer repellent tests (unpublished data) and as an attractant for coyotes (Linhart et al., 1977). Since FEP is a manufactured item and can be readily purchased, we selected it for further development.

In the patented process for preparing FEP (Mulla and Hwang, 1974), a mixture of powdered whole egg and water is held in open contact with the air at room temperature for 7–14 days. Microorganisms from the air decompose the fat and protein. The egg–water mixture becomes a flowable slurry, which after aging is complete, is converted to a yellow powder (FEP) by freeze-drying.

Unfortunately, this method is subject to changing conditions, and batch-to-batch variation in quality occurs. Such variations in turn influence the behavioral response of animals and cause variable results. Another difficulty in the preparation of FEP is that pathogenic organisms may be cultured inadvertently, posing a health hazard to handlers.

Hwang and Mulla (1971) previously identified ten carboxylic acids in FEP, only three of which were considered to be key volatile components. Our objectives in this work were to identify other important volatile constituents, determine their relative concentrations, and formulate a synthetic preparation that duplicates the human odor panel response of FEP.

EXPERIMENTAL SECTION

It was apparent from the beginning of our work that information needed for the synthetic blending process must come from several analytical methods. For example, fatty acids were known to be predominant, and they could not be collected and analyzed under the same conditions as the other volatiles. Another procedure was required for the amines. However, most of the volatiles that were identified came from a cryogenic trapping procedure.

All analyses were conducted on samples taken from the FEP batches that had been used in the 1971–1975 surveys

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