Nitsch, J. P., Nitsch, C., *Plant Physiol.* 31, 94 (1956).

Pilet, P. E., in "Les Phytohormones de Croissance", Masson et Cie Editeurs, Paris, France, 1961.

Sekita, S., Yoshihira, K., Natori, S., Kuwano, H. *Tetrahedron Lett.*, 2109 (1973).

Springer, J. P., Cox, R. H., Cutler, H. G., Crumley, F. G. unpublished results.

Wells, J. M., Cutler, H. G., Cole, R. J., *Can. J. Microbiol.* 22, 1137

Clearance of the Synthetic Prostaglandin Cloprostenol ("Estrumate") from the Milk of Cows

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The concentration of the synthetic prostaglandin cloprostenol ("Estrumate") has been measured in the milk of three cows following an intramuscular dose of 0.5 mg of [^{14}C]cloprostenol. The maximum observed concentration of cloprostenol in milk was 0.270 ng/mL (2.7×10^{-4} ppm) at 4 h after dosing. At 24 h after dosing, the concentration of cloprostenol in milk had declined to 0.002 ng/mL (2×10^{-6} ppm). The excretion of radioactivity in milk and urine and the proportion of cloprostenol in milk at doses of 0.5 mg and 10 mg of cloprostenol were similar, demonstrating that the drug is cleared by apparent first-order processes over the dose range studied. The similarity in the time course of the clearance from milk of cloprostenol and the endogenous prostaglandin, PGF$_{2\alpha}$, is discussed.

Cloprostenol ("Estrumate", trademark the property of Imperial Chemical Industries Ltd.) (see Figure 1) is a synthetic prostaglandin which is of commercial benefit for the synchronization of estrus and treatment of infertile conditions in cows (Cooper and Furr, 1974; Cooper and Rowson, 1975; Jackson and Cooper, 1976). Studies of the clearance of this potent agent from the milk and edible tissues of the cow have been previously reported (Reeves, 1978). This author reported that the elimination of total radioactivity in milk was a minor route of excretion following intramuscular administration of [^{14}C]cloprostenol. Milk is a tissue of importance with respect to residues for new animal drugs (Perez, 1977), and studies have been undertaken to characterize the components which constitute the radioactivity in milk after dosing [^{14}C]cloprostenol. This paper describes studies undertaken to determine the concentration of the drug in milk using isotope dilution analysis. These studies have been carried out following intramuscular dosing of [^{14}C]cloprostenol at the therapeutic dose (0.5 mg) and an elevated dose (10 mg).

METHODS

Animal Studies. Three Friesian dairy cows (body weight approximately 500 kg) were used. [^{14}C]Cloprostenol prepared as previously described with a radiochemical purity >99% (White, 1977) was administered intramuscularly to the cows at two dose levels. The higher dose was chosen to facilitate the determination of cloprostenol in milk at 24 h after dosing. Each cow received a single injection of 0.5 mg of [^{14}C]cloprostenol (sp act., 91.9 μCi/mg) in 2 mL of citrate buffer. Urine (via a urethral catheter) and milk were collected for 24 h after dosing. Following a 48-h recovery period, the procedures were repeated following administration of 10 mg of [^{12}C/^{14}C]-cloprostenol (ratio ^{12}C/^{14}C forms, 1:2; sp act. of [^{14}C]clo-

prostenol, 91.9 μCi/mg). Urine and milk were collected for 24 h after dosing.

Determination of the Proportion of [^{14}C]Cloprostenol in Milk. Milk (20 mL) collected from cows up to 8 h after dosing with [^{14}C]cloprostenol was spiked with nonlabeled drug (5 mg) as carrier and [^{3}H]cloprostenol (generally labeled in the phenyl ring; sp act., 33 μCi/mg) such that the ratio of ^{3}H/^{14}C in each sample was known. Standards were prepared in the same manner. The milk was acidified with sulfuric acid (0.5 M, 2 mL), and diethyl ether (25 mL) was added. The sample was mixed and centrifuged and the supernatant removed and concentrated. The extract was redissolved in ether (10 mL) and washed with dipotassium hydrogen phosphate (0.05 M, 10 mL). The aqueous phase was removed and acidified with orthophosphoric acid (1 M, 0.5 mL), and the sample was extracted with ether. After mixing and centrifuging, the organic phase was removed, concentrated, and redissolved in phosphate buffer (pH 6).

For milk samples collected during 8–24 h after dosing, 1 L of milk was taken for analysis. The procedures were modified accordingly.

Aliquots of the buffered solution were assayed for ^{3}H and ^{14}C by liquid scintillation counting. The remainder of the sample was analyzed by high-pressure liquid chromatography using "Spherisorb O.D.S." as column packing (solvent, methanol/water/acetic acid, 60:40:0.5, v/v). The peak eluted with a retention time equivalent to cloprostenol was concentrated and counted for ^{3}H and ^{14}C. The chromatographic procedure was then repeated for each sample using methanol/water/acetic acid, 55:45:0.5 (v/v), then 50:50:0.5 (v/v). The proportion of cloprostenol in each sample was estimated from the changing ^{3}H/^{14}C ratio with successive chromatographic analysis.

Radiochemical Techniques. Urine and milk were assayed for radioactivity as previously described (Reeves, 1978). Samples containing ^{3}H and ^{14}C were assayed using the Triton scintillant described by Reeves and a Packard 3320 or Intertechnique SL40 scintillation counter adjusted for counting dual-labeled samples. All samples were

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Clearance of Cloprostenol from Cow Milk

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Table I. Analysis of Milk[a]

dose, mg	collection period after dosing, h	concn of total ^{14}C in milk, ng/mL	% cloprostenol rel to total ^{14}C	concn of cloprostenol, ng/mL
0.5	0–4	0.419 ± 0.152	63.6 ± 13.9	0.270 ± 0.131
	4–8	0.146 ± 0.036	48.5 ± 10.3	0.069 ± 0.017
	8–24	0.011 ± 0.006	16.6 ± 10.0	0.002 (range, 0.0006–0.005)
10.0	0–4	7.009 ± 1.339	52.0 ± 5.8	3.673 ± 0.972
	4–8	2.223 ± 0.767	43.8 ± 6.5	0.987 ± 0.446
	8–24	0.154 ± 0.079	16.1 ± 5.8	0.026 ± 0.020

[a] Results are mean ± SD of single determinations for three animals.
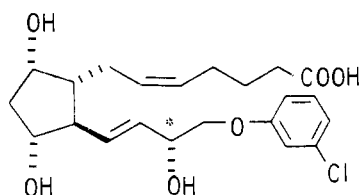

Figure 1. Structure of [^{14}C]cloprostenol ("Estrumate"). The asterisk denotes the position of ^{14}C.

counted for 40 min or to 10^4 accumulated counts. Quench correction was by internal standardization using ^{3}H or ^{14}C hexadecane (10 000 dpm).

RESULTS

Excretion of Radioactivity in Urine. Urinary excretion during 0–24 h after dosing accounted for 58.2 ± 16.0% and 56.3 ± 9.8% (mean result ± SD; $n = 3$) of the dosed radioactivity following intramuscular administration of 0.5 and 10 mg of [^{14}C]cloprostenol, respectively. Excretion was rapid and the major portion of the radioactivity was excreted during 0–4 h (0.5-mg dose, 40.1%; 10-mg dose, 44.5%; mean result $n = 3$) and 4–8 h (0.5-mg dose, 11.5%; 10-mg dose, 8.5%) after dosing. There was no difference either in the percentage dose excreted in urine during 24 h after dosing or in the rate of excretion of radioactivity between the two dose levels of cloprostenol.

Analysis of Milk. Milk production during 24 h after dosing cloprostenol was the same at both dose levels. The production of milk during each collection period after dosing 0.5 mg of cloprostenol was: 0–4 h, 2.6 ± 1.5 L (mean ± SD; $n = 3$); 4–8 h, 1.8 ± 0.8 L; 8–24 h, 7.7 ± 2.1 L; total milk production during 24 h, 12.1 ± 4.3 L. Following a 10-mg dose of cloprostenol, milk production was as follows: 0–4 h, 2.5 ± 0.6 L; 4–8 h, 2.0 ± 0.1 L; 8–24 h, 9.0 ± 0.7 L; total milk production during 24 h, 13.5 ± 1.2 L.

Following intramuscular dosing of 0.5 mg of [^{14}C]cloprostenol to cows, excretion of radioactivity in milk during 24 h after dosing accounted for 0.330 ± 0.193% (mean ± SD; $n = 3$) of the dose. The major portion of the radioactivity excreted by this route was eliminated during the first 4 h after dosing (0.252 ± 0.183% equivalent to 76.4% of the material finally excreted by this route). During 4–8 and 8–24 h after dosing 0.057 ± 0.016 and 0.020 ± 0.013% of the dose was excreted in milk.

After dosing 10 mg of [^{14}C]cloprostenol, excretion of radioactivity in milk was similar to that seen at the lower dose. During 24 h after dosing, 0.248 ± 0.025% of the dose was excreted in milk. The percentage of dose excreted during the first 4-h collection period was 0.185 ± 0.023% (74.6% of total), 0.048 ± 0.007 during 4–8 h after dosing, and 0.015 ± 0.007% during 8–24 h after dosing.

It is concluded that there is no difference in the fraction of the dose excreted in milk nor in the rate of excretion of radioactivity in milk after dosing 0.5 or 10 mg of [^{14}C]cloprostenol to cows.

Samples of milk collected during 0–4, 4–8, and 8–24 h after intramuscular administration of [^{14}C]cloprostenol (0.5

or 10 mg) were analyzed by the isotope dilution method to determine the proportion of unchanged drug present. Predose milk samples spiked with [^{14}C]cloprostenol were stored with the milk from dosed cows and analyzed to assess the stability of cloprostenol under the chosen storage conditions (−20 °C).

For samples of milk which had been spiked with cloprostenol and stored together with the milk from dosed cows, no change in the percentage of cloprostenol during storage was detected. The proportion of cloprostenol detected in spiked milk collected from cows prior to the 0.5-mg dose was estimated to be 103.6 ± 4.5% (mean ± SD, $n = 3$) and 105.3 ± 6.4% for spiked milk collected before the 10-mg dose of cloprostenol.

The rapid elimination of radioactivity in milk was characterized by a rapid change in concentration of cloprostenol in milk during 24 h after dosing (Table I). Isotope dilution analysis demonstrated that 63.6% of the radioactivity in 0–4-h milk samples was present as cloprostenol. The proportion of cloprostenol in milk had fallen to 16.6% in the 8–24 h milk (Table I). The concentration of cloprostenol had fallen from 0.270 ng/mL (2.7×10^{-4} ppm) after 4 h to 0.002 ng/mL (2×10^{-6} ppm) at 24 h. The proportion of cloprostenol in milk after a 10-mg dose was equivalent to that seen at the lower dose for each time point (Table I).

It is concluded that the percent dose excreted in milk and the proportion of cloprostenol in milk samples collected during 24 h after dosing is the same following intramuscular administration of 0.5 or 10 mg of [^{14}C]cloprostenol to cows.

DISCUSSION

The elimination of dosed radioactivity in milk and urine measured during this study following administration of the therapeutic dose of cloprostenol to cows (0.5 mg) was equivalent to that reported by Reeves (1978). The change in concentration of total radioactivity in milk was also similar to that previously reported. During this study we have measured, for the first time, the concentration of unchanged cloprostenol in milk, demonstrating that during 24 h after dosing the concentration of cloprostenol falls by a factor of 100. Cloprostenol constitutes the bulk (64%) of the radioactivity in milk collected during 0–4 h after dosing and accounts for a decreasing proportion of the total ^{14}C with increasing time after dosing. Endogenous prostaglandins are rapidly metabolized in vivo, thus it is likely that the remainder of the radioactivity present in milk is due to metabolites of cloprostenol. Cloprostenol was administered at two dose levels. The rate of elimination of radioactivity in milk and urine and the proportion of cloprostenol in milk was equivalent at the higher dose to that seen at the therapeutic dose, demonstrating that the clearance of cloprostenol from cows is by apparent first-order processes over the dose range studied.

The clearance of the endogenous prostaglandin $PGF_{2\alpha}$ from cows' milk has been reported (Manns, 1975). For $PGF_{2\alpha}$ the maximum observed concentration of drug in

milk was 0.8–0.9 ng/mL at 1–3 h after dosing 30 mg of $PGF_{2\alpha}$. The concentration of $PGF_{2\alpha}$ in milk declined to predose values (0.2 ng/mL) at 21 h after dosing. Thus for both cloprostenol and $PGF_{2\alpha}$, the clearance of the parent drug from milk follows a similar time course. For cloprostenol the clearance of the total drug derived residue is very rapid (Reeves, 1978). Similar data have not been reported for $PGF_{2\alpha}$.

ACKNOWLEDGMENT

LITERATURE CITED

Cooper, M. J., Furr, B. J., *Vet. Rec.* **94**, 161 (1974).
Cooper, M. J., Rowson, L. E. A., *Ann. Biol. Anim. Biochim. Biophys.* **15**, 427 (1975).
Jackson, P. S., Cooper, M. J., *Int. Congr. Dis. Cattle* **2**, 903 (1976).
Manns, J. G., *Prostaglandins* **9**, 463 (1975).
Perez, M. K., *J. Toxicol. Environ. Health* **3**, 837 (1977).
Reeves, P. R., *J. Agric. Food Chem.* **26**, 152 (1978).
White, D. F., *J. Label Compd.* **13**, 23 (1977).

Chlor-Phosphate Process for Production of Chlorine, Hydrogen, and Potassium Phosphates

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An electrolytic process for the production of chlorine, hydrogen, and potassium phosphates as solution, solid, or slurry was developed in a bench-scale continuous microplant. Potassium amalgam made by the decomposition of pure potassium chloride solution in a mercury-cathode electrolyzer was reacted with a solution of potassium phosphates to which controlled amounts of ortho- or polyphosphoric acid and water were supplied. Solutions were produced over a wide pH range. A 0–32–32 solution of pH 7.5 was prepared, but solutions with total plant food in excess of 70% are anticipated. The solution contained <100 ppm Cl and <0.01 ppm Hg. The polyphosphate levels of the products were slightly lower than those of the starting acids. Fertilizer grades of potassium chloride and wet-process phosphoric acid required special treatment to avoid operational problems.

Potassium chloride is the most abundant and least expensive source of potassium for fertilizer use. It contains 63% plant food as K_2O and is an excellent low-cost fertilizer for most applications. Because potassium salts usually have high solubilities, it is perhaps surprising that at 0 °C a saturated aqueous solution of potassium chloride contains the equivalent of only 14% K_2O (Noyes, 1966). For this reason the solubility of KCl is limiting for many solution fertilizers wherein high solubility is desirable. Furthermore, the chlorine associated with the potassium is not a nutrient and is toxic to some plants. The conversion of potassium chloride to other fertilizer materials, such as potassium phosphate, can be justified if the new potassium compound is a more desirable fertilizer and valuable coproducts are produced.

Nearly all processes for the production of potassium phosphates employ potassium chloride as the source of potassium. Potassium hydroxide, derived from the chloride, is usually regarded as too expensive for fertilizer use. Most of the processes produce monopotassium phosphate or a mixture of mono- and dipotassium phosphates as intermediates. Both solid and fluid fertilizers are produced.

The general expression for the phosphoric acids—ortho-, pyro-, tripoly-, tetrapoly-, etc.—which exist as an homologous series of chain phosphates is $H_{n+1}P_nO_{3n+1}$. A similar but much more complex homologous series of potassium phosphates is described by the formula $K_mH_{n-m+2}P_nO_{3n+1}$. The respective corresponding oxide formulas for the above expressions more conveniently describe the materials in terms of plant nutrients and water of constitution:

$$n/2P_2O_5 + (n + 2)/2H_2O$$

$$n/2P_2O_5 + m/2K_2O + (n - m + 2)/2H_2O$$
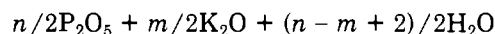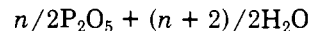
Although hydrated acids and salts also exist, water of hydration is not included in these formulas. Figure 1 shows some of the parent acid species and the many anhydrous salts that can be postulated by stoichiometry. These acids contain 72–84% plant nutrient as P_2O_5, and their salts contain 87–100% plant nutrients as K_2O and P_2O_5. Although only a few of the salts indicated exist as equilibrium solid phases, many hydrated forms of the potassium phosphates do exist, and double salts and others described by the inclusion of one or more moles of acid in some of the above salts have been reported (Van Wazer, 1958).

The potassium phosphates are very soluble in water, and the solubilities vary with the relative proportions of K_2O and P_2O_5 present. Potassium phosphates have maximum solubilities at 0 °C when the weight ratio $K_2O:P_2O_5$ is slightly greater than unity (Potts et al., 1961). At this temperature a solution with about 45% plant food is attainable with the ortho species (Van Wazer, 1958). Because mixtures of orthophosphates with polyphosphates are much more soluble than the orthophosphates alone (Farr and Williard, 1971; Frazier et al., 1972), it is understandable that solutions of even >70% plant food, crystal free at 0 °C, have been prepared. So in saturated potassium phosphate solutions there are P–K plant food concentrations that compare very favorably with those of solid fertilizers.

Potassium phosphates and their solutions are concentrated, chloride-free plant foods; they have low salt indices (Rader et al., 1943) and are especially suited to no-nitrogen and low-nitrogen applications. In spite of their higher

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