Metabolism of Triamcinolone Acetonide-21-phosphate in Dogs, Monkeys, and Rats

K. J. KRIPALANI ˣ, A. I. COHEN, I. WELIKY, and E. C. SCHREIBER

Abstract □ The absorption, distribution, and metabolic fate of triamcinolone acetonide-^{14}C-21-phosphate were studied in the dog, monkey, and rat. A comparison of levels of radioactivity in blood or plasma, reached after intramuscular or intravenous administration, indicated that the drug was completely absorbed from the site of intramuscular injection within 10–15 min in all three species. Within 1–5 min after intramuscular or intravenous administration, the 21-phosphate ester was completely hydrolyzed to triamcinolone acetonide, which was present in the blood. The radioactivity was eliminated rapidly ($t_{1/2}$ = 1–2 hr) from plasma (dogs, monkeys, and rats) and tissues (rats) after intramuscular or intravenous administration. In the three species, the major route of excretion was *via* the bile; however, the ratio of biliary to urinary excretion among the species varied considerably (from 1.5 to 15). In rats, excretion of radioactivity as expired carbon dioxide accounted for only 2–3% of the dose. 6β-Hydroxytriamcinolone acetonide was the major metabolite in urine of the three species. Hydrolytic cleavage of the acetonide group did not appear to be significant.

Keyphrases □ Triamcinolone acetonide-21-phosphate, ^{14}C-labeled—absorption, distribution, and elimination after intravenous and intramuscular administration, dog, monkey, rat □ Absorption—triamcinolone acetonide-^{14}C-21-phosphate after intravenous and intramuscular administration, dog, monkey, rat □ Distribution—triamcinolone acetonide-^{14}C-21-phosphate after intravenous and intramuscular administration, dog, monkey, rat □ Elimination—triamcinolone acetonide-^{14}C-21-phosphate after intravenous and intramuscular administration, dog, monkey, rat □ Metabolism—triamcinolone acetonide-^{14}C-21-phosphate after intravenous and intramuscular administration, dog, monkey, rat

Esterification of steroid alcohols has a marked influence on their absorption and excretion. After intramuscular administration, water-soluble esters (*e.g.*, phosphates, succinates, and glycinates) are absorbed and excreted rapidly (1–4), whereas water-insoluble esters (*e.g.*, acetates) are absorbed and excreted more slowly (1, 5). After administration, the steroid esters seem to be readily hydrolyzed to the corresponding steroid alcohols.

Hydrolysis of the phosphate esters of hydrocortisone, prednisolone, and dexamethasone appears to be rapid and complete, possibly as a result of the activity of phosphomonoesterases in plasma (2). The steroid alcohol resulting from such hydrolysis may then be metabolized as if it had been administered directly. In dogs, 6-methylprednisolone-^{3}H-21-acetate was metabolized in a manner similar to that of prednisolone (5).

No data are available for the relative blood levels obtained after intravenous and intramuscular administration of water-soluble esters of synthetic anti-inflammatory steroids, nor has the distribution of such drugs in tissues been reported. It is important to determine the distribution in blood and other tissues at various times after administration, because the increased water solubility of a steroid ester may alter its distribution in the body and, hence, influence its concentration at the target site.

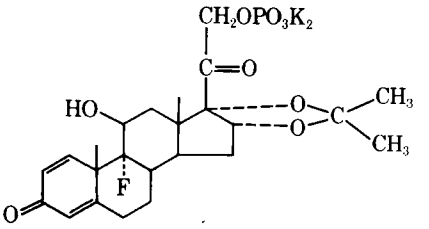
Triamcinolone acetonide-21-dipotassium phosphate (I) is a water-soluble 21-phosphate ester of triamcinolone acetonide (9-fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone, 21-phosphate, dipotassium salt). It is about 80 times more active than cortisone acetate in the liver glycogen deposition assay[1], and 10–50 times more active than hydrocortisone acetate in the antigranuloma assay in the rat[2].

Metabolic transformations of triamcinolone acetonide-21-phosphate may be similar to those of triamcinolone acetonide. Triamcinolone was metabolized in dogs and humans to the 6β-hydroxy compound (6), and triamcinolone acetonide was converted in rat liver *in vitro* to 6β-hydroxytriamcinolone acetonide (7). However, biotransformation *in vivo* of triamcinolone acetonide or of its phosphate ester has not been reported.

The 17-hydroxy group, considered necessary for the glucocorticoid activity of hydrocortisone, is blocked by the presence of the 16,17-acetonide group in the triamcinolone acetonide molecule. Inasmuch as triamcinolone acetonide has greater glucocorticoid activity than does hydrocortisone, it is of interest to find out whether the 16,17-acetonide group is hydrolyzed *in vivo* to give rise to a 17-hydroxy group. The present studies were carried out to determine this hydrolysis as well as the absorption, excretion, tissue distribution, and biotransformation of triamcinolone acetonide 21-phosphate after intramuscular or intravenous administration to dogs, monkeys, and rats.

EXPERIMENTAL

Measurement of Radioactivity—All samples were counted in a liquid scintillation spectrometer[3], using the method of automatic external standardization. Feces were homogenized in a volume of



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[1] L. J. Lerner, Endocrinology Department, Squibb Institute for Medical Research, Apr. 18, 1961, unpublished data.

[2] A. R. Turkheimer, Endocrinology Department, Squibb Institute for Medical Research, May 31, 1960, unpublished data.

[3] Packard Tri-Carb model 3375 or 3380.

methanol equivalent to half the weight of the sample. After portions of fecal homogenate, blood, plasma, and tissues were combusted in an oxygen atmosphere and the resulting carbon dioxide was trapped in a solution containing phosphor, the samples were counted.

Portions of bile and urine were counted in Bray's solution (8). Nonaqueous samples were counted in a solution containing 5 g of 2,5-diphenyloxazole and 0.3 g of 1,4-bis(4-methyl-5-phenyloxazolyl)benzene in 1 liter of spectral grade toluene. Portions (0.5 ml) of solutions containing trapped, expired $^{14}CO_2$ were counted after the addition of 2 ml of a solubilizer[4] and 15 ml of toluene phosphor solution.

Purity and Specific Activity of Triamcinolone Acetonide-^{14}C-21-phosphate—Triamcinolone acetonide-21-phosphate (dipotassium salt), labeled with ^{14}C in the 2-position of the acetonide group, was synthesized[5] and had a specific activity of 5.8 μCi/mg and a radiochemical purity of 96%, as determined by paper chromatography[6] in *n*-butanol–ethanol–2 *N* ammonium hydroxide (10:2:4).

Extraction of "Unconjugated" Metabolites—Plasma samples were extracted once with 10 volumes of methylene chloride. Urine and bile samples were extracted four times with an equal volume of ethyl acetate; the pooled extracts were washed with 0.1 volume of water.

Extraction of "Conjugated" Metabolites—Conjugated metabolites were separated from the extracted urine or bile by use of a nonion-exchange resin[7], according to the procedure described by Bradlow (9). Hydrolysis of the conjugated steroid fraction was carried out by incubation at 37° for 48 hr in 0.1 *M* acetate buffer, pH 4.6, containing 10% (v/v) of a solution containing β-glucuronidase[8].

After incubation, samples were extracted with ethyl acetate by the procedure described for urine and bile. The ethyl acetate extracts represented hydrolyzed metabolites originally present as glucuronides. The resulting aqueous fraction was subjected to solvolysis (10) to cleave and isolate the steroids originally present as sulfate conjugates.

Chromatographic Methods—TLC was carried out on precoated 0.25- and 2.0-mm silica gel H and silica gel F-254 plates[9]. Prior to use for preparative purposes, the plates were predeveloped, first in benzene and then in methanol. Three solvent systems were used for developing the thin-layer chromatograms: A, methanol–chloroform (8:92); B, methanol–ethyl acetate (2:98); and C, acetone–benzene (35:65). The radioactive zones on TLC plates were located by use of a scanner[10].

Paper chromatography[6] was carried out with 50% aqueous methanol–benzene (1:1) as the developing solvent. Radioactive zones were located by uniform sectioning of a lengthwise portion of the paper chromatogram, followed by liquid scintillation counting of each section in Bray's solution.

Silica gel[11] (100–200 mesh) was used for column chromatography. The eluting solvents were 10-ml portions of mixtures that contained increasing amounts of ethyl acetate in benzene (5% increase per successive 10-ml portion up to 100%). The elution was then continued with similar mixtures containing increasing amounts of methanol in ethyl acetate. Portions of the fractions collected were counted to locate the labeled compounds.

Partition chromatography[12] was carried out in a 1.5 × 60-cm column, with chloroform–toluene–ethylene glycol (1:6:7) as the solvent system. Fractions (10 ml) were collected at a rate of three per hour. Portions of the fractions collected were counted to locate the labeled compounds.

Drug Administration—The ^{14}C-labeled drug was dissolved in a vehicle formulated for the injectable product. The vehicle consisted of saccharin sodium, 40 mg; sodium citrate, 10 mg; sodium bisulfite, 3 mg; polyethylene glycol 300, 250 mg; benzyl alcohol, 15 mg; and water for injection to make 1.0 ml. Triamcinolone acetonide-21-phosphate was stable in this vehicle.

Purebred beagles (9.2–9.6 kg), female rhesus monkeys (*Macacca mulatta*, 3.6–5.6 kg), and Sprague–Dawley rats[13] (0.2 kg) were used. Dogs and rats were placed in individual metabolism cages, and monkeys were seated in restraining chairs[14]. Dogs and monkeys were fitted with urinary catheters. All animals were deprived of food, but not of water, for 24 hr before dosing.

At 60 and 30 min prior to drug administration, the dogs and monkeys were hydrated by oral administration of 20 ml of water/kg. A bile-cannulated dog was maintained under gaseous anesthesia (methoxyflurane) and infused with isotonic saline at a rate of 3 ml/min; thiamylal sodium[15], 0.4 ml of 4% solution/kg iv, was used as the preanesthetic. Intramuscular doses were injected into the gastrocnemius muscle of dogs, monkeys, and rats. The saphenous vein of dogs and monkeys and the tail vein of rats were used as the sites of intravenous injections.

Sampling Procedure—Heparinized syringes were used to withdraw blood (5–10 ml) from the jugular vein of dogs or the saphenous vein of monkeys. A portion of the blood sample was saved for radioassay, and the remainder was centrifuged under refrigeration at 2000 rpm for 10 min to separate the plasma.

During the first 8 hr after administration to dogs, urine was collected through catheters; thereafter, urine was collected in metabolism cages. After ligation of the gallbladder of the dog, bile was collected through a catheter inserted into the common bile duct. With monkeys, urine was collected through catheters throughout the experiment. Feces were collected every 24 hr from dogs and monkeys.

Urine and feces from rats were collected from the metabolism cages. To measure the $^{14}CO_2$ evolved, each rat was placed in a plastic metabolism cage enclosed in a plastic bag. Air was drawn from the cages through two carbon dioxide-absorbing traps, connected in series with a vacuum line and containing a solution of monoethanolamine and 2-methoxyethanol (1:3). Tissues were excised from rats after decapitation.

Incubation with Dog Blood—Triamcinolone acetonide-^{14}C-21-phosphate (0.2 mg) was incubated with 50 ml of freshly drawn heparinized blood at 37° with gentle shaking. Blood samples (5 ml) were removed at various times and centrifuged to separate plasma. Portions of plasma (1 ml) were extracted with 5 ml of methylene chloride. Portions of blood, plasma, extracted plasma, and methylene chloride extracts were assayed for radioactivity. Triamcinolone acetonide-^{14}C-21-phosphate was incubated with phosphate buffer (0.15 *M*, pH 7.4) as a control.

Incubation with Rat Muscle—Femoral muscles from four female rats were minced in a meat grinder, and a 10% suspension of this minced muscle in 0.25 *M* sucrose was prepared. Muscle homogenate was prepared from a portion of the minced muscle suspension (11). Minced muscle suspension or muscle homogenate, each containing the equivalent of 1.2 g of minced muscle and 0.2 μmole of glucose, was incubated with 40 μg of triamcinolone acetonide-^{14}C-21-phosphate at 37° for 24 hr. An equivalent amount of muscle homogenate, immersed for 30 min in boiling water, was used as a control.

Portions of each mixture (1 ml) were removed at various times during the incubation period. Each portion was extracted with 5 ml of methylene chloride, and 0.5 ml of each methylene chloride extract was assayed for radioactivity.

Isolation of Triamcinolone Acetonide (Metabolite 1) and 6β-Hydroxytriamcinolone Acetonide (Metabolite 2) from Urine—*Dog*—An ethyl acetate extract of the first 8-hr urine sample from Dog Q9-191 was subjected to preparative TLC in Solvent System B. Metabolite 1 was eluted from silica gel in an area corresponding in R_f value to that of authentic triamcinolone acetonide and was purified by paper chromatography.

Metabolite 2 was isolated by preparative TLC (Solvent System A) of an ethyl acetate extract of an 8-hr urine sample from a bile-cannulated dog (Q9-156). The radioactive material, isolated from an area corresponding to R_f 0.3 (triamcinolone acetonide, R_f 0.45), was purified by TLC in Solvent System B.

Monkey—Silica gel column chromatography of the combined ethyl acetate extracts of the 24-hr urine samples (from the four ex-

[4] NCS, Amersham-Searle.
[5] By Dr. L. High, Squibb Institute for Medical Research.
[6] Whatman No. 1.
[7] Amberlite XAD-2.
[8] Ketodase, Warner-Chilcott.
[9] Brinkmann Instruments.
[10] Actigraph III, Nuclear Chicago.
[11] Matheson Coleman & Bell.
[12] Celite Filter-Aid, Johns-Manville.
[13] Charles River.
[14] PCP-1208 primate chair, BRS Foringer.
[15] Surital, Parke-Davis.

Table I—Plasma Levels (Micrograms per Milliliter) of Radioactivity[a] in Dogs and Monkeys after Intravenous or Intramuscular Administration of Triamcinolone Acetonide-^{14}C-21-phosphate[b]

	Dogs		Monkeys			
Minutes	Q9-191, Intramuscular	Q9-156, Intravenous	MF-65, Intramuscular	MF-66, Intramuscular	MF-65, Intravenous	MF-66, Intravenous
5	—[c]	—	—	0.82	3.48	—
10	0.61	—	0.48	1.47	0.96	1.13
15	—	—	—	1.63	0.55	—
20	—	0.68	—	—	—	—
30	0.60	—	—	—	—	—
40	—	—	—	0.52	—	—
50	0.42	0.31	—	—	—	—
60	—	—	0.36	0.43	0.39	0.28
70	0.29	0.23	—	—	—	—
90	0.22	0.19	—	—	—	—
120	0.15	0.14	—	0.28	0.29	—
180	—	—	0.16	—	—	0.13
240	0.044	0.04	—	0.12	0.18	—
360	0.023	0.021	0.063	0.07	0.09	0.05
480	0.017	0.013	0.044	0.06	0.07	0.04

[a] Expressed as microgram equivalents of triamcinolone acetonide. [b] Dose: 0.9 mg/kg for dogs and 1.3 mg/kg for monkeys. [c] Indicates no sample taken.

periments with Monkeys 65 and 66) yielded two peaks of radioactivity. Metabolites 1 and 2 were isolated by TLC in Solvent System A from the fractions corresponding to the first and second peaks off the column, respectively. Metabolite 2 was further purified by TLC in Solvent System B.

Rat—For isolation of metabolites from urine, 10 mg of triamcinolone acetonide-^{14}C-21-phosphate was administered intramuscularly daily to each of four female rats (0.25–0.30 kg) for 4 days. Urine collected for 4 days from the four rats was pooled. TLC (Solvent System A) indicated that radioactive metabolites in this pooled urine were the same as those in the urine collected after administration of a single dose (1.5 mg/kg).

The pooled urine was extracted with ethyl acetate. After evaporation of the solvent from the ethyl acetate extract, the residue was partitioned between benzene and water. The aqueous fraction was then extracted with ethyl acetate (ethyl acetate fraction). Metabolite 1 and a portion (20%) of Metabolite 2 were isolated from the benzene extract by preparative TLC in Solvent System A. Metabolite 1 was crystallized from methanol. Metabolite 2, isolated from the benzene fraction, was purified by silica gel column chromatography and crystallized from ethyl acetate. The major portion of Metabolite 2 (80% of the total) was isolated from the ethyl acetate fraction by silica gel column chromatography and then crystallized from ethyl acetate.

NMR and Mass Spectrometry—The 100-MHz NMR spectra were obtained on an NMR spectrometer[16] homolocked to the protons in the tetramethylsilane reference. All chemical shifts are given in tau (τ) values. The low- and high-resolution mass spectra were obtained on a mass spectrometer[17], using the direct insertion probe. The source temperature was maintained at 180° above ambient; data were collected on a frequency-modulated analog tape recorder and were subsequently processed on a computer using Squibb programs (12).

RESULTS

The dose of triamcinolone acetonide-21-phosphate and the levels of radioactivity are expressed as triamcinolone acetonide equivalents.

Hydrolysis of Triamcinolone Acetonide Phosphate to Triamcinolone Acetonide by Incubation with Dog Blood—Based on an hematocrit value of 0.44 and relative concentrations in blood and plasma, it was established that at least 98% of the radioactivity in blood was present in the plasma. One minute after incubation of triamcinolone acetonide-^{14}C-21-phosphate with blood (4 μg/ml), 46% of plasma radioactivity was extractable into methylene chloride. No radioactivity was extracted into methylene chloride from the phosphate buffer control incubation. During the 2-hr incubation, methylene chloride extracts of plasma samples, obtained at nine different times, contained 44–62% of the radioactivity in plasma, whereas extracts of control incubation contained no more than 3% of the total radioactivity.

TLC (Solvent System A) of the methylene chloride extracts of plasma samples showed a single peak of radioactivity, which had the same R_f value (0.45) as did triamcinolone acetonide.

A portion (8000 dpm) of the combined methylene chloride extracts of plasma samples was mixed with unlabeled triamcinolone acetonide (8.0 mg), and the mixture was crystallized from ethyl acetate three successive times. The specific activity of each successive batch of crystals was 930, 1005, and 962 dpm/mg.

These results demonstrate that triamcinolone acetonide-21-phosphate is hydrolyzed to triamcinolone acetonide by plasma esterases.

Hydrolysis of Triamcinolone Acetonide-21-phosphate to Triamcinolone Acetonide by Incubation with Rat Muscle—One minute after incubation of triamcinolone acetonide-^{14}C-21-phosphate with rat minced muscle or rat muscle homogenate (33 μg/g of muscle), 3% of the radioactivity in the incubation mixture could be extracted with methylene chloride. Successively greater quantities were extractable: 28% in 2 hr and 42% after 4 hr. The percentage of radioactivity extracted from a control incubation with a boiled muscle homogenate was 0.6–1.1% during the first 4 hr of incubation.

Methylene chloride extracts of the incubation mixtures contained only one radioactive compound, which had the same R_f as did triamcinolone acetonide (Solvent System A).

These results show that hydrolysis of the 21-phosphate ester of triamcinolone acetonide could occur in the rat muscle. The rate of hydrolysis in the rat muscle incubations appeared to be much slower ($t_{1/2}$ = 4.5 hr) than that in plasma, in which case 46% was hydrolyzed after 1 min of incubation.

Plasma Levels—Levels of radioactivity in the plasma of two dogs and four monkeys are shown in Table I. In addition, plasma levels in a bile-cannulated dog (Q9-156)[18] are shown in Fig. 1. Plasma samples collected during the first 10 or 15 min after intravenous administration to Dog Q9-156 (bile cannulated) and Monkey 65 showed a rapid and precipitous drop in the level of radioactivity during this interval, indicating rapid distribution of the drug into the extravascular compartments. The plasma half-life of radioactivity during this distribution phase was calculated[19] to be 1 min for the dog and 1.5 min for the monkey.

After the first 10 or 15 min, elimination of radioactivity from plasma of the three dogs and the four monkeys followed a biphasic pattern. The half-life values, calculated by the method of residuals, are shown in Table II. The plasma half-life during the first phase (10–240 min) was 35 min in each of the three dogs and 65–85

[16] Varian Associates XL-100.
[17] Model MS-902, Associated Electrical Industries.
[18] This dog had been used as an intact dog 7 weeks earlier.
[19] Calculated by methods of residuals as shown in Fig. 1 for Dog Q9-156.

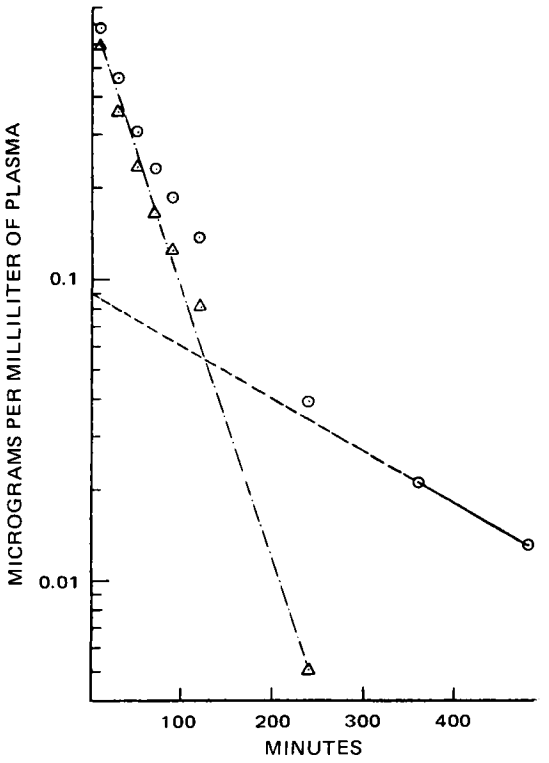


Figure 1—*Levels of radioactivity in plasma of Dog Q9-156 after intravenous administration (0.9 mg/kg) of triamcinolone acetonide-^{14}C-21-phosphate. Key: O, experimental values; and Δ, residual values, calculated by subtracting from the experimental value the value obtained at the corresponding time on the extrapolated part of the curve. The ordinate values are expressed as microgram equivalent of triamcinolone acetonide based on radioassay.*

min in the four monkeys. In the same animals, the rate of disappearance during the second phase (240–480 min) was slower and more variable than that during the first phase. The half-life values during the second phase were 170–270 min in the dogs and 250–720 min in the monkeys; however, the levels of radioactivity during this phase were less than 5% of the initial radioactivity in plasma observed after intravenous administration to dogs or monkeys.

Levels of radioactivity in rats after intramuscular or intravenous administration are shown in Tables III and IV (with tissue distribution data). The elimination of radioactivity from plasma followed first-order kinetics during the interval of 30–360 min after dosing. The half-life for disappearance of radioactivity during this phase was 95 or 110 min after intramuscular or intravenous administration, respectively[20]. After this initial phase, the plasma levels declined at a slower rate ($t_{1/2}$ = 420 min) between 360 and 1440 min. Plasma levels during the latter phase were less than 3% of the initial levels seen after intravenous administration.

In all three species, peak levels of radioactivity in plasma were observed 10–15 min after intramuscular administration, and the subsequent plasma levels were substantially the same as those observed after intravenous administration. These results indicate a rapid and complete absorption of the drug from the site of intramuscular injection.

Distribution in Rat Tissues—Concentrations of the labeled drug in different tissues at various times between 1 and 1440 min after intravenous or intramuscular administration are shown in Tables III and IV. After intramuscular administration, peak levels of radioactivity were measured at 15 min in the plasma, liver, adrenals, ovary, and stomach and at 30 min in other tissues (except the intestine plus contents). The levels of radioactivity between 30 and 1440 min after intramuscular or intravenous administration were approximately equal in all tissues, indicating rapid and complete distribution of the drug when given by the intramuscular route.

[20] Due to insufficient data points on the plasma level curves, the half-life values were calculated from the slope of the semilogarithmic plots and not by the method of residuals.

Table II—Half-Life Values for Disappearance of Radioactivity from Plasma of Dogs and Monkeys after Intramuscular or Intravenous Administration of Triamcinolone Acetonide-^{14}C-21-phosphate

Animal	Route of Administration	Dose, mg/kg	Half-Life, min: Between 10 and 240 min	Half-Life, min: Between 240 and 480 min
Dog Q9-191	Intramuscular	0.9	35	270
Dog Q9-156	Intravenous	0.9	35	250
Dog Q9-156[a]	Intravenous	1.2	35	170
Monkey 65	Intramuscular	1.3	65	250
Monkey 66	Intramuscular	1.3	65	720
Monkey 65	Intravenous	1.3	85	380
Monkey 66	Intravenous	1.3	70	360

[a] Anesthetized bile-cannulated dog; 7 weeks after previous dosing.

At 1 min after drug administration (intravenous or intramuscular), as one might expect, plasma had a higher concentration of radioactivity than did any of the tissues examined. At subsequent times, the concentrations in liver were 3–10 times greater than those in plasma; those in adrenals were 1.3 to 8 times greater. Concentrations in the kidneys increased from 0.7 to 2 times those in plasma. The concentration in the brain was the lowest (less than one-tenth the concentration in plasma) of all tissues. The amount of radioactivity in the intestines and their contents increased continuously throughout the 1440-min period, suggesting biliary excretion of the drug; this suggestion was confirmed by excretion studies.

The half-life values for disappearance of labeled drug from tissues were calculated. Although the concentrations in various tissues varied widely, the half-life values for disappearance of labeled drug from all tissues (except the intestine plus contents) were in good agreement with the half-life in plasma. Between 120 and 360 min, the half-life values after intravenous or intramuscular administration ranged from 96 to 120 min in plasma and from 84 to 180 min in the tissues other than intestine plus contents.

The half-life for transfer of radioactivity from the site of injection after intramuscular administration was 12 min during the first 30 min; this half-life represents the rate of drug transfer. After 120 min, when most of the injected drug would be expected to have left the injection site, the half-life observed, *i.e.*, 102 min, was in good agreement with the measurements of half-lives in the other tissues.

Characterization of Radioactive Material in Plasma—The percentage of radioactivity extracted into methylene chloride from plasma samples obtained at various times from the three species is shown in Table V. When triamcinolone acetonide-^{14}C-21-phosphate was added to plasma (4 μg/ml) *in vitro*, no radioactivity was extracted into methylene chloride; however, under the same conditions, triamcinolone acetonide-^{14}C was completely extractable. For the plasma samples obtained within the first 10 min after administration of the labeled drug, 91–98% of the radioactivity in plasma was extracted into methylene chloride; with subsequent samples, the extractable radioactivity generally decreased with time.

Radioscans after TLC of methylene chloride extracts of plasma samples, obtained during the first 60 min after dosing of dogs, monkeys, and rats, showed one major peak (90–95% of the radioactivity in the extracts), corresponding in R_f values to triamcinolone acetonide in Solvent Systems A (R_f 0.45) and B (R_f 0.40). At later times (up to 24 hr), smaller peaks with R_f values lower than that of triamcinolone acetonide were also seen; these peaks accounted for 15–25% of the radioactivity in the plasma extracts.

The radioactivity present in the first 60-min plasma samples of each species was eluted with ethyl acetate from the silica gel plates. The isolated material for each species (approximately 4000–8500 dpm) was mixed with an authentic sample of unlabeled triamcinolone acetonide (6–7 mg) and crystallized from ethyl acetate to constant specific activity. For labeled material isolated from dog plasma, the specific activity of each successive batch of

Table III—Levels of Radioactivity[a] Found in Tissues of Female Rats after Intravenous Administration of Triamcinolone Acetonide-^{14}C-21-phosphate[b]

Tissue	Micrograms per Gram[c]				
	1 min	30 min	120 min	360 min	1440 min
Adipose	0.57	0.93 ± 0.09	1.84 ± 0.08	0.14 ± 0.02	0.04 ± 0.01
Adrenals	4.65	2.52 ± 0.70	2.41 ± 1.22	0.91 ± 0.28	0.12 ± 0.10
Bone	1.18	0.43 ± 0.01	0.34 ± 0.0	0.10 ± 0	0.01 ± 0
Brain	0.48	0.12 ± 0.01	0.08 ± 0.02	0.02 ± 0	0
Eye	1.04	0.34 ± 0.10	0.18 ± 0.01	0.05 ± 0	0
Heart	2.99	1.18 ± 0.04	0.71 ± 0.21	0.19 ± 0.01	0.02 ± 0
Intestine plus contents	0.74	3.20 ± 0	9.40 ± 6.70	8.75 ± 1.60	13.7 ± 8.00
Kidney	4.41	1.97 ± 0.05	1.10 ± 0.03	0.39 ± 0.03	0.06 ± 0.01
Liver	3.56	5.93 ± 0.20	5.32 ± 1.62	2.13 ± 0.37	0.42 ± 0.04
Lung	3.38	1.19 ± 0.01	0.81 ± 0.26	0.28 ± 0.02	0.04 ± 0.01
Skeletal muscle	0.61	0.64 ± 0.03	0.44 ± 0.12	0.13 ± 0.01	0.02 ± 0
Skin	0.44	0.66 ± 0.06	0.42 ± 0.34	0.16 ± 0.03	0.03 ± 0.01
Spleen	0.53	0.73 ± 0.03	0.49 ± 0.16	0.18 ± 0.01	0.04 ± 0.01
Stomach	0.39	0.69 ± 0.50	0.44 ± 0.02	0.21 ± 0.08	0.08 ± 0.03
Ovary	2.21	1.11 ± 0.05	1.20 ± 0.02	0.23 ± 0.03	0.04 ± 0.03
Thymus	0.85	0.69 ± 0.01	0.50 ± 0.13	0.18 ± 0.02	0.03 ± 0.01
Uterus	0.90	1.15 ± 0.11	0.65 ± 0.13	0.22 ± 0.01	0.03 ± 0.01
Plasma[d]	18.63	1.96 ± 0.01	0.97 ± 0.15	0.26 ± 0.04	0.04 ± 0
Blood[d]	11.08	1.26 ± 0	0.75 ± 0.11	0.18 ± 0.03	0.04 ± 0

[a] Expressed as micrograms of triamcinolone acetonide per gram of tissue. [b] 1.5-mg equivalents of triamcinolone acetonide/kg. [c] Each value shown is the average and range of the mean for two determinations, each in a separate rat, with the exception of the 1-min values. [d] Micrograms per milliliter.

crystals was 729, 736, 709, and 710 dpm/mg; calculated specific activity was 731 dpm/mg. Similar values obtained in plasma samples from the monkey were 1290, 1245, 1179, and 1220 dpm/mg; those for the rat were 954, 942, and 941 dpm/mg. Calculated values for specific activity were 1214 and 1095 dpm/mg for the monkey and rat, respectively.

Based on chromatography in the two solvent systems and on isotope dilution, the major radioactive metabolite found in the plasma of dog, monkey, and rat was identified as triamcinolone acetonide.

These results show that as early as 1–10 min after its administration, triamcinolone acetonide-21-phosphate is almost completely hydrolyzed to triamcinolone acetonide, probably by plasma esterases, and is then further metabolized. Other water-soluble esters of corticosteroids have also been shown to undergo hydrolysis to corresponding steroid alcohols after being administered to humans (1–4).

Excretion in Urine and Feces of Dogs, Monkeys, and Rats—Table VI shows the data for excretion in urine and feces. Elimination of radioactivity from the body took place rapidly. In all but one animal (Monkey 65 after intravenous or intramuscular administration), two-thirds of the total excretion in urine occurred during the first 6–8 hr after administration. For Monkey 65, 24 hr was required for elimination of two-thirds of the total excretion in urine.

In all species, excretion in urine was essentially complete after 48 hr and excretion in feces was essentially complete between 48 and 96 hr. Total excretion in urine and feces accounted for 95, 85, and 87% of the intravenously or intramuscularly administered dose in dogs, monkeys, and rats, respectively. Excretion of radioactivity

Table IV—Concentration of Radioactivity[a] Found in Tissues of Female Rats after Intramuscular Administration of Triamcinolone Acetonide-^{14}C-21-phosphate[b]

Tissue	Micrograms per Gram[c]					
	1 min	15 min	30 min	120 min	360 min	1440 min
Adipose	0.18 ± 0.06	0.77 ± 0.06	0.81 ± 0.05	0.32 ± 0.15	0.10 ± 0.01	0.07 ± 0.04
Adrenals	0.31 ± 0.10	3.10 ± 1.20	2.64 ± 0.18	1.30 ± 0	0.39 ± 0.08	0.23 ± 0
Bone	0.11 ± 0.10	0.46 ± 0.01	0.53 ± 0.08	0.30 ± 0.02	0.07 ± 0.01	0.01 ± 0
Brain	0.04 ± 0.02	0.07 ± 0.01	0.10 ± 0.01	0.07 ± 0.01	0.01 ± 0	0
Eye	0.22 ± 0.06	0.25 ± 0.03	0.27 ± 0.04	0.14 ± 0.01	0.05 ± 0	0
Heart	0.24 ± 0.07	0.97 ± 0.05	1.15 ± 0.10	0.71 ± 0.07	0.13 ± 0.02	0.02 ± 0
Intestine plus contents	0.04 ± 0.03	0.92 ± 0	1.69 ± 0.36	5.60 ± 0.50	5.20 ± 1.40	23.0 ± 7.5
Kidney	0.39 ± 0.01	1.40 ± 0.02	1.80 ± 0.08	1.28 ± 0.12	0.28 ± 0.03	0.26 ± 0.01
Liver	0.40 ± 0.31	8.60 ± 0.90	6.73 ± 0.68	4.39 ± 0.07	1.24 ± 0.12	0.26 ± 0.01
Lung	0.21 ± 0	0.97 ± 0.08	1.15 ± 0.11	0.77 ± 0.06	0.17 ± 0.02	0.06 ± 0.01
Muscle at site of injection	40.4 ± 1.70	18.4 ± 2.60	7.35 ± 0.67	0.68 ± 0.08	0.14 ± 0.03	0.02 ± 0
Skeletal muscle	0.05 ± 0.03	0.45 ± 0.02	0.63 ± 0.07	0.45 ± 0.05	0.08 ± 0.02	0.03 ± 0.01
Skin	0.03 ± 0.02	0.47 ± 0	0.69 ± 0.07	0.49 ± 0.03	0.10 ± 0.02	0.03 ± 0
Spleen	0.03 ± 0.03	0.57 ± 0.01	0.73 ± 0.09	0.51 ± 0.06	0.14 ± 0	0.05 ± 0
Stomach	0.03 ± 0.03	1.30 ± 0.60	0.42 ± 0.15	0.68 ± 0.02	0.10 ± 0.02	0.05 ± 0
Ovary	0.12 ± 0.06	1.14 ± 0.54	1.03 ± 0.04	0.67 ± 0.02	0.18 ± 0	0.03 ± 0.01
Thymus	0.05 ± 0.01	0.42 ± 0.02	0.65 ± 0.03	0.51 ± 0	0.11 ± 0.01	0.04 ± 0.02
Uterus	0.11 ± 0.04	0.72 ± 0.07	1.03 ± 0.01	0.66 ± 0.09	0.23 ± 0.06	0.08 ± 0.03
Plasma[d]	1.60 ± 0.20	1.90 ± 0.15	1.88 ± 0.18	1.04 ± 0.08	0.17 ± 0.03	0.03 ± 0
Blood[d]	0.81 ± 0.13	1.13 ± 0.03	1.28 ± 0.17	0.71 ± 0	0.12 ± 0.02	0.04 ± 0.01

[a] Expressed as micrograms of triamcinolone acetonide per gram of tissue. [b] 1.5-mg equivalents of triamcinolone acetonide/kg. [c] Each value shown is the average and range of the mean for two determinations, each in a separate rat. [d] Micrograms per milliliter.

Table V—Percentage of Radioactivity Extracted into Methylene Chloride after Intravenous or Intramuscular Administration of Triamcinolone Acetonide-^{14}C-21-phosphate to Dogs, Monkeys, and Rats[a]

Time after Administration, min	Percent Extracted					
	Dogs		Monkeys		Rats	
	Intravenous	Intramuscular	Intravenous	Intramuscular	Intravenous	Intramuscular
1	—	—	—	—	98	95
5	—	—	—	98	—	—
10	95	91	98	—	—	—
15	—	—	93	89	90	—
30	79	87	—	—	92	92
40	—	—	—	81	—	—
50	78	75	—	—	—	—
70	69	64	—	—	—	—
90	63	89	—	—	—	—
120	49	90	81	74	94	92
240	53	72	72	55	—	—
360	—	77	58	35	92	92
480	—	86	48	37	—	—
1440	—	—	52	41	76	51

[a] Dose: 0.9 mg/kg for dogs (male), 1.2 mg/kg for monkeys (female), and 1.5 mg/kg for rats (female).

in feces was always greater than that in urine. The ratio of fecal to urinary excretion varied among the species, however; it was 15:1 for dogs, 4.7:1 for monkeys, and 1.4:1 for rats. The results indicate that excretion *via* bile is the major route of elimination in all three species.

Excretion in Bile and Urine of a Dog—Excretion of radioactivity in bile and urine after intravenous administration (1.2 mg/kg) of triamcinolone acetonide-^{14}C-21-phosphate is shown in Table VII. A total of 81% of the dose was accounted for during the 8-hr period, 10% in urine and 71% in bile. The apparent half-life for excretion of radioactivity in bile and urine was 2 hr during the first 3 hr and 5 hr between 3 and 8 hr after dosing. The results show that excretion *via* bile is a major route of elimination of this drug in the dog.

Based on the findings that *(a)* the plasma $t_{1/2}$ of radioactivity in the intact dogs and the bile-cannulated dog was the same (Table II) and *(b)* the ratio of excretion of radioactivity in urine to that in feces was no higher for intact dogs than for the bile-cannulated animal, it appears that the drug is not reabsorbed after excretion *via* bile in the canine species.

Excretion of Radioactivity as Respiratory $^{14}CO_2$ in Rats—During 48 hr after intravenous and intramuscular administration (1.5 mg/kg) of triamcinolone acetonide-^{14}C-21-phosphate to rats, the administered radioactivity found in the expired air was 2.6 and 2.4%, respectively. Of this total radioactivity expired as $^{14}CO_2$, 90% was eliminated during the first 8 hr.

Table VII—Excretion of Radioactivity in Urine and Bile of Dog Q9-156[a] after Intravenous Administration of Triamcinolone Acetonide-^{14}C-21-phosphate (1.2 mg/kg)

Collection Period, min	Cumulative Percent of Dose	
	Urine	Bile
0–6	0	0
6–15	0	1.7
15–30	1.1	7.2
30–45	—	19.0
45–60	2.0[b]	27.0
60–90	3.8	39.0
90–180	6.2	56.0
180–300	8.0	65.0
300–420	9.3	69.0
420–480	9.8	71.0

[a] Seven weeks after the previous dose. [b] A 30–60-min collection.

The triamcinolone acetonide-21-phosphate used in these studies was labeled with ^{14}C in the acetonide group (2-position). The appearance of only 2 or 3% of the administered radioactivity as $^{14}CO_2$ indicates that hydrolysis and subsequent oxidation of the acetonide group are not significant metabolic reactions in the rat. In addition, because the total recovery in urine and feces (in 4 or 7 days) accounted for 95 and 85% of the dose in dogs and monkeys, respectively, elimination of radioactivity as $^{14}CO_2$ and, hence, degradation of the acetone-derived portion also may not be significant in these species.

Isolation and Identification of Metabolites in Urine—TLC (Solvent System A) of urine or ethyl acetate extract of urine from the dog, monkey, and rat showed three peaks of radioactivity, A, B, and C. Peak A had the same R_f value (0.45) as that of triamcinolone acetonide, peak B had an R_f value of 0.30, and peak C remained at the origin. TLC of ethyl acetate extracts of urine, collected sequentially from dogs and monkeys, revealed a pattern of change in the levels of each peak with time. The data in Table VIII show the percent of urinary radioactivity associated with peaks A and B. In addition, Table VIII shows the percent of radioactivity as X, which represents the radioactivity in peak C plus that not extracted with ethyl acetate.

In the rat, TLC was carried out on urine samples prior to any extraction. The mean values (intramuscular or intravenous administration) for percent of urinary radioactivity associated with peaks A, B, and C were 17 ± 1, 73 ± 1, and 10 ± 1%, respectively, in the 0–6-hr urine, and 10 ± 2, 76 ± 4, and 15 ± 1% in the 6–24-hr urine, respectively. The pattern of urinary metabolites in each species, as seen in these chromatograms, was the same after intravenous or intramuscular administration.

In characterization studies of metabolites in the test species, pooled urine was collected during the first 8 hr from Dog Q9-156 (bile cannulated) and during the first 24 hr from each monkey (65 and 66, four experiments) and each of four rats; these samples

Table VI—Excretion of Radioactivity by Dogs, Monkeys, and Rats after Administration of Triamcinolone Acetonide-^{14}C-21-phosphate[a]

Animal	Route of Administration	Cumulative Percent of Dose					
		Urine			Feces		Total
		0–8 hr	0–24 hr	0–48 hr	0–24 hr	0–48 hr	0–96 or 0–144 hr[b]
Dog Q9-156	Intravenous	4	5	6	82	88	96
Dog Q9-191	Intramuscular	3.6	6	6	83	86	94
Monkey 65[c]	Intravenous	6.6	14	19	2	19	102
Monkey 66[c]	Intramuscular	7.1	9	11	0.1	56[d]	75
Monkey 65	Intramuscular	4.8	8	12	1	56	78
Monkey 66	Intravenous	7.8	9	10	11	63	76[e]
Rat 1	Intravenous	24	38	38	43	47	85
Rat 2	Intramuscular	26	29	29	49	52	81
Rat 3	Intramuscular	28	35	36	47	49	74

[a] Dose: 0.9 mg/kg for dogs (male), 1.2 mg/kg for monkeys (female), and 1.5 mg/kg for rats (female). [b] 0–96 hr for dogs and rats, and 0–144 hr for monkeys. [c] Received second dose 7 weeks later. [d] 0–72 hr. [e] Feces sample between 48 and 72 hr was lost.

Table VIII—Percent Distribution of Radioactivity[a] in Urine Collected at Various Times after Administration of Triamcinolone Acetonide-^{14}C-21-phosphate to Dogs, Monkeys, and Rats

Time of Urine Collection	Intravenous Administration			Time of Urine Collection	Intramuscular Administration		
	A	B	X		A	B	X
Minutes		Dog Q9-156		Minutes		Dog Q9-191	
0–1	96.5	0	3.5	0–20	25.3	13.7	61.0
1–3	38.5	0	61.5	20–40	25.7	19.7	60.1
3–7	26.6	5.4	68.0	40–60	13.0	22.5	64.5
7–20	13.6	25.4	61.0	60–80	11.9	26.0	62.6
20–30	9.8	33.4	56.8	80–105	14.5	19.9	65.6
30–50	6.8	29.5	63.8	105–180	10.1	21.3	68.6
50–90	0	30.3	69.7	180–300	10.8	22.1	67.1
90–180	11.1	21.8	67.1	300–420	4.6	29.2	66.3
180–300	0	38.8	61.2	420–480	1.6	10.6	87.8
300–420	0	24.9	75.1				
420–480	0	35.5	64.5				
Hours		Monkey 66		Hours		Monkey 65	
0–4	5.6	27.8	66.8	0–4	5.4	30.7	63.9
4–8	5.0	42.0	53.0	4–8	1.2	41.7	57.1
8–24	3.9	41.6	54.5	8–24	1.8	46.6	51.6
24–48	1.8	26.2	72.0	24–48	2.0	15.4	82.6
48–72	0.5	10.6	88.9	48–72	1.4	7.0	91.6

[a] A and B represent percent of urinary radioactivity associated with peaks A and B (described in text) calculated by measuring areas under the peaks seen in radioscans after TLC (Solvent System A) of ethyl acetate extracts of urine; X represents percent radioactivity associated with peak C in the same radioscans plus radioactivity in urine not extracted in ethyl acetate.

were extracted with ethyl acetate. The ethyl acetate extract accounted for 53% of the radioactivity in urine of the dog. In the monkeys and rats, the ethyl acetate urinary extracts accounted for 41 ± 7 and 90 ± 3% of the radioactivity, respectively.

Radioscans after TLC (Solvent System A) of these ethyl acetate extracts showed the three peaks of radioactivity noted previously, *i.e.*, A, B, and C. Peak B was the major peak in all three species. Based on the area under the peak, peak B accounted for 30, 29, and 72% of the radioactivity in the urine of the dog, monkey, and rat, respectively. Similarly, peak A accounted for 8, 2, and 17% in the dog, monkey, and rat, respectively.

Metabolites 1 and 2, associated with peaks A and B, respectively, were isolated from the urine of the three species, as described under *Experimental*.

Chromatography of a portion of Metabolite 1, isolated from urine samples of each species, showed a single peak of radioactivity, with R_f values of 0.45 and 0.40 in Solvent Systems A and B, respectively. Authentic triamcinolone acetonide had R_f values of 0.45 and 0.40 in Solvent Systems A and B, respectively.

Samples of Metabolite 1, isolated from urine of each species, were mixed with an authentic sample of triamcinolone acetonide and crystallized from ethyl acetate to constant specific activity (Table IX).

A low-resolution mass spectrum of Metabolite 1, isolated from the urine of rats, exhibited a fragmentation pattern similar to that of authentic triamcinolone acetonide. A molecular ion peak at *m/e* 434 and a base peak at *m/e* 375, resulting from the loss of $C_2H_3O_2$ (side chain) through cleavage between carbon atoms 17 and 20, were seen in the mass spectra of both triamcinolone acetonide and Metabolite 1. The mass spectrum of triamcinolone acetonide was discussed by Florey (13).

On the basis of data from chromatography, isotope dilution, and mass spectrometry, Metabolite 1 was identified as triamcinolone acetonide.

TLC of Metabolite 2 from the urine of rats showed a single radioactive peak with R_f values of 0.30 and 0.28 in Solvent Systems A and B, respectively. A triamcinolone acetonide standard had R_f values of 0.45 and 0.40 in these systems, respectively.

A mass spectrum of Metabolite 2 from the urine of rats showed a molecular ion peak at *m/e* 450 and an intense peak at *m/e* 391. If the peaks resulting from low molecular weight impurities (*m/e* 41–44) are excluded, the peak at *m/e* 391 was the most intense in the spectrum. The relative intensity of the molecular ion peak at *m/e* 450 was 6% that of the peak at *m/e* 391. Compared with the mass spectrum of triamcinolone acetonide, the spectrum of Metabolite 2 showed an increase of 16 amu which suggested addition of one oxygen atom. The mass spectrum also demonstrated an M−18 ion at *m/e* 432, corresponding to the loss of a hydroxy group through dehydration on electron impact. The mass spectrum of triamcinolone acetonide did not exhibit a significant M−18 ion, even though there are two hydroxy groups in the compound (13).

The 100-MHz NMR spectrum of Metabolite 2 was obtained in deuteromethanol containing tetramethylsilane as internal reference. Based on this analysis, the following proton assignments were made: C-1H, 2.63 τ (doublet, 10 Hz); C-2H, 3.72 τ (multiplet, 10 Hz); C-4H, 3.80 τ (multiplet); C-18H, 9.07 τ; C-19H, 8.25 τ; α-acetonide methyl, 8.59 τ; and β-acetonide methyl, 8.85 τ. The NMR spectrum of triamcinolone acetonide was discussed by Florey (13). Comparison of the two spectra indicated that the C-19 proton resonance had been shifted downfield by 0.21 ppm. The introduction of a hydroxy group in the *cis* 1,3-axial position to the axial methyl group will cause a downfield shift of this magnitude (14).

In a C-19 steroid, four positions can have a 1,3-diaxial relationship to the C-19 methyl group: C-2, C-6, C-8, and C-11. In triamcinolone acetonide, C-2 is not available and there already is a C-11 hydroxy in a 1,3-diaxial relationship to the C-19 methyl group. C-8 is excluded, because the C-8 β-hydroxy group causes a downfield

Table IX—Specific Activity after Isotopic Dilution of Metabolite 1 Isolated from Urine after Administration of Triamcinolone Acetonide-^{14}C-21-phosphate to Dogs, Monkeys, and Rats

Source of Metabolite	Metabolite 1, dpm	Unlabeled Triamcinolone Acetonide, mg	Calculated Specific Activity	Specific Activity after Crystallization, dpm/mg			
				1	2	3	4
Dog	4,000	7.3	545	624	469	456	470
Monkey	10,050	9.9	1015	1025	1010	1015	—
Rat	10,470	9.0	1115	1140	1125	1135	—

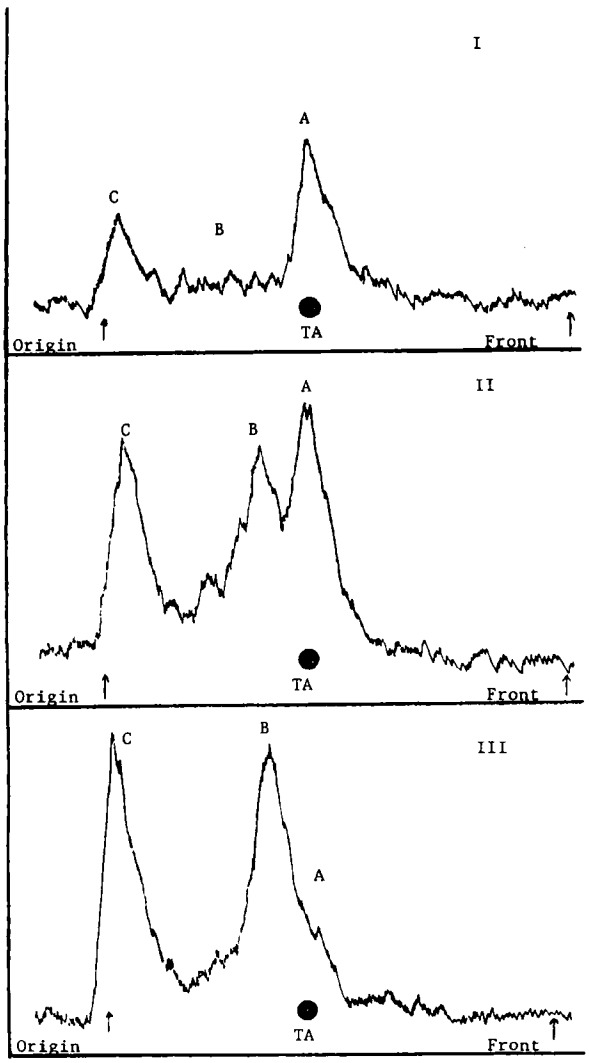
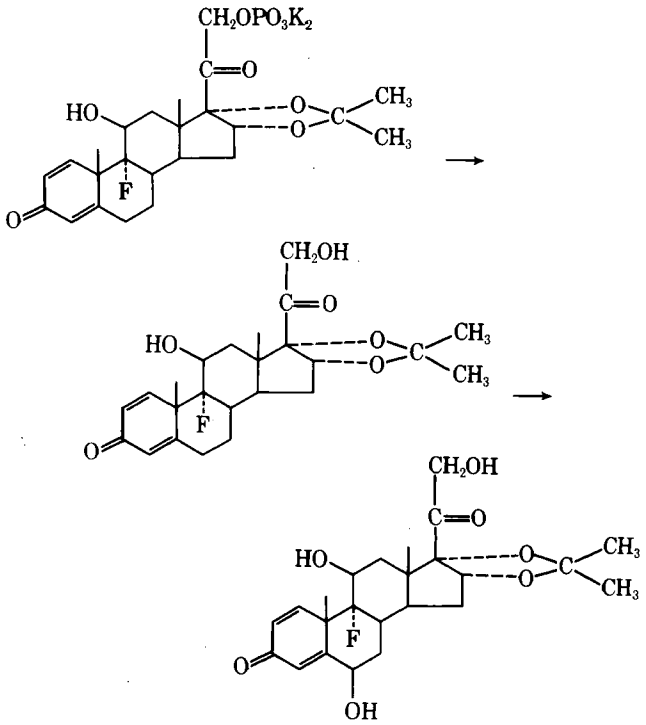


Figure 2—*Radioscans after TLC (Solvent System A) of ethyl acetate extracts of bile collected at various times after intravenous administration of triamcinolone acetonide-^{14}C-21-phosphate (1.2 mg/kg) to Dog Q9-156. Time of bile collection after dosing was: I, 6–15 min; II, 30–45 min; and III, 60–90 min. TA represents authentic triamcinolone acetonide chromatographed in the same solvent system.*



Scheme I—Biotransformations of triamcinolone acetonide-21-dipotassium phosphate

shift of both the C-18 and C-19 protons (15). Therefore, the downfield shift (0.21 ppm) of the C-19 protons must be due to a hydroxy group in the 6β-position.

Based on evidence from mass and NMR spectra, Metabolite 2, isolated from the urine of rats, was identified as 6β-hydroxytriamcinolone acetonide. Fifty-nine percent of the total urinary radioactivity was associated with the isolated compound, but this value is a minimum based only on the material recovered.

TLC of Metabolite 2 from the urine of the monkey showed a single radioactive peak in Solvent Systems A (R_f 0.30) and B (R_f 0.28). These R_f values were similar to those of 6β-hydroxytriamcinolone acetonide isolated from the rat urine. A portion of Metabolite 2 (6000 dpm) isolated from the monkey urine was mixed with an equal amount (6000 dpm) of 6β-hydroxytriamcinolone acetonide isolated from the rat urine. TLC of the mixture in Solvent Systems A, B, and C showed only a single radioactive peak in each system.

The mass spectrum of Metabolite 2 from the monkey urine was similar to that of 6β-hydroxytriamcinolone acetonide from the rat urine; both spectra showed a molecular ion peak at *m/e* 450 and a base peak at *m/e* 391.

On the basis of chromatographic characteristics and mass spectral analysis, Metabolite 2 from the monkey urine was identified as 6β-hydroxytriamcinolone acetonide. A total of 12% of the radioactivity in urine was recovered in the isolated 6β-hydroxytriamcinolone acetonide.

Metabolite 2 isolated from the urine of the dog had the same R_f values as did 6β-hydroxytriamcinolone acetonide in Solvent Systems A, B, and C. This metabolite was not obtained in a sufficiently pure form to allow analysis by mass spectrometry.

6β-Hydroxylation was reported (6) to occur in dogs and humans after dosing with triamcinolone and in studies *in vitro* (rat liver) with triamcinolone acetonide as the substrate (7). The results of the present study show that triamcinolone acetonide undergoes this biotransformation (6β-hydroxylation) in the rat and monkey and, apparently, in the dog as well.

Metabolites present in the urine as glucuronide conjugates accounted for 21, 6, and 4% of the radioactivity in urine of dogs, monkeys, and rats, respectively. In each species, incubation of the glucuronide fraction with β-glucuronidase yielded equal amounts of radioactive metabolites corresponding to peaks B and C (Solvent System A). Sulfate conjugates, which accounted for 11% of the radioactivity in the dog urine, remained at the origin when chromatographed in Solvent System A.

Characterization of Metabolites in Dog Bile—TLC (Solvent System A) of ethyl acetate extracts of bile samples that had been collected sequentially (Dog Q9-156) showed a pattern of radioactive peaks that was similar to that of ethyl acetate extracts of urine samples from the same dog; *i.e.*, with time, peak A decreased, whereas peaks B and C increased. This finding is illustrated by radioscans shown in Fig. 2 for bile samples collected at 15, 45, and 90 min after dosing.

Metabolites extracted into ethyl acetate (unconjugated fraction) accounted for 23% of the radioactivity in total bile collected during the first 8 hr after dosing. Another 23% of the remaining radioactivity was extracted into ethyl acetate after incubation with β-glucuronidase (glucuronide fraction). Radioscans after TLC (Solvent System A) showed peaks A, B, and C in the ratio of 1:3:2 for the unconjugated fraction and peaks B and C (4:1) for the glucuronide fraction. The TLC (Solvent System A) of radioactive material not extracted by ethyl acetate showed only peak C.

From the unconjugated fraction, Metabolite 1, which corresponded to peak A, was isolated by column chromatography, followed by preparative TLC (Solvent System A) of the radioactive material eluted in fractions 32–62 from the column. Metabolite 1 was identified as triamcinolone acetonide, based on evidence from TLC (Solvent Systems A and B), mass spectrometry, and isotope

dilution. Three percent of the total radioactivity in bile was associated with the isolated triamcinolone acetonide.

A metabolite corresponding to peak B in the unconjugated fraction was eluted from the silica gel plate; it had the same R_f value as that of 6β-hydroxytriamcinolone acetonide in Solvent Systems A and B.

The sequence of biotransformations is shown in Scheme I.

CONCLUSIONS

Within 10–15 min after intramuscular administration of triamcinolone acetonide-^{14}C-21-phosphate to dogs, monkeys, and rats, levels of radioactivity in blood and other tissues were approximately equal to those obtained after intravenous administration. These results indicate that triamcinolone acetonide-21-phosphate is absorbed rapidly from the site of intramuscular injection.

Plasma half-life values of 0.5–2 hr in dogs, monkeys, and rats and the rate of excretion in a bile-cannulated dog ($t_{1/2}$ = 2 hr) suggest a short biological half-life for triamcinolone acetonide-21-phosphate after intramuscular or intravenous administration. Excretion *via* the bile seems to be the major route of drug elimination; in all species studied, excretion in the feces after intramuscular or intravenous administration was higher than that in the urine, although the ratio of fecal to urinary excretion varied among the species. Reabsorption of the drug after its excretion in the bile did not appear to take place in the dog.

In the rat, only 2–3% of the ^{14}C label in the acetonide portion of the molecule appeared as $^{14}CO_2$. Most of the remaining radioactivity (81–94%) was recovered in the urine and feces. These results indicate that hydrolysis of triamcinolone acetonide to give acetone-^{14}C and, therefore, a subsequent yield of $^{14}CO_2$ is not a significant metabolic reaction in the rat. Although $^{14}CO_2$ was not measured in dogs or monkeys, 95 and 85%, respectively, of the labeled dose was recovered in urine and feces, suggesting the existence of a metabolic pathway similar to that in the rat.

Triamcinolone acetonide-21-phosphate was rapidly and extensively hydrolyzed to triamcinolone acetonide after intramuscular or intravenous administration. This hydrolysis apparently occurred prior to any other metabolic transformation. Unchanged triamcinolone acetonide-21-phosphate was not found in plasma, urine, or bile samples collected as early as 1–5 min after its administration to dogs, monkeys, and rats.

Hydrolysis of triamcinolone acetonide phosphate also could be determined *in vitro* in dog plasma and rat muscle. Triamcinolone acetonide was identified in the plasma and urine of all species studied and in the bile of the dog. 6β-Hydroxytriamcinolone acetonide was a major metabolite in the urine of rats and monkeys and apparently in the urine of dogs as well.

The presence of 6β-hydroxytriamcinolone acetonide as a major metabolite in the urine of rats and monkeys and the absence of any metabolites resulting from the reduction of Δ^4-3-keto or C-20-keto groups in any of the species studied indicate that the Δ^1, 9α-fluoro, and 16,17-acetonide substituents may block the normal reduction reactions undergone by hydrocortisone. Similar results were reported by other investigators for different corticosteroids possessing Δ^1 or 9α-fluoro substituents (16, 17).

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[x] To whom inquiries should be directed.