diographs shows that a considerable amount of carbon-14 persisted in the body. In addition, as shown previously (12), a lag period might exist before the elimination of the polymer.

The long period of nanoparticle accumulation at the injection site after intramuscular administration correlates well with the previously observed prolonged adjuvant activity of these particles (1, 6). This prolonged activity was especially pronounced after incorporation into larger amounts of polymer and in comparison to other adjuvants (1).

The attraction of the nanoparticles to the immune system has already been mentioned. However, transporation of the adjuvant from the administration site is not necessary for a good immune response. This is also the case with other adjuvants (16, 17). In contrast to these other adjuvants (16–18), poly(methyl methacrylate) nanoparticles did not induce granulomas in guinea pigs within 1 year (6). Histological reactions were the same as with the fluid control. In spite of the long-term persistence at the injection site, poly(methyl methacrylate) nanoparticles might be an improvement over the presently used adjuvants from a toxicity standpoint; the other adjuvants also persist at the site of injection, but they exhibit much stronger adverse histological reactions (16–19).

REFERENCES

(1) J. Kreuter and P. P. Speiser, *Infect. Immun.*, **13**, 204 (1976).
(2) G. Birrenbach and P. P. Speiser, *J. Pharm. Sci.*, **65**, 1763 (1976).
(3) J. J. Marty, R. C. Oppenheim, and P. Speiser, *Pharm. Acta Helv.*, **53**, 17 (1978).
(4) J. Kreuter, *ibid.*, **53**, 33 (1978).
(5) J. Kreuter and E. Liehl, *Med. Microbiol. Immunol.*, **165**, 111 (1978).
(6) J. Kreuter, R. Mauler, H. Gruschkau, and P. P. Speiser, *Exp. Cell Biol.*, **44**, 12 (1976).
(7) J. Charnley, "Acrylic Cement in Orthopaedic Surgery," Livingstone, Edinburgh, Scotland, 1970.
(8) H. Nothdurft, *Naturwissenschaften*, **42**, 106 (1955).
(9) F. Bischoff and G. Brynson, *Progr. Exp. Tumor Res.*, **5**, 85 (1964).
(10) N. E. Stinson, *Br. J. Exp. Pathol.*, **45**, 21 (1964).
(11) *Ibid.*, **46**, 135 (1965).
(12) B. S. Oppenheimer, E. T. Oppenheimer, I. Danishefsky, A. P. Stout, and F. R. Eirich, *Cancer Res.*, **15**, 333 (1955).
(13) L. Tomatis, *Tumori*, **52**, 165 (1966).
(14) J. Urban, *Collect. Czech. Chem. Commun.*, **24**, 4050 (1959).
(15) J. Kreuter and H.-J. Zehnder, *Radiat. Eff.*, **35**, 161 (1978).
(16) W. J. Herbert, *Immunology*, **14**, 301 (1968).
(17) R. Haas and R. Thommssen, *Ergebn. Bakteriol. Immunitätsforsch. Exp. Ther.*, **34**, 27 (1961).
(18) F. M. Davenport, *Ann. Allergy*, **26**, 288 (1968).
(19) H. Raskova and K. Masek, in "International Symposium on Adjuvants on Immunity, Utrecht 1966," Symposia Series Immunobiological Standardization, vol. 6, R. H. Regamey, W. Hennessen, D. Ikic, and J. Ungar, Eds., Karger, Basel, Switzerland, 1967, p. 115.
(20) A. Roe and J. B. Finlay, *J. Am. Chem. Soc.*, **74**, 2442 (1952).

Analysis of Cyclazocine in Plasma

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Abstract □ The analysis of plasma cyclazocine by two methods is described. The radioimmunoassay employed a ^{125}I-labeled radioligand, rabbit antiserum, and separation of bound from free cyclazocine with a second antibody. The radioimmunoassay was specific for cyclazocine and had a detection limit of ~20 pg/ml. The GLC method employed a mass spectrometer as the detector and had a detection limit of ~109 pg/ml. Both techniques had acceptable accuracy and precision when used to quantitate cyclazocine in dog and human plasma. The methods were used successfully to quantitate cyclazocine from beagle hounds receiving 0.5 mg of ^{3}H-cyclazocine/kg iv. The decline in plasma cyclazocine fitted a two-compartment body model with a mean plasma clearance rate of 39.2 liters/hr.

Keyphrases □ Cyclazocine—analysis, GLC–mass spectrometry, radioimmunoassay, human and dog plasma, pharmacokinetics □ Analgesics—cyclazocine, GLC–mass spectrometric analysis, radioimmunoassay, human and dog plasma, pharmacokinetics □ GLC–mass spectrometry—analysis, cyclazocine, human and dog plasma □ Radioimmunoassay—analysis, cyclazocine, human and dog plasma

Cyclazocine, (2α,6α,11R*) - (±) - 3 - (cyclopropylmethyl)-1,2,3,4,5,6-hexahydro -6,11- dimethyl-2,6-methano-3-benzazocin-8-ol (I), is a member of the benzomorphan series (1). It is a relatively long-acting, orally effective narcotic antagonist (2) and may find clinical usefulness in the treatment of opiate dependence (3). Doses as low as 0.1–0.25 mg po have provided effective pain relief in humans (4).

Previously reported analytical methods for cyclazocine include TLC (5) and GLC (6). These methods have a detection limit of ~10 ng/ml. From these laboratories, a radioimmunoassay using ^{3}H-cyclazocine was previously reported (7) with a sensitivity of ~3 ng/ml. The antibody dilution was 1:50, and the antiserum for this assay was soon exhausted.

To estimate accurately the cyclazocine concentration in plasma following low oral doses, a sensitive measurement method is needed. This article reports the development of two new analytical methods: a radioimmunoassay using a ^{125}I-labeled cyclazocine derivative and a a GLC method using a mass spectrometer as the detector. Both techniques demonstrate sensitivity in the picogram range. These assays have been used to measure cyclazocine added to human and dog plasma. The techniques successfully measured cyclazocine in the plasma of dogs receiving 0.5 mg/kg iv. The pharmacokinetic parameters were determined from the radioimmunoassay data.

EXPERIMENTAL

Radioimmunoassay—*Solutions*—Phosphate-buffered saline was prepared by dissolving 8.17 g of NaCl, 0.50 g of $NaH_2PO_4{\cdot}H_2O$, 1.07 g of Na_2HPO_4, and 0.095 g of thimerosal[1] in 1 liter of distilled water and adjusting to pH 7.0. The assay buffer contained 0.1% (w/v) gelatin dissolved in phosphate-buffered saline. Nonimmune normal rabbit serum was diluted 1:500 in phosphate-buffered saline containing 0.05 *M* edetate

[1] Sigma Chemical Co., St. Louis, Mo.

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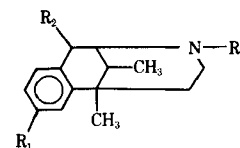
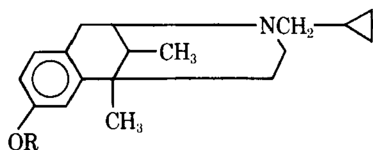


Table I—Relative Affinities of Cyclazocine and Related Compounds to the Antiserum

Compound (Trivial Name)	R_1	R_2	R_3	Relative Affinity
I (cyclazocine)	—OH	—H	$-CH_2CH-CH_2$ (cyclopropyl, CH_2)	1.000
IV (volazocine)	—H	—H	$-CH_2CH-CH_2$ (cyclopropyl, CH_2)	0.4500
V	—OH	—H	$-CH_2-CH$ (cyclobutyl, CH_2, CH_2, CH_2)	0.2000
VI	—OH	—H	$-CH_2-CH{=}CH_2$	0.2000
VII (ketazocine)	—OH	=O	$-CH_2CH-CH_2$ (cyclopropyl, CH_2)	0.0200
VIII (pentazocine)	—OH	—H	$-CH_2-CH{=}C(CH_3)_2$	0.0080
IX	—OH	—OH	$-CH_2CH-CH_2$ (cyclopropyl, CH_2)	0.0020
X (norcyclazocine)	—OH	—H	—H	0.0008
XI	—H	—H	—H	0.0006
XII	$-OCH_3$	—H	—H	0.0004



I: R = —H
II: R = $-(CH_2)_3COOH$
III: R = $-(CH_2)_3CONHCH(p\text{-hydroxyphenyl})CO_2CH_3$

disodium[2]. Iodination buffer contained 0.5 *M* Na_2HPO_4–0.5 *M* NaH_2PO_4 (4:1) adjusted to pH 7.5. Dilute iodination buffer was the same buffer diluted 1:10 with distilled water.

Preparation of Cyclazocine Antiserum—A carbodiimide condensation (8) was performed to conjugate a cyclazocine hapten[3] (II) with bovine serum albumin. A trinitrobenzenesulfonic acid determination of free amine groups (9) indicated that approximately 14 hapten molecules were attached to each albumin molecule. The hapten–protein conjugate (1.0–2.0 mg) was emulsified in complete Freund's adjuvant[4] and injected intradermally at multiple sites on the backs of Dutch belted rabbits. Conjugate (1.0 mg/rabbit) in incomplete Freund's adjuvant was administered as a booster immunization at ~3-week intervals. The rabbits were bled periodically *via* the marginal ear vein, and serum was obtained.

Preparation of Second Antibody—Anti-rabbit γ-globulin was raised in two castrated rams. Rabbit γ-globulin[5] (0.5 mg) emulsified in complete Freund's adjuvant was injected intradermally at multiple sites in the rump. Booster immunizations in incomplete adjuvant were administered at monthly intervals. The sheep were bled at 2-week intervals, and serum was obtained. Serum from numerous bleedings was pooled, and the titer of the second antibody (1:16) was determined by a danazol assay (10).

Radioiodination—A modification of the Greenwood and Hunter (11) method was used to radioiodinate a methyltyrosinate derivative (III) of cyclazocine at room temperature. A methanol solution of III (10 μg) was mixed with 50 μl of iodination buffer. Sodium ^{125}I-iodide[6] (1.0 mCi) was added, the vial was stoppered, and the contents were mixed gently. Dilute iodination buffer, 15 μl, containing 30 μg of chloramine-T[7] was added, and the mixture was allowed to react for 2 min. The reaction was stopped by 60 μg of sodium metabisulfite in 30 μl of dilute iodination buffer.

One hundred microliters of a solution containing sucrose (16% w/v) dissolved in assay buffer was added, and the contents of the vial were transferred to a gel filtration[8] column previously equilibrated with assay buffer. A solution containing 8% (w/v) sucrose dissolved in assay buffer (70 μl) was used to rinse the vial, and the wash solution was added to the column. The column eluate was collected in 1.0-ml fractions, which were counted to locate the radioiodinated derivative.

Assay—Duplicate unknown or standard plasma samples (2.0 ml) were measured into extraction tubes (15 × 85 mm). The standards contained 50–1000 pg of cyclazocine/ml. Extraction buffer (0.2 ml), consisting of a solution saturated with both sodium bicarbonate and sodium carbonate, was added to each tube. A solution containing 5% (v/v) of ethanol in toluene (7 ml) was added, and the tubes were shaken mechanically for 15 min. The tubes were centrifuged, and aliquots of the organic phase were transferred to assay tubes (12 × 75 mm). The solvent was evaporated under a nitrogen stream, and the residue was suspended in 0.5 ml of assay buffer. The tubes were mixed, covered, and allowed to remain overnight at 4°.

The cyclazocine antiserum (200 μl) at a 1:12,000 dilution in normal rabbit serum was added to each tube. Radioiodinated III (100 μl) was added, and the tubes were incubated for 4–6 hr at 4°. Appropriately diluted second antibody (200 μl) was added, and the incubation was continued for an additional 12–18 hr. After incubation, 2.5 ml of cold phos-

Table II—Estimated Cyclazocine Concentration by Radioimmunoassay in Human Plasma Samples Supplemented with Cyclazocine

Cyclazocine Added, pg/ml	Assayed Mean[a], pg/ml: Day 1	Day 2
50	50.2 (6.0, +0.3)[b]	51.2 (8.2, +2.5)
100	98.4 (5.3, −1.6)	99.8 (6.6, −0.3)
200	209 (4.3, +4.5)	198 (7.0, −0.8)
500	520 (2.9, +3.9)	517 (2.0, +3.4)
800	799 (2.7, −0.1)	867 (2.4, +8.3)
1000	931 (1.7, −6.9)	1010 (2.7, +0.8)

[a] $n = 12$. [b] Numbers in parentheses are the percent standard error and the percent difference from expected, respectively.

[2] Edetate disodium, Sigma Chemical Co., St. Louis, Mo.
[3] 4-[3-(Cyclopropylmethyl)-1,2,3,4,5,6-hexahydro-*cis*-6,11-dimethyl-2,6-methano-3-benzazocin-8-yloxy]butanoic acid methanesulfonate.
[4] Difco Laboratories, Detroit, Mich.
[5] Fraction II, Miles Laboratories, Kankakee, Ill.
[6] New England Nuclear, Boston, Mass.
[7] Eastman Kodak, Rochester, N.Y.
[8] Sephadex G-25-150, Sigma Chemical Co., St. Louis, Mo.

Table III—Estimated Cyclazocine Concentration by GLC–Mass Spectrometry in Human Plasma Samples Supplemented with Cyclazocine

Cyclazocine Added, pg/ml	Assayed Mean[a], pg/ml	
	Day 1	Day 2
300	342 (2.0, +13.9)[b]	307 (9.1, +2.2)
450	449 (1.7, −0.3)	506 (0.8, +12.5)
600	530 (4.7, −11.7)	609 (5.4, +1.5)
800	760 (0.2, −5.0)	844 (15.7, +5.5)
1500	1320 (1.0, −12.2)	1560 (2.0, +4.2)

[a] $n = 3$. [b] Numbers in parentheses are the percent standard error and the percent difference from expected, respectively.

phate-buffered saline was added, and the tubes were centrifuged at 2200×*g* for 30 min; the supernates were decanted, and the precipitates were counted. The computer program, based on a weighted least-squares regression analysis (12), used the reciprocal of the variance as the weight for the individual points on the standard curve.

GLC–Mass Spectrometry—*Glassware and Solutions*—All test tubes were thoroughly cleaned with concentrated chromic acid; rinsed several times with water, 5% aqueous ammonia, deionized water, and methanol[9]; and oven dried. All tubes were silanized using 5% dichlorodimethylsilane in toluene. Saturated potassium carbonate–potassium bicarbonate buffer was extracted twice with an equal volume of toluene prior to use.

Assay—Cyclazocine (100 ng/ml of methanol) was added to plasma to produce duplicate 300–3000-pg/ml standards. Plasma (1.0 ml) was added to a tube, followed by 3 ng of internal standard[10] and 0.3 ml of buffer. The sample was extracted twice with 5 ml of benzene[11], and the combined extracts were evaporated to dryness. Trimethylamine, 100 μl of a 0.002 *M* solution in benzene, and pentafluorobenzoyl chloride, 20 μl of 1:100 (v/v) in benzene, were added to the tube. The tube was mixed and allowed to stand at room temperature for 15 min. Benzene (0.3 ml) was added, the tube was mixed, and the benzene was evaporated as described.

The residue was dissolved in 20 μl of toluene, and a 5-μl aliquot was analyzed on a GLC–mass spectrometer[12] operated in the chemical-ionization mode, employing a selected ion monitor program. The conditions were as follows: 91.44-cm glass column[13]; column temperature, 260°; injection port temperature, 300°; source temperature, 200°; methane carrier/reactant gas, 10 ml/min at 0.8 torr; 230-ev electron energy; and 50-mamp emission. The two programs were: (*a*) *m/e* 466, MH^+, pentafluorobenzoyl derivative of cyclazocine, 50-msec dwell time; and (*b*) *m/e* 482, MH^+, pentafluorobenzoyl derivative of internal standard, 50-msec dwell time.

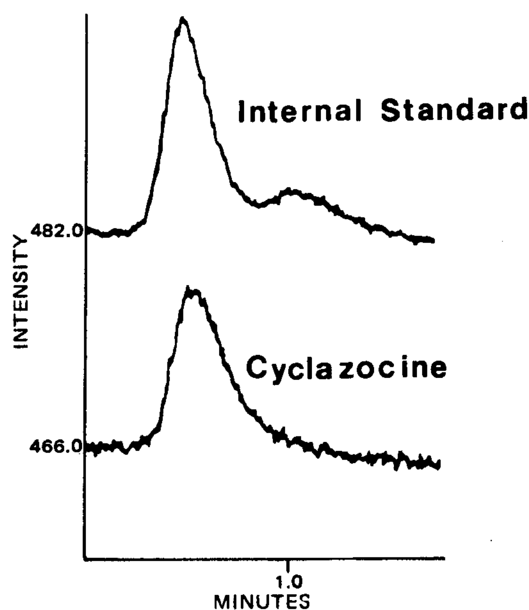


Figure 1—*Selected ion chromatogram of processed human plasma containing 3.0 ng of internal standard/ml and 1.2 ng of cyclazocine/ml.*

Table IV—Mean[a] Cyclazocine Concentration in Dog Plasma after 0.5 mg of ^{3}H-Cyclazocine/kg iv

Hours	Values Obtained by Radioimmunoassay, ng/ml	Values Obtained by GLC–Mass Spectrometry, ng/ml	Values Obtained by Extractable Radioactivity, ng/ml
0.016	171 ± 26	164 ± 37	130 ± 19
0.050	126 ± 21	119 ± 23	107 ± 14
0.083	94 ± 6.1	73 ± 13	95 ± 6.8
0.166	99 ± 7.3	85 ± 14	78 ± 5.2
0.250	93 ± 4.8	87 ± 2.0	65 ± 4.4
0.500	56 ± 10	60 ± 21	36 ± 3.5
0.750	35 ± 3.4	49 ± 5.0	23 ± 1.8
1.00	21 ± 1.5	22 ± 2.0	16 ± 1.9
1.50	11 ± 2.8	9.5 ± 1.6	8.0 ± 0.9
2.00	9.6 ± 0.6	9.0 ± 0.96	4.9 ± 0.7
4.00	1.5 ± 0.03	—[b]	—[c]
6.00	0.8 ± 0.05	0.89 ± 0.05	—[c]
24.0	0.22 ± 0.09	—[c]	—[c]

[a] Mean ± *SE* of three animals. [b] Not analyzed. [c] Below detection limits.

Pharmacokinetic Study in Dogs—Three female beagle hounds were medicated intravenously with 0.5 mg of ^{3}H-cyclazocine/kg[14]. The dose was prepared as a 1-mg/ml solution of cyclazocine in physiological saline containing 1% lactic acid. The specific activity of the dose was 1.12×10^9 dpm/mg.

Blood samples were collected from an indwelling venous catheter at appropriate intervals, using potassium oxalate as the anticoagulant. The samples were centrifuged to separate the plasma. An aliquot was counted, and the remainder was frozen until analyzed. When necessary, plasma samples were diluted with control dog plasma prior to analysis.

Another plasma sample (0.2–0.5 ml) was extracted as described for the radioimmunoassay procedure. Cyclazocine (5 μg) and norcyclazocine (5 μg) were added to each extract, which was evaporated to dryness. The residue was dissolved in 100 μl of toluene–methanol (1:1 v/v). A 10-μl aliquot was counted in Biofluor[6]; radioactivity was measured, and the counting efficiency was determined by the channels-ratio method. The remaining extract was analyzed by TLC[15] with a solvent system of toluene–methanol–isopropylamine (80:20:3 v/v/v).

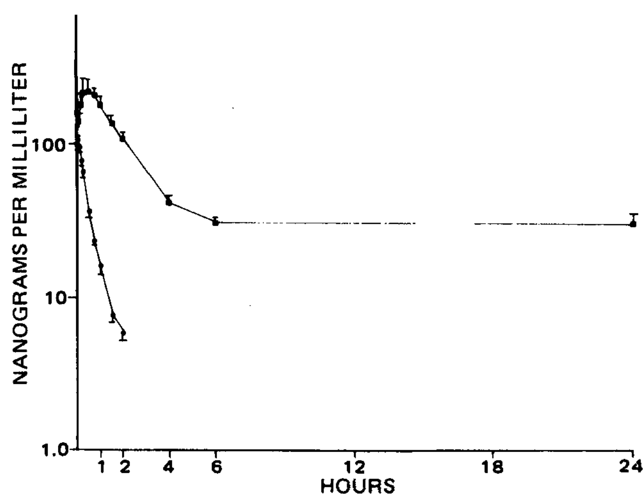


Figure 2—*Plasma levels of total (■) and extractable (●) radioactivity after intravenous administration of 0.5 mg of ^{3}H-cyclazocine/kg to beagle hounds* (n = 3, *mean* ± SE).

[9] Analytical reagent, Mallinckrodt, St. Louis, Mo.
[10] 1,2,3,4,5,6-Hexahydro-6(*eq*),11(*ax*)-dimethyl-3-(3-methylbutyl)-2,6-methano-3-benzazocin-8-ol.
[11] Nanograde, Mallinckrodt, St. Louis, Mo.
[12] Model 5982, Hewlett-Packard.
[13] Three percent SP-2100 on 100–200-mesh Gas Chrom Q, Supelco, Bellfonte, Pa.
[14] Amersham, Arlington Heights, Ill.
[15] Silica gel, E. M. Laboratories, Elmsford, N.Y.

Table V—Pharmacokinetic Parameters for Cyclazocine Disappearance from Plasma of Beagle Hounds following an Intravenous Dose of ^{3}H-Cyclazocine (0.5 mg/kg)

Animal Weight, kg	Rate Constants, hr^{-1} k_{12}	k_{21}	k_{10}	Plasma Clearance Rate, liters/hr	Apparent Volume of Distribution at Steady State, liters	Terminal $t_{1/2}$[a], min	Area under Curve, ng hr^{-1} ml
7.0	0.46	0.08	1.50	33.2	152	702	99.8
7.4	0.37	0.11	1.52	43.6	122	460	86.5
8.2	0.13	0.13	1.22	41.0	67.5	349	108
Mean	0.32	0.11	1.41	39.2	114	504	98.0
±*SE*	±0.09	±0.01	±0.09	±3.13	±24.8	±104	±6.21

[a] Calculated from $\frac{0.693}{1/2\{(k_{12}+k_{21}+k_{10}) - [(k_{12}+k_{21}+k_{10})^2 - 4k_{12}k_{21}]^{1/2}\}}$.

RESULTS AND DISCUSSION

The rabbits produced antiserum within 5 months, and the antiserum titer ranged from 1:1000 to 1:15,000. The antiserum dilution that was chosen as the most sensitive and specific was 1:12,000, equivalent to a 1:60,000 final dilution in the assay tube; this dilution resulted in 45–50% radioligand binding.

The antiserum specificity was assessed by determining the ability of related compounds to compete with the radioiodinated III for binding to the antibody. The amounts of each compound required for 50% displacement of radioactivity were determined by comparison with cyclazocine standard curves to arrive at a relative affinity (13). The antiserum exhibiting the least cross-reactivity was selected for all subsequent work. The relative affinities of the related compounds for this antiserum are shown in Table I. Three compounds (IV–VI) demonstrated affinity for the cyclazocine antibody. However, these compounds are not metabolites and, therefore, pose no problem. The known metabolite (norcyclazocine, X) (14, 15) showed little affinity for the cyclazocine antiserum.

The sensitivity or detection limit of the assay is defined as the smallest amount of compound that is significantly different from zero at the 95% confidence limits (16). Thus, the detection limit in human plasma was ~20 pg/ml; it was ~25 pg/ml in dog plasma.

Exogenous cyclazocine was quantitated accurately when varying amounts were added to human plasma, extracted, and assayed in the cyclazocine radioimmunoassay system (Table II). One set of samples was analyzed immediately after preparation (Day 1), and a second set was analyzed after storage in the frozen state for several days (Day 2). No statistical difference was found between standards prepared in dog and human plasma. The assay accuracy ranged from 6.9 to 8.3% over 50–1000 pg/ml. The assay had acceptable precision with a maximum within-concentration standard error of 8.2%.

The GLC–mass spectrometric analysis of plasma standards indicated that the relationship between peak area ratio and cyclazocine concentration fitted a linear model over the 300–3000-pg/ml range. The retention times for cyclazocine and the internal standard, as their pentafluorobenzoyl derivatives, were both ~0.9 min; separation was dependent on the selective ion monitoring capability of the instrument (Fig. 1).

Exogenous cyclazocine was quantitated accurately (Table III) when varying amounts were added to human plasma. The accuracy ranged from 12.2 to 13.9%, and the method had acceptable precision with a maximum within-concentration standard error of 15.7%. The detection limit as determined from a "robust" statistical analysis (17) of the plasma standards were 110 and 146 pg/ml in human and dog plasma, respectively. The average cyclazocine recovery from plasma was 96%, as determined by comparison of direct and extracted standards.

Total and extractable radioactivities from the pharmacokinetic study are presented in Fig. 2. In contrast to the anticipated decline in plasma tritium, the amount of tritium in the systemic circulation increased, reaching a maximum after 30 min. After a moderate decline, total radioactivity was similar after 6 and 24 hr. The pattern with extractable tritium was different; there was a rapid decline during the first 2 hr. TLC of plasma extracts collected during the first 45 min revealed a single radioactive zone with migration characteristics identical to authentic cyclazocine. Later samples lacked sufficient radioactivity for TLC.

The higher levels of total radioactivity as compared to extractable cyclazocine radioactivity imply that the difference is the result of polar conjugated drug or metabolite. It was reported that dogs excrete ~30–38% of cyclazocine as a glucuronide conjugate in the urine (15). The initial rise in total radioactivity, which is accompanied by a concomitant decline in free cyclazocine, suggests rapid formation of a slowly cleared metabolite.

The mean cyclazocine concentrations in dog plasma after ^{3}H-cyclazocine are shown in Table IV. There was good agreement between the analytical methods. The radioimmunoassay values were used to generate pharmacokinetic parameters (Table V) by a modified computer program (18) using a two-compartment body model. The high plasma clearance rate, 39.2 liters/hr, supports the hypothesis of fast elimination of free cyclazocine from the plasma.

Cyclazocine disappearance from dog plasma during the first 2 hr followed apparent first-order kinetics. The half-lives were determined by linear regression from the logarithm of the concentration data; the half-life values were 25, 28, and 20 min for the radiotracer, radioimmunoassay, and GLC–mass spectrometry techniques, respectively.

REFERENCES

(1) S. Archer, N. F. Albertson, L. S. Harris, A. K. Pierson, J. G. Bird, A. S. Keats, J. Telford, and C. N. Papadopoulous, *Science*, **137**, 541 (1962).

(2) L. S. Harris and A. K. Pierson, *J. Pharmacol. Exp. Ther.*, **143**, 141 (1964).

(3) R. B. Resnick, M. Fink, and A. M. Freedman, *Compr. Psychiatry*, **12**, 491 (1971).

(4) L. Lasagna, T. J. DeKornfeld, and J. W. Pearson, *J. Pharmacol. Exp. Ther.*, **144**, 12 (1964).

(5) S. J. Mule, *Anal. Chem.*, **36**, 1907 (1964).

(6) R. F. Kaiko and C. E. Inturrisi, *J. Chromatogr.*, **100**, 63 (1974).

(7) K. A. Pittman and T. A. Williams, *Pharmacologist*, **15**, 167 (1973).

(8) B. F. Erlanger, *Pharmacol. Rev.*, **25**, 271 (1973).

(9) A. F. S. A. Habeeb, *Anal. Biochem.*, **14**, 328 (1966).

(10) J. E. Peterson, M. E. King, W. F. Banks, J. F. Baker, A. F. Jensen, R. W. Ross, Jr., S. Clemans, and J. Edelson, *J. Pharm. Sci.*, **67**, 1425 (1978).

(11) F. C. Greenwood and W. M. Hunter, *Biochem. J.*, **89**, 114 (1963).

(12) D. Rodbard and J. E. Lewald, *Acta Endocrinol., Suppl. 147*, **64**, 79 (1970).

(13) G. E. Abraham, *ibid., Suppl. 183*, **75**, 1 (1974).

(14) S. J. Mule and C. W. Gorodetzky, *J. Pharmacol. Exp. Ther.*, **154**, 632 (1966).

(15) S. J. Mule, T. H. Clements, and C. W. Gorodetzky, *ibid.*, **160**, 387 (1968).

(16) A. R. Midgley, Jr., G. D. Niswender, and R. W. Rebar, *Acta Endocrinol. Suppl. 142*, **63**, 163 (1969).

(17) M. Hollander and D. H. Wolfe, "Nonparametric Statistical Methods," Wiley, New York, N.Y., 1973, p. 200.

(18) M. Pfeffer, *J. Pharmacokinet. Biopharm.*, **1**, 137 (1973).

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