FHKnapp Conf-8306109--1

BIODISTRIBUTION AND PHARMACOKINETICS OF ^{195m}Pt-LABELED CIS-DICHLORO-TRANS-DIHYDROXO-BIS(ISOPROPYLAMINE)PLATINUM(IV), CHIP, IN THE NORMAL FEMALE FISCHER 344 RAT

CONF-8306109--1

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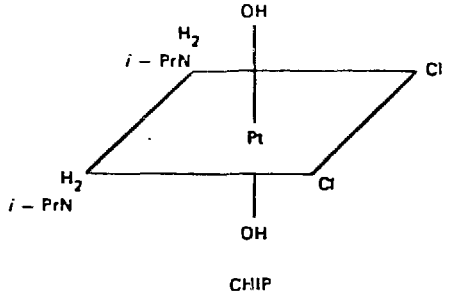
1. INTRODUCTION

The discovery and successful clinical application of the potent anti-tumor compound, cis-Dichlorodiammineplatinum(II), cis-DDP* has stimulated considerable interest in developing effective but less toxic second-generation platinum antitumor drugs. One such candidate drug is cis-Dichloro-trans-dihydroxo-bis-(isopropylamine)platinum(IV), cis-trans-$[PtCl_2(OH)_2(i\text{-}PrNH_2)_2]$, (CHIP), the molecular structure of which is shown in Fig. 1. An important feature of this Pt(IV) agent is that in addition to exhibiting a generally milder clinical toxicity than cisplatin, the dose-limiting toxicity of CHIP is the more common myelosuppression rather than the less desirable nephrotoxicity. Also, CHIP has been reported recently to be more effective than cisplatin against both alkylating agent sensitive and resistant strains of the Yoshida sarcoma.[1] That CHIP is indeed a promising candidate drug is underscored by its selection by the National Cancer Institute as one of four new platinum analogs to enter clinical trials in the U.S.A.



CHIP

FIGURE 1. Molecular structure of CHIP

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As part of our continuing interest in platinum antitumor agents, we have developed a microscale synthesis for ^{195m}Pt-labeled CHIP and have studied the tissue distribution and pharmacokinetic properties of this radiolabeled agent in normal female Fischer 344 rats. The purpose of this paper is to report the details of the microscale synthesis, biodistribution, and pharmacokinetic studies of ^{195m}Pt-CHIP including a comparison with similar data for ^{195m}Pt-*cis*-DDP.[2]

2. MATERIAL AND METHODS

2.1. Microscale synthesis of ^{195m}Pt-labeled CHIP

2.1.1. ^{195m}Pt radiolabel. ^{195m}Pt ($t_{1/2}$ = 4.02d) was produced by neutron irradiation (n,γ) of highly enriched ^{194}Pt (97.41%) in the High Flux Isotope Reactor (HFIR) at Oak Ridge National Laboratory at a flux of 2.5 x 10^{15} neutrons/cm^2/sec. The high burn-up cross-section of ^{195m}Pt (13,000 barns) limits the specific activity to ~ 1.2 mCi of ^{195m}Pt/mg Pt metal. Details of the reactor production of ^{195m}Pt and hot-cell processing of irradiated targets are published elsewhere.[3] The ^{195m}Pt nuclide is an ideal radiolabel for studying the biodistribution and pharmacokinetic properties of Pt antitumor drugs because (1) ^{195m}Pt emits penetrating γ radiation (99 and 129 keV) which provides the capability of direct and sensitive detection (~ 2.7 x 10^2 disintegrations/min/ng Pt°) of Pt at low concentrations in biological specimens and (2) ^{195m}Pt decays directly to stable ^{195}Pt by isomeric transition, which simplifies quantitation by γ-ray spectrometry since no daughter activities are produced.

2.1.2. Reaction scheme. The eight-step reaction scheme for synthesizing ^{195m}Pt-CHIP is schematically outlined in Fig. 2.

Reaction conditions have been optimized at a 0.2 mmole reaction scale in order to obtain the highest yield and purity of CHIP. Steps 1, 2, 4, 5 (after substituting *i*-PrNH_2 for NH_4OH) and 6 are are carried out exactly as described in the refined microscale synthesis of *cis*-DDP.[3] Brief comments on the remaining steps are as follows:

(Step 3) A stoichiometric amount of KCl is added to the Na_2PtCl_4 solution from Step 2 and K_2PtCl_4 precipitated on adding 2 volumes of EtOH (absolute). The crude K_2PtCl_4(s) is dissolved in 0.1M HCl and then

1. ^{195m}Pt-target $\xrightarrow{aqua\ regia + 2NaCl}$ $\xrightarrow{\Delta,\ dryness}$ $Na_2PtCl_6(s)$
2. $Na_2PtCl_6 + 0.5\ N_2H_4 \cdot 2HCl \xrightarrow{5°C\ 5\ min;\ 85°C} Na_2PtCl_4$
3. $Na_2PtCl_4 + KCl + EtOH \xrightarrow{25°C} K_2PtCl_4(s)$ + recrystallize
4. $K_2PtCl_4 + 6KI \xrightarrow{25°C} K_2PtI_4$
5. K_2PtI_4 + 2A BELOW $\xrightarrow{25°C}$ *cis*-$PtA_2I_2(s)$
6. *cis*-$PtA_2I_2(s) + 2.2\ AgNO_3 \xrightarrow{60°C}$ *cis*-$[PtA_2(H_2O_2)]^{2+}$
7. *cis*-$[PtA_2(H_2O)_2]^{2+} + HCl \xrightarrow{45°C}$ until Ag^+-free
   $\xrightarrow{60°C;\ 12M\ HCl}$ *cis*-$PtA_2Cl_2(s)$
8. *cis*-$PtA_2Cl_2(s) + H_2O_2$ (30%) $\xrightarrow{5\ min;\ 95°C}$ *cis-trans*-$[PtCl_2(OH)_2A_2]$(CHIP)

FIGURE 2. Scheme for the microscale synthesis of ^{195m}Pt-labeled-CHIP. Non-essential reaction products are omitted. Denotations: A for i-propylamine, Pt for ^{195m}Pt-labeled Pt.

reprecipitated with EtOH (absolute) as before. The use of pure K_2PtCl_4 instead of Na_2PtCl_4 generated in situ leads to a substantially purer CHIP product and usually eliminates the need to recrystallize the CHIP precursor, cis-Pt(i-$PrNH_2)_2Cl_2$.

(Step 8) The addition of 1 ml of 30% H_2O_2 to the yellow CIP residue from Step 7 followed by reaction at 95°C for 5 minutes converts the precursor to CHIP (crude). Reaction times longer than 5 minutes lead to reduction in the yield and quality of product. Under these reaction conditions at least five minute but detectable components are produced in this step.

2.1.3. Purification by preparative TLC. The crude ^{195m}Pt-CHIP was purified by preparative thin-layer chromatography (TLC) employing silica gel G (2000 μ thickness) as the support and a solvent mixture of Me_2CO: EtOAc:H_2O (45:45:10) as the developer. A saturated solution of ^{195m}Pt-CHIP in H_2O (1.5 ml at ~ 22 mg/ml) was loaded onto the TLC plate. After drying and development, the band containing CHIP was scraped off the plate and the CHIP eluted with MeOH. After evaporating the MeOH solution to dryness, the residue was dried in high vacuum at ambient temperature. TLC analysis of the purified CHIP (40% yield) using Whatman analytical LHP-K plates confirmed that the product was pure [single,

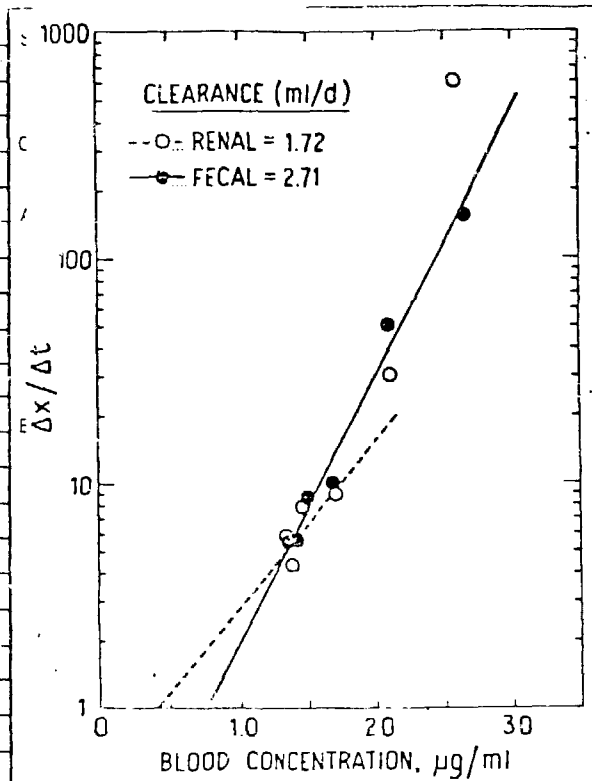

FIGURE 3. A graph of the $\Delta x/\Delta t$ vs [CHIP] in blood (μg/ml). x = amount of drug in urine or feces; t = time

homogeneous spot; R_f 0.43]. The uv-visible absorption spectra of the labeled and unlabeled reference samples were virtually the same. The molar absorptivities, ε, at the wavelengths of 206, 300, and ~ 384 nm are 2.8×10^4, 5.64×10^2, and 5.6×10^1 M^{-1} cm^{-1}, respectively.

Elemental, TLC, and spectroscopic analyses (^{13}C and ^1H-NMR) of a non-radioactive non-radioactive sample of CHIP which was prepared and purified as described above confirm that (1) the sample is pure (no extraneous peaks by NMR and TLC) and exists in the anhydrous form (i.e., no associated H_2O_2).

2.2. Biodistribution studies. The in vivo distribution studies were carried out in two separate experiments employing normal female Fischer 344 rats (137-175 g). Each rat received a single dose of ^{195m}Pt-labeled CHIP (7.5 mg drug/kg) via either the intraperitoneal (i.p.) or intravenous (lateral tail vein) route. Only four rats were treated i.v. ^{195m}Pt-CHIP was administered in saline (0.85% NaCl solution) and ~ 75-100 μCi of ^{195m}Pt was administered per rat. Rats were sacrificed at seven different times post-injection: 0.25, 1, and 6 h; 1, 3, 8, and 13 d. In general, a total of six to eight rats were used for each observation time. Fourteen tissues/organs were excised, rinsed with saline, and weighed. Urine and feces from four rats were collected in metabolism cages out to 8 d. ^{195m}Pt radioactivity was determined in the whole organ by means of an automatic gamma counter

s (Packard, Model 522) set as follows: lower level at 390, window at 110, and a gain setting of 0.25 keV/channel.

2.3 Pharmacokinetic parameters. Clearance from the blood (Cl_b) and biological half-life ($t_{1/2}(b)$) were calculated according to model-independent methods described by Gibaldi and Perrier.[4] The apparent terminal-elimination phase rate constant (λ_z) was calculated by linear regression analysis using the 3 and 8 day post-injection data (Table 1).

TABLE 1. Pharmacokinetic parameters

Parameter	Value	Parameter	Value
$AUC_{0-\infty}$	31.9 µg d $ml^{-1}kg^{-1}$	Cl_b	236.1 ml $min^{-1}kg^{-1}$
λ_z	0.0676 d^{-1}	Cl_r	8.60 ml $min^{-1}kg^{-1}$
$t_{1/2}(b)$	10.4 d	Cl_f	13.5 ml $min^{-1}kg^{-1}$

Area under the blood concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) was calculated by the trapezoidal method with extrapolation to infinity. The apparent renal (Cl_r) and fecal (Cl_f) clearance values were determined from the slopes of graphs relating the natural logarithmic values of the incremental amount of drug in urine or feces/increment of time (i.e., $\Delta x/\Delta t$), respectively, plotted against the concentration of drug in the blood at the midpoint of the collection interval (cf. Fig. 3). Biological half-times $t_{1/2}(b)$ were also evaluated from a plot of the cumulative % dose of Pt excreted in the urine and feces as a function of time.

3. RESULTS AND DISCUSSION

3.1. Biodistribution data. The distribution and retention of ^{195m}Pt determined in 14 tissues as a function of time following administration of ^{195m}Pt-labeled CHIP to female Fischer rats is compiled in Tables 2 and 3. The data are expressed in terms of the % injected dose/organ and % injected dose/g tissue, respectively. The combined average uncertainty (mean % standard deviation) in these data is 13% for all tissues other than the stomach, small intestine, and colon. The combined average %

uncertainty for the latter three tissues is 31%. Unless stated otherwise, reference to the distribution data should be interpreted as the % injected dose/g tissue. Also, reference to the level or concentration of CHIP should be interpreted as the level or concentration of Pt, as measured by ^{195m}Pt, since the nature of the retained species is unknown. For purposes of data comparison, a retention of 1% of the injected dose/g tissue for a 0.15 kg rat corresponds to 0.27 μmoles or 5.2 μg Pt/g tissue.

Profiles of the tissue distribution of ^{195m}Pt-CHIP are shown in Figure 4. Examination of this data shows that there is initially a relatively rapid loss of ^{195m}Pt from all tissues followed by a slower loss out to 8 d for all tissues and blood with the exception of the spleen. Levels (% dose/g tissue) appear to rise anomalously between 8 to 13 d although the % dose/organ in general shows a uniform decline with time. The apparent increase results from the loss of organ weight without loss of the % Pt retained. Necropsy on Day 13 revealed nearly total fat depletion in the peritoneal cavity which may signal a potentially serious side effect. While the greatest ^{195m}Pt concentration is in the kidney and the lowest in the brain, the greatest amounts of ^{195m}Pt CHIP are retained in the liver and kidney. Examination of the tissue distribution data at 24 h shows that the general order of decreasing tissue retention is:

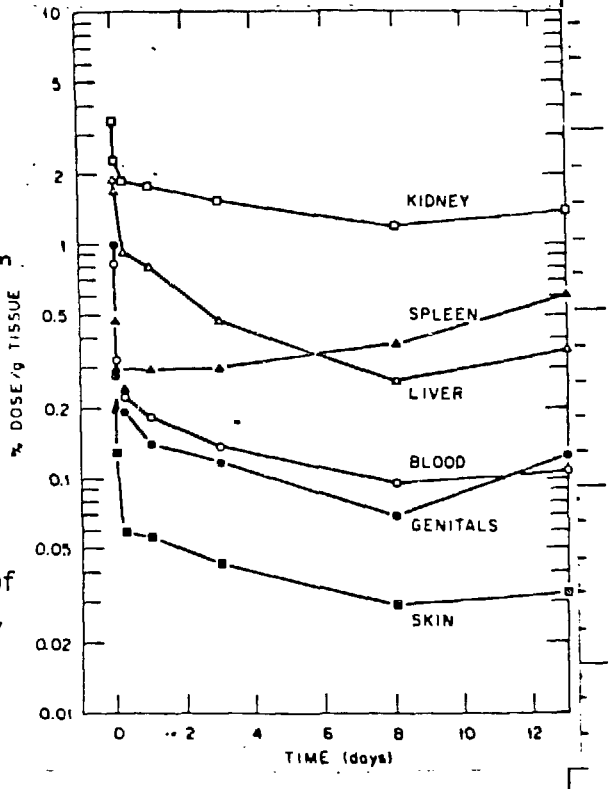

FIGURE 4 Distribution of ^{195m}Pt-labeled CHIP in selected tissues of the Fischer 344 (female) rat

Kidney > colon > liver > pancreas > spleen > stomach > blood > adrenals > small intestine > lungs > genitals > heart > skin > brain.

This order is to be compared with that for cis-DDP at 24 h[2] which is:

Kidney > genitals > liver > adrenals > spleen > bladder > lung > pancreas > heart > brain (ten tissues).

TABLE 2. Distribution of ^{195m}Pt in (female) Fischer 344 rat tissues[a,b] as a function of time after administration (i.p.) of ^{195m}Pt-labeled CHIP[c]

Time, Post-Injection	% Injected Dose/g Tissue							% Deviation (Mean)
	0.25 h	1.0 h	6.0 h	24.0 h	3.0 d	8.0 d	13.0 d	
Tissue								
Blood	0.778	0.378	0.232	0.194	0.139	0.109	0.110	9.95
Liver	1.67	1.87	0.899	0.739	0.476	0.257	0.367	12.0
Spleen	0.478	0.316	0.232	0.262	0.300	0.366	0.613	8.92
Pancreas	1.29	0.633	0.396	0.296	0.234	0.120	0.169	17.0
Stomach	0.299	0.199	0.0953	0.233	0.0500	0.0157	0.0240	51.6
S. Intestine	0.784	1.31	0.299	0.167	0.0535	0.0215	0.0275	31.0
Colon	0.225	0.0898	1.83	0.879	0.104	0.0411	0.0325	27.8
Adrenals	0.926	0.377	0.173	0.162	0.190	0.0845	0.180	18.9
Kidneys	3.46	2.73	2.03	1.74	1.56	1.32	1.43	11.4
Genitals	1.09	0.363	0.197	0.151	0.118	0.0679	0.125	22.3
Heart	0.328	0.190	0.129	0.107	0.0780	0.0518	0.0532	7.07
Lungs	0.663	0.306	0.192	0.151	0.101	0.0610	0.0655	10.7
Brain[d]	2.14	1.23	0.812	0.631	0.525	0.330	0.400	11.6
Skin	0.301	0.131	0.0588	0.0531	0.0438	0.0295	0.0330	14.5

a, b, c, d; see Table 3 for explanation.

A comparison of i.p. vs i.v. route for the 24 h data (Table 4) shows that levels following i.p. injection are slightly but uniformly higher than those following i.v. injection.

The persistence of ^{195m}Pt activity in the tissues at longer times (8 to 13 d) is consistent with the long $t_{1/2}$(b) of CHIP (and/or perhaps a long-lived metabolite). Similar data were reported by Harrison, et al,[5] who speculated that the higher tissue levels in their longer-term studies could be artificially high due to the potential retention of extraneous Ir daughter activity resulting from ^{191}Pt decay. While the retention of Ir activity is possible, the two sets of data are in qualitative accord, suggesting that the contribution of the Ir activity to the total tissue activity may be less significant than was first thought.

TABLE 3. Distribution of ^{195m}Pt in (female) Fischer 344 rat tissues[a,b] as a function of time after administration (i.p.) of ^{195m}Pt-labeled CHIP[c]

Time, Post Injection	% Injected Dose/Organ							% Deviation (Mean)
	0.25 h	1.0 h	6.0 h	24.0 h	3.0 d	8.0 d	13.0 d	
Tissue								
Blood	6.22	2.88	1.85	1.55	1.16	0.895	0.941	8.43
Liver	9.06	9.76	4.50	3.61	2.46	1.41	1.08	11.4
Spleen	0.185	0.121	0.0951	0.105	0.114	0.155	0.177	14.3
Pancreas	0.358	0.181	0.124	0.0992	0.0648	0.0507	0.0770	14.3
Stomach	0.670	0.443	0.173	0.418	0.082	0.0461	0.0390	29.8
S. Intestine	5.44	7.97	2.10	0.842	0.323	0.151	0.0990	21.0
Colon	1.53	0.659	11.2	5.65	0.820	0.279	0.145	27.5
Adrenals[d]	3.75	1.51	0.879	0.749	0.630	0.383	0.650	18.6
Kidneys	4.19	3.26	2.43	2.12	1.82	1.46	1.37	8.68
Genitals	0.468	0.144	0.0854	0.0687	0.0508	0.0303	0.0375	20.1
Heart	0.164	0.0932	0.0652	0.0533	0.0385	0.0271	0.0235	11.7
Lungs	0.499	0.254	0.167	0.126	0.0813	0.0553	0.0550	20.0
Brain[d]	3.40	2.00	1.31	1.04	0.850	0.550	0.600	18.0
Skin[e]	4.52	1.97	0.88	0.80	0.66	0.44	0.50	

[a] Rats ranged in weight from 137-175 g

[b] Eight rats/group except as follows: three and 13 d (four rats); 0.25, 1.0, and 6.0 h for stomach, intestine, colon, and skin (four rats)

[c] 7.5 mg/kg

[d] Divide data by 100 to obtain true values

[e] Based on 10% of body weight for a 150 g rat

A comparison of the tissue distribution of CHIP vs cis-DDP at 24 h postinjection (i.v.) shows that the tissue levels of CHIP are higher than those of cis-DDP in the colon, pancreas, liver (approximately twice as high) and to a lesser extent than that of the heart. Levels for all other tissues are either approximately the same or lower. If there is an association between tissue levels and chemotherapeutic effectiveness against cancers of the same tissue of origin, then CHIP might show a significantly better response than cis-DDP against colonic cancer since the levels of CHIP in the colon are approximately twice as high as those of cis-DDP.

Pt levels in the reproductive organs (uterus, fallopian tubes, ovaries) are substantially lower than for those of cis-DDP. On the same basis, then, one could speculate that CHIP may not be as potentially effective as cis-DDP against genito-urinary cancers. Although CHIP levels

TABLE 4. Distribution of ^{195m}Pt in (female) Fischer 344 rat tissues[a,b] 24 h after administration of ^{195m}Pt-labeled cis[dichlorotrans-dihydroxy-bis (isopropylamine)platinum(IV)], CHIP[c]

Route of Injection	I.P.	I.V.	Route of Injection	I.P.	I.V.
Tissue	% Dose g Tissue	% Dose g Tissue	Tissue	% Dose g Tissue	% Dose g Tissue
Blood	0.187	0.164	Adrenals	0.185	0.113
Liver	0.801	0.591	Kidneys	1.79	1.62
Spleen	0.297	0.202	Genitals	0.140	0.103
Pancreas	0.308	0.267	Heart	0.109	0.0985
Stomach	0.230	0.0908	Lungs	0.154	0.136
Intestine	0.166	0.102	Brain	0.0068	0.0070
Colon	0.967	0.669			

[a] 151-175 g

[b] Four rats/time period

[c] Dose = 7.5 mg/kg

in the kidney are higher than those of cis-DDP, CHIP is substantially less nephrotoxic suggesting that kidney toxicity is not solely a function of the amount of bound Pt in the kidney but rather is related to the intrinsic nature of the administered drug. Thus, one can continue to be optimistic about finding second-generation drugs which are less.

The urine and fecal excretion data demonstrate that CHIP is excreted rapidly and exhibits at least biphasic excretion kinetics typical of most labeled Pt complexes studied to date. A semi-log plot of the cumulative % dose retained (urine plus feces) vs time is shown in Figure 5. Resolution of this curve into two components provides a graphical estimate of the fast ($t_{1/2}$ = 8 h) and slow ($t_{1/2} \cong$ 15 d) phases. Loss of ^{195m}Pt-CHIP via the feces is considerably slower than via the urine and amounts to 23% of the injected activity on day 8. It is significant to note that the rate of fecal elimination is ten-fold higher for CHIP (23%) compared to cis-DDP (~2-3%).

3.2. Pharmacokinetics. Interpretation of the radioactivity data obtained in this study requires caution because total rather than compound-specific radioactivity in the blood, urine, feces, and tissues was measured. Interpretation of the pharmacokinetic parameters (Table 1) is subject to the following assumptions/limitations: (a) It was assumed that absorption of CHIP from the peritoneal cavity was complete. (b) The short sampling period (8 days) relative to the apparent $t_{1/2}$(b) suggests that the apparent $t_{1/2}$(b) and the extrapolated area under the CHIP concentration-time curve from 8 days to infinity may be poorly determined. Consequently, the value for Cl_b, which is derived from the $AUC_{0-\infty}$ may also be poorly estimated. A longer sampling period was somewhat impractical in view of the short physical half-life of 195mpt (4.02 d); also, the need for such could not have been anticipated a priori.

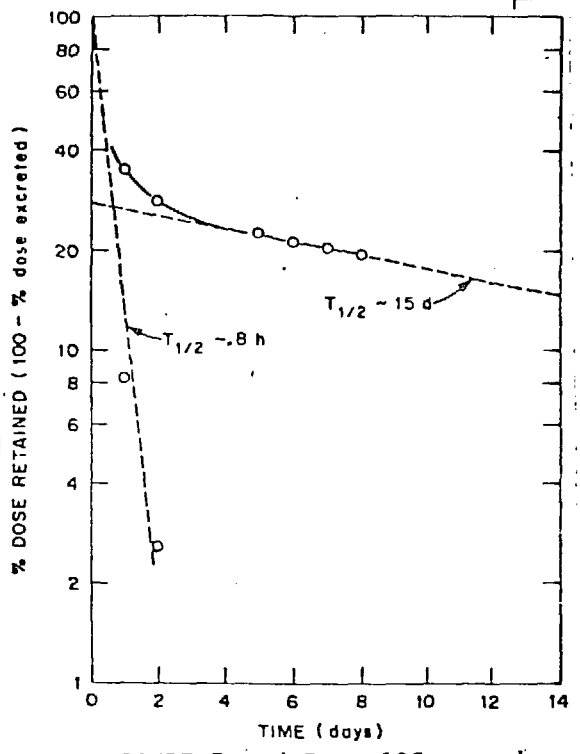

FIGURE 5 % Dose 195mpt-CHIP retained in the rat; graphical estimate of $t_{1/2}$ (b)

The concentration-time profile of CHIP in blood (figure not included) shows an initially rapid decline in CHIP concentration followed by a slower decline with an apparent $t_{1/2}$(b) of 10.4 d. Comparison of the renal and fecal clearance data (Fig. 3) shows that the fecal clearance of CHIP is linear (i.e., first order) and that renal clearance is nonlinear. The renal clearance data is, however, inconsistent with Michaelis-Menten (capacity-limited) kinetics of renal elimination. The nonlinearity of the renal clearance may be attributable to the relatively more rapid renal elimination of CHIP during the early sampling periods (i.e., at high blood concentrations) and less rapid renal elimination of a metabolite during the later sampling periods (i.e., at low blood concentrations) and/or due-

stochastic time-dependent decrease in renal function. The latter possibility appears less likely since CHIP is much less nephrotoxic than cisplatin and also because there was no apparent decrease in urine output with time.

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