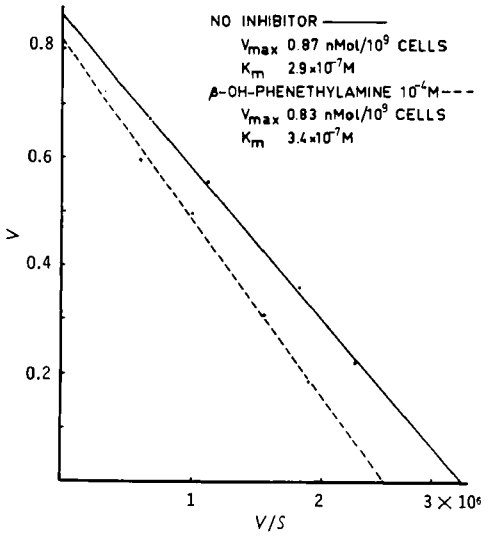


**Figure 6**—*Kinetics of 5-hydroxytryptamine uptake with β-hydroxy-β-phenethylamine as an inhibitor. (Mean of four duplicate experiments.)*

In conclusion, it is felt at this phase of the investigation that partly noncompetitive uptake inhibitors with large nonpolar groups may attach to the transport carrier in a relatively nonspecific manner and, in some cases, only through the amino group, which seems to be the only absolute requirement for the inhibition. The relatively high activity in the cases studied might be explained by the accumulation of the inhibitor in high concentrations at the cell membrane. To prove conclusively that one conformation of phenethylamine-type inhibitors is actually the most effective, relatively small molecular weight compounds that possess a lipid solubility not too different from the parent compound should be investigated.

## REFERENCES

(1) J. Tuomisto, E. E. Smissman, T. L. Pazdernik, and E. J. Walaszek, *J. Pharm. Sci.*, **63**, 1708(1974).

(2) J. Tuomisto, *Ann. Med. Exp. Fenn.*, **46**, 441(1968).

(3) T. L. Pazdernik, Ph.D. thesis, University of Kansas, 1971.

(4) E. E. Smissman and W. H. Gastrock, *J. Med. Chem.*, **11**, 860(1968).

(5) H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.*, **56**, 658(1934).

(6) G. S. Eadie, *Science*, **116**, 688(1952).

(7) B. H. J. Hofstee, *ibid.*, **116**, 329(1952).

(8) J. Tuomisto, E. J. Walaszek, and E. E. Smissman, *J. Pharm. Sci.*, **63**, 15(1974).

## ACKNOWLEDGMENTS AND ADDRESSES

Received May 3, 1973, from the *Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103, and the [1]Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66044

Accepted for publication May 23, 1974.

Supported in part by a Public Health Service International Postdoctoral Research Fellowship (TW 1549) to J. Tuomisto and National Institutes of Health Grants GM-09605 and NS 90399.

The authors gratefully acknowledge the technical assistance of Miss Maire Urvas.

[§] Present address: Department of Pharmacology, University of Helsinki, Helsinki, Finland.

[†] Deceased.

[x] To whom inquiries should be directed. Present address: Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103

# Distribution of $^{14}$C-Lomustine ($^{14}$C-CCNU)-Derived Radioactivity following Intravenous Administration of Three Potential Clinical Formulations to Rabbits

**CHARLES L. LITTERST *x, EDWARD G. MIMNAUGH *, ALAN C. COWLES [‡], THEODORE E. GRAM *, and ANTHONY M. GUARINO ***

**Abstract** □ $^{14}$C-Lomustine (CCNU) was administered intravenously to rabbits in three potential clinically useful vehicles of propylene glycol–ethanol (4:1), fat emulsion, and vegetable oil emulsion; the organ distribution of the radioactivity was followed for 24 hr. Bile and fat were the only tissues showing a consistent vehicle-dependent change in the distribution of radioactivity, and in these two instances the fat emulsion and the vegetable oil emulsion both produced the same distribution pattern. Liver, kidney, and lung tissue consistently contained the highest amounts of radioactivity. By 12 hr after dosing, essentially all radioactivity had been eliminated by the animals. Binding of radioactive drug to plasma proteins was found to be twice as great *in vitro* (93%) as *in vivo* (51%).

**Keyphrases** □ Lomustine, radiolabeled—distribution of radioactivity, three vehicles tested, intravenous administration, rabbits □ 1-(2-Chloroethyl)-3-(cyclohexyl-1-$^{14}$C)-1-nitrosourea (lomustine)—distribution of radioactivity, three vehicles compared, intravenous administration, rabbits □ Antineoplastic agents—radiolabeled lomustine, effect of three vehicles on distribution of radioactivity, intravenous administration, rabbits

Lomustine, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU, I), is a potent antineoplastic drug utilized in the treatment of Hodgkins disease and other solid tumors (1). Although it shares the nitrosourea structure with several other drugs, I differs from other nitrosoureas in having no satisfactory parenteral dose formulation. In experimental animals and humans, I has been administered in propylene glycol–

**Table I**—Tissue–Blood Ratio of Radioactivity in Tissues Containing More Drug-Derived Radioactivity than Was Present in Blood

| Tissue | Vehicle[a] | Hours 0.2 | 1 | 4 | 12 | 24 |
|---|---|---|---|---|---|---|
| Liver | C | 1.86 | 2.78 | 2.04 | 1.00 | 0.86 |
| | B | 1.77 | 2.51 | 4.31 | 0.91 | 1.07 |
| | A | 2.00 | 2.63 | 3.67 | 0.91 | 0.76 |
| Lungs | C | 1.42 | 1.37 | 1.20 | 0.67 | 0.64 |
| | B | 1.36 | 1.17 | 1.25 | 0.64 | 0.53 |
| | A | 1.41 | 1.39 | 1.36 | 0.73 | 0.44 |
| Kidneys | C | 3.37 | 3.51 | 2.56 | 2.44 | 1.43 |
| | B | 3.18 | 3.80 | 3.44 | 1.41 | 1.20 |
| | A | 4.01 | 3.44 | 2.64 | 1.50 | 0.88 |
| Adrenal | C | 1.65 | 0.98 | 0.67 | 0.44 | 0.50 |
| | B | 1.54 | 0.84 | 0.81 | 0.68 | 0.47 |
| | A | 1.58 | 0.73 | 0.69 | 0.64 | 0.44 |
| Mucosa | C | 1.27 | 0.92 | 1.42 | 0.63 | 0.64 |
| | B | 1.24 | 1.12 | 1.53 | 0.82 | 0.60 |
| | A | 1.27 | 1.09 | 1.83 | 0.64 | 0.36 |
| Bile | C | 1.50 | 13.56 | 12.82 | 5.44 | 1.36 |
| | B | 1.55 | 10.28 | 8.50 | 18.23 | 0.73 |
| | A | 3.35 | 30.33 | 23.53 | 20.45 | 6.08 |
| Serosa | C | 0.86 | 0.91 | 1.42 | 0.52 | 0.36 |
| | B | 0.89 | 0.86 | 1.00 | 0.54 | 0.53 |
| | A | 0.86 | 0.91 | 1.11 | 0.54 | 0.36 |
| Fat | C | 0.64 | 1.22 | 1.33 | 1.30 | 0.36 |
| | B | 0.63 | 1.15 | 1.03 | 0.82 | 0.34 |
| | A | 0.51 | 0.58 | 0.67 | 0.68 | 0.36 |
| Urine | C | 13.18 | 26.11 | 176.67 | 69.82 | 37.28 |
| | B | 3.51 | 793.88 | 183.56 | 159.86 | 28.00 |
| | A | 2.65 | 238.16 | 229.95 | 102.26 | 61.71 |
| **Absolute Amounts of Recovered Drug Equivalents in Blood, μg/ml** | | | | | | |
| | Vehicle A | 1.56 ± 0.03 | 0.79 ± 0.06 | 0.36 ± 0.03 | 0.22 ± 0.04 | 0.25 ± 0.04 |
| | Vehicle B | 1.80 ± 0.33 | 0.81 ± 0.07 | 0.36 ± 0.05 | 0.22 ± 0.04 | 0.15 ± 0.04 |
| | Vehicle C | 1.68 ± 0.05 | 0.87 ± 0.14 | 0.45 ± 0.06 | 0.27 ± 0.07 | 0.14 ± 0.04 |

[a] A = propylene glycol–ethanol (4:1); B = fat emulsion (Intralipid)–ethanol (9:1); and C = vegetable oil emulsion (Emulphor)–ethanol–0.85% NaCl (1:1:18).

ethanol (4:1); in humans it is given either orally or intravenously utilizing a fat emulsion[1]. The effectiveness of the oral formulation of I is limited by the drug-related toxicity seen at therapeutic doses, and the utility of the emulsion is hampered by such a short stability that the drug must be prepared for administration at the bedside using a cumbersome mixing procedure.

Recently, Davignon *et al.* (2) experimented with a polyethoxylated vegetable oil emulsifier[2] as a possible vehicle for parenteral administration of nitrosoureas in humans. Preliminary studies in humans utilizing this vehicle are already commencing[3], based in part on results reported here on the similarity of drug distribution between the vegetable oil emulsifying agent[2] and traditional clinical vehicles. The present investigation was conducted to provide information concerning the distribution of radioactivity derived from $^{14}$C-I after intravenous administration of the drug to rabbits in each of three vehicles.

## EXPERIMENTAL

**Drug**—$^{14}$C-I was labeled in the 1-position of the cyclohexyl ring[4] (specific activity 12.626 mCi/mmole). The radiochemical purity of the drug was in excess of 99%, and the drug was chemically pure. The drug was made up weekly in absolute ethanol at a concentration of 0.37 mg/ml (54.05 μCi/mg) and stored at −30° until use. In solution, I has been shown (2) to be stable for 7 days at 0°. On the day of the experiment, 0.5 ml of the ethanol solution was mixed with 9.8 mg of nonradioactive I and diluted to 10 ml with one of three vehicles: Vehicle A, ethanol–propylene glycol (1:4 v/v); Vehicle B, ethanol–fat emulsion[5] (1:9 v/v); and Vehicle C, ethanol–polyethoxylated vegetable oil[6]–0.85% NaCl (1:1:18 v/v).

**Animal Handling**—Compound I was administered to male New Zealand rabbits, 1.4–1.9 kg, through a marginal ear vein at a dose of 1 mg/kg; this dose approximates the clinical dosage of this drug. Each milligram of drug contained 1 μCi of radioactivity, and 1 ml of the drug solution was administered/kg of body weight. At 10 min or 1, 4, 12, or 24 hr after injection, the rabbits were killed and the organ distribution of radioactivity was determined. Five rabbits received I dissolved in each vehicle at each time point.

Approximately 2 min prior to sacrifice, blood was drawn from the heart, using ethylenediaminetetraacetic acid-coated evacuated blood collection tubes[7]. The animal then was killed by the injection of a 30-ml bolus of air into the marginal ear vein contralateral to that used for drug injection. The following tissues were removed: testes, kidneys, adrenals, perirenal fat, spleen, stomach, small intestine, liver, suprasternal muscle, lungs, heart, brain, and lumbar spinal cord. An incision was made along the lesser curvature of the stomach and the organ was everted and washed free of food. The contents of the small intestine were manually expressed, a 20–25-cm segment of jejunum was incised, and the mucosa was removed using a glass microscope slide. In addition to the mucosa,

[1] Intralipid, Vitrum Corp., Stockholm, Sweden.
[2] Emulphor, GAF Corp., New York, N.Y.
[3] M. D. Walker, Baltimore Cancer Research Center, National Cancer Institute, Baltimore, Md., personal communication.
[4] Synthesized by Monsanto Chemical Co. under Contract NIH-NCI-72-3715 and provided by Dr. R. Engle, National Cancer Institute.
[5] Fat emulsion containing, in water, soybean oil (10% w/v), glycerol (2.25% w/v), and egg yolk phospholipid (1.2% w/v); Vitrum Corp., Stockholm, Sweden.
[6] Polyethoxylated vegetable oil; Emulphor, EL 620, GAF Corp, New York, N.Y.
[7] Vacutainer, Becton-Dickinson Co., Rutherford, N.J.

**Table II**—Tissue–Blood Ratio[a] of Radioactivity in Tissues Containing Less Drug-Derived Radioactivity than Was Present in Blood

| Tissue | Vehicle[b] | Hours 0.2 | 1 | 4 | 12 | 24 |
|---|---|---|---|---|---|---|
| Heart | C | 0.68 | 0.72 | 0.58 | 0.37 | 0.29 |
| | B | 0.62 | 0.62 | 0.78 | 0.45 | 0.53 |
| | A | 0.65 | 0.66 | 0.28 | 0.32 | 0.28 |
| Spleen | C | 0.65 | 0.70 | 0.73 | 0.59 | 0.50 |
| | B | 0.52 | 0.60 | 0.75 | 0.41 | 0.33 |
| | A | 0.53 | 0.59 | 0.61 | 0.45 | 0.28 |
| Testes | C | 0.67 | 0.77 | 0.73 | 0.56 | 0.50 |
| | B | 0.56 | 0.72 | 0.78 | 0.54 | 0.60 |
| | A | 0.68 | 0.73 | 0.69 | 0.59 | 0.44 |
| Brain | C | 0.40 | 0.46 | 0.51 | 0.37 | 0.21 |
| | B | 0.38 | 0.52 | 0.67 | 0.36 | 0.33 |
| | A | 0.42 | 0.52 | 0.61 | 0.45 | 0.16 |
| Spinal cord | C | 0.51 | 0.44 | 0.47 | 0.41 | 0.29 |
| | B | 0.48 | 0.33 | 0.58 | 0.41 | 0.20 |
| | A | 0.51 | 0.38 | 0.53 | 0.45 | 0.20 |
| Muscle | C | 0.54 | 0.52 | 0.58 | 0.41 | 0.21 |
| | B | 0.46 | 0.58 | 0.69 | 0.32 | 0.53 |
| | A | 0.46 | 0.57 | 0.56 | 0.45 | 0.28 |
| Stomach | C | 0.58 | 0.63 | 0.73 | 0.41 | 0.43 |
| | B | 0.46 | 0.56 | 0.78 | 0.36 | 0.53 |
| | A | 0.57 | 0.68 | 0.92 | 0.50 | 0.28 |
| Small intestine | C | 0.81 | 0.99 | 0.93 | 0.63 | 0.57 |
| | B | 0.59 | 1.00 | 1.28 | 0.73 | 0.40 |
| | A | 0.89 | 0.99 | 1.06 | 0.68 | 0.36 |

[a] Absolute amounts of drug products per milliliter of blood are the same as listed in Table I. [b] See Footnote *a* in Table I for vehicle identification.

the underlying submucosal–serosal portion of the intestine also was assayed. Each organ was weighed and a random sample was analyzed for radioactivity.

Urine and bile were collected from their respective bladders at the time of sacrifice. For time points longer than 4 hr, passed urine was collected and measured and the catch pans were rinsed. Rinses and urine were pooled prior to counting and calculations were based on the original volume of urine. Although determined separately, bladder urine data were combined with passed urine data for presentation in Table I.

**Analysis of Radioactivity**—Tissue samples (90–150 mg) were placed in glass scintillation vials containing 2 ml of a tissue solubilizer[8]. The tissue was minced with scissors and the capped vials were placed in a shaking water bath at 38° until the tissues dissolved. Vials were cooled to room temperature; then 18 ml of a toluene-based scintillation solution containing 4.2 mg/ml of 2,5-diphenyloxazole and 52.5 mg/ml of 1,4-bis[2-(5-phenyloxazolyl)]benzene was added and the radioactivity was determined[9]. Background disintegrations per minute were subtracted from each sample prior to any mathematical manipulation. The data were punched on cards and calculations were done by computer[10]. Differences between the two experimental vehicles and the clinically used propylene glycol vehicle were determined by a two-tailed *t* test with a level of significance of $p \leq 0.05$.

**Plasma Binding**—*In Vivo*—$^{14}$C-I in each vehicle was administered intravenously to four rabbits; 30–40 ml of blood was drawn by cardiac puncture 30 min later. The plasma was separated by centrifugation and a 3-ml aliquot of plasma was placed in an ultrafiltration membrane cone[11] and centrifuged at 1000×*g* for 30 min as described previously (3). The whole plasma and the resulting ultrafiltrate were analyzed for radioactivity as described for the other rabbit tissues.

*In Vitro*—Blood was drawn from an untreated rabbit and the plasma was separated. Then 8.3 μg of $^{14}$C-I was added per milliliter of plasma, and the protein binding was analyzed as described. A range (4.0–8.3 μg/ml) of concentrations was studied *in vitro* with equivalent results; because of the associated better counting statistics, the higher values are reported here. The *in vitro* binding was determined on four replicate samples for each vehicle.

[8] NCS, Amersham Searle Corp., Arlington Heights, Ill.
[9] Radioactivity was determined in a Nuclear Chicago Mark II liquid scintillation spectrometer.
[10] FORTRAN IV program run on an IBM 370 computer.
[11] Centriflo, Amicon Corp., Lexington, Mass.

**Table III**—Binding of Radioactivity to Plasma Protein of Rabbits following Intravenous Administration of $^{14}$C-I in Three Vehicles[a]

| Vehicle[b] | Percent Bound *In Vitro* | *In Vivo* |
|---|---|---|
| A | 92.3 ± 1.2 | 52.9 ± 7.4 |
| B | 93.9 ± 1.1 | 51.7 ± 2.1 |
| C | 93.5 ± 1.4 | 49.7 ± 2.0 |

[a] Data are means ± standard deviation of radioactivity bound to proteins for four replicates where the drug was added *in vitro* or administered *in vivo* as described under *Experimental*. [b] See Footnote *a* in Table I for vehicle identification.

## RESULTS

**Vehicle Influence on Disposition of Radioactivity following Injection of $^{14}$C-I**—In general, there were no marked differences in the disposition of radioactivity from $^{14}$C-I as a function of the vehicle employed (Tables I and II). Although statistically significant differences were noted at isolated time points in various organs, there were, with two notable exceptions, no consistent differences in tissue levels of drug-derived radioactivity among Vehicles A, B, and C.

The exceptions to this generalization were substernal fat and bile. The biliary excretion of radioactivity was significantly greater with Vehicle A than for either emulsion. In addition, the two emulsions partitioned radioactivity more readily into fat than did Vehicle A. The explanation for these differences in distribution is obscure, but an alteration in disposition of metabolites is unlikely because tissues such as brain and spinal cord, which are essentially lipid depots, showed no change in distribution.

**Organ Distribution of Radioactivity following $^{14}$C-I Administration**—When considered in terms of tissue–blood ratios, it is clear that only the organs of excretion (liver, kidneys, and lungs) concentrated radioactivity to any appreciable extent after intravenous administration of $^{14}$C-I (Table I). In addition, at selected times after dosing, the adrenal glands and the mucosal surface of the small intestine also showed amounts of drug-derived radioactivity that were appreciably greater than the radioactivity found in the blood. The reason for the great difference in amounts of drug products localized in mucosal as opposed to serosal surfaces of the small intestine is unknown but may reflect relative blood flow because radioactivity in the serosal portion reflects radioactivity seen in muscle (Table II). The radioactivity in heart, spleen, adrenals, testes, brain, spinal cord, fat, muscle, and gut did not differ appreciably from blood at most time points.

The organs accounting for the majority of the radioactivity were those having large concentrations of radioactivity (*e.g.*, kidneys), those of large mass but small concentration of radioactivity (*e.g.*, small intestine), and those with a large concentration of radioactivity and a large mass (*e.g.*, liver). The largest amounts of radioactivity were found mainly in organs associated with the excretion of I and its biotransformation products.

**Binding of $^{14}$C-I to Plasma Proteins**—In addition to determining the tissue distribution of radioactivity from I, the plasma binding of radioactivity derived from parent and biotransformation products was studied (Table III). Although all three vehicles gave identical results both *in vivo* and *in vitro*, the *in vivo* binding results differed considerably from the *in vitro* binding. When $^{14}$C-I was added to plasma *in vitro*, only 7% of the radioactivity was recovered in the ultrafiltrate, indicating that the label was 93% bound to plasma proteins. When the drug was administered to rabbits and the binding was determined in the resulting plasma, 49% of the radioactivity was recovered in the ultrafiltrate, indicating that 51% of the label was bound in the plasma.

## DISCUSSION

Relative to Vehicle A, neither Vehicle B nor Vehicle C appreciа-

bly altered the distribution pattern of the nitrosourea-derived radioactivity. In the two instances where differences were noted between Vehicle A and the emulsifiers, both emulsifiers produced similar results. Because Vehicle A is not preferred clinically, the fact that Vehicles B and C produced equivalent distribution patterns for the radioactivity should be encouraging to those interested in the practical usefulness of Vehicle C as a parenteral formulation for nitrosoureas and other lipid-soluble drugs.

Other investigators (4, 5) showed that urinary excretion is a major route by which biotransformation products of I and other nitrosoureas are cleared from the body in mice, monkeys, and humans, and this is confirmed by the finding of large tissue–blood ratios of radioactivity in the kidneys and urine of rabbits, especially during the first 4 hr after drug administration. Moreover, relatively high concentrations of the isotope in bile (Table I) indicate that this clearance route is of significance in the rabbit. Similar observations have been made for other species (6).

Knowledge of the tissue concentrations of antitumor drugs might be used to help explain specific organ toxicity or unique tissue uptake by certain organs and to suggest hitherto unconsidered therapeutic implications. Thus, the high concentrations of drug-derived radioactivity in the lungs support the use of I in the treatment of lung neoplasms (7, 8). The high levels of radioactivity recovered in the liver and kidneys correlate well with organ toxicity data for nitrosoureas. Thus, Oliverio (9) reported hepatic and renal damage in dogs after a single oral dose of I, and Thompson and Larson (10) showed liver damage in rats following a single oral dose of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, II). In addition, Lu and Larson (11) showed a decrease in hepatic microsomal metabolism of pentobarbital in male rats following intraperitoneal administration of II.

The chemical instability of the nitrosourea antitumor drugs as a class has been abundantly documented (5, 12, 13), and numerous studies have established that these agents are orally effective in the treatment of certain kinds of human neoplastic disease (14, 15). For example, a recent investigation (5) considered the disposition of I and its methyl analog in humans following their oral administration and found that with neither of these agents could unchanged parent drug be detected in the plasma at any time after drug administration. These studies imply that unchanged I *per se* is not the active carcinostatic chemical species (5) but that this activity may reside in one or more of its metabolites and/or degradation products. Unfortunately, I is known to give rise to numerous metabolic or degradation products, and the active carcinostatic species has not yet been identified (5). For these reasons, the data from this study are reported in terms of the tissue–blood ratio of total radioactivity, and an attempt was not made to differentiate this radioactivity into unchanged I and a number of chemically distinct fractions.

The results reported here for protein binding of I-derived radioactivity agree well with other results from nitrosoureas. Loo *et al.* (13) reported that II was bound to the extent of 80% to proteins from human plasma *in vitro*, while Oliverio *et al.* (6) estimated an initial binding of I to dog plasma protein of 45% which increased to 60% 6 hr after treatment. The discrepancy found between *in vitro* and *in vivo* binding of I to plasma components is of interest (Table III). More drug or drug products appear to be bound *in vitro* than *in vivo*, suggesting that a dynamic process is responsible for the *in vivo* decrease in binding. The rapid metabolism and clearance of I might be suggested as the basis for the binding decrease *in vivo*.

The clinical usefulness of I could be greatly enhanced with a convenient vehicle for intravenous administration. Propylene glycol-based vehicles are common for I administration but possess obvious toxicity problems. The fat emulsion has little of the propylene glycol toxicity, but the emulsion it forms with I has an extremely short stability and must be prepared and administered immediately. Vehicle C is a potentially useful vehicle for human intravenous administration of I due to its convenience, its low acute toxicity (16), and a pattern of drug distribution consistent with that produced by currently used parenteral vehicles.

## REFERENCES


(1) J. H. Burchenal and S. K. Carter, *Cancer*, **30**, 1639(1972).

(2) J. P. Davignon, K. W. Yang, H. B. Wood, and J. C. Craddock, *Cancer Chemother. Rep.*, **4**, 7(1973).

(3) A. M. Guarino, J. B. Anderson, D. K. Starkweather, and C. Chignell, *ibid.*, **57**, 125(1973).

(4) V. T. DeVita, C. Denham, J. D. Davidson, and V. T. Oliverio, *Clin. Pharmacol. Ther.*, **8**, 556(1967).

(5) R. W. Sponzo, V. T. DeVita, and V. T. Oliverio, *Cancer*, **31**, 1154(1973).

(6) V. T. Oliverio, W. M. Vietzke, M. K. Williams, and R. H. Adamson, *Cancer Res.*, **39**, 1330(1970).

(7) S. K. Carter, *Cancer*, **30**, 1402(1972).

(8) H. Takita and A. Brugarolas, *J. Nat. Cancer Inst.*, **50**, 49(1973).

(9) V. T. Oliverio, *Cancer Chemother. Rep.*, **4**, 13(1973).

(10) G. R. Thompson and R. E. Larson, *J. Pharmacol. Exp. Ther.*, **166**, 104(1969).

(11) I. Lu and R. E. Larson, *Proc. West. Pharmacol. Soc.*, **13**, 78(1970).

(12) J. A. Montgomery, R. James, G. S. McCalek, and T. P. Johnston, *J. Med. Chem.*, **19**, 668(1967).

(13) T. L. Loo, R. L. Dion, R. L. Dixon, and D. P. Rall, *J. Pharm. Sci.*, **55**, 492(1966).

(14) R. C. Young, M. D. Walker, G. P. Canellos, P. S. Schein, B. A. Chabner, and V. T. DeVita, *Cancer*, **31**, 1164(1973).

(15) L. E. Broder and H. H. Hansen, *Eur. J. Cancer*, **9**, 147(1973).

(16) U. Schaeppi and R. S. Phelan, U.S. Department of Commerce National Technical Information Service, PB-220-052 (1972).


## ACKNOWLEDGMENTS AND ADDRESSES


Received March 11, 1974, from the **Laboratory of Toxicology and the ‡ Membrane Transport Section, Experimental Therapeutics, National Cancer Institute, National Institutes of Health, Bethesda, MD 20014*

Accepted for publication June 11, 1974.

The authors thank Dr. V. T. Oliverio and Dr. R. L. Dedrick for their helpful discussions during the preparation of this manuscript.



ˣ To whom inquiries should be directed.