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The Pharmacokinetics of 4-Acetyl Tritium Vinblastine in Two Patients[1]

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SUMMARY

Vinblastine, labeled with tritium in the 4-acetyl group, was given to two patients with malignant disease, and the pharmacokinetic behavior of the drug was determined. Clearance of radioactivity from the blood was biphasic, with $t_{1/2}$ values for a first rapid phase of 4.25 and 4.78 min, and for a slower phase of 185 and 195 min. The volume of the central compartment was calculated as 29.7 and 39.4 liters, while the total fictive volume of distribution was 86.4 and 111.4 liters. Binding to blood components occurred in the order: plasma > platelets > red blood cells > white blood cells. Excretion of radiolabel occurred via the stool and the urine so that, after 72 hr, 25 and 41% of the total dose had appeared in the former and 19 and 23% had appeared in the latter. Appreciable amounts of unchanged drug appeared in the urine, while very little appeared in the stool, suggesting hepatic metabolism, consistent with prior animal studies.

INTRODUCTION

Although the *Catharanthus* alkaloids, VCR[3] and VLB, have been widely used clinically in the treatment of many neoplastic disorders, nothing is known of their distribution, metabolism, or excretion in humans. Avaliable data concerning the pharmacokinetics of these agents come from studies in laboratory animals (1-3, 8, 10, 14) or studies *in vitro* (4, 7, 17), using tritium-labeled drugs. It has been determined, for instance, that both drugs were rapidly cleared from the blood of the rat, and that the major route of excretion was biliary (3, 8, 10, 14). For VLB, only a small amount of radioactivity was excreted in the urine, mostly as unchanged drug (1, 3). The chemical nature of the metabolic products was not elaborated.

Creasey and Marsh (5) recently described the distribution and excretion of [^{3}H]VLB in the dog. They found that clearance from the blood was biphasic ($t_{1/2}$ values of 40 to 281 min for the 2 phases), that the leukocyte fraction had drug levels higher than plasma, and that excretion was mostly via the stool (~30 to 36% of the label in 9 days), although significant amounts of label also appeared in the urine (12 to 17% in 9 days). Most of the drug in the stool was metabolically altered, with initially only 18% of the label as unchanged VLB. Urinary radioactivity initially was mostly free VLB (72% of the label), although the amount of free VLB fell rapidly with time (to 2% of the label).

Distribution of [^{3}H]VLB has been measured *in vivo* and *in vitro* among rat blood components (8, 10, 17), and while plasma, WBC's, and RBC's bound 15, 15, and 10% of the label, respectively, most of the radioactivity (60%) was found in the platelet fraction.

We have recently reported that VLB and VCR bind to human serum components, and we have characterized the parameters of this interaction (7). Binding to whole serum increased linearly both with increasing drug concentration and with increasing protein concentration. However, this association was not strong enough to hinder rapid distribution into other body compartments. We observed that the drugs were bound primarily to the α- and β-globulins.

As a continuation of our studies of these alkaloids, we have prepared and purified 4-acetyl [^{3}H]VLB and administered it to 2 patients, one with metastatic adenocarcinoma of the lung and the other with metastatic hypernephroma. Our studies were designed to determine blood clearance and urine and stool excretion, and to examine the distribution of drug among the various blood components.

MATERIALS AND METHODS

VLB sulfate was a gift from Eli Lilly and Company, Indianapolis, Ind. Deacetylvinblastine was prepared by the method of Hargrove (9) and reacetylated with [^{3}H]acetic anhydride (New England Nuclear, Boston, Mass.), as reported earlier (7, 8). The labeled alkaloid was purified by column chromatography on Whatman P-11 cellulose phosphate and eluted with a 10% urea-2.5% glycine-0.05% NH_4Cl buffer, adjusted to pH 3.1 with HCl (13). The major fraction was found to be 93% pure using the TLC analysis method described earlier (7).

Labeled VLB sulfate (45.0 μCi/mg) in aqueous solution was sterilized by passage through a 0.45-μm Millipore filter. The labeled drug was then mixed with unlabeled VLB sulfate (Eli Lilly Company) to achieve the desired dose level, and administered i.v. In calculating the dose, ideal body weight (6) was used for the 1 patient who was obese, as it is known from studies of the rat that the drug does not distribute significantly into fat (3). Blood samples were

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[3] The abbreviations used are: VCR, vincristine; VLB, vinblastine; WBC, white blood cell; RBC, red blood cell; TLC, thin-layer chromatography.

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drawn from the arm opposite the site of injection, through an indwelling heparin lock, and preserved with EDTA. The blood sampling was initiated at the times indicated and required no more than 30 sec to complete. To determine whole-body radioactivity, triplicate 0.50- or 1.00-ml samples were oxidized in a Packard 305 sample oxidizer, and the ^3H_2O was counted in 15 ml of Instagel (Packard Instrument Company, Downers Grove, Ill.). Portions of blood were centrifuged at 60 × *g* for 15 min in Wintrobe tubes, and the plasma and cell fractions were carefully removed by pipetting. The plasma and cell fractions were centrifuged again at 1000 × *g* for 15 min and the clear plasma, platelet, WBC, and RBC fractions were separated. All 4 fractions were transferred to combustocones (Packard) (rinsing the transfer pipets with a small amount of 0.9% NaCl solution), and oxidized as described above. Weights of each fraction were determined by the difference in weight of the Wintrobe tube before and after each fraction transfer.

Samples of blood (5 ml) were made basic to pH 10 with 10 N NaOH and extracted 3 times with 10 ml benzene. The benzene was dried with Na_2SO_4, evaporated to dryness, and the residue was taken up to 1.00 ml of benzene. A portion of the benzene was spotted on a TLC plate (silica gel, 13181 chromagram; Eastman Kodak Company, Rochester, N. Y.), unlabeled VLB was added, and the plate was developed in acetone (7). The VLB spot was identified by UV and the plate was cut into 1-cm segments and counted for radioactivity directly in 15 ml Instagel.

Urine was collected at timed intervals after the labeled drug was administered and was stored at 4° in the dark. The total volume of each sample was measured and the radioactivity was determined by counting triplicate 1.00-ml samples in 15 ml of Instagel. To evaluate free tritium water and volatile tritium-labeled organic acid content, separate 10-ml aliquots of the urine were made basic (pH 10) with NaOH or acid (pH 1) with HCl. Triplicate 0.50-ml samples were then absorbed on filter paper, air dried, and oxidized as before. Another 10-ml portion of urine was made basic (pH 10) with NaOH, saturated with NaCl, and extracted with 10 ml benzene. Any emulsion that formed was broken by centrifugation at 10,000 × *g* for 15 min. The organic layer was separated and evaporated to dryness, and the residue was taken up in 1.00 ml of benzene. A portion (0.50 ml) of the benzene was added to 15 ml of Instagel for counting, while another portion (100 μl) was spotted on silica gel for TLC as before.

All stools were collected in opaque plastic bags, weighed, and stored at 4°. For liquid stools, 0.50-g samples were placed on paper or in combustocones, and oxidized as above. Solid stools were homogenized in 4 volumes of distilled water, and 1.00-g portions were oxidized as above. A separate 15-ml portion of homogenized stool was made basic with NH_4OH and extracted 3 times with 10-ml portions of benzene, breaking any emulsion by centrifugation. The benzene was evaporated to dryness, and the residue was taken up in 1.00 ml benzene. Both radioactivity and TLC purity were determined as for the urine described above.

RESULTS

Two patients with progressive cancers were administered [^{3}H]VLB i.v. over a 1-min period, and timing was marked from the end of the infusion. Their pertinent data are shown in Table 1. Both patients had normal renal and liver function studies. Blood samples were drawn at timed intervals, beginning as early as 4 min after the dose of [^{3}H]VLB had been administered. In Chart 1, the radioactivity in the blood is displayed as a semilog plot of concentration *versus* time. Analysis of the data was performed assuming an open 2-compartment kinetic system (18), and the values are listed in Table 1. The initial rapid phase had a $t_{½}$ of 4.78 and 4.25 min. The volume of the central compartment was 29.7 and 39.4 liters. For the 2nd phase, the $t_{½}$ was 185 and 195 min, while the total fictive volume of distribution was 86.4 and 111.4 liters. The elimination constant was calculated at 343 and 414 ml/min. Regression analyses of these data provided a good fit for a straight line (semilog), and the *r* values are listed in Table 1. After 4 hr, the data no longer followed 1st-order kinetics out through 30 hr, and further analysis was not performed.

Recovery of [^{3}H]VLB from biological fluids, especially blood, was at best a difficult job. Emulsions were common when benzene (or other organic solvents) extraction was done at basic pH. Routinely, some of the alkaloid adhered to the glass walls of the apparatus, amounting at times to as much as 15% in control studies. We suspect that losses also occurred by occlusion within precipitated protein. Extraction of [^{3}H]VLB from blood was done on 1.00-ml samples with benzene after making them basic, and the extract was analyzed by TLC. In control studies, 30 to 60% of the radioactivity was recovered by this extraction process after known amounts of [^{3}H]VLB were added to blood, but of that recovered, the purity on TLC examination was unchanged from the original [^{3}H]VLB used. From the *in vivo* samples, approximately 50% of the radioactivity was recovered, and of the radioactivity recovered, 87% cochromatographed with VLB in the 4-min sample, and 76% in the 45-min sample. We suspect that this decrease represents drug metabolism.

Since binding to both WBC's and platelets had been reported (4, 8, 10, 17), we fractionated blood samples taken at 4 and 20 min into plasma, RBC, WBC, and platelets by differential centrifugation. The data shown in Table 2 demonstrate that, while much of the alkaloid is present in the plasma fractions, a very significant percentage was bound to the platelet fraction. The amount of radioactivity per mg of tissue was also of interest. The platelet fraction had the highest values, some 30 to 170 times the plasma or RBC activity, while the WBC fraction was 12 to 15 times that of the plasma or RBC's. To satisfy ourselves that the distribution was representative, we repeated the differential centrifugation on whole normal blood that had been incubated with [^{3}H]VLB *in vitro*, and these results also appear in Table 2. Here, most of the total activity is in the plasma and RBC fractions, with the highest specific activity in the platelet and WBC fractions. Compared with the *in vivo* data, there were larger amounts of radioactivity in the RBC

Table 1

Blood clearance and distribution of [³H]VLB

Two patients with progressive malignant disease were treated with therapeutic doses of [³H]VLB. Both had a clinical antitumor response. Regression analysis was done with a Hewlett Packard 9810A calculator.

	Patient	
	S. A.	B. J.
Diagnosis	Adenocarcinoma of the lung	Hypernephroma
Age (yr)	42	31
Sex	Male	Female
Weight (kg)	64.2	60.6[a]
VLB dose (total mg)	15.9	12.1
VLB dose (mg/kg)	0.25	0.20
Total radioactive dose (μCi)	176	122.5
$t_{1/2}$ in min, 1st phase	4.25 (4-25)[b]	4.78 (4-20)
V_1[c] in liters	39.4	29.7
$t_{1/2}$ in min, 2nd phase	195 (25-180)	185 (40-240)
V_f[d] in liters	111.4	86.4
r[e] values from regression analysis	0.954 and 0.9775	0.9636 and 0.9928
Concentration at equilibrium (moles/liter)	1.44×10^{-7}	1.38×10^{-7}
k_{el}[g] (ml/min)	414	343

[a]Since VLB does not distribute into fat (in the rat), ideal body weight was used (actual weight, 85.9 kg).
[b]Numbers in parentheses, time-span.
[c]Volume of central compartment.
[d]Total fictive volume of distribution.
[e]r, correlation coefficient.
[f]Based on y intercept.
[g]Elimination constant.

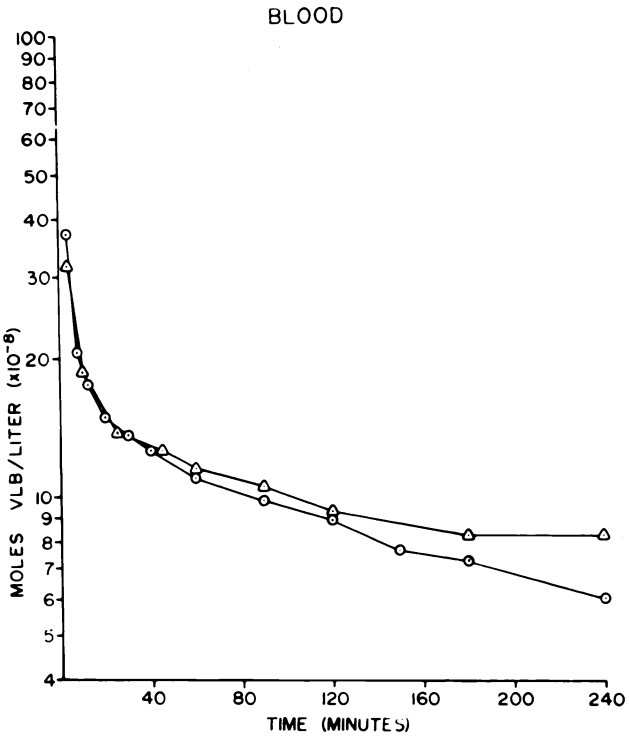

Chart 1. Blood levels of total radioactivity in 2 patients after an i.v. dose of [³H]VLB expressed as moles of VLB per liter *versus* time in min.

fraction and less in both the WBC and platelet fractions. The reason for this is not clear, but may have to do with several factors. It is possible that the impurities of the original [³H]VLB are not cleared from the blood as rapidly as the original alkaloid and are more highly concentrated in plasma, WBC's, and platelets. A more reasonable possibility is that the drug is rapidly metabolized in a manner parallel to that of the rat (10), where the by-products of [³H]VLB metabolism are more concentrated in these fractions. The latter possiblity is supported by the progressive rise in the percentages of the total radioactivity in the WBC and platelet fractions, in the order: 20 min > 4 min > "instant," *in vitro* values. Although there are also differences in the specific activities of these fractions, since the weights of the WBC and platelet pellets were small and variable, we feel that this information is unreliable for quantitative comparison.

Excretion of radioactive material in the urine is shown in Chart 2. After an initial pulse of excreted label in the 1st 8 hr of 10 and 13%, respectively, of the total administered dose, the rate of excretion progressively decreased. In the 1st 24 hr, a total of 14 and 19% of the total dose had appeared, while in the 1st 4 days, 19 and 25% of the radioactivity had been cleared in the urine. Samples of urine were made either basic or acidic, and portions were allowed to air dry on filter paper prior to combustion. For the 1st 48 hr, about 80 to 85% of the radioactivity was recovered, compared with direct counting in Instagel, with no significant difference between acid- and base-treated samples. These values were identical to controls obtained by air drying samples of normal urine with added known amounts of [³H]VLB. By 72 hr, the recovery had fallen to 59% and,

Table 2

Binding of [³H]VLB to blood components

Blood was drawn and preserved in EDTA at the times indicated after an i.v. bolus dose of [³H]VLB (*in vivo*), or samples at the times shown after thorough mixing of [³H]VLB with heparinized blood (*in vitro*). Centrifugation at 60 × *g* allowed separation of platelet-rich plasma from the WBC and RBC fractions. Recentrifugation at 1,000 × *g* separated the platelet and plasma fractions.

	Time (min)		dpm/mg	% of whole blood
In vivo	4[a]	Plasma	4.6 ± 0.1[b]	33.9 ± 1.1
		RBC	5.8 ± 0.64	25.5 ± 2.5
		WBC	68.6 ± 2.7	9.5 ± 1.8
		Platelets	176 ± 36	31.1 ± 2.3
	20	Plasma	2.35 ± 0.20	34.5 ± 2.5
		RBC	1.96 ± 0.05	18.0 ± 0.7
		WBC	28.1 ± 6.0	13.2 ± 3.1
		Platelets	332 ± 212	34.2 ± 2.4
In vitro	5 and 90[c]	Plasma	24.4 ± 1.1	29.8 ± 1.2
		RBC	34.2 ± 0.6	50.9 ± 0.5
		WBC	162 ± 35	6.3 ± 1.0
		Platelets	180 ± 9.5	13.0 ± 0.7

[a]Calculated concentration of [³H]VLB, 3.71×10^{-7} M.

[b]Mean ± S.E.

[c]Added concentration of [³H]VLB was 3.74×10^{-7} M, with a specific activity 4.07 times that of the *in vivo* dose. Since the data at 5 and 90 min were not different, they were averaged together.

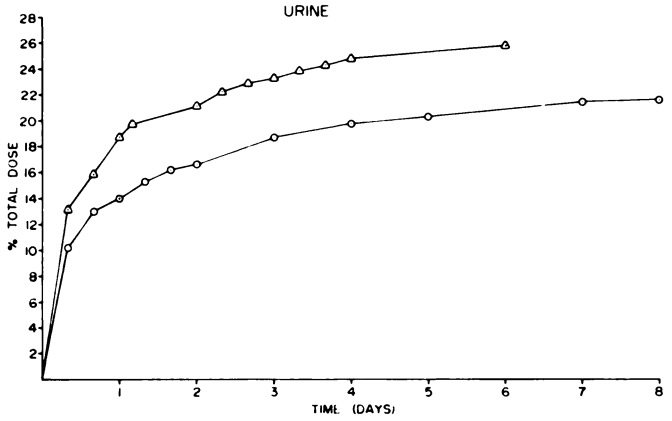

Chart 2. Appearance of total radioactivity in the urine of 2 patients after an i.v. dose of [³H]VLB. The radioactivity is expressed cumulatively as the percentage of the total dose administered *versus* time in days.

by 88 hr, it had fallen to 41%, with again no difference between acid- and base-treated samples.

Estimates of the amount of unchanged drug in the urine were done on samples that were made basic, extracted with benzene, and the benzene spotted on a TLC plate and developed as described above. Our results here were better than with blood and are given in Table 3. Benzene extraction recovered from 26 to 52% of the counts that were in the urine, and between 67 and 90% of this radioactivity cochromatographed with VLB. From these data, we estimate that a total of 4.5 to 6.5% of the original dose is eliminated in the urine as unchanged drug in the 1st 8 hr, 8.4% in the 1st 24 hr, and 9.1% in the 1st 48 hr.

Stool samples were oxidized and counted as above. The results are shown in Chart 3. While only 5.3 and 9.5% of the total radioactivity were excreted in the 1st day, by 72 hr, 25 and 41% had appeared. After this, very little further activity was eliminated. Extraction with benzene of stool samples that had been made basic, followed by TLC examination, allowed determination of the amount of unchanged [³H]VLB, as seen in Table 3. Here, in contrast to the urine, very little unchanged drug was found, amounting to only 0.6% of the total dose at 24 hr, and 0.6 and 0.7% at 48 hr.

Table 3

Excretion of [³H]VLB and TLC data

Samples of urine and stool were extracted with benzene under basic conditions, and the amount of unchanged VLB was determined by TLC.

	Patient	Time (hr)	% recovery into benzene[a]	% VLB by TLC[b]	Total % VLB[c]
Urine	B. J.	8	52	84	44
		72	26	67	17
	S. A.	8	55	90	50
		24	43	79	34
		48	41	72	29
Stool	B. J.	19.5	8	65	5
		22.5	18	62	11
		32.5	4	57	2
	S. A.	44	13	43	6
		96	2	40	1
		144	23	31	7

[a] Ten-ml samples of urine and 10.0 ml of stool homogenized 5 to 1 with distilled water were made basic with NH_4OH and extracted with benzene 3 times. The percentage of the original dpm found in the benzene extract is recorded.

[b]Determined from TLC plates, as the percentage of total cpm on the plate located within the VLB spot.

[c]Product of the 2 preceding columns.

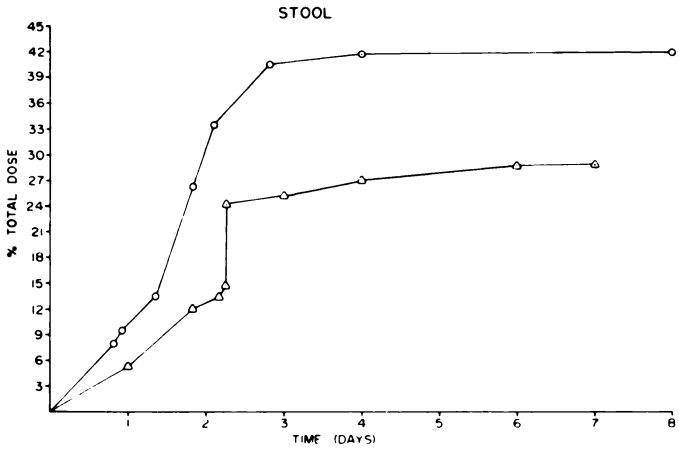

Chart 3. Appearance of total radioactivity in the stool of 2 patients after an i.v. dose of [^{3}H]VLB. The radioactivity is expressed cumulatively as the percentage of the total dose administered *versus* time in days.

DISCUSSION

The rapid clearance of [^{3}H]VLB from the blood in both our patients was similar to the data previously obtained in animals (2, 3, 5, 17). The data in Chart 1 are consistent with a 2-compartment distribution pattern (18), with a rapid transfer of drug into a central compartment very close in volume to calculated total body water [60% of body weight (6), representing 38.5 and 36.4 liters]. This rapid 1st phase, combined with the large total fictive volume of distribution (calculated from the 2nd phase, and roughly equal to 2.5 to 3 times the total body water), indicates that VLB is preferentially and avidly bound in extravascular body tissue (1, 3). This is consistent with tissue distribution measurements done in rats. These data, considered with our prior plasma studies (7), demonstrate that, although binding of VLB to blood components occurs, binding does not prevent rapid distribution into the other organs of the body. Deviation of the curves from 1st-order kinetics after about 4 hr is presumed to be related to the release of radioactive label by metabolic conversion, or perhaps by further redistribution processes, *e.g.*, reabsorption by the gut of drug and/or metabolites released into the bile.

Significant binding of VLB to platelets has been reported in rats (10, 17) and we now confirm platelet binding in humans. The specific activity of the platelet pellet was much higher than for the other blood fractions and may, indeed, be even higher than measured, since separation and weight determination of the platelet pellet, with our technique, tended to overestimate the weight of the platelet fraction. Although there is significant binding of VLB to platelets, we have no evidence that the drug interferes with platelet function. In VLB-treated patients, bleeding problems are not a recorded toxicity of the drug, except when due to thrombocytopenia (12). Binding of VLB to the WBC fraction is consistent with the *in vitro* data of Creasey *et al.* (4). We suggest that, as reported earlier, the VLB is localized in the cellular elements because of binding to tubulin in the cell.

The percentage of total administered radioactivity excreted in the urine is higher in humans that that previously reported for the dog (5), and much greater than that reported for the rat (2). Although a significant amount of the radioactivity is initially excreted in the urine as unchanged VLB, this percentage falls with time as metabolism of the drug occurs. Since only 50% or less of the radioactivity in the urine is benzene extractable under basic conditions, and as there is little or no loss of radioactivity as volatile products, we conclude that the major metabolic product excreted in the urine is not deacetylvinblastine, but acidic or amphoteric material(s). Increasing amounts of volatile radioactivity appear after the 1st 2 days, and this is probably free tritium water.

The major excretory route in humans for the VLB radiolabel is the stool. Very small amounts of unchanged drug are found in the stool, and most of the radioactivity is acidic or amphoteric in nature, and may be in the form of glucuronides or sulfates. The metabolic fate of [^{3}H]VLB in the stool has not yet been determined. Possibly, it was converted to deacetylvinblastine, as occurs in the dog (4). We did not look for volatile radioactive products in the stool, and any deacetylvinblastine formed would be unlabeled and too small in amount to be seen in our TLC examination. Evaluation of this possible mode of metabolism is now being pursued in our laboratory, utilizing VLB labeled in the aromatic ring. Ring-labeled VLB enables metabolic products to be more readily traced and isolated, since the tritium is located in a part of the molecule where the normal mechanisms of metabolism would not affect it. VLB administered p.o. has been reported as active in neoplastic disease (11), and therefore, the drug must be absorbed from the gastrointestinal tract to some extent. After an i.v. dose, it is reasonable to assume that a portion of that drug and its metabolites that were cleared by the liver were later reabsorbed through the gut. This enterohepatic recirculation may account for the break from 1st-order kinetics for the blood radioactivity levels.

Binding of VLB to tubulin, a phenomenon known to occur at pharmacological drug levels (15, 16), and probably the means whereby cells are arrested in metaphase and whereby peripheral neuropathy occurs, is probably the explanation for the cellular localization of the drug that we observed. However, tubulin binding does not, in itself, explain why some tissues take up more VLB or VCR than others. For example, the brain has a very high level of tubulin, yet no labeled VLB or VCR has been found within the brains of treated animals (1, 14). We suggest that membrane binding (15) and/or membrane transport are also important factors, yet to be fully investigated, that play a major role in *Vinca* alkaloid localization *in vivo*.

We conclude that VLB is rapidly cleared from the blood and localized in body tissues. Binding to the blood components is not sufficient to interfere with this rapid distribution. The platelet fraction binds far more than the other blood fractions, although there is some binding to RBC and WBC fractions. The drug is rapidly excreted in both stool and urine, with appreciable amounts of unchanged VLB appearing in the urine, but very little in the stool. Metabo-

lism is extensive, but the nature of the conversion products has yet to be determined.

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