Comparative Metabolism of 2-[Bis(2-chloroethyl)amino]tetrahydro-
-2H-1,3,2-oxazaphosphorine-2-oxide (Cyclophosphamide) and Its
Enantiomers in Humans

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ABSTRACT

The comparative metabolism of the enantiomers of cyclophos-
phamide and of the racemate has been studied in humans. Four
patients were each given, sequentially, the racemate, the (+)-enantiomer, and its (-)-antipode. The plasma levels of
parent drug and the urinary output (24 hr) of unchanged drug
and of two enzymatically produced metabolites, 4-ketocyclo-
phosphamide and carboxyphosphamide, were determined using
mass spectrometry-stable isotope dilution. There was no
significant difference between the three forms of cyclo-
phosphamide with respect to plasma half-life (β phase) or in the
urinary outputs of the drug or of carboxyphosphamide. The
output of 4-ketocyclophosphamide after administration of
(+)-cyclophosphamide was significantly greater than that pro-
duced from the racemate. Cyclophosphamide recovered from
the urine of patients given the racemate was either racemic or
only slightly enriched in the (-)-enantiomer. The two enantiom-
ers were almost equally bound to plasma protein.

Based on these metabolic studies alone, there is little reason
to predict that the enantiomers will differ from each other or
from the racemate in their therapeutic effects in humans, but
there are other factors, e.g., stereoselective uptake of the
intermediary 4-hydroxylated metabolites by neoplastic cells,
which could elicit such differences.

INTRODUCTION

Since its introduction into clinical practice 20 years ago,
cyclophosphamide has become one of the most commonly
used antineoplastic drugs with a broad spectrum of activity
against solid tumors and hematological cancers. Despite the
wealth of clinical and experimental knowledge generated dur-
ing this time, one feature of this compound that has not previ-
ously been explored in humans relates to the structure of the
molecule itself. Cyclophosphamide is a dissymmetrical mole-
cule by virtue of the chiral phosphorus atom, and optical
isomers are therefore possible. Hitherto, the drug has been
administered clinically as the racemate. However, there are
numerous reports of therapeutic differences between enan-
tiomers of drugs, and this fact has prompted the synthesis and
biological evaluation of the enantiomers of cyclophosphamide.

As examples of this phenomenon may be cited the greater
central nervous system stimulant activity of (+)-amphetamine
compared with that of its (-)-isomer (23), the superior anti-
oculant activity of S(-)-phenprocoumon compared with the
R(+)-isomer (10), the inactivity of (+)-methadone in the main-
tenance of opiate-dependent patients (21), and the superior
analgesic activity of (+)-propoxyphene compared with the
(-)-antipode (4). In the area of cancer chemotherapy, mel-
phalan (3-[4-bis(2-chloroethyl)aminophenyl]-L-alanine) was se-
lected for clinical use in preference to the D isomer (medphalan)
on the basis of the superior potency of the L isomer against the
Walker 256 carcinoma in rats (2). The L-mannitol analog of
mannitol myleran (1,6-dimethanesulfonyl-1,6-dideoxy-D-
mannitol) has been reported to have very low antitumor activity
compared with the D antipode (3). These examples concern
molecules which are dissymmetrical by virtue of a chiral carbon
atom. As an example of a biological difference based on
chirality at phosphorus may be cited the greater enzyme inhi-
bition by the S(-)-isomers of some anticholinesterases, the
alkyl S-alkylmethylphosphonothioates compared with their R-
(+)-antipodes (9).

Recent syntheses of the enantiomers of cyclophosphamide
(14) and of isotopically labeled (deuterated) variants thereof
(5) have enabled their antitumor activity and metabolism to be
separately evaluated. Therapeutic differences have been re-
ported in tests against 2 murine tumors. Against the ADJ/
PC6A plasma cell tumor, (-)-cyclophosphamide had approxi-
mately twice the therapeutic index (50% lethal dose/90%
inhibitory dose) of the (+)-antipode (6). Smaller differences
were observed in tests against the L1210 leukemia; male mice
survived longest when treated with (-)-cyclophosphamide,
whereas female mice showed the best response with the (+)-
isomer (24). The metabolism of the enantiomers has been
compared in several species (mouse, rabbit, and rat), both in
vivo, using liver microsomes, and in vivo (5, 7). Particular
attention has been paid to the influence of chirality upon the
rate of the initial 4-hydroxylation step (Chart 1) and upon the
yields of the enzymatically produced detoxification products 4-
ketocyclophosphamide and carboxyphosphamide. It has been
suggested that the relatively selective toxicity of cyclophos-
phamide toward tumor tissue is related to the more efficient detox-
ification thereto of 4-hydroxycyclophosphamide and aldophos-
phamide by normal host tissues (8). If this hypothesis is valid,
then differences between the enantiomers regarding the rela-
tive yields of these products could result in different therapeutic
efficacies, as could differences between the rates of metabo-
lism of the enantiomers in the initial 4-hydroxylation step.

The main objective of this study in humans has therefore

1 This investigation was supported by grants to the Institute of Cancer Re-
search from the Medical Research Council (Grant G973/786 K) and to the Polish
Academy of Sciences, Centre for Molecular and Macromolecular Studies, from
the National Cancer Program.
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Received December 5, 1978; accepted March 29, 1979.

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CANCER RESEARCH VOL. 39
were measured by mass spectrometry-stable isotope dilution using tetradeuterated cyclophosphamide as an internal standard. The method was similar to that described previously for the determination of cyclophosphamide in blood (12). Cyclophosphamide-4-d₄ (10 μl; 1.00 mg/ml in methanol) was added to duplicate aliquots (1 ml) of plasma which was then extracted with ethyl acetate (2 ml). The organic phase (separated by centrifugation where necessary) was concentrated to a small volume before final concentration onto the direct insertion probe of the mass spectrometer. The ratios between cyclophosphamide and its d₄ analog were determined by comparison of the peak heights at m/e 211 and 215 (from at least 10 scans) in the mass spectrum ([M—CH₂Cl]+) for the d₀ and d₄ forms, respectively). Plasma levels were fitted (by Dr. L. I. Hart, Institute of Cancer Research, Sutton, England) to a 2-compartment open model by digital computer using a nonlinear least-squares regression program (18), and pharmacokinetic parameters were calculated therefrom.

**Quantification of Cyclophosphamide, 4-Ketocyclophosphamide, and Carboxyphosphamide in Urine.** The following deuterated internal standards (1.00 mg/ml in methanol) were added to duplicate aliquots (1 ml) of the urine: cyclophosphamide-d₄ (50 μl); 4-ketocyclophosphamide-d₄ (10 μl); and carboxyphosphamide-d₄ (50 μl). The procedure used for the isolation of cyclophosphamide and its metabolites was essentially that applied to their isolation and quantification in mouse urine (5). After extraction with ethyl acetate (2 ml), the organic phase was concentrated before subjection to TLC* on glass plates (20 x 20 or 5 x 20 cm) coated with silicic acid (Merck Kieselgel G, 0.25 mm thick) developing with CHCl₃:CH₃OH (9:1). Cyclophosphamide was run as a standard (cyclophosphamide and carboxyphosphamide-d₄, 50 μl). The procedure used for the isolation of cyclophosphamide and 4-ketocyclophosphamide have the same Rₜ in this solvent) and the band corresponding to the cyclophosphamide marker was detected by exposure to iodine vapor and eluted with methanol (approximately 0.5 ml). The methanol eluate was treated for 10 min with ethereal diazomethane (0.5 ml) to convert 4-ketocyclophosphamide into its N-methyl derivative. After concentration, the ratios between cyclophosphamide, 4-ketocyclophosphamide, and carboxyphosphamide and its d₄ analogs were measured by mass spectrometry as above ([M—CH₂Cl]+ ions at m/e 239 and 243 for the d₀ and d₄ forms, respectively, of the 4-ketocyclophosphamide derivatives).

The aqueous phase remaining after the above extraction with ethyl acetate was acidified to approximately pH 2 by the addition of 0.1 M HCl (1 ml) and extracted with ethyl acetate (4 ml). The organic phase was concentrated to dryness, and a solution of the residue in methanol was treated with ethereal diazomethane as above to yield the methyl esters of carboxyphosphamide and its d₄ analog. The concentrate was subjected to TLC as above, alongside authentic carboxyphosphamide which had been similarly methylated. Bands were located by iodine staining. Where several bands of closely similar Rₜ’s were present at the appropriate region, the major portion of each was removed and the location of the desired component was confirmed using the Epstein reagent (20). The ratio of carboxyphosphamide to its d₄ analog was determined by mass spectrometry ([M—CH₂Cl]+ ions at m/e 257 and 261 for the methyl esters and the d₀ and d₄ forms, respectively). A linear

* The abbreviation used is: TLC, thin-layer chromatography.

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**MATERIALS AND METHODS**

**Patients.** The 4 patients in this study had squamous cell carcinoma of the lung. Their mean age was 61 (range 54 to 68). Patient E. D. was female. All patients had normal renal and hepatic function as measured by standard biochemical tests. None had received cyclophosphamide previously.

Doses (1 g i.v.) of racemic cyclophosphamide, the (+)- and the (−)-enantiomer, were administered as a bolus, sequentially, with a 3-week interval between doses. Blood samples were taken at 5, 10, and 30 min and at 1, 2, 4, 6, and 12 hr. Urine was collected between 0 and 24 hr after administration of the drug. Patients were catheterized, and urine was passed into collection bags which were emptied at intervals of approximately 2 hr into a refrigerated (4°) container. Plasma and urine samples were stored at −30° prior to assay.

**Quantification of Cyclophosphamide in Plasma.** Drug levels...
relationship was demonstrated for the concentration range
encountered between the composition of mixtures of d<sub>1</sub> and d<sub>-1</sub>
forms and peak intensities for cyclophosphamide, 4-ketocyclo-
phosphamide, and carboxyphosphamide after recovery from
urine by the procedures described above.

Mass Spectrometry. Electron impact mass spectra were
determined by the direct insertion technique with an AEI-MS12
spectrometer, using an ionizing voltage of 70 eV, a trap current
of 100 μA, and an ion source temperature of 80-100°C.

Determination of the Enantiomeric Composition of Cyclo-
phosphamide Recovered from Patients' Urine. Unmetabo-
лизированный циклосфосфамид был восстановлен из мочи всех 4
пациентов, получавших препарат с разными энантиомерами. Моча (0-24 час после
лечения) подверглась анализу на циклосфосфамид, изолированный, затем был
разведен и высущен на вакууме через фильтр под вакуумом. Взвешенный на Na<sub>2</sub>SO<sub>4</sub> органический
экстракт был растворен в хлороформе (2 x 500 ml). Где образовался
емulsion, его выделяли, осушали и концентрировали.

The band containing cyclophosphamide (R<sub>f</sub> 0.5) was detected
by exposure to iodine vapor and its location confirmed by edge
spraying with the Dragendorff reagent (Bismuth tiniodate, 10%
w/v in 5 M HCl) which gave a positive reaction (orange colon
against a yellow background) with cyclophosphamide. The
band was removed, eluted with methanol, and the concentrated
eluate was treated with charcoal before concentration to dry-
ness. The residues from urines of patients receiving the race-
mate were crystallized from water (0.3 ml) and those recovered
from urine following administration of the enantiomers were
dissolved in CCl<sub>4</sub> (0.1 ml) and the solutions were treated with
n-hexane (0.2 ml). In each case, large crystals were slowly
deposited (usually during 2 days) and these were transferred
onto filter paper and washed with the appropriate solvent (water
or CCl<sub>4</sub> : n-hexane).

The products were chromatographically homogeneous on TLC in CHCl<sub>3</sub>:CH<sub>3</sub>OH (9:1), but TLC in ethyl
acetate, which resolves cyclophosphamide (R<sub>f</sub> 0.1) from 4-
ketocyclophosphamide (R<sub>f</sub> 0.3), revealed a trace of the latter
(blue color with the Epstein reagent) as contaminant. The
enantiomeric composition of the cyclophosphamide in each
sample was determined by the method of Zon et al. (27)
utilizing nuclear magnetic resonance spectroscopy in the pre-

cence of a chiral shift reagent (see below).

31P Nuclear Magnetic Resonance Spectroscopy. The cyclo-
phosphamide samples (3.45 to 10.5 mg) and [tris(3-trifluoro-
methylhydroxymethylene)-d-camphorato]europium(III) were
dissolved in CDCl<sub>3</sub> (2 ml). Spectra were recorded on a Jeol FX-
60 spectrometer at 24.3 MHz with proton noise decoupling and
deuterium internal lock; repeating time was 3 sec. Chemical
shifts were relative to external 85% H<sub>3</sub>PO<sub>4</sub> using high-
frequency positive convention.

Determination of Plasma Protein Binding of Cyclophos-
phamide and Its Enantiomers. Membrane ultrafiltration (Ami-
on Centriflo CF25; M.W., 25,000 cut off) was performed by
centrifugation (2500 rpm; 20 min). Levels of cyclophosphamide in
plasma and ultrafiltrate were determined under "Quantifi-
cation of Cyclophosphamide in Plasma." (+)-Cyclophos-
phamide (20 μl; 0.89 mg/ml in water) was added to normal plasma
(2 ml; Dr. M. Jarman) which was then incubated at 37°C for 20
min. After ultrafiltration, cyclophosphamide-d<sub>4</sub> (10 μl; 1.00 mg/
ml in methanol) was added to the ultrafiltrate (1 ml), and the
level of cyclophosphamide was determined. The experiment
was repeated using (-)-cyclophosphamide (20 μl; 0.87 mg/ml
in water). In a further experiment, (-)-cyclophosphamide (30
μl) and (+)-cyclophosphamide-d<sub>4</sub> (30 μl; 1.19 mg/ml) were
added to normal plasma (3 ml). After incubation and ultrafiltra-
tion as above, the ratio of cyclophosphamide-d<sub>4</sub> to cyclophos-
phamide-d<sub>0</sub> was determined in both plasma and ultrafiltrate.

RESULTS

The pharmacokinetic parameters (Table 1) and the urinary
outputs of cyclophosphamide, 4-ketocyclophosphamide, and
carboxyphosphamide (Table 2), following sequential adminis-
tration of racemic cyclophosphamide and its enantiomers to
each of the 4 patients, are presented. With the exception of 2
sets of plasma level determinations, the data points were fitted
to a biexponential function, C = Ae<sup>-αt</sup> + Be<sup>-βt</sup>, by computer
(18). For the remaining sets, data points after 30 min were
fitted to monoeponential function, C = Be<sup>-μt</sup>, by least-squares
regression (r = 0.92, 0.98).

There was a remarkable similarity in the mean t<sub>1/2</sub> β values
(Table I) for the racemate, the (+)-enantiomer, and the (-)-
enantiomer. There was no significant overall difference be-
tween the 3 forms in the outputs of cyclophosphamide (range,
39 to 104 mg) or of the principal detoxification product car-
boxyphosphamide (range, 22 to 114 mg). The output of 4-
ketocyclophosphamide (range, 6 to 29 mg) was significantly
greater (p < 0.05) from (+)-cyclophosphamide than from the
racemate.

Table 1

<table>
<thead>
<tr>
<th>Composition of administered cyclophosphamide</th>
<th>A (μg/ml)</th>
<th>α (hr⁻¹)</th>
<th>B (μg/ml)</th>
<th>β (hr⁻¹)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (hr)</th>
<th>Vd</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)</td>
<td>19.2 ± 2.6</td>
<td>4.1 ± 0.3</td>
<td>23.6 ± 6.1</td>
<td>0.096 ± 0.016</td>
<td>7.45 ± 1.4</td>
<td>45.9 ± 0.0</td>
</tr>
<tr>
<td>(±)</td>
<td>19.2 ± 2.6</td>
<td>4.1 ± 0.3</td>
<td>23.6 ± 6.1</td>
<td>0.096 ± 0.016</td>
<td>7.45 ± 1.4</td>
<td>45.9 ± 0.0</td>
</tr>
<tr>
<td>(-)</td>
<td>21.0 ± 2.6</td>
<td>4.0 ± 1.8</td>
<td>20.0 ± 4.5</td>
<td>0.100 ± 0.024</td>
<td>7.25 ± 1.75</td>
<td>50.6 ± 11.4</td>
</tr>
<tr>
<td>(-)</td>
<td>22.6 ± 9.9</td>
<td>4.7 ± 3.6</td>
<td>20.0 ± 3.3</td>
<td>0.092 ± 0.024</td>
<td>7.9 ± 1.7</td>
<td>49.6 ± 8.5</td>
</tr>
</tbody>
</table>

*Mean ± S.D.
Unchanged cyclophosphamide, recovered from the urine after administration of the racemate, was little different from the racemate in enantiomeric composition (Table 3). Cyclophosphamide recovered from the urines of one patient after administration of each enantiomer remained enantiomerically pure.

Plasma protein binding of cyclophosphamide was not stereoselective at the concentrations chosen. The percentage binding of the enantiomers when incubated individually was almost identical, 35% for the (+)-cyclophosphamide and 36% for the (−)-isomer. When unlabeled (−)-cyclophosphamide and (+)-cyclophosphamide-d4 were incubated in admixture (total concentration of the "pseudoracemate", 20.6 μg/ml), the intensity ratio of the ions m/e 211 and 215 was the same in the mass spectra of extracts from both plasma and ultrafiltrate.

No intrapatient differences in either clinical antitumor response or host toxicity were observed following the administration of the 3 forms of cyclophosphamide used in this study.

**DISCUSSION**

Inasmuch as the enantiomers of cyclophosphamide differed little from each other and from the racemate in their plasma half-lives (β phase) and in the urinary outputs of unchanged drug and of the principal detoxification product, carboxyphosphamide, there is little evidence that metabolism of cyclophosphamide is stereoselective in humans.

The results may be compared with those for the 3 animal species thus far studied (mouse, rabbit, and rat), since these studies were undertaken, in part, to determine whether there exists a good animal model for metabolism of the enantiomers of cyclophosphamide in humans. Evidence for stereoselectivity in the initial 4-hydroxylation step in these animals was afforded by comparing the rates of metabolism of the enantiomers by liver microsomes (7). On this evidence, metabolism by the mouse is only marginally stereoselective, (+)-cyclophosphamide being slightly more rapidly metabolized than (−)-cyclophosphamide. Rat microsomes showed some stereoselectivity for the (−)-enantiomer, whereas those from the rabbit metabolized this isomer 3- to 5-fold more rapidly than the (+)-form. The mouse and the rabbit exhibited stereoselectivity in the formation of urinary metabolites (5), particularly 4-ketocyclophosphamide. Thus, the yield of 4-ketocyclophosphamide from (−)-cyclophosphamide was 4- to 11-fold greater than that from the (+)-isomer when racemic cyclophosphamide was given to rabbits. In mice, the reverse stereoselectivity was observed and was particularly pronounced when the yield of 4-keto derivative was compared after separate administration of the enantiomers, being nearly 10-fold greater from (+)-cyclophosphamide than from the (−)-antipode.

Inasmuch as there was evidence (Table 2) in humans for stereoselective formation of 4-ketocyclophosphamide from (+)-cyclophosphamide, but little indication of stereoselectivity...
in the initial metabolism of cyclophosphamide, the mouse could afford a model for human metabolism of the cyclophosphamide enantiomers. Because of large interpatient variations in metabolism, more patients would have to be examined to determine whether the trend toward greater output of 4-ketocyclophosphamide from (+)-cyclophosphamide is general in humans. However, even if confirmed, it seems unlikely that this relatively small degree of stereoselectivity in the formation of a minor detoxification product (the yield of the 4-keto derivative in no case represents >3% of the administered dose) could elicit clinically observable differences in response between the enantiomers.

The validity of the carboxyphosphamide levels requires comment, in view of evidence that this metabolite decomposes significantly to ornitrogen mustard under mild conditions (11). Indeed, we found that carboxyphosphamide was 50% decomposed when a solution (concentration, 200 μg/ml) in urine (pH 5.5) was kept at room temperature for 24 hr. In the present study, catheterization prevented more than a brief exposure of the urine to in vivo temperatures, and samples were exposed for only 2 hr at room temperature before refrigeration. In the cited study (11), urine was collected on ice in 4-hr batches. Decomposition was thereby minimized, although probably not eliminated. In view, however, of the proven instability of carboxyphosphamide, it is highly probable that some of the carboxyphosphamide formed enzymatically from aldophosphamide (Chart 1) is subsequently lost by decomposition in vivo and that this detoxification pathway is more important in humans than measurements of urinary levels of carboxyphosphamide would indicate.

The finding (Table 3) that there was no marked preponderance of one enantiomer in the urine recovered after racemic cyclophosphamide was given requires further comment, in view of a previous report to the contrary from these laboratories (6). Thus, based on their specific optical rotations, samples of cyclophosphamide isolated from the urines of 2 of 3 patients in this earlier study were markedly enriched in the (−)-isomer, the percentage of this isomer being 83 and 91%. Since the specific optical rotation of the enantiomers of cyclophosphamide ([α]D20 ± 2.3°) is small compared with that of many chiral substances of natural origin which may be present in urine, minor contamination by such substances could have been responsible for the observed optical rotation of these samples. Hence, the enantiomeric composition of 2 of the 3 samples from the earlier study, which had apparent (−)-cyclophosphamide contents based on optical rotation of 57 and 91%, was re-determined by the presently described method of 31P nuclear magnetic resonance spectroscopy in the presence of a chiral shift reagent, which is not subject to the possible source of error of the earlier method. The new values for the content of the (−)-enantiomer were, respectively, 50 and 55.2%. These findings provide further evidence that the initial metabolism (mainly 4-hydroxylation) of cyclophosphamide is either not stereoselective or only slightly so and show that the previously reported conclusion to the contrary was wrong.

It was important to show whether either enantiomer underwent inversion of configuration in vivo, and it was for this reason that the enantiomeric composition of cyclophosphamide recovered from the urine of one patient after administration of each enantiomer was determined. Several examples of metabolic inversions of configuration of compounds chiral at carbon have been reported (13, 16, 26), although none, as far as the present authors are aware, of inversion at phosphorus. This consideration is also important in connection with the use of pseudoracemates, in which one enantiomer is labeled with a stable isotope, which serves to distinguish its metabolic fate from that of its antipode. Such mixtures have been used in our studies on stereoselective metabolism of cyclophosphamide in animals (5, 7). The procedure is clearly valid only in the absence of metabolic inversion. The present study shows that, at least in humans, inversion at phosphorus in cyclophosphamide does not occur. This finding does not exclude the possibility that metabolites might undergo spontaneous inversion, particularly the acyclic chiral metabolites carboxyphosphamide and aldophosphamide (Chart 1). Inversion of the latter would also affect the cyclic tautomer 4-hydroxy-cyclophosphamide and its oxidation product, 4-ketocyclophosphamide. However, the appropriate synthetic enantiomers required to test these possibilities were not available at the time of this study, although syntheses are reported to be in progress (24).

Binding of the enantiomers of cyclophosphamide to plasma proteins was compared because of a recent report (11) that 24% of racemic cyclophosphamide was bound in contrast with earlier reports that there was no detectable adsorption of cyclophosphamide by serum proteins (17) and that the drug was only 2.4% bound to human serum albumin (22). The presently determined values of between 35 and 44% binding are more consistent with the later report (11). It is noteworthy that these higher values for protein binding were found when drug concentrations were within the range of those encountered clinically, whereas concentrations used in the earlier studies were much higher, respectively, 0.7 to 1.4 × 10−2 M (1.9 to 3.9 mg/ml) (17) and 3 × 10−2 M (8.3 mg/ml) (22). At these high concentrations, protein binding might be saturated. The present results also show that there is no stereoselectivity in plasma protein binding of the enantiomers of cyclophosphamide, administered either separately or in a racemic mixture.

In summary, there is insufficient stereoselectivity in the enzymatic pathways of metabolism of cyclophosphamide in humans to support a prediction that either enantiomer would be superior to its antipode or to the racemate in cancer chemotherapy. However, other factors could elicit different therapeutic responses between the enantiomers; for example, preferential uptake of one of the stereoisomers of 4-hydroxy-cyclophosphamide into neoplastic cells could confer selective toxicity. In this connection, it is noteworthy that 4-hydroxylation introduces a second chiral center into the cyclophosphamide molecule and that one of the 4 stereoisomers of 4-methylcyclophosphamide, which is similarly additionally chiral at C-4, is metabolized differently from the other 3 by rat liver microsomes (1). The superior activity of melphalan against L1210 leukemia cells in culture compared with its D isomer [cf. the earlier report (2) cited herein] has been tentatively ascribed to a preferential uptake by the L1210 cells of the L antipode (25). Stereoselective uptake among the stereoisomers of 4-hydroxy-cyclophosphamide could result in different intracellular concentrations of the spontaneously formed cytotoxic decomposition product phosphoramide mustard (see Chart 1) despite the absence of stereoselectivity in the enzymatic pathways of metabolism. Finally, the conclusions reached in the present study regarding
the relative lack of evidence for stereoselective metabolism of cyclophosphamide enantiomers in humans do not necessarily apply to analogs chiral at phosphorus, and the comparative metabolism of the enantiomers of one such analog, isophosphamide (15), is therefore currently under investigation.

ACKNOWLEDGMENTS

The authors wish to thank Professor A. B. Foster for his interest and encouragement and M. H. Baker for skilled technical assistance.

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