SUMMARY

The human pharmacology of cyclophosphamide was investigated in 26 patients who received cyclophosphamide-\textsuperscript{14}C in doses of 6 to 80 mg/kg i.v. Levels of the intact drug in plasma and urine and excretion of \textsuperscript{14}C label in breath and stools were determined by liquid scintillation counting. Plasma and urine alkylating activity was measured by reaction with \textsuperscript{4}(4-nitrobenzyl)pyridine. Protein binding of cyclophosphamide and plasma alkylating metabolites were determined by plasma ultrafiltration.

Injected cyclophosphamide distributed rapidly into 64\% of body weight, and plasma cyclophosphamide half-life in patients without prior drug exposure was 6.5 hr. Not more than 20\% of injected cyclophosphamide was excreted intact in urine at any dose level.

Plasma alkylating metabolites were 56\% bound to plasma proteins. After a 40-mg/kg dose, peak unbound alkylating activity averaged 13.3 \textmu mol/ml, and in most patients at this dose alkylating activity in the plasma was measurable for 24 hr. Sixty-eight \% of injected \textsuperscript{14}C label was excreted in urine. Breath and fecal excretion were negligible.

In a regimen of five consecutive daily cyclophosphamide administrations, cyclophosphamide half-life was shorter and peak alkylating levels were constantly higher on the 5th day than on the 1st day. Prior patient treatment with allopurinol resulted in significantly longer cyclophosphamide half-life, but concomitant prednisolone treatment had no effect. The effect of hepatic metastases on cyclophosphamide metabolism was unclear. Moderate renal failure in one patient resulted in prolonged retention of alkylating materials in plasma and severe toxicity.

Although patients with and without prior exposure to microsomal enzyme-inducing drugs demonstrated marked variation in plasma cyclophosphamide half-life and peak alkylating levels, the total concentration \times time product remained relatively constant for a given cyclophosphamide dose, suggesting that alterations in the rate of cyclophosphamide metabolism by drugs or liver metastases in the absence of renal failure will not change toxicity or therapeutic effect.

INTRODUCTION

Cyclophosphamide is an anticancer agent with antitumor effects against a wide variety of human neoplasms for which it is widely used (18). More recently, its immunosuppressive properties have been exploited successfully in the treatment of nonneoplastic diseases such as rheumatoid arthritis, lupus erythematosus, and Wegener's granulomatosis (19-21, 25). This drug is unique among antitumor drugs in that it requires activation to an alkylating metabolite by the liver. Several investigators have shown that activation occurs on hepatic microsomes and that microsomes from other organs and tumors are largely incapable of activating cyclophosphamide (4, 11, 15).

Hepatic microsomal drug metabolism may be altered by previous and simultaneous exposure to drugs, many of which are commonly used in the care of patients with cancer (7). Although the effects of prior drug therapy on cyclophosphamide activation have been studied in animals, there are few similar studies in man. Likewise, the effect of liver and renal disease, prior cyclophosphamide therapy, and pharmacological differences inherent in p.o. cyclophosphamide administration have not been the subject of most of the published reports. In an attempt to clarify the role of these and other variables, cyclophosphamide metabolism and distribution were studied in patients with the use of radiolabeled compound to follow intact drug levels and the NBP\textsuperscript{1} assay to determine levels of alkylating activity.

MATERIALS AND METHODS

Patients. Cyclophosphamide-\textsuperscript{14}C was administered i.v. 43 times and nonradioactive cyclophosphamide 37 times (i.v., 26; p.o., 11) to 26 patients who received cyclophosphamide as therapy for advanced cancers (non-Hodgkin's lymphoma, 12; carcinoma of ovary, 6; melanoma, 5; others, 3). Whenever possible, patients were studied while on no medication of any sort for in excess of 1 week; but, in most cases, clinical care required the administration of other drugs before and during study. If these drugs were known to affect microsomal metabolism in animals or man, the patients' data were considered separately and correlations were sought. Prophylactic antiemetics were not given.

Cyclophosphamide doses varied from 6.7 to 80 mg/kg body weight. After labeled drug administration, urine was collected for 1 to 4 days whenever possible, and stools were collected for 4 days in selected patients. Endogenous creatinine clearance and liver function tests were used to evaluate the clinical status of the kidneys and liver. Expired air samples were obtained at intervals over 4 days in 2 patients. After every cyclophosphamide administration, venous blood samples

\textsuperscript{1} The abbreviations used are: NBP, \textsuperscript{4}(4-nitrobenzyl)pyridine; CVP, cyclophosphamide (40 mg/sq m/day for 5 days, p.o. or i.v.); vincristine (1.4 mg/sq m), and prednisone (100 mg/sq m/day for 5 days p.o.); C \times T, concentration \times time product.

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were taken every 1 to 2 hr for 4 hr and then every 4 hr for up
to 24 hr in heparinized syringes, and they were centrifuged at
once at 4°C to obtain plasma. All samples were stored frozen at
-10°C, unless specified otherwise, and assays were done within
1 week of collection.

Preparation of Cyclophosphamide Dose. Cyclophosphamide
uniformly labeled with 14C on the bischloroethyl side chain
(specific activity, 9.6 Ci/mole) was obtained from Monsanto
Research Corporation, Dayton, Ohio [Lot 131-5(a)] under
contract with the National Cancer Institute, Bethesda, Md.,
and was prepared with sterile water in 5-ml vials by the
Radiopharmaceutical Service, National Institutes of Health,
Bethesda, Md. Vials remained frozen until use. Purity of
cyclophosphamide-14C was determined by ascending
chromatography on silica gel plates with the use of 100% ethanol.
An aliquot of every patient’s dose was also assayed
for purity. For determination of the amount of radioactive
nornitrogen mustard in the stock cyclophosphamide-14C, 2.0
ml of stock solution were acidified to pH 1.30 with HCl and
extracted twice with 5.0-ml volumes of chloroform. Then the
pH was adjusted to 7.50 with NaOH, and a 3rd chloroform
extraction was done. Radioactivity present in the 3rd
chloroform layer was presumed to be nornitrogen mustard or
its hydrolysis products on the basis of these solubility
characteristics.

Unlabeled cyclophosphamide for clinical use in 200- or
500-mg vials (Cytoxan; Mead-Johnson Laboratories,
Evansville, Ind.) was dissolved in appropriate diluent at a
dosage based on the patient’s actual weight. From 30 to 100
μCi of cyclophosphamide-14C were added, these were mixed,
and a weighed aliquot was removed for specific activity
determination. The drug was then administered i.v. in less than
10 min.

Assay for Cyclophosphamide. Two-ml aliquots of plasma or
urine were placed in 45-ml stoppered tubes with subsequent
addition of 2 mg unlabeled stock cyclophosphamide (in 0.2
ml) and 5.0 ml chloroform. (The pH of urine samples was
adjusted to 7.5 with 0.25 M NaOH, if necessary.) The tubes
were shaken mechanically for 15 min and centrifuged to
separate layers; the aqueous layer was discarded. Then 1.5 ml
of 0.05 M HCl were added, and the mixture was shaken for 15
min, centrifuged, and removed. Two ml of the remaining
chloroform layer were dried in counting vials at room
temperature and redissolved in 10 ml of dioxane counting
fluid. Plasma cyclophosphamide levels were plotted as
μmoles/ml on semilog paper, and the half-life and theoretical
zero-hr drug level were obtained. In all cases, the
cyclophosphamide levels on semilog plot were well fitted by a
straight line for at least 8 hr.

Protein Binding Studies. Determination of the fraction of
plasma cyclophosphamide bound to protein was done by
centrification in dialysis tubing, as described by Dixon and
Adamson (9) with the use of cyclophosphamide-14C after
extraction with 0.05 M HCl to remove impurities.
Radioactivity in the original plasma, the filtrate, and the
unfiltered residue within the tubing was determined by
addition of 0.5 ml of each directly to Aquasol scintillation
fluid (New England Nuclear, Boston, Mass.)

For determination of protein binding by urinary
metabolites, urine with high alkylating activity was obtained
from a patient after cyclophosphamide-14C administration,
adjusted to pH 7.5, and extracted exhaustively with
chloroform to remove cyclophosphamide and nornitrogen
mustard. The extracted urine was then added to 3 heparinized
plasma samples to achieve final alkylating activities
compable to those found in vivo, and quadruplicate aliquots
of each plasma were placed in dialysis tubing and handled as
above.

Protein binding of plasma alkylating activity was
determined by comparison of total and free alkylating levels in
4 patients, with correction for solute trapping and for
activation of cyclophosphamide during NBP assay (see below).

Assay for Plasma Alkylating Metabolites. For determination
of total plasma alkylating metabolite levels, plasma and ascites
samples were deproteinized before assay with perchloric acid
and KOH neutralization. When titrated water was added to
control plasma, 73.8% (range, 72.0 to 75.5%) of tritium label
was recovered after this deproteinization procedure. Apparent
plasma alkylating metabolite values of plasma samples assayed
after deproteinization were corrected by this factor. When
plasma from a patient who had received cyclophosphamide
was ultrafiltered to remove protein (see below) and then
deproteinized, there was no detectable destruction of
alkylating ability due to the perchloric acid deproteinization.

Alkylating activity was measured by reaction with NBP, as
described by Friedman and Boyer (12), except that ethyl
acetate volumes varied from 2 to 5 ml according to anticipated
quantity of alkylating activity to improve sensitivity. It was
presumed that the plasma alkylating metabolite values
determined by this method (with the correction for trapping in
protein precipitate) represented the total amount of plasma
alkylating metabolites, bound or unbound, in the patient’s
plasma.

To determine free plasma alkylating metabolite levels, 3- to
7-ml plasma (or ascites) samples were placed in
Centriflo ultrafiltration cones (Amicon Corporation,
Lexington, Mass.) and centrifuged at 1000 X g at 4°C for 1 to
15 hr. The resultant ultrafiltrate was assayed directly. The
filters passed 95% of plasma alkylating metabolite intact.
Because of the large volumes of plasma required at frequent
intervals for these assays, only free plasma alkylating
metabolite levels were measured in most patients. Urine
alkylating levels were measured by the NBP reaction directly.

Radiolabel Excretion Assays. Radiolabel content of stool
was determined by combustion with collection of 14CO2 in
modified Cardinal solution (Burdick and Jackson Laboratories,
Muskegon, Mich.) (22, 23). Total urine 14C content was
determined by pipetting 0.50 ml of urine directly into 15 ml
Aquasol counting fluid. Timed 1-min breath collections in
Douglas bags were obtained at frequent intervals on the 1st
day of drug administration and daily thereafter for 4 days. The
breath was then aspirated out of the bag through a 14CO2 trap
containing 20 ml of the modified Cardinal solution. The
breath bubbled through the solution from a fine pipet at a rate
that averaged 300 ml/min. For determination of the efficiency
of trapping, 5 breath samples were aspirated through 2 such
14CO2 traps in succession; 93.9% (range, 92.8 to 96.5%) of
total counts were contained in the 1st trap.

February 1973
Scintillation Counting. Liquid scintillation counting was done with a Packard Tri-Carb Model 4322 liquid scintillation counter. Quench correction curves were prepared for each scintillation medium with the use of chloroform, nitromethane, plasma, and/or urine for quenching. Counting efficiency for dioxane and Aquasol counting solutions approached 80%, and for the combustion method, it approached 70%.

Variation in results is expressed as ±1 S.D. with range in parentheses. Test for significance of differences was done with the Student t test.

RESULTS

Purity of Cyclophosphamide-\textsuperscript{14}C. On chromatography in 100% ethanol cyclophosphamide migrated as a single spot (R\textsubscript{F}, 0.67) containing 86.4% of spotted radioactivity. Assay of an aliquot of each patient’s dose by chloroform extraction yielded an average purity of 87.1 ± 5.6%. Cyclophosphamide specific activity was corrected for this impurity. Nornitrogen mustard or its hydrolysis products composed 2.5% (range, 1.3 to 3.3%) of total radioactivity.

Cyclophosphamide and Plasma Alkylating Metabolite Assays. Assay of plasma containing known additions of cyclophosphamide-\textsuperscript{14}C yielded 94.0% (range, 91.4 to 97.3%) recovery by this method. Chromatography of saline solutions of cyclophosphamide-\textsuperscript{14}C and of patient’s urine, before and after chloroform extraction, showed complete removal by chloroform of the spot corresponding to cyclophosphamide and appearance of this spot in the chromatograms of the chloroform layers. Other radioactive and NBP-reactive spots in the chromatograms of patient’s urine were not affected by chloroform extraction. Levels of radioactivity in patient’s plasma were too low to be analyzed by radiochromatography.

With weighed standards, the molar extinction coefficients of the alkylated NBP molecule after alkalinization and ethyl acetate extraction were found to be 3.38 × 10\textsuperscript{4} (range, 3.32 to 3.45 × 10\textsuperscript{4}) cm/mole for nitrogen mustard and 3.42 × 10\textsuperscript{4} (range, 3.37 to 3.47 × 10\textsuperscript{4}) sq cm/mole for nornitrogen mustard and were presumed to be identical. Intact cyclophosphamide reacted with NBP to the extent of 0.6 to 1.0%, probably due to hydrolysis to nornitrogen mustard during assay. Deproteinization and assay of intact cyclophosphamide solutions yielded absorbance equivalent to 1.6% (range, 1.4 to 1.7%) of added drug.

Protein Binding of Cyclophosphamide, Plasma Alkylating Metabolite, and Urinary Metabolites. At plasma cyclophosphamide-\textsuperscript{14}C concentrations of 10 and 200 \textmu mmoles/ml, protein bindings were 14 ± 2.5% and 12 ± 5%, respectively. Urinary cyclophosphamide metabolites at concentrations of 2.3 to 110 \textmu mmoles/ml were not detectably protein bound. Following 5 cyclophosphamide doses of 40 mg/kg to 4 patients, plasma was assayed for both total and free plasma alkylating metabolite levels. At free plasma alkylating metabolite levels of 5.7 to 27 \textmu mmoles/ml, metabolite was bound 56 ± 4% (range, 52 to 61%) to plasma proteins.
### Table 1

_Data from 43 patient studies of cyclophosphamide-\(^1\)\(^*\)C_

<table>
<thead>
<tr>
<th>Patient</th>
<th>Other medications(^a) Before study</th>
<th>During study</th>
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<th>Alkylation activity</th>
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<td></td>
<td>Dose (mg/kg)</td>
<td>Half-life (hr)</td>
<td>% in urine(^b)</td>
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<td>17.4</td>
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<td>14.5</td>
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<td>10.9</td>
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<td>3.5</td>
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<td>18.1</td>
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<td>18.7</td>
</tr>
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<td>4.7</td>
<td>9.8</td>
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<td>-</td>
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<td>40f</td>
<td>7.2</td>
<td>11.2</td>
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<tr>
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<td>4.0</td>
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</table>

\(^a\) Drug and dosage in mg/day.  
\(^b\) Percentage of total cyclophosphamide dose appearing in urine in 1st 24 hr.  
\(^c\) Liver dysfunction at time of study (see text).  
\(^d\) –, datum not available. Data from other studies with nonradioactive cyclophosphamide are not shown.  
\(^e\) Calculated from total plasma alkylating metabolite levels assuming 56% plasma binding.  
\(^f\) Received 2nd dose of cyclophosphamide 12 to 24 hr later. Only data from 1st dose considered in this report.  
\(^g\) Receiving CVP (1) regimen. Actual cyclophosphamide dose, 400 mg/sq m.
Chart 1. Plasma levels of cyclophosphamide and of free alkylating activity in patients following injection of cyclophosphamide-14C on Day 1 and again on Day 5 of a daily i.v. cyclophosphamide regimen. Prednisone, 100 mg/sq m/day was given simultaneously with each dose of cyclophosphamide.

to 80% of peak plasma levels and subsequently declined more slowly than did plasma levels.

These patients without prior drug exposure excreted 16.0 ± 5% (8 to 23%) of injected cyclophosphamide as alkylating activity in the urine in 24 hr, without correlation with endogenous creatinine clearance or total dose of cyclophosphamide.

Excretion of Radiolabel. Following cyclophosphamide-14C administration, an average of 62 ± 10% (range, 41 to 82%) of administered 14C appeared in the urine in 2 days, and 68 ± 11% (59 to 82%) appeared in 4 days. A further 1.1% of the dose appeared in urine from the 4th to the 10th day after therapy in the 1 patient thus studied. Six patients had 4-day stool collections, excreting 1.8 ± 0.53% (range, 0.92 to 2.5%) of 14C label by this route. In 2 patients, 4-day breath collections yielded 0.9 and 1.4% of administered 14C label, respectively. Excretion of radiolabel by all routes was maximal in the 1st 24 hr after administration and fell rapidly thereafter.

Effect of Prior or Concomitant Drug Exposure. Nine patients with non-Hodgkin’s lymphomas were treated with a combination drug regimen, CVP (1). In 3 of these patients, who had received no medication for over 2 weeks, the regimen was modified, so that cyclophosphamide only was given in the above dosage daily for 5 days as a single morning injection or as a p.o. dose. Labeled drug was given on the 1st and 3rd or 5th days, and no other drugs were allowed during the study. (Vincristine and prednisone were given on the day after completion of study.) Plasma free plasma alkylating metabolite levels were measured daily. In these 3 patients, plasma cyclophosphamide half-life averaged 6.1 ± 2 hr on the 1st day, and the half-life decreased by an average of 31 ± 11% when remeasured on the 3rd or 5th day. Peak plasma alkylating metabolite levels averaged 2.6 ± 1.6 μmoles/ml on the 1st day and increased by an average of 73 ± 33% by the 3rd day of therapy, without significant change thereafter. This increase in peak plasma alkylation metabolite levels was not due to accumulation of plasma alkylation metabolite from the doses of the previous day, since in most patients there was no detectable plasma alkylation metabolite 24 hr after each dose; and in the few in which plasma alkylation metabolite was detectable, the difference in base line levels could not wholly account for the higher peak levels on Days 3 to 5 (of Chart 1).

Five patients were treated with the complete CVP regimen, receiving p.o. prednisone daily, simultaneously with each cyclophosphamide dose and other medications were withheld. Three of these patients had received no previous medications for over 2 weeks, and 2 patients had received only allopurinol, 300 mg/day for 5 to 10 days. In these 5 patients, plasma cyclophosphamide half-life averaged 6.4 ± 1 hr and decreased by 41 ± 9% on the 5th day of therapy. Peak plasma alkylation metabolite levels rose by 96 ± 30% by the 3rd day of therapy and did not change thereafter. These changes in cyclophosphamide half-life and plasma alkylation metabolite with 5 days of combined cyclophosphamide and prednisone therapy are not significantly different from those changes found with 5 days of cyclophosphamide alone.

Chart 2. Plasma peak free alkylation activity (μmoles/ml) compared to plasma cyclophosphamide half-life (hr) in 12 patients with no prior drug exposure for over 1 week. Each point represents both the cyclophosphamide half-life and peak alkylation activity measured during a single cyclophosphamide-14C study. Actual peak alkylation levels were multiplied by 40/[actual cyclophosphamide dose (mg/kg)] to allow comparison of alkylation levels after different cyclophosphamide doses. In 6 of these patients, after 3 to 5 days of therapy (arrows) with cyclophosphamide or cyclophosphamide and prednisoone, cyclophosphamide half-life is shorter and peak alkylation levels are higher.
For determination of whether large doses of synthetic steroids interfered with cyclophosphamide metabolism, 4 patients were given identical cyclophosphamide-\(^{14}\)C doses during each of 2 studies, separated by 2 to 30 days, special precautions being taken to ensure that all patients were receiving the same other medications before and during each administration. At the time of the 2nd study in 3 patients, 1 g of prednisolone was given rapidly i.v., followed in 2 min by the cyclophosphamide-\(^{14}\)C dose (6.8, 12, or 40 mg/kg). The 4th patient was given 1 g of prednisolone divided into 5 boluses, 2 hr apart. In each of the 1st 3 patients, plasma cyclophosphamide half-life with prednisolone administration differed by less than 2% from the same patient's earlier control study; in R. S., the half-life decreased by 30%. Peak free plasma alkylating metabolite levels were 15 ± 13% higher with prednisolone administration. The differences are not statistically significant.

Four patients received allopurinol in full dosage for 3 to 10 days prior to cyclophosphamide-\(^{14}\)C therapy and during this time received no other medications known to affect microsomal function. Plasma cyclophosphamide half-life in these patients was 8.1 ± 1.4 hr, significantly longer than in patients without prior medication (\(p < 0.01\)). However, the fraction of administered doses appearing as intact cyclophosphamide in the urine (8.8 ± 7%) was not different from controls, when the patients' creatinine clearances are considered. Due to technical error, plasma free plasma alkylating metabolite levels are available only on 2 patients. One patient treated at 9.5 mg/kg had a peak level of 2.7 \(\mu\)mole/ml (versus 3.2 in control patients) and 1 patient had peak levels of 26 \(\mu\)mole/ml, which is appropriate for his dose of 60 mg/kg. The fraction of injected cyclophosphamide appearing as alkylating metabolites in urine in 24 hr was 17 ± 5%, identical to the value for controls.

Other medications received by patients during or before cyclophosphamide therapy are shown in Table 1. Insufficient studies were done with pretreatment with other drugs to reach firm conclusions regarding their effect on cyclophosphamide metabolism. However, if one considers all studies performed following patient exposure to drugs thought to stimulate microsomal activity (i.e., barbiturates, diphenylhydantoin, cyclophosphamide, and prednisone), the peak plasma alkylating metabolite levels were 70% higher than in the same or similar patients without prior drug exposure, but the 8-hr plasma alkylating metabolite levels showed a more rapid decline (to 44 ± 20% of peak) than in unexposed patients (to 77 ± 15% of peak) (\(p < 0.01\)).

In 9 patients receiving a single administration of cyclophosphamide, with or without prior drug exposure, the plasma alkylating metabolite C \(\times\) T could be calculated with reasonable accuracy. The average value following a dose of 40 mg/sq m was 139 ± 50 \(\mu\)mole hr, and after 80 mg/kg it was 282 ± 211 \(\mu\)mole hr. There was no correlation of C \(\times\) T with cyclophosphamide half-life (over the range of 1.8 to 8.2 hr) or with prior drug exposure at either dose level.

Cyclophosphamide Therapy p.o. In 6 patients, the cyclophosphamide doses of the CVP regimen was given i.v. on Days 1, 3, and 5 and p.o. [as 50-mg tablets (Mead-Johnson)] on Days 2 and 4. Peak plasma alkylating metabolite levels were determined on every day. The expected rise in peak plasma alkylating metabolite levels from Day 1 to Day 3 was observed, and in every case the Day 2 (p.o.) level was intermediate between Day 1 and Day 3 (i.v.) levels. After p.o. dosage on Day 4, peak plasma alkylating metabolite levels were essentially identical to those of Days 3 and 5 (i.v.). Peak levels occurred 1 to 3 hr after p.o. administration and declined at the same rate as after i.v. administration.

Liver and Renal Disease. Patient D. V., with Hodgkin's disease, filling defects on liver scan, jaundice, and markedly elevated alkaline phosphatase, had the longest cyclophosphamide half-life (8.4 hr) and lowest peak plasma alkylating metabolite level (4.2) of any patient treated with cyclophosphamide, 40 mg/kg. The data were insufficient to calculate his C \(\times\) T. Patient K. C. had widespread hepatic metastases on liver scan, but liver function tests were normal. She had a normal cyclophosphamide half-life (7.0 hr), but the 2nd lowest plasma alkylating metabolite peak (4.8 \(\mu\)mole/ml) and lowest C \(\times\) T (95 \(\mu\)mole/hr) of patients receiving 40 mg/kg. Urinary excretion of intact cyclophosphamide and alkylating activity was not different from controls. Patient M. F. had severe jaundice and elevated alkaline phosphatase levels, presumed to be due to extrahepatic duct obstruction with ovarian carcinoma, since a liver scan was normal. Her cyclophosphamide half-life was normal (6.3 hr) and free plasma alkylating metabolite levels (27 \(\mu\)mole/ml) and C \(\times\) T (600 \(\mu\)mole/hr) were the highest found at the 80-mg/kg dose level. Incontinence prevented evaluation of her urinary metabolite excretion. Only 1 patient (A. S.) had severely depressed renal function (due to renal invasion with lymphocytic lymphoma). She was treated with full doses of CVP (cyclophosphamide, 9.7 mg/kg, for 5 days) while her creatinine clearance was 18 ml/min. Initial plasma cyclophosphamide half-life was 3.1 hr (possibly due to the patient's many prior medications since her \(t_1/2\) was 6.0 hr when she was restudied later while on no medication). Urinary cyclophosphamide excretion was appropriate for her creatinine clearance. Peak free plasma alkylating metabolite levels (\(\mu\)mole/ml) were: Day 1, 7.8, Day 2, 12.5; Day 3, 17, and Day 5, 23. The plasma alkylating metabolite level 24 hr after Day 1 therapy was 0.5 \(\mu\)mole/ml and after Day 2 it was 2.2 \(\mu\)mole/ml. Unfortunately, 24-hr level assays were not obtained after later doses. Of 7 other patients assayed, who received 9 to 11 mg/kg of drug, and in 2 repeat studies of Patient A. S. after renal function had improved, none had detectable plasma alkylating metabolite remaining 24 hr later.

**DISCUSSION**

The metabolic pathway for the activation of cyclophosphamide, as proposed by Hill et al. (15), is shown in Chart 3. "Aldophosphamide" inhibited growth of cultured human epidermoid carcinoma No. 2 cells by 50%, at a drug level of 0.2 g/ml (0.7 \(\mu\)mole/ml) and is thought to be the active metabolite of cyclophosphamide (15, 26). Subsequently, aldophosphamide may be oxidized in the liver by the nonmicrosomal enzyme, aldehyde oxidase, to "carboxyphosphamide," which is the major metabolite...
as being inducers of microsomal enzymes. A correlation of studies were receiving medications that were not appreciated. HOOC-CH₂-CH₂-O-P-O-N(CH₂CH₂Cl)₂ Aldophosphamide

Chart 3. Metabolic pathway of cyclophosphamide leading to formation of the active alkylating metabolite, aldophosphamide, and its subsequent oxidation to carboxyphosphamide, as proposed by Hill et al. (15).

appearing in the urine (2, 27). Carboxyphosphamide inhibited human epidermoid carcinoma No. 2 cells by 58% at 1.0 g/ml (4 μmole/μl) (27). Metabolism of cyclophosphamide appears to be qualitatively identical in man and animal.

Aldophosphamide, carboxyphosphamide, and nonnitrogen mustard all alkylate NBP (15, 27). However, Hohorst et al. (16) have reported that the cyclophosphamide metabolite with the highest serum levels is uncharged and gives aldehyde reactions. This is compatible with aldophosphamide. Other investigators have shown that, following cyclophosphamide therapy in humans, plasma contains substances capable of completely inhibiting Ehrlich ascites cell cultures and that peak inhibition occurred 2 hr after therapy (28), which is the time when we have most consistently found peak plasma alkylating metabolite levels on NBP assay. Therefore, we feel that plasma alkylating levels, as measured by the NBP assay, give a good indication of the levels of toxic and tumoricidal metabolites resulting from cyclophosphamide administration.

We found negligible cyclophosphamide binding to plasma proteins, as has been reported (6). The half-life of intact plasma cyclophosphamide that we found in 12 patients without prior exposure to drugs capable of inducing microsomal enzymes is longer than previously reported from this laboratory (6.45 versus 5.3 hr) (8). Mellett et al. (20) have described a cyclophosphamide half-life of 4.1 hr in cancer patients. It is possible that patients in some of these earlier studies were receiving medications that were not appreciated as being inducers of microsomal enzymes. A corollary of plasma cyclophosphamide half-life with drug dosage was not seen in this study. The maximal initial cyclophosphamide metabolic rate found in this study (average, 486 mg/hr after 80-mg/kg dose) is similar to that predicted from direct assay of human liver microsomes (440 mg/hr) (3). The fraction of intact cyclophosphamide appearing in urine was also independent of drug dosage.

Peak total plasma alkylating metabolite levels in control patients receiving cyclophosphamide, 40 mg/kg, averaged 22.5 μmole/μl, which is lower than the average levels of 47 μmole/μl found by Brock (3) after 60-mg/kg doses, even when the data are adjusted for the difference in dosage. However, it is not stated whether Brock's patients were receiving enzyme-inducing drugs.

Our previous studies of patients with Burkitt's lymphoma in Africa and the United States did not show acceleration of cyclophosphamide metabolism after heavy pretreatment with phenobarbital (8), but Mellett et al. (20) reported shortening of cyclophosphamide half-life from 4.1 to 1.6 hr after 2 weeks of phenobarbital therapy. Two studies with single or multiple doses of cyclophosphamide in rats have suggested that cyclophosphamide itself causes a decrease in microsomal oxidative functions for up to 21 days, which might be expected to inhibit subsequent metabolism of cyclophosphamide (10, 29). However, Sladek found no such inhibition (24). Our data show that, in fact, cyclophosphamide in the doses used here stimulated its own metabolism within the 1st 2 days of consecutive daily therapy and then continued to be metabolized more rapidly until at least the 5th day of treatment. Low-dose daily cyclophosphamide therapy (50 to 200 mg/day) has not been studied and enhanced metabolism may not necessarily occur. Two animal studies have suggested that simultaneous treatment with prednisone inhibits cyclophosphamide metabolism (14, 15), but this has been rebutted by others (13). In our present study, massive single doses of prednisolone just before cyclophosphamide administration did not inhibit cyclophosphamide metabolism. However, we have not studied the effect of prolonged pretreatment with steroids.

Allopurinol has become an essential drug in the prevention of hyperuricemia during therapy of drug-sensitive cancers and has been reported to inhibit microsomal metabolism of other drugs in man, when given for several days prior to testing (30). We found that, although allopurinol pretreatment resulted in longer cyclophosphamide half-life, plasma alkylating activity and urinary metabolite and cyclophosphamide excretion were unchanged. Also allopurinol pretreatment did not prevent self-induction of more rapid metabolism by cyclophosphamide.

Our observations suggest that prior exposure to drugs with an inducing or repressing effect on microsomal oxidase should have little effect on the antitumor and toxic effects of cyclophosphamide. In patients having received inducing drugs, we found higher peak plasma alkylating metabolite levels, which declined more rapidly, and the plasma alkylating metabolite C×T factor, when calculable after a given drug dosage, did not differ significantly over a 4-fold range of cyclophosphamide half-lives in several patients. Since intact cyclophosphamide excretion is slow, it is probable that patients with even greatly prolonged drug half-lives would still be able to metabolize a large fraction of administered drug. Thus, it would appear that cyclophosphamide-alkylating metabolite therapeutic effect and toxicity should be a function of administered dose only and should be largely independent of the rate of metabolic activation. Sladek (24) has made similar observations in rats. Drugs that inhibit aldehyde oxidase, such as chlorpromazine, might be expected to inhibit a aldophosphamide metabolism, increase the C×T product, and result in increased toxicity (17). However, our 1 patient given large doses of the related drug, prochlorperazine, did not have a higher C×T product than other patients.
receiving the same cyclophosphamide dose.

Several investigators have shown that the presence of transplanted tumors in s.c. and i.m. sites results in diminution of microsomal enzyme activity (5, 24). Although we have only 12 non-drug-exposed patients, those with considerable tumor bulk appeared to metabolize cyclophosphamide as rapidly as those with minimal tumor. Our 1 patient with renal function impairment had accelerated cyclophosphamide metabolism on her 1st day of therapy, probably due to prior exposure to a large number of drugs. She subsequently manifested much higher peak plasma alkylating metabolite levels than were seen in any other patient at that dose level. She was the only large number of drugs. She subsequently manifested much her 1st day of therapy, probably due to prior exposure to a impairment had accelerated Cyclophosphamide metabolism on those with minimal tumor. Our 1 patient with renal function aldophosphamide, prolonged carboxyphosphamide exposure alkylating activity from 8 to 24 hr. Although less active than have a rapid rise and fall in plasma aldophosphamide but that Cyclophosphamide half-life. It is possible that in fact she did have a rapid rise and fall in plasma aldophosphamide but that carboxyphosphamide was retained in plasma for a prolonged period due to renal failure and contributed most or all of the alkylating activity from 8 to 24 hr. Although less active than aldophosphamide, prolonged carboxyphosphamide exposure to the marrow may have caused the unusually severe hematological toxicity that this patient endured for the 4 weeks following therapy. Until further studies can be done on the effect of renal failure on cyclophosphamide metabolism, such patients should receive the drug in low initial dosage with close observation for toxicity.

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Clinical Pharmacology of Cyclophosphamide

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