

# Preliminary Studies on the Distribution and Fate of TEM, TEPA, and Myleran in the Human\*

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The clinical use of alkylating drugs represents an important contribution toward the chemotherapeutic management of certain forms of human cancer. Several recent reports summarize the palliative responses obtained with triethylenemelamine (TEM), triethylene phosphoramidate (TEPA), and Myleran (Busulfan) in patients with malignant neoplasias of lymphatic or myelogenous origin (2, 3, 11, 12). The clinician is interested in knowing the fate of drugs administered to the patient and length of time for which the drugs circulate in the blood as a guide in determining adequate dosage. Few studies concerning the metabolic fate of alkylating agents in the human have been reported previously. Maller *et al.* reported the first detailed study on the distribution and metabolism of radioactive N-(3-oxapentamethylene)-N', N''-diethylenethiophosphoramidate (OPSPA-C<sup>14</sup> and OPSPA-P<sup>32</sup>) in the human (6, 7).

It seemed pertinent to study the distribution, metabolic alterations, and eventual excretory fate of these drugs following administration to cancer patients. The purpose of this study was to measure the concentrations in blood, rates of disappearance of drugs, and the urinary excretion following drug administration. A comparison of the excretory patterns following an oral as against intravenous administration was of interest, since such information might be of value in improving existing therapeutic regimens.

The high toxicity of alkylating drugs to the hematopoietic system limits the amounts which may be administered with safety. Furthermore,

\* Aided by grants from the National Cancer Institute, National Institutes of Health, a grant from the Damon Runyon Memorial Fund for Cancer Research (DRIR-42), and from the American Cancer Society (T-14). A preliminary report has been presented and published in *Proc. Am. Assoc. Cancer Research*, Vol. 2, 1957.

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Received for publication January 20, 1959.

physicochemical methods for the analysis of these drugs or their probable metabolites are inadequate to detect such minute amounts as are present under pharmacologic experimentation in mammals. Therefore, radioisotopic tracer technics were employed in the present experiments. The molecular structures of the drugs studied and the type of the isotopic label employed are shown in Chart 1.

The chemical structure of each of these drugs consists of two distinct portions: (a) the "alkylating" moiety and (b) the "carrier" moiety to which the alkylating portion of molecule is attached. It should be noted that in TEPA-P<sup>32</sup> and in Myleran-S<sup>35</sup> the isotopic element is present in the "carrier" portion, while TEM-C<sup>14</sup> and Myleran-C<sup>14</sup> contained the C<sup>14</sup> label in the alkylating portion of the molecule with no label on the triazine or dimethanesulfonyl "carrier" group.

## MATERIALS AND METHODS

*Radioactive drugs.*—Tris(1-aziridinyl-C<sup>14</sup>)-s-triazine (TEM-C<sup>14</sup>), methanesulfonic acid, tetramethylene 1,4-C<sup>14</sup>-ester (Myleran-C<sup>14</sup>), and methanesulfonic-S<sup>35</sup>-acid, tetramethylene ester (Myleran-S<sup>35</sup>), were synthesized in this laboratory by methods previously described in literature with modifications to suit preparation on a semimicro scale (4, TEM; 13, Myleran). Tris-(1-aziridinyl) phosphine-P<sup>32</sup> oxide (TEPA-P<sup>32</sup>) was obtained from the research laboratories of the Calco Division, American Cyanamid Company.<sup>1</sup> The chemical and radio-purity of each of the four drugs was established by paper chromatography. The specific activities of the drugs were as follows: TEPA-P<sup>32</sup>, 40/ $\mu$ c/mg; TEM-C<sup>14</sup>, 1.2  $\mu$ c/mg.; Myleran-S<sup>35</sup>, 10  $\mu$ c/mg.; Myleran-C<sup>14</sup>, 4.0  $\mu$ c/mg.

*Clinical material.*—Subjects of this study were selected patients from the Warwick Memorial Cancer Clinic and the George Washington University Hospital, Washington, D.C.<sup>2</sup>

Case histories of individual patients are not described, since they are not essential for purposes of this report. In total, 21 separate experiments were made on twelve patients. The diagnoses included three patients with lymphosarcomas, two with Hodgkin's disease, two with chronic myelogenous leukemia, three with adenocarcinoma of the breast, one with reticulum-cell sarcoma, and one with epidermoid carcinoma

<sup>1</sup> Courtesy Dr. R. P. Parker.

<sup>2</sup> We are indebted to Dr. Louis K. Alpert and his staff for their cooperation and help during the conduct of these experiments.

of the pharynx. There was no evidence of gross dysfunction of the kidney or liver in any of the subjects. Most patients had been previously treated with one or more alkylating drugs, and a few had received x-ray therapy as well. Patients were ambulatory and capable of visiting the out-patient clinic for routine check-up and maintenance therapy. Most of the patients were hospitalized for the purposes of these studies.

**Experimental procedure.**—Breakfast was withheld from the patient on the day of experiment. Each radioactive drug was administered as a single dose and at a level which is generally accepted as its therapeutic range for a single dose for humans (5). Thus, TEPA was administered at a total single dose of 5–10 mg., TEM at 2–3 mg., and Myleran at 3–7 mg./patient. Only one drug was given to a patient at any one time. For oral administration it was diluted with dextrose for bulk and given in a gelatin capsule. For intravenous

excreted radioactivity computed from aliquot assay. Separation and identification of urinary metabolites were achieved by the application of various analytical technics including paper chromatography in various solvent systems, ion exchange chromatography, and inverse isotope dilution.

**Radioactivity measurements.**—Radioactive phosphorus was counted by using a Geiger-Müller tube (Tracerlab Type-TGC-2 window thickness 1.8 mg/sq cm) attached to a Radiation Counter Laboratories scaler (Mark 13 Model 1). A windowless, gas flow-type proportional counter (RCL, Chicago) was employed for C<sup>14</sup> and S<sup>35</sup> counting. Aliquots of samples to be counted (plasma, urine, or tissue homogenates) were plated uniformly on aluminum discs in duplicate, and the radioactivity of the sample was determined at constant geometry for sufficiently long periods to achieve less than 5 per cent variation. Corrections were made for self-absorption and radioactive decay. From these data the proportion of radioactivity in blood and urine and the uptake of radioactivity per gram of wet tissue were computed. These values were expressed as the percentage of the injected radioactivity. The latter was determined from standard plates from an aliquot of the solution employed for injection.

## RESULTS

For brevity in presentation no attempt is made here to record individual dosages and observed

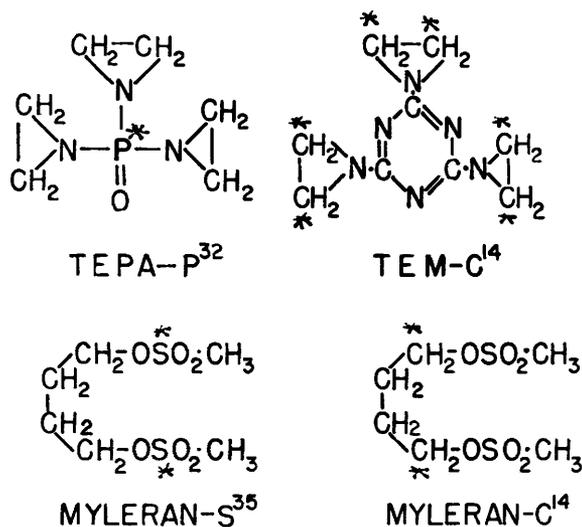


CHART 1.—Chemical structures of TEM, TEPA, and Myleran indicating location of radio-isotopic element.

administration TEM was dissolved in physiological saline, while Myleran was dissolved in a small volume of acetone and then diluted with 10 volumes of saline and injected immediately into the antecubital vein. In comparisons of the oral against the intravenous route, the patient usually served as his own control. The drug was given to the patient by the alternate route after a prolonged period without isotope administration.

**Blood concentrations.**—The blood concentrations were followed over a period of 48 hours following drug administration. Beginning immediately after injection, several 5-ml. blood samples were withdrawn at predetermined times. Plasma was separated by centrifugation at 2000 r.p.m., the cells were washed twice with saline and resuspended in a known volume of physiological saline solution. Aliquots of plasma and red cells were assayed for radioactivity by usual procedures.

**Tissue distribution.**—In general, the specific activity of the drugs was too low to achieve measurable levels of radioactivity in the tissues. Because of the relatively high specific activity of TEPA-P<sup>32</sup>, in three patients, two with lymphosarcomas and one with Hodgkin's disease, it was feasible to measure the radioactive uptake of the enlarged cervical or axillary lymph nodes 24 hours after administration. For comparison with a nonspecific tissue, muscle biopsy was made at the same time, and the relative specific activities were computed.

**Urinary metabolites.**—Urinary output was collected at 6, 12, 24, and 48 hours following drug administration and the

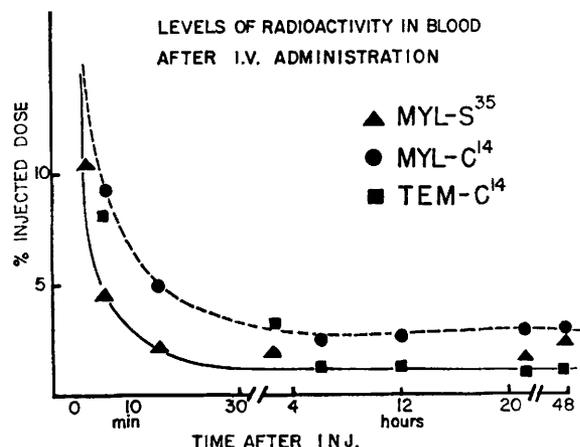


CHART 2.—Composite curves showing proportions of injected radioactivity circulating in total blood of patients at various intervals following intravenous administration of radioactive TEM, TEPA, or Myleran.

values in each separate experiment. Charts 2–5 represent composite curves obtained from observations in individual patients. Variations among the subjects were minor and of an order which may be expected in experiments of this type. The range of dosage in terms of radioactivity may be computed from the dose level administered to the patient and the specific activity of the drugs mentioned earlier.

**Blood concentrations.**—Chart 2 shows the proportions of total injected radioactivity circulating in the total blood at various intervals following intravenous administration of the drugs. The disappearance of radioactivity from the blood was

rapid, and between 90 and 95 per cent of the drug was removed from the circulating blood within 3–5 minutes after injection. After this initial fall, however, a steady low level of radioactivity of the order of 1–3 per cent of the injected dose was maintained for the rest of the 48-hour period of observation. Chart 3 shows corresponding values when the drugs were administered orally in a capsule. Under these conditions an initial lag of approximately  $\frac{1}{2}$ –2 hours was observed before measurable blood levels could be obtained. However, at periods longer than four hours the circulating activity was comparable to that obtained by the intravenous route. In Charts 2 and 3, the dotted and the continuous lines indicate the maximum and the minimum limits of radioactivity levels circulating in the blood following administration of the drugs. With TEM-C<sup>14</sup>, Myleran-C<sup>14</sup>, and Myleran-S<sup>35</sup>, between 88 and 93 per cent of the total blood radioactivity was associated with the plasma, and this relative distri-

drop in plasma radioactivity in patients given TEPA-P<sup>32</sup> by the oral route.

In three patients (two with lymphosarcomas and one with Hodgkin's disease) a biopsy of the metastatically involved lymph node and of muscle in proximity was made 24 hours after TEPA-P<sup>32</sup> dose. The relative specific activities of the tissues are shown in Table 1. In each

LEVELS OF RADIOACTIVITY IN BLOOD AFTER ORAL ADMINISTRATION

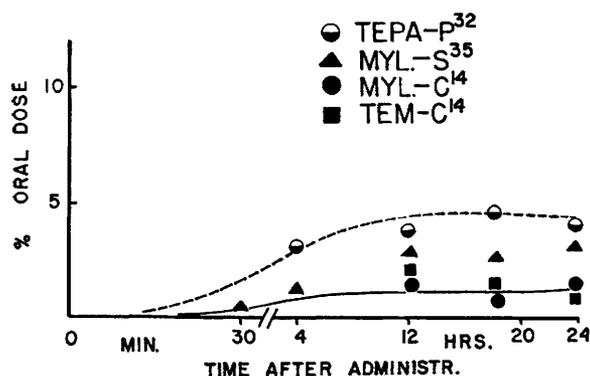


CHART 3.—Composite curves showing proportions of injected radioactivity circulating in total blood of patients at various intervals following oral administration of radioactive TEM, TEPA, or Myleran.

bution between plasma and washed red cells remained constant over a period of 48 hours. Only blood samples obtained within 5 minutes after intravenous injections contained enough radioactivity to estimate further the location of the label. Over 90 per cent of the radioactivity could be precipitated by additions of acetone to a final concentration of 80 per cent, indicating that most of the plasma radioactivity was associated with the protein fraction. In the case of TEPA-P<sup>32</sup>, however, the proportion of radioactivity in plasma decreased with the time of sample withdrawal, indicating a general transfer of P<sup>32</sup> from the plasma to the red cells. Chart 4 shows the relative marked

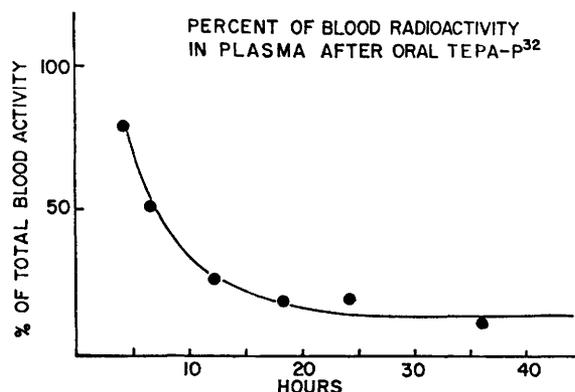


CHART 4.—Proportion of radioactivity in whole blood associated with the plasma fraction at various intervals after oral administration of TEPA-P<sup>32</sup>.

TOTAL URINARY EXCRETION OF RADIOACTIVITY 24 AND 48 HRS. AFTER DOSE

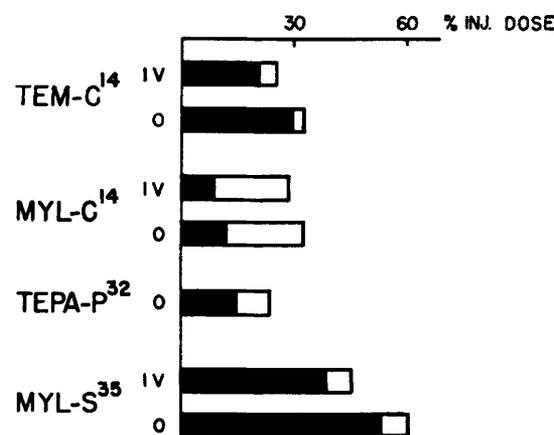


CHART 5.—Average cumulative urinary excretion of radioactivity 24 and 48 hours following intravenous or oral administration of radioactive TEM, TEPA, or Myleran. The dark portion of the bar indicates excretion during the first 24 hours.

case the metastatic node was of a higher specific activity than that of the nonproliferating tissue.

*Urinary excretion.*—The average cumulative 24- and 48-hour urinary excretions of radioactivity following intravenous or oral administration of the labeled drugs are illustrated in Chart 5, the dark portions of the bar indicating excretion during the first 24 hours. With TEPA-P<sup>32</sup>, TEM-C<sup>14</sup>

and Myleran-C<sup>14</sup>, the average urinary excretion was of the order of 25–30 per cent of the injected dose. Radioactivity from Myleran-S<sup>35</sup>, however, was excreted to the extent of 45–60 per cent. No marked differences were evident between the total excretions at 24- or 48-hour periods whether the drugs were given orally or intravenously. TEM-C<sup>14</sup> and Myleran-S<sup>35</sup> were metabolized more rapidly during the first 24 hours than in the second 24-hour period. The quantitative difference between the excretions of sulfur-labeled and carbon-labeled Myleran suggested a more rapid elimination of the sulfonoxymethyl moieties as compared with the four-carbon chain.

*Urinary metabolites.*—Lyophilized urine samples from various experiments were subjected to paper and ion exchange chromatography procedures for

as an organic phosphorus compound assumed to be an intermediate between TEPA and inorganic phosphate. No TEPA was excreted unchanged.

*Metabolite of Myleran-S<sup>35</sup>.*—A single radioactive spot was obtained on a Whatman No. 1 paper chromatogram in each of two solvent systems.

#### URINARY METABOLITES OF:

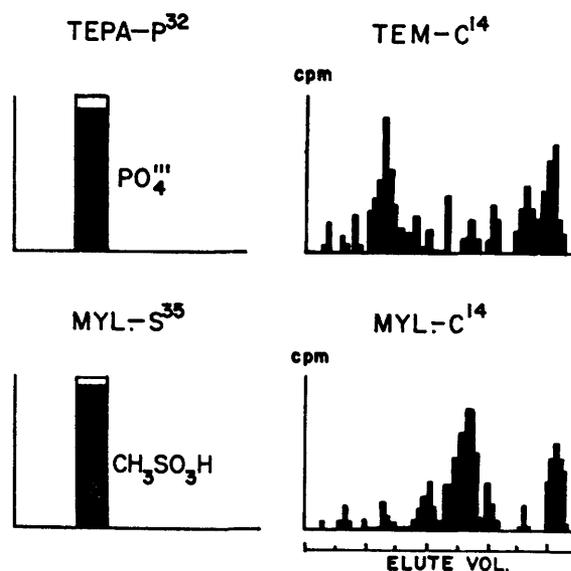


CHART 6.—Relative number and proportions of radioactive components excreted in the urine of patients administered radioactive TEM, TEPA, or Myleran.

TABLE 1  
SPECIFIC ACTIVITIES OF LYMPH NODE AND  
MUSCLE AT BIOPSY 24 HOURS AFTER  
ORAL DOSE OF TEPA-P<sup>32</sup>

Total dose (counts/min)	Lymph node (counts/min/ gm wet wt)	Muscle (counts/min/ gm wet wt)
1.07×10 <sup>6</sup>	140	0
2.68×10 <sup>6</sup>	537	118
3.50×10 <sup>6</sup>	1620	90

identification of metabolites. Whenever the mixture of metabolites could be resolved and separated into two or more major metabolites, attempts were made to identify the compounds by inverse isotope dilution. In case the paper chromatogram resulted in continuous smears of radioactivity the urine was chromatographed on ion exchange columns (4) and attempts made to identify the compounds contained in the major radioactive fractions. Findings from experiments on urine obtained after administration of each labeled drug are illustrated in Chart 6.

*Metabolites of TEPA-P<sup>32</sup>.*—Paper chromatography in either of two solvent systems yielded two well defined radioactive spots. Neither of these corresponded to the R<sub>f</sub> value of unmetabolized drug. Fractional extraction with ether, chloroform, and ethyl alcohol and analysis for inorganic and total phosphates (10) showed that between 88 and 93 per cent of the radioactivity was present as inorganic phosphate. The remaining “organic” form of radioactivity could be readily converted to the inorganic phosphate form by alkaline or acid hydrolysis. Thus, a major proportion of the administered drug was converted to the inorganic phosphate, while a small percentage was excreted

TABLE 2

R<sub>f</sub> VALUES OF MYLERAN, MYLERAN-HYDROLYSATE,  
AND URINARY METABOLITE OF MYLERAN,  
IN THREE SOLVENT SYSTEMS

TEST MATERIAL	SOLVENT SYSTEM*		
	I	II	III
Myleran	0.83	0.68	0.96
Myleran-hydrolysate	0.09	0.40	0.17
Urinary metabolite	0.10	0.38	0.17
NH <sub>4</sub> -methane sulfonate	0.10	0.42	0.18

\* Solvent systems (proportions by volume):

- I. *n*-Butanol: acetic acid: water (4: 1: 1).
- II. Methyl alcohol: water (19: 1).
- III. Acetone: acetic acid: water (8: 1: 1).

The metabolite was identified as an alkali salt of methanesulfonic acid by co-chromatography with several reference compounds and hydrolytic product of the drug itself (Table 2). Further confirmation was obtained by inverse isotope dilution in which the unknown was diluted with 1

ml. of methanesulfonic acid and precipitated as phenylhydrazonium salt (8). Repeated recrystallization resulted in a derivative with constant specific activity (40.0, 41.8, 40.0 counts/min/mg). Quantitatively, methanesulfonic acid accounted for over 95 per cent of the excreted radioactivity.

*Metabolites of TEM-C<sup>14</sup>.*—Paper chromatography of the urine from patients given TEM-C<sup>14</sup> failed to separate the metabolites into distinct entities. The majority of the metabolites were insoluble in nonpolar solvents. Ion exchange elution chromatography yielded fourteen separate radioactive peaks. Approximately 8 per cent of the total radioactivity excreted in the urine was present as urea and creatinine. These normal metabolites were isolated by standard procedures for analysis of urea as urea-xanthidrol derivative and creatinine directly by the method of Bloch and Schoenheimer (1). Attempts to correlate the  $R_f$  values of the radioactive peaks to respective  $R_f$  values of amino acids normally excreted in the urine were unsuccessful. No unchanged drug could be detected.

*Metabolites of Myleran-C<sup>14</sup>.*—The ion exchange elution of urine collected after Myleran-C<sup>14</sup> treatment also resulted in the separation of twelve radioactive components. The major proportion of radioactivity resided in two fractions: one eluted at pH 2.0 and the other eluted at pH 6.8. These two peaks accounted for 75 per cent of the total urinary excretion. The metabolite represented by the second major peak was found to be volatile.

From a study of the temperature gradient on the residual radioactivity of aluminum planchets plated with the fraction, the volatile component was estimated to have a boiling point of 130°–160° C. Individual tests and isolation procedures indicated little or no radioactivity in compounds which might be expected to be probable metabolites of Myleran, e.g., succinic acid, glucose, butanediol, oxalate, ethanolamine, and urea. So far, all attempts to identify the major metabolites have failed. No unchanged drug was found to be present in the urine.

## DISCUSSION

In spite of attempts to control variables, evaluation of data pertaining to patients, as in the present study, entails certain limitations; minor variations are to be expected among individual readings of quantitative data from one subject to another or even from the same subject at different times.

The levels of radioactivity following administration of each of the labeled alkylating drugs follow a common pattern in that the drug or its

radioactive metabolite is rapidly removed from the blood within a very short time after injection. This is followed by a low but prolonged level maintained for the next 48 hours. In all instances the radioactive metabolite was rapidly excreted through the kidneys. In view of this and the fact that in no instance was the unchanged drug excreted in the urine, it appears that these drugs are catabolized at a fairly rapid rate. The maintenance of a low but prolonged level of radioactivity might be the result of slow release of a metabolite after initial binding with some tissue. The radioactivity in the blood was associated with plasma proteins as in the case of observations in animal experiments (9). These observations parallel the findings in animal studies with HN<sub>2</sub>, TEM, and TEPA. The therapeutic effect of these drugs may be manifested by the small amount of intact drug which remains in the body. The apparent selective incorporation of P<sup>32</sup> radioactivity in the lymph node, as compared with the muscle tissue in proximity to the node (Table 1), needs cautious interpretation, since the metastatic lymph node represents highly proliferative mass of cells and, therefore, higher phosphorus turnover could be expected in this tissue as compared with the muscle.

A comparison of the metabolic excretion patterns following oral and intravenous administration of the drugs indicated very slight quantitative or qualitative differences. However, striking differences were obvious in the total number of urinary metabolites and their relative proportions, as illustrated in Chart 6. Whenever the "carrier" moiety is labeled, e.g., P atom in TEPA or the methylsulfonyl unit in Myleran, there is primarily a single metabolite which accounts for almost complete excretion. This demonstrates a severance of the alkylating portion of the drug from the carrier part. However, when the alkylating moiety is labeled (ethylenimino-labeled TEM or butylene-C<sup>14</sup> in Myleran) the drug results in a large number of metabolites in the urine. Some of these, though a relatively minor proportion, are urinary constituents of normal metabolism, probably as the result of a part of the detached alkylating moiety entering the carbon pool. Many of the major metabolites have eluded attempts at identification. It is probable that these represent alkylated excretion products of normal metabolism.

From the observed precipitous drop in radioactivity circulating in blood immediately following single intravenous administration, it appears unfeasible to attain or maintain a high "blood level" with TEM, TEPA, or Myleran. However, in their report on the metabolism of radioactive

OPSPA in man, Maller *et al.* concluded that, although the administered radioactivity was excreted rapidly in the urine, repeated dosage with this compound produced consistently higher blood levels than those observed with single injections (7). In this connection, it seems appropriate to point out the following pertinent observation. In our studies on the physiological disposition of TEM, TEPA, and Myleran in laboratory animals and in the human, the drugs were shown to be excreted in the urine mostly as inert catabolic products which possessed no known anti-tumor action. Heidelberger and his associates, on the other hand, have demonstrated that, in the human, OPSPA is first transformed into its principal metabolite, MEPA, which is a tumor-inhibitory compound of demonstrable potency.

#### SUMMARY

1. Triethylenemelamine- $C^{14}$ , Myleran- $C^{14}$ , Myleran- $S^{35}$ , and triethylenephosphoramidate- $P^{32}$  were administered individually to twelve cancer patients, and their blood concentrations, urinary excretion, and distribution were studied.

2. All drugs disappeared rapidly from the blood after intravenous administration. Maintenance of relatively high blood levels appeared impossible to achieve. The drugs administered by oral and intravenous routes produced similar responses with respect to blood levels and urinary excretion patterns.

3. None of the drugs was excreted unchanged in the urine.

4. The studies indicated a common pattern of metabolism of alkylating drugs in the human. A study of urinary metabolites of each drug suggested detachment of the alkylating moiety from the "carrier" portion of the drug molecule.

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*Cancer Res* 1959;19:713-718.

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