Metabolism of Human Leukocytes in Vitro

I. Effects of A-Methopterin on Formate-C\textsuperscript{14} Incorporation*

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A considerable amount of evidence exists to show that various types of human leukocytes may differ markedly in their content of certain enzymes and in their metabolic behavior. Much of this work has been previously reviewed (2, 3, 5, 6, 11, 13, 14, 18, 25–27).

Additional biochemical studies with human leukocytes are reported here. Biochemical differences between normal leukocytes and those from the several types of leukemic patients are of particular importance, since such dissimilarities may be responsible for the variations in clinical response to chemotherapeutic agents used in the treatment of leukemia. It is likewise possible that development of resistance by leukemic patients to a given therapeutic agent may reflect metabolic changes in morphologically similar leukocytes.

Our approach to this problem has been to study the incorporation of isotopic precursors into the ribonucleic acid and protein of isolated leukocytes. These studies have provided further evidence that leukocytes isolated from the blood of normal individuals and from patients with chronic lymphocytic (CLL), chronic granulocytic (CGL), or acute leukemia (AL) differ biochemically.

Certain chemotherapeutic agents have proved moderately effective in bringing about temporary remission of some leukemias. The clinical use of several such compounds including 4-amino-N\textsuperscript{10}-methyl-pteroylglutamic acid (A-methopterin) (10), 1,4-dimethanesulfonylbutane (Myleran) (15), 6-mercaptopurine (Purinthal) (8), and O-diazo-acetyl-l-serine (azaserine) (12) has prompted a study of the effects of these drugs, and others, on certain aspects of the metabolism of normal and leukemic human leukocytes. In the present report, the effects of one of these agents, A-methopterin, on the incorporation, in vitro, of formate-C\textsuperscript{14} into the gross protein fraction, into serine, and into the purine ribonucleotides of human leukocytes will be described.

MATERIALS AND METHODS

Separation of leukocytes.—Types of leukemia were identified by usual clinical criteria (20). For purposes of analysis, the acute leukemias have been considered as a single group and include predominantly individuals over 15 years of age suffering from acutely granulocytic or undifferentiated acute leukemia. Leukocytes from peripheral blood samples of normal individuals and leukemic patients were obtained\textsuperscript{1} by a method (Chart 1) which is essentially that reported by Beck and Valentine (3). A 60–80 per cent recovery of leukocytes was obtained by this method. The preparations frequently contained equal numbers of leukocytes and erythrocytes. This red cell contamination affected the results to be reported here only negligibly. Preliminary studies showed that detectable amounts of formate-C\textsuperscript{14} were not incorporated by erythrocytes. However, a small error was introduced by the contribution of the red cells in the incubation mixture to the planchet weights. The error was estimated to average about 25 per cent with CLL cells and about 15 per cent with other types of cells. Usually, 300 ml. of blood was drawn for the isolation of leukocytes from normal individuals. The amount needed from leukemic patients depended upon the peripheral cell count, about 30 ml. being obtained from pa-

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patients with a white count of 100,000/mm. Sixty to 90 minutes were required to prepare the leukocytes for incubation.

Incubations.—A calculated volume of freshly drawn or once-frozen serum from normal donors, supplemented with 3 mg of glucose/ml, was added to the concentrated leukocytes to yield a suspension containing $1 \times 10^8$ white cells/ml. One-mL aliquots of this suspension were distributed into 10-mL beakers for incubation. Further additions to the incubation vessels consisted of 1 mL of normal human serum supplemented with 3 mg of glucose/ml for control studies, or 1 mL of the same serum containing A-methopterin and supplemented with 8 mg of glucose/ml. The concentrations of A-methopterin used are indicated in the charts and tables. The vessels were shaken at 100–120 oscillations per minute in a Dubnoff metabolic shaking incubator at 37° C. in an atmosphere of 95 per cent $O_2$:5 per cent $CO_2$. After a 15-minute preincubation period, 0.2 mL of 0.9 per cent saline containing 2 $\mu$g (usually 0.1 mg) of sodium formate-C$^{14}$ was added, and the vessels were incubated for 4 hours. In some cases Krebs-Ringer-bicarbonate (94) containing 6 mg of glucose/ml (KRBG) was used instead of serum. This modification and other variations are indicated in the specific charts and tables in which results are given. A minimum of three and usually four or more replicate incubations was carried out for each variable in a single experiment.

Separation of "gross protein fraction."—After incubation the cells were recovered and washed twice with 0.9 per cent saline by centrifugation. They were then resuspended in 2 mL of 5 per cent trichloroacetic acid (TCA) at 5° C. to obtain the TCA-insoluble fraction. It was found that five washes with 5-mL portions of cold 5 per cent TCA were necessary to remove acid-soluble radioactivity from the insoluble fraction. The TCA-insoluble residue was extracted twice at 60° C. with 5 mL of a 3:1 ethanol-ether mixture, and, after a final ether wash, the insoluble residue was dried. The resulting lipid-free residue is designated the "gross protein fraction" (GPF). It was dissolved in 1 mL of 90 per cent formic acid and dried under an infrared lamp on tared copper planchets for determination of radioactivity. After drying, the planchets were counted for a sufficient time to reduce the counting error to less than 5 per cent. The standard error of the mean of three to five replicate incubations was carried out for each variable in a given experiment. The values shown in the tables represent the mean and standard deviations of separate experiments carried out at different times with leukocytes from different sources. The results for the GPF were expressed as $\mu$moles of formate incorporated/mg of protein and as per cent inhibition by A-methopterin, with the following relations:

$$\mu\text{moles formate incorporated}$$
$$= \frac{\mu\text{g formate/mg protein} \times \mu\text{moles formate added}}{\mu\text{g formate added}}$$

We wish to acknowledge generous gifts of A-methopterin, folic acid, and leucovorin from the Lederle Company, Pearl River, New York.

Separation of ribonucleic acid purine nucleotides.—Ribonucleic acid (RNA) was extracted from a portion of the GPF and was hydrolyzed to the free ribonucleotides with previously described technics (98). Following a preliminary separation of the ribonucleotides on a Dowex-1 formate anion exchange column (17), the purine ribonucleotides were further purified by filter paper ionophoresis and by filter paper chromatography as described previously (29). Quantitative measurement of purine nucleotides was made spectrophotometrically, and the radioactivity of infinitely thin deposits of the purine nucleotides on copper planchets was determined. The specific activities of RNA adenylc and guanylic acids were expressed as $\mu$moles of formate incorporated/ $\mu$ mole of the compound.

Separation of protein-bound serine.—Serine was isolated from another portion of the GPF which had been hydrolysed
in 6 N HCl by being autoclaved at 115°-120° C. for 1½ hours. After removal of excess HCl by evaporation to dryness in vacuo, the residue was dissolved in 0.01 N HCl and the amino acids were absorbed on a 10 X 1 cm. Dowex-50 column (hydrogen form). The column was washed with 80 ml. of 0.01 N HCl and was then eluted with three 10-ml. fractions of 1 N NH₄OH, the second fraction containing all the amino acids (90). Small portions of the concentrated amino acid mixture were applied as a 10-cm. band on Whatman No. 1 filter paper and developed with a water-saturated phenol solvent (9). Parallel strips from each edge of the paper were sprayed with ninhydrin solution and dried to locate the various amino acid bands. The serine band, well separated from other amino acids, was eluted with water. One aliquot of the eluate was used for quantitative amino acid assay by the ninhydrin reaction (22), while another aliquot was counted at infinite thinness for radioactivity. The specific activity of serine was expressed as amoles of formate incorporated/µmole of serine. The major portion of the radioactivity in the amino acid mixture was found in the serine band. An average of 2.5 mg of serine/100 mg of GPF was recovered from CGL and CLL cells.

**RESULTS**

**Influence of medium.**—Preliminary experiments were carried out with cells from patients with chronic granulocytic leukemia incubated for 4 hours in various media to determine some of the factors influencing formate uptake. Some of these results are summarized in Table 1. It is of interest that formate-C₁⁴ was incorporated into the GPF more rapidly when cells were incubated in KRBG solution than when they were incubated in normal human serum. However, the cells usually clumped in the KRBG solution and in various modifications thereof. Since it was desired to maintain the cells in as normal a physiological state as possible, most of the work to be reported in this paper was carried out with cells incubated in serum from normal donors, the serum being used immediately or kept in the frozen state until used. Leukocytes incubated with formate-C₁⁴ in serum which previously had been dialyzed against KRBG buffer resulted in an increased rate of formate uptake (Table 1). The effect of anaerobiosis on formate incorporation has been somewhat variable. However, in only one instance has more than a 25 per cent inhibition been observed in the absence of oxygen. Erythrocytes incubated under the conditions described for leukocytes did not incorporate formate-C₁⁴ into the GPF to a significant extent. Preparations made by exposing leukocytes in KRBG buffer to sonic vibrations (Raytheon-2KW, 3 minutes), in which less than 2 per cent of the leukocytes remained intact, just prior to incubations, did not fix formate into a protein-bound state. Variations of the numbers of cells from 0.5 X 10⁸ to 2.0 X 10⁸ per incubation vessel had little influence on the specific activity of the isolated GPF. Formate-C₁⁴ incorporation was reduced in the absence of glucose.

### Table 1

**Influence of Medium on Formate-C₁⁴ Uptake by Chronic Granulocytic Leukemia Cells**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Per cent of normal</th>
<th>Exp. serum control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum control</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>Dialyzed normal serum</td>
<td>6</td>
<td>104 ± 101</td>
</tr>
<tr>
<td>Krebs-Ringer-bicarbonate-glucose</td>
<td>7</td>
<td>240 ± 50</td>
</tr>
<tr>
<td>Normal serum—95 per cent N₂; 5 per cent CO₂</td>
<td>4</td>
<td>48 to 130</td>
</tr>
</tbody>
</table>

*10⁸ cells incubated 4 hours at 37° C. in 2.2 ml. normal serum containing 6 mg. of glucose and 1.47 amoles formate-C₁⁴ (2 µC.) in an atmosphere of 95 per cent O₂; 5 per cent CO₂, unless otherwise indicated.

† Dialed 24 hours at 5° C. against Krebs-Ringer-bicarbonate-glucose solution.

‡ Standard deviation.

**Separation of acid-soluble adenylic acid.**—Most of the TCA was removed from the acid-soluble fraction by extraction with ether. The nucleotides were separated by chromatography on Dowex-1 formate ion exchange columns essentially by the method described by Hurlbert et al. (17). The ultraviolet-absorbing peaks eluted from the column were located spectrophotometrically, and the tubes from the adenine peak (AMP) peak were pooled and concentrated by lyophilization for further purification by chromatographic techniques described previously (20). The specific activity of AMP was expressed as amoles of formate incorporated/µmole of the compound.

### Table 2

**Effect of A-Methopterin on Formate-C₁⁴ Incorporation by Leukocytes**

<table>
<thead>
<tr>
<th>Type</th>
<th>Isotope</th>
<th>Per cent inhibition of cell incorporation† by A-methopterin‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4.9 ± 0.62 (37)</td>
<td>3.9 ± 4.1 (37)</td>
</tr>
<tr>
<td>CLL</td>
<td>11.1 ± 5.1 (35)</td>
<td>6.7 ± 5.4 (25)</td>
</tr>
<tr>
<td>CGL</td>
<td>21.2 ± 7.2 (49)</td>
<td>80.5 ± 8.2 (49)</td>
</tr>
<tr>
<td>AL</td>
<td>60.0 ± 15.8 (13)</td>
<td>35.0 ± 4.5 (13)</td>
</tr>
</tbody>
</table>

*10⁸ cells incubated 4 hours at 37° C. in 2.2 ml. of normal serum containing 6 mg. of glucose and 1.47 amoles (2 µC.) formate-C₁⁴ in an atmosphere of 95 per cent O₂; 5 per cent CO₂. Number of experiments indicated in parentheses.

† Amoles formate incorporated X 10⁻⁶/mg of protein in 4 hours.

‡ A-methopterin concentration, 100 µg/ml.

§ Standard deviation.

**Formate-C₁⁴ incorporation into the “gross protein fraction” (GPF) of different types of leukocytes.**—Marked differences were observed in the rates of formate-C₁⁴ incorporation into the GPF of leukocytes from normal individuals, from patients with chronic lymphocytic leukemia (CLL) or with chronic granulocytic leukemia (CGL), and from adults with acute leukemia (AL). The incorporation rate increased in the order: normal <CLL <CGL <AL, with only slight overlapping between groups. This is shown in Table 2 for all the data thus far available. Also included in this
table are the effects of A-methopterin on formate-\textsuperscript{14}C incorporation by these cells. These results will be discussed later. The uptake of formate was linear with time, as is shown in Chart 2, for CGL cells in the presence and absence of A-methopterin.

It is evident that the standard deviations for formate incorporation were relatively large in the leukemic series. The relation of this variability to variations in the average maturity of cellular populations was considered. Chronic granulocytic leukemia was chosen for this comparison because of the larger number of studies which were available for analysis and because of the greater range of relative cellular maturity in this series. Adequate data for analysis were available in 37 studies on twenty patients with chronic granulocytic leukemia. Cellular maturity was expressed as the percentage of myelocytes plus progranulocytes plus myeloblasts, based on differential counts of 100 cells on peripheral smears.

Chart 3 shows the relation of formate uptake to cellular maturity in five patients studied serially. Consecutive tests are joined by lines characteristic for each patient. A positive slope indicates that immature populations tended to incorporate formate more rapidly than did mature populations during that segment of the patient's disease encompassed by the consecutive tests. On this graph there are fourteen positive slopes and three negative slopes. If there were no relation between cellular maturity and formate uptake in consecutive tests, there should be an equal number of positive and negative slopes. The probability of the observed differences occurring on the basis of chance alone is less than 1 in 100. Chart 4 combines the first tests on these five patients with single tests on the other fifteen patients. The scatter of values

\begin{chart}
\textbf{Chart 2.}—Time course of formate incorporation into CGL leukocytes in the presence and absence of A-methopterin.

10\textsuperscript{6} CGL cells incubated at 37° C. in normal human serum containing 2 \textmu C. (0.1 mg.) sodium formate-\textsuperscript{14}C in the presence and absence of 100 \textmu g A-methopterin/ml.

\begin{chart}
\textbf{Chart 3.}—The effect of cellular maturity on formate incorporation into CGL leukocytes (serial studies in five patients)

\begin{chart}
\textbf{Chart 4.}—Relation of Leukocyte Immaturity to Leukocyte Incorporation of Formate (Serial tests)

Therapy at time of test:
- None
- Myleran
- Cortisone
- X-ray

Status six mos. after test:
- Alive
- Dead
- Unknown

Tests linked sequentially
- EG, 47 f
- J.B, 43 f
- T.R, 56 g
- D.O, 39 f
- O.Y, 49 f

Patients linked sequentially
- First of serial tests

\begin{chart}
\textbf{Chart 5.}—The effect of cellular maturity on formate incorporation into CGL leukocytes (serial studies in five patients)
suggests a crude correlation, and this is confirmed by calculation of a correlation coefficient, which is 0.54 with a P value less than .02. This graph includes data for one patient who was in preterminal acute exacerbation of his disease. One might object to inclusion of acute exacerbations on the grounds that the process has undergone some fundamental change other than just a change in relative maturity. Of the entire 37 tests, including repeats, two tests were made during acute exacerbation. A correlation coefficient on the remaining 35 tests is 0.45 with P less than 0.01. Thus, there appears to be little doubt that formate uptake is related to relative cellular maturity. Charts 3 and 4 indicate therapy at time of testing as well as subsequent fate of the patients. No relationship between formate uptake and concomitant therapy is evident. There is a suggestion, which cannot be statistically validated with the present data, that, as the course of the disease progresses, formate uptake becomes greater, even at the same level of cellular maturity. Thus, there appears to be little doubt that a direct correlation exists between formate uptake and leukocyte maturity.

The specific activity of the labeled cellular components depends on the specific activity of the exogenous formate pool and the concentration of formate in the medium. Since the specific activity has been expressed as μmoles of formate incorporated/mg of protein at this saturation level of formate, unless otherwise stated. In some cases this involved correction from observations made at lower formate concentrations with the use of the curves in Chart 5.

Formate-C\textsuperscript{14} incorporation into serine and into acid-soluble and RNA purine nucleotides.—Table 3 shows the specific activities of the gross protein fraction, protein-bound serine, RNA adenine and guanine, and acid-soluble adenylic acid (AMP) of an experiment with CGL cells incubated in KRBG medium. The results are expressed as μmoles of formate incorporated per μmole of the compound.
except for the gross protein fraction. The specific activities of serine and the RNA purines were comparable, while that of the acid-soluble AMP was some tenfold higher.

Seventy to 80 per cent of the total radioactivity could be accounted for as protein-bound serine. Very little radioactivity was observed in other amino acids, including methionine or histidine, into which formate may be incorporated in some systems. The radioactivity in RNA adenine and guanine accounted for part but not for all the remaining radioactivity in the GPF.

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Effect of A-methopterin.—The effect of A-methopterin on formate incorporation by CGL cells was immediate and nonprogressive. In the experiment shown in Chart 2, CGL leukocytes were equilibrated with A-methopterin for 15 minutes prior to the addition of the radioactive formate. Pre-incubation of the cells in the presence of A-methopterin for as long as 2 hours before the addition of the labeled substrate did not increase the inhibitory effect of the folic acid antagonist. This absence of a latent period for the A-methopterin effect suggests that neither permeability barriers nor conversion to active derivatives is involved in A-methopterin inhibition of formate uptake by CGL cells.

The fact that formate uptake could be only partially inhibited (80 per cent in CGL and 50 per cent in AL cells, Table 2) even at high levels of A-methopterin was of particular interest. Previous in vitro studies with a transplantable mouse leukemia (29) showed that a similar A-methopterin-resistant formate uptake occurred in this tissue, and that development of A-methopterin resistance was paralleled by an increase in the A-methopterin-insensitive formate incorporation. Others have shown a folic acid antagonist-resistant formate uptake in leukemic mouse spleen (1) and rabbit bone marrow (22).

Typical dose-response curves, in which formate uptake is plotted as a function of A-methopterin

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**TABLE 3**

**EFFECT OF A-METHOPTERIN ON UPTAKE OF FORMATE INTO SEVERAL COMPONENTS OF CHRONIC GRANULOCYTIC LEUKEMIA LEUKOCYTES**

<table>
<thead>
<tr>
<th>Tissue component</th>
<th>Control</th>
<th>A-Methopterin*</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gross protein fraction</td>
<td>4.0 × 10⁻⁴</td>
<td>8.9 × 10⁻⁴</td>
<td>80</td>
</tr>
<tr>
<td>Protein-bound serine</td>
<td>0.06 × 10⁻⁴</td>
<td>0.16 × 10⁻⁴</td>
<td>30</td>
</tr>
<tr>
<td>RNA adenine</td>
<td>0.05 × 10⁻⁴</td>
<td>0.05 × 10⁻⁴</td>
<td>10</td>
</tr>
<tr>
<td>RNA guanine</td>
<td>1.47 × 10⁻⁴</td>
<td>0.11 × 10⁻⁴</td>
<td>22.5</td>
</tr>
<tr>
<td>Acid-soluble AMP</td>
<td>1.00 μg A-methopterin/ml.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* μmole formate incorporated/mg GPF.
† μmole formate incorporated/μmole of component.

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**CHART 5.—The effect of formate concentration on formate incorporation into normal, CLL, CGL, and AL leukocytes. 10⁶ cells incubated in normal human serum for 4 hours at 37°C.**
concentration, are shown in Chart 6 for normal, CLL, CGL, and AL leukocytes. This figure shows that the maximum effect of A-methopterin on CGL and AL cells was reached at concentrations of about 2–4 \( \mu g/ml \), and that higher concentrations of A-methopterin did not increase the inhibition. The low level of A-methopterin necessary for the maximum inhibitory effect is in accord with those affecting formate incorporation in vitro into purines of rabbit bone marrow suspension (22) and into the protein fraction of mouse leukemia (29).

The same series of patients with chronic granulocytic leukemia analyzed in Charts 3 and 4 were examined for a relationship between A-methopterin inhibition and cellular immaturity. No significant correlation was found between inhibition by A-methopterin of formate incorporation and the percentages of myelocytes plus progranulocytes plus myeloblasts in 35 tests on these twenty patients. Consideration of therapy and subsequent clinical course in these patients did not reveal any apparent relationship to per cent inhibition by A-methopterin.

**Effect of leucovorin.**—It has been shown in other systems (16) that citrovorum factor more effectively reverses the effects of folic acid antagonists than does folic acid (21). This same relationship was found in the present experiments. Chart 7 shows that there was a marked stimulation of formate-C\(^{14} \) incorporation by CGL cells incubated with leucovorin \(^2 \) in the absence of A-methopterin. There was a reversal of the inhibitory effect of A-methopterin by rather high concentrations of leucovorin in a manner suggestive of a competitive relationship. Folic acid \(^2 \) produced neither the stimulating nor the A-methopterin-reversing effects shown by leucovorin.

**DISCUSSION**

It is interesting that the rate of formate incorporation into leukocytes was faster in Krebs-Ringer-bicarbonate-glucose (KRBG) medium than in normal serum (Table 1). Some of this effect might have been due to a dilution of the specific activity of the exogenous formate pool by one-
cells, although the inhibitory effect of A-methopterin was considerably greater in CGL cells.

The incorporation of formate by leukocytes in the absence of oxygen was studied in four experiments with CGL cells. In these cases the per cent uptake ranged from 48 to 120 per cent of that observed in control aerobic experiments (Table 1). These results suggest that the high glycolysis rates of leukocytes (2, 4) are sufficient to provide whatever energy is required for the incorporation reactions.

The data reported in Table 2 are consonant with other work which indicates consistent differences in the metabolism and composition of various types of human leukocytes (2–6, 11, 13, 14, 18, 25–27). It is evident that human leukocytes may be as different biochemically and metabolically as they are morphologically.

The available evidence is not sufficient to determine whether the different rates of formate incorporation by the several types of leukocytes are due to differences in net de novo synthesis or to differences in exchange reaction rates involving active one-carbon units. Buchanan (7) and others have demonstrated such exchange reactions by purine nucleotides in cell-free systems. The question of de novo synthesis is one of considerable significance. The formate uptake into serine and RNA-purines resulted in specific activities which corresponded to an uptake of \( 0.06 \times 10^{-14} \text{ moles of formate/mole} \) at the end of a 4-hour incubation period in CGL cells (Table 3). Thus, the de novo synthesis of about 0.06 per cent of the amount of serine, RNA adenine, or guanine initially present would account for the radioactivity found in these compounds. De novo synthesis of this slight magnitude is certainly a possibility. Measurement of the relative incorporation of formate into the carbon 2 and carbon 8 positions of RNA-purines should aid in distinguishing between de novo synthesis and exchange reactions, and such experiments are currently being carried out in our laboratory.

The physiological and biochemical basis for the differences in the A-methopterin sensitivity of different types of cells is a problem of fundamental biological and clinical interest. Such differences may underlie the variations seen in the clinical effectiveness of this drug and also the development of resistance during a course of therapy.

High concentrations of A-methopterin result in only partial inhibition of formate incorporation of CGL and AL cells. This raises the question of whether there are two populations of cells in these conditions, one sensitive and the other insensitive to the drug, or whether A-methopterin-insensitive pathways for formate incorporation exist in all cells. It is not yet possible to distinguish between these possibilities, since in both situations all the components could (as is found in the data shown in Table 3) be inhibited to an equal extent.

Although formate incorporation by leukocytes from CGL patients was markedly inhibited by A-methopterin in these in vitro studies, it should be noted that this drug was not used in the treatment of these individuals. A-methopterin has been most effectively used clinically in the treatment of acute leukemia in children, but studies with this type of cell in this in vitro system are difficult to carry out owing to the leukopenia commonly present in these patients. No correlation can be made at this time between the clinical effectiveness of A-methopterin and the in vitro effects of the compound on formate incorporation by leukocytes. It would be advantageous to extend the studies described here to cells from patients under treatment with A-methopterin.

Biochemical situations responsible for differences in sensitivity to A-methopterin by the various cell types may include differences in citrovorum factor synthesis, permeability to A-methopterin, affinities of the drug for certain one-carbon transferring enzymes, and alternative metabolic pathways of one-carbon metabolism (19). It is hoped that this most fundamental problem will attract continued attention.

**SUMMARY**

The effects of A-methopterin on the rate of incorporation of formate-\(^{14}C\), in vitro, by human leukocytes have been studied.

The rate of formate incorporation by different types of human leukocytes increases in the following order: normal, chronic lymphocytic, chronic granulocytic, and acute leukemic cells, with little overlapping between values obtained with the various cell types. In the granulocytic series a linear relationship existed between the relative maturity of circulating leukocytes and the rate of formate incorporation by these cells, the more immature populations taking up more formate. Leukovorin stimulated this rate of formate uptake, whereas folic acid did not.

In chronic granulocytic cells, protein-bound serine and ribonucleic acid adenine and guanine had similar specific activities, whereas the specific activity of acid-soluble adenylate acid was much higher. A-methopterin inhibited incorporation into all the components equally.

The inhibitory effect of A-methopterin on formate incorporation into all fractions of chronic granulocytic cells was greatest (70–90 per cent inhibition), with somewhat less effect on formate
uptake into acute leukemic cells and little or no effect on formate incorporation into the protein fraction of chronic lymphocytic or normal leukocytes.

REFERENCES

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