Comparative Incorporation of $S^{35}$ L-Cystine and $S^{35}$ Sodium Sulfate by Normal and Leukemic Leukocytes

By Austin S. Weisberger and Leif G. Suhrland

L-CYSTINE OR RELATED COMPOUNDS may have an important role in the metabolism of leukocytes. Studies with sulfur (S$^{35}$) labeled l-cystine demonstrate that this amino acid is readily incorporated by leukocytes both in vivo and in vitro. Distinctive patterns of incorporation and differences in rates and levels of uptake of radioactive l-cystine by normal and leukemic leukocytes have been observed. Preliminary comparison of the uptake of S$^{35}$ sodium sulfate with that of S$^{35}$ l-cystine by normal and leukemic leukocytes indicates that there may also be certain differences in the incorporation of these compounds. The following studies were therefore undertaken to extend these observations. The data demonstrate certain alterations in the metabolic turnover of cystine sulfur and inorganic sulfur by leukemic leukocytes both in vivo and in vitro.

Methods

1. Comparative Incorporation of S$^{35}$ L-Cystine and S$^{35}$ Sodium Sulfate by Leukocytes In Vivo*

Tracer amounts of S$^{35}$ l-cystine were administered orally to 22 individuals and the radioactivity present in circulating leukocytes was determined at intervals by methods previously described. Blood was withdrawn every ten minutes for one hour, hourly for eight hours and daily thereafter. The leukocytes were separated, washed with normal saline and 1 per cent acetic acid and the radioactivity present determined in a pre-flush gas flow counter. The radioactivity present in the leukocytes was expressed as counts per minute (c/m) per ml. packed leukocytes. All counts were corrected for background and decay.

Leukocytes which were washed with 1 per cent acetic acid contained less radioactivity than those washed only with saline. This loss appears to represent a constant fraction of the total amount of radioactivity present. Since the values obtained were used for comparison, no correction factor was applied for the loss of this acid-soluble radioactivity. The values reported do not, therefore, represent the absolute amount of radioactivity incorporated.

Ten of the patients studied had no hematologic abnormalities, five had acute leukemia, four had chronic myeloid leukemia, and three had chronic lymphoid leukemia. Three microcuries of S$^{35}$ l-cystine per Kg. body weight were administered to each patient. This amount of radioactivity was contained in approximately 5 to 10 mg. of carrier l-cystine.

In addition to the radioactivity present in the leukocytes, plasma levels of radioactivity, as well as the amount of S$^{35}$ excreted in the urine during the first 24 hours, were also determined by methods previously described.

* S$^{35}$ l-cystine and S$^{35}$ sodium sulfate were obtained from Abbott Laboratories, North Chicago, Ill. Atomic Energy Commission authorization for radioisotope procurement No. 13996.
Similar studies were carried out with $^{35}$S sodium sulfate in ten patients. Approximately the same amount of radioactivity contained in approximately the same amount of carrier was administered orally in both instances. Three of the patients had no hematologic abnormality, two had acute leukemia, three had chronic myeloid leukemia, and two had chronic lymphoid leukemia.

2. **Comparative Incorporation of $^{35}$S L-Cystine and $^{35}$S Sodium Sulfate by Leukocytes In Vitro**

Studies on the incorporation of $^{35}$S L-cystine and $^{35}$S sodium sulfate by normal and leukemic leukocytes were carried out in vitro by methods previously described. Freshly drawn heparinized whole blood was incubated with either radioactive L-cystine or radioactive sodium sulfate for 45 minutes at 37°C. The leukocytes were then separated, washed, and the radioactivity present determined in a pre-flush gas flow counter. The radioactivity was expressed as c/m per ml. packed leukocytes and all counts were corrected for background and decay.

The comparative uptakes of both radioactive compounds were always determined on cells from the same patient on the same day. The same amount of radioactivity contained in approximately the same amount of carrier was used in both instances. Although blood was incubated with several concentrations of either $^{35}$S L-cystine or $^{35}$S sodium sulfate, the values reported here are those obtained when incubation was carried out with approximately 5 to 10 micrograms of either compound (200,000 c/m) per ml. whole blood and are the mean of at least five pairs of observations in each instance.

The effect of various physical and chemical factors on the uptake of radioactive amino acids by leukocytes in vitro has previously been determined. The effect of varying all these factors on the uptake of $^{35}$S sodium sulfate was not repeated. The effect of increasing concentrations of sodium sulfate and of varying temperatures on the uptake of $^{35}$S was, however, studied.

**Results**

1. **Comparative Incorporation of $^{35}$S L-Cystine and $^{35}$S Sodium Sulfate by Normal and Leukemic Leukocytes In Vitro**

The results are summarized in the accompanying figures and tables. The curves represent the mean values obtained in each condition studied and are, in general, representative of the patterns of incorporation which occurred in each individual.

The incorporation of $^{35}$S from L-cystine into normal leukocytes proceeds slowly during the initial phase. Maximum radioactivity appears in the peripheral blood between the fifth and twelfth days. The incorporation of $^{35}$S into normal leukocytes from orally administered sodium sulfate roughly parallels that of $^{35}$S L-cystine but is quantitatively less (fig. 1).

In acute leukemia the incorporation of $^{35}$S L-cystine into leukocytes is much more rapid than in normals and maximum levels of radioactivity are present within one hour (fig. 2). Maximum levels of radioactivity are maintained for only two days following which there is a gradual disappearance of radioactivity from the leukocytes. In comparison, the incorporation of $^{35}$S sodium sulfate into acute leukemic leukocytes is considerably less on a quantitative basis. Significant amounts of radioactivity are present for only a brief period compared with the more prolonged presence of cystine sulfur.

The incorporation of $^{35}$S L-cystine into the leukocytes of chronic myeloid leukemia closely parallels that found in acute leukemia during the initial phase. Unlike acute leukemia, however, there is a progressive increase in the incorporation of $^{35}$S after the initial phase with maximum levels of radioactivity appearing...
INCORPORATION OF $^{35}$SODIUM SULFATE AND $^{35}$S-CYSTINE BY ACUTE LEUKEMIC LEUKOCYTES IN VIVO

Fig. 1.—Comparative incorporation of $^{35}$Sodium sulfate and $^{35}$S-Cystine by normal leukocytes in vivo. The incorporation of $^{35}$S from sodium sulfate roughly parallels that of 1-cystine but is quantitatively less.

Fig. 2.—Comparative incorporation of $^{35}$Sodium sulfate and $^{35}$S-Cystine by acute leukemic leukocytes in vivo. The incorporation of $^{35}$Sodium sulfate is much less than that of $^{35}$S-Cystine and significant amounts of radioactivity are present for only a brief period.

In the leukocytes between the fourth and eighth days (fig. 3). In comparison, the incorporation of $^{35}$Sodium sulfate in chronic myeloid leukemia is markedly diminished. The uptake of $^{35}$Sodium sulfate in chronic myeloid leukemia is greater than that in acute leukemia but is not as good as that in normals.

In chronic lymphatic leukemia a low flat level of incorporation of $^{35}$S-Cystine and a slow degradation of this radioactivity is characteristic. In comparison, there is almost no incorporation of $^{35}$Sodium sulfate as shown by a consistent absence of significant amounts of radioactivity in the lymphocytes at all times.

In these experiments, the plasma levels of radioactivity with $^{35}$S-Cystine were...
similar in the different conditions. Minor differences were present only during the first few hours, after which the plasma levels were almost identical in each group of patients. The plasma levels of radioactivity with S\textsuperscript{35} sodium sulfate also were similar after the first few hours (fig. 4). The radioactivity disappeared from the plasma more rapidly with S\textsuperscript{35} sodium sulfate than with S\textsuperscript{35} l-cystine.

The urinary excretion of S\textsuperscript{35} was determined only during the first 24 hours. With S\textsuperscript{35} l-cystine 3 per cent to 13 per cent of the administered radioactivity was excreted in 24 hours (table 1). There was no significant difference in the amount excreted by normals and by patients with acute or chronic leukemia. With S\textsuperscript{35} sodium sulfate the urinary excretion of radioactivity was significantly greater. In normals, 25 per cent of the administered radioactivity was excreted in 24 hours.
INCORPORATION OF $^{35}$ L-CYSTINE AND $^{35}$ SODIUM SULFATE

### Table 1.—Urinary Excretion of $^{35}$

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases</th>
<th>$^{35}$ Compd. administered</th>
<th>Av. Per cent excreted in 24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>L-Cystine</td>
<td>9</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>5</td>
<td>L-Cystine</td>
<td>8</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td>4</td>
<td>L-Cystine</td>
<td>3</td>
</tr>
<tr>
<td>Chronic lymphoid leukemia</td>
<td>3</td>
<td>L-Cystine</td>
<td>13</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>Sod. sulfate</td>
<td>25</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>2</td>
<td>Sod. sulfate</td>
<td>53</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td>3</td>
<td>Sod. sulfate</td>
<td>49</td>
</tr>
<tr>
<td>Chronic lymphoid leukemia</td>
<td>2</td>
<td>Sod. sulfate</td>
<td>63</td>
</tr>
</tbody>
</table>

**EFFECT OF CONCENTRATION OF $^{35}$ L-CYSTINE AND $^{35}$ SODIUM SULFATE ON UPTAKE BY CHRONIC LYMPHATIC LEUKEMIC LEUKOCYTES IN VITRO**

Fig. 5.—Effect of concentration of $^{35}$ l-cystine and $^{35}$ sodium sulfate on uptake by chronic lymphatic leukemia leukocytes in vitro. Incubation with increasing amounts of either compound results in an increased uptake by leukocytes. Saturation occurs when 20 to 25 micrograms (between 150,000 and 200,000 c/m) of either l-cystine or sodium sulfate are added per ml. blood. The uptake of l-cystine is greater than that of sodium sulfate.

In acute and chronic leukemia the urinary excretion of $^{35}$ within 24 hours ranged from 49 per cent to 63 per cent of the amount administered.

2. **Comparative Incorporation of $^{35}$ L-Cystine and $^{35}$ Sodium Sulfate by Normal and Leukemic Leukocytes In Vitro**

When leukocytes are incubated in vitro with either $^{35}$ l-cystine or $^{35}$ sodium sulfate, they readily incorporated $^{35}$. Incubation with increasing amounts of either compound results in an increased uptake by the leukocytes. Saturation occurs when approximately 20 to 25 micrograms (between 150,000 and 200,000 c/m) of either l-cystine or sodium sulfate are added per ml. blood (fig. 5).

The optimum incorporation of both compounds occurs at 37 C. There is a marked reduction in uptake above or below this temperature (fig. 6).
FIG. 6.—The effect of temperature on the incorporation of $^{35}$S-l-cystine and $^{35}$S sodium sulfate by chronic myeloid leukemia leukocytes. Maximum incorporation of $^{35}$S occurs at 37°C with either compound.

### Table 2.—Comparative Incorporation of $^{35}$S-L-Cystine and $^{35}$S Sodium Sulfate by Normal and Leukemic Leukocytes in Vitro

<table>
<thead>
<tr>
<th></th>
<th>$^{35}$S-l-cystine (C/M per ML packed WBCs)</th>
<th>$^{35}$S sodium sulfate (C/M per ML packed WBCs)</th>
<th>Ratio of sulfate to cystine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9050</td>
<td>1920</td>
<td>19%</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td>7665</td>
<td>5055</td>
<td>66%</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>8200</td>
<td>670</td>
<td>8%</td>
</tr>
<tr>
<td>Chronic lymphatic leukemia</td>
<td>8050</td>
<td>1350</td>
<td>17%</td>
</tr>
</tbody>
</table>

L-cystine sulfur is readily incorporated by all types of leukocytes and after one hour of incubation both normal and leukemic leukocytes contain equivalent amounts of radioactivity. In comparison, only the leukocytes of chronic myeloid leukemia incorporate comparable amounts of $^{35}$S from sodium sulfate. All other leukocytes exhibit low levels of incorporation of inorganic sulfur (Table 2). It was also observed that in the blastic stage of chronic myeloid leukemia the uptake of inorganic sulfur is markedly impaired and is of the same magnitude as that occurring in acute leukemia.
The data demonstrate certain differences in the metabolism of both organic and inorganic sulfur by normal and leukemic leukocytes, both in vivo and in vitro. The differences observed in the metabolic turnover of l-cystine as compared with that of sodium sulfate are further evidence of the possible importance of l-cystine (or l-cysteine) in leukocyte metabolism.

The patterns of incorporation obtained with orally administered S\textsuperscript{35} l-cystine appear to be distinctive. The different patterns observed in normal and leukemic patients are not due to differences in the amount of S\textsuperscript{35} available. Thus there were no significant differences either in the plasma levels of S\textsuperscript{35} or in the excretion of S\textsuperscript{35} in the various conditions. It is possible, therefore, that these distinctive patterns are the result of factors peculiar to the metabolism of leukocytes in these disorders.

The rapid incorporation of S\textsuperscript{35} from l-cystine by leukocytes in acute leukemia and in chronic myeloid leukemia may be due to turnover of cystine by immature cells in the peripheral blood. In comparison, the delayed peak of radioactivity observed in normal leukocytes may be explained by assuming that most of the S\textsuperscript{35} is incorporated by immature cells in the marrow and that this radioactivity is subsequently released into the peripheral blood as maturation occurs. Similarly, the secondary rise in radioactivity observed in chronic myeloid leukemia may represent release into the peripheral blood of partially matured cells which have incorporated S\textsuperscript{35} during the initial phase.

S\textsuperscript{35} l-cystine appears to be best utilized in chronic myeloid leukemia. This may be correlated with the observation that l-cysteine has the unique action of preventing the characteristically severe neutropenia induced by nitrogen mustard. Baldini and Sacchetti\textsuperscript{8} have also observed that both l-cysteine and l-cystine exert a protective effect against degeneration of granulocytes in bone marrow cultures. L-cystine (or l-cysteine) may therefore have an important role in the metabolism of granulocytes.

The differences observed in the patterns of incorporation of orally administered S\textsuperscript{35} sodium sulfate do not parallel the variations observed with l-cystine. In comparison with the incorporation of l-cystine, the uptake of S\textsuperscript{35} with sodium sulfate is poor in acute leukemia, almost completely absent in chronic lymphatic leukemia, and is only moderate in chronic myeloid leukemia (table 3). It roughly parallels that of S\textsuperscript{35} l-cystine in normal leukocytes but is at a lower level quantitatively. These differences are not due to differences in the amount of S\textsuperscript{35} available. Thus the relatively minor differences in the plasma levels of S\textsuperscript{35} are not sufficiently great to account for the variations in the patterns of incorporation.

<table>
<thead>
<tr>
<th></th>
<th>In vivo</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Chronic myeloid</td>
<td>Moderate</td>
<td>Good</td>
</tr>
<tr>
<td>Chronic lymphatic</td>
<td>Poor</td>
<td>Poor</td>
</tr>
</tbody>
</table>
The comparatively large amounts of inorganic \( ^{35}S \) excreted in the urine of patients with leukemia (table 1) may be due to an impairment in the ability of leukemic leukocytes to incorporate inorganic sulfur. It is unlikely that \( ^{35}S \) sodium sulfate is rapidly metabolized and excreted by leukemic leukocytes since high levels of radioactivity were not present in the leukocytes at any time. It is also unlikely that the poor uptake by leukemic leukocytes is due to the rapid excretion of inorganic sulfur since the amount of \( ^{35}S \) available for incorporation in the plasma was similar during the first six to eight hours in both normal and leukemic patients. It therefore appears likely that the differences observed with inorganic sulfur may also be associated with factors peculiar to the metabolism of leukocytes in these disorders.

The incorporation of \( ^{35}S \) l-cystine by leukocytes in vitro appears to be an active process and not solely dependent upon simple diffusion. Similarly the lack of direct correlation between temperature gradient and uptake of sodium sulfate (fig. 6) indicates that the incorporation of this compound is also an active process.

Although equivalent amounts of cystine sulfur are incorporated by all types of leukocytes after incubation for 1 hour in vitro, only the leukocytes of chronic myeloid leukemia incorporate comparable amounts of \( ^{35}S \) from inorganic sulfur (table 2). All other leukocytes exhibit low levels of incorporation of inorganic sulfur. Since the uptake of inorganic \( ^{35}S \) is markedly impaired in the blastic stage of chronic myeloid leukemia, it appears likely that the incorporation of inorganic sulfur in vitro is associated primarily with the presence of immature granulocytes. Although the in vivo incorporation of inorganic sulfur is only moderate in chronic myeloid leukemia compared with that of normals (table 3), it may be assumed that the comparatively greater incorporation of inorganic sulfur in these two instances is due to the presence of large numbers of immature granulocytes in either the marrow or peripheral blood.

Neither the form in which sodium sulfate is incorporated into leukocytes nor the function of sulfate in leukocyte metabolism is known. It has been repeatedly shown that neither elemental sulfur nor sulfate can be utilized in animals to form sulfur containing amino acids. Singher and Marinelli, however, found a high concentration of \( ^{35}S \) in the bone marrow of animals following the injection of \( ^{35}S \) sodium sulfate. Other tissues took up much less sulfur and the fall in \( ^{35}S \) concentration was slower in the marrow than in other tissues. The nature of the substance responsible for the high \( ^{35}S \) activity was not identified in these experiments but radioactivity was present in both the acid soluble and acid insoluble or protein fraction of the marrow tissue. The high uptake of sulfate by marrow tissue and by immature granulocytes indicates that sulfate turnover may be of importance in leukocyte metabolism.

**Summary and Conclusions**

1. Certain differences are demonstrated in the metabolic turnover of l-cystine sulfur and inorganic sulfur by normal and leukemic leukocytes. These differences are apparent both in vivo and in vitro and may be associated with factors peculiar to the metabolism of leukemic leukocytes.

2. The incorporation of \( ^{35}S \) l-cystine or \( ^{35}S \) sodium sulfate by leukocytes appears to be associated primarily with the presence of immature granulocytes.
3. Both l-cystine (or l-cysteine) and sulfate may be of importance in the metabolism of leukocytes.

**SUMMARIO E CONCLUSIONES IN INTERLINGUA**

1. Es demonstrate certe differentias in le metabolismo leucocytic de l-cystina e de sulfure inorganic in le caso de leucocytos normal e de leucocytos leucemic. Iste differentias es apparente tanto in vivo como etiam in vitro, e il pare permisse associar los con factores characteristic in le metabolismo de leucocytos leucemic.

2. Le incorporation, del parte de leucocytos, de l-cystina etiquettate per $\text{S}^{35}$ e de sulfato de natrium equalmente etiquettate pare esser associate primarimente con le presentia de immatur granulocytos.

3. Tanto l-cystina (o l-cysteina) como etiam sulfato es possibilemente de importantia in le metabolismo de leucocytos.

**REFERENCES**


Comparative Incorporation of $^{35}S$ L-Cystine and $^{35}S$ Sodium Sulfate by Normal and Leukemic Leukocytes

AUSTIN S. WEISBERGER and LEIF G. SUHRLAND