Studies on Analogues of L-Cysteine and L-Cystine

I. Some Structural Requirements for Inhibiting the Incorporation of Radioactive L-Cystine by Leukemic Leukocytes

By Austin S. Weisberger, Leif G. Suhrland and Joseph Seifert

THE SULFHYDRYL amino acid L-cysteine (L-cystine) appears to have an important role in the metabolism of leukocytes. Thus L-cysteine modifies the severe leukopenia characteristically induced by nitrogen mustard and this protective effect is distinctive in that a highly specific structural and spatial molecular configuration is required.2-4

The effects of L-cysteine depletion are also suggestive of the importance of this amino acid in the metabolism of leukocytes. Thus animals fed on diets deficient in L-cysteine or its metabolic precursor, L-methionine, develop leukopenia.5,6 Lack of cystine in the diet reduces the incidence of leukemia in animals, whereas addition of cystine increases the incidence.7 Similar restrictions of lysine and tryptophan have no effect on the incidence of leukemia. Cultures of human bone marrow on synthetic media deficient in either L-cysteine or L-cystine exhibit rapid cellular degeneration.8 Addition of either L-cysteine or L-cystine to the media results in a protective effect, especially on granulocytic leukocytes.

These observations suggest that a decreased availability of L-cysteine (L-cystine) may have important effects on leukocytes. Leukocytes of acute leukemia and chronic myeloid leukemia exhibit a more rapid turnover of radioactive L-cystine than do normal leukocytes8,10 and as a result may be more susceptible to depletion of this amino acid than normal leukocytes. The following experiments were therefore undertaken to study the possibility of decreasing the influx of radioactive L-cystine into leukemic leukocytes in vitro. Leukemic leukocytes were exposed to various compounds with structures closely related to cysteine (cystine) prior to incubation with radioactive L-cystine. It was found that only compounds with a structural and spatial configuration closely related to either

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* Since the reversible interconversion of cystine and cysteine is readily accomplished, these two compounds are considered as a single amino acid in metabolism1.
L-cystine or L-cysteine were capable of decreasing the influx of radioactive L-cystine into leukemic leukocytes.

METHODS

All studies were carried out in vitro with whole blood using heparin* as an anticoagulant. All glassware, needles and syringes were coated with non-wettable agents.† Incorporation studies were carried out in a water bath at 37 C. with blood from patients with acute leukemia, chronic myeloid leukemia or chronic lymphatic leukemia. The majority of these studies was performed with leukocytes obtained from patients with chronic myeloid leukemia. Whole blood from these patients was incubated with the substance to be tested prior to adding sulfur35 (S35) L-cystine.‡ After further incubation with the S35 L-cystine the leukocytes were separated and the amount of S35 incorporated per ml packed leukocytes was determined by methods previously described.§ All experiments were performed in duplicate and were repeated with various cell populations. Each group of experiments on a given day was compared with a control value for that particular day as follows:

<table>
<thead>
<tr>
<th>Control</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Whole blood</td>
<td>1. Whole blood</td>
</tr>
<tr>
<td>2. Add saline</td>
<td>2. Add compound to be tested</td>
</tr>
<tr>
<td>3. Incubate 20 minutes</td>
<td>3. Incubate 20 minutes</td>
</tr>
<tr>
<td>4. Add S35 L-cystine</td>
<td>4. Add S35 L-cystine</td>
</tr>
<tr>
<td>5. Incubate 45 minutes</td>
<td>5. Incubate 45 minutes</td>
</tr>
<tr>
<td>6. Determine incorporation of S35 in leukocytes</td>
<td>6. Determine incorporation of S35 in leukocytes</td>
</tr>
</tbody>
</table>

The amount of S35 incorporated was calculated as a percentage of the uptake observed in the control determination. Thus each experiment served as its own control. Furthermore, values could then be compared with those obtained on different days and from different patients, thereby minimizing the effects of therapy or variations in the disease.

The comparative effect of preincubation with increasing amounts of unlabeled D-cysteine¶ and of L-cysteine on the incorporation of S35 L-cystine was first investigated. The amount of these amino acids added to test inhibition ranged from 0.1 to 2.0 micromoles per ml whole blood and was dissolved in .02 to 0.1 ml of 0.85 per cent saline. Twenty to 25 micrograms of S35 L-cystine (approximately 0.1 micromole containing 150,000 counts per minute), dissolved in .05 ml of 0.85 per cent saline, were added per ml whole blood after incubation with the unlabelled amino acids. The comparative uptakes were determined as described above.

Compounds containing different combinations of the reactive groups present in cysteine (sulphydryl, amino and carboxyl radicals) as well as various substituted analogues of cysteine and cystine were then investigated for their ability to interfere with the incorporation of S35 L-cystine (tables 2 and 3). In addition, substances known to affect cysteine metabolism or known to produce a leukopenia were studied. Equimolar amounts (1.0 micromole per ml whole blood) of the substances tested were used whenever possible.

* Heparin, sodium. 1000 USP units per ml., Upjohn and Co., Kalamazoo, Michigan.
† Desicote, Beckman Instruments, Inc., South Pasadena, California. Tris Dodecyl Ammonium Chloride and Isopropanol, Armour Laboratories, Chicago, Illinois.
‡ S35 L-cystine was obtained from Abbott Laboratories, Chicago, Illinois.
¶ C/m per ml packed leukocytes = \( \frac{\text{Hematocrit of sample}}{\text{c/m per ml sample}} \)
§ D-cysteine hydrochloride was obtained from the California Foundation for Biochemical Research, Los Angeles, Calif., and showed a specific rotation (a)D = -3.4° in water. D-cysteine was also obtained from H&M Chemical Company, Ltd., Santa Monica, Calif., Lot No. 11617-30.
RESULTS

1. Comparative effects of unlabeled D- and L-cysteine on the incorporation of $^{35}$S

L-cysteine

Incubation of leukemic leukocytes with increasing amounts of unlabeled L-cysteine prior to adding $^{35}$S L-cystine resulted in a progressive decrease in the amount of $^{35}$S incorporated by the leukocytes (table 1, fig. 1). In contrast, preincubation with similar amounts of unlabeled D-cysteine did not appreciably decrease the amount of $^{35}$S incorporated by the same cell population. Thus preincubation with 2 micromoles of L-cysteine per ml. blood reduced the incorporation of the subsequently added $^{35}$S L-cystine to 24 per cent of the control value. Following incubation with the same amount of D-cysteine, the subsequent incorporation of $^{35}$S L-cystine was 91 per cent of the control value. The decreased influx of $^{35}$S L-cystine obtained when cells were preincubated with L-cysteine was independent of the type of leukemic leukocytes used in the study.

2. Effect of modification or substitution of the sulfhydryl, amino or carboxyl groups of L-cysteine (L-cystine)

a). Compounds ineffective in decreasing the influx of $^{35}$S L-cystine into leukemic leukocytes.

Substitution or modification of any of the vicinal reactive groups present in cysteine (cystine) resulted in a complete loss of any inhibitory effect on the incorporation of radioactive cystine as determined by these methods (table 2, fig. 2). Any alteration in the spatial configuration of the sulfhydryl or amino groups likewise renders an analogue ineffective in decreasing the influx of $^{35}$S L-cystine. Thus preincubation with D,L-isocysteine, * which is identical with

* Furnished through the generosity of Dr. William Wingo, School of Medicine, Department of Biochemistry, University of Alabama.

Table 1.—Comparative Effect of Non-Isotopic D and L-cysteine on the Incorporation of $^{35}$S L-Cystine by Leukemic Leukocytes

<table>
<thead>
<tr>
<th>Amount of Non-Isotopic Amino Acid Added Per ML Blood</th>
<th>Incorporation of $^{35}$S (c/m per ML Packed WBC’s)</th>
<th>Incorporation of $^{35}$S (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu$M L-cysteine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10,420</td>
<td>100</td>
</tr>
<tr>
<td>0.10</td>
<td>7,930</td>
<td>76</td>
</tr>
<tr>
<td>0.5</td>
<td>5,610</td>
<td>53</td>
</tr>
<tr>
<td>0.9</td>
<td>4,100</td>
<td>39</td>
</tr>
<tr>
<td>2.0</td>
<td>2,540</td>
<td>24</td>
</tr>
<tr>
<td>$\mu$M D-cysteine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>9,770</td>
<td>94</td>
</tr>
<tr>
<td>0.3</td>
<td>10,010</td>
<td>96</td>
</tr>
<tr>
<td>0.6</td>
<td>9,700</td>
<td>94</td>
</tr>
<tr>
<td>0.9</td>
<td>9,540</td>
<td>92</td>
</tr>
<tr>
<td>2.0</td>
<td>9,450</td>
<td>91</td>
</tr>
</tbody>
</table>

* Serial determinations were obtained with five different cell populations and the mean values are presented.
L-cysteine except for reversal of the positions occupied by the sulfhydryl and amino groups within the molecule (fig. 2), did not decrease the influx of S\textsuperscript{35} L-cystine. Similarly, in contrast to L-cysteine, D-cysteine had no inhibitory effect. Homocysteine, which differs from cysteine only in containing an added methylene group, was completely ineffective. Glutathione also was ineffective even though cysteine is present in the molecule in conjugation with glycine and glutamic acid.

L-serine and L-alanine, which are similar to L-cysteine except for substitution of the sulfhydryl group, had no inhibitory effect. \(\beta\)-mercapto-ethylamine, which may be obtained from cysteine by removal of the carboxyl group, also had no inhibitory effect. Similarly, compounds containing a sulfhydryl and carboxyl group but no amino group were not effective in decreasing the influx of radioactive L-cysteine (table 2, fig. 2).

Allylglycine, in which a vinylene group is substituted for the sulfhydryl group of cysteine, was ineffective even though this compound is a blocking analogue of cysteine for bacteria.\textsuperscript{11}

Many of these compounds appeared to increase rather than to decrease the incorporation of S\textsuperscript{35} L-cystine. Thus following preincubation with some compounds, the subsequent incorporation of S\textsuperscript{35} L-cystine exceeded the control value.

b). Compounds effective in decreasing the influx of S\textsuperscript{35} L-cystine into leukemic leukocytes.

Of the compounds studied, only those with structures closely related to L-cysteine, in which the sulfhydryl, amino and carboxyl group are relatively unmodified, were effective in decreasing the incorporation of radioactive cystine
TABLE 2.—Compounds Ineffective in Decreasing the Influx of $^{35}$S L-Cystine into Leukemic Leukocytes*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Cysteine</td>
<td>1.0 micromole per ml. blood whenever possible.</td>
</tr>
<tr>
<td>D,L-Isocysteine</td>
<td>0.1 micromole per ml.</td>
</tr>
<tr>
<td>D,L-Homocysteine</td>
<td>0.05 mg. per ml.</td>
</tr>
<tr>
<td>Djenkolic Acid</td>
<td>1 mg per ml.</td>
</tr>
<tr>
<td>Glutathione</td>
<td>10,000 U per ml.</td>
</tr>
<tr>
<td>L-Serine</td>
<td>0.1 mg per ml.</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>0.1 mg per ml.</td>
</tr>
<tr>
<td>3-Mercaptoethylamine</td>
<td>3.0 mg per ml.</td>
</tr>
<tr>
<td>S-Ethyl L-Cysteine</td>
<td>3.0 mg per ml.</td>
</tr>
<tr>
<td>S-Benzyl L-Cysteine</td>
<td>3.0 mg per ml.</td>
</tr>
<tr>
<td>Allylglycine</td>
<td>3.0 mg per ml.</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>3.0 mg per ml.</td>
</tr>
<tr>
<td>Ethionine</td>
<td>3.0 mg per ml.</td>
</tr>
</tbody>
</table>

* Uptake of $^{35}$S after preincubation with these substances varied from 85 per cent to 140 per cent of the control values.

† 1 micromole per ml. blood used whenever possible.

**COMPOUNDS INEFFECTIVE IN DECREASING THE INCORPORATION OF RADIOACTIVE L-CYSTINE BY LEUKEMIC LEUKOCYTES**

![Diagram of compounds effective in decreasing the influx of $^{35}$S L-cystine into leukemic leukocytes.](image)

**Fig. 2.**—Structural configuration of compounds ineffective in decreasing the incorporation of $^{35}$S L-cystine into leukemic leukocytes. Despite the close similarity of these compounds to L-cysteine, none of these compounds decrease the influx of $^{35}$S L-cystine. Alteration of the spatial location of the sulfhydryl or amino group as compared to L-cysteine results in failure to inhibit the incorporation of $^{35}$S L-cystine (D-cysteine, isocysteine and homocysteine). Modification, substitution or omission of either the sulfhydryl, amino or carboxyl groups present in L-cysteine also results in failure to inhibit the incorporation of radioactive L-cystine.

by leukemic leukocytes. The effective compounds were unlabeled L-cysteine, L-selenium cystine, D-selenium cystine and phenyl selenium cysteine* (table 3.

* The initial supplies of L-selenium cystine and D-selenium cystine used in these experiments were furnished through the kindness of Dr. Arne Fredga, Professor of Organic
TABLE 3.—Compounds Effective in Decreasing the Influx of S\textsuperscript{35} L-Cystine into Leukemic Leukocytes

<table>
<thead>
<tr>
<th>Compound*</th>
<th>Uptake of S\textsuperscript{35} L-Cystine (% of Control)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. L-Cysteine (unlabeled)</td>
<td>45</td>
</tr>
<tr>
<td>2. L-Selenium Cystine</td>
<td>45</td>
</tr>
<tr>
<td>3. D-Selenium Cystine</td>
<td>50</td>
</tr>
<tr>
<td>4. Phenyl Selenium Cysteine</td>
<td>50</td>
</tr>
</tbody>
</table>

* 1 micromole per ml. blood used for preincubation prior to adding S\textsuperscript{35} L-Cystine.
† Per cent of Control = \frac{Uptake after preincubation with compound}{Uptake after preincubation with saline}

COMPOUNDS EFFECTIVE IN DECREASING THE INCORPORATION OF RADIOACTIVE L-CYSTINE BY LEUKEMIC LEUKOCYTES

![Structural formula of compounds](image)

L-CYSTEINE  SELENIUM CYSTEINE  PHENYL SELENIUM CYSTEINE

Fig. 3.—Structural configuration of compounds effective in decreasing the influx of S\textsuperscript{35} L-cystine into leukemic leukocytes. Note the close similarity in the structure of the selenium compounds to that of L-cysteine. The diselenide compound, selenium cystine (\(\beta,\beta'\) diseleno-dialanine), was used in these studies instead of selenium cysteine (\(\beta\) seleno-alanine) which is represented in the above figure for purposes of comparison.

and fig. 3). L-selenium cystine (diseleno-dialanine) is structurally closely related to L-cysteine and differs only in that selenium replaces sulfur within the molecule. Preincubation of leukemic leukocytes with 1.0 micromole of L-selenium cystine per ml. blood consistently reduced the incorporation of S\textsuperscript{35} to approximately 45 per cent of the control uptake values (table 3). This was as effective as preincubation with unlabeled L-cystine in decreasing the influx of S\textsuperscript{35} L-cystine.

D-selenium cystine was almost as effective as L-selenium cystine in decreasing the incorporation of radioactive L-cystine. This is in marked contrast to the ineffectiveness of unlabeled D-cysteine in diminishing the uptake of S\textsuperscript{35} L-cystine. Phenyl selenium cysteine (fig. 3) was also an effective inhibitor. However, other selenium compounds, which do not have structures related to cysteine or cystine, were ineffective. These include selenic acid, selenic oxide and diphenyl diselenide.

3. Effect of compounds known to modify the leukocyte count

Irradiation (5,000 r total), or such substances as urethane, aminopterin, potassium arsenite (Fowler's solution) or nitrogen mustard were ineffective in decreasing the incorporation of S\textsuperscript{35} L-cystine by these methods and in the
amounts employed. Excessive amounts of nitrogen mustard (0.5 mg. per ml. blood), however, decreased the incorporation of \(^{35}\)S L-cystine to 20 per cent of the control value. Neither cortisone nor citrovorum factor had any appreciable effect on the incorporation of the radioactive L-cystine.

**Discussion**

A highly specific structural configuration is apparently required to inhibit the incorporation of radioactive L-cystine by leukemic leukocytes in vitro. Although preincubation with unlabeled L-cysteine decreases the influx of \(^{35}\)S L-cystine, many reactive compounds with structures closely related to cysteine or cystine are ineffective. In general, any modification or substitution of the sulfhydryl, amino or carboxyl group present in cysteine results in a complete loss of inhibitory activity. An exception to this observation is that the sulfur of cystine may be replaced by selenium without loss of inhibitory activity.

A specific spatial configuration involving the sulfhydryl and amino groups is apparently also required to decrease the influx of \(^{35}\)S L-cystine. Thus D-cysteine, which differs from L-cysteine only in the spatial location of the sulfhydryl group, and D,L-isocysteine, which differs only in that the sulfhydryl and amino groups occupy reversed positions in the molecule, are completely ineffective in decreasing \(^{35}\)S L-cystine incorporation. Of the compounds studied and in the concentrations employed, only unlabeled L-cysteine, selenium cysteine and benzylselenium cysteine were effective in decreasing the influx of \(^{35}\)S L-cystine.

Such structural and spatial requirements for decreasing the incorporation of \(^{35}\)S L-cystine suggest that specific intracellular receptors are involved in the incorporation of L-cysteine or L-cystine. According to Heinz, preincubation with an unlabeled amino acid should result in a decreasing influx of the corresponding radioactive amino acid only if there is a binding process involving these substances within the cell. Thus unlabeled L-cysteine may diffuse through the cell membrane and combine with some specific cytoplasmic constituents or receptors so as to compete with or inhibit the uptake of the subsequently added \(^{35}\)S L-cysteine. An opposite effect was obtained by Heinz in kinetic studies on the transport of C\(^{14}\) glycine into Ehrlich mouse ascites tumor cells. With these cells an increased influx of labeled glycine occurred after preincubating the cells with unlabeled glycine. This was interpreted as signifying that intracellular binding of glycine is limited in these cells.

It is possible that the reduced uptake of \(^{35}\)S L-cystine following exposure of the cells to unlabeled L-cysteine may be due to extracellular dilution of the radioactive amino acid by the unlabeled amino acid during the period of incubation. No such dilution effect apparently occurs with similar amounts of unlabeled D-cysteine or equivalent amounts of other sulfhydryl containing compounds. The possibility of interference of influx of \(^{35}\)S by extracellular dilution was investigated by removing the excess unlabeled L-cysteine prior to adding \(^{35}\)S L-cystine. This was achieved by washing the cells before adding the radioactive L-cystine. When this was done, preincubation with unlabeled L-cysteine still resulted in decreasing the influx of \(^{35}\)S L-cystine as compared with control preparations handled in the same manner. The decrease in the incorporation of \(^{35}\)S was not, however, as great as when the excess unlabeled
L-cysteine was not removed. This may be due in part to back diffusion of intracellular cysteine during the saline wash. The possibility of some dilution of $^{35S}$L-cystine in the extracellular incubation phase is not entirely excluded. It seems more likely, however, that intracellular binding or saturation accounts for most of the decreased influx following preincubation with unlabeled L-cystine.

The failure of D-cysteine to interfere with the incorporation of radioactive L-cystine may be due to lack of incorporation of the D-amino acid by leukemic leukocytes. It has been shown that D-amino acids in general are not utilized to an appreciable extent in metabolism. It is possible that D-cysteine does pass through the cell membrane but fails to combine with cytoplasmic constituents because of specific metabolic requirements or receptors for L-amino acids. It is also possible that D-cysteine is either rapidly excreted or destroyed. Similar experiments performed with L-methionine tend to confirm the relative ineffectiveness of D-amino acids. Thus unlabeled L-methionine decreases the influx of $^{35S}$L-methionine whereas unlabeled D-methionine has no effect on the incorporation of $^{35S}$L-methionine.

In contrast to the lack of inhibitory effect of D-cysteine, D-selenium cystine is as effective as L-selenium cystine in decreasing the influx of $^{35S}$L-cystine into leukemic leukocytes. D-selenium cystine may enter the cell and exert a toxic effect by virtue of the presence of selenium, even though it is not utilized in metabolic processes. Both D and L-selenium cystine may, however, exert their effect on the cell membrane rather than intracellularly.

The inhibitory effect of both D and L-selenium cystine is not due solely to a non-specific toxic effect of selenium. Thus other selenium compounds which do not have a structural configuration similar to cysteine or cystine are ineffective in decreasing $^{35S}$L-cystine incorporation. Both organic and inorganic selenium compounds are known to inactivate certain enzymes. It is possible that selenium cystine is effective because its structural relationship to cystine makes it possible for sufficient selenium to enter the cell so as to inactivate some enzymes necessary for normal cell function or to inactivate other essential compounds. In any event, the effectiveness of selenium cystine appears to depend to a great extent upon the structural relationship of this selenium compound to cystine.

Previous studies have indicated that L-cystine (L-cysteine) and related compounds may have an important role in leukopoiesis. Analogues of cystine or cysteine which interfere with the incorporation of these amino acids may therefore have a desirable effect in certain disorders of leukocytes. Since low concentrations of selenium analogues of cystine inhibit the incorporation of L-cystine by leukemic leukocytes in vitro, these compounds may also have an effect on the leukemic process in vivo.

**Summary and Conclusions**

The amino acids L-cysteine and L-cystine appear to have an important role in the metabolism of leukocytes. Decreased availability of these amino acids may therefore have important effects on leukocytes.

The possibility of decreasing the influx of radioactive L-cystine into leukemic leukocytes was investigated by exposing the leukocytes to various analogues of...
cysteine (cystine) prior to incubation with $^{35}$S L-cystine. It was found that a highly specific structural and spatial configuration is required to decrease the influx of $^{35}$S L-cystine. Thus unlabeled L-cysteine is effective in decreasing the incorporation of radioactive L-cystine. However, analogues of cysteine in which there is modification or substitution of the sulfhydryl, amino or carboxyl group do not decrease the influx of $^{35}$S L-cystine. Furthermore, any alteration in the spatial relationship of the sulfhydryl and amino groups of L-cysteine also results in a loss of the ability of an analogue to decrease the incorporation of $^{35}$S L-cystine.

Of the compounds studied and in the concentrations employed, only unlabeled L-cysteine, selenium cystine and phenyl selenium cysteine were effective. Selenium cystine is identical with cystine except that selenium replaces the sulfur in the molecule. Phenyl selenium cysteine is also closely related structurally to cysteine.

The mechanism of action of selenium cystine and phenyl selenium cysteine in decreasing the influx of $^{35}$S L-cystine is not known. Other selenium compounds tested were ineffective. These compounds may exert their inhibitory effect by (a) competitive combination with specific intracellular receptors for L-cysteine (L-cystine), (b) inactivation of enzymes or compounds essential for normal cellular function, (c) alteration in membrane permeability or (d) a toxic effect of selenium.

Since selenium cystine and phenyl selenium cystine are inhibitory in low concentrations in vitro, these compounds may have important effects on leukemic leukocytes in vivo.

**Summario e Conclusiones in Interlingua**

Le aminoacidos L-cysteina e L-cystina pare haber importante functiones in le metabolismo del leucocytos. Ergo il es a supponer que le reducere disponibilitate de ille aminoacidos va resultar in significative effectos super le leucocytos.

Le possibilitate de reduce le influxo de radioactive L-cystina in leucemic leucocytos esseva investigate per exponer le leucocytos a varie analogos de cysteina (cystina) ante lor incubation con L-cystina a $^{35}$S. Il eseva constatate que un specificissance configuration structural e spatial es requisite pro reduce le influxo de L-cystina a $^{35}$S. Non-etiquettate L-cystina, per exemplo, ha le effecto de reduce le incorporation de radioactive L-cystina. Tamen, analogos de cystina con modificationes o substitutiones del gruppo sulfhydrylic, aminic, o carboxyl non reduce le influxo de L-cystina a $^{35}$S. In plus, omne alteration in le relation spatial del gruppos sulfhydrylic e aminic intra le molecula de L-cysteina resulta etiam in un reduction del capacitate del analogo a reduce le incorporation de L-cystina a $^{35}$S.

Inter le compositos studiate, e in le concentrationes empleate, solmente non-etiquettate L-cysteina, selenio-cystina, e phenylo-selenio-cysteina esseva efficace. Selenio-cystina es identic con cystina excepte que selenium reimplacia le sulfure in le molecula. Phenyl-selenio-cysteina es etiam de structura multo affin a illo de cysteina.

Le mecanismo del reduce influxo de L-cystina a $^{35}$S sub le influentia de selenio-cystina e phenylo-selenio-cystina non es cognoscite. Altere compositos a
selenium que eseva examine per nos se provava inefficace. Il es possibile que iste compositos exerce lor effecto inhibitori (a) per usurpar un placia in le combination con receptores intracellular specific pro L-cysteina (L-cystina), (b) per inactivar enzymas o compositos que es indispensabile pro le normal functionamento cellular, (c) per effectuar un alteration del permeabilitate membranal, o (d) per le effecto toxic de selenium.

Proque selenio-cystina e phenylo-selenio-cystina exerce lor effecto inhibitori in vitro in basse concentrationes, il pare possibile que iste compositos ha effectos significative super leucocytos leucemic in vivo.

REFERENCES

5 ——: Unpublished observations.
Studies on Analogues of L-Cysteine and L-Cystine: I. Some Structural Requirements for Inhibiting the Incorporation of Radioactive L-Cystine by Leukemic Leukocytes

AUSTIN S. WEISBERGER, LEIF G. SUHRLAND and JOSEPH SEIFTER