INFLUENCE OF IODOACETIC ACID ON SULFUR METABOLISM. GROWTH STUDIES IN THE YOUNG RAT

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The extensive experimental use of iodoacetic acid for altering normal reactions has disclosed suggestive relationships between this compound and certain substances containing the sulfhydryl group. Iodoacetic acid inhibition of methylglyoxalase is reversed by the tripeptide glutathione, or by cysteine (1); the poisoning effect of iodoacetic acid on the carbohydrate metabolism of brain tissue can be almost completely prevented by the addition of glutathione or cysteine (2); inhibition of development of sea urchin eggs, produced by iodoacetic acid, may be overcome and growth reinitiated under the influence of sulfhydryl (3). These experiments and many others strongly suggest that a portion of the effect produced by iodoacetic acid in many systems may be explained on the basis of a combination of the halogen-containing acid with sulfhydryl, a reaction which has been demonstrated to occur in vitro (1, 3, 4) and which may be represented as follows:

\[ R-\text{SH} + \text{ICH}_2\text{COOH} \rightarrow R-\text{SCH}_2\text{COOH} + \text{HI}. \]

In view of the evidence suggesting a reaction between sulfhydryl and iodoacetic acid, the present study was initiated to examine the influence of iodoacetic acid on the reactions in the living organism which involve sulfur-containing compounds. It has previously

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been demonstrated that a wide variety of organic compounds inhibits the growth of young rats subsisting on a diet of relatively low content of sulfur-containing amino acids (5, 6). The data obtained have led to the suggestion that these growth-inhibiting substances exert their effect by imposing on the organism an abnormally high demand for the sulfur-containing amino acids for detoxication mechanisms. Thus the organism is deprived of essential elements required for the synthesis of the new tissue necessary for growth.

With a similar experimental approach, a study has been made of the effect of iodoacetic acid on the growth of the rat. The influence of various supplements on the inhibited growth rate has been determined.

EXPERIMENTAL

Male rats, at weaning, were placed in individual cages and fed, ad libitum, a basal diet having the following composition: casein\(^1\) 6, starch 50, lard 24, sucrose 15, cod liver oil 1, and salt mixture\(^2\) 4 per cent. In addition, each animal received a daily supplement of 400 mg. of dried yeast.\(^3\) When the animals had reached a body weight of approximately 75 to 85 gm., 100 mg. of iodoacetic acid\(^4\) were incorporated into each 100 gm. of the basal diet. Growth was immediately inhibited. After the animals had subsisted for at least 4 weeks on the iodoacetic acid-containing diet, a supplementary compound was incorporated into the ration already containing iodoacetic acid. The supplementary compounds and the amounts of each incorporated singly into each 100 gm. of the iodoacetic acid-containing basal diet were l-cystine (360 mg.), l-cysteine hydrochloride (470 mg.), dl-methionine (450 mg.), dl-homocystine (402 mg.), d-cystine (360 mg.), taurine (375 mg.), anhydrous sodium sulfate (426 mg.), and l-phenyluraminocysteine (717 mg.). The l-cystine was obtained from hair; l-cysteine hydrochloride was prepared by reduction of l-cystine (8); dl-methionine was obtained from the Organic Chemical Manufactures Division,

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\(^1\) Casein No. 453, Casein Company of America.

\(^2\) Osborne and Mendel salt mixture (7).

\(^3\) Northwestern Yeast Company.

\(^4\) Eastman Kodak Company product, recrystallized from warm petroleum ether until a melting point of 81-82° (uncorrected) was obtained.
University of Illinois. The $dl$-homocystine was prepared from $dl$-methionine by the method of Butz and du Vigneaud (9), with the modification suggested by Brand, Cahill, and Block (10). Optically pure $d$-cystine was obtained by resolution of $dl$-cystine (11); taurine was prepared by decarboxylation of cysteic acid (12); $l$-phenyluraminocysteine was obtained by the reduction of $l$-diphenyluraminocysteine with thioglycolic acid, with subsequent purification by repeated precipitation from ethyl acetate solution by the addition of 5 volumes of petroleum ether. Analysis for sulfhydryl sulfur indicated that 75 per cent of the isolated product was in the reduced form.

A second group of supplements was injected subcutaneously into animals ingesting the growth-inhibiting, iodoacetic acid-containing basal diet. Solutions of $l$-cysteine hydrochloride (38 mg. per rat per day), $dl$-methionine (36 mg. per rat per day), and glutathione (56 mg. per rat per day) were made immediately prior to injection, each solution being adjusted to approximately pH 7 with 0.1 N sodium hydroxide. Solutions of thioglycoler, riboflavin, and riboflavinphosphoric acid were made in quantities sufficient for not more than 1 week. 20 mg. of thioglycerol, and 40 $\gamma$ each of riboflavin and of riboflavinphosphoric acid were injected daily. Glutathione was obtained from Hoffmann-La Roche, Inc.; thioglycerol (60 per cent solution in glycerol) was kindly furnished by the Abbott Laboratories, Chicago. Riboflavin was obtained from the S. M. A. Corporation, Cleveland. Riboflavinphosphoric acid was synthesized from riboflavin by the method of Kuhn and Rudy (13). All of the compounds employed were analytically pure. The required quantity of each of the injected supplements mentioned above was contained in an administered volume of 0.5 cc. The effect of cortin supplements was determined by injection of 1 cc. of a cortin solution (Upjohn) three times daily. The quantity of cortin administered is sufficient to maintain an adrenalectomized animal in a healthy condition.

Results

A summary of the average daily weight changes and food consumptions under the various dietary conditions of the study is given in Table I and representative graphs illustrating the
growth rates of the animals are presented in Figs. 1 to 3. The data show the inhibitory effect on growth resulting in young rats weighing about 80 gm. when iodoacetic acid is incorporated into

<table>
<thead>
<tr>
<th>Diet</th>
<th>Designation of diet in Figs. 1 to 3</th>
<th>No. of animals in group</th>
<th>Average daily weight change gm.</th>
<th>Average daily food consumption gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>C-6</td>
<td>83</td>
<td>+1.8</td>
<td>6.8</td>
</tr>
<tr>
<td>&quot; + iodoacetic acid</td>
<td>I</td>
<td>95</td>
<td>0.0</td>
<td>3.6</td>
</tr>
<tr>
<td>&quot; + injected iodoacetic acid</td>
<td>I'</td>
<td>8</td>
<td>+0.3</td>
<td>5.3</td>
</tr>
<tr>
<td>&quot; + &quot; &quot; &quot; &quot; &quot; + injected cysteine</td>
<td>I'-CH'</td>
<td>3</td>
<td>+1.5</td>
<td>5.7</td>
</tr>
<tr>
<td>Iodoacetic acid-containing basal diet + each of the following growth-promoting supplements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary l-cystine</td>
<td>I-lC,</td>
<td>14</td>
<td>+1.0</td>
<td>5.5</td>
</tr>
<tr>
<td>&quot; l-cysteine hydrochloride</td>
<td>I-CH</td>
<td>7</td>
<td>+1.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Injected &quot;</td>
<td>I-CH'</td>
<td>17</td>
<td>+1.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Dietary dl-methionine</td>
<td>I-M</td>
<td>9</td>
<td>+1.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Injected &quot;</td>
<td>I-M'</td>
<td>8</td>
<td>+0.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Dietary dl-homocystine</td>
<td>I-H</td>
<td>4</td>
<td>+1.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Injected glutathione</td>
<td>I-GSH'</td>
<td>8</td>
<td>+1.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Iodoacetic acid-containing basal diet + each of the following ineffective supplements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary d-cystine</td>
<td>I-dC</td>
<td>5</td>
<td>-0.2</td>
<td>4.2</td>
</tr>
<tr>
<td>&quot; taurine</td>
<td>I-T</td>
<td>5</td>
<td>+0.1</td>
<td>4.3</td>
</tr>
<tr>
<td>&quot; sodium sulfate</td>
<td>I-S</td>
<td>4</td>
<td>-0.4</td>
<td>4.0</td>
</tr>
<tr>
<td>&quot; l-phenylalamino-cysteine</td>
<td>I-PC</td>
<td>7</td>
<td>-0.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Injected thioglycerol</td>
<td>I-TG'</td>
<td>5</td>
<td>+0.2</td>
<td>4.1</td>
</tr>
<tr>
<td>&quot; riboflavin</td>
<td>I-F</td>
<td>3</td>
<td>-0.05</td>
<td>4.0</td>
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<tr>
<td>&quot; riboflavin-phosphoric acid</td>
<td>I-FP'</td>
<td>6</td>
<td>-0.13</td>
<td>4.1</td>
</tr>
<tr>
<td>&quot; cortical extract</td>
<td>I-CE'</td>
<td>4</td>
<td>0.0</td>
<td>4.1</td>
</tr>
</tbody>
</table>

the basal diet. This observation is similar to the finding of Laszt and Verzar (14); the latter investigators succeeded in arresting growth by the inclusion of iodoacetic acid at a level of 1 part in 5000 parts of diet. The addition of l-cystine to the basal diet
containing iodoacetic acid results in a marked stimulation of growth as well as an increase in food consumption. The growth-

![Graph showing typical growth results obtained on basal diet, on basal diet with added iodoacetic acid, and on iodoacetic acid-containing basal diet as influenced by various supplements. The diet employed in any portion of an experiment is indicated between two downward arrows representing the beginning and end of a period. For interpretation of the abbreviations designating the diets in various periods, see the second column of Table I. The quantities of the supplements administered are given in the text. The average daily food consumption in gm. for the corresponding interval is shown by the figures between the upward arrows. The initial and final body weights are presented in parentheses.]

stimulating ability of L-cystine in animals fed iodoacetic acid is strikingly similar to previous studies (5, 6) in which this amino
acid stimulated the growth of animals ingesting one of a wide variety of toxic, growth-inhibitory compounds.

The experimental conditions under which iodoacetic acid stimulated growth did not preclude a possibility which might modify the interpretation of the observed effect. Inasmuch as both l-cysteine and iodoacetic acid were incorporated into the diet, it was possible that the two substances combined to form the known thio ether (1, 3, 4) before absorption from the gastrointestinal tract. In order to test this point, iodoacetic acid was fed in the diet, while l-cysteine\(^5\) was administered subcutaneously. Since l-cysteine hydrochloride was used rather than l-cystine because of the greater solubility of the former in water. The solution was prepared and neutralized as described in the text.

Fig. 2. For the dietary abbreviations used in this chart, see the second column of Table I. The other designations are the same as in Fig. 1.
the injected amino acid stimulated growth as well as did dietary cystine, it is concluded that the growth-promoting action of cystine supplements is not dependent on a preliminary combination of iodoacetic acid with cystine prior to the absorption of these compounds. In other experiments, iodoacetic acid and \(l\)-cysteine were injected subcutaneously on opposite sides of the animal further to demonstrate that the intestine, or an intestinal factor, does not detoxicate iodoacetic acid before absorption. The data in these instances were similar to those obtained with orally administered supplements. Young rats exhibit an extreme sensitivity to injected iodoacetic acid, a fact previously noted by Genevois and Brisou (15). After a considerable number of

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**Fig. 3.** For the dietary abbreviations used in this chart, see the second column of Table I. The other designations are the same as in Fig. 1.
trials, it was found that the daily subcutaneous injection of an aqueous solution containing 0.4 mg. of iodoacetic acid, neutralized to pH 7.0 with 0.1 N sodium hydroxide, produced a growth inhibition similar in extent to that observed with orally administered iodoacetic acid (1 part in 1000 parts of the diet). Cysteine injections under these circumstances caused a prompt resumption in growth, despite continued simultaneous injection of the halogen-containing fatty acid. It may be mentioned that rats obtained from different colonies, the Yale laboratory and the Connecticut Agricultural Experiment Station, exhibited striking differences in their ability to tolerate the toxic effects of injected iodoacetic acid. Young rats from the Connecticut Agricultural Experiment Station could not be employed for studies of the effects of injected iodoacetic acid, inasmuch as their growth was not inhibited to any appreciable extent by the quantities of iodoacetic acid used to stunt animals in the laboratory colony.

Further suggestive evidence indicating that the relationship between iodoacetic acid and cystine is metabolic in nature is seen in the restricted pathological changes found on histological examination. The lungs, intestine, adrenals, pancreas, and spleen of iodoacetic acid-fed animals exhibited no abnormalities. However, in the four animals examined in detail, there was present marked necrosis of the convoluted tubules of the kidneys; destruction of the cellular structure had occurred with no evidence of regeneration. The localization of the pathological symptoms emphasizes the fact that the effects of iodoacetic acid observed in the present study cannot be accounted for by wide-spread toxic symptoms in the animal organism.

The sulfur-containing amino acids dl-methionine, dl-homocystine, and l-cysteine have all been demonstrated to substitute for l-cystine in the conventional cystine-low diets (16–18). Further, methionine functions in lieu of cystine in promoting growth in rats stunted by a wide variety of toxic, organic compounds (5, 6). In animals ingesting the iodoacetic acid-containing basal diet, all the aforementioned sulfur-containing amino acids support growth as well as does cystine. In addition, glutathione, which had previously been found to stimulate growth in animals given

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a cystine-low diet (19), and in animals stunted by naphthalene (20) or by methylcholanthrene feeding (5), also actively promoted growth in the present experiments.

In contrast to the above results, the unnatural isomer of cystine was unable to cause growth of rats stunted by means of iodoacetic acid. Two explanations may be suggested. It is possible that \( d \)-cystine may not be readily reduced to \( d \)-cysteine, a reaction which might be expected to be a prerequisite for the postulated combination of the sulphydryl group with iodoacetic acid. The failure of the organism completely to oxidize \( d \)-cystine (21) indicates that metabolism of this isomer may proceed in part along lines differing from those believed to predominate in the case of the naturally occurring isomer of cystine. A second explanation is based on the hypothesis of Stekol (22), suggesting that cystine for detoxication is drawn from the tissues. The failure of \( d \)-cystine to stimulate growth in iodoacetic acid-inhibited animals would thus be related to the inability of the dextrorotatory isomer to enter into the tissue protein as a structural component.

The compounds \( l \)-phenyluraminocysteine, taurine, thioglycerol, and sodium sulfate were tested as sources of various forms of sulfur. The failure of these substances to promote growth demonstrates that the ability to stimulate growth in animals ingesting the iodoacetic acid-containing basal diet is not a general property of sulfate or sulphydryl sulfur, but rather depends upon the specific chemical requirement for cystine or methionine or both or for compounds yielding one or both of these sulfur-containing amino acids in metabolism.

Laszt and Verzar (14) have reported that riboflavinphosphoric acid was capable of stimulating growth in rats stunted with iodoacetic acid; riboflavin was without effect under these conditions. In the studies reported here, the growth-promoting properties of these two compounds were compared. The effects on growth rate produced by the injection of either riboflavin or riboflavinphosphoric acid into animals ingesting the iodoacetic acid-containing basal diet were practically identical (0.05 and 0.13 gm. average daily weight decline, respectively). It is concluded that riboflavinphosphoric acid does not promote growth in animals ingesting iodoacetic acid under the experimental conditions employed. Laszt and Verzar (14) also claimed that the
administration of a cortical extract (eucortone) was effective in promoting growth in iodoacetic acid-fed animals. The latter investigators postulated that the cortex of the adrenal functions antagonistically to iodoacetic acid and that an excess of the adrenal cortical hormone allows the organism again to resume normal phosphorylation processes. In the present study, however, when cortin was injected in an amount sufficient to maintain a completely adrenalectomized animal in a healthy condition, no stimulus to growth was observed in rats stunted by iodoacetic acid. It may be pointed out that the level at which iodoacetic acid was fed in the present study was considerably higher (1 part of iodoacetic acid in 1000 parts of basal diet) than that employed by Laszt and Verzar (1 part of iodoacetic acid added to 5000 parts of a milk-grain diet). Moreover, the latter investigators' animals weighed only 50 gm. at the time that the halogen-containing acid was incorporated into the diet.

The data which have been obtained support the general hypothesis (5) that the inhibition of growth produced in young rats by a wide variety of toxic compounds is related to the demands placed upon the organism by detoxication mechanisms involving either of the sulfur-containing amino acids, cystine and methionine. The detoxication requirements of the organism for these amino acids apparently take precedence over the needs for growth, and on a relatively low protein diet, with a limited supply of cystine and methionine, a wide variety of compounds may then produce growth inhibition. The addition of either of these two sulfur-containing amino acids, or of compounds which may yield either cystine or methionine in the organism, makes available material for both detoxication mechanisms and for the synthesis of new tissue protein. Under these conditions, a prompt resumption in growth occurs, despite the continued presence in the diet of the toxic foreign compound. It is realized that convincing proof for this hypothesis includes the demonstration by isolation of a detoxication product, involving one of the sulfur-containing amino acids, from the urine of the experimental animal following the administration of the toxic substance. Evidence of this type has been obtained only after the administration of bromobenzene (22, 23), naphthalene (22, 24), and anthracene (25).
SUMMARY

Growth inhibition has been produced in young rats by the addition of iodoacetic acid to a basal diet of relatively low protein content. The superimposition of l-cystine, l-cysteine hydrochloride, dl-methionine, or dl-homocystine on this iodoacetic acid-containing basal diet results in a prompt stimulation of growth with a resulting weight gain comparable to that observed in animals ingesting the basal diet alone. Subcutaneously injected l-cysteine hydrochloride or glutathione was also capable of stimulating growth in animals stunted with iodoacetic acid. On the other hand, the addition of d-cystine, taurine, anhydrous sodium sulfate, or l-phenyluraminocysteine to the basal diet containing the iodoacetic acid did not stimulate growth under the experimental conditions employed. Subcutaneously administered thioglycerol, riboflavin, riboflavinphosphoric acid, or cortical extract was also without effect on the inhibited growth rate. It is concluded that iodoacetic acid probably imposes a specific demand on the organism for the sulfur-containing amino acids, cystine or methionine, for detoxication purposes. Either of the latter compounds, or compounds yielding cystine or methionine in metabolism, may stimulate growth in the presence of iodoacetic acid by fulfilling the requirements for detoxication mechanisms and for the synthesis of tissue protein.

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