BIOSYNTHESIS OF THE PURINES

XV. THE EFFECT OF AZA-L-SERINE* AND 6-DIAZO-5-OXO-L-NORLEUCINE ON INOSINIC ACID BIOSYNTHESIS DE NOVO†

By BRUCE LEVENBERG,† IRVING MELNICK,§ AND JOHN M. BUCHANAN

(From the Division of Biochemistry, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts)

(Received for publication, August 27, 1956)

The compound aza-L-serine (δ-diazoacetyl-L-serine), which has been isolated from cultures of Streptomyces, has been shown to exhibit inhibitory action against Enro scelesteus in eggs, Crocke mouse sarcoma 180, and various types of mouse leucemia. It has also been shown to be effective against various Gram-positive and Gram-negative bacteria and certain fungi in vitro (1-4). Skipper and his colleagues have demonstrated that aza-L-serine, when injected into tumor-bearing mice, causes a diminution of the incorporation of either radioactive formate or glycine into the purines of the nucleic acid of several tissues (5). This led to the finding in this laboratory that azaserine inhibits the synthesis of inosinic acid de novo by pigeon liver extract and results in the accumulation of formylglycinamide ribotide1 by acting as an antimetabolite of glutamine (6, 7).

This communication reports detailed information on the site and mechanism of the inhibition by this compound, together with more recent data on the action of 6-diazo-5-oxo-L-norleucine on inosinic acid biosynthesis.

Materials and Methods

Aza-L-serine, 6-diazo-5-oxo-L-norleucine, and other glutamine analogues were generously provided by Dr. Alexander Moore of The Mellon Institute and Parke, Davis and Company.

The barium salt of FGAR was prepared by enzymatic synthesis as pre-

* The position of the prefix in the name aza-L-serine conforms with the rules of nomenclature of amino acids approved by the International Union of Pure and Applied Chemistry.
† Supported by a grant-in-aid from the Damon Runyon Memorial Fund for Cancer Research, Inc.
§ Research Fellow of the National Institute of Neurological Diseases and Blindness, United States Public Health Service (1954-55).
§ Karl Taylor Compton Fellow of the Nutrition Foundation in Biochemistry.
1 The following abbreviations are used: FGAR, formylglycinamide ribotide; FGAM, formylglycinamidine ribotide; AIR, 5-aminoimidazole ribotide; DON, 6-diazo-5-oxo-L-norleucine; IMP, inosinic acid; ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide; TCA, trichloroacetic acid; Tris, tris(hydroxymethyl)aminomethane; ADP, adenosine diphosphate.
Table I

Effect of Azaserine on Five Representative Reactions Concerned with Inosinic Acid Synthesis de Novo

<table>
<thead>
<tr>
<th>Reaction No.</th>
<th>Vessel</th>
<th>Azaserine</th>
<th>Glutamine</th>
<th>Product</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>0</td>
<td>1</td>
<td>124</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>1</td>
<td>1</td>
<td>103</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>10</td>
<td>1</td>
<td>71</td>
<td>48</td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>0</td>
<td>2</td>
<td>17.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.2</td>
<td>2</td>
<td>6.7</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>2.0</td>
<td>2</td>
<td>0.9</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>a</td>
<td>0</td>
<td>0</td>
<td>24.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>0.2</td>
<td>0</td>
<td>22.8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>2.0</td>
<td>0</td>
<td>15.0</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>a</td>
<td>0</td>
<td>0</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>2</td>
<td>0</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>0</td>
<td>0</td>
<td>63</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>1</td>
<td>0</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>2.5</td>
<td>0</td>
<td>69</td>
<td>0</td>
</tr>
</tbody>
</table>

Reaction 1: Glutamine + 5-phosphoribosylpyrophosphate → glutamic acid + 5-phosphoribosylamine + pyrophosphate. Measurement made of formation of glutamic acid spectrophotometrically by conversion to α-ketoglutarate by glutamic acid dehydrogenase in presence of DPN. The incubation medium contained, expressed in micromoles, glutamine, 1; 5-phosphoribosylpyrophosphate, 1; MgCl₂, 1; tris(hydroxymethyl)aminomethane buffer, pH 8.8, 15; and 0.05 ml. of enzyme; total volume, 0.3 ml. Incubation time, 30 minutes; 38 °C. This experiment was kindly performed by Mr. Standish C. Hartman.

Reaction 2: Formylglycinamide ribotide + glutamine + ATP → formylglycinamidine ribotide + ADP + orthophosphate + glutamic acid. FGAM synthesized from FGAR was measured by conversion to AIR. Incubation medium contained 0.06 pmole of FGAR, 2 μmoles of ATP, 20 μmoles of K₂SO₄, 7 μmoles of MgCl₂, 10 μmoles of sodium phosphate buffer, pH 7.4, and 0.06 ml. of purified enzyme; final volume, 0.35 ml. Incubation time, 30 minutes; 38 °C.

Reaction 3: Formylglycinamidine ribotide + ATP → 5-aminoimidazole ribotide. Measurement was made of conversion of FGAM to AIR by reaction of product with Bratton-Marshall reagents (11). Vessels contained, expressed in micromoles, barium salt of FGAM, 0.055; dipotassium salt of ATP, 1; 0.1 ml. (2 mg.) of enzyme fraction of pigeon liver. Salts and buffer were identical to those of Reaction 2; total volume, 0.5 ml. Incubation time, 30 minutes; 38 °C.

Reaction 4: 5-Aminoimidazole ribotide + aspartic acid + CO₂ + ATP → 5-amino-4-imidazolecarboxamide ribotide. Measurement made by Bratton-Marshall reaction (11) of the amount of 5-amino-4-imidazolecarboxamide ribotide formed. Materials, expressed in micromoles, were, in a final volume of 0.42 ml., barium salt of AIR, 0.062; potassium aspartate, 5; KHCO₃, 1.3; disodium salt of ATP, 1; sodium phosphate buffer, pH 7.4, 10; K₂SO₄, 11; MgSO₄, 3.5; 3 mg. of lyophilized “13 to 33” per cent ethanol fraction of pigeon liver extract. Azaserine added as indicated. Incubation time, 30 minutes; 38 °C.

Reaction 5: 5-Amino-4-imidazolecarboxamide ribotide + serine + TPN → inosinic acid + glycine + TPNH + H⁺. Measurement made of disappearance of 5-amino-4-imidazolecarboxamide ribotide by Bratton-Marshall reaction (11). Vessels co-
Table I—Continued

tained, expressed in micromoles, in a final volume of 0.50 ml., dipotassium salt of
5-amino-4-imidazolecarboxamide ribotide, 0.1; L-serine, 10; TPN, 0.1; isoleucovorin
chloride, 0.01; Tris buffer, pH 7.4, 30; and aliquots of three enzyme fractions, de-
scribed later. Incubation time, 15 minutes; 38°; terminated by addition of 0.5 ml.
of 10 per cent TCA. This experiment was kindly performed by Mr. Joel G. Flaks.

viously described (8). The sample of FGAR employed in most of the fol-

Enzymatic Synthesis and Assay of Formylglycinamidine Ribotide—The
enzyme preparation used for the study of the effect of azaserine or DON on
the conversion of FGAR to FGAM was either the partially purified Frac-
tion I (9) or a more purified enzyme (10), both of which are described in
preceding papers. The total volume of the incubation solution was usually
between 0.4 and 0.6 ml. Incubation was carried out for 10 minutes unless
otherwise noted and was terminated by heating the vessels in a boiling
water bath for 30 to 40 seconds. The tubes were then immediately chilled
in an ice bath. Fraction II, which contains the enzyme activity (FGAM →
AIR), was sometimes included in the incubation medium, and analysis
was made directly for AIR. In some instances, however, the FGAM
formed in the presence of Fraction I was converted to AIR by Fraction II
after denaturation of Fraction I by heat. The details of the method of
analysis of FGAM and AIR are included in Paper XIV (10).

Results

Site of Action of Azaserine on Inosinic Acid Biosynthesis—Although it
was known from experiments previously cited that azaserine inhibits spe-
cifically some enzyme system concerned with the conversion of FGAR to
IMP, it has been possible recently to make a survey of all the reactions
concerned with the biosynthesis of inosinic acid individually, since adequate
assay methods are now available with relatively purified enzymes. Spe-
cial note was made in two of the reactions which require glutamine as a
substrate to relate azaserine and glutamine concentration since preliminary
experiments had indicated that azaserine exerts its inhibitory action as an
antimetabolite of glutamine. The results of studies on five representative
enzymes of this biosynthetic sequence are shown in Table I. It may be
seen that in the case of only one reaction, the conversion of FGAR to
FGAM (Reaction 2), may inhibition by obtained at a low concentra-
tion of azaserine (0.2 µmole per 0.35 ml. of incubation volume). As will be shown
later, the degree of azaserine inhibition at this level is directly related to the
glutamine concentration. Thus, at a lower concentration of glutamine an
equivalent effect may be obtained at a lower concentration of inhibitor.

In the four remaining reactions, either inhibition could not be obtained
at higher concentrations of azaserine or the inhibition produced was substantially less than that obtained on Reaction 2 at an equivalent concentration of antibiotic. A comparison of the action of azaserine on the two reactions involving glutamine is of particular note. At a level of 10 μmoles, azaserine had less effect on Reaction 1 than did 0.2 μmole of inhibitor on Reaction 2. This corresponds with a recent report by Bentley and Abrams (12) that azaserine has a competitive action on glutamine in the enzymatic conversion of xanthyllic to guanylic acid, but only at relatively high concentrations of inhibitor. It is thus seen that, although azaserine may compete with glutamine in several reactions, its most pronounced effect enzymatically and probably also physiologically is its action on the enzyme responsible for the conversion of FGAR to FGAM.

**Table II**

Effect of Increased Levels of Glutamine on Inhibition of Reaction (FGAR → FGAM) Caused by Azaserine

The incubation system contained the following quantities of materials expressed in micromoles: FGAR, 1.2; ATP, 2; K₂SO₄, 20; MgCl₂, 7; sodium phosphate buffer, pH 7.4, 10; 0.02 ml. of purified enzyme of Fraction I, and 0.05 ml. of Fraction II. The final volume was 0.65 ml. The vessels were incubated for 10 minutes at 38°.

<table>
<thead>
<tr>
<th>Azaserine present (μmole)</th>
<th>Glutamine present (μmoles)</th>
<th>FGAM formed (μmole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15</td>
<td>2</td>
<td>56.5</td>
</tr>
<tr>
<td>0.15</td>
<td>4</td>
<td>38.0</td>
</tr>
<tr>
<td>0.15</td>
<td>6</td>
<td>44.0</td>
</tr>
<tr>
<td>0.15</td>
<td>8</td>
<td>46.5</td>
</tr>
<tr>
<td>0.15</td>
<td>10</td>
<td>46.5</td>
</tr>
</tbody>
</table>

Mechanism of Action of Azaserine—The competitive relationship of glutamine and azaserine is shown in Tables II and III. In the experiment in Table II, the concentration of azaserine was maintained constant but that of glutamine varied. Although in the uninhibited system 2 μmoles of glutamine were more than adequate to permit an optimal response of reaction, concentrations 3 to 4 times greater than this were required in the presence of 0.15 μmole of azaserine. Even at the highest concentration of glutamine, the rate of reaction in the presence of azaserine was substantially less than the optimal response obtained in the absence of inhibitor. This was one of the first indications that the mechanism of azaserine inhibition of this enzyme system was more complicated than at first suspected.

One feature of a competitive inhibitor is that the degree of inhibition of a reaction should be a function of the ratio of inhibitor to substrate rather than of the concentration of inhibitor itself. In Table III is shown an ex-
periment in which the concentrations of both azaserine and glutamine were varied 100-fold but their ratio maintained constant. The relative constancy of the degree of inhibition obtained under these circumstances is further evidence in support of the belief that azaserine and glutamine are structurally and metabolically related to each other.

A more quantitative appraisal of the action of azaserine on the enzyme system has been obtained by treatment of the data by the method of Line-weaver and Burk as modified by Waley (13) to account for substrate utilization. At optimal concentrations of the various auxiliary factors involved in the reaction (ATP, 3 μmoles per ml., K+, 3 μmoles per ml., Mg++, 10 μmoles per ml., glutamine, 3 μmoles per ml.), measurement was made of the Michaelis constant ($K_m$) of the substrate, formylglycinamide ribotide. In Fig. 1 are plotted the functions

$$\frac{1}{(S_0 - S_t)} \text{ versus } \ln \frac{S_o}{S_t} \left/ (S_0 - S_t) \right.$$ 

from the equation

$$1/(S_0 - S_t) = \frac{1}{\bar{V}_t} + \frac{K_m}{\bar{V}_t} \left( \ln \frac{S_o}{S_t} \right) \left/ (S_0 - S_t) \right.$$ 

where $S_o$ and $S_t$ are, respectively, the concentrations of substrate initially and at time, $t$. $\bar{V}$ is the maximal reaction velocity.

A value of $6.4 \times 10^{-5}$ M has been calculated as the $K_m$ of formylglycinamide ribotide.

**Determination of $K_m$ and $K_I$ of Glutamine and Azaserine, Respectively—**

In experiments designed to determine the relative binding power of glutamine and azaserine, each vessel contained, in a total volume of 0.50 ml., the following materials expressed in micromoles: barium salt of FGAR, 0.06; ATP, 2; K$_2$SO$_4$, 20; MgCl$_2$, 7; sodium phosphate buffer, pH 7.4, 10; and 0.06 ml. of Fraction I. Glutamine and azaserine were added as indicated.

<table>
<thead>
<tr>
<th>Vessel No.</th>
<th>Glutamine (μmoles)</th>
<th>Azaserine (μmoles)</th>
<th>FGAM synthesized (μmoles)</th>
<th>Inhibition (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.15</td>
<td>0.015</td>
<td>20.0</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>0.015</td>
<td>10.0</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
<td>0.15</td>
<td>24.0</td>
<td>57</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>0.60</td>
<td>10.4</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>6.0</td>
<td>1.50</td>
<td>8.7</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>15.0</td>
<td>1.50</td>
<td>7.4</td>
<td>60</td>
</tr>
</tbody>
</table>
tamine and azaserine for the enzyme, a concentration of the substrate, formylglycinamide ribotide, was used, which was approximately 30 times greater than the $K_m$ value. Under these circumstances the concentration of glutamine has been varied at three different concentrations of azaserine (Fig. 2). From the plot of $1/V$ versus $1/S$, where $V$ and $S$ are the velocity of the reaction and concentration of glutamine, respectively, the values $1/K_p$ may be estimated graphically and the values $K_p$, the apparent constants of glutamine in the presence of inhibitor, calculated. When these $K_p$ values were plotted against azaserine concentration (Fig. 3), a value of $6.2 \times 10^{-4} \text{ M}$ was obtained for the $K_m$ of glutamine and $3.4 \times 10^{-5} \text{ M}$ for the $K_I$ of azaserine. The ratio of these two values, $K_m/K_I$, is 18, a value which indicates that azaserine is a fairly potent inhibitor of the reaction.

**Effect of Preincubation of Azaserine with Enzyme**—In spite of the competitive nature of glutamine and azaserine demonstrated above, further experiments to be described indicated that a simple competitive relationship of these two compounds did not explain all aspects of azaserine inhibition of the enzyme. In Fig. 4 are described experiments in which the order of addition of the two compounds to the incubation mixture was studied. In these experiments all the vessels containing at least enzyme and buffered...
salts were preincubated at 38° for 2 minutes. In addition, some vessels contained either azaserine or glutamine or both during the preincubation period. At the conclusion of the preincubation period the substrates, ATP and formylglycinamide ribotide, were added, together with amounts of azaserine or glutamine required to bring all vessels (except the control,
first column) to the same composition of enzyme, substrates, and inhibitor. All the vessels were then incubated for 10 minutes, during which time reaction of FGAR was permitted to take place. The control vessel did not contain azaserine during either the preincubation or the incubation period. The values in parentheses in Fig. 4 indicate that substances were added in the preincubation period and carried over into the main incubation. As shown in the first two columns, the expected inhibition of reaction occurred during the incubation period when both azaserine and glutamine were in-

![Fig. 4. Effect of preincubation of azaserine with the enzyme; relationship of glutamine to this inhibition. In the preincubation period, 0.02 ml. of the purified enzyme of Fraction I was incubated with 0.1 ml. of a solution containing 10 μmoles of sodium phosphate buffer, pH 7.4, 10 μmoles of MgCl₂, and 20 μmoles of K₂SO₄, and with 0.1 ml. of either water or additions of azaserine or glutamine as indicated. This solution (0.22 ml.) was incubated for 2 minutes at 38°. At this point the main incubation was begun for a period of 10 minutes after adding 0.6 μmole of FGAR, 2 μmoles of ATP, 0.05 ml. of Fraction II, and quantities of azaserine or glutamine as indicated.](http://www.jbc.org/)

cluded in the reaction system as compared to when glutamine was present alone. However, when azaserine alone was included in the vessel with enzyme during the preincubation period, addition of glutamine together with the other substrates at a later time did not result in reaction of FGAR. However, if glutamine were added first (during the preincubation period) and azaserine later (fourth column), the extent of reaction was about the same as in the vessel represented by the second column. Again, if both azaserine and glutamine were included during the preincubation period, the enzyme was protected sufficiently to permit considerably greater reaction in the vessel represented by the fifth column than that in the vessel shown by the third column.
These results demonstrate that, if azaserine is permitted to react with the enzyme in the absence of glutamine, the enzyme is inactivated irreversibly. Glutamine has no effect on the course of this inhibition if it is added after the azaserine. On the other hand, if glutamine is incubated simultaneously with the azaserine, it competes with the inhibitor for the enzyme site and delays the inactivation of the enzyme.

**Time Study of Azaserine Effect on Enzyme at Different Concentrations of FGAR**—The fact that glutamine delays rather than prevents the irreversible inactivation of the enzyme caused by azaserine is shown in the experiments reported in Fig. 5, in which a study was made of the effect of the inhibitor on the course of the reaction at two concentrations of FGAR but at a constant concentration of glutamine or azaserine. At the lower concentration of FGAR (0.03 μmole), the reaction was essentially complete after

![Time study of azaserine effect on enzyme reaction at different levels of FGAR.](http://www.jbc.org/)

**Fig. 5.** Time study of azaserine effect on enzyme reaction at different levels of FGAR. The vessels represented by Curves A and B each contained, in a final volume of 0.43 ml., 0.03 μmole of Ba-FGAR, 2 μmoles of ATP, 1 μmole of glutamine, and 0.10 ml. of "buffer-salts solution," containing 10 μmoles of sodium phosphate buffer, pH 7.4, 20 μmoles of K₂SO₄, and 7 μmoles of MgSO₄. In addition, the vessels of Curve A contained 0.075 μmole of azaserine. The vessels represented by Curves C and D each contained 0.15 μmole of Ba-FGAR. Other materials were added at the same levels as in the vessels of Curves A and B. Azaserine (0.075 μmole) was present in the vessels of Curve C. All the vessels contained Fraction I.
10 minutes, the level of total reaction in the vessel which contained azaserine being about one-half that of the controls which contained no inhibitor. The cessation of the uninhibited reaction at a level (12 mMoles) below the theoretical value of complete reaction (30 mMoles) is always observed when Fraction I is incubated alone with the substrates and then Fraction II is added later to convert the FGAM formed into AIR, as required by the method of assay. When Fractions I and II are incubated simultaneously and direct determination is made for the formation of AIR,

Fig. 6. Comparison of the effect of azaserine and DON as inhibitors of the enzyme system (FGAR → FGAM). The basic system consisted of 2 μmoles of ATP, 2 μmoles of glutamine, 0.1 μmole of FGAR, 10 μmoles of sodium phosphate buffer, pH 7.4, 20 μmoles of K₂SO₄, 7 μmoles of MgCl₂, and 0.1 ml. of Fraction I and 0.05 ml. of Fraction II in a final volume of 0.65 ml. The vessels were incubated for 10 minutes at 38°. (○) vessels containing azaserine; (●) vessels containing 6-di Zoe-5-oxo-1-norleucine.

the quantities of FGAR converted to AIR approach the theoretical quantities (in the absence of azaserine).

When incubation was carried out at a higher level of FGAR (0.15 μmole), a different pattern of results was obtained. In the case of the vessels with azaserine, reaction was again finished approximately after a 10 minute incubation period. However, in the control vessel without inhibition, reaction continued for at least 30 minutes. These data are interpreted as indicating that the enzyme remains active under the conditions of incubation for a period of time greater than 30 minutes, but that in the presence of azaserine there is a progressive and irreversible denaturation of the enzyme,
the rate of which is primarily a function of the ratio of the concentrations of glutamine to azaserine.

Action of 6-Diazo-5-oxo-L-norleucine—Studies outlined above for azaserine have been repeated with the inhibitor, DON. DON, which has been both synthesized (14, 15) and isolated (16) from natural sources as an antibiotic, has many properties in common with azaserine (17-20) as an antimetabolite in physiological systems. It has been found that an equivalent response may be obtained in a variety of physiological systems with DON when administered in approximately one-fortieth the quantities required for azaserine.

When a comparison was made of the effect of DON and azaserine on the

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Quantity of inhibitor</th>
<th>Inhibition of reaction (FGAR → FGAM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aza-L-serine</td>
<td>0.15</td>
<td>46</td>
</tr>
<tr>
<td>Aza-D-serine</td>
<td>0.15</td>
<td>0</td>
</tr>
<tr>
<td>O-Carbamyl-L-serine</td>
<td>1.00</td>
<td>0</td>
</tr>
<tr>
<td>&quot;</td>
<td>0.20</td>
<td>0</td>
</tr>
<tr>
<td>γ-Glutamylhydrazine</td>
<td>0.50</td>
<td>54</td>
</tr>
</tbody>
</table>

enzyme system (FGAR → FGAM), it was found that DON was likewise considerably more effective as an inhibitor than was azaserine (Fig. 6). Upon comparison of the extent of inhibition at three levels of inhibitor and treatment of the data by the method of Lineweaver and Burk, a value of $8 \times 10^{-4} M$ has been obtained for the $K_m$ of glutamine and a value of $1.1 \times 10^{-6}$ for the $K_I$ of DON. The values for the $K_m$ of glutamine as determined in the DON and azaserine experiments are in good agreement. The value of 727 for the ratio of $K_m$ to $K_I$ in the DON experiments is approximately 40 times the value of the corresponding ratio obtained in the experiments on azaserine. This correspondence of the relative effectiveness of DON and azaserine in the physiological and enzymatic experiments provides strong evidence for the belief that the point of action of these compounds in the physiological system is concerned with the conversion of FGAR to FGAM.
DON behaves like azaserine when preincubated with the enzyme. In the absence of glutamine the enzyme is inactivated irreversibly. However, if the preincubation is carried out in the presence of both glutamine and DON, the enzyme is partially protected against inactivation, at least during the time interval of the experiment.

Effect of Other Inhibitors—O-Carbamylserine and glutamylhydrazine, two compounds which bear a structural resemblance to glutamine, were compared to azaserine and DON for their action on the enzyme system (FGAR $\rightarrow$ FGAM). The enzyme system has also been incubated with aza-D-serine. As seen in Table IV, aza-D-serine in contrast to aza-L-serine has no effect on the reaction. O-Carbamylserine has a small effect at higher concentration and $\gamma$-glutamylhydrazine was the most effective inhibitor other than aza-L-serine and DON. These comparisons indicate that the azide moiety of DON and of aza-L-serine is probably the reactive site of these inhibitors, but that the structure of the remaining part of the molecule plays an important role in the specificity of the inhibitor as a competitor of glutamine.

DISCUSSION

There are two points partially discussed above which require further clarification in future studies. One is the need for more precise information on the mechanism of interaction of the inhibitors with the enzyme system (FGAR $\rightarrow$ FGAM). Present studies have shown that aza-L-serine and 6-diazo-5-oxo-L-norleucine are metabolic and structural analogues of glutamine, and that they are uniquely reactive with only one enzyme so far studied in the purine synthetic system de novo. There are, however, at least two other examples in which milder reactions of inhibitor with enzyme systems have been observed. Apparently the inhibitors react irreversibly with the enzyme (FGAR $\rightarrow$ FGAM) to cause its denaturation, and glutamine, the natural substrate of the reaction, competes with the inhibitors to prevent this reaction. It would be of value to determine whether there is reaction of the inhibitors involving the amino acids of the enzyme or some as yet unidentified coenzyme bound to the enzyme surface.

The second point of discussion concerns the question of whether the several effects of azaserine on biological systems can be explained in terms of its action on enzymatic systems as a competitor of glutamine. It seems probable that, in the animal tissues studied, both normal and cancerous, the effect of azaserine is due to its effect on the enzyme (FGAR $\rightarrow$ FGAM). The correspondence of enzymatic and physiological response in comparison of DON and aza-L-serine is cited as evidence for this possibility.

In certain microbiological systems, azaserine has been shown by Tomisiek, Kelly, and Skipper (21) to cause the inhibition of growth of cells by
blocking this enzymatic system and effecting the accumulation of formyl-
glycinamide ribotide or the riboside. Kaplan and Stock (22), employing
Escherichia coli, have observed that azaserine toxicity can be prevented
most effectively by tryptophan, phenylalanine, or tyrosine. Schabel and
Skipper2 have confirmed these observations and further noted that, when
minimal inhibiting levels of azaserine were employed, methionine or purines
were effective reversal agents. The further comment has been made by
Tomisek, Kelly, and Skipper (21) that disturbance of amino acid metab-
olism occurs in their microbiological system at a concentration of azaserine
10 times greater than that required to inhibit nucleic acid synthesis. In
their experiments it would seem, therefore, that nucleic acid synthesis is the
primary site of azaserine action.

Regardless of the point of action of azaserine in metabolism, it is probable
that azaserine is inhibiting a reaction involving glutamine. Thus, Meister
and Tice (23) have described an enzyme system in which glutamine reacts
with a variety of α-keto acids to form the corresponding amino acids.
Phenylpyruvic acid and p-hydroxyphenylpyruvic acid were among those
compounds listed as amino acceptors. It is, therefore, possible that the
biosynthesis of phenylalanine and tyrosine is inhibited (22) at this trans-
amination step involving glutamine, and that growth of these microorgan-
isms can be restored by adding the amino acids whose syntheses have been
blocked.

The lack of success in attempting to reverse inhibition of azaserine or
DON with glutamine (20) is explained by experiments in this paper. Glu-
tamine only delays the inhibition caused by the inhibitors. Addition of
this compound after inhibition has taken place would not be expected to
cause a reversal of the reaction. Thus, under certain experimental condi-
tions it might be difficult to establish readily the relationship of these in-
hibitors to glutamine even though it exists.

SUMMARY

Two metabolic inhibitors, aza-L-serine and 6-diazo-5-oxo-L-norleucine,
have been studied for their action on the enzymes concerned with inosinic
acid biosynthesis in vitro. Both compounds specifically inhibit one reaction
of the series, the conversion of formylglycinamide ribotide to formylglycin-
amidine ribotide in the presence of glutamine and adenosine triphosphate.
They behave as competitive inhibitors of glutamine. 6-Diazo-5-oxo-L-nor-
leucine is the more effective inhibitor, being required at only one-fortieth the
concentration as azaserine to give an equivalent response. If either com-

2 Unpublished data, cited by F. M. Schabel, Jr., and H. E. Skipper, in Progress
report of the Kettering-Meyer Laboratory of the Southern Research Institute,
August 15, 1955.
pound is allowed to react with the enzyme in the absence of glutamine, there is an irreversible inactivation of the enzyme which cannot be overcome by the addition of glutamine. Glutamine, the natural substrate of the reaction, apparently competes with the inhibitors in delaying the enzyme inactivation caused by them. It is believed that the action of these inhibitors on this enzyme system is responsible in many instances for the effects of these compounds on physiological systems.

BIBLIOGRAPHY
BIOSYNTHESIS OF THE PURINES: XV.
THE EFFECT OF AZA-L-SERINE AND
6-DIAZO-5-OXO-L-NORLEUCINE ON
INOSINIC ACID BIOSYNTHESIS DE
NOVO
Bruce Levenberg, Irving Melnick and John M.
Buchanan


Access the most updated version of this article at
http://www.jbc.org/content/225/1/163.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/225/1/163.citation.full.html#ref-list-1