Effect of Azaserine on the Biosynthesis of Diphosphopyridine Nucleotide in Mouse*

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The biosynthetic pathway of diphosphopyridine nucleotide has been formulated through a study of yeast autolysates, erythrocytes, and liver homogenates. Two such pathways have been described by those procedures. Each system ultimately utilizes the enzyme diphosphopyridine nucleotide pyrophosphorylase. In the first pathway, the enzyme produces diphosphopyridine nucleotide directly by coupling adenosine triphosphate to nicotinamide mononucleotide (1); in the second pathway, the enzyme couples adenosine triphosphate to nicotinic acid mononucleotide to form the nicotinic acid analogue of diphosphopyridine nucleotide (2). The nicotinic acid analogue of diphosphopyridine nucleotide is then amidated to produce diphosphopyridine nucleotide (3).

The enzymes involved in both pathways appear to be fairly ubiquitous in biological systems (1, 4) and it is difficult to evaluate the importance of each system. Studies on diphosphopyridine nucleotide levels in erythrocytes after the administration of nicotinamide or nicotinic acid indicate that the main pathway of diphosphopyridine synthesis is through the formation of the nicotinic acid analogue of diphosphopyridine nucleotide, as only the latter compound can elevate the diphosphopyridine nucleotide level (5). Similar studies in mouse liver indicate the converse of the results obtained with erythrocytes; here nicotinamide was found to be much more active than nicotinic acid (6). Although the effects of nicotinamide in the mouse suggest that a pathway involving nicotinamide mononucleotide may be the main system in the liver, recent studies (7) have shown that the nicotinic acid analogue of diphosphopyridine nucleotide is also elevated after nicotinamide injections.

Normal levels of diphosphopyridine nucleotide may be also altered by the administration of azaserine (8). The subcutaneous injection of this compound will reduce markedly the level of diphosphopyridine nucleotide in mouse liver in 2 hours. The mechanism by which this decrease occurs is not known; however, it appears likely that the turnover of diphosphopyridine nucleotide is affected.

A study of the incorporation of the various moieties of diphosphopyridine nucleotide in the presence and absence of azaserine have been carried out in an effort to give some evaluation of the possible pathways of diphosphopyridine nucleotide formation in mouse liver as well as the mechanism of action of azaserine in reducing the diphosphopyridine nucleotide concentration of this tissue.

EXPERIMENTAL PROCEDURE

Nicotinamide-7-C¹⁴ and nicotinic acid-7-C¹⁴ were purchased from Research Specialties Corporation. The other radioactive compounds were secured from the following companies: N¹⁵H₄Cl, The Isomet Corporation; H₃P₃O₄ converted to the sodium salt before use, Oak Ridge National Laboratories; and n-ribose-1-C¹⁴, the Volk Radiochemical Company. DPN was purchased from the Pabst Laboratories, and deamino-DPN was prepared from it by nitrous acid treatment (9). Azaserine was generously supplied by Parke, Davis and Company. Adenine-H² was prepared by the New England Nuclear Corporation.

Experiments were conducted with 2- to 3-month-old BAF mice obtained from the Jackson Memorial Laboratories. The mice, each weighing 18 to 22 g, were raised on a diet of Purina laboratory chow ad libitum; feed was withdrawn at time of injection as previously described (10). Azaserine injections were made subcutaneously, and all other injections were made intraperitoneally. Animals were killed by cervical dislocation. The livers were removed rapidly, blotted, weighed, and immediately homogenized in 5 volumes of cold 5% trichloroacetic acid. The homogenates were centrifuged at low speed and aliquots of the supernatant were removed for analysis of total DPN. Four animals were used for each determination where azaserine was used; two animals were used at other points, except where indicated.

Nucleoside di- and triphosphates, dinucleotides, and glycerogen were precipitated from the trichloroacetic extract by the addition of 5 volumes of cold acetone (-20°C). The precipitate was removed by centrifugation at 1000 x g after being allowed to stand overnight at -20°C. The precipitate was dissolved in a minimal amount of distilled water, and the solution was adjusted to pH 7.5 with 0.5 n NaOH, then applied to a Dowex 1-formate column. The column was then washed with distilled water until free of 260 μm-absorbing materials. The pyridine nucleotides were then eluted with 0.1 n formic acid (11). The fractions were measured at 260 μm; the peak fractions were combined and the volume reduced in a vacuum at 30°C. Although this fraction has been reported as pure DPN (12), we found when chromatographing the fraction in a system of 0.1 M phosphate, pH 6.8, ammonium sulfate, and n-propanol (13) that a very faint spot of material capable of quenching ultra-
violet light, corresponding to CMP, appeared in addition to a similar spot corresponding to DPN. When the quantity of DPN in these fractions was diminished, as by prior treatment of the mice with azaserine, an increased amount of this substance (relative to DPN concentration) appeared and was isolated by elution from the paper. The material gave the same absorption spectrum as CMP and gave as well similar \( R_F \) values in several other chromatographic systems. Therefore, the combined DPN fraction from column chromatography was chromatographed on paper with the phosphate-propanol-ammonium sulfate solvent (13) and the DPN spot eluted with distilled water before the DPN was measured in those experiments involving the injection of \( \text{P}^{32} \) or ribose-C\(^{14} \). These compounds could have been incorporated into CMP, resulting in elevated specific activity of DPN.

The synthesis of nicotinamide-8-N\(^{15} \) was carried out by a modification of the method of Murray et al. (14). This involved reaction of \( \text{N}^{14}\text{H}_3 \) with nicotinoyl chloride, the ammonia being produced by the action of KOH pellets on an aqueous solution of \( \text{N}^{14}\text{H}_4\text{Cl} \) (67% excess of \( \text{N}^{14} \)). The \( \text{N}^{14}\text{H}_4 \) was generated in a Thunberg tube and allowed to pass through the side arm into a second tube containing the nicotinoyl chloride. Diffusion of ammonia to the tube containing nicotinoyl chloride was facilitated by placing that tube at \(-40^\circ \) while the temperature of the generating tube was held at \(40^\circ \). After 2 hours, the tubes were sealed and separated. The tube containing the mixture of nicotinoyl chloride and \( \text{N}^{14}\text{H}_4 \) was held an additional 16 hours at \(-5^\circ \), after which 10 ml of distilled water were added. The pH was adjusted to 7.5 and the solution filtered. The filtrate was then chromatographed on Dowex 1-formate and the eluate containing nicotinamide-8-N\(^{15} \) was collected free of nicotinuric acid resulting from hydrolysis of nicotinoyl chloride. The eluate was then brought to dryness under reduced pressure and the constant melting point.

The DPN in \( \text{N}^{15} \) experiments was first isolated as described above, then cleaved by Neurospora DPNase and the adenosine-diphosphate ribose removed on a Dowex 1-formate column. Free nicotinamide was isolated as described previously, and the amide nitrogen of nicotinamide was then converted to nitrogen gas by the method of Sprinson and Rittenberg (15). The nitrogen gas was analyzed for \( \text{N}^{15} \) in a Consolidated mass spectrophotometer, type 21-103C, the use of which was kindly made available to us by the Department of Chemistry, Harvard University.

Radioactivity was measured by plating aliquots on sintered glass planchets and counting at "infinite thinness" in a proportional gas flow counter (University series, Baird Atomic). Adenine-\(^{15}\)H was counted in a liquid scintillation spectrometer (Packard Instrument Company, Inc., model 314-P).

### RESULTS

**Effect of Azaserine on Liver DPN Levels**—As reported previously (8), the effects of azaserine were found to diminish the pre-existing liver DPN as well as inhibit the increased levels of DPN caused by the injection of nicotinamide. The effects of nicotinamide and azaserine are antagonistic to each other, depending upon time of injection. As shown in Fig. 1, when mice were given injections of azaserine first, the DPN level quickly fell. This reduction could be rapidly halted with an increase in DPN if these animals were treated with nicotinamide one-half hour after azaserine was administered. The converse of this experiment is also shown in Fig. 1. If nicotinamide was injected first, the DPN level quickly rose, and after 5 hours, the effects were neutralized and reversed with the administration of azaserine.

The effects of azaserine were found to be reversed only by nicotinamide. As shown in Table I, the almost simultaneous injection of nicotinamide and azaserine does not change the level of DPN. However, the almost simultaneous injection of either nicotinic acid, glutamine, deamino-DPN, or DPN with the azaserine has little effect on antagonizing the azaserine action on the liver DPN.

**Injection of Radioactive Phosphate, Ribose, and Nicotinic Acid**

The injection of radioactive ribose or phosphorus with high levels of nicotinamide will cause an increased incorporation of radioactivity into liver DPN in agreement with the findings of Shuster et al. (12). If the azaserine is administered 2 hours after either ribose-C\(^{14} \) or P\(^{32} \) with nicotinamide, the total radioactivity falls with the concurrent reduction of the DPN liver levels. Surprisingly, the effect of azaserine on incorporation of ribose-C\(^{14} \) or P\(^{32} \) is to reduce the amount of radioactivity incorporated into DPN before azaserine treatment. This expresses itself by a reduction in specific activity as shown in Table II. A similar effect of azaserine on the incorporation of nicotinic acid into DPN is also shown in Table II. In this case, 50 mg per kg of nicotinic acid-C\(^{14} \), which is the maximal stimulatory dosage.

#### Table I

**Reversal of azaserine effect on DPN level of mouse liver**

Values were determined on pooled liver extracts of two animals. Azaserine (200 mg per kg of body weight) was injected at time 0 and the other compounds (4.1 mmoles per kg of body weight), 30 minutes later. Animals were killed 2 hours after azaserine injection.

<table>
<thead>
<tr>
<th>Compounds injected</th>
<th>DPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>415</td>
</tr>
<tr>
<td>Azaserine</td>
<td>143</td>
</tr>
<tr>
<td>Azaserine + nicotinamide</td>
<td>430</td>
</tr>
<tr>
<td>Azaserine + nicotinic acid</td>
<td>142</td>
</tr>
<tr>
<td>Azaserine + glutamine</td>
<td>181</td>
</tr>
<tr>
<td>Azaserine + DPN</td>
<td>284</td>
</tr>
<tr>
<td>Azaserine + deamino-DPN</td>
<td>185</td>
</tr>
</tbody>
</table>
Effect of azaserine on specific activity of DPN after injection of nicotinic acid-7-C\textsuperscript{14}, P\textsuperscript{32}, or ribose-1-C\textsuperscript{14}

The values were determined on isolated DPN from pooled liver extracts of two animals. Each animal was given an injection of 5 μc of nicotinic acid-7-C\textsuperscript{14} (50 mg per kg of body weight), 50 μc of P\textsuperscript{32}, or 5 μc of n-ribose-1-C\textsuperscript{14}. Unlabeled nicotinamide was administered together with P\textsuperscript{32} or ribose-1-C\textsuperscript{14} at 500 mg per kg of body weight. Azaserine was administered at 200 mg per kg of body weight 2 hours after the injection of radioactive compounds.

<table>
<thead>
<tr>
<th>Time after azaserine</th>
<th>Nicotinic acid-C\textsuperscript{14}</th>
<th>P\textsuperscript{32} + nicotinamide</th>
<th>Ribose-1-C\textsuperscript{14} + nicotinamide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control + Azaserine</td>
<td>Control + Azaserine</td>
<td>Control + Azaserine</td>
</tr>
<tr>
<td>hours</td>
<td>c.p.m./pmole DPN</td>
<td>c.p.m./pmole DPN</td>
<td>c.p.m./pmole DPN</td>
</tr>
<tr>
<td>0</td>
<td>27,800</td>
<td>175,220</td>
<td>14,200</td>
</tr>
<tr>
<td>1</td>
<td>27,200</td>
<td>14,450</td>
<td>23,100</td>
</tr>
<tr>
<td>2</td>
<td>30,200</td>
<td>22,200</td>
<td>21,300</td>
</tr>
</tbody>
</table>

Effect of azaserine on specific activity of DPN after injection of nicotinamide-7-C\textsuperscript{14}

The values were determined on isolated DPN from pooled liver extracts of two animals. Nicotinamide-7-C\textsuperscript{14}, in above dosage, was administered by injection to all the animals, followed by extracts of two animals. Nicotinamide-7-C\textsuperscript{14}, in above dosage, was administered by injection to all the animals, followed by extracts of two animals. Nicotinamide was administered with 500 mg per kg and asazaserine at 200 mg per kg of body weight.

<table>
<thead>
<tr>
<th>Time after azaserine</th>
<th>Nicotinic acid-C\textsuperscript{14}</th>
<th>P\textsuperscript{32} and nicotinamide</th>
<th>P\textsuperscript{32} + azaserine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control + Azaserine</td>
<td>Control + Azaserine</td>
<td>Control + Azaserine</td>
</tr>
<tr>
<td>hours</td>
<td>c.p.m./pmole DPN</td>
<td>c.p.m./pmole DPN</td>
<td>c.p.m./pmole DPN</td>
</tr>
<tr>
<td>0</td>
<td>65,000</td>
<td>60,000</td>
<td>10,332</td>
</tr>
<tr>
<td>1</td>
<td>117,000</td>
<td>130,000</td>
<td>11,050</td>
</tr>
<tr>
<td>2</td>
<td>252,000</td>
<td>248,000</td>
<td>11,800</td>
</tr>
<tr>
<td>3</td>
<td>8,950</td>
<td>6,030</td>
<td>12,100</td>
</tr>
</tbody>
</table>

Effect of azaserine on incorporation of adenine-H\textsuperscript{3} into mouse liver DPN

All figures represent the pooled analyses of four animals. Each animal was given an injection of 3.3 × 10\textsuperscript{-2} μmoles of adenine-H\textsuperscript{3} with a specific activity of 2.97 × 10\textsuperscript{5} c.p.m. per μmole.

<table>
<thead>
<tr>
<th>Time after adenine</th>
<th>Adenine-H\textsuperscript{3}</th>
<th>Adenine-H\textsuperscript{3} + azaserine</th>
<th>c.p.m./pmole DPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>33,810</td>
<td>39,690</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>29,665</td>
<td>39,920</td>
<td></td>
</tr>
</tbody>
</table>

Effect of azaserine on specific activity of DPN after the simultaneous injection of P\textsuperscript{32}

Each value was determined on isolated DPN from pooled liver extracts from two animals. Animals were given injections of 50 μc of nicotinic acid (50 mg per kg of body weight) or 5 μc of ribose-1-C\textsuperscript{14} and nicotinamide (500 mg per kg). Azaserine was administered at 200 mg per kg of body weight.

<table>
<thead>
<tr>
<th>Time after azaserine</th>
<th>Nicotinic acid-7-C\textsuperscript{14}</th>
<th>Ribose-1-C\textsuperscript{14} and nicotinamide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control + Azaserine</td>
<td>Control + Azaserine</td>
</tr>
<tr>
<td>hours</td>
<td>c.p.m./pmole DPN</td>
<td>c.p.m./pmole DPN</td>
</tr>
<tr>
<td>1</td>
<td>127,120</td>
<td>51,200</td>
</tr>
<tr>
<td>2</td>
<td>121,821</td>
<td>28,666</td>
</tr>
<tr>
<td>3</td>
<td>53,692</td>
<td>12,000</td>
</tr>
</tbody>
</table>

Effect of azaserine on specific activity of DPN after the simultaneous injection of P\textsuperscript{32}

Each value was determined on isolated DPN from pooled liver extracts from two animals. Animals were given injections of 50 μc of nicotinic acid (50 mg per kg of body weight) or 5 μc of ribose-1-C\textsuperscript{14} and nicotinamide (500 mg per kg). Azaserine was administered at 200 mg per kg of body weight.

<table>
<thead>
<tr>
<th>Time after azaserine</th>
<th>P\textsuperscript{32} + azaserine</th>
</tr>
</thead>
<tbody>
<tr>
<td>hours</td>
<td>c.p.m./pmole DPN</td>
</tr>
<tr>
<td>1</td>
<td>153,305</td>
</tr>
<tr>
<td>2</td>
<td>311,999</td>
</tr>
<tr>
<td>3</td>
<td>185,705</td>
</tr>
</tbody>
</table>

Effect of azaserine on incorporation of adenine-H\textsuperscript{3} into mouse liver DPN

All figures represent the pooled analyses of four animals. Each animal was given an injection of 3.3 × 10\textsuperscript{-2} μmoles of adenine-H\textsuperscript{3} with a specific activity of 2.97 × 10\textsuperscript{5} c.p.m. per μmole.

<table>
<thead>
<tr>
<th>Time after adenine</th>
<th>Adenine-H\textsuperscript{3} + azaserine</th>
<th>c.p.m./pmole DPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>hours</td>
<td></td>
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<tr>
<td>1</td>
<td>33,810</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>29,665</td>
<td></td>
</tr>
</tbody>
</table>

Unpublished observations.
amide nitrogen of nicotinamide. Azaserine was injected at 200
N's was given as 67 atom % excess as either N^5H & Cl or as the
were used. Each animal received 12.5 mg of nicotinamide. Nicotinamide-7-Cl^4 was administered in 5 µ amounts per animal;
N^15 was given as 67 atom % excess as either N^1HCl or as the
amide nitrogen of nicotinamide. Azaserine was injected at 200
mg per kg of body weight.

<table>
<thead>
<tr>
<th>Compounds injected</th>
<th>Specific activity in DPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinamide-7-Cl^4</td>
<td>94</td>
</tr>
<tr>
<td>Nicotinamide (amide N^15)</td>
<td>25</td>
</tr>
<tr>
<td>N^1HCl + nicotinamide</td>
<td>15</td>
</tr>
<tr>
<td>N^1HCl + nicotinamide + azaserine</td>
<td>13</td>
</tr>
</tbody>
</table>

N^15 and nicotinamide-7-Cl^4 are administered together at 500
mg per kg C^4 enters the liver DPN with relatively little
dilution in specific activity while the N^15 is diluted in atom
per cent excess of N^15 approximately 1 to 4, as seen in Table
VII. An exchange of amide nitrogen before incorporation of
nicotinamide into DPN is also seen when N^1HCl is administered
with unlabeled nicotinamide. In this case, the amide group of
the nicotinamide moiety of DPN becomes labeled with N^15.
Although azaserine reduces the total amount of N^15 incorpora-
tion, it does not appear to greatly alter the atom per cent excess of N^15 into the nicotinamide moiety of DPN.

**DISCUSSION**

Langan et al. (7) have shown that the nicotinamide moiety of
nicotinamide is incorporated into nicotinic acid *DPN in the
mouse liver, thus indicating the possible presence of the Preiss-
Handler pathway of DPN synthesis in liver in vivo. The
conversion of nicotinamide to the acid before its incorporation into
DPN is also suggested by the dilution of the labeled amide group in
a ratio of 1:4 as compared with the incorporation of the la-
beled carbonyl group, the latter being relatively undiluted.
It should be pointed out that the incorporation of labeled nico-
tinamide into nicotinic acid *DPN and finally into DPN, does
not indicate whether the loss of the amide group occurs at the
free base or at the mononucleotide or nucleoside level.

Azaserine lowers the total concentration of liver DPN. At
least two possible mechanisms can be postulated for this obser-
vation: (a) the inhibition of a synthetic step in the normal turn-
over or (b) by an enhancement of DPN breakdown. The first
mechanism is the most attractive in view of the studies of Preiss
and Handler, who demonstrated that azaserine would inhibit
the amidation reaction of nicotinic acid *DPN (3). However, no
significant accumulation of nicotinic acid *DPN was observed
after azaserine administration, as would be anticipated if such
a block were operating in vivo (7). Furthermore, glutamine, the
compound for which azaserine has been shown as a competitive
inhibitor in amidation reactions (16), was not found to reverse
the effects of azaserine on liver DPN levels.

At first consideration, the enhancement of DPN breakdown
was not considered a likely cause of azaserine action in reducing
DPN levels (8), the observed turnover of DPN in the liver being
too slow to account for the relatively rapid effects of azaserine
on the DPN concentration. The turnover of liver DPN based on
radioactive incorporation has been shown in this report and
others (1, 2, 8) to be highly susceptible to alteration. Thus,
the possibility that azaserine alters the turnover exists and
could explain the action of azaserine upon DPN levels in the liver.

In substantiation of the latter postulate, the specific activity of
liver DPN after the incorporation of P^32, nicotinic acid-7-C^4,
or ribose-1-C^4 was found to be lowered after azaserine adminis-
tration. If there were no enhancement of DPN turnover with
azaserine treatment, the amount of radioactive DPN per unit of
DPN would remain the same when a block in DPN synthesis
occurred or the amount would rise if only a partial block were
present. This mechanism would necessarily preclude an elimina-
tion of radioactive moieties of DPN to prevent recyclization.
 Studies of the elimination of radioactive nicotinamide have
shown that the vitamin is excreted more rapidly in various met-
abolic forms after the administration of azaserine (17).

It has been shown that the total incorporation as well as the
specific activity of P^32 or adenine-^H into DPN is raised in ani-
mals treated simultaneously with P^32 or adenine-^H and azaser-
ine. A similar observation was made by Fernandes et al. (18)
in studying the utilization of adenine-8-C^4 by ascites tumor
cells. In their studies, they found that the rate of adenine
utilization was increased after azaserine administration, and
they concluded that these effects were due to the inhibition of
de novo adenine formation by azaserine, thereby causing an
increased utilization of pre-existing or added adenine.

The experiments reported here suggest that azaserine induces
a more rapid turnover of the DPN. The marked decrease in
DPN levels resulting from azaserine injection would then result
from a more rapid rate of destruction of the coenzyme as com-
pared to the rate of synthesis. This hypothesis may also ac-
count for the more rapid excretion of nicotinamide metabolites
observed after the administration of azaserine, as the nicotin-
amide arising from DPN breakdown would reenter the dinucleo-
otide form at a rate slower than it was excreted.

Although the total radioactive incorporation of nicotinamide-
7-C^4 is reduced by azaserine treatment, there is no change in
specific activity compared to nonazaserine treated animals,
despite altered time of treatment or dosage. This suggests an
equilibrium of the nicotinamide incorporated into DPN with
the free nicotinamide existing in the liver by a reaction insensi-
tive to azaserine. The DPNase described by Kaplan (19) can
mediate this reaction and is not affected by azaserine.

The action of DPNase might also account for the somewhat
decreased effect of azaserine on the specific activity of DPN
when animals were injected with nicotinic acid-7-C^4. When
ribose-1-C^4, P^32, or nicotinic acid-7-C^4 was injected into mice,
followed by azaserine 2 hours later (Table II), there was a rapid
drop in specific activity of DPN over the respective control
animals; however, this effect continued for at least the next hour,
even in the case of nicotinic acid-7-C^4 injection. In the latter
case, the specific activity increased in the next hour, presumab-
ly until it equaled that of the nonazaserine control group. Thus,
the incorporation of nicotinic acid into DPN with its subsequent
destruction would lead to an increase in the free nicotinamide
pool, the amide in this case being labeled with C^4 in position 7.
This, in turn, could exchange with existing DPN and increase the
specific activity of DPN, as observed with nicotinamide-7-C^4 ad-
ministration (Table III) and where there is no diminution of
specific activity.
SUMMARY

1. Azaserine lowers the diphosphopyridine nucleotide level of mouse liver to approximately one-fourth of its normal content in 2 hours. This effect can be neutralized or reversed by nicotinamide. Glutamine, nicotinic acid, deamino diphosphopyridine nucleotide or diphosphopyridine nucleotide have little or no effect on this action of azaserine.

2. The synthesis of liver diphosphopyridine nucleotide in vivo appears to proceed, at least in part, via the formation of the nicotinic acid analogue of diphosphopyridine nucleotide. This is shown by the marked dilution of the amide group of nicotinamide-N\(^5\) before it is incorporated into diphosphopyridine nucleotide. On the other hand, the carbonyl group of nicotinamide-7-C\(^{14}\) is relatively undiluted when incorporated into diphosphopyridine nucleotide.

3. The turnover of diphosphopyridine nucleotide is influenced by the presence of both nicotinamide and azaserine. Radioactive ribose, phosphorus, nicotinic acid, or nicotinamide is rapidly incorporated into liver diphosphopyridine nucleotide. The simultaneous injection of azaserine (200 mg per kg of body weight) and \(\text{P}^{32}\) or adenine-\(\text{H}^3\) increases the specific activity of diphosphopyridine nucleotide as well as the total radioactive phosphorus incorporated.

When the injection of radioactive phosphorus, ribose, nicotinic acid, or nicotinamide was followed 2 hours later by azaserine, the total incorporation of radioactivity was reduced. In addition, the specific activities of all moieties injected with the exception of nicotinamide were found to be reduced in the presence of azaserine.

4. Although azaserine can alter the total incorporation of radioactive nicotinamide into diphosphopyridine nucleotide, the specific activity of the nicotinamide moiety of diphosphopyridine nucleotide is not altered by azaserine. Evidence has been obtained that the diphosphopyridine nucleotide-catalyzed exchange reaction, in which free nicotinamide exchanges with the bound nicotinamide of the diphosphopyridine nucleotide, occurs in vivo.

5. Unlike the action of a true or partial blocking agent, azaserine decreases the specific activity of diphosphopyridine nucleotide after injection of radioactive ribose or nicotinic acid. The specific activity of diphosphopyridine nucleotide increases when azaserine is injected simultaneously with \(\text{P}^{32}\), during the early time intervals when \(\text{P}^{32}\) incorporation is still rapidly increasing in the control group. These results suggest that azaserine enhances the rate of diphosphopyridine nucleotide turnover.

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