Adenocarcinoma of the Pancreas in Azaserine-treated Rats

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Summary

Development of a model of carcinoma of the pancreas in rats was approached by attempting to identify chemicals that (a) behave as mutagens and (b) localize in the pancreas following systemic administration; and then to study the effects of long-term administration. Azaserine was selected because it behaves as a direct-acting mutagen in two bacterial test systems and because tissue distribution studies showed concentration especially in kidney and pancreas. Groups of rats have been given i.p. injections once or twice weekly for 6 months, and rats have been autopsied after 6 to 18 months. During the first year pancreases developed (a) nodules of atypical exocrine cells which seem to represent hyperplastic foci and (b) encapsulated adenomas. After 1 year most pancreases from treated rats are diffusely abnormal and contain many hyperplastic nodules and adenomas, while more than one-quarter have had pancreatic adenocarcinoma. Metastases have been observed in lymph nodes, liver, and lung. No carcinomas or adenomas have been observed in control rats. No other organ shows as high an incidence of involvement as pancreas, but renal neoplasms were frequent.

Studies with another chemical O-(N-methyl-N-nitroso-β-alanyl)-L-serine, are at an earlier stage. The tissue distribution of radioactivity following injection of a 14C-labeled sample is similar to that of azaserine; however, this compound is not a direct-acting bacterial mutagen. Rats treated for 6 months twice weekly i.p. have a higher incidence of nodules of atypical acinar cells than did controls, although the number of nodules per rat is few. No adenomas or carcinomas have been found during 13 months of the study.

We conclude that azaserine is a carcinogen in rats and causes major abnormalities of growth and differentiation of the exocrine pancreas, including adenocarcinoma in some rats. O-(N-Methyl-N-nitroso-β-alanyl)-L-serine had less effect than azaserine on pancreatic growth and differentiation.

Materials and Methods

Wistar rats (Charles River Breeding Laboratories, Wilmington, Mass.) fed Purina rat chow ad libitum and caged in groups of 2 to 5 were used in all experiments.

Azaserine (Calbiochem, La Jolla, Calif.) dissolved in 0.9% NaCl solution was injected i.p. at a dose of 5 mg/kg once or twice weekly for 6 months (Table I). The higher dose, 5 mg/kg 2 times/week proved to be close to a chronic LD10.

Alanoser, synthesized by a method which will be described in a separate publication, was injected i.p. twice weekly at a dose of 10 or 50 mg/kg (Table I). Preliminary toxicity studies showed that rats tolerated up to 1-g/kg doses of alanoser injected i.p. without morbidity or mortality. Solutions of both agents were made immediately prior to injection, and solutions appear to be stable for at least 1 day. The structures of azaserine and alanoser are shown in Chart I.

Puromycin (Nutritional Biochemicals Corp., Cleveland, Ohio), 40 mg/kg, was given in courses of 4 hourly doses in the 3rd and 4th months to one-half of the rats in Groups 2 and 3 (Table I) to cause cycles of pancreatic acinar cell necrosis and regeneration (6).
In addition, a sample of alanoser was submitted to Herbert S. Rosenkranz, Department of Microbiology, Columbia University, for evaluation in the bacterial systems.

Tissue localization studies were done using $[^3H]$azaserine, $[^4C]$alanoser, and $[^4C]$serine (New England Nuclear, Boston, Mass.). The first was obtained by submitting a 500-mg sample of azaserine for commercial tritium gas exposure labeling. The product, returned in aqueous solution, was purified by recrystallizing from 90% ethanol after adding unlabeled azaserine, followed by preparative thin-layer chromatography on silica gel plates using a butanol-acetic acid-water solvent system. The zone coinciding with azaserine was extracted with water. An analytical thin-layer chromatogram of the final product was scanned in a radiochromatogram scanner and showed more than 90% of radioactivity migrating in the position of azaserine. $[^4C]$-Alanoser was synthesized from commercially obtained $[^4C]$serine. A thin-layer radiochromatogram showed greater than 96% radiochemical purity.

Radioactivity in trichloroacetic acid-soluble and -insoluble fractions of several tissues was measured following i.v. injection of the agents. We followed our previously described procedures (4) except that we omitted the hydrolysis of nucleic acids in preparation of the acid-insoluble fraction so that these should contain both nucleic acids and proteins.

**Results**

**Mutagenicity.** We have previously reported that azaserine behaves as a direct-acting mutagen in the *Escherichia coli* repair test but that alanoser does not (7). Data in Table 2 indicate that azaserine is also a direct-acting mutagen in the *S. typhimurium* reversion test system whereas alanoser is not.

**Distribution Studies.** Radioactivity levels found in tissues following administration of $[^3H]$azaserine or $[^4C]$-alanoser are summarized in Table 3. The kidney consistently contained the highest levels of acid-soluble

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### Table 2

**Assay of mutagenic potential of azaserine and alanoser in the *S. typhimurium* reversion system**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>μg</th>
<th>n</th>
<th>Strain 1535</th>
<th>Strain 1536</th>
<th>Strain 1537</th>
<th>Strain 1538</th>
</tr>
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<tr>
<td>None</td>
<td></td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Azaserine</td>
<td></td>
<td>2</td>
<td>22</td>
<td>14</td>
<td>61</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>189</td>
<td>14</td>
<td>39</td>
<td>500</td>
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<td>10</td>
<td>2</td>
<td>98</td>
<td>94</td>
<td>368</td>
<td>226</td>
</tr>
<tr>
<td>Alanoser</td>
<td></td>
<td>2</td>
<td>14</td>
<td>10</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>14</td>
<td>7</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4</td>
<td>12</td>
<td>0</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>

* Direct activity. No microsomal activation system was used. Counts of fewer than 50 revertants/plate are regarded as negative for mutagenic activity. The count reported in the average of $n$ plates.
Table 3

Specific radioactivity of trichloroacetic acid-soluble and -insoluble fractions of tissues from rats following injections of [\(^{1}H\)]azaserine, \([^{14}C\)]alanoser, and \([^{14}C\)]serine

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Time (min)</th>
<th>Dose (μCi)</th>
<th>Rat wt (g)</th>
<th>Route</th>
<th>Pancreas</th>
<th>Kidney</th>
<th>Liver</th>
<th>Intestine</th>
<th>Spleen</th>
<th>Testis</th>
<th>Muscle</th>
<th>Thymus</th>
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<tr>
<td><strong>Soluble fractions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[(^{1}H)]Azaserine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>4</td>
<td>15</td>
<td>2.6</td>
<td>8</td>
<td>i.v.</td>
<td>71,892 ± 2,180</td>
<td>295,473 ± 14,437</td>
<td>26,642 ± 1,151</td>
<td>27,393 ± 1,889</td>
<td>28,703 ± 746</td>
<td>13,947 ± 719</td>
<td>20,631 ± 662</td>
</tr>
<tr>
<td>Group 2</td>
<td>3</td>
<td>15</td>
<td>5.2</td>
<td>16</td>
<td>i.v.</td>
<td>139,707 ± 8,506</td>
<td>548,977 ± 18,279</td>
<td>51,738 ± 4,226</td>
<td>57,134 ± 2,212</td>
<td>51,485 ± 3,233</td>
<td>26,146 ± 1,939</td>
<td>36,322 ± 1,793</td>
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<tr>
<td>[(^{14}C)]Alanoser</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>281 i.v.</td>
<td>249,255 ± 10,341</td>
<td>603,293 ± 23,000</td>
<td>31,253 ± 1,190</td>
<td>30,625 ± 990</td>
<td>35,986 ± 1,266</td>
<td>11,721 ± 605</td>
<td>11,644 ± 430</td>
</tr>
<tr>
<td>Group 4</td>
<td>4</td>
<td>15</td>
<td>2</td>
<td>3</td>
<td>288 i.p.</td>
<td>97,647 ± 17,713</td>
<td>311,647 ± 51,750</td>
<td>28,326 ± 3,690</td>
<td>18,859 ± 3,535</td>
<td>29,229 ± 4,281</td>
<td>8,775 ± 1,559</td>
<td>11,479 ± 3,131</td>
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<tr>
<td>[(^{14}C)]Serine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 5</td>
<td>5</td>
<td>15</td>
<td>2</td>
<td>0.02</td>
<td>83 i.v.</td>
<td>55,815 ± 2,625</td>
<td>47,789 ± 3,666</td>
<td>47,787 ± 1,523</td>
<td>28,084 ± 2,420</td>
<td>41,999 ± 1,772</td>
<td>11,193 ± 351</td>
<td>18,426 ± 829</td>
</tr>
<tr>
<td>Group 6</td>
<td>5</td>
<td>15</td>
<td>2</td>
<td>0.01</td>
<td>165 i.p.</td>
<td>89,622 ± 14,370</td>
<td>33,408 ± 3,928</td>
<td>60,403 ± 8,308</td>
<td>34,392 ± 5,186</td>
<td>36,120 ± 3,803</td>
<td>9,836 ± 1,497</td>
<td>16,843 ± 1,719</td>
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<tr>
<td><strong>Insoluble fractions</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>[(^{1}H)]Azaserine</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1'</td>
<td>18.2 ± 2.4</td>
<td>9.8 ± 0.4</td>
<td>2.2 ± 0.6</td>
<td>7.6 ± 0.5</td>
<td>7.4 ± 3.5</td>
<td>1.4 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>2.5 ± 0.8</td>
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</tr>
<tr>
<td>Group 2</td>
<td>19.7 ± 2.5</td>
<td>17.2 ± 0.5</td>
<td>3.0 ± 0.2</td>
<td>10.1 ± 0.7</td>
<td>5.6 ± 3.8</td>
<td>1.7 ± 0.3</td>
<td>0.9 ± 0.1</td>
<td>2.6 ± 0.1</td>
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<tr>
<td>[(^{14}C)]Alanoser</td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>17 ± 1</td>
<td>20 ± 1</td>
<td>13 ± 1</td>
<td>12 ± 3</td>
<td>43 ± 4</td>
<td>4 ± 1</td>
<td>3 ± 0</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Group 4</td>
<td>38 ± 7</td>
<td>20 ± 1</td>
<td>20 ± 3</td>
<td>11 ± 1</td>
<td>46 ± 5</td>
<td>3 ± 0</td>
<td>2 ± 0</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>[(^{14}C)]Serine</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Group 5</td>
<td>314 ± 68</td>
<td>115 ± 2</td>
<td>120 ± 27</td>
<td>312 ± 76</td>
<td>178 ± 30</td>
<td>33 ± 4</td>
<td>8 ± 1</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Group 6</td>
<td>532 ± 80</td>
<td>45 ± 5</td>
<td>129 ± 30</td>
<td>177 ± 31</td>
<td>96 ± 16</td>
<td>56 ± 22</td>
<td>7 ± 1</td>
<td></td>
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</tr>
</tbody>
</table>

* Interval from injection to killing.

\(^a\) Approximate dose of azaserine, alanoser, or serine.

\(^b\) Average weight/rat. Within groups the weight range was 10 to 20 g.

\(^c\) Data have been corrected for varying weight of rats by the formula:

\[
\text{Observed cpm/g} \times \text{(wt of rat/100)} = \text{"corrected" cpm/g}
\]

\(^d\) Mean ± S.E.

\(^e\) Experimental data for all groups under "Insoluble fractions" are the same as those for corresponding groups under "Soluble fractions."
Groups 1 and 2 autopsied to date. Pancreases from at least 6.5 cm in greatest diameter. The weight of the total pancreas was 2.94 ± 0.48 (S.E.) g, whereas the mean weight of 12 such pancreases was 2.94 ± 0.48 (S.E.) g, whereas the mean weight of 12 such pancreases was 1.35 ± 0.19 g. Individual nodules measure from 2 to 10 mm in diameter, and they are found in all portions of the pancreas. This change in pancreas seems to merit a descriptive designation of diffuse adenomatous hyperplasia.

The acid-insoluble fraction from pancreas ranks 3rd in radioactivity at 5 min and 2nd at 15 min following \[^{14}C\]alanoser. Since the molecule was labeled in serine, the acid-insoluble radioactive may reflect hydrolysis, release of \[^{14}C\]serine, and its incorporation into protein. We collected urine from these rats at sacrifice and found increasing radioactivity at 5, 15, and 60 min after injection. Thin-layer chromatograms of the urine showed more radioactivity in the alanoser spot (R_F 0.66) than in the serine spot (R_F 0.27) at all intervals.

The distribution of \[^{14}C\]serine is also given in Table 3. With the natural amino acid, the levels of both acid-soluble and acid-insoluble radioactivity in pancreas exceed those in the kidney as well as in other tissues in both groups. The specific radioactivity achieved in the acid-insoluble fraction exceeds that found following either alanoser or azaserine suggesting that the latter are not readily incorporated into proteins.

Rat Autopsy Findings. Hyperplastic nodules of acinar cells such as we have previously described have been found in the pancreas of all azaserine-treated rats (Table 1, Groups 1 and 2) autopsied to date. Pancreases from at least 25% of the rats have contained small encapsulated tumors (adenomas) of acinar cell origin. However, beginning 12 months after treatment, increasing numbers of rats have had diffusely abnormal pancreases with multiple nodules such as that illustrated in Fig. 1. The mean weight of 12 such pancreases was 2.94 ± 0.48 (S.E.) g, whereas the mean weight of 5 pancreases from similar-sized control rats was 1.35 ± 0.19 g. Individual nodules measure from 2 to 10 mm in diameter, and they are found in all portions of the pancreas. This change in pancreas seems to merit a descriptive designation of diffuse adenomatous hyperplasia. It seems fatuous to designate individual nodules in such pancreases as “adenomas” although they might be several mm in diameter and encapsulated. A representative histological field from such a diffusely abnormal gland is shown in Fig. 2. Some foci in such pancreases clearly become malignant as evidenced by lymph node invasion, vascular invasion (Figs. 3 and 4), or metastasis to liver (Fig. 5) or lung (Figs. 6 and 7). In other diffusely abnormal pancreases, individual nodules or adenomas become large and exhibit marked changes in cellular differentiation that are like those seen in the clearly malignant tumors; however, no vascular or lymphatic invasion or metastatic spread has been detected in several such rats. We have designated these as “probable carcinomas.”

Individual malignant tumors have measured as much as 6.5 cm in greatest diameter. The weight of the total pancreas in this rat was 37 g. Malignant tumors have been found in all portions of the pancreas [head, body, tail (Fig. 8)]. Of the 23 Group 1 azaserine-treated rats autopsied 12 or more months after initial treatment, 9 had had adenocarcinomas of the exocrine pancreas and 3 more had tumors that were probably carcinomas. Of 18 Group 2 azaserine-treated rats, 5 had adenocarcinomas and 1 had probable carcinoma. Five of the 14 rats with pancreatic carcinomas had metastases in the liver, 1 had diffuse spread in peritoneal fat, and 1 had a pulmonary metastasis. In general, the metastatic foci were similar in differentiation to the most abnormal tumor nodule in the pancreas, e.g., Figs. 4 and 5, or 6 and 7. Of the 18 rats with malignant or probably malignant pancreatic neoplasms, 16 were males. Among Group 2 rats, only 1 of 5 with pancreatic carcinoma had been treated with puromycin.

Differentiation. Cells in hyperplastic nodules have retained acinar cell differentiation with varying degrees of zymogen production and marked variation in nuclear size as had been noted before (5). Cells often remain organized into acini with central lumens (Fig. 8).

The adenomas are characteristically less differentiated than nodules, but they usually retain evidence of zymogen production. Duct-like areas appear within some adenomas and nodules in the diffusely normal pancreases (Fig. 10). Cystic tumors were found that seem to merit designation as cystadenomas (Fig. 11).

The adenocarcinomas (and the tumors designated as probable carcinomas) have varied widely in differentiation. The most highly differentiated clearly produce zymogen (Fig. 14), even in metastatic sites (Figs. 12 and 13); whereas poorly differentiated tumors, although clearly epithelial, have lost all glandular pattern and show no zymogen production (Figs. 2, 4, and 6). Electron microscopy of several such tumors has failed to reveal evidence of zymogen production. Some carcinomas have contained duct-like structures (Fig. 15), but none has been exclusively a ductal carcinoma.

Ultrastructural Characteristics. The most highly differentiated cells in the hyperplastic nodules retain ultrastructural appearance and organization of normal acinar cells. Cells in some nodules characteristicly have enlarged nuclei as has been apparent by light microscopy. In less-differentiated tumors there is decreased zymogen production and loss of cell polarity coincident with loss of acinar organization. Even cells with little or no zymogen tend to retain moderate amounts of rough endoplasmic reticulum and prominent Golgi vesicles. Zymogen granules sometimes assume bizarre cylindrical or angular shapes or are smaller than normal. In the poorly differentiated tumors, we have encountered cytoplasmic inclusions such as annulate lamellae and what appear to be aggregates of microtubules.

Tumors in Other Tissues of Azaserine-treated Rats. Neoplasms encountered in other tissues are summarized in Table 4. No effort has been made to classify the renal tumors as benign or malignant. Papillary epithelial tumors, clear-cell tumors, and tubular adenomas have been seen. Only one had metastasized (to liver). The smallest were microscopic, and the largest weighed 44 g. The renal tumors will be more completely described in a separate report. Nor have we classified the breast tumors regarding...
It has been shown to alkylate protein (2), but DNA alkylation has apparently not been evaluated following azaserine treatment.

Breast tumors are relatively common in Wistar rats (10), and the incidence in our control and azaserine-treated groups is similar. Spontaneous renal adenomas and carcinomas are less frequent (10), and since we have seen none among control rats we attribute them to azaserine.

The progression of changes in the pancreas suggests that many cells are affected by azaserine and that some abnormality persists in some cells which alters their growth rate and differentiation. This alteration is evidenced as early as 2 months (D. S. Longnecker, unpublished observations) after treatment by the presence of nodules of atypical acinar cells. Apparently, some such foci represent clones that will ultimately grow to become "adenomas," whereas a few have or acquire malignant growth potential.

In the experiment reported here, one-half of the Group 2 azaserine-treated rats received 2 courses of puromycin injections as a stimulus for promotion, since it has been shown that such treatment induces a cycle of cell regeneration in acinar cells (6). At this point there is no indication that this treatment has increased the incidence of pancreatic cancer in the group that received it.

The spectrum of changes encountered in the pancreases of azaserine-treated rats leaves no question regarding the likely cell of origin for most tumors; it is the acinar cell. Many adenomas and some carcinomas retain a high degree of acinar cell differentiation. Thus, it is clear that this model of chemical carcinogenesis in rat pancreas does not simulate the majority of spontaneously occurring human neoplasms, which appear to be ductal.

Azaserine, which is not a direct-acting bacterial mutagen, has a much smaller effect on pancreatic growth and differentiation than does azaserine. At this point, it seems unlikely that azaserine will prove to be a carcinogen, although it does seem to induce a few nodules of atypical acinar cells. We have reported small zones of inhibition of a DNA polymerase-deficient E. coli mutant in the presence of a liver microsomal system, i.e., evidence that azaserine is apparently mutagenic following metabolic activation (7). However, subsequently, it has been found that azaserine is apparently nonmutagenic for S. typhimurium strains in the Ames system in the presence of a liver microsomal activating mixture (H. S. Rosenkranz, personal communication).

We believe that additional amino acid derivatives that are direct- or indirect-acting bacterial mutagens should be evaluated with regard to their effect on pancreatic growth.

Acknowledgments

The authors acknowledge the technical assistance of Barbara Crawford, Douglas S. Daniel, Maureen Devine, Susan James, Janice French, Herman Lilja, and Richard Markley.

References


Table 4

<table>
<thead>
<tr>
<th>Tumor site and type</th>
<th>Azaserine-treated</th>
<th>0.9% NaCl solution-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas, adenocarcinoma*</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Kidney, adenomas and adenocarcinomas</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Breast, epithelial tumors</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Neck, epidermoid carcinoma*</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Liver, hepatoma</td>
<td>3</td>
<td>0</td>
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<tr>
<td>Pituitary, adenoma</td>
<td>3</td>
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<tr>
<td>Subcutaneous</td>
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<tr>
<td>Fibrosarcoma</td>
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</tr>
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<td>Lipoma</td>
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<tr>
<td>Rectum, leiomyosarcoma</td>
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<tr>
<td>(No. of autopsies 6-18 mo.)</td>
<td>54</td>
<td>17</td>
</tr>
</tbody>
</table>

*Includes 4 neoplasms rated as "probable adenocarcinomas" (see text) but not glands with adenomas or diffuse adenomatous hyperplasia.

* Possibly ear duct carcinomas.

The progression of changes in the pancreas suggests that many cells are affected by azaserine and that some abnormality persists in some cells which alters their growth rate and differentiation. This alteration is evidenced as early as 2 months (D. S. Longnecker, unpublished observations) after treatment by the presence of nodules of atypical acinar cells. Apparently, some such foci represent clones that will ultimately grow to become "adenomas," whereas a few have or acquire malignant growth potential.

In the experiment reported here, one-half of the Group 2 azaserine-treated rats received 2 courses of puromycin injections as a stimulus for promotion, since it has been shown that such treatment induces a cycle of cell regeneration in acinar cells (6). At this point there is no indication that this treatment has increased the incidence of pancreatic cancer in the group that received it.

The spectrum of changes encountered in the pancreases of azaserine-treated rats leaves no question regarding the likely cell of origin for most tumors; it is the acinar cell. Many adenomas and some carcinomas retain a high degree of acinar cell differentiation. Thus, it is clear that this model of chemical carcinogenesis in rat pancreas does not simulate the majority of spontaneously occurring human neoplasms, which appear to be ductal.

Azaserine, which is not a direct-acting bacterial mutagen, has a much smaller effect on pancreatic growth and differentiation than does azaserine. At this point, it seems unlikely that azaserine will prove to be a carcinogen, although it does seem to induce a few nodules of atypical acinar cells. We have reported small zones of inhibition of a DNA polymerase-deficient E. coli mutant in the presence of a liver microsomal system, i.e., evidence that azaserine may be mutagenic following metabolic activation (7). However, subsequently, it has been found that azaserine is apparently nonmutagenic for S. typhimurium strains in the Ames system in the presence of a liver microsomal activating mixture (H. S. Rosenkranz, personal communication).

We believe that additional amino acid derivatives that are direct- or indirect-acting bacterial mutagens should be evaluated with regard to their effect on pancreatic growth.

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References


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All light micrographs are stained with H & E.

Fig. 1. Pancreas from a rat treated twice weekly with azaserine for 6 months and autopsied after 18 months. The gland weighed 5.9 g. Multiple nodules are apparent.

Fig. 2. Pancreas (same as Fig. 1) shows diffuse adenomatous hyperplasia. Multiple nodules of acinar cell overgrowth are present. Some nodules have fibrous capsules. × 18.

Fig. 3. Poorly differentiated adenocarcinoma from the pancreas of a rat that had been treated twice weekly with azaserine, 5 mg/kg, for 6 months and autopsied after 18.5 months. Most of the tumor appears to be within vascular spaces. × 170.

Fig. 4. Portions of a poorly differentiated carcinoma of the pancreas from a rat that had been treated once weekly with azaserine, 5 mg/kg for 6 months. There is vascular invasion (top center). Several mitoses are evident. × 180.

Fig. 5. Metastatic adenocarcinoma from the pancreas in liver of the same rat illustrated in Fig. 4. This nodule was subserosal. × 190.

Fig. 6. Pancreas with an undifferentiated carcinoma composed of large cells from a rat treated with azaserine twice weekly for 6 months and autopsied 14.5 months after 1st treatment. There was no evidence of zymogen production by either light microscopy or electron microscopy in this tumor. × 170.

Fig. 7. Lung with metastatic carcinoma from the same rat illustrated in Fig. 6. The carcinoma is poorly differentiated and mimics the tumor found in pancreas. × 90.

Fig. 8. Pancreas with carcinoma and spleen (right) from a Group 1 azaserine-treated rat autopsied after 13 months. The gland weighed 26 g and contained tumors in both head (left) and tail.

Fig. 9. Portion of a hyperplastic nodule from a rat that had been treated once weekly with azaserine for 6 months and autopsied after 18 months. Normal pancreas is at left. Cells of the hyperplastic nodule with large nuclei show evidence of acinar organization (arrows). × 350.

Fig. 10. Portion of a pancreatic nodule from a gland that showed diffuse adenomatous hyperplasia in a rat that had been treated twice weekly with azaserine and autopsied at 18.5 months. There are duct-like epithelial elements (upper right) with a slight desmoplastic response. × 250.

Fig. 11. Portion of the lining of a cyst from a pancreas that showed diffuse adenomatous hyperplasia. The rat had been treated once weekly with azaserine for 6 months and was autopsied 18 months after 1st treatment. The cyst was lined by columnar or cuboidal epithelium, which showed occasional areas of nodular overgrowth such as is illustrated. These areas contained occasional zymogen-bearing cells. × 65.

Fig. 12. Portion of a pancreatic adenocarcinoma from a rat that was treated once weekly with azaserine for 6 months and autopsied at 18 months. This tumor retained a somewhat glandular, ribbon-like pattern. Electron microscopy revealed zymogen granules in the tumor cells (see Fig. 13). × 170.

Fig. 13. Metastatic adenocarcinoma of pancreas in liver from the same rat shown in Fig. 12. Although the cancer cells lack normal acinar cell organization, zymogen granules, rough endoplasmic reticulum, and Golgi are conspicuous in the binucleate cell at the center. Lead citrate and uranyl acetate, × 7000.

Fig. 14. Adenocarcinoma of pancreas from an azaserine-treated rat autopsied after 16.5 months. Tumor cells contain zymogen, rough endoplasmic reticulum, Golgi, and surface microvilli (lower left) but have a small cytoplasmic mass and lack normal polarity and organization of cell organelles. Lead citrate and uranyl acetate, × 3900.

Fig. 15. Metastatic carcinoma from pancreas in lymph node of an azaserine-treated rat (once weekly) autopsied after 17 months. Some epithelial elements in the node appear duct-like. × 190.

Fig. 16. Pancreas from a rat treated with alanoser for 6 months and autopsied 12 months after 1st treatment. A subserosal nodule of atypical acinar cells (center) has slightly larger nuclei than did surrounding cells. × 130.
Pancreatic Carcinoma in Azaserine-treated Rats
Adenocarcinoma of the Pancreas in Azaserine-treated Rats

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