Adenocarcinoma of the Pancreas in Azaserine-treated Rats[1]

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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 ^{14}C-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.

Introduction

Investigation of possible causes of carcinoma of the pancreas is receiving increased attention (8, 12), and there has been recent success in the development of new animal models of pancreatic neoplasms (3, 5, 9, 11). Experimental induction of adenocarcinoma of the exocrine pancreas in rats is the goal of work in progress in our laboratory. Our approach has been to study the long-term effects of chemicals (a) that are mutagenic or carcinogenic and (b) that localize in the pancreas following systemic administration. We initially selected azaserine (NSC 742) as a potential pancreatic carcinogen because (a) it is a bacterial mutagen and (b) it has a high affinity for pancreas following i.v. injection. We have also studied alanoser,[3] a newly synthesized compound that localized similarly to azaserine following injection. Alanoser is not a direct-acting bacterial mutagen.

In studies still in progress, pancreatic adenocarcinomas have developed in azaserine-treated rats but not in rats treated with alanoser, although the latter has induced a higher incidence of hyperplastic nodules of acinar cells than is observed in control rats. These experiments are described here.

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 1). The higher dose, 5 mg/kg 2 times/week proved to be close to a chronic LD_{10}.

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 1). 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 1.

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 1) to cause cycles of pancreatic acinar cell necrosis and regeneration (6).

Table 1

Summary of treatment regimens

Group	Treatment[a]	Dose/ injection (mg/kg)	Injections/ wk	No. of rats[b]
1	Azaserine	5	2	60
2	Azaserine[c]	5	1	60
3	0.9% NaCl solution[c,e]		2	60
4	Alanoser	50[d]	2	60
5	0.9% NaCl[e]		2	30

[a] All rats were treated for 6 months. All injections were i.p.

[b] One-half of each group were female.

[c] One-half of Groups 2 and 3 received, in addition, 2 series of 4 hourly 40-mg/kg injections of puromycin given at 3.5 and 4.5 months during azaserine treatment.

[d] During Weeks 2 to 6 of treatment, injections were 10 mg/kg for 9 doses because of short supply of alanoser.

[e] Group 3 were controls for Groups 1 and 2. Group 5 were controls for Group 4.

$$N_2CHCO-OCH_2\ CHCO_2^-$$
$$NH_3^+$$

AZASERINE

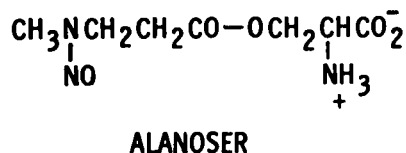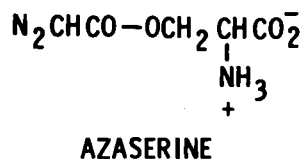$$CH_3NCH_2CH_2CO-OCH_2CHCO_2^-$$
$$NO \qquad NH_3^+$$

ALANOSER

Chart 1. Formulas of azaserine and alanoser.

Rats were killed and autopsied at 6, 9, 12, and 18 months following 1st treatment and, in addition, whenever (a) there was weight loss of 15% or 100 g, or (b) when there was obvious morbidity so that survival was questionable. The pancreas was weighed, fixed *in toto*, and embedded as slices 1 to 2 mm thick. Representative sections of liver, kidney, intestine, stomach, testis or ovary, and salivary glands were routinely taken. Thymus, prostate, lung, pituitary, breast, and obvious lesions in other tissues were sampled in selected rats. Tissues were fixed in Susa's solution, embedded in paraffin, and routinely stained with hematoxylin and eosin. Prior to sacrifice, selected rats were laparotomized under ether anesthesia, and sections of pancreas, liver, or kidney were excised and fixed for electron microscopy in 4% cacodylate-buffered glutaraldehyde, pH 7.4. These tissues were postfixed in 2% osmium tetroxide in cacodylate buffer and prepared for electron microscopy as has been previously described (6). Sections were examined in a Siemans-Elmiskop 1A electron microscope or Philips EM 201 with 60 kV.

The mutagenic activity of azaserine and alanoser was evaluated in 2 bacterial test systems (1, 7). *Salmonella typhimurium* test strains were supplied by Dr. Bruce Ames.

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, [¹⁴C]alanoser, and [¹⁴C]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. [¹⁴C]-Alanoser was synthesized from commercially obtained [¹⁴C]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 [¹⁴C]-alanoser are summarized in Table 3. The kidney consistently contained the highest levels of acid-soluble

Table 2

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

Chemical	μg	n	Revertants/plate[a] Strain 1535	Strain 1536	Strain 1537	Strain 1538
None		6	10	0	5	9
Azaserine	1	2	22	14	61	105
	5	2	189	14	39	500
	10	2	98	94	368	226
Alanoser	1	2	14	10	20	18
	10	5	14	7	14	14
	100	4	12	0	5	11
	200	1	9	0	4	13

[a] 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 [³H]azaserine, [¹⁴C]alanoser, and [¹⁴C]serine

	No. of rats	Time (min)[a]	Dose µCi	Dose mg/kg[b]	Rat wt (g)[c]	Route	Pancreas	Kidney	Liver	Intestine	Spleen	Testis	Muscle	Thymus
									cpm/g tissue[d]					
Soluble fractions														
[³H]Azaserine														
Group 1	4	15	2.6	8	160	i.v.	71,892 ± 2,180[e]	295,473 ± 14,437	26,642 ± 1,151	27,393 ± 1,889	28,703 ± 746	13,947 ± 719	20,631 ± 662	33,826 ± 1,230
Group 2	3	15	5.2	16	161	i.v.	139,707 ± 8,506	548,977 ± 18,279	51,738 ± 4,226	57,134 ± 2,212	51,485 ± 3,233	26,146 ± 1,939	36,322 ± 1,793	64,884 ± 3,254
[¹⁴C]Alanoser														
Group 3	5	5	2	3	281	i.v.	249,255 ± 10,341	603,293 ± 23,000	31,253 ± 1,190	30,625 ± 990	35,986 ± 1,266	11,721 ± 605	11,644 ± 430	
Group 4	4	15	2	3	288	i.p.	97,647 ± 17,713	311,647 ± 51,750	28,326 ± 3,690	18,859 ± 3,535	29,229 ± 4,281	8,775 ± 1,559	11,479 ± 3,131	
[¹⁴C]Serine														
Group 5	5	15	2	0.02	83	i.v.	55,815 ± 2,625	47,789 ± 3,866	47,787 ± 1,523	28,084 ± 2,420	41,999 ± 1,772	11,193 ± 351	18,426 ± 829	
Group 6	5	15	2	0.01	165	i.p.	89,622 ± 14,370	33,408 ± 3,928	60,403 ± 8,308	34,392 ± 5,186	36,120 ± 3,803	9,836 ± 1,497	16,843 ± 1,719	
Insoluble fractions														
[³H]Azaserine														
Group 1[f]							18.2 ± 2.4	9.8 ± 0.4	2.2 ± 0.6	7.6 ± 0.5	7.4 ± 3.5	1.4 ± 0.2	0.6 ± 0.1	2.5 ± 0.8
Group 2							19.7 ± 2.5	17.2 ± 0.5	3.0 ± 0.2	10.1 ± 0.7	5.6 ± 3.8	1.7 ± 0.3	0.9 ± 0.1	2.6 ± 0.1
[¹⁴C]Alanoser														
Group 3							17 ± 1	20 ± 1	13 ± 1	12 ± 3	43 ± 4	4 ± 1	3 ± 0	
Group 4							38 ± 7	20 ± 1	20 ± 3	11 ± 1	46 ± 5	3 ± 0	2 ± 0	
[¹⁴C]Serine														
Group 5							314 ± 68	115 ± 2	120 ± 27	312 ± 76	178 ± 30	33 ± 4	8 ± 1	
Group 6							532 ± 80	45 ± 5	129 ± 30	177 ± 31	96 ± 16	56 ± 22	7 ± 1	

[a] Interval from injection to killing.
[b] Approximate dose of azaserine, alanoser, or serine.
[c] Average weight/rat. Within groups the weight range was 10 to 20 g.
[d] Data have been corrected for varying weight of rats by the formula:

Observed cpm/g × (wt of rat/100) = "corrected" cpm/g

[e] Mean ± S.E.
[f] Experimental data for all groups under "Insoluble fractions" are the same as those for corresponding groups under "Soluble fractions."

radioactivity, and the pancreas consistently ranked 2nd with both compounds. The radioactivity of pancreas-derived acid-insoluble fractions from [³H]azaserine-treated rats exceeded that in all other tissues, suggesting that macromolecules were alkylated or that azaserine or [³H]serine derived from [³H]azaserine had entered into protein synthesis.

The acid-insoluble fraction from pancreas ranks 3rd in radioactivity at 5 min and 2nd at 15 min following [¹⁴C]alanoser. Since the molecule was labeled in serine, the acid-insoluble radioactivity may reflect hydrolysis, release of [¹⁴C]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 [¹⁴C]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 abnormal 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 characteristically 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

Table 4

List of primary neoplasms encountered in azaserine-treated and control rats

Tumor site and type	No. of rats	
	Azaserine-treated	0.9% NaCl solution-treated
Pancreas, adenocarcinoma[a]	18	0
Kidney, adenomas and adenocarcinomas	18	0
Breast, epithelial tumors	8	3
Neck, epidermoid carcinoma[b]	5	0
Liver, hepatoma	3	0
Pituitary, adenoma	3	1
Subcutaneous		
Fibrosarcoma	1	0
Lipoma	0	1
Rectum, leiomyosarcoma	1	0
(No. of autopsies 6 18 mo.)	54	17

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

[b] Probably ear duct carcinomas.

state of malignancy. Some were fibroadenomas and some appeared to be adenocarcinomas, although none had metastasized. There was no evidence of metastatic spread of any of the other tumors, and they are classified by histological appearance. Animals survive in all groups so that it is premature to state an incidence of any tumor. About 3 times more azaserine-treated than control rats have been examined to date.

Alanoser-treated Rats. Pancreases from 6 alanoser-treated rats contained 1 to 4 hyperplastic nodules. These rats were examined 6 to 12 months after 1st treatment. The nodules were small to medium sized (Fig. 16) even in the 2 rats killed at 12 months, and the pancreases were not increased in weight. The number of nodules per pancreas was in the range encountered occasionally in control rats, but the incidence of nodule-bearing pancreases (5 of 6) was greater than in the appropriate control group where the incidence was 2 of 9. No adenomas, glands with diffuse adenomatous hyperplasia, or adenocarcinomas have been encountered in the alanoser-treated rats during 13 months of observation following 1st treatment.

NaCl Solution (0.9%)-treated Control Rats. No adenomas or adenocarcinomas of the exocrine pancreas have been encountered in control rats although we have found 1 islet cell tumor. About 40% (8 of 19) of controls for the azaserine-treated group have contained nodules of atypical cells like those described above, but they are few. Five rats had a single nodule, 2 rats had 2 nodules, and 1 rat had 6 nodules. The incidence of rats with such nodules in the pancreas seems to increase with age.

Discussion

On the basis of data reported here we conclude that azaserine has induced carcinomas in the pancreas and kidneys of rats. These 2 target sites correspond to the major sites of tissue concentration following i.v. administration of azaserine. The fact that azaserine is a direct-acting bacterial mutagen suggests that it may be a direct-acting carcinogen.

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.

Alanoser, 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 alanoser 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 alanoser may be mutagenic following metabolic activation (7). However, subsequently, it has been found that alanoser 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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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.
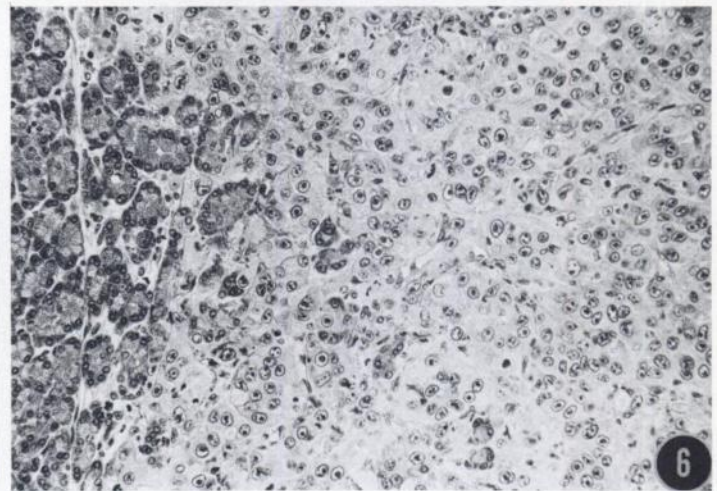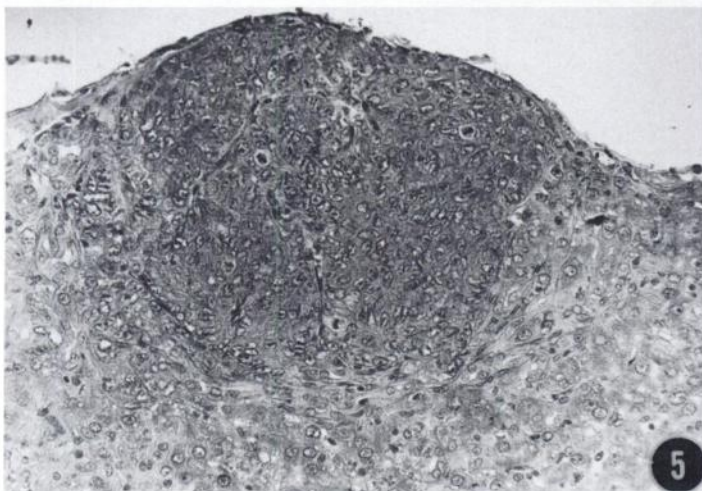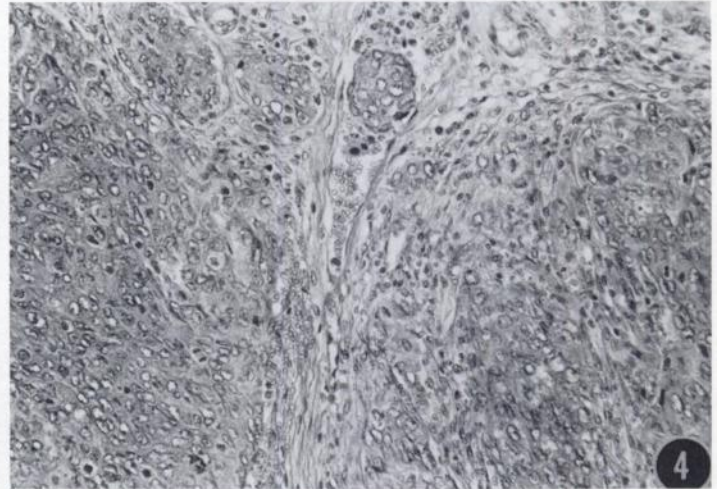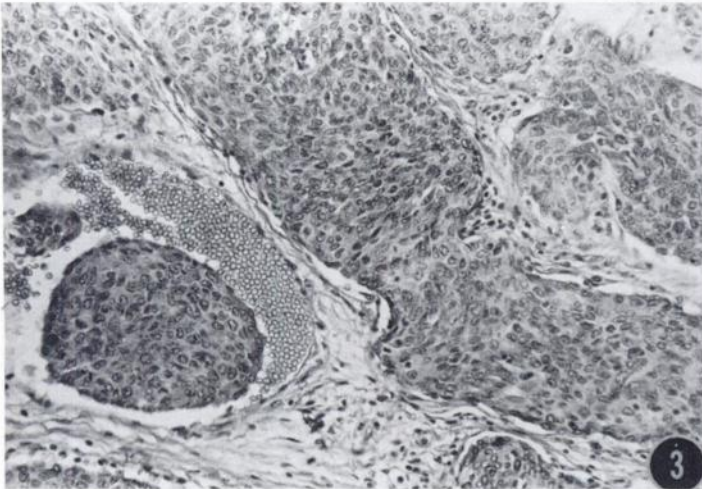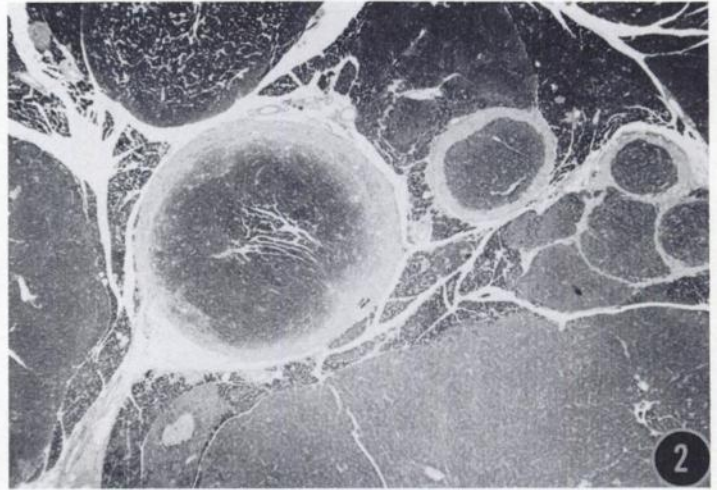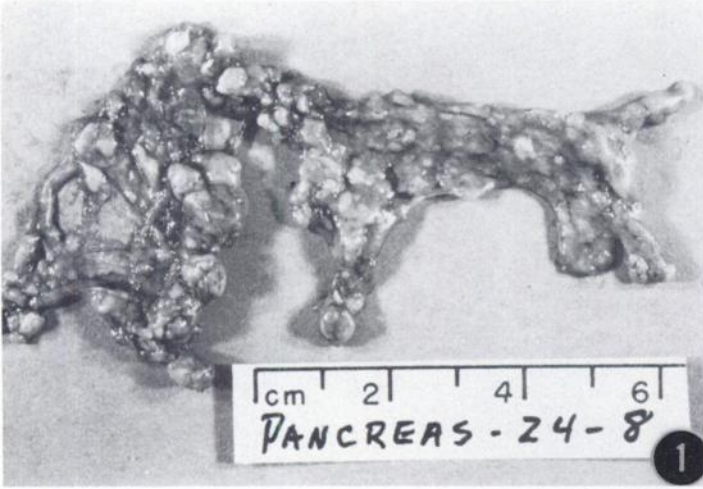
Fig. 13. Metastatic adenocarcinoma of pancreas in liver from the same rat shown in Fig. 12. Although the cancer cells lack normal acinar cell organization, zymogen granules, rough endoplasmic reticulum, and Golgi are conspicuous in the binucleate cell at the *center*. Lead citrate and uranyl acetate, × 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.
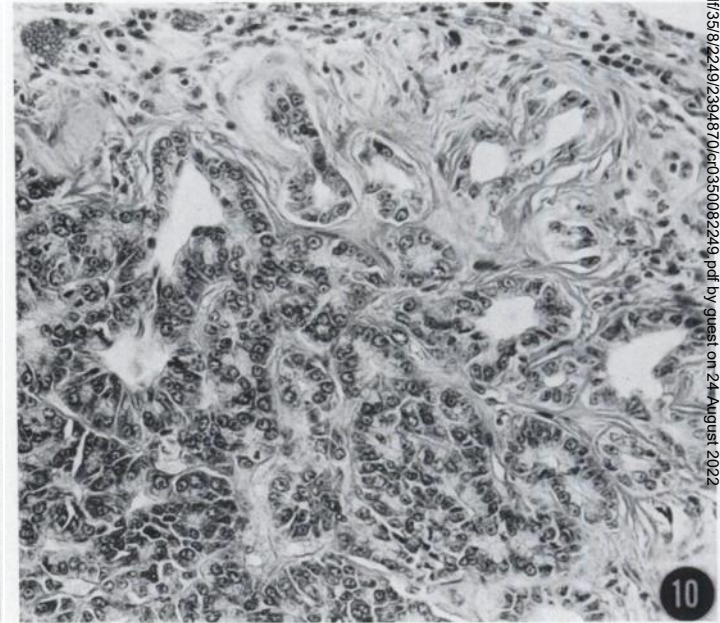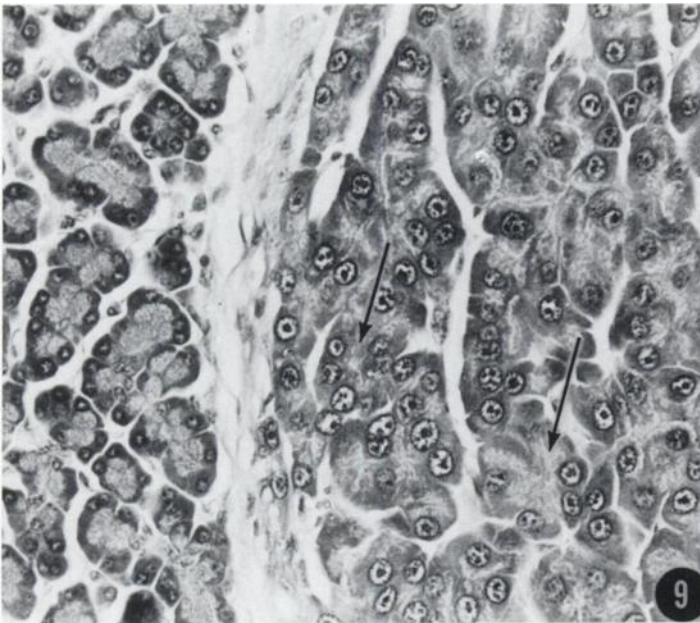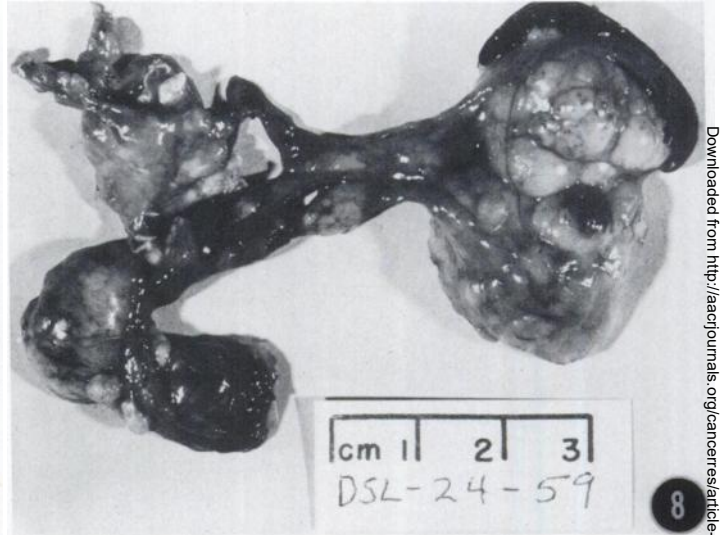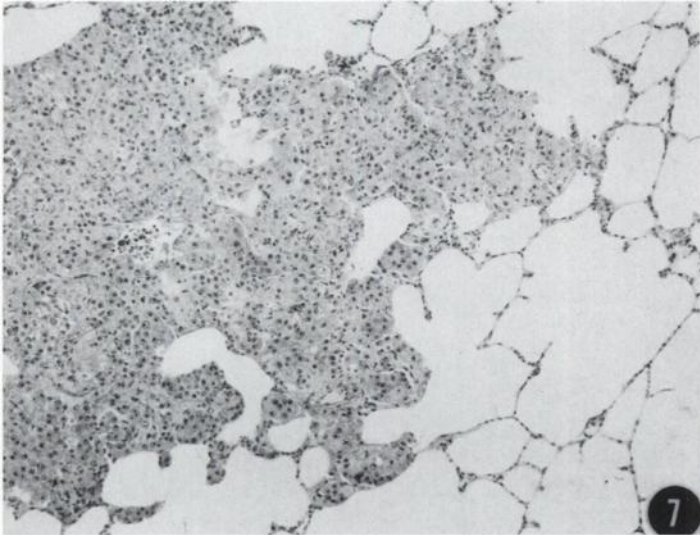
Fig. 15. Metastatic carcinoma from pancreas in lymph node of an azaserine-treated rat (once weekly) autopsied after 17 months. Some epithelial elements in the node appear duct-like. × 190.

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.