Metabolism of the Pancreatic Carcinogen N-Nitroso-2,6-dimethylmorpholine by Hamster Liver and Component Cells of Pancreas

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ABSTRACT

N-Nitroso-2,6-dimethylmorpholine (NNDM) is an indirect nitrosamine carcinogen which induces ductal adenocarcinoma of the pancreas in the Syrian golden hamster. NNDM is oxidized by normal male hamster pancreas postmitochondrial supernatants (S-9) in the presence of reduced nicotinamide adenine dinucleotide phosphate and oxygen to give three major identifiable metabolites as measured by high-pressure liquid chromatography. The major products are N-nitrosobis(2-hydroxypropyl)amine, N-nitrosod(2-hydroxypropyl)(2-oxopropyl)amine, and N-nitroso(2-oxopropyl)amine. Two other major metabolites have been detected, one which elutes near the solvent front and is referred to as X1, and one which is more hydrophobic than N-nitrosod(2-oxopropyl)amine and is called X2. The rates of formation of N-nitrosobis(2-hydroxypropyl)amine, N-nitrosod(2-hydroxypropyl)(2-oxopropyl)amine, and N-nitroso(2-oxopropyl)amine and X1, and X2 were linear with protein up to a final concentration of 15 mg of pancreatic S-9 per ml. The in vivo metabolism of NNDM by liver and pancreas S-9 and microsomes was compared. Although the liver subcellular fractions only produced two major metabolites, N-nitrosod(2-hydroxypropyl)(2-oxopropyl)amine and X1, under initial rate conditions the liver enzymes were greater than 40 times more active than the pancreatic enzymes with respect to the overall metabolism of NNDM. The enzyme(s) for the initial oxidative metabolism of NNDM are associated with the microsomes in the liver and the pancreas. However, the metabolite profiles obtained with pancreatic S-9 preparations are different from those observed with pancreatic microsomes, suggesting the involvement of cytosolic proteins in modulating the pancreatic metabolism of NNDM. Acinar and islet cell S-9 fractions metabolize NNDM at similar rates and exhibit a 2-fold increase in NNNDM metabolism over whole pancreas S-9. However, the metabolite profiles for the two cell types are very different. Pretreatment of hamsters with 2,3,7,8-tetrachlorodibenzo-p-dioxin results in a marked increase in the rate of NNDM metabolism in both acinar and islet cells. However, the two cell types exhibit different patterns of induction as measured by changes in the metabolite profiles. Pretreatment of hamsters with 2,3,7,8-tetrachlorodibenzo-p-dioxin results in a greater than 20-fold induction of aryl hydrocarbon hydroxylase activity in acinar cell S-9, whereas there is no induction of aryl hydrocarbon hydroxylase activity in the islet cell S-9. These results suggest the presence of different forms of the microsomal mixed-function oxidases in these two pancreatic cell types. Duct cells can also metabolize NNDM to an active form as demonstrated by unscheduled DNA synthesis in the nuclei of pancreatic duct epithelial cells after exposure to NNDM. These results provide additional evidence that pancreatic carcinogens are activated within the target cell(s) of the pancreas.

INTRODUCTION

The Syrian golden hamster represents a major experimental model for the study of pancreatic cancer (23). Hamster pancreas is susceptible to the carcinogenic effects of a variety of N-nitrosamines such as NNDM3 (19, 27), BOP (22), BHP (25), and HPOP (26). The lesions induced are ductal adenocarcinomas which closely resemble the neoplasms encountered with greatest frequency in humans (24). Exploitation of this model will require a detailed knowledge of the role of the pancreas in the metabolism of these carcinogens. Previous studies of their metabolism in hamsters have dealt almost exclusively with identification of metabolites in blood, urine, bile, and pancreatic juice of whole animals. Such studies have demonstrated that NNDM is metabolized to BHP and HPOP as major metabolites in blood and urine (8). Similar metabolites are also excreted in the urine when BOP is administered (9), and HPOP was identified as the major metabolite in blood and pancreatic juice (7). HPOP has been shown to be a more potent pancreatic carcinogen in the hamster than either NNDM or BHP, suggesting that it may be a proximate carcinogen (26). Although much has been learned from such studies about the metabolism of N-nitrosamines by the hamster, little is known about their metabolism by the target organ, pancreas. Previous work in this laboratory (30) indicates that hamster pancreas has the requisite enzyme(s) for activation of NNDM and BOP to mutagens, that these enzymes can be induced, that there may be multiple pathways for their metabolism in pancreas, and that some of the enzymes metabolize NNDM to nonmutagenic forms. The present studies were undertaken to ascertain the capacity of hamster liver and pancreas, and the component cells of pancreas, namely acinar, islet, and ductal, to metabolize NNDM.

MATERIALS AND METHODS

Isolation of S-9 and Microsomal Fractions from Liver and Pancreas

Male Syrian golden hamster (Charles River, Wilmington, Mass.)
weighing 100 to 120 g were maintained on hamster diet (Teklad test diets, Madison, Wis.) and starved overnight before sacrifice. The animals were induced with TCDD (a generous gift from Dr. A. Poland, Madison, Wis.) by an i.p. single injection of 100 μg TCDD in dioxane per kg body weight 4 days prior to sacrifice. Control and TCDD-treated animals were anesthetized under ether. Livers from 4 or more hamsters were removed, trimmed, weighed, sliced, and placed in chilled beakers. All subsequent procedures were carried out at 4°. Postmitochondrial fractions (S-9) from liver and pancreas were prepared using the methods described previously by Scarpelli et al. (30). The S-9 fractions were withdrawn from the centrifuge tubes with a sterile syringe, placed into sterile polystyrene tubes (0.5 ml per 12- x 75-mm tube), immediately frozen, and stored at -80°. For the preparation of the microsomal fractions, freshly prepared liver and pancreas S-9 preparations were placed in ultracentrifuge tubes and centrifuged at 105,000 x g (38,000 rpm in a Beckman 40 rotor) for 60 min. The 105,000 x g supernatant and lipid on the side of the tube were removed and discarded, and the pellets were resuspended in a volume of 0.1 M potassium phosphate buffer (pH 7.4) with 0.25 M sucrose, equal to the volume of supernatant removed. Microsomal suspensions were homogenized in a Teflon glass homogenizer, frozen in polyethylene tubes (0.5 ml/tube), and stored at -80° until use.

**Isolation of Acinar Cells and Preparation of Acinar Cell Subcellular Fractions.** Acinar cells were isolated using the method of Amsterdam and Jamieson (1). Using this procedure, yields were obtained based on cell counts and DNA content of 18.5 x 10⁷ acinar cells per pancreas with a purity of about 92 to 95% and a viability of >95%, as measured by cell counts and trypan blue exclusion. Amylase was measured by the method of Bernfeld et al. (2), and DNA was measured by the method of Burton (5) to determine enrichment of the acinar cell preparation as compared to whole pancreas homogenates. Acinar cells from 10 to 15 animals were pooled, suspended in 2 to 3 ml of 0.155 M KCl:0.05 M Tris-HCl containing 0.02% STI, and homogenized by 10 to 15 strokes in a Potter-Elvehjem homogenizer. Postmitochondrial and microsomal fractions were isolated by centrifugation and stored using the methods described previously for the preparation and storage of these fractions from whole liver and pancreas homogenates.

**Isolation of Islet Cells and Preparation of the S-9 Fractions.** Islet cells were prepared using the method of Lacy and Kostanovsky (14), as modified by Buitrago et al. (4). The pancreas was excised, trimmed, washed, and injected with Hanks’ solution containing 0.1% glucose. Minced pieces from 3 to 4 pancreata were suspended in 3 ml Hanks’ solution containing 15 mg collagenase (type IV, 150 to 200 units/mg) and 3 mg STI in a sterile culture tube and incubated at 37° for 18 to 20 min with vigorous shaking every 2 min. Following this digestion step, the tissue slurry was diluted with 3 ml of ice-cold Hanks’ solution, poured through a No. 60 stainless steel sieve, and allowed to sediment for 2 to 3 min in the original tube. The supernatant was carefully removed with a syringe, and the islet aggregate-containing sediment was resuspended in fresh Hanks’ solution. This procedure of washing and allowing the heavier islet cell aggregates to settle by gravity was repeated 8 to 10 times to obtain a progressive enrichment and purification of the islet cell population. During various stages of this part of the isolation, aliquots of the sediment were monitored by examination for the presence of the islet cell population. During various stages of this part of the isolation, aliquots of the sediment were monitored by examination for the presence of the islet cell population.

For the preparation of the S-9 fractions, freshly prepared liver and pancreas S-9 preparations were placed in ultracentrifuge tubes and centrifuged at 105,000 x g for 20 min. The 9000 x g supernatant was withdrawn with a sterile syringe, placed in a polypropylene tube, and stored at -80° until use. Chemical determination of the enrichment of the islet cell preparation was established by measuring the insulin content of whole pancreas and isolated islets by radioimmunoassay according to the method of Morgan and Lazarow (20), modified by Dr. A. LaBarbera as follows. The incubation conditions for the antibody reaction were 48 hr at 4° in an incubation volume of 600 μl and the antigen-antibody complex was then precipitated with 11% polyethylene glycol. The standard was purified rat insulin obtained from Novo Labs, Copenhagen. These were compared to the DNA content of the various preparations determined by the method of Kissane and Robinson (12). Examination of islet cell preparations by light and electron microscopy indicated that they were free from contamination by other components of the pancreas.

**In Vitro Metabolism.** Incubations of S-9 or microsomal fractions from whole pancreas or purified acinar cells and S-9 from islet cells were performed at 37° in a shaking incubator for 30 min. Unless otherwise stated, incubation mixtures (0.6 ml) contained 0.6 mM NAPDH, 1.0 mM [3H]NDM (labeled at the α-carbon), 3 mM glucose-6-phosphate, 6 mM MgCl₂, 6 units glucose-6-phosphate dehydrogenase, and protein not exceeding 20 mg/ml, as determined by the method of Lowry et al. (18), in 0.2 M potassium phosphate buffer (pH 7.4). Each set of experiments included 2 types of controls; in one case, the protein was omitted and replaced with a solution of 0.155 M KCl:0.05 M Tris-HCl containing 0.02% STI, and in another the complete system was placed in ice for a period equal to the incubation of the regular samples. Reactions were initiated by addition of the protein and terminated by filtering the incubation mixture through a 0.22-μm Millex GS filter (Millipore Corp., Bedford, Mass.) followed by immediate freezing of the filtrates at -20°.

Samples were thawed prior to analysis, and 50-μl aliquots were injected onto the HPLC. The HPLC system consisted of a Zorbax ODS column (4.6 mm x 25 cm) (DuPont Co., Wilmington, Del.), a Varian 5020 liquid chromatography unit (Varian, Palo Alto, Calif.), a Varco (Valco Instruments, Houston, Texas) loop injector with a 50-μl sample loop, an Aerograph UV absorbance detector (254 nm), a Varian 9176 strip chart recorder interfaced with a Varian CDS 111 LC chromatography data system. The metabolites of NDM were separated by isocratic elution with water-acetonitrile (73:27) at a flow rate of 1.2 ml/min. Samples were collected every 12 sec in scintillation vials, mixed with 6 ml 3aTQ/3 complete counting cocktail (Research Products International Corp., Elk Grove Village, Ill.), and counted in a Beckman LS9000 microprocessor-controlled liquid scintillation system (Beckman Instruments, Inc., Fullerton, Calif.). Duplicate determinations from 2 different preparations were used to derive the mean ± S.E., and results are given as pmol formed per 30 min per mg protein.

**DNA Repair.** Small segments of main pancreatic duct were preincubated in Krebs-Ringer bicarbonate buffer (KRB; pH 7.4) at 37° for 30 min. NDNM (2.5 mM), [3H]thymidine (50 μCi/5 ml; specific activity of 57.5 Ci/mmol), and hydroxyurea (5 mM) were added, and the segments were incubated for an additional 90 min. The ducts were washed 4 times in KRB buffer, fixed in 2.5% glutaraldehyde buffered phosphoric acid-cadaverine (pH 7.4) for 4 hr, embedded in plastic, and sectioned into 1-μm-thick sections. The sections were coated with Kodak NTB₂ emulsion, exposed for 4 weeks at 4°, and lightly stained with 1% toluidine blue. Controls contained only hydroxyurea and [3H]thymidine during the 90-min incubation.

**AHH Assay.** AHH activity was assayed by measuring the benzo(a)pyrene hydroxylase (EC 1.14.14.2) activity using the fluorimetric method of Nebert and Gelboin (21) as described previously (30). The

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* Dr. A. LaBarbera, personal communication.
assays were linear with time and protein concentration under the conditions described.

Chemicals. NNDM, BOP, HPOP, BHP, and [3H]NNDM (specific activity, 1.68 mCi/mmol), were generously supplied by Dr. William Lijinsky of the Frederick Cancer Research Center. The NADPH, benzo(a)pyrene, bovine serum albumin, STI, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from the Sigma Chemical Company (St. Louis, Mo.). Acetonitrile (Gold Label) was obtained from Aldrich. Distilled water was adjusted to pH 7.4 with sodium carbonate and filtered through a 0.42-μm pore size Millipore filter. All other chemicals used were analytical reagent grade from commercial suppliers.

RESULTS

HPLC Separation of the Major Metabolites of NNDM. β-Hydroxylation of NNDM gives the cyclic form of HPOP, which readily undergoes a nonenzymic ring opening to give the open form of HPOP, which can subsequently be metabolized to form BOP and BHP (Chart 1; Refs. 8 and 9). All of these metabolites have been detected in the urine of hamsters exposed to NNDM, and their interrelationships have been deduced from studies on the metabolism of NNDM and BOP in vivo (8, 9).

A HPLC system was developed to assay for the major metabolites expected to be formed as a result of incubation of NNDM with hamster pancreas S-9 or microsomal fractions and a NADPH-generating system. HPLC elution profile for a standard mixture containing BOP, HPOP, BHP, and NNDM is shown in Chart 2. BHP is the first standard to elute (Rf = 3.4 min), followed by HPOP (Rf = 4.4 and 5.0 min), and BOP (Rf = 5.3 min); NNDM elutes last (Rf = 12.8 and 14.9 min). Two isomers of NNDM and HPOP can be readily separated by HPLC (6, 31). A typical HPLC separation of the metabolites formed upon the incubation of [3H]NNDM with pancreatic acinar cell microsomes in the presence of a NADPH-generating system is superimposed on the elution profile for the standards. Since the pancreatic metabolism of NNDM does not result in the production of large amounts of metabolites, the HPLC analyses of NNDM metabolism by pancreatic preparations were performed with [3H]NNDM, and the quantitation of the separated peaks was determined by liquid scintillation counting. Qualitatively similar elution patterns were obtained with all of the pancreatic preparations tested. Due to the low levels of products formed, their identification was difficult except by comparison of their retention times with those of known standards. In this respect, 3 of the products cochromatographed with BHP, HPOP, and BOP, and are tentatively identified accordingly. An additional hydrophilic metabolite of unknown structure appears near the solvent front and is called X1, while another major metabolite of apparent hydrophobic nature appears after BOP and is called X2. The other minor peaks eluting between X2 and NNDM (see Chart 2) are unidentified, and are quantitated collectively and added to the amounts of X1, BHP, HPOP, BOP, and X2 to give total metabolism.

In Vitro Metabolism of NNDM by Hamster S-9 Fractions. The effect of S-9 protein concentration on the rates of formation of the 5 major metabolites is shown in Chart 3. The formation of all of the products increased linearly with increasing protein concentration up to 15 mg protein per ml of reaction mixture. The metabolite ratios (product distribution) also remained relatively constant up to 15 mg/ml. The major metabolite, representing almost 50% of the total products formed, was the highly soluble X1, while BOP was barely detectable. HPOP was always distributed between 2 characteristic peaks having retention times of 4.4 and 5 min. Since the isomeric forms of HPOP are readily interconvertible,5 the amounts of HPOP found in both peaks were added together to give a total value for HPOP formed. Since HPOP is thought to be the initial product formed during the metabolism of NNDM, and is an intermediate in the pathway for the formation of BHP and BOP, the rates of HPOP formation determined by this assay, in which significant

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5 Dr. D. Kokkinakis, unpublished observation.
amounts of BHP and BOP are formed, may be artificially low. A more accurate picture of the rate of formation of HPOP from NNDM might be obtained by adding the amounts of HPOP, BHP, and BOP formed. However, this would only provide an accurate value for HPOP if all of the BHP and BOP detected were formed from HPOP and were not metabolized further. We have not yet established this to be true for this system.

The activity of liver and pancreas subcellular fractions for the NADPH-supported metabolism of NNDM is shown in Table 1. Although pancreas fractions metabolized NNDM to give all 3 of the known products (BHP, HPOP, and BOP) as well as the 2 major unidentified product peaks, the total metabolism of NNDM by pancreas was less than that observed with liver subcellular fractions by at least a factor of 40. In the pancreas, the pattern of metabolites produced by S-9 was different from those formed by microsomes. This is not due to the presence in the 100,000 x g supernatant of an enzyme which has enzymic activity for the metabolism of NNDM since the 100,000 x g supernatant fractions from either pancreas or liver do not exhibit metabolic activity towards NNDM. However, the difference may be due to soluble proteins which play a role in the metabolism and interconversion of the metabolites of NNDM. The metabolism of NNDM by liver subcellular fractions results primarily in the formation of HPOP with some formation of X1. Although the amounts of metabolites formed using the S-9 fraction are less per mg of protein than those formed by the microsomal fraction, the ratios of the products are similar. It is noteworthy that the specific activity (24,600 pmol/30 min/mg protein or approximately 0.8 nmol/min/mg protein) for total product formation of liver microsomal metabolism of NNDM is of the same order of magnitude as that observed for the metabolism of other substrates by liver microsomal mixed-function oxidases (13).

**Metabolism of NNDM by Subcellular Fractions of Isolated Pancreatic Acinar and Islet Cells.** We have demonstrated previously that hamster pancreas S-9 preparations are capable of metabolically activating NNDM to a form(s) mutagenic to Salmonella typhimurium TA-1535 (30). Since the pancreas is composed of 3 major cell types (acinar, islet, and duct cells), the measurement of the metabolism of NNDM by enriched preparations of these 3 different cell types could provide information concerning which cell type, or types, are involved in the metabolic activation of the carcinogen. Methods for the preparation of duct cells of high purity in quantities sufficient for these types of studies are not yet available (10). However, methods have been developed for the isolation of hamster pancreatic acinar (1) and islet (4, 14) cells. Enrichment of these 2 cell preparations, as measured by marker proteins, is shown in Table 2; in the case of the acinar cell fraction, α-amylase was increased 1.68-fold, and the islet cell fraction radioimmune assay for insulin showed a 198-fold increase.

Metabolism of NNDM by the isolated pancreatic acinar and islet cell preparations is shown in Table 3. The data for the whole pancreas homogenate are included for comparison. Both the acinar and islet cell S-9 fractions metabolize NNDM equally well and exhibit significantly greater metabolic activity than whole pancreas preparation. The profile of metabolites formed by the acinar S-9 preparation was qualitatively similar to that of the whole homogenate. This is not surprising since acinar cells

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**Table 1**

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>X1</th>
<th>BHP</th>
<th>HPOP</th>
<th>BOP</th>
<th>X2</th>
<th>Total†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas S-9</td>
<td>199 ± 5 °</td>
<td>34 ± 2</td>
<td>64 ± 5</td>
<td>33 ± 5</td>
<td>43 ± 3</td>
<td>470 ± 14</td>
</tr>
<tr>
<td>Pancreas microsomes</td>
<td>142 ± 11</td>
<td>81 ± 6</td>
<td>28 ± 6</td>
<td>27 ± 1</td>
<td>47 ± 7</td>
<td>462 ± 32</td>
</tr>
<tr>
<td>Liver S-9</td>
<td>1,990 ± 120</td>
<td>ND *</td>
<td>8,610 ± 720</td>
<td>ND</td>
<td>ND</td>
<td>15,600 ± 1,210</td>
</tr>
<tr>
<td>Liver microsomes</td>
<td>4,430 ± 320</td>
<td>ND</td>
<td>14,900 ± 1,150</td>
<td>ND</td>
<td>ND</td>
<td>24,600 ± 2,520</td>
</tr>
</tbody>
</table>

* Includes X1, BHP, HPOP, BOP, X2, and all other unidentified metabolites.
° Mean ± S.E.
* ND, not detectable.
are the predominant cell type in the pancreas. In acinar cells, BHP, HPOP, and BOP were formed in essentially equal amounts. In the islet cell preparations, on the other hand, BHP was the major identified metabolite produced, HPOP was present at approximately one-half the amount of BHP, and only a small amount of BOP was formed. Although the preparation of microsomes from whole pancreas S-9 did not result in a marked increase in product formation, there was a marked increase in product formation for microsomes prepared from acinar S-9. In acinar microsomes, there was a change in the metabolite profile from that observed with the S-9 since the formation of BHP was approximately 3 times greater than that of HPOP and BOP. Although acinar and islet cells exhibited similar enzymic capabilities for the metabolism of NNDM, the profiles of the metabolites formed were quite different. Taking into account the high percentage of acinar cells in the pancreas, the bulk of the metabolism by whole pancreas primarily reflects metabolism by these cells. Since the 100,000 × g supernatant fractions of whole pancreas and acinar cell preparations do not metabolize NNDM to any products detectable by HPLC, the initial pancreatic metabolism of NNDM is carried out by the microsomes. However, the formation of BHP and BOP, as measured in Table 3, represents not only microsomal metabolism, but also cytosolic metabolism of HPOP.

**Induction of Enzymes for Metabolism of NNDM by Pancreatic Acinar and Islet Cells.** We have shown previously that pretreatment of hamsters with TCDD, a potent inducer of the liver microsomal cytochrome P-450-dependent mixed-function oxidases, results in a marked increase in the activation of NNDM to a mutagen for *S. typhimurium* TA-1535 by hamster pancreas S-9 fractions with a concomitant increase in AHH activity. As shown in Table 4, pretreatment with TCDD also causes an increase in the metabolism of NNDM by whole pancreas S-9. The most significant increases are in the levels of BHP and BOP (almost 100% increases). In microsomes, the increases are somewhat greater with the major differences being a marked increase in HPOP. The inductions are even more dramatic when the isolated cell preparations are investigated. Acinar cells exhibit a 78% increase in overall metabolism with a 262% increase in BHP formation. Islet cells exhibit a 72% increase with a 215% in the formation of HPOP. An even more marked difference in the cellular response to TCDD was apparent when AHH activity was assayed; acinar cell activity was increased 23-fold while that of islet cells was increased only 1.17-fold as shown in Table 5. Thus, different pancreatic cell types appear to respond differently to TCDD in terms of the induction of the enzyme activities responsible for the metabolism of NNDM. In addition, induction of NNDM metabolism in a given cell type appears to involve more than one enzyme system since different metabolite patterns are observed with different subcellular fractions.

### Table 2

*Enrichment of cell fractions isolated from hamster pancreas*

<table>
<thead>
<tr>
<th>Marker protein</th>
<th>Whole pancreas</th>
<th>Acinar cells</th>
<th>Islet cells</th>
<th>-Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Amylase (IU/μg DNA)</td>
<td>251 ± 12.7</td>
<td>421 ± 13.6</td>
<td>—b</td>
<td>1.68</td>
</tr>
<tr>
<td>Insulin (μ units/μg DNA)</td>
<td>455 ± 211</td>
<td>—</td>
<td>90,400 ± 4,300</td>
<td>198.0</td>
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</tbody>
</table>

*a* Mean ± S.E.  
*b* Not determined.

### Table 3

*Metabolism of NNDM by isolated cell preparations*

The isolated cells and the S-9 and microsomal fractions were prepared as described in "Materials and Methods." The reaction mixtures, incubation conditions, sample preparation, and HPLC analyses were the same as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>X₁</th>
<th>BHP</th>
<th>HPOP</th>
<th>BOP</th>
<th>X₂</th>
<th>Totala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas S-9</td>
<td>199 ± 5b</td>
<td>34 ± 2</td>
<td>64 ± 5</td>
<td>33 ± 5</td>
<td>43 ± 3</td>
<td>470 ± 14</td>
</tr>
<tr>
<td>Acinar S-9</td>
<td>451 ± 15</td>
<td>80 ± 4</td>
<td>94 ± 6</td>
<td>93 ± 5</td>
<td>106 ± 2</td>
<td>1070 ± 30</td>
</tr>
<tr>
<td>Islet cell S-9</td>
<td>200 ± 24</td>
<td>191 ± 6</td>
<td>102 ± 19</td>
<td>12 ± 5</td>
<td>141 ± 6</td>
<td>1060 ± 55</td>
</tr>
<tr>
<td>Pancreas microsomes</td>
<td>142 ± 11</td>
<td>81 ± 6</td>
<td>28 ± 6</td>
<td>27 ± 1</td>
<td>47 ± 7</td>
<td>462 ± 32</td>
</tr>
<tr>
<td>Acinar microsomes</td>
<td>548 ± 41</td>
<td>250 ± 14</td>
<td>75 ± 11</td>
<td>56 ± 1</td>
<td>181 ± 28</td>
<td>1750 ± 31</td>
</tr>
</tbody>
</table>

*a* Includes X₁, BHP, HPOP, BOP, X₂, and all other unidentified metabolites.  
b Mean ± S.E.

### Table 4

*Effect of TCDD pretreatment on the pancreatic metabolism of NNDM*

The isolated cells and the S-9 and microsomal fractions were prepared as described in "Materials and Methods." The reaction mixtures, incubation conditions, sample preparation, and HPLC analyses were the same as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Subcellular fraction</th>
<th>X₁</th>
<th>BHP</th>
<th>HPOP</th>
<th>BOP</th>
<th>X₂</th>
<th>Totala</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas S-9</td>
<td>203 ± 23b (21)</td>
<td>97 ± 5 (185)</td>
<td>57 ± 16</td>
<td>31 ± 3</td>
<td>43 ± 3</td>
<td>572 ± 14 (22)</td>
</tr>
<tr>
<td>Acinar S-9</td>
<td>713 ± 52 (58)</td>
<td>290 ± 15 (262)</td>
<td>115 ± 12 (23)</td>
<td>104 ± 14 (12)</td>
<td>229 ± 16 (115)</td>
<td>1912 ± 62 (78)</td>
</tr>
<tr>
<td>Islet S-9</td>
<td>328 ± 17 (64)</td>
<td>283 ± 16 (48)</td>
<td>322 ± 24 (215)</td>
<td>32 ± 12 (166)</td>
<td>201 ± 10 (43)</td>
<td>1812 ± 54 (72)</td>
</tr>
<tr>
<td>Pancreas microsomes</td>
<td>376 ± 6 (164)</td>
<td>201 ± 7 (148)</td>
<td>87 ± 6 (210)</td>
<td>57 ± 13 (111)</td>
<td>87 ± 11 (85)</td>
<td>1073 ± 31 (132)</td>
</tr>
<tr>
<td>Acinar microsomes</td>
<td>876 ± 131 (60)</td>
<td>400 ± 45 (60)</td>
<td>150 ± 25 (100)</td>
<td>88 ± 12 (57)</td>
<td>235 ± 32 (30)</td>
<td>2463 ± 230 (41)</td>
</tr>
</tbody>
</table>

*a* Includes X₁, BHP, HPOP, BOP, X₂, and all other unidentified metabolites.  
b Mean ± S.E.  
c Numbers in parentheses, percentage of increase over corresponding values for untreated animals.

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DNA Repair by Isolated Main Pancreatic Duct of Hamster. Although sufficient quantities of highly enriched duct cell preparations could not be prepared for investigation of NNDM metabolism using the HPLC assay, we were able to determine whether duct cells can metabolize NNDM to a form(s) capable of triggering unscheduled DNA synthesis (DNA repair). As shown in Figure 1, nuclear labeling of isolated main pancreatic duct epithelium was obtained with sparse grains characteristic of DNA repair synthesis, suggesting that these cells are also capable of metabolizing NNDM. However, this type of experiment does not provide adequate quantitative data; therefore, comparisons of specific activities with those of acinar and islet cells, and the determination of the metabolite profiles cannot be performed until adequate methods are available for preparing enriched fractions of isolated duct cells.

DISCUSSION

The induction of neoplasia by chemicals is generally accepted to involve their metabolic activation to electrophilic forms which react with cellular macromolecules and initiate heritable alterations which, in turn, lead to uncontrolled growth. In view of the high reactivity of electrophilic forms of carcinogens with appropriate macromolecules, it is presumed that, in the majority of instances, their metabolic activation occurs in the organs in which they induce cancer. Accordingly, it seems reasonable to attempt to understand the mechanism(s) involved in carcinogenesis and their modulation by focusing on metabolism of the carcinogen by cells of the target organ. As noted previously, in the case of hamster pancreas, which is the target organ for a variety of N-nitrosamines, the majority of studies detailing their metabolic activation have been limited to whole animals. Only 2 reports deal directly with metabolism of these compounds by the pancreas, one by Scarpe1 et al. (30) documenting activation of NNDM to electrophilic form(s) mutagenic for S. typhimurium strain TA-1538, and a second by Whalley et al. (32) describing the metabolism of BOP by hamster pancreas microsomes.

In the present study, it is established that acinar, islet, and ductal cells, the 3 cell types of which comprise pancreas, are capable of metabolizing NNDM. Because it is possible to isolate relatively pure fractions of acinar and islet cells from the pancreas, we were able to directly study metabolism of NNDM by S-9 and microsomes, by HPLC. On the other hand, metabolic activation by ductal cells had to be approached indirectly by autoradiographic demonstration of unscheduled DNA (repair) synthesis due to our inability to isolate fractions of isolated ducts and ductules of sufficient purity. S-9 and microsomal fractions derived from acinar and islet cells were capable of oxidizing NNDM in the presence of NADPH and oxygen to 3 metabolites. These results only indicate that BHP, HPOP, and BOP 2 major as yet unidentified metabolites designated as X, and X2. By contrast, liver S-9 and microsomes produced only 2 metabolite peaks, HPOP and X,. Since hamster liver microsomes can metabolize HPOP (1 HIM) to BOP or BHP in the presence of NADP+ or NADPH, respectively, these results should not be interpreted as indicating that the liver is incapable of forming these 2 metabolites. These results only indicate that BHP, BOP, or other metabolites such as MOP are not formed in significant quantities under the initial rate conditions used for these studies where less than 2% of NNDM is converted to HPOP (i.e., the final concentration of HPOP in the reaction mixture is less than 0.02 mm). MOP, a potential methylating metabolite of BOP found in hamster pancreas after the administration of BOP (16), is a potent carcinogen in the Syrian hamster exhibiting a greater potency for the pancreas than BOP (23). Although MOP standards elute close to the X, in the standard HPLC analysis described here, analysis of pancreatic and liver incubation mixtures using an HPLC gradient elution profile which separated X, from MOP standards indicated that MOP was formed by the liver in very small amounts. This method failed to detect any formation of MOP by pancreas preparations. The differences between the metabolism of NNDM by the liver and pancreas subcellular fractions may be due to differences in the types and/or concentrations of enzymes present in the 2 tissues, or to differences in their catalytic properties (e.g., the Km for HPOP). These possibilities are currently under investigation. In view of the fact that the liver-derived subcellular fractions were 40 times more active than the corresponding ones from pancreas in their capacity to metabolize NNDM, the involvement of hepatic metabolism in

Table 5

Effect of TCDD pretreatment on pancreatic AHH activity

<table>
<thead>
<tr>
<th>Cell preparation</th>
<th>Control</th>
<th>TCDD treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinar cell S-9</td>
<td>0.96 ± 0.08a</td>
<td>22.1 ± 0.63a</td>
</tr>
<tr>
<td>Islet cell S-9</td>
<td>10.5 ± 1.29</td>
<td>12.4 ± 1.62</td>
</tr>
</tbody>
</table>

a Mean ± S.E.
Significantly increased over control group; p < 0.001.
carcinogenesis of hamster pancreas by this compound must be considered seriously. It may be that HPOP produced from NNDM by the liver is presented to the pancreas which then metabolizes it further to the ultimate carcinogen. There is ample precedent for the involvement of hepatic metabolism in the carcinogenesis of more distant organs such as that of urinary bladder by N-hydroxyarylamines (11) and colon by the β-glucoside, cycasin (15). That such is the case for pancreatic carcinogenesis by NNDM remains to be established.

In the pancreas, metabolism of NNDM by S-9 differs from that obtained from microsomes, suggesting that cytosolic proteins may modulate activation of this N-nitrosamine carcinogen. Although total metabolism of NNDM by S-9 derived from isolated acinar cells was quantitatively comparable to that from isolated islet cells, the profiles of metabolites differed considerably. Basic differences between these 2 pancreatic cell types were further emphasized by their disparate response to the stimulatory effects of TCDD on their synthesis of AHH. Acinar cell levels of AH were increased 23-fold following their exposure to TCDD as contrasted to essentially no effect on AH levels of islet cells. The capacity of acinar cells to metabolize NNDM and their high sensitivity to induction by TCDD of NNDM metabolism and of AH activity coupled with the fact that they are the predominant cells of the pancreas (3) suggest that they may play an important role in pancreatic carcinogenesis. This possibility is further strengthened by the increased responsiveness of pancreatic acinar cells of the hamster to BHP and BOP as evidenced by their injury, death, and subsequent regeneration characterized by sustained mitosis during carcinogenesis (17, 28), while ductal and islet cells were significantly more quiescent.

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Metabolism of the Pancreatic Carcinogen N-Nitroso-2,6-dimethylmorpholine by Hamster Liver and Component Cells of Pancreas


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