Induction of Pancreatic Acinar Pathology via Inhalation of Nicotine (43494B)

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Abstract. **This study was conducted to determine the effects of nicotine inhalation on the onset, progression, and sequential development of pancreatic lesions. Male Sprague-Dawley rats in groups of five were exposed to saline or nicotine aerosol twice daily for 15, 30, 45, and 60 min for 21 days. After sacrifice, blood samples were analyzed for plasma levels of nicotine, glucose, gastrin, and cholecystokinin. Pancreatic tissues were examined for pathological lesions. While there were no significant differences in plasma levels of glucose, gastrin, and cholecystokinin in all groups, there was a steady increase in plasma levels of nicotine with increased exposures to nicotine. Histopathological examination of pancreatic tissue revealed definitive pancreatic injuries that also appeared to be directly correlated with increased duration of nicotine exposure. The pathological changes of the pancreas were confined only to acinar cells of the exocrine pancreas. Two main types of cellular changes were observed: cellular swelling/vacuolation and nuclear condensation/cellular pyknosis. Both of these changes indicated tissue injuries in the pancreas. Transformation of the glandular acini to solid masses of epithelial cells was also observed. The results from our present study strongly suggest that the exocrine pancreas is very sensitive and susceptible to nicotine toxicity. Our data further indicate that early morphological changes in the pancreas induced by nicotine may occur without functional or metabolic alterations; however, such changes could occur at a later stage, when tissue and cellular changes become more extensive.**
[P.S.E.B.M. 1992, Vol 201]

Chronic inhalation of nicotine through cigarette smoking has been suggested to be one of the major factors in the induction of pancreatitis, pancreatic dysfunction, and even pancreatic carcinoma (1–4). The mechanisms by which nicotine induces such disorders in the pancreas, however, are still unclear. Although it has been shown that cigarette smoking and nicotine inhibit pancreatic enzyme secretion in dogs (5) and rats (6) and bicarbonate secretion in rabbits (7), no histopathological correlations have been demonstrated. Despite the suspicion that the impairment of exocrine pancreatic secretions may reflect pathological changes in the pancreas (8), the nature of the lesions and the onset and progression of such lesions are yet to be defined.

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Received January 10, 1992. [P.S.E.B.M. 1992, Vol 201]
Accepted May 6, 1992.

0037-9727/92/2012-0159$3.00/0

In our previous studies, we demonstrated that rats, when given nicotine via drinking water for 16 weeks, developed pathological lesions in the exocrine pancreas (9). However, in those experiments, the route of nicotine exposure did not represent exposure conditions as seen in cigarette smoking. Our current study was designed to determine the nature and progressive development of pathological lesions in the rat pancreas as a result of inhalation of nicotine via aerosol. Enzymatic and hormonal changes in the blood were also simultaneously measured to account for changes in pancreatic function. Plasma nicotine levels were monitored for correlation with histopathology development.

Materials and Methods

Description of the Aerosol Chamber. The aerosol chamber was made of Plexiglas, with an opening to introduce the aerosol generated in a nebulizer. The chamber was designed with an automated vent system that maintained the inside pressure of the chamber close to atmospheric pressure. A flow meter was placed to monitor the flow of aerosol into the chamber. The humidity of the exposure chamber was not controlled.

A schematic representation of the aerosol chamber is shown in Figure 1.

Animals. Male Sprague-Dawley rats with an average body weight of 180 g were used in the study. The animals were divided into six groups of five ($n = 5$). Rats were acclimatized in animal quarters equipped with a 12:12-hr light:dark cycle for 2 weeks before initiation of the study. All animals were maintained under the same conditions throughout the experimental period.

Exposure Schedules and Experimental Procedures. Two groups of the animals, Groups 1 and 2, were exposed to saline via aerosol for 15 or 60 min, respectively, twice daily for 21 days. The remaining four groups of animals, Groups 3 through 6, were exposed to nicotine via aerosol (concentration of nicotine maintained in the nebulizer was 57 μg/liter) for 15, 30, 45, or 60 min, respectively, twice daily for 21 days. Throughout the experimental period, the animals were maintained with food and water *ad libitum*. Body weight gain, food, and fluid intakes were regularly monitored during the treatment period.

At Day 21, the animals were fasted overnight, anesthetized, and sacrificed. Blood samples were collected, centrifuged, plasma separated, and stored in the freezer at −20°C until plasma analysis was performed.

The pancreas was carefully isolated, trimmed of fat, and fixed in 10% buffered formalin. The formalin-fixed pancreases were then dehydrated with graded ethanol and embedded in paraplast. Sections of 5–6 μm were cut and stained with hematoxylin and eosin according to standard procedures as stated in a histotechnology manual (10).

Plasma glucose was measured by an orthotoluidine method (11). Plasma levels of gastrin (12) and cholecystokinin (CCK) (13) were measured by specific radioimmunoassays developed in our laboratory. Validation of these assays has been reported in dogs (13) and in rats (6, 14).

Nicotine levels in the plasma were measured employing high-performance liquid chromatography (Waters Associates, Milford, MA). Prior to high-performance liquid chromatography, plasma samples were extracted with equal volumes of chloroform. The extract was dried by nitrogen and supplemented in 70% methanol in water (pH of water adjusted to pH 9.5–9.7 by concentrated ammonium hydroxide; 15). Plasma levels of nicotine were determined from a standard curve developed with graded doses of pure nicotine.

Data Analysis. All data were presented as mean ± SE and analyzed by using two-tailed Student's unpaired t test and one-way analysis of variance. A P-value < 0.05 was considered significant.

Results

There were no significant differences in body weights, food, and fluid intakes between animals exposed to saline and those exposed to nicotine via aerosol during the 21-day period. There were also no significant changes in plasma levels of glucose, gastrin, and CCK in rats exposed to saline (Groups 1 and 2) and in nicotine-aerosol-exposed animals (Groups 3 through 6).

Nicotine levels in blood measured at 24 hr after exposure to nicotine averaged 21 ± 0.6 ng/ml in Group 3 animals. The plasma nicotine levels increased to 37 ± 2.2, 35 ± 1.1, and 40 ± 2.2 ng/ml in Groups 4, 5, and 6 animals, respectively.

Histopathological examination of the pancreas revealed no significant changes in animals exposed to saline via aerosol (Groups 1 and 2) nor in animals exposed to nicotine via aerosol for 15 min (Group 3) (Fig. 2).

Increasing pathological changes in the pancreas, however, were observed in animals exposed to higher dosing regimens of nicotine (Groups 3 and 4). Morpho-

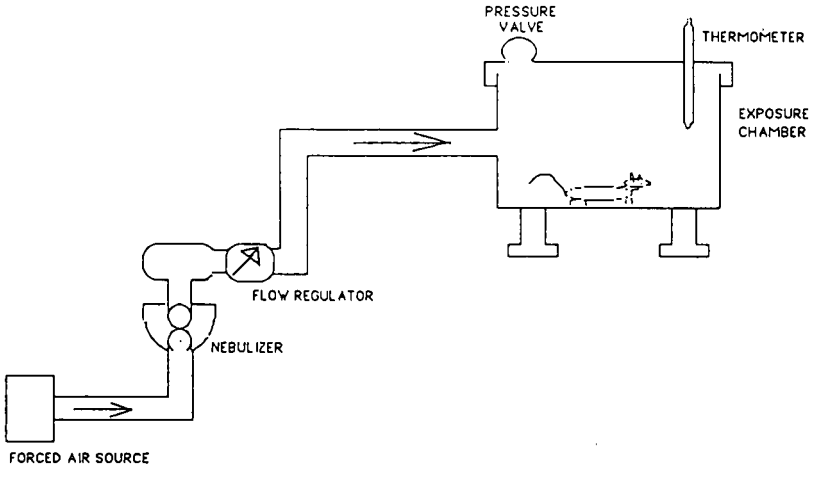

Figure 1. Sketch of aerosol chamber.

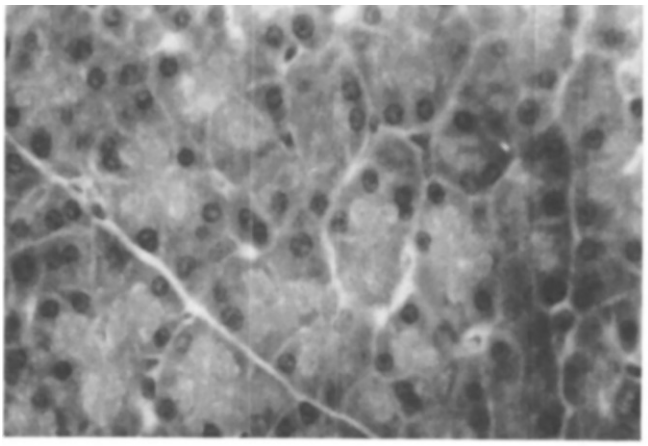

Figure 2. Pancreas, nicotine aerosol, 15-min regimens (Group 3). The histology was undistinguishable from those of control or saline aerosol-exposed animals. No histopathology was found (hematoxylin and eosin, ×400).

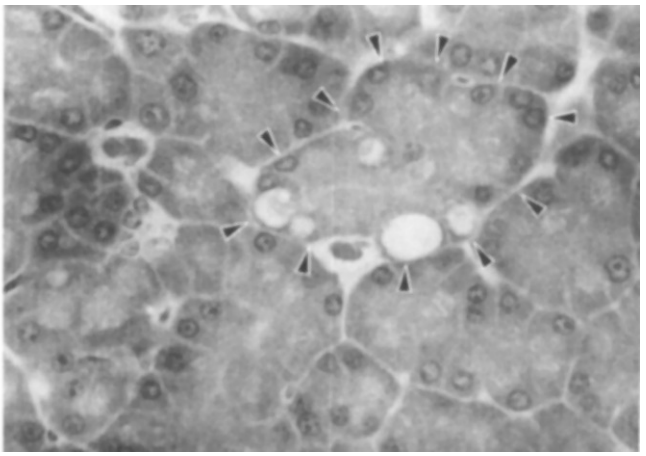

Figure 5. Pancreas, nicotine aerosol, 45-min regimens (Group 5). Vacuolar changes of several epithelial cells in the same acinar gland (outlined by arrows) can be demonstrated (hematoxylin and eosin, ×400).

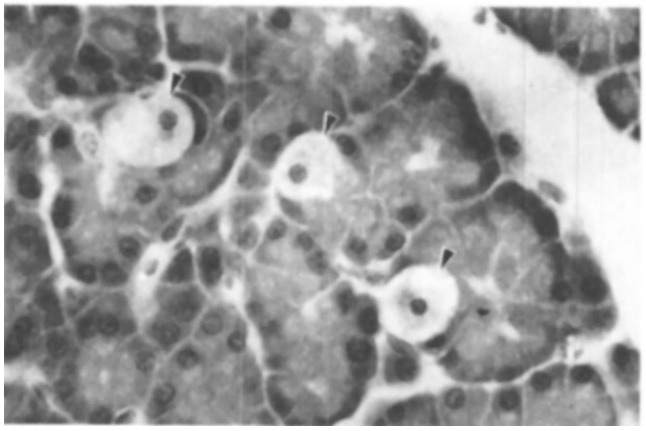

Figure 3. Pancreas, nicotine aerosol, 30-min regimens (Group 4). Edematous swelling was found in isolated acinar cells (arrows) in the pancreas (hematoxylin and eosin, ×400).

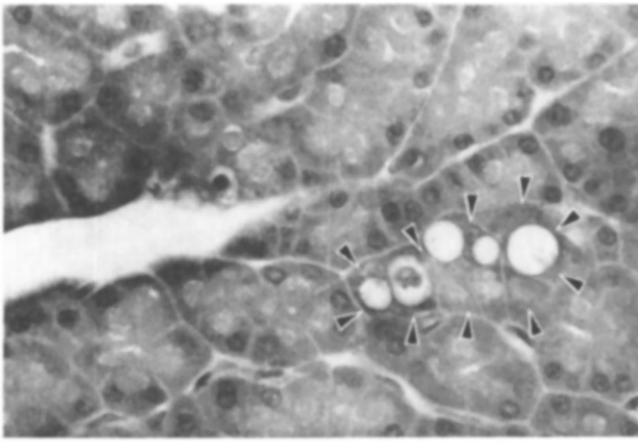

Figure 6. Pancreas, nicotine aerosol, 60-min regimens (Group 6). Severe vacuolization of an acinar gland (outlined by arrows) (hematoxylin and eosin, ×400).

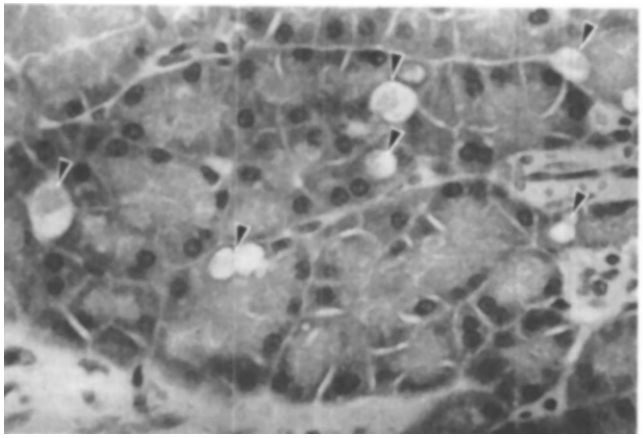

Figure 4. Pancreas, nicotine aerosol, 45-min regimens (Group 5). Scattered vacuolar changes of the acinar cells (arrows) were observed throughout the pancreas (hematoxylin and eosin, ×400).

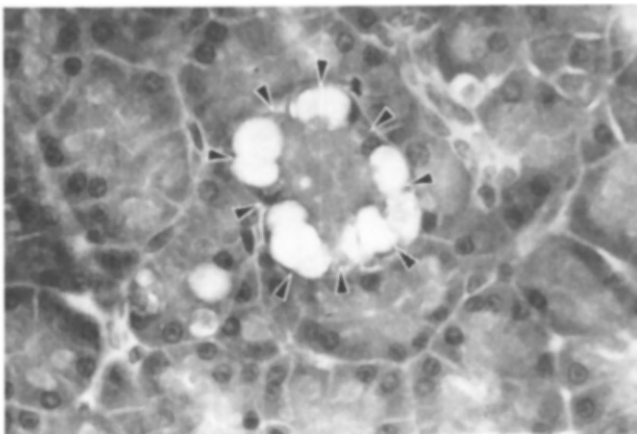

Figure 7. Pancreas, nicotine aerosol, 60-min regimens (Group 6). Total vacuolarization of an acina (outlined by arrows) (hematoxylin and eosin, ×400).

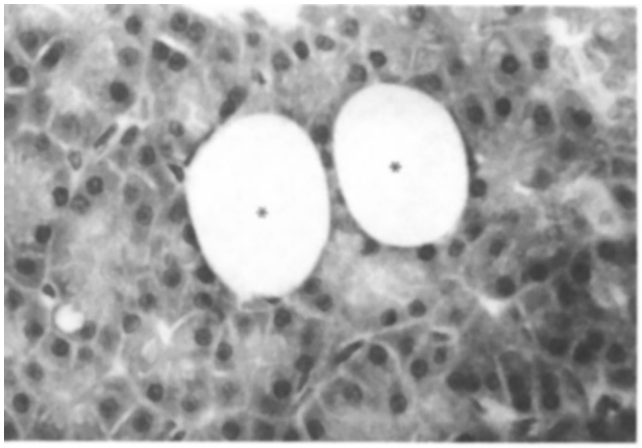

Figure 8. Pancreas, nicotine aerosol, 60-min regimens (Group 6). Large vacuoles (asterisks), probably representing the end result of total vacuolarization of the acini, could be found (hematoxylin and eosin, ×400).

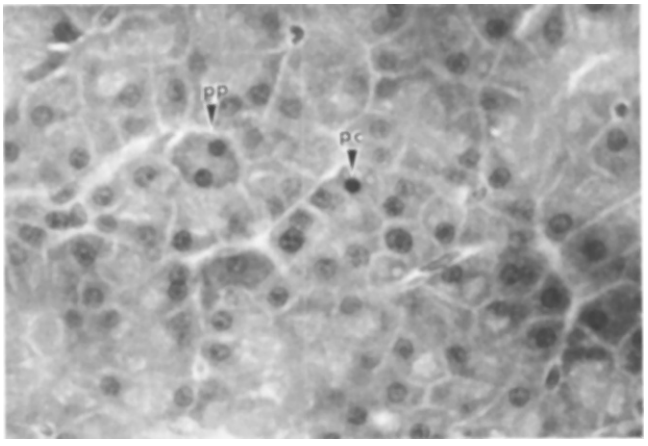

Figure 9. Pancreas, nicotine aerosol, 45-min regimens (Group 5). Prepyknotic changes of acinar cells (pp) showing nuclear condensation and hyperchromatic cytoplasm were demonstrated. A pyknotic cell (pc) is also shown (hematoxylin and eosin, ×400).

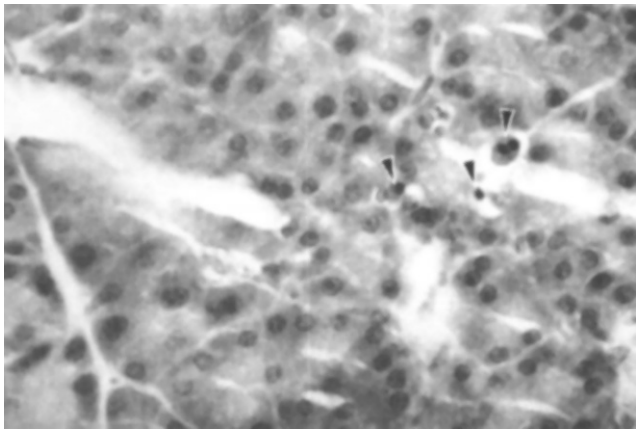

Figure 10. Pancreas, nicotine aerosol, 60-min regimens (Group 6). Groups of pyknotic (necrotic) acinar cells (arrows) were demonstrated (hematoxylin and eosin, ×400).

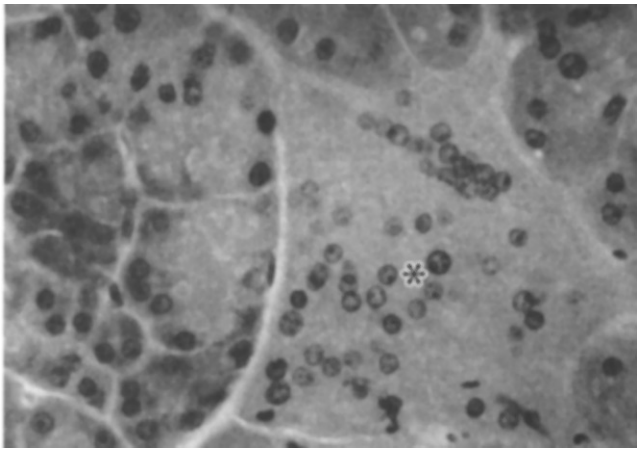

Figure 11. Pancreas, nicotine aerosol, 60-min regimens (Group 6). Large area (asterisk) of the pancreas was found to be devoid of any acinar glandular structures (hematoxylin and eosin, ×400).

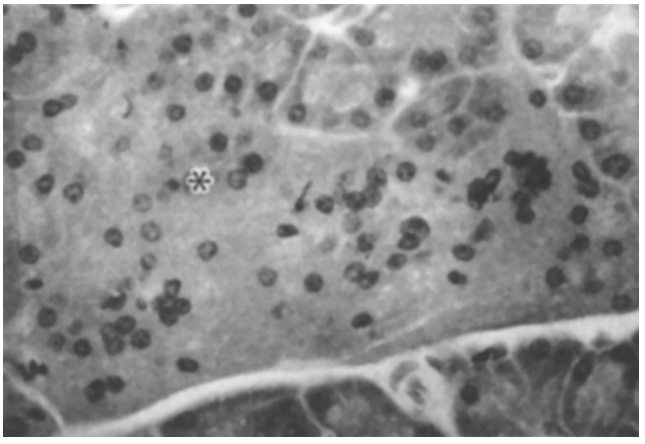

Figure 12. Pancreas, nicotine aerosol, 60-min regimens (Group 6). The area (asterisk) devoid of acinar glands was occupied by cells resembling epithelial cells. Thus, these areas may represent areas of collapsed acini and transformed acinar cells (hematoxylin and eosin, ×400).

Table I. Plasma Nicotine Levels and Histopathology

Time of exposure (min)	Plasma nicotine (ng/ml)	Extent of pancreatic lesions[a]
15	21 ± 0.6	0
30	37 ± 2.2	+
45	35 ± 1.1	++
60	40 ± 2.2	+++

[a] +, Mild lesions; ++, moderate lesions; +++, extensive lesions.

logically, the changes observed may be divided into two main categories: cellular swelling and vacuolation, and nuclear condensation (clumping) and cellular necrosis. All the histopathological changes were found to be confined only in the exocrine pancreatic acini. The endocrine pancreatic islet and other organs were spared from any damage.

Edematous swelling of isolated acinar cells (Fig. 3)

probably represented the earliest morphological changes in the pancreas, and this was the most prominent finding in the pancreases of Group 4 animals. With increased time of exposure to nicotine (Groups 5 and 6 animals), groups of cellular vacuolation (Figs. 4, 5) and extensive vacuolar destruction of the entire acinus (Figs. 6, 7) were found. Large vacuoles (Fig. 8) in the pancreas were found in some of the animals in Groups 5 and 6. These large vacuoles probably represented the result of this extensive vacuolization process of the acini.

Nuclear condensation, a recognized prepyknotic condition, could also be detected in scattered acinar cells. These cells displayed condensed nuclei and darkly stained (hyperchromatic) cytoplasm (Fig. 9). This type of cellular change could be found in the pancreases of animals exposed to ≥30-min exposures to nicotine via aerosol (Groups 4 through 6 animals). Necrotic cells with pyknotic nuclei (Figs. 9 and 10) were formed with increasing numbers and frequency in higher exposure groups (Groups 5 and 6).

In two of the Group 6 animals, large areas of the pancreases were found to be totally devoid of acinar structures (Figs. 11 and 12). Although they had no glandular structures, these areas still consisted of cells similar to epithelial cells. Based on the morphological appearance of these structures, we believe that they probably represented collapsed acini, with transformation of the acinar cells. Although the significance of this observation is still uncertain, it is worth reporting.

Progression of the lesion developments, either from cellular swelling to vacuolar changes or from nuclear condensation to cellular necrosis, tended to be well correlated with time of exposure to nicotine. That is, more severe lesions and more extensive involvements of the pancreas were seen in animals exposed to higher regimens of nicotine (Group 4 < Group 5 < Group 6).

Discussion

The results from our present study indicated that nicotine exposure of rats via aerosol inhalation at the dose levels studied could induce significant and progressive pathological changes in the pancreatic acinar cells without induction of significant physiological changes, as reflected by plasma levels of glucose, gastrin, and CCK. These histopathological findings are consistent with those that we reported earlier with rats exposed to nicotine via drinking water (9). The induction of pathological changes in the pancreas via inhalation of nicotine, however, appears to be even more rapid than the changes found in rats exposed via drinking water. The lesions, as those observed previously, were confined only to exocrine pancreatic acinar cells.

From our present investigation, it is apparent that a threshold level of plasma nicotine may be required for the induction of significant histopathological changes in the pancreas, because pathological lesions were only observed in animals exposed to a nicotine regimen of 30 min or more. This required a steady state plasma level of nicotine exceeding 35 ng/ml (Table I).

The appearance of cytoplasmic vacuoles in pancreatic acinar cells has been considered to be one of the earliest stages of pancreatitis development in rats and mice (16–22). These vacuoles have been shown to colocalize both digestive and lysosomal enzymes (20, 23–26) with an acidic internal environment (27). Niderau and colleagues (27, 28) have recently shown that in both choline-deficient ethionine-induced hemorrhagic pancreatitis and in cerulein-induced edematous pancreatitis, there was markedly reduced secretion of digestive enzymes and zymogen granules. In those studies of experimentally induced pancreatitis, intracellular vacuoles also appeared early in the course of pancreatic changes (27, 28).

In addition, experimental pancreatitis induced by cerulein, choline-deficient diet, and taurocholate showed an increasing resistance to CCK stimulation by the exocrine pancreas (27). At the time of maximal histological damage, CCK-stimulated secretion was almost abolished. In our earlier studies in which rats were given nicotine in drinking water for a period of 4 months, we also observed histological changes in the pancreas accompanied by significant changes in plasma levels of CCK and glucose (9). In addition, secretagogue-stimulated secretion of amylase was markedly inhibited (29) without affecting the CCK-receptor binding (30). However, in our current study, in which rats were exposed to nicotine for only 3 weeks, the development of histopathology in the pancreas was found without significant changes in the plasma levels of CCK and glucose. Our present observations may represent early morphological changes in the pancreas prior to significant functional alterations.

Although cigarette smoking has been linked to chronic pancreatitis (31), the time course and the precise agents from smoking involved in the progression of such pathology remain essentially undefined. The structural changes (cellular edema, intracellular vacuoles, and cellular necrosis) after nicotine inhalation in our current study certainly suggest that nicotine in the cigarette, when exposed for a duration of time, is hazardous to the pancreas.

Our present study, therefore, lends support to the hypothesis that cigarette smoking can induce pancreatitis and nicotine in cigarettes probably plays a major role in that process. As demonstrated in our previous studies, nicotine via ingestion (drinking water) can also lead to similar changes of the pancreas (9). Thus, the effects of nicotine on the pancreas via tobacco chewing as well as via nicotine-containing chewing gum should also be a concern. Further investigation will be required to elucidate its toxic mechanism.

This study was supported in part by NIH Grant 30415.

The authors wish to acknowledge the assistance of Danny McKay, Sandra McCullough, and Carla Marie LeCroy for their technical assistance, Francis Miller for histology, and Mae Agnew for excellent typing of the manuscript.

A portion of this study was presented at the American Gastroenterological Association meeting held in San Antonio, TX, 1990, and appeared in abstract form in Gastroenterology **98**:A214, 1990.

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