Acute Hemorrhagic Pancreatic Necrosis in Mice

Induction in Male Mice Treated With Estradiol

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Acute hemorrhagic pancreatic necrosis (AHPN) is induced in young female mice fed for 4 days a choline-deficient diet containing diet 0.5% DL-ethionine (CDE). Contrary to females, male mice do not develop AHPN when fed the same diet. For determination of whether estrogens are involved in the induction of AHPN, estradiol-treated male mice were fed the CDE diet. In such estrogen-treated male mice, the mortality rate, incidence of AHPN, and alterations in biochemical parameters of the pancreas and of serum were similar to those induced by the CDE diet in females. (Am J Pathol 1982, 109:8-14)

PANCREATITIS and various alterations in pancreatic functions have been reported to occur with estrogen therapy.[1-3] However, there is no experimental evidence that directly assesses the role that estrogens might play in the pathogenesis of pancreatitis. Previously we showed that feeding a choline-deficient diet containing 0.5% DL-ethionine (CDE) to mice results in pancreatitis, the induction and severity of which is conditioned by several factors. These include the sex of the animals and the choline content of the diet.[4,5] Indeed, a fatal acute hemorrhagic pancreatitis with fat necrosis (AHPN) develops in 100% of young female mice fed the CDE diet for 4 days. However, when the animals are fed a choline-supplemented diet or an unpurified diet both containing 0.5% DL-ethionine, the incidence of AHPN is only 10%.[5] On the other hand, when the same diets are fed to male mice, a chronic, nonhemorrhagic and nonfatal pancreatitis is induced, which is characterized, at worst, by focal necrosis and atrophy of acinar cells.[4,5] Thus, the sex of the animals and the choline content of the diet appear to be critical factors in the induction of AHPN by this dietary model.[5] Therefore, male mice fed a CDE diet seemed to be a good experimental system with which to test whether estrogens mediate or are otherwise involved in determining the severity of the pancreatic disease that develops in female mice. For this reason, we fed a CDE diet to estrogen-treated and untreated male mice and compared the severity of the pancreatic disease elicited. The results of these studies are the subject of the present communication.

Material and Methods

Experimental Design

Two experiments were performed, and their basic design is shown schematically in Figure 1. We performed the first experiment to determine the incidence of, and rate of mortality from, AHPN in estrogen-treated male mice fed the CDE diet. We performed the second experiment to evaluate the effects of the diet and of estrogen treatment on biochemical parameters of pancreas and serum, as well as on the severity of the elicited pancreatic histopathologic changes.

Animals and Diets

Male mice, 24 days old and 34-day-old female mice (Hill-Top Laboratory Animals, Inc., Scottdale, Pa)

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were used. The animals were fed an unpurified diet[6] (Laboratory Chow, Ralston Purina Co., St. Louis, Mo) for an adjustment period of 3 days before beginning any treatment, and had access to feed and water *ad libitum* throughout the period of study. The following purified diets were used: CS, choline supplemented; CD, choline deficient; CSE, CS plus 0.5% DL-ethionine; and CDE, CD, plus 0.5% DL-ethionine. CDE is the experimental diet, and CD its control. The CS diet was used to assess any effect that choline deficiency *per se* might have in the model, and the CSE diet to assess the effect of ethionine alone. Preparation of the diets and housing of the animals were as reported previously.[7,8]

Estrogen Treatment of Male Mice

The animals were anesthetized with halothane, and a small incision was made in the skin overlying the vertebral column. A Silastic capsule (2 mm inside diameter/2 cm length) containing estradiol or a control capsule without hormone was implanted under the skin. The wound was closed with stainless steel staples, and the animals were returned to their cages. The animals were maintained on laboratory chow for the next 7 days. During this period, all mice were free of infection and showed no sign of discomfort. Estradiol capsules were prepared as previously described.[9] Each capsule was filled with 0.063 ml of a solution of estradiol (300 μg/ml) in peanut oil.

First Experiment

Groups of 10 female mice, 10 non-estrogen-treated male mice, and 10 estrogen-treated male mice were fed the CDE diet up to the time of death or sacrifice (Figure 1). The animals were observed daily, and autopsies or necropsies were performed immediately after death. At necropsy, the gross appearance of the abdominal organs was noted, and representative pieces of pancreas and of abdominal fat tissues were taken and processed for histologic examination as reported previously.[4]

Second Experiment

Non-estrogen-treated and estrogen-treated male mice were divided into 4 groups, and each pair of groups was fed, for 3 days, one of the purified diets (Figure 1). The mice were sacrificed either by decapitation or by exsanguination through the retroorbital plexus. The collected blood was used to obtain serum for analysis. The abdominal cavity was opened, and

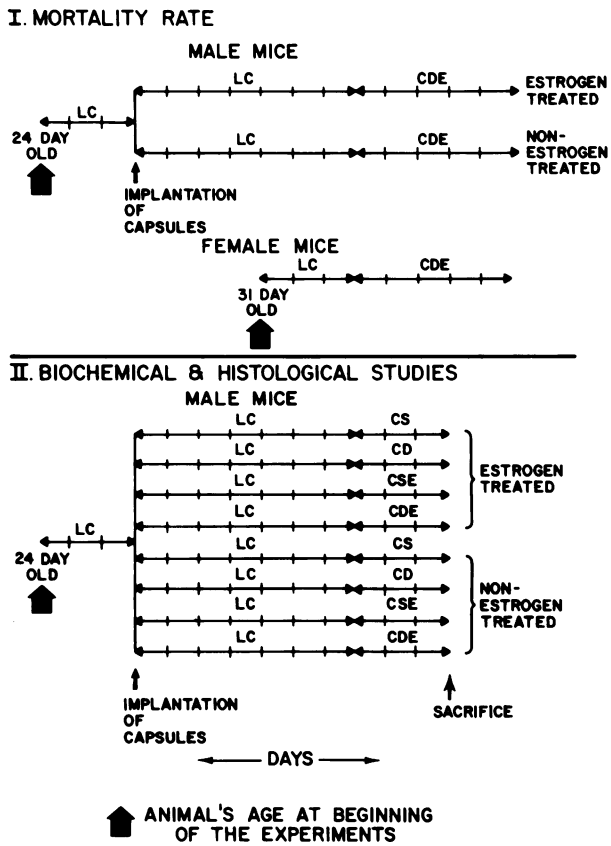

Figure 1 – Experimental design. *LC*, Laboratory Chow; *CS*, choline-supplemented; *CD*, choline-deficient; *CSE*, choline-supplemented ethionine; and *CDE*, choline-deficient ethionine.

the gross appearance of the pancreases and of the intraabdominal fat tissues was noted. The pancreases were removed, weighed, and chilled on ice. In one experiment, a piece of pancreas was taken and processed for histologic examination. Pancreatitis was scored blindly from 1+ to 4+. The scoring was performed as follows: 1 +, decrease in the basophilia of the cytoplasm of the acinar cells; 2+, vacuolation of the acinar cells; 3+, lobular necrosis of the parenchyma; and 4+, massive hemorrhagic necrosis. Intermediate stages were also scored. Pancreatic tissue remaining (about 80%) after sampling for histologic examination or whole pancreases, were homogenized without delay in preparation for biochemical analyses as described below. All enzyme assays, except those of trypsinogen, were performed without delay.

Determination of Amylase, Cathepsin B, Trypsinogen, and Trypsin

We pooled pancreas remnants of 2 mice from each group in order to have sufficient tissue to perform all

the analyses. We homogenized the pooled tissue in 9 volumes of 0.25 M sucrose with a Potter-Elvejhem glass homogenizer and a Teflon pestle, at 1500 rpm, using three up-and-down strokes. One aliquot of the homogenate was diluted 200-fold with a 20 mM phosphate buffer, pH 6.9, containing 6 mM NaCl. One hundred to two hundred microliters of the diluted homogenates were used to determine amylase.[7] Three hundred microliters of nondiluted homogenates were used to determine[10] cathepsin B, using α-N-benzoyl-DL-arginine-β-naphthylamine HCl (BANA) as the substrate. Units of enzyme activities were as follows: amylase, milligrams of maltose released per minute, and cathepsin B, nanomoles of BANA hydrolyzed per minute. As in previous communications from this laboratory, the results were expressed as units per pancreas, rather than units per milligram protein, in view of the marked changes, caused by the CDE diet, in the weight and protein content of the pancreas. Similarly, with the onset of pancreatitis and of acinar cell necrosis, expression of results as units per milligram DNA would not be reliable.

The pancreases were homogenized in 9 volumes of 0.1 M Tris-HCl buffer, pH 8.5, containing 0.1% Triton-X-100 and 0.9% NaCl. The homogenates were diluted 10-fold with the same buffer, and aliquots of the diluted homogenates were mixed with equal volumes of a 0.05% enterokinase solution in 0.1 M Tris-HCl buffer (pH 7.8) containing 0.1 M $CaCl_2$. The homogenates were activated for 2 hours at 37 C and were then used for determination[7] of trypsin, with p-toluenesulfonyl-L-arginine methyl ester hydrochloride (TAME) as the substrate. Units of trypsin activity were expressed as nanomoles of TAME hydrolyzed per minute, and the results were expressed again as units per pancreas.

Serum Analyses

Serum from individual mice was separated into three aliquots. The first aliquot was used immediately for estimation of amylase, and the others were stored at −70 C until used for protein electrophoresis and estradiol determination. Serum aliquots for amylase estimation[7] were diluted 200-fold with 20mM PO_4 buffer (pH 6.9) containing 6mM NaCl, and 0.2 ml of the diluted serum were used for assay. Serum estradiol was determined by a radioimmunoassay (RIA) method[11,12] with an intraassay coefficient of variation of less than 6% and an interassay variation of less than 8%. The detection limit of the method is 1.0/pg estradiol/tube. We performed agarose gel zone electrophoresis[13,14] to detect fractions known to contain major serum proteinase inhibitors.[15] Particular attention was directed toward the resolution of the α_1-antitrypsin and α_2-macroglobulin fractions. Native C3 was assessed similarly.[16]

Other Procedures

Proteins were estimated by the method of Lowry et al.[17] Statistical analysis of data was performed using an analysis of variance[18] with appropriate multiple comparison procedures, and differences between means were considered significant if P was less than 0.05.

Reagents and Chemicals

Estradiol was purchased from Sigma Chemical Co., St. Louis, Missouri. 2-4-6,7,16,17-^{3}H(N)-Estradiol (E_2), 150 Ci/mmole, was obtained from New England Nuclear Corporation, Chicago. Its purity was checked periodically by thin-layer chromatography on silica gel, using ethyl acetate : hexane : ethanol (85 : 10 : 5) as the development solvent. The component was used only when its purity was 97% or greater. Phenylmethylsulfonyl fluoride (PMSF); Triton-X-100; BANA, TAME, and crystalline trypsin were purchased from Sigma Chemical Co. Enterokinase was obtained from Worthington Biochemical Corporation, Freehold, New Jersey, and Norit A and Dextran C from Fisher Scientific Co., Pittsburgh, Pennsylvania. All other reagents and chemicals were of purity grade and were obtained from standard commercial sources.

Results

In mice fed the CDE diet up to the time of their death or sacrifice, the following mortality rates were observed: 8 females died on the fourth day of the experiment, 2 on the fifth; 6 estrogen-treated male mice died on the fourth day, 1 on the fifth day, and 3 on the sixth day; and 2 non-estrogen-treated male mice died on the fourth day, 1 each on the eighth, eleventh, and thirteenth days, and the surviving five were sacrificed on the fourteenth day. Death of female mice and of estrogen-treated males, but not of the non-estrogen-treated male mice, was preceded, in all cases, by a shocklike state characteristic of this model.[4,5] On inspection of the abdominal organs at necropsy, a swollen, edematous, and hemorrhagic pancreas, as well as necrosis of the peritoneal fat tissues, was noted in all of the female mice and in all of the estrogen-treated males. In marked contrast, the

Table 1—Body Weight and Wet Weight and Histopathologic Changes of the Pancreas of Non-Estrogen-Treated and Estrogen-Treated Male Mice Fed Semipurified Diets for 3 Days

Diet	Group	Estrogen	Body weight (g)	Pancreas	
				Wet weight (mg)	Histopathologic changes (degree of pancreatitis*)
CS	a	–	(6)25.00 ± 0.41	(6)203 ± 6	(3)0.00 ± 0.00
	b	+	(6)26.67 ± 0.65	(6)220 ± 9	(3)0.00 ± 0.00
CD	c	–	(14)26.07 ± 0.87	(14)198 ± 9	(10)0.08 ± 0.05
	d	+	(14)29.0 ± 1.42[a†]	(12)219 ± 15	(12)0.00 ± 0.00
CSE	e	–	(14)24.19 ± 1.03[d]	(14)310 ± 23[a–d]	(10)0.82 ± 13[a–d]
	f	+	(14)24.64 ± 0.98[d]	(14)326 ± 16[a–d]	(14)0.91 ± 0.10[a–d]
CDE	g	–	(13)22.0 ± 0.58[a–d]	(13)375 ± 21[a–e]	(10)2.10 ± 0.17[a–f]
	h	+	(13)21.92 ± 0.98[b–d]	(13)398 ± 22[a–f]	(10)3.10 ± 0.20[a–g]

* Pancreatitis was scored as described in Materials and Methods.

† Each value represents the mean ± SE and is significantly different when compared with the group indicated. $P < 0.05$ is considered significant. The number of animals is shown in parenthesis.

CS, choline-supplemented; CD, choline-deficient; CSE, CS plus 0.5% DL-ethionine; CDE, CD plus 0.5% DL-ethionine.

typical finding in non estrogen-treated male mice was a milky white pancreas of reduced size and abdominal fat tissues of normal appearance. Histologic examination confirmed the presence of an AHPN and of necrosis of the peritoneal fat tissues in females and estrogen-treated males, while in the non-estrogen-treated male mice only atrophy of the acinar parenchyma of the pancreas was present.

As shown in Table 1, feeding the CS and CD diets for 3 days caused only minor variations in the body weight and the wet weight of the pancreas. On the other hand, the CSE diet caused a slight but significant decrease in the body weight of both non-estrogen-treated and estrogen-treated male mice. A further significant decrease was present in mice fed the CDE diet. No difference in the wet weight of the pancreases was observed between non-estrogen-treated and estrogen-treated mice fed any of the diets. However, the CSE and CDE diets resulted in a significant increase in the wet weight of the pancreases. Notable histopathologic features were absent in the pancreases of mice fed the CS and CD diet. Loss of cytoplasmic basophilia and increased granularity of the cytoplasm of the acinar cells were the predominant lesions in the pancreases of non-estrogen-treated and estrogen-treated mice fed the CSE diet. However, in non-estrogen-treated male mice fed the CDE diet, the pancreatic disease was more advanced and included a marked vacuolation of the acinar cells. In contrast, colliquative necrosis, involving entire lobules of the pancreas, was the typical finding in estrogen-treated male mice fed the same diet.

The results of the biochemical analyses are shown in Tables 2 and 3. Analyses of serum and tissues of male mice fed the CS diet were omitted, since the body weight, pancreas weight, and gross and histologic appearance of the pancreases of these mice were essentially the same as in male mice fed the CD diet. Total protein per pancreas was significantly higher in both non-estrogen-treated and estrogen-treated mice

Table 2—Pancreas and Serum Proteins and Serum Amylase of Non-Estrogen-Treated and Estrogen-Treated Male Mice Fed Purified Diets for 3 Days

Diet	Group	Estrogen	Pancreas (mg protein)	Serum	
				Protein (mg/ml)	Amylase (units/ml)
CD	a	–	(5)23.45 ± 2.40	(5)60.26 ± 1.97	(4)12.37 ± 1.60
	b	+	(5)25.59 ± 2.17	(4)58.92 ± 2.65	(3)8.04 ± 0.91
CSE	c	–	(5)48.58 ± 3.59[a,b*]	(5)59.03 ± 1.04	(5)14.64 ± 7.67
	d	+	(6)46.85 ± 3.67[a,b]	(5)62.75 ± 2.93	(5)22.72 ± 7.90
CDE	e	–	(5)41.24 ± 3.26[a,b]	(5)52.15 ± 2.06[a,d]	(5)97.34 ± 9.35[a–d]
	f	+	(5)43.12 ± 6.16[a,b]	(3)54.57 ± 2.85	(5)109.72 ± 10.00[a–d]

* Each value represents the mean ± SE and is significantly different when compared with the group indicated. $P < 0.05$ is considered significant. The number of animals is shown in parenthesis.

CD, choline-deficient; CSE, CS plus 0.5% DL-ethionine; CDE, CD plus 0.5% DL-ethionine.

Table 3—Pancreas Trypsinogen, Trypsin, and Cathepsin B of Non-Estrogen-Treated and Estrogen-Treated Male Mice Fed Purified Diets for 3 Days

Diet	Group	Estrogen	Trypsinogen (units)	Trypsin (units)	Cathepsin B (units)
CD	a	–	(3)54.70 ± 9.17	(3)2.83 ± 0.30	(5)32.63 ± 3.08
	b	+	(3)73.39 ± 7.19	(3)3.52 ± 0.25	(6)42.07 ± 4.59
CSE	c	–	(4)96.85 ± 23.37	(4)2.83 ± 0.30	(5)48.99 ± 5.87
	d	+	(4)103.06 ± 18.34[a–c]*	(4)3.68 ± 0.22[c]	(6)54.43 ± 6.47
CDE	e	–	(3)188.51 ± 8.13[a–c]	(3)3.81 ± 0.25[a,c]	(3)36.93 ± 1.86[d]
	f	+	(3)262.16 ± 24.30†	(3)6.17 ± 0.02†	(5)65.43 ± 8.26[a–e]

* Each value represents the mean ± SEM and is significantly different when compared with the group indicated. $P < 0.05$ is considered significant. The numbers in parenthesis refer to pools of two pancreases analyzed (see Materials and Methods).

† Significantly different from all groups.

CD, choline-deficient; CSE, CS plus 0.5% DL-ethionine; CDE, CD plus 0.5% DL-ethionine.

fed the CSE and CDE diets. No significant alteration, due either to diet or hormonal status, was present in the serum level of proteins, with the exception of a small decrease observed in non-estrogen-treated mice fed the CDE diet (Table 2). Non-estrogen-treated and estrogen-treated male mice fed the CSE diet had the highest content of amylase per pancreas. However, male mice fed the CDE diet had serum amylase levels 5–10-fold greater than any other group of male mice. Feeding the CSE and CDE diets resulted in an increased pancreatic content of trypsinogen (Table 3). Moreover, the content of trypsinogen was significantly greater in estrogen-treated mice fed the CDE diet than in any other group of mice. Trypsin activity was similar in all groups of male mice, except for the estrogen-treated male mice fed the CDE diet, in which a 2-fold increase was found. In the latter animals, the activity of cathepsin B was also significantly greater than in any other group of mice.

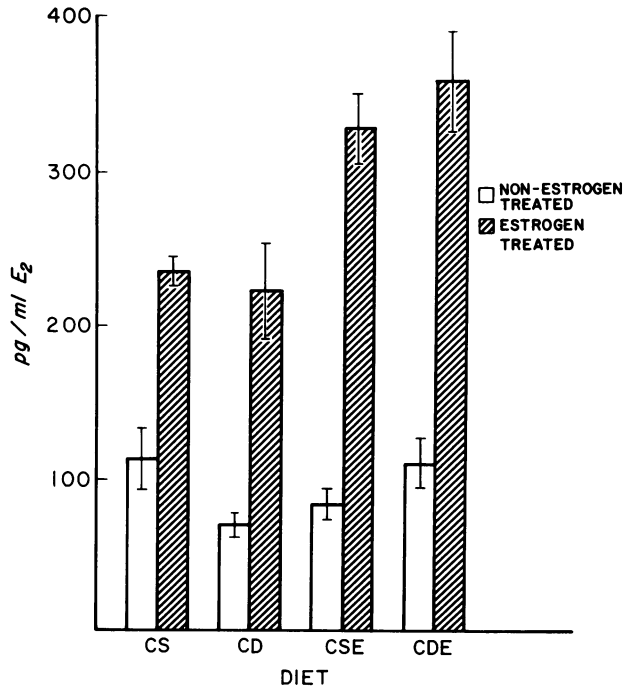

Figure 2—Serum estradiol levels in male mice fed various diets. Estradiol levels are expressed as picograms per 1 ml of serum. *Bars* represent mean values, and *brackets* represent SEM. *CS*, choline-supplemented; *CD*, choline-deficient; *CSE*, choline-supplemented ethionine; and *CDE*, choline-deficient ethionine.

The data illustrated in Figure 2 show that hormone treatment of male mice, irrespective of the diet, resulted in serum levels of estradiol two to four times higher than those observed in the respective non-estrogen-treated mice. In addition, serum estradiol levels were significantly higher in animals receiving both estrogen and ethionine than in those receiving only estrogen.

A representative electrophoretogram of serum proteins is shown in Figure 3. No differences were apparent in the concentrations of albumin, α_1-globulins, and α_2-globulins between non-estrogen-treated and estrogen-treated mice fed any of the diets. However, decreases were existent in the concentrations of α_1-globulins, which contain α_1-antitrypsin; α_2-globulins, which contain α_2-macroglobulin; and transferrin, whch contains C3, in non-estrogen-treated mice fed the CSE diet, and even more so in those fed the CDE diet. Hormonal treatment produced even further decreases in these proteins.

Discussion

In agreement with previous reports,[4,5] male mice, contrary to female mice, were found to be rather insensitive to the induction of an AHPN by a choline-deficient diet containing DL-ethionine. The results of the present studies clearly indicate that the sex difference in the response to the dietary regimen is mediated, at least in part, by estrogens. Indeed, feeding the diet to estrogen-treated males resulted in marked increases in the mortality rate, incidence of

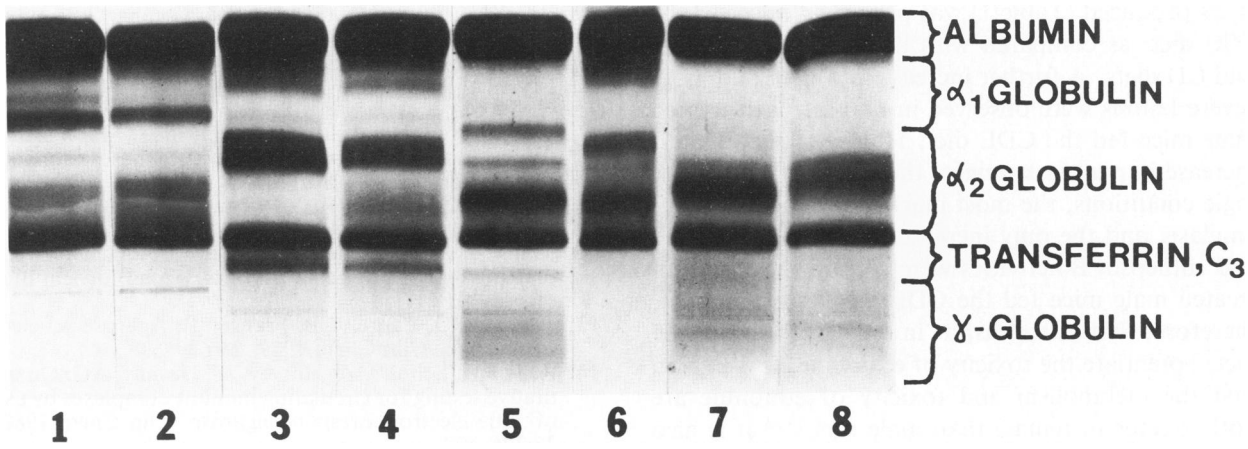

Figure 3—Serum proteins of male mice fed various diets. Agarose gel electrophoresis of serum proteins was performed as described under Materials and Methods. *CS*, choline-supplemented; *CD*, choline-deficient; *CSE*, choline-supplemented ethionine; and *CDE*, choline-deficient ethionine. 1) CS-non-estrogen-treated. 2) CS-estrogen-treated. 3) CD-non-estrogen-treated. 4) CD-estrogen-treated. 5) CSE-non-estrogen-treated. 6) CSE-estrogen-treated. 7) CDE-non-estrogen-treated. 8) CDE-estrogen-treated.

AHPN, and in alterations of biochemical parameters of pancreas and serum similar to those previously described to occur in female mice fed the same diet.[4,5,7,8,16] In the latter animals, the hallmark lesions and their progression, leading to the onset of AHPN, are an increase in the weight of the pancreas, due mostly to engorgement of the acinar cells with zymogens; an increase in the activity of the lysosomal enzyme cathepsin B; an intraparenchymal activation of zymogens, probably triggered by cathepsin B, with formation of active trypsin and other proteinases; an increased level of serum amylase; spillage of active proteinases into the circulation, as shown by decreases in the serum levels of α_1-antitrypsin, α_2-macroglobulin, and C3; onset of a shocklike state, in all likelihood caused by input into the circulation of active proteinases; and, finally, massive necrosis of the pancreas due to autodigestion of the organ, with the ultimate death of the animals.[4,5,7,8,10,16] Evidence of the occurrence of all these alterations in estrogen-treated male mice fed the CDE diet was obtained in the present studies (Tables 1–3).

Implantation of estradiol capsules resulted in a 2–3-fold increase in the level of circulating estradiol in male mice fed the CS or CD diet (Figure 2), and in a 3–4-fold increase in those fed the same diets containing ethionine. These results suggest that ethionine either facilitates estradiol assimilation or interferes with its metabolism and/or clearance. The latter possibility seems to be the more plausible, because ethionine produces liver injury,[4,19] and the liver is intimately involved in the clearance of estrogens from the circulation.[20] Furthermore, chronic ingestion of ethanol, another hepatotoxin, is known to result in increased levels of serum estrogens, as well as of hepatic estrogen receptors in the cytosol of rats.[12] Mammalian pancreatic acinar cells are known to possess estrogen receptors, and exogenously administered estrogens have been shown to accumulate in the pancreas.[21,25] In preliminary findings we have also observed that estrogen treatment results in enhanced pancreatic cytosolic estrogen receptor levels in male mice fed the CS or CD diets, and that ethionine supplementation of these diets results in yet further increases.

Estrogen administration to both rats and dogs has been shown to produce an increase in the weight of the pancreas, alterations in the flow rate and composition of pancreatic juice, and histopathological changes.[24] Furthermore, pancreatitis and other changes in pancreatic functions have been reported to occur in patients taking estrogens for clinical reasons.[1–3] However, the results of the present studies suggest that under the experimental conditions used, estrogens *per se* are not pancreatotoxic. Indeed, estrogen treatment of male mice fed the CS or CD diets did not influence the weight, the anatomic and histologic features, and the content of trypsinogen, trypsin, and cathepsin B of the pancreas (Table 3) or the protein and amylase content of the serum (Table 2). Results of previous studies with this experimental model of AHPN have indicated clearly that the principal pancreatotoxic agent in the inducing regimen is ethionine, and that the role played by choline deficiency is to enhance the toxicity of ethionine. Evidence of this potentiating effect was obtained also in the present studies. An increase in the weight of the pancreas and in the severity of the histopathologic le-

sions produced (Table 1) was present in mice fed the CSE diet, as compared with male mice fed the CS and CD diets. A further increase in weight and more severe lesions were observed in non-estrogen-treated male mice fed the CDE diet. However, the greatest increase in pancreas weight, the severest histopathologic conditions, the most marked increase in serum amylase, and the only increase in pancreatic trypsin and cathepsin B activities were seen in the estrogen-treated male mice fed the CDE diet. It is apparent, therefore, that estrogens, as in the case with the CD diet, potentiate the toxicity of ethionine. It is known that the metabolism and toxicity of ethionine are both greater in female than male rats.[19,26] It is also known that estrogens induce changes in the lysosomal content of the uterus.[27] It seems possible that a combined action of estrogens and of ethionine on the lysosomes of the pancreas might induce an activation of cathepsin B and of zymogens, while neither agent alone can do so.

References

1. Glueck CJ, Scheel D, Fishback J, Steiner P: Estrogen induced Pancreatitis with previously covert familial V hyperlipoproteinemia. Metabolism 1972, 21:657-666
2. Davidoff F, Tishler S, Rosoff C: Marked hyperlipidemia and pancreatitis associated with oral contraceptive therapy. N Engl J Med 1973, 289:552-555
3. Foster ME, Powell DEB: Pancreatitis, multiple infarcts and oral contraception. Postgraduate. Med J 1975, 51: 667-669
4. Lombardi B, Estes LW, Longnecker DS: Acute hemorrhagic pancreatitis (massive necrosis) with fat necrosis induced in mice by DL-ethionine fed with a choline deficient diet. Am J Pathol 1975, 79:465-478
5. Lombardi B, Rao KN: Acute hemorrhagic pancreatic necrosis in mice. Influence of age and sex of the animals and of dietary ethionine, choline, methionine and adenine sulfate. Am J Pathol 1975, 81:87-100
6. AIN: Report of the American Institute of Nutrition. Ad Hoc Committee on Standards for Nutritional Studies. J Nutr 1977, 107:1340-1348
7. Rao KN, Tuma J, Lombardi B: Acute hemorrhagic pancreatic necrosis in mice: Intraparenchymal activation of zymogens, and other enzyme changes in pancreas and serum. Gastroenterology 1976, 70:720-726
8. Rao KN, Katyal SL, Immarino RM, Lombardi B: Acute hemorrhagic pancreatic necrosis in mice: Alterations in pancreatic lipase activity, pancreas lipids, and serum lipoproteins. Digestion 1980, 20:314-322
9. Gay VL, Hanger RL: A sex related pattern of gonodotropin secretion in the castrated rat: Effect of changing the inhibitory steroid pituitary LH content. Biol Reprod 1977, 16:527-535
10. Rao KN, Zuretti MF, Baccino FM, Lombardi B: Acute hemorrhagic pancreatic necrosis in mice: Activity of lysosomal enzymes in the pancreas and the liver. Am J Pathol 1980, 98:45-59
11. Hotchkiss J, Atkinson LE, Knobil E: Time course of serum estrogen and lutenizing hormone (LH) concentrations during menstrual cycle of the Rhesus monkey. Endocrinology 1971, 89:117-183
12. Eagon PK, Zdunek JR, Van Thiel DH, Singletary BK, Egler KM, Gavaler JS, Porter LE: Alcohol induced changes in estrogen binding proteins: A mechanism explaining feminization in alcoholics. Arch Biochem Biophys 1981, 211:48-54
13. Jeppson JO, Laurell CB, Franzen B: Agarose gel electrophoresis. Clin Chem 1979, 25:629-638
14. Kelly RH, Scholl MA, Harvey VS, Devenyi AG: Quantitative testing for circulating immune complexes by use of zone electrophoresis on agarose. Clin Chem 1980, 26:396-402
15. Clausen J, Hermans J: An immunologic and chemical study of the similarities between mouse and human serum proteins. J Immunol 1960, 84:128-134
16. Kelly RH, Rao KN, Harvey VS, Lombardi B: Acute hemorrhagic pancreatic necrosis in mice: Alterations of serum complement. Digestion 1981, 22:1-7
17. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 1951, 193:265-275
18. Steel RGD, Torrie JH: Principles and procedures of statistics: A biometrical approach. 2nd edition. New York, McGraw-Hill, 1980, pp 137-195
19. Farber E, Koch-wiser DK, Popper H: Influence of sex and of testosterone upon fatty liver due to ethionine. Endocrinology 1951, 48:205-212
20. Adlercreutz H, Martin F: Biliary excretion and intestinal metabolism of progesterone and estrogens in man. J Steroid Biochem 1980, 13:231-244
21. Ullberg S, Bengtsson G: Auto-radiographic studies with natural estrogens. Acta Endocrinol 1963, 43:75-86
22. Kirdani RY, Sandberg AA, Murphy GP: Estrogen binding to pancreas. Surgery 1973, 74:84-90
23. Sandberg AA, Kirdani RY, Varkarakis MJ, Murphy GP: Estrogen receptor protein of pancreas. Steroids 1973, 22:259-271
24. Sandberg AA, Rosenthal HE: Steroid receptors in the pancreas. J Steroid Biochem 1974, 5:969-975
25. Sandberg AA, Rosenthal HE: Steroid receptors in exocrine glands: The pancreas and prostate. J Steroid Biochem 1979, 11:293-299
26. Farber E, Segaloff A: Effect of androgens and growth and other hormones on ethionine fatty liver in rats. J Biol Chem 1955, 216:471-477
27. Sloane BF: Lysosomal appartus in uterine muscle: Effects of estrogen and of ovariectomy. Biol Reprod 1980, 23:867-876

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