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Folate Deficiency and Pancreatic Acinar Cell Function (41938)

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Abstract. The present study was designed to determine the effect of folate deficiency on pancreatic acinar cell function. In the first series of experiments, three groups of rats were fed *ad libitum* regular rat feed, folate-deficient diet, or an equivalent amount of folate-sufficient diet. In the second series of experiments, rats were either fed *ad libitum* or rendered folate deficient by a purified folate-deficient diet; half of the folate-deficient group was replenished with oral folate. Body weight, pancreatic weight, DNA, [*methyl*-^{14}C]thymidine incorporation into DNA, RNA, [8-^{14}C]adenine incorporation into RNA, protein content, synthesis of proteins, amylase content, and basal and bethanechol-stimulated amylase secretion were determined. The parameters were the same in the rats fed a folate-sufficient diet as in those fed a regular rat feed. Feeding a folate-deficient diet resulted in impaired DNA synthesis as evidenced by diminished incorporation of [*methyl*-^{14}C]thymidine into DNA. There was no change in secretion of amylase. Similar results were obtained in the second series of experiments. These studies indicate that folate deficiency (rather than antibiotic content of the diet) impaired pancreatic function. Folate deficiency may therefore contribute to pancreatic injury in malnutrition and alcoholism.

In malnourished alcoholics with liver disease, deficiency of folate (as evidenced by low serum folate, increased urinary excretion of formiminoglutamic acid, and macrocytic anemia) is frequently observed clinically (1–3). Moreover, in this population of patients reduction in circulating levels of B-complex factors, including folate, is commonly observed in the absence of clinical stigmata of vitamin deficiency (1). Besides a diminished intake, patients with liver disease such as fatty liver and cirrhosis have a decreased hepatic storage capacity for folate, pyridoxine, and thiamin (4). Low levels of tissue folate are paralleled by decreased *in vitro* incorporation of tritiated thymidine into hepatic DNA as determined from percutaneous liver biopsy specimens, and administration of folate restores hepatic DNA synthesis patterns to normal (1). Previous studies in our laboratory show that deficiencies of vitamin B_6 and thiamin lead to remarkable changes in the pancreatic function of Sprague–Dawley rats (5, 6). There are no reports of the effects of folate deficiency on the pancreatic acinar cell function. The present study was undertaken to extend our previous observations in order to determine the effect of relatively short-term folate deficiency on pancreatic acinar cell metabolism in the rat.

Materials and Methods. *Experimental design.* Male Sprague–Dawley rats weighing 50 to 74 g were purchased from Harlan–Sprague–Dawley, Indianapolis, Indiana. Folate-deficient diet (Diet 1406) and corresponding control diet (Diet 1405) were purchased from Bio Serve, Inc., Frenchtown, New Jersey, and regular stock diet (Wayne Laboratories Blox MRH 22/5) was purchased from Allied Mills, Inc., Chicago, Illinois. The folate-deficient diet consisted of casein 18%, corn oil 6%, dextrose 22%, cornstarch 6%, succinyl sulfathiazole 0.1%, vitamin mixture 1%, salt mixture 4%, choline dihydrogen citrate 0.25%, methionine 0.3%, fiber 5%, and sucrose 37.35%. The folate-sufficient diet had the same constituents except succinyl sulfathiazole. The folate-sufficient diet contained 1.56 mg kg^{-1} of folate compared to 16 μg kg^{-1} of folate in the deficient diet (Lot 1733). Wayne Lab-Blox contained 22% crude protein, 5% crude fat, and 4.5% crude fiber and consisted of soybean meal, ground yellow

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corn, corn and wheat flakes, wheat middlings, fish meal, dried whey, brewers dried yeast, soybean oil, cane molasses, vitamin mixture, and salt mixture. All diets were solid and had energy density roughly equivalent to 4 kcal/g. The animals were fed these diets for 43.5 ± 1.5 days and weighed once a week. Rats fed folate-deficient food (average intake of 286 ng folic acid) were rate limiting for the amount of folate-sufficient diet (average daily intake of 32 μg folic acid) fed to pair-fed control, whereas other controls were given Wayne Lab-Blox feed *ad libitum*. In other experiments, one-third of the rats were fed control diet *ad libitum;* two-thirds of the rats were fed folate-deficient diet and this group was further divided into two groups designated folate deficient and folate replenished. Folate-replenished rats were pair fed with folate-deficient rats, using a folate-deficient diet; however, the animals were supplemented with 1 mg of folate dissolved in saline by oral gavage once weekly. All rats were housed under identical conditions in stainless steel wire net cages with elevated mesh floors to avoid coprophagy. At the end of the feeding period a deficient rat, its pair-fed control, and an *ad libitum* control were sacrificed in batches. The animals were decapitated and bled into heparinized beakers. A part of the liver (~2 g) was excised, folded into foil, and frozen in liquid nitrogen. After staying in liquid nitrogen for approximately 1 min, the tissue was stored at −20°C for later folate determinations.

Preparations of tissue lobules for incubations. The pancreas was removed, trimmed of excess fat and connective tissue, and weighed on a Roller–Smith precision balance. Pancreatic lobules were prepared by the method described by Scheele and Palade (7, 8). All *in vitro* incubations were performed in Krebs Henseleit bicarbonate buffer containing 5 m*M* glucose and amino acids as specified for Eagle's tissue culture medium (subsequently referred to as KHB buffer). The buffered suspension was put in 25-ml Erlenmeyer flasks and then placed in a shaker water bath (90 oscillations min^{-1}) at 37°C with the gas phase of 95% O_2:5% CO_2.

Studies of DNA; RNA; protein content; accumulation and incorporation of L-[^{14}C]-phenylalanine or a mixture of fifteen ^{14}C-amino acids (Algal profile, Schwarz/Mann, Orangeburg, N.Y.) into total tissue, microsomes, and nascent proteins; [8-^{14}C]adenine accumulation and incorporation into total RNA; and [*methyl*-^{14}C]thymidine accumulation and incorporation into DNA were done by methods described in detail previously (1, 2, 8, 9).

Extraction and separation of RNA, DNA, and protein from tissue pellets were done by methods described by Schmidt and Thannhauser (10) and Schneider (11), modified by Webster and Tyor (12). RNA was assayed by the diphenylamine method using calf thymus DNA as the standard (13). Protein was assayed by the biuret method (14). Additional aliquots were counted in glass vials containing 10 ml of scintillation mixture (containing toluene, Triton X and ethanol, 8/4/3 v/v/v).

Studies of tissue amylase content. A separate 100-mg piece of pancreas obtained from each animal was homogenized with 2.5 ml of 0.02 *M* phosphate buffer containing 6.7 m*M* NaCl (pH 6.9) and assayed for amylase activity.

Studies of amylase secretion. For basal amylase secretion, lobules weighing 100 mg were obtained from appropriate groups of animals and incubated in 3 ml KHB buffer for 60 min (15). Aliquots of media and tissue homogenized in phosphate buffer were saved for amylase content. To determine stimulated amylase secretion, control and experimental flasks were incubated in parallel in the manner outlined above except that the experimental flasks contained 10^{-4} *M* bethanechol (Merck Sharp & Dohme, West Point, Pa.).

Assay of amylase activity. Amylase activity was assayed in aliquots of the media and in homogenized tissue by the method of Bernfeld using Lintner's starch (Merck & Co., Rahway, N.J.) as a substrate (16). One unit of amylase activity represents that amount which catalyzes the formation of 1 mg of maltose in 3 min at 37°C (15).

Studies of amino acid content. Two experiments were performed to measure free amino acid content of the tissue from rats. Pancreases from three animals in each feeding group were pooled, homogenized in 1% picric acid, centrifuged, and excess picric acid was removed from the supernate by passage through a Dowex 2-X10 column. The samples were

lyophilized, buffered to pH 2.2, and stored at −20°C until the time of assay. Amino acids were assayed by the technique of Spackman, Stein, and Moore using a Beckman 120-B amino acid autoanalyzer (11). These methods have been used previously in our laboratories (9).

Folate determination. Folate assays were performed by methods previously employed in our laboratories (18). Tissue samples were thawed, homogenized in 9 vol of 50 m*M* phosphate buffer (pH 7.0) containing 2 m*M* sodium ascorbate, and heated at 90°C for 5 min to stop metabolism and to release particulate folate. To the cooled samples, 50 μl dialyzed chick pancreas extract (Difco Laboratories, Detroit, Mich.) was added. The samples were incubated at 37°C for an hour to cleave the folylpolyglutamates to folylmonoglutamates. Tissue samples as well as plasma samples were then diluted appropriately and assayed microbiologically using lactobacilli casei in folic acid casei medium (Difco Laboratories). The optical density of the 18-hr growth was measured at 660 nm. All samples were measured in duplicate at two dilutions.

Statistical analysis. Data were analyzed by one-way analysis of variance with replication (one time × three treatments). Group means were analyzed by the Tukey honestly significant difference procedure for the 0.05 level (19). Values of $P < 0.05$ were considered to be statistically significant. Folate effect was considered to be operative when analysis of variance showed that the folate-deficient group was different from both the *ad libitum* fed group and the isocaloric controls in the first series of experiments or from both the *ad libitum* fed group and the folate-supplemented group in the second series of experiments. Control diet effect was considered to be operative when the isocaloric controls or folate-supplemented controls were different from the *ad libitum* fed controls. With this statistical design, overinterpretation of folate effect would be avoided if control diet effect and folate effects were to occur simultaneously.

Results. Liver folate activity was markedly reduced in folate-deficient rats. In the first series of experiments, liver folate levels were 73% in isocaloric control animals and 4% in folate-deficient animals; serum folate levels were 113% in isocaloric control animals and 21% in folate-deficient animals when compared with liver and serum folate levels in controls fed Wayne Lab-Blox *ad libitum.* In the second series of experiments, liver folate levels were 81% in folate-replenished animals and 5% in folate-deficient animals; serum folate levels were 110% in folate-replenished animals and 23% in folate-deficient animals when compared with controls fed a folate-sufficient diet.

The mean starting body weight of folate-deficient, isocaloric control, and *ad libitum* fed control rats in the first series of experiments was 69.9 (±1.55 SEM), 69.4 (±1.50 SEM), and 69.8 (±1.49 SEM), respectively. Folate-deficient, isocaloric control, and *ad libitum* fed control rats gained weight at an equal rate with the result that the mean body weights of folate-deficient, isocaloric control, and *ad libitum* fed animals increased to 279 (±9 SEM), 281 (±6 SEM), and 272 (±11 SEM), respectively, after feeding for 43.5 (±1.5 SEM) days. Signs and symptoms typical of folate deficiency (20) (loss of weight, loss of fur, spots of rough coat, dry scabby tail with dark brown patches, etc.) were not observed in the present study.

The effects of dietary treatment on body weight; pancreas weight; DNA; [*methyl*-^{14}C]thymidine accumulation and incorporation into DNA; RNA; [8-^{14}C]adenine accumulation and incorporation into RNA; protein content; total amylase content; amylase secretion under basal conditions and following stimulation with 10^{-4} *M* bethanechol; and protein synthesis in the first series of experiments are shown in Tables I and II. Decreases in parameters that could clearly be attributed to the isocaloric control diet (control diet effects) were not observed. An effect of dietary treatment that could clearly be attributed to folate lack was the diminished [*methyl*-^{14}C]thymidine incorporation into DNA. Total RNA, RNA synthesis, total content of amylase and secretion of amylase under both basal and bethanechol stimulated conditions, and protein synthesis were not altered. Phenylalanine content of the pancreas was similar in the three dietary groups. L-Phenylalanine content of the medium was 12 μ*M* or about 20 times greater than the

TABLE I. EFFECT OF DIET TREATMENT ON BODY WEIGHT, PANCREAS WEIGHT, DNA, [METHYL-^{14}C]THYMIDINE ACCUMULATION AND INCORPORATION INTO DNA, RNA, [8-^{14}C]ADENINE ACCUMULATION AND INCORPORATION INTO RNA, TOTAL PROTEIN, L-[^{14}C]PHENYLALANINE INCORPORATION INTO PROTEIN, AND AMYLASE CONTENT AND SECRETION IN RAT PANCREAS

Parameter[a]	Mode of reference	Treatment group[b]		
		Folate deficient	Isocaloric control	Fed *ad libitum* control
Body weight (10)	g	279 ± 9	281 ± 6	272 ± 11
Pancreas weight (10)	mg/organ	956 ± 32	894 ± 33	960 ± 24
DNA (10)	μg/organ	961 ± 61	930 ± 59	927 ± 41
[*methyl*-^{14}C]Thymidine accumulation into tissue (8)	cpm/100 μg DNA	11,786 ± 1,768	13,198 ± 2,856	14,285 ± 2,142
[*methyl*-^{14}C]Thymidine incorporation into DNA (8)	cpm/100 μg DNA	1,330 ± 210[c]	1,980 ± 370	2,170 ± 330
RNA (10)	μg/organ	4,416 ± 301	4,201 ± 292	4,147 ± 321
[8-^{14}C]Adenine accumulation in tissue (8)	cpm/μg RNA	220 ± 17	250 ± 21	281 ± 29
[8-^{14}C]Adenine incorporation into RNA (8)	cpm/μg RNA	38 ± 4	41 ± 3	40 ± 4
DNA/RNA ratio (8)		.21 ± .02	.23 ± .03	.24 ± .02
Protein (10)	mg/organ	69.9 ± 10.1	72.9 ± 9.1	95.2 ± 6.1
L-[^{14}C]/Phenylalanine incorporation	cpm/μg DNA	172 ± 24	286 ± 53	255 ± 35
into protein (10)	cpm/μg RNA	44 ± 7	68 ± 8	73 ± 8
Total amylase (8)	U/100 mg tissue	2,102 ± 328	2,216 ± 381	2,667 ± 381
Pancreatic secretion Basal (10)	% of total in tissue + medium	6.7 ± 0.9	7.9 ± 0.9	5.5 ± 0.9
10^{-4} *M* bethanechol (10)	% of total in tissue + medium	26.6 ± 3.5	28.0 ± 4.2	27.1 ± 2.5

[a] Number of experiments given in parentheses.
[b] Results expressed as means ± SEM.
[c] Folate effect ($P < 0.05$); see Materials and Methods.

TABLE II. EFFECT OF DIET TREATMENT ON PROTEIN SYNTHESIS IN RAT PANCREAS

Parameter[a]	Mode of reference	Treatment group[b]		
		Folate deficient	Isocaloric control	Fed *ad libitum* control
L-[^{14}C]Phenylalanine accumulation into tissue	cpm/100 μg DNA	6329 ± 478	6598 ± 623	6139 ± 893
L-[^{14}C]Phenylalanine incorporation into microsomal protein (8)	cpm/mg protein	1095 ± 178	1140 ± 241	1011 ± 178
L-[^{14}C]Phenylalanine incorporation into exportable protein (8)	cpm/mg protein	540 ± 94	511 ± 176	423 ± 56
L-Phenylalanine content (2)	nmole phenylalanine/g tissue wet wt	51	49	47

[a] Number of experiments given in parentheses.
[b] Results expressed as means or means ± SEM.

concentration of free L-phenylalanine in the tissue water.

Results of the second series of experiments using folate-deficient, folate-replenished, and *ad libitum* fed rats were similar to those of the first series of experiments. DNA and protein synthesis studies using a mixture of fifteen ^{14}C-amino acids were also similar (Table III).

Discussion. Dietary regimens used in the present study were adequate to achieve folate deficiency as evidenced by the low folate levels in the liver (4–5% of control) and in the serum (21–23% of control) in both series of experiments. Since DNA content and pancreatic weight were not changed, there was no decrease in the cell numbers. Moreover there was no change in cell size since DNA per milligram of protein was not changed. The folate-deficient diet caused a specific change in incorporation of [*methyl*-^{14}C]thymidine into DNA. In previous studies, lack of vitamin B_6 diminished DNA synthesis whereas lack of thiamin did not impair DNA synthesis in the pancreases of Sprague–Dawley rats (1, 2). These results are in agreement with studies by Leevy *et al.* (24) which indicated that deficiencies of folate, vitamin B_{12}, and vitamin B_6 diminished DNA synthesis whereas deficiencies of thiamin, riboflavin, nicotinic acid, pantothenic acid, and biotin did not impair DNA synthesis in the liver (5). There was no significant change in total RNA, protein content, and amylase content or amylase secretion. Moreover there was no significant change in the synthesis of RNA and total and exportable proteins. These observations are based on the assumption that the changes in incorporation are not due to changes involving pool size of either precursor or product. Uptake of [*methyl*-^{14}C]thymidine, [8-^{14}C]adenine, and L-[^{14}C]phenylalanine into tissue was not altered as was the total DNA, RNA, and protein content. Phenylalanine content of the tissue was not altered in the three feeding groups. Moreover, by studying incorporation *in vitro,* intracellular pools of precursors are rapidly equilibrated with higher concentrations of the amino acids in the incubating media. No differences in protein synthesis were observed using a mixture of fifteen ^{14}C-amino acids rather than L-[^{14}C]phenylalanine. Intracellular concentrations of amino acids in tissue slices have been shown to rapidly equilibrate with that of the surrounding medium (21).

The present study indicates that folate deficiency impairs DNA synthesis, but unlike pyridoxine and thiamin deficiency, folate deficiency did not significantly change the enzyme content. Moreover, unlike pyridoxine deficiency which decreased enzyme secretion and thiamin deficiency which increased enzyme secretion, folate deficiency did not alter enzyme secretion. Both B_6 and thiamin-deficient animals had characteristic stigmata coincident with vitamin deficiency (1, 2). The changes observed in the present study may therefore indicate the early effect of moderate folate deficiency since these animals did not show any physical evidence of prolonged folate deficiency (20). These animals were studied before the development of physical signs and symptoms to determine the effect of early folate deficiency on pancreatic acinar cell function. In the present study impaired pancreatic DNA synthesis is due to folate deficiency per se and not due to thiamin

TABLE III. EFFECT OF DIET TREATMENT ON DNA AND PROTEIN SYNTHESIS IN RAT PANCREAS

Parameter[a]	Mode of reference	Treatment group[b]		
		Folate deficient	Folate replenished	Fed *Ad Libitum* control
[*methyl*-^{14}C]Thymidine incorporation into DNA (5)	cpm 100 μg DNA	1461 ± 211[c]	1879 ± 365	2011 ± 367
Algal profile incorporation into microsomal protein (5)	cpm/mg protein	18804 ± 1614	21789 ± 248	15659 ± 3827
Algal profile incorporation into exportable proteins (5)	cpm/mg protein	51735 ± 8281	54263 ± 5398	47239 ± 5009

[a] Number of experiments given in parentheses.
[b] Results expressed as means ± SEM.
[c] Folate effect ($P < 0.05$); see Materials and Methods.

or vitamin B_{12} deficiency which may accompany folate deficiency (21, 22). Changes in the acinar cell function did not suggest that thiamin lack (2) and B_{12} lack have not been shown to alter DNA synthesis in the presence of a marked reduction of tissue folate (24). Lack of response to uridylic acid and significant increase in DNA labeling with thymidylic acid in liver and other tissues with low folate stores provide evidence that folate facilitates conversion of uridylic acid to thymidylic acid in the mammalian systems (25). Impaired synthesis of pancreatic DNA in the present study suggests that folate is essential for conversion of uridylic acid to thymidylic acid which is rate limiting for pancreatic DNA synthesis. Although synthesis of DNA was impaired, total DNA was not significantly changed. This suggests either early deficiency or altered rate of turnover of DNA in folate-deficient animals. Long-term studies to determine the effect of folate deficiency on the pancreas are presently being carried out. Changes with respect to vitamin B_{12}, thiamin, and choline deficiency have been reported in the liver and brain of long-term folate-deficient animals (21, 22); therefore, thiamin and vitamin B_{12} levels will also be monitored. The present data, however, show that interference with the pancreatic DNA synthesis by folate deficiency alone constitutes a significant functional alteration which may prevent repair of the pancreas damaged by alcohol and malnutrition.

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