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An *in Vitro* Study of Pancreatic Ductal Cells[1] (39982)

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Introduction. The exocrine pancreas is divided into acinar cells which synthesize and secrete digestive enzymes and ductal cells which secrete water and electrolytes. Our knowledge of acinar cell function has increased tremendously during the past 10 years because techniques are available for *in vitro* studies of acinar cells as a part of the whole pancreas, as slices, as isolated lobules, as isolated cells, or as subcellular fractions. On the other hand, our knowledge of ductal cell function has remained relatively static. Possible impediments to accumulation of knowledge concerning ductal cell function are: (i) the complexities involved in the isolation of ductal cells from surrounding connective and acinar tissue; (ii) the small number of ductal cells compared to acinar cells; and (iii) the efforts required to obtain sufficient amounts of ductal tissue for study. Thus, it is not surprising that most of our information concerning ductal cell function has been derived from physiologic studies of water and electrolyte secretion utilizing the intact organ *in situ* or the perfused organ *in vitro*.

Ductal cells have been well characterized morphologically and histochemically (1-8). Wizemann *et al*. have shown that the epithelial lining of intermediate and large size ducts isolated by dissection was rich in a number of enzymes (9). However, extensive metabolic or biochemical characterization of ductal cells has not been accomplished. Moreover, there is no information to indicate effects of fasting, feeding, or gastrointestinal hormones on ductal cell function *in vitro*.

This paper reports studies on intact pancreatic ducts isolated by quick dissection and incubated under appropriate *in vitro* conditions. Fasting for 72 hr was associated with decreases in [^{14}C]glucose oxidation to $^{14}CO_2$ and alkaline phosphatase activity. There were no changes in the following: protein, RNA, or DNA content; ^{14}C-labeled amino acid incorporation into protein; [^{14}C]glucose or [^{14}C]glucosamine incorporation into glycoproteins; Mg^{2+}-ATPase, HCO_3^--ATPase, 5′-nucleotidase, or leucine aminopeptidase activities.

Methods. Male Sprague-Dawley rats (initial weights 300–350 g) housed in temperature-controlled quarters were used for all studies. Animals referred to as "fasted" were denied food for 72 hr; rats referred to as "fed" had free access to Purina Rat Chow (Ralston Purina Co., St. Louis, Mo.). All rats were allowed free access to tap water.

The following materials were used: D-[U-^{14}C]glucosamine hydrochloride (237.7 mCi/mmole), D-[1-^{14}C]glucose (8.72 mCi/mmole), and L-[U-^{14}C]-labeled amino acid mixture (New England Nuclear Corp., Boston, Mass.); materials for incubation media, NCTC-109, nonessential amino acids (Microbiological Associates, Inc., Bethesda, Md.); calf thymus DNA and D-ribose (Nutritional Biochemical Corp., Cleveland, Ohio); orcinol (Fisher Scientific Co., Pittsburg, Pa.); diphenylamine (Eastman Organic Chemicals Division, Eastman Kodak Co., Rochester, N.Y.); bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.); hydroxide of hyamine 10-X, 1 *M* solution in methanol (Packard Instrument Co. Inc., Downers Grove, Ill.). Reagent kits for alkaline phosphatase (Cat. No. 15990) and leucine aminopeptidase (Cat. No. 15952) were obtained from Boehringer Mannheim Corp., New York.

Treatment of tissue. Rats were killed by

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cervical dislocation. The pancreatic duct was located, and acinar tissue was trimmed away with sharp microsurgical scissors. Approximately 4 cm of duodenum were left attached to the duct to facilitate handling; however, after acinar tissue was removed, the pancreatic duct was transected and the duodenum discarded. The pancreatic duct was placed on filter paper and kept moist with cold saline. Residual acinar tissue was removed by dissection and scraping with the blunt side of scissors. The duct was then cut longitudinally for maximal exposure to the incubation medium. The isolation procedure required 20 min for 12 ducts which were pooled, weighed, and placed in a flask containing freshly oxygenated tissue culture medium.

Studies of total protein, RNA, and DNA. Pooled ducts were weighed and homogenized in a ground-glass homogenizer using cold perchloric acid (PCA). The homogenate was centrifuged and the pellet washed twice with cold PCA. Methods used for separation of RNA, DNA, and protein were modifications of methods developed by Schmidt and Thannhauser (10) and Schneider (11). RNA was determined by the orcinol method using ribose as the standard (12, 13). DNA was determined by the diphenylamine method using calf thymus DNA as a standard (12, 13). Protein was determined by the method of Lowry *et al.* using bovine serum albumin as the standard (14).

Studies of ^{14}C-labeled mixed amino acid incorporation into protein. The pooled ducts were incubated for 30 min in a 25-ml Erlenmeyer flask containing 1 ml of incubation medium with 0.2 μCi of ^{14}C-labeled mixed amino acids. Methods used for *in vitro* incubation and determination of radioactive incorporation have been described (15, 16). Radioactivity was measured in a Packard liquid scintillation counter using a phosphor developed by Patterson and Greene (17).

Studies of [^{14}C]glucose oxidation. Pooled ducts were incubated in a 25-ml Erlenmeyer flask modified to contain a center well. Each flask contained 6.94 μmoles (294,000 cpm) of [^{14}C]glucose and 1 ml of incubation medium. Incubations were performed for 60 min at 37° in a shaking water bath. These methods were described previously (18).

Studies of [^{14}C]glucose and [^{14}C]glucosamine incorporation into protein. Pooled ducts were incubated in a 25-ml Erlenmeyer flask containing 1 ml of incubation medium with 0.05 μCi of [^{14}C]glucose or 1 μCi of [^{14}C]glucosamine for 60 and 30 min, respectively. Glycoproteins were precipitated with PCA and dissolved in KOH, and measurements were performed as described (15, 19).

Studies of ductal enzymes. Pooled ducts were homogenized with a ground-glass homogenizer in 2 ml of cold buffer containing 0.25 *M* sucrose and 10 m*M* triethanolamine-HCl, pH 7.4. An aliquot of the homogenate was taken for measurement of DNA and protein. Mg^{2+}-ATPase was assayed by the method used by Simon *et al.* and Wizemann *et al.* (20, 9); HCO_3^--ATPase by the method of Simon *et al.* (20); 5'-nucleotidase by the method of Dixon and Purdom (21). Alkaline phosphatase was assayed by the method of Hausamen *et al.* (22); leucine aminopeptidase by the method of Nagel *et al.* (23). Reagent kits for some assays were obtained from the Boehringer Mannheim Corporation. Enzymes were assayed under conditions which gave linear results with respect to both quantity of tissue and time of incubation.

Results. Table I shows protein, RNA, and DNA content of pancreatic ducts from fed and fasted rats. The data are expressed in terms of tissue wet weight or micrograms of DNA (mean ± SE). There were no significant differences between fed and fasted animals.

Table II shows values for incorporation of ^{14}C-labeled mixed amino acids into proteins by pancreatic ducts from fed or fasted animals. The results are expressed as counts per minute incorporated per milligram of protein or as counts per minute incorporated into protein per 100 micrograms of DNA. There were no significant differences between fed and fasted rats.

Table III shows values for [^{14}C]glucose oxidation to $^{14}CO_2$. The results are expressed as nanomoles of [^{14}C]glucose oxidized per milligram of protein or per 100 micrograms of DNA. There were significant

TABLE I. EFFECTS OF FEEDING AND FASTING ON PROTEIN, RNA, AND DNA.

	Protein		RNA		DNA
	μg/mg tissue wet wt	μg/100 μg of DNA	μg/mg tissue wet wt	μg/100 μg of DNA	μg/mg tissue wet wt
Fed	66 ± 4[a]	830 ± 78	0.55 ± 0.44	10.3 ± 1.2	7.92 ± 0.27
Fasted	65 ± 5	830 ± 49	0.49 ± 0.05	9.1 ± 1.2	7.91 ± 0.21

[a] Values are means ± SE of three experiments.

TABLE II. ^{14}C-LABELED MIXED AMINO ACID INCORPORATION INTO PROTEIN.[a]

	cpm/milligram of protein	cpm/100 μg of DNA
Fed	60.7 ± 15.9[b]	23.7 ± 2.5
Fasted	60.0 ± 8.3	20.3 ± 2.3

[a] Time of incubation, 30 min.
[b] Values are means ± SE of three experiments.

TABLE III. [^{14}C]GLUCOSE OXIDATION.[a]

	Nanomoles/mg of protein	Nanomoles/100 μg of DNA
Fed	$1.48 \times 10^{-2} \pm 0.35$*,[b]	$9.7 \times 10^{-3} \pm 1.0$**
Fasted	$0.46 \times 10^{-2} \pm 0.11$	$3.2 \times 10^{-3} \pm 0.5$

[a] Time of incubation, 60 min.
[b] Values are means ± SE of seven experiments.
* $P < 0.05$.
** $P < 0.005$.

TABLE IV. INCORPORATION OF [^{14}C]GLUCOSE AND [^{14}C]GLUCOSAMINE.

	[^{14}C]Glucose[a]		[^{14}C]Glucosamine[b]	
	cpm/mg of protein	cpm/100 μg of DNA	cpm/mg of protein	cpm/100 μg of DNA
Fed	118 ± 8[c]	90 ± 7	249 ± 8	215 ± 15
Fasted	112 ± 8	86 ± 11	244 ± 11	208 ± 24

[a] Time of incubation, 60 min.
[b] Time of incubation, 30 min.
[c] Values are means ± SE of four experiments.

decreases in [^{14}C]glucose oxidation with fasting when the data were expressed in terms of protein ($P < 0.05$) or DNA ($P < 0.005$).

Table IV shows values for [^{14}C]glucose or [^{14}C]glucosamine incorporation into glycoproteins. There were no differences between fed and fasted rats.

Table V shows mean values for Mg^{2+}-ATPase, HCO_3^--ATPase, 5′-nucleotidase, leucine aminopeptidase, and alkaline phosphatase activities expressed as a function of protein or DNA. No significant differences were found in Mg^{2+}-ATPase, HCO_3^--ATPase, 5′-nucleotidase, or leucine aminopeptidase activities.

Alkaline phosphatase activity in ductal tissue from fasted animals was significantly lower than in fed animals. Values for alkaline phosphatase activity in pancreatic juice from fed and fasted animals were essentially similar. Moreover, there was no difference between fed and fasted rats in total alkaline phosphatase activity of the whole pancreas. We concluded that the decrease associated with fasting resulted from changes in the ductal epithelium where alkaline phosphatase activity is mainly located.

Studies were conducted to determine the degree of contamination of ductal tissue with acinar tissue. Morphologic examination using light microscopy revealed no acinar elements in the pellets of ductal tissue. Measurements of amylase activity in ductal tissue isolated from fed or fasted animals were essentially similar (0.385 ± 0.58 and 0.355 ± 0.39 units/mg tissue wet weight). The results of morphologic studies, the paucity of amylase activity in ductal tissue (<1% of amylase activity in acinar tissue per milligram tissue wet weight), and the lack of differences between fed and fasted animals would indicate that little or no acinar tissue contaminated the ductal preparation.

Discussion. These studies grew out of efforts to obtain more definitive information of a biochemical nature regarding the function of pancreatic ductal epithelium. Such information is particularly relevant since it is recognized that pancreatic ductal epithelium may be the primary site of origin for pancreatic adenocarcinoma.

The fed-fasted model was used to determine if there were differences in metabolic activities of pancreatic ductal epithelium

TABLE V

Enzyme	No. of experiments	μmoles of phosphate/hr/mg of protein: Fed	μmoles of phosphate/hr/mg of protein: Fasted	μmoles of phosphate/hr/100 μg of DNA: Fed	μmoles of phosphate/hr/100 μg of DNA: Fasted
Mg^{2+}-ATPase	3	16.2 ± 6.7[a]	16.0 ± 6.9	19.4 ± 7.3	18.6 ± 6.9
HCO_3^--ATPase	3	15.1 ± 5.7	15.5 ± 4.6	17.5 ± 6.3	17.6 ± 5.8
5′-Nucleotidase	7	5.0 ± 0.6	5.0 ± 0.6	8.9 ± 1.3	8.5 ± 0.8
		μmoles/hr/mg of protein		μmoles/hr/100 μg of DNA	
Leucine aminopeptidase	7	0.06 ± 0.01	0.06 ± 0.01	0.109 ± 0.2	0.103 ± 0.2
Alkaline phosphatase	7	9.74 ± 1.27	6.14 ± 0.62*	16.4 ± 1.4	11.9 ± 1.1**

[a] Values are means ± SE.
* $P < 0.005$.
** $P < 0.05$.

with decidedly different states of nutrition. We have shown in earlier works using acinar tissue that fasting was associated with decreases in protein synthesis, specifically amylase, RNA synthesis, activities of polymerases I and II enzymes, as well as decreases in [^{14}C]glucose oxidation to $^{14}CO_2$ and [^{14}C]palmitate oxidation and incorporation into phospholipids. In addition, we demonstrated that increases observed in metabolic activities with refeeding were simulated by the administration of cholecystokinin–pancreozymin (16, 18, 24, 25). These observations have led us to conclude that many of the changes observed in the exocrine pancreas with feeding were under the control of gastrointestinal hormones (26).

The studies presented here show no differences between fed and fasted animals in protein content, RNA content, amino acid incorporation into protein, or amounts of [^{14}C]glucose or [^{14}C]glucosamine incorporation into glycoproteins. Electron microscopic studies of ductal epithelium have shown little endoplasmic reticulum or evidences of active protein synthesis (1, 2). Thus, we did not anticipate great changes in rates of protein or RNA synthesis. Since the duct epithelial cells in rat, unlike those in mouse and hamster, did not appear to be active in mucin secretion (27), we did not anticipate changes in glycoprotein synthesis.

Significant decreases in glucose oxidation with fasting support other observations that ductal epithelium has an active metabolic rate (7). The decreased glucose oxidation suggests that such functions may be under hormonal control.

We selected the five enzymes under study because of their possible role in secretory or transport activities. Bicarbonate-ATPase was shown to be localized to both plasma membrane and mitochondria in the pancreas (20). Wizemann found greater amounts of HCO_3^--ATPase in the small duct system, whereas Mg^{2+}-ATPase was significantly higher in large ducts, i.e., 700 to 1000 μm (9). 5′-Nucleotidase and leucine aminopeptidase were selected because of reported presence in ductal structures (9, 28). Alkaline phosphatase was demonstrated histochemically in ductal and transport epithelium (29–31).

The decreases in alkaline phosphatase activity in ductal tissue with fasting were of interest. Alkaline phosphatase activity was demonstrated in large ducts (700–1000 μm) and in small ducts (25–60 μm) by Wizemann *et al.* (9). Amounts of alkaline phosphatase activity in main ducts were 2.6 times greater than amounts in the whole pancreas. We found no differences in alkaline phosphatase activity with fasting or feeding in whole pancreas or in pancreatic juice.

We did not find differences with feeding or fasting in Mg^{2+}-ATPase, HCO_3^--ATPase, 5′-nucleotidase, or leucine aminopeptidase activities. Similar levels of activity with fasting or feeding would suggest that these enzymes were not inducible and, therefore, were not under control of gastrointestinal hormones.

Considerable technological difficulties were encountered in efforts to isolate and study pancreatic duct cells. At present, there appears to be two methodologic approaches to the isolation of ductal cells from acinar tissue. The first depends on the dissociation of cells by the use of collagenase

or other enzymes and the separation by the use of density gradient techniques (32–34). The second method depends on the separation of ductal and acinar tissue by the use of mechanical methods. The techniques used in this study, as well as the study by Wizemann *et al.*, depended on the mechanical removal of acinar tissue (9).

We have used methods for cellular dissociation developed by Amsterdam and Jamieson (32, 33). We then applied the dissociated cells to discontinuous Ficoll gradients according to the method of Spalsbury *et al.* (34) or on albumin gradients according to the method of Sykes *et al.* (35). To date, we have been unable to satisfactorily separate ductal cells from acinar cells using such methods. In the course of the dissociation studies, we observed that larger and smaller ducts remained intact and could be studied as such.

Difficulties were expected in the separation of ductal, centroacinar, and acinar cells since ductal cells represent less than 5–8% of the total cellular mass (36). Nevertheless, *in vitro* studies of ductal cells will be necessary for a better understanding of mechanisms of water and electrolyte secretion and metabolic changes associated with such functions.

Summary. Although pancreatic duct cells have been characterized morphologically and histochemically, information on their biochemistry and mechanism is lacking. The aim of the present work was to study selected metabolic parameters in the pancreatic duct of fed and fasted rats. The intact duct was quickly isolated by dissection and opened longitudinally. Twelve ducts were pooled for each study. Fasting for 72 hr was associated with decreases in [^{14}C]glucose oxidation to $^{14}CO_2$ and alkaline phosphatase activity. There were no changes with fasting in the following parameters: protein, RNA, or DNA content; ^{14}C-labeled amino acid incorporation into proteins; [^{14}C]glucose or [^{14}C]glucosamine incorporation into glycoproteins; Mg^{2+}-ATPase, HCO_3^--ATPase, 5′-nucleotidase, or leucine aminopeptidase activities. In contrast to acinar cells, duct cells did not actively synthesize proteins. This study points out that pancreatic duct cells are metabolically active and that fasting was associated with reductions in glucose oxidation and alkaline phosphatase activity. Whether these metabolic activities are under the control of gastrointestinal hormones remains to be determined. Moreover, the significance of the reduction in alkaline phosphatase activity with fasting is not known.

1. Ekholm, R., and Edlund, Y., J. Ultrastruct. Res. **2,** 453 (1959).
2. Ekholm, R., Zelander, T., and Edlund, Y., J. Ultrastruc. Res. **7,** 73 (1962).
3. Herman, L., and Fitzgerald, P. J., J. Cell Biol. **12,** 277 (1962).
4. Churg, A., and Richter, W. R., Amer. J. Pathol. **68,** 23 (1972).
5. Laitio, M., Lev, R., and Orlic, D., J. Anat. **117,** 619 (1974).
6. Riches, D. J., J. Anat. **111,** 157 (1972).
7. McMinn, R. M. H., and Kugler, J. H., J. Anat. **95,** 1 (1961).
8. Wang, C. C., Wang, K. J., and Grossman, M. I., Amer. J. Physiol. **160,** 115 (1950).
9. Wizemann, V., Christian, A.-L., Weichmann, J., and Schultz, I., Pfluegers Arch. **347,** 39 (1974).
10. Schmidt, G., and Thannhauser, S. J., J. Biol. Chem. **161,** 83 (1945).
11. Schneider, W. C., J. Biol. Chem. **161,** 293 (1945).
12. Volkin, E., and Cohn, W. E., Methods Biochem. Anal. **1,** 287 (1954).
13. Schneider, W. C., Methods Enzymol. **3,** 680 (1957).
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193,** 265 (1951).
15. Mainz, D. L., Black, O., and Webster, P. D., J. Clin. Invest. **52,** 2300 (1973).
16. Morisset, J. A., and Webster, P. D., J. Clin. Invest. **51,** 1 (1972).
17. Patterson, M. S., and Greene, R. C., Anal. Chem. **37,** 854 (1965).
18. Webster, P. D., and Tyor, M. P., Amer. J. Physiol. **210,** 1076 (1966).
19. Kawasaki, T., and Yamashina, I., Biochim. Biophys. Acta **225,** 234 (1971).
20. Simon, B., Kinne, R., and Sachs, G., Biochim. Biophys. Acta **282,** 293 (1972).
21. Dixon, T. F., and Purdom, M., J. Clin. Pathol. **7,** 341 (1954).
22. Hausamen, T. U., Helger, R., Rich, W., and Gross, W. Clin. Chim. Acta **15,** 241 (1967).
23. Nagel, W., Willig, F., and Schmidt, F. H., Klin. Wochenschr. **42,** 447 (1964).
24. Webster, P. D., Singh, M., Tucker, P. C., and Black, O., Gastroenterology **62,** 600 (1972).

25. Morisset, J. A., and Webster, P. D., Amer. J. Physiol. **219,** 1286 (1970).
26. Webster, P. D., Black, O., Mainz, D. L., and Singh, M., Gastroenterology (in press).
27. Yamada, K., J. Anat. **105,** 511 (1969).
28. Simon, B., and Thomas, L., Biochim. Biophys. Acta **288,** 434 (1972).
29. Jacoby, F., and Martin, B. F., J. Anat. (London) **85,** 391 (1951).
30. Warnes, T. W., and Bulmer, D., J. Anat. (London) **106,** 410 (1970) (Abstract).
31. Haussler, M. E., Nagode, L. A., and Rasmussen, H., Nature (London) **228,** 1199 (1970).
32. Amsterdam, A., and Jamieson, J. D., Proc. Nat. Acad. Sci. USA **69,** 3028 (1972).
33. Amsterdam, A., and Jamieson, J. D., J. Cell Biol. **63,** 1037 (1974).
34. Spalsbury, C., Brodetsky, A. M., Teplitz, R. L., Acta Cytol. **17,** 522 (1973).
35. Sykes, J. A., Whitescarver, J., Briggs, L., and Anson, J. H., J. Nat. Cancer Inst. **44,** 855 (1970).
36. Bolender, R. P., J. Cell Biol. **61,** 269 (1974).

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