PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 156, 340-344 (1977)

Effect of Fasting and Refeeding on Pancreatic DNA Synthesis and Content[1] (39932)

D. L. MAINZ, N. M. PARKS, AND P. D. WEBSTER, III

Medical Research Service, Veterans Administration Hospital (FHD), and Department of Medicine, Medical College of Georgia, Augusta, Georgia 30904

Introduction. Considerable information is available concerning the effects of food deprivation on the liver, stomach, and small intestine; less is available concerning the pancreas (1-14). We have demonstrated progressive decreases in total protein, amylase, ribonucleic acid, and water content in the pancreas of animals fasted 24, 48, 72, or 96 hr. Refeeding for 24, 48, and 72 hr was associated with increases in values under consideration to levels equal to or greater than the basal fed state (12). It is now recognized that the lack of foodstuffs in the gut not only deprives the organ of substrate, but also reduces stimulation by gastrointestinal hormones which, in part, control secretion, synthesis, metabolism, and trophism in target tissues (4, 5, 13).

The studies presented here are designed to provide information concerning the effects of fasting and refeeding on pancreatic DNA content and on rates of [^{14}C]thymidine incorporation into DNA. In addition to studies on the pancreas, we report similar studies on kidney, thigh muscle, and spleen which served as control tissues.

Materials and methods. Male albino rats (Sprague-Dawley), weighing approximately 300-380 g, were purchased from Holtzman Company, Madison, Wisc. They were fed Purina Rat Chow (Ralston Purina Company, St. Louis, Mo.), and had free access to water. The animals were divided into groups of 12 rats each and fasted for 2, 4, and 6 days. Refed animals were fasted 6 days and then allowed free access to food for 1, 2, or 3 days. Control animals were allowed free access to food and were sacrificed at the beginning of the fast (Day 0).

Animals were sacrificed at appropriate times by cervical dislocation. The organs were rapidly removed, fat and connective tissue were excised, and the organs were weighed on a Roller-Smith balance. For studies using the pancreas, a 500-mg piece of pancreas was incubated at 37° for 90 min in KRP medium containing 2 μCi of [^{14}C]thymidine. This procedure was described previously (13). At the conclusion of incubation, the tissue was removed and washed in cold KRP buffer and placed in 5 ml of 0.2 *N* PCA containing cold thymidine. Studies on "control" or nondigestive organs were performed using kidney, thigh muscle, and spleen. These organs were passed through a tissue press and the brei was transferred to tissue culture medium (200 mg of tissue per 1 ml of medium) for incubation. Incubation and subsequent treatment were similar to that used for pancreas except that 10 μCi of [^{14}C]thymidine was used to increase levels of incorporated radioactivity.

RNA, DNA, and protein were separated by methods of Schmidt and Thannhauser as modified by Munro and Fleck (15). RNA was measured by the orcinol method using ribose as the standard (16). DNA was measured by the diphenylamine method using calf thymus DNA as the standard (17). Protein was measured by the biuret method using albumin as the standard (18). [^{14}C]Thymidine incorporation into DNA was determined using methods previously described (14). All data were expressed as means ± 1 SD. Student's *t*-test was used to determine statistical significance. As shown in the tables and indicated by the differences in control weights, the experiments on pancreas and nondigestive control tissues were performed at different times and on slightly differently aged rats. Changes in fasted animals were compared with fed *ad libitum* controls; refed animals were compared with 6-day fasted ones.

Results. Table I shows the results derived from rats that were fasted 2, 4, and 6 days

[1] This study was supported by the Veterans Administration (MRIS No. 5442 and 9274) and by Research Grant No. AM 13131 from the National Institutes of Health.

or fasted 6 days and refed 1, 2, and 3 days. Body weight (column 2) decreased by 17, 21, and 33% on days 2, 4, and 6 of the fast. Refeeding increased body weight 19, 14, and 25%. Pancreatic weight (column 3) decreased with fasting 8, 28, and 43%, and increased with refeeding 23, 12, and 36%. All changes were statistically significant.

Protein content per gram of tissue wet weight (column 4) increased by 5% on day 2 and decreased by 19 and 19% on Days 4 and 6. Refeeding decreased weight 5% on Days 1 and 2, but, by Day 3, protein content increased 12% over the 6-day fasted level. The last increase was statistically significant. Protein content expressed as milligrams of protein per 100 μg of DNA declined 6, 32, and 42% on Days 2, 4, and 6; refeeding increased protein/DNA ratios by 36% on Day 3. Pancreatic wet weight and total milligrams of protein decreased to a greater degree than total milligrams of DNA. Thus, DNA content is the more stable and preferred reference measurement for data.

Ratios of RNA to DNA (micrograms of RNA/100 μg of DNA) are shown in column 6. RNA decreased by 20, 27, and 32% with fasting; refeeding increased RNA by 7, 0, and 29%. These data suggested that DNA template activity was not adequate on Days 1 and 2 of refeeding to effect synthesis of RNA to levels equivalent to controls. The slow return seen in protein and RNA paralleled the slow recovery seen in pancreatic weight.

Columns 7 and 8 express changes in DNA content and synthesis. Fasting decreased total DNA content by 2, 9, and 20%; refeeding increased DNA content by 6, 4, and 16%. The increase in DNA content at Day 3 of refeeding compared to Day 6 of fasting was statistically significant. DNA synthesis as determined by [^{14}C]thymidine incorporation decreased 40, 69, and 86% with fasting and increased 70, 267, and 511% with refeeding. All changes were statistically significant.

Table II shows results obtained from similar studies on different animals using kidney. Two, four, and six days of fasting were associated with 14, 21, and 24% decreases in body weight. With 2 days of refeeding, body weight increased 8% compared with 6-day fasted animals. Kidney weight (column 3) decreased 14, 17, and 27% with fasting and increased 14% above the 6-day fasted levels with 2 days of refeeding. Similar changes were observed in protein content per gram of tissue wet weight (column 4); milligrams of protein/100 μg of DNA (column 5); and micrograms of RNA/100

TABLE I. EFFECT OF FASTING AND REFEEDING ON PANCREATIC DNA.[a]

Treatment	Body weight (g)	Pancreatic weight (mg)	Protein (mg/g tissue wet weight)	Protein (mg/100 μg of DNA)	RNA (mg/100 μg of DNA)	Total DNA (mg)	[^{14}C]Thymidine (cpm/100 μg of DNA)
Control							
Fed *ad lib.*	313 ± 13	906 ± 94	0.21 ± 0.03	3.1 ± 0.5	133 ± 10	6.2 ± 0.7	187 ± 70
Fasted							
2 Days	261 ± 34[c]	822 ± 87[b]	0.22 ± .02	2.9 ± 0.6	107 ± 14[b]	6.1 ± 1.4	133 ± 68[c]
	(−17%)	(−8%)	(+5%)	(−6%)	(−20%)	(−2%)	(−40%)
4 Days	247 ± 12[c]	652 ± 46[d]	0.17 ± .04[c]	2.1 ± 0.4[d]	97 ± 9[d]	5.6 ± 0.7	62 ± 32[d]
	(−21%)	(−28%)	(−19%)	(−32%)	(−27%)	(−9%)	(−69%)
6 Days	209 ± 18[d]	517 ± 44[d]	0.17 ± .003[c]	1.8 ± 0.2[d]	90 ± 16[d]	5.0 ± 0.8[c]	27 ± 14[d]
	(−33%)	(−43%)	(−19%)	(−42%)	(−32%)	(−20%)	(−86%)
Refed							
1 Day	249 ± 18[d]	688 ± 66[d]	0.16 ± .04	1.8 ± 0.3	97 ± 24	5.3 ± 0.8	46 ± 10[d]
	(+19%)	(+23%)	(−5%)	(0%)	(+7%)	(+6%)	(+70%)
2 Days	238 ± 25[b]	587 ± 72[c]	0.16 ± .03	1.7 ± 0.4	90 ± 14	5.2 ± 0.4	99 ± 53[d]
	(+14%)	(+12%)	(−5%)	(−6%)	(0%)	(+4%)	(+267%)
3 Days	261 ± 28[d]	802 ± 77[d]	0.19 ± .02[c]	2.8 ± 0.4[d]	127 ± 15[d]	5.8 ± 0.7[c]	165 ± 63[d]
	(+25%)	(+36%)	(+12%)	(+36%)	(+29%)	(+16%)	(+511%)

[a] Whole pancreases (500 mg) were incubated in 2.5 ml of KRP medium containing 2 μCi of [^{14}C]thymidine for 90 min. Protein, RNA, and DNA were extracted as described in Materials and Methods. All numbers represent 12 experiments and are expressed as means ± 1 SD.

[b] Significance, $P < 0.05$.

[c] Significance, $P < 0.025$.

[d] Significance, $P < 0.001$.

TABLE II. EFFECT OF FASTING AND REFEEDING ON KIDNEY DNA.[a]

Treatment	Body weight (g)	Kidney weight (mg)	Protein (mg/g tissue wet weight)	Protein (mg/100 μg of DNA)	RNA (μg/100 μg of DNA)	Total DNA (mg)	[^{14}C]Thymidine (cpm/100 μg of DNA)
Control							
Fed *ad lib.* (N = 18)	414 ± 28.0	1357 ± 137.0	0.14 ± 0.02	1.7 ± 0.16	12.9 ± 2.1	7.9 ± 0.9	241 ± 50.8
Fasted							
2 Days	357 ± 16.9[d]	1173 ± 84.7[c]	0.13 ± 0.01	1.6 ± 0.12	12.2 ± 1.5	7.7 ± 0.6	151 ± 35.3[d]
(N = 6)	(−14%)	(−14%)	(−7%)	(−6%)	(−5%)	(−3%)	(−37%)
4 Days	326 ± 17.1[e]	1122 ± 73.4[d]	0.12 ± 0.01	1.5 ± 0.60	9.7 ± 1.2[d]	7.9 ± 0.6	101 ± 14.7[e]
(N = 6)	(−21%)	(−17%)	(−14%)	(−12%)	(−25%)	(0%)	(−58%)
6 Days	315 ± 25.7[e]	990 ± 60.8[c]	0.09 ± 0.004[b]	1.4 ± 0.57	10.3 ± 0.6[b]	6.8 ± 0.5	103 ± 27.5[c]
(N = 3)	(−24%)	(−27%)	(−34%)	(−18%)	(−20%)	(−14%)	(−57%)
Refed							
2 Days	340 ± 34.6	1127 ± 102.6	0.11 ± 0.02	1.5 ± 0.1	10.2 ± 0.3	7.8 ± 0.6	164 ± 20.2[c]
(N = 3)	(+8%)	(+14%)	(+18%)	(+7%)	(−1%)	(+15%)	(+59%)

[a] Kidney brei (500 mg) was incubated in 2.5 ml of KRP medium containing 10 μCi of [^{14}C]thymidine for 90 min. Protein, RNA, and DNA were extracted as described in Materials and Methods. All numbers are expressed as the mean ± 1 SD.

[b] Significance, $P < 0.05$.

[c] Significance, $P < 0.025$.

[d] Significance, $P < 0.005$.

[e] Significance, $P < 0.001$.

μg of DNA (column 6). DNA content decreased only 3 and 0% after 2 and 4 days of fasting, but decreased 14% after 6 days. [^{14}C]Thymidine incorporation decreased 37, 58, and 57%, but increased 59% with refeeding.

Changes in [^{14}C]thymidine incorporation in pancreas and kidney were greater than were changes in DNA content. Specifically, after 6 days of fasting, [^{14}C]thymidine incorporation decreased 86 and 57%, respectively, but DNA content decreased only 20 and 14%. Refeeding for 2 days was associated with a striking increase in [^{14}C]-thymidine incorporation in both pancreas and kidney: 267 and 59%, respectively.

Muscle DNA content was unchanged after 4 days of fasting; however, [^{14}C]-thymidine incorporation decreased 74% (68 ± 26 compared with 18 ± 4 cpm/100 μg of DNA). Refeeding for 3 days was associated with no changes in DNA content, but a 180% increase in [^{14}C]thymidine incorporation (17.6 ± 4 compared with 49.2 ± 4 cpm/100 μg of DNA).

The changes observed in the spleen were similar to those seen in the pancreas and muscle. DNA content decreased with 4 days of fasting by 29% (22.5 ± 2.3 compared with 15.9 ± 1.8 mg), and [^{14}C]thymidine incorporation decreased 31% (38.2 ± 4.7 compared with 26.5 ± 4.3 cpm/100 μg of DNA).

Fasting resulted in decreased protein, RNA, and DNA in pancreas, kidney, spleen, and muscle. Changes in DNA content were minimal during the first 2 to 4 days of fasting, but were striking by Day 6. The increases in [^{14}C]thymidine incorporation in the pancreas were much greater with refeeding than in kidney, spleen, or muscle.

Discussion. Extensive metabolic changes were observed in the pancreas following fasting and refeeding. Fasting resulted in decreased protein content and synthesis, RNA content and synthesis, [^{14}C]palmitate oxidation, [^{14}C]glucose oxidation, tissue respiration, and cellular water (12). The greatest changes in these parameters were seen when the data were expressed in relation to DNA content; however, as shown in these studies, DNA content was also decreased. Studies of protein synthesis *in vitro* revealed that microsomes and supernatant prepared from fasted animals incorporated fewer counts of [^{14}C]phenylalanine than microsomes and supernatant prepared from fed animals (19). Studies of polysomes isolated from pigeons revealed that fasting resulted in: (a) decreased amounts of polysomes, (b) disaggregation of polysomes to monosomes, and (c) decreased capability of polysomes to synthesize peptides and to initiate additional peptide synthesis (20).

Ju and Nasset demonstrated a decrease in the nitrogen content of the stomach,

pancreas, small gut, and liver of rats after 24, 48, 96, and 192 hr of fasting. Decreases of approximately 60 to 70% were observed in nitrogen content of the pancreas with 192 hr of fasting. With refeeding, there were rapid increases (10).

The studies presented here point out that the pancreas undergoes striking changes which extend to the nuclear level of cell function. The decreases in [^{14}C]thymidine incorporation were of a magnitude similar to that observed in other tissues; i.e., kidney, muscle, and spleen. However, the increases following refeeding were much greater in the pancreas than in these organs.

The effects of fasting and refeeding on the pancreas closely paralleled changes observed in the stomach and small intestine. Willems and co-workers demonstrated that 36 hr of fasting was associated with decreases in DNA synthesis and in the mitotic index in dog fundic mucosa. The administration of gastrin, but not histamine, was associated with increased DNA synthesis and mitotic activity in fundic glands of the dog stomach. These changes were observed 12 hr after administration of gastrin and appeared to be quite sudden. By contrast, no significant variations in the proliferative parameters were observed after maximal stimulation of acid secretion with histamine (21, 22). Lichtenberger *et al.* demonstrated a decrease in immunoreactive gastrin in antral tissue following 4 days of fasting (6). Pentagastrin administration prevented many of the changes associated with food deprivation in both the stomach and small intestine (5, 23). These studies suggested that the role of gastrin as a trophic agent may be as important as foodstuffs or nutrients in the gut.

Altmann demonstrated progressive decreases during starvation in the numbers of intestinal epithelial cells per villus and crypt, as well as decreases in the numbers of mitotic figures per crypt. The decreases in villus size were more pronounced in the duodenum than in the ileum. Refeeding increased the number of cells undergoing mitosis in every region. The calculated turnover time of intestinal epithelium was longer than normal during starvation (24). Brown and co-workers demonstrated that the rate of cell renewal of intestinal epithelium was reduced to about one-half following extreme starvation (25). Morphologic changes and impaired differentiation of epithelial cells accompanied this change. McNeill and Hamilton demonstrated decreases in mucosal protein, maltase, and sucrase activities in rats fasted for 120 hours (9). Steiner *et al.* demonstrated decreases in small intestinal weight, RNA, protein, and total tissue water following starvation (8).

Summary. These studies were designed to determine the effects of fasting and refeeding on pancreatic DNA content and rates of [^{14}C]thymidine incorporation into DNA. The following changes were observed following fasting for 2, 4, and 6 days or fasting for 6 days with refeeding for 1, 2, and 3 days. Pancreatic weight decreased 8, 28, and 43% and then increased 23, 12, and 36%; DNA content decreased 2, 9, and 20% and then increased 6, 4, and 16%. [^{14}C]Thymidine incorporation into DNA decreased 40, 69, and 86% and then increased 70, 267, and 511%. These studies show the influence of fasting and refeeding on rates of pancreatic DNA synthesis and on DNA content. Control studies demonstrated similar decreases in kidney, muscle, and spleen. These changes represent complex metabolic adaptive responses to food deprivation. The changes may be mediated in part by lack of substrate but also likely reflect alterations in the hormonal milieu.

The authors wish to acknowledge the secretarial and editorial assistance of Ms. Jane Long and Ms. Hazel Wall.

1. Saudek, C. D., Felig, P., Amer. J. Med. **60,** 117 (1976).
2. Hayashi, T. T., and Kazmierowski, D., Biochemistry **11,** 2371 (1972).
3. Platt, B. S., Heard, C. R. C., and Stewart, R. J. C., *in* "Mammalian Protein Metabolism" (H. N. Munro and J. B. Allison, eds), Vol. 2, p. 445. Academic Press, New York (1964).
4. Johnson, L. R., Copeland, E. M., Dudrick, S. J., Lichtenberger, L. M., and Castro, G. A., Gastroenterology **68,** 1177 (1975).
5. Johnson, L. R., Lichtenberger, L. M., Copeland, E. M., Dudrick, S. J., and Castro, G. A., Gastroenterology **68,** 1184 (1975).
6. Lichtenberger, L. M., Lechago, J., and Johnson, L. R., Gastroenterology **68,** 1473 (1975).

7. Hopper, A. F., Rose, P. M., and Wannemacher, R. W., J. Cell Biol. **53,** 225 (1972).
8. Steiner, M., Bourges, H. R., Freedman, L. S., and Gray, S. J., Amer. J. Physiol. **215,** 75 (1968).
9. McNeill, L. K., and Hamilton, J. R., Pediatrics **47,** 65 (1971).
10. Ju, J. S., and Nasset, E. S., J. Nutr. **68,** 633 (1959).
11. Nevalainen, T. J., and Janigan, D. T., Virchows Arch. Abt. B. Zellpathol. **15,** 107 (1974).
12. Webster, P. D., Singh, M., Tucker, P. C., and Black, O., Gastroenterology **62,** 600, (1972).
13. Morisset, J. A., and Webster, P. D., J. Clin. Invest. **51,** 1 (1972).
14. Mainz, D. L., Black, O., and Webster, P. D., J. Clin. Invest. **52,** 2300 (1973).
15. Munro, H. N., and Fleck, A., Analyst **91,** 78 (1965).
16. Schneider, W. C., *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. 3, p. 680 (1957).
17. Volkin, E., and Cohn, W. E., Methods Biochem. Anal. **1,** 287 (1954).
18. Gornall, A. G., Bardawill, C. J., and David, M. D., J. Biol. Chem. **177,** 751 (1949).
19. Morisset, J. A., Black, O., and Webster, P. D., Proc. Soc. Exp. Biol. Med. **140,** 1308 (1972).
20. Black, O., and Webster, P. D., J. Cell Biol. **57,** 1 (1973).
21. Willems, G., Vansteenkiste, Y., and Smets, Ph., Gastroenterology **61,** 323 (1971).
22. Willems, G., Vansteenkiste, Y., and Limbosch, J. M., Gastroenterology **62,** 583 (1972).
23. Lichtenberger, L., Welsh, J. D., and Johnson, L. R., Dig. Dis. **21,** 33, (1976).
24. Altmann, G. G., Amer. J. Anat. **133,** 391 (1972).
25. Brown, H. O., Levine, M. L., and Lipkin, M., Amer. J. Physiol. **205,** 868 (1963).

Received July 2, 1976. P.S.E.B.M. 1977, Vol. 156.