Changes with Fasting in Pigeon Pancreas Alkaline and Acid Ribonuclease (36186)

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Modest food deprivation results in decreased content as well as rates of synthesis of pancreatic ribonucleic acid (RNA) (1, 2). The rapidity of decrease in pancreatic RNA suggested that such changes resulted from enhanced rates of RNA degradation, as well as decreased rates of synthesis. There is suggestive evidence that intracellular alkaline ribonuclease (RNase) may be involved in control in intracellular RNA content and function. In turn there is suggestive evidence that the activity of alkaline RNase is controlled, at least in part, by binding with an alkaline RNase inhibitor (3, 4).

We have determined free and total RNase activity measured at alkaline and acid pH as well as RNase inhibitor activity in pancreata of pigeons fed *ad libitum* or fasted 72 hr. Compared with fed controls, free and total alkaline RNase activities were increased in pancreata obtained from 72-hr fasted pigeons. In addition, with fasting there were decreases in amounts of RNase inhibitor. Free and total acid RNase activities were not significantly different between fed or fasted pigeons.

Materials and Methods. White Carneau pigeons 6–8 weeks of age, weighing 450–500 g, were purchased from Palmetto Pigeon Farm, Sumter, South Carolina. "Fed" birds were fed *ad libitum;* "fasted" birds were denied food for 3 days prior to study. All birds had free access to water.

Crystalline pancreatic RNase and yeast RNA were obtained from Worthington Biochemical Laboratories, Freehold, NJ.

Preparation of homogenate and supernatant fractions of pancreas. Fed and fasted pigeons were killed, the pancreas was removed, minced, homogenized (Teflon homogenizer, 0.01 mm clearance) and made up in 0.44 *M* sucrose (RNase-free) for a final concentration of 1:10 (w/v). A supernatant fraction was prepared from the homogenate by centrifugation in a Spinco preparative centrifuge at 81,000*g* for 60 min (Beckman L2-65B, Spinco preparative centrifuge, No. 65 rotor). The supernatant resulting from this centrifugation was used undiluted for RNase inhibitor assay. A portion of the homogenate was precipitated, washed with 10% perchloric acid, and DNA content was determined as described (1). The remainder of the homogenate was frozen and thawed twice to rupture lysosomes. A fivefold dilution of this preparation was made using 0.44 *M* sucrose and prepared for assay of RNase activity.

Assay of free and total alkaline and acid RNase activity. Assay of free and total acidic and basic RNase activity was carried out by methods described by Shortman (5) and Quirin-Stricker *et al.* (6). The incubation mixture consisted of: 0.1 ml of a suitable dilution of sample mixed with 0.2 ml of buffer solution (0.03 *M* sodium barbital buffer (pH 7.8) for assay of alkaline RNase activity and 0.03 *M* acetate buffer (pH 5.8) for acidic RNase assay); 0.1 ml of either water or 2% Triton solution; 0.02 ml of 1% solution of purified RNA; mixed and incubated with agitation at 37° for 30 min. The reaction was stopped by addition of 0.6 ml of acidic-ethanol mixture (1 *N* HCl in 76% ethanol). The tubes were allowed to stand in ice water bath for 30 min, then centrifuged at 27,000*g* for 15 min. One milliliter of the resulting supernatant was added to 5 ml of water, mixed, and absorption was determined using a Beckman DU at 260 nm. Suitable sample and reagent blanks were run simultaneously.

RNase activity was compared to activity of a standard preparation of crystalline pancreatic RNase.

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TABLE I. Effect of Fasting on Free and Total Ribonuclease Activities in the Pigeon Pancreas.[a]

	Ribonuclease activity (μg of RNase/mg of DNA)		
	Fed	Fasted	p value
Acidic ribonuclease			
Free	0.034 ± 0.006	0.039 ± 0.009	NS
Total	0.044 ± 0.005	0.050 ± 0.012	NS
Basic ribonuclease			
Free	0.114 ± 0.006	0.166 ± 0.018	$< .05$
Total	0.179 ± 0.040	0.271 ± 0.036	$< .05$

[a] Values are means ± SE of 8 experiments. p value determined by use of Student's t test.

Assay of RNase inhibitor activity. Assay of RNase inhibitor activity was carried out by the method described by Shortman with the exception that samples were allowed to stand in ice for 30 min after reaction was stopped to obtain complete precipitation. The sample was then centrifuged at 27,000g for 15 min (5).

One unit of RNase-inhibitor activity was defined as that which gave 50% inhibition of 0.005 μg of crystalline pancreatic RNase under these conditions.

Results. Results of these experiments are shown in Table I. Neither free nor total RNase activity, measured at an acidic pH, was altered by fasting. However, RNase activity measured at alkaline pH was significantly increased with fasting; free RNase activity was increased 46% and total RNase activity was increased 52% above fed levels. Differences in basic RNase activity between fed and fasted animals were significant.

RNase inhibitor activity was reduced 51% with fasting; fed birds, 1.355 units/100 μg of DNA; fasted birds, 0.692 units/100 μg of DNA. Differences in RNase inhibitor activity between fed and fasted pigeons were significant at the 0.01 level.

Discussion. These results show that in pigeon pancreas fasting resulted in increased activity of both free and total alkaline RNase activity. There was a concomitant in RNase inhibitor activity. Quirin-Stricker *et al.* (6) reported similar changes with protein deprivation in rat liver. They reported during protein deprivation that activities of both free and latent RNase were not significantly changed when tested at pH 5. On the other hand, activities of both free and latent RNases were increased fivefold and two- to threefold, respectively, when tested at pH 8. Concomitant activity of RNase inhibitor was decreased. In contrast, Onishi (7) showed that, in rats fasted for 9 days, both free and total acid and alkaline RNase were decreased in activity; RNase inhibitor activity decreased likewise.

Roth (4) and later Barnard (3) have reviewed current understanding and methodology concerning studies of ribonucleases. Furthermore, Roth (8) has pointed out problems involved with comparisons of results of different techniques obtained in different laboratories (5).

Biological functions of ribonuclease enzyme systems have not been well defined. Suggested functions for various intracellular RNase include the following: (a) removal of informational RNA molecules; (b) defense against foreign (viral) RNA; (c) general intracellular digestive function; (d) synthesis of polynucleotides (3).

Alkaline RNase activity seems to be controlled in the cell by complexing of the enzyme with a soluble inhibitor. Such an inhibitor is generally found in mammalian tissue cells.

There is some indication of a correlation of RNase activity to cellular RNA content and development. Bresnick *et al.* (9) have shown that RNase activity measured at alkaline pH decreases with development. Brody (10) has shown a significant decrease in activity of the enzyme as a function of age of placental tissue. Enwonwu *et al.* (11) have reported increased hepatic RNA degradation in animals subjected to food deprivation.

In the pigeon pancreas, decreases in amounts of RNA were associated with increases in alkaline RNase activity. Although not proven conclusively, it is tempting to speculate that the reason for decreased amino acid incorporation observed in fasting animals in previous studies might be due to increased RNase activity resulting in degradation of one or more species of RNA required for protein synthesis.

Summary. Modest food deprivation results in decreased pancreatic RNA content as well as rates of synthesis. The rapidity of decrease in RNA content suggested that enhanced degradation may play a role. Measurements of free and total alkaline RNase activity showed 46 and 52% increases in alkaline RNase activity in pancreata of pigeons fasted 3 days compared to fed controls. In addition, there was a decrease in amounts of alkaline RNase-inhibitor activity. The data suggest reductions in pancreatic RNA content observed with fasting may be due, at least in part, to enhanced RNA degradation.

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