EVIDENCE FOR DEGRADATION OF INTRACELLULAR PROTEIN IN LIVER LYSOSOMES OF FASTED RATS

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Summary: Evidence that intracellular protein degradation occurs in lysosomes has been indirect and derived from liver perfusion (1) or the inhibitor studies (2,3). We report here that liver lysosomes of greater purity are obtained from fed rats than from fasted rats. Lysosomes of less purity may contain an enlarged pool of partially degraded intracellular protein; on the other hand, less purity could be due to less marker enzyme, NAβGase. Measurements of NAβGase activity and lysosomal protein of rat livers showed that both NAβGase and lysosomal protein increased upon fasting but protein more so (3.5 and 6.5x, in 2 days). The increase in lysosomal protein is direct evidence that liver lysosomes are involved in intracellular protein degradation during fasting of rats.

The known role of lysosomes in cellular functions is broadening to include more than their pathological functions (4). Previous studies of endocytosis have demonstrated the deposition of extracellular material inside the lysosome (5,6). However, little has been done to lend weight to the hypothesis that lysosomes are sites of intracellular protein degradation (1-3). This report presents direct evidence that lysosomes are inextricably involved in intracellular protein degradation *in vivo*.

Materials and Methods

Male, albino rats were injected with Triton WR-1339 and lysosomes were prepared by the method of Leighton *et al.* (7). Fasting was initiated by removal of food. The rats were maintained on a 12 hour light-dark cycle: the lights were turned on at 7 A.M. and all animals were sacrificed at 10 A.M. except that those not fasted at all (0 hr) were sacrificed at 7 A.M. N-acetyl-β-glucosaminidase (NAβGase) was assayed essentially as described (8) although 0.1% Triton X-100

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was added to assure maximum activity. Para-Nitrophenyl-N-acetyl-β-D-glucosaminide was obtained from Sigma Chemical Corp. Protein determinations were made by the method of Lowry (9) with the following modifications: sucrose interference was eliminated by precipitation of protein with 10% trichloroacetic acid and centrifugation and 0.4% sodium deoxycholate was added to the hydroxide solution to clarify membranous preparations.

Results

When rats were fasted for increasing period of time, the condition of liver lysosomes changed significantly. As can be seen in Figure 1, the apparent

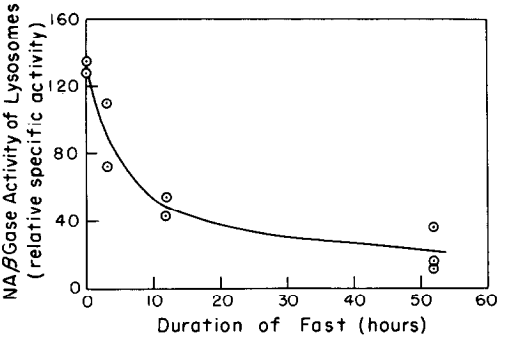

Figure 1. Effect of Fasting on Rat Liver Lysosome Purification.

Rats were fasted for increasing periods as indicated. Lysosomes were prepared using Triton WR-1339 (7) and NAβGase and protein determinations were performed as described in Materials and Methods. Relative specific activity was calculated from the amount of marker enzyme and the amount of protein as previously described (12) and is equal to the fold purification.

purity of lysosomes, judged by relative specific activity of NAβGase, decreased 6x. Table I shows that comparable purities are calculated on basis of specific activity. Furthermore the specific activity of total liver (E + N) and of lysosomes were both affected.

These changes could depend on a decrease in marker enzyme activity (NAβGase) or an increase in lysosomal protein or both. Figure 2 shows that the amount of NAβGase increased, rather than decreased, with fasting. Two-thirds of the increase

Table 1. Decrease in Purity of Liver Lysosomes Upon Fasting of Rats.

Rats were fasted, lysosomes were isolated, and NAβGase and protein were measured as described in Figure 1.

Duration of Fast (hours)	Relative Specific Activity	Lysosome Purity		
		Specific Activity		
		E + N	Lysosomes	Enrichment
		(μmole/min/mg)		
0	128	0.0162	2.13	131
0	135	0.0161	1.37	85
3	110	0.0125	1.59	127
3	72	0.0379	2.23	59
12	54	0.0278	1.72	62
12	43	0.0432	1.83	42
52	36	0.0615	1.80	29
52	16	0.0325	0.59	18
52	12	0.0379	1.01	27

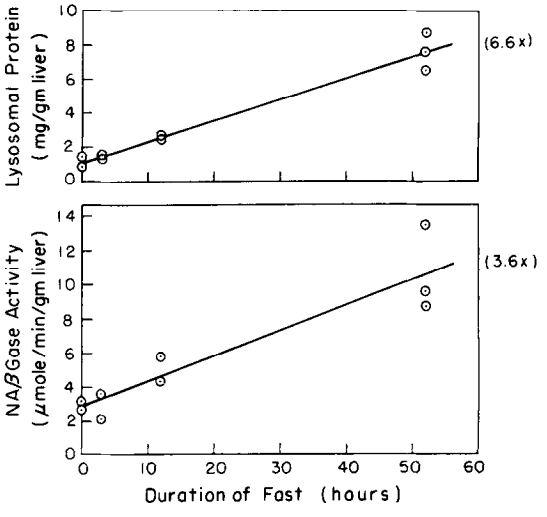

Figure 2. Effect of Fasting on Liver NAβGase and Lysosomal Protein.

Rats were fasted, lysosomes were isolated, and NAβGase (lower panel) and protein (upper panel) were measured as described in Figure 1. The NAβGase values are those of total liver (E + N) whereas lysosomal protein is that of the isolated lysosomes after correcting for yield. After 2 days of fasting NAβGase increases 3.5x and protein 6.5x.

in NAβGase activity upon fasting is attributable to a decrease in liver weight (data not shown). The remainder represents net increase due to increased synthesis or decreased degradation of NAβGase enzyme. However, during fasting lysosomal protein increased more dramatically than NAβGase (Figure 2). Thus, upon fasting of rats, isolation of liver lysosomes results in less enrichment of marker enzyme because more protein is associated with lysosomes. Since fasting is known to promote degradation of intracellular protein in liver, the increase in protein associated with lysosomes presumably represents an expanded pool of intracellular protein in the degradative system.

Discussion

The 6.5x increase in lysosomal protein after 2 days of fasting constitutes direct evidence for intracellular protein degradation in the lysosome. Whether degradation occurs in other locations is unknown, and these results do not exclude a role for neutral protease(s). The possibility of initiation of degradation by neutral protease and of completion by lysosomal proteases is being considered. The pathway which brings intracellular protein to the lysosomes is biochemically obscure and may involve autophagy (4).

The 3.5x increase in lysosomal marker enzyme, NAβGase, that occurs during 2 days of fasting reflects both a decrease in liver mass with conservation of NAβGase activity and an increase in NAβGase activity. A similar situation occurs in regression of tumor growth (10) and cardiac hypertrophy (11). The conservation of and net increase of NAβGase during fasting point to the essential role of lysosomal hydrolases in catabolism of intracellular material.

Acknowledgements

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