J. Biosci., Vol. 10, Number 1, March 1986, pp. 49–55. © Printed in India.

Influence of starvation, Triton WR-1339 and [^{131}I]-human serum albumin on rat liver lysosomes

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MS received 22 April 1985; revised 20 August 1985

Abstract. The response of rat liver lysosomes to starvation and administration of lysosomotropic agents *viz*. Triton WR-1339 and [^{131}I]-human serum albumin, was assessed in terms of their distribution pattern after isopycnic sucrose density gradient centrifugation. Starvation induced changes in lysosomes appeared to be similar to that produced by the detergent uptake. Both the treatments caused a distinct decline in the equilibration densities of the organelles. On the other hand, injected labelled protein failed to comigrate with the lysosomal markers in starved as well as Triton treated rats and conspicuously remained in a region of high specific gravity in the gradient. These findings indicate retarded fusion between secondary lysosomes and [^{131}I]-human serum albumin containing phagosomes in the livers of rats subjected to starvation or detergent treatment.

Keywords. Lysosome; starvation; Triton WR-1339; [^{131}I]-human serum albumin; isopycnic centrifugation.

Introduction

Susceptibility of lysosomes to dietary restrictions such as starvation is often manifested in terms of specific alterations in size, shape and density of the organelles (Swift and Hruban, 1964; Harikumar and Ninjoor, 1979) which have been attributed to the stimulated autophagic uptake of cellular constituents. Thus, the increase in lysosomal density noted under deprivation induced autophagy in perfused livers of rats has been shown to be due to the sequestration of glycogen and smooth endoplasmic reticulum (Neely *et al*., 1977). We have demonstrated earlier that the equilibration densities of heterogeneous populations of liver lysosomes in starved rats decrease markedly due to an apparent enhancement in the accumulation of lipid particles in lysosomes (Harikumar and Ninjoor, 1979). In view of the finding that the uptake of Triton WR-1339 by lysosomes promote lipid accumulation (Hayashi *et al*., 1981) and thereby reduces lysosomal specific gravity (de Duve, 1975; Warburton and Wynn, 1977) an attempt is made in the present work to compare the starvation induced lysosomal changes with that produced by the detergent, to deduce evidence substantiating the above hypothesis. Data on the influence of another lysosomotropic agent, [^{131}I]-

Abbreviations used: [^{131}I]-HSA, [^{131}I]-Human serum albumin; NAG, N-acetyl -β-glucosaminidase; AS, arylsulphatase; EDTA, ethylene diamine tetraacetic acid; ML, mitochondria lysosome rich; L_1, low density lysosome; L_2, high density lysosome.

labelled human serum albumin ([^{131}I]-HSA) on lysosomes are also provided to enable further comparison.

Materials and methods

Para - nitrocatechol sulphate, *p* - nitro - pheny - 2 - acetamido - 2 - deoxy - β - D - glucopyranoside, *p*-nitrophenol and *p*-nitrocatechol were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. Analar sucrose and Triton WR-1339 were obtained from British Drug House, Bombay and Ruger Chemical Co., Irvington, New Jersey, USA respectively. [^{131}I]-HSA was supplied by the Isotope Division, Bhabha Atomic Research Centre. All other chemicals were of analytical grade quality.

Preparation of denatured labelled protein

[^{131}I]-HSA (specific activity 39–46 μCi per mg protein) was denatured (Mego and McQueen, 1967) by treating with 4% formaldehyde solution in 0·05 M sodium carbonate buffer, pH 10·0. After standing in cold (0–4°C) for 3 days, the protein solution was dialysed exhaustively against 0·1 M NaCl. The protein content and the radioactivity were then determined.

Treatment of animals

Male albino rats of Wistar strain weighing 250–300 g, reared on a laboratory stock diet *ad libitum* were used in the present investigation. Animals were starved for indicated time intervals by withdrawing the diet, while they had free access to water throughout the experimental period. Triton WR-1339 was administered intraperitoneally to rats in 0·9% saline at a dose of 85mg/100g body weight (Leighton *et al*., 1968). After administering the detergent, the animals were either fed the stock diet for 5 days or fasted for indicated periods. Formaldehyde denatured [^{131}I]-HSA was injected into the tail veins of rats (0·25 mg/100 g body weight). The animals were sacrificed 30 min after the administration of labelled protein. Five rats were included in each of these experiments.

Tissue fractionation

Rats were killed by decapitation. Livers were rapidly excised, washed quickly with the homogenizing medium, blotted and weighed. Liver homogenates (10%, w/v) were prepared in 0·25 M sucrose containing 1 mM EDTA (disodium salt) by two up and down strokes in a Potter-Elvehjem glass homogenizer and filtered through 2 layers of surgical gauge (Harikumar *et al*., 1978). Fractionation of liver homogenates was carried out as described earlier (Harikumar and Ninjoor, 1979) employing differential and isopycnic sucrose density gradient centrifugation procedures. A mitochondrial-lysosome rich (ML) fraction isolated from liver homogenate by differential centrifugation was resuspended in 10 % (w/v) sucrose and layered on the top of a continuous gradient (42 ml) of 80 % (w/v) and 35 % (w/v) sucrose solutions. The samples were spun at 25,000 rev/min in a Beckman-Spinco Model L2-65 B ultracentrifuge using SW 25-2 rotor for 90 min and the fractions (2 ml) were collected from the top of the gradient by aspiration with a glass syringe.

Analytical procedures

Distribution of radioactivity in tissue preparations was measured employing a Gamma Counter (Medical Spectrometer, Type NI-2123, BARC). Lysosomal marker enzymes, arylsulphatase (AS) [EC 3.1.6.1] and N-acetyl-β-D-glucosaminidase (NAG) [EC 3.2.1.30] were assayed as described by Barrett (1972) after subjecting tissue samples to 8 freeze thaw cycles. Protein was measured according to the method of Lowry *et al.* (1951).

Results and discussion

In the present investigation the characteristic properties of Triton WR-1339 and [^{131}I]-HSA in decreasing (Warburton and Wynn, 1977) and increasing (Davies, 1973) lysosomal densities respectively, are taken advantage of in eliciting information regarding the starvation induced alterations in rat liver lysosomes. As shown in figure 1, fasting for 5 days as well as administration of the detergent led to a pronounced shift in the equilibration densities of lysosomal marker enzymes NAG (left panel) and AS (right panel). Starvation resulted in a distinct bimodal distribution of NAG with nearly 85 % of the enzyme activity concentrated in two regions of the gradient *i.e.* fractions 9–13 (ρ = 1·059–1·146) and fractions 15–21 (ρ = 1·166–1·230). These two regions apparently

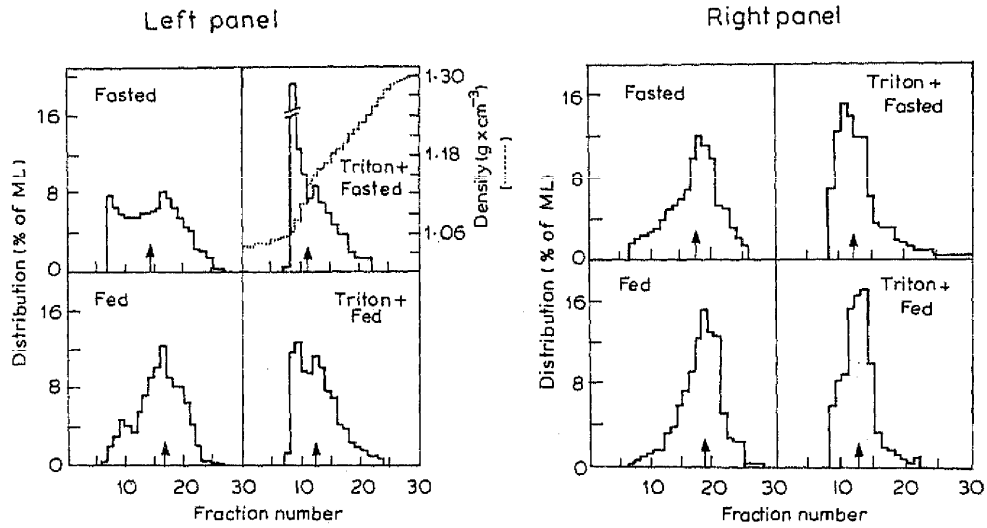

Figure 1. Influence of starvation (5 days) and Triton WR-1339 on the distribution profiles of NAG (left panel) and AS (right panel) after isopycnic density gradient centrifugation. While fractions 1–11 exhibited a non-linear density distribution between 1·038 and 1·04 g × cm^{-3}, fractions 12–30 showed a linear distribution in the range of 1·132–1·298 g × cm^{-3}. The histograms show averages of results obtained from 3 independent experiments. The arrows indicate the position of median densities.

Table 1. Yield of marker enzymes in lysosomal populations isolated by isopycnic density gradient centrifugation.

	Yield of enzymes (%)			
	AS		NAG	
Treatments	L_1	L_2	L_1	L_2
Control	9·2	57·1	12·4	72·6
Fasted, 5 days	14·0	63·6	30·6	57·2
Triton + fed, 5 days	55·4	19·7	56·0	30·0
Triton + fasted, 5 days	60·7	22·0	64·6	25·1

Yield of marker enzymes is expressed as % of the enzyme activity in ML fraction. L_1 represents populations equilibrated in fractions 9–13 (ρ = 1·059–1·146 g × cm^{-3}). L_2 represents populations equilibrated in fractions 15–21 (ρ = 1·166–1·230 g × cm^{-3}).

representing dual populations of lysosomes, were termed as low density lysosomes (L_1) and high density lysosomes (L_2) respectively. Density alterations in lysosomes induced by starvation and Triton WR-1339 were evaluated in terms of the relative yield of marker enzymes in these two populations. Unlike NAG, AS did not exhibit bimodal distribution. However, in view of the marked shift in median density of the enzyme from high density region to lower densities (figure 1), the distribution of this enzyme was also assessed in L_1 and L_2. The density lowering effect of the detergent (Warburton and Wynn, 1977; de Duve, 1975) is obvious from the decline noted in the median densities (1·204 to 1·142g × cm^{-3} for AS and 1·195 to 1·139 g × cm^{-3} for NAG) of the lysosomal populations (figure 1) concomitant with the perceptible increase in the yield of L_1 (table 1). It is interesting to note that despite differences in the degree of increase in the yield of L_1 during starvation (4–18 %) and Triton treatment (45 %), a translocation of lysosomal populations to markedly reduced density regions (figure 1) is a characteristic feature of both starvation and detergent loading. It may therefore be pointed out that Triton loading simulates the effects of starvation at least with respect to causing changes in lysosomal densities. These results assume significance in the light of available information on the involvement of lipids even in the presence of other cellular materials like proteins (Gregoriadis and Ryman, 1972) in lowering the lysosomal density, and their relatively longer retention in lysosomes (Glaumann *et al*., 1979) and lead us to conclude that both the treatments promote accelerated accumulation of lipid particles in the organelles. When Triton administration was coupled with 5 days starvation, a further narrowing of the distribution histograms (figure 1) and an enhancement in the yield of L_1 (table 1) was observed suggesting that the effects of fasting and detergent are inter-related and additive in nature. This explanation is valid in view of the reported observations that Triton filled lysosomes fuse with preformed phagolysosomes rather than with newly synthesized lysosomes (Tsung *et al*., 1975). While the property of Triton WR-1339 in triggering rapid inclusion of lipids in lysosomes is undisputed, the origin of lipids and the significance of their

enhanced accumulation in lysosomes during starvation remain obscure. It is possible that the extra energy demand of the cell in the absence of dietary supply is met by the mobilization of lipids from the fatty resources (Nepolitano, 1963) and/or by the direct utilization of cellular constituents (Levy and Elliot, 1968) subsequent to their sequestration into lysosomes. Since lipid degradation in lysosomes is comparatively a slower process (Glaumann *et al*., 1979) both the above events could lead to a progressive accumulation of undigested lipids affecting the buoyant densities of the organelles. We have observed (Brij Bhushan *et al*., 1981) that fasting enhances lipid storage in rat liver, mainly in the form of triglycerides. How much of this increase could be attributed to inclusion inside lysosomes is however, not known.

Unlike Triton WR-1339, the endocytosis of [^{131}I]-HSA seems to exert hardly any influence on the equilibration densities of lysosomes isolated either from fasted or fed livers (figure 2). In both the groups, the labelled protein appears as a single sharp peak at the high density region (1·166–1·230 g × cm^{-3}). The starvation effect is discernible to the extent that there is a 13 % increase in the yield of radioactivity accompanied by an elevation in the peak height of the label. It is however, noteworthy that the marker enzymes tend to migrate towards lower densities, away from the protein peak as a consequence of starvation indicating an inadequate fusion of protein containing phagosomes with the existing lysosomes. This was further substantiated by the data incorporated in figure 3 and table 2 where the lysosomal response elicited by both Triton WR-1339 and [^{131}I]-labelled protein was ascertained in livers of rats fed or fasted for varying periods. It was observed that the particulates containing labelled

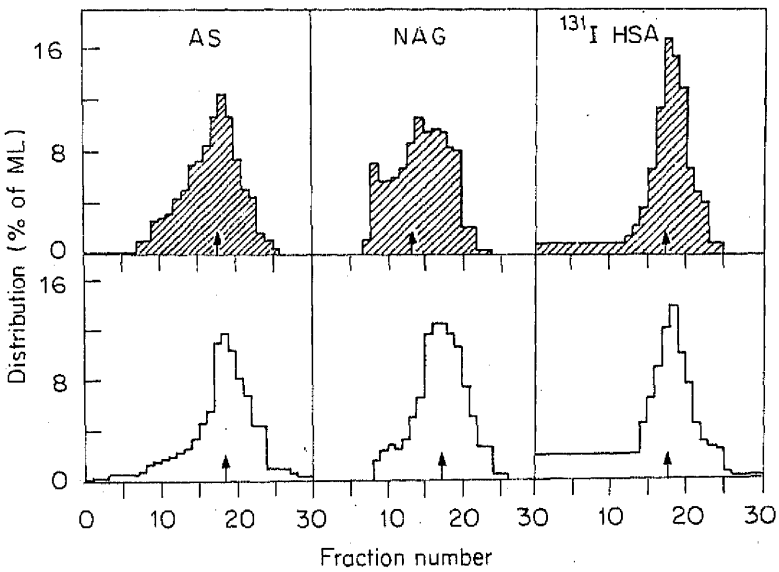

Figure 2. Equilibration profiles of [^{131}I]-HSA and liver lysosomal marker enzymes during isopycnic density gradient centrifugation in fed, control □ and fasted (5 days) ▨ rats. The distribution of [^{131}I]-HSA represents total radioactivity and is expressed on the basis of radioactivity in ML fraction which is considered as 100%.

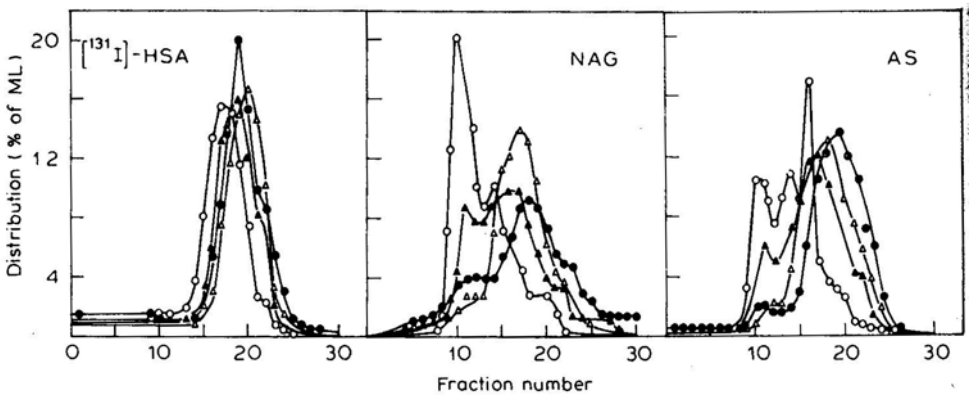

Figure 3. Alterations in the distribution profiles of lysosomal marker enzymes in response to starvation for varying periods in rat livers treated with Triton WR-1339 and [^{131}I]-HSA. Rats were administered Triton WR-1339 and fasted for 1 day (Δ); 2 days (▲)and 5 days (O). These animals along with those belonging to fed control (●) group have been administered [^{131}I]-HSA exactly 30 min prior to sacrifice. The distribution of [^{131}I]-HSA represents total radioactivity and is expressed on the basis of radioactivity in ML fraction which is considered as 100%.

Table 2. Influence of starvation and lysosomotropic agents on the median equilibration densities of lysosomes.

Treatment	Median density ($g \times cm^{-3}$) [^{131}I]-HSA	AS	NAG
Control	1·195	1·201	1·189
Triton + fasted, 1 day	1·204	1·190	1·180
Triton + fasted, 2 days	1·196	1·177	1·167
Triton + fasted, 5 days	1·181	1·153	1·120

The median equilibration densities of the lysosomal marker enzymes and [^{131}I]-HAS were computed from the results presented in figure 3.

protein consistently remain as a single peak in the region of higher specific gravity away from lysosomal marker enzymes in response to starvation and Triton treatment. Administration of labelled protein does not seem to influence the density lowering efects of fasting and detergent treatment. This is apparent from the progressive shift in median densities of NAG and AS to lower values as the period of starvation advanced from 1–5 days (figure 3 and table 2). It is evident from these results that autophagosomes formed in response to starvation fuse readily with tritosomes but not with phagosomes containing [^{131}I]-HSA. These findings taken together with our observation on the diminished intracellular degradation of labelled protein in livers of starved rats (Harikumar and Ninjoor, 1985) are in conformity with the reports on the selectivity associated with the fusion process between secondary lysosomes and vesicles containing externally administered macromolecules (Tsung *et al.*, 1975) and cellular structures

(Glaumann and Trump, 1975). No obvious explanation for the nonfusion of phagosomes containing [^{131}I]-labelled protein with the existing phagolysosomes is available at present. Nevertheless, it is conceivable that the accentuated accumulation of lipids in tritosomes and fasted lysosomes could be responsible for the delayed transfer of labelled protein to secondary lysosomes, possibly due to charge variations in the lysosomal microenvironment. Recently, Hostetler *et al*. (1985) have demonstrated that in chloroquine induced fatty liver, the surface charges of the lysosomes are markedly altered as a consequence of lipid inclusion. Warburton and Wynn (1977) have in fact reported that newly endocytosed material preferentially enters those lysosomes which contain low levels of lipids. Also the functional specificity of the heterogeneous populations of lysosomes (Harikumar and Ninjoor, 1979; Pertoft *et al*., 1978) as well as disturbances in the cytoplasmic endowments such as microtubules which regulate the intracellular translocation and fusion of vesicles (Dunn *et al*., 1980; Collot *et al*., 1984) could influence the phagolysosome formation.

Acknowledgement

The authors thank Dr. G. B. Nadkarni, Head, Biochemistry and Food Technology Division, for his helpful suggestions and critical evaluation of the manuscript.

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