The Effect of Intralysosomal Sucrose Storage on the Turnover of Hamster Fibroblast Lysosomal and Golgi-Apparatus Enzymes

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1. The effect of overloading of hamster fibroblast lysosomes with sucrose on the turnover of lysosomal and Golgi-apparatus enzymes was studied. Arylsulphatase B and UDP-galactose-*N*-acetylglucosamine galactosyltransferase were chosen as appropriate marker enzymes. The relative contributions of changes in the rates of synthesis and degradation to the increased activities of these enzymes after uptake of sucrose were examined by isotopic-labelling experiments. The effects of sucrose uptake on the degradation of total cellular protein and of the cytoplasmic enzyme, alkaline ribonuclease, were determined for comparative purposes. 2. The rates of enzyme synthesis in the presence and absence of sucrose were compared by pulse-labelling the cells with ^{14}C -labelled amino acids, followed by isolation of purified enzymes and determination of their radioactivity. Sucrose uptake produced increases of 270% and 90% respectively in the rates of synthesis of arylsulphatase B and galactosyltransferase, whereas the rate of synthesis of alkaline ribonuclease was not affected. 3. The rates of degradation of the enzymes were estimated by measuring the decay with time of the radioactivity of purified enzymes from prelabelled cells. In the absence of sucrose, the apparent half-lives of arylsulphatase B and galactosyltransferase were about 30 days and 40h respectively. After uptake of sucrose, the half-life of arylsulphatase B decreased to about 10 days and that of galactosyltransferase to 10h. Neither the half-life of alkaline ribonuclease (4 days) nor the rate of degradation of total cellular protein was affected by the uptake of sucrose. 4. These results indicate that the hyperactivity of lysosomal and Golgi-apparatus enzymes after the uptake of sucrose is accompanied by increases in the rates of both synthesis and degradation, and that the increased rates of degradation are insufficient to prevent accumulation of the excess of enzymes synthesized; also that the effects of sucrose uptake are restricted to the vacuolar apparatus.

Under normal physiological conditions, mammalian cells are capable of degrading most of the material entering the lysosomal system (Vaes, 1973). However, under certain circumstances, non-degradable material may enter the vacuolar apparatus. Pathological situations resulting in part from the intralysosomal accumulation of non-digestible material include the lysosomal storage diseases (Hers & Van Hoof, 1973), silicosis (Allison, 1969), gout (Weissmann *et al.*, 1971) and atherosclerosis (Peters & de Duve, 1974). The inherent variability of these diseases necessitates the use of simpler model systems in preliminary studies of the effects of lysosomal overloading on the functioning of the vacuolar apparatus.

One example of such a model system is the intralysosomal accumulation of sucrose (Lloyd, 1973), since sucrose can be degraded only very slowly by lysosomal enzymes (Horvat, 1973). After injection into rats, sucrose is taken into the liver by endocytosis and enters the lysosomal system, producing

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greatly enlarged lysosomes (Brewer & Heath, 1963; Rohr & Zollinger, 1966). The increased size of the lysosomes is possibly due to an influx of water to maintain the osmotic balance between the interior of the lysosome and the cytosol (Wattiaux, 1966), and leakage of acid hydrolases from the enlarged lysosomes results in liver damage (Janigan *et al.*, 1960). Other cell types in which the effects of sucrose have been studied include cultured chick-embryo skeletal tissue (Dingle *et al.*, 1969), mouse peritoneal macrophages (Cohn & Ehrenreich, 1969), mouse leukaemic cells (Bernacki & Bosmann, 1971) and Chinese-hamster ovary fibroblasts (Munro, 1968). In general the extent of vacuolation depends on the amount of sucrose taken up. The vacuoles arise through pinocytosis and the inability to hydrolyse sucrose rather than a stimulation of the rate of ingestion. Other compounds which have been used to study the effects of lysosomal overloading include dextran (Maunsbach, 1969), various peptides (Axline & Cohn, 1970) and Trypan Blue (Lloyd *et al.*, 1972).

The intralysosomal storage of non-digestible material often results in increases in the activities of

acid hydrolases. Lysosomal-enzyme hyperactivity has been noted during the storage of dextran (Goldberg *et al.*, 1960), polyvinylpyrrolidone (Meijer & Willighagen, 1963) and sucrose (Wattiaux, 1966; Cohn & Ehrenreich, 1969; Dingle *et al.*, 1969; Bernacki & Bosmann, 1971). The most striking example of lysosomal hyperactivity is provided by the lysosomal storage diseases, with increases in excess of tenfold (Van Hoof, 1973). The increases in enzyme amounts are believed to be due to changes in both the rates of synthesis (Axline & Cohn, 1970) and the rates of degradation (Van Hoof & Hers, 1972).

We have previously shown that overloading of the hamster fibroblast lysosomal system with sucrose produces increases in the activities of lysosomal, Golgi-apparatus and plasma-membrane enzymes (Warburton & Wynn, 1976a). In the present paper the relative contributions of changes in the rates of synthesis and degradation to the hyperactivity of Chinese-hamster fibroblast lysosomal and Golgi-apparatus enzymes after lysosomal overloading with sucrose are assessed.

Experimental

Materials

U-¹⁴C-labelled amino acids (algal protein hydrolysate, specific radioactivity 57 mCi/mg-atom of C) and UDP-[U-¹⁴C]galactose (sp. radioactivity 272 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Sepharose 4B was supplied by Pharmacia Fine Chemicals AB, Uppsala, Sweden. Saccharo-1,4-lactone and UDP-galactose were purchased from Sigma (London) Chemical Co. Ltd., Kingston-upon-Thames, Surrey, U.K. α -Lactalbumin and methylumbelliferyl sulphate were supplied by Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. Agarose-5'-(4-aminophenyl-phosphoryl)uridine 2':3'-phosphate was purchased from Miles-Seravac, Maidenhead, Berks., U.K.

Cell culture

Chinese-hamster CH-23 fibroblasts were cultured as described previously (Warburton & Wynn, 1976b). In experiments to study the effects of sucrose, sucrose was added to the growth medium to a final concentration of 0.08 M.

Effects of sucrose uptake on total intracellular protein degradation

Approx. 10⁵ cells were inoculated into culture flasks of 50 cm² growth surface, containing 20 ml of medium (see Warburton & Wynn, 1976b); 1 μ Ci of U-¹⁴C-labelled amino acids was added to each flask and the cells were grown to confluency. On reaching

confluency, the cell sheet was washed twice with 20 ml of unlabelled medium. The cells were then incubated with 20 ml of unlabelled medium and harvested at various time-intervals. The radioactivity of intracellular proteins and amino acids was measured by the procedure of Poole & Wibo (1973).

Measurement of turnover rates of specific proteins

(a) *Rate of incorporation of labelled amino acids.* About 5 $\times$ 10⁵ cells, in exponential growth, were inoculated into 5 ml of medium in 1-litre Roux bottles. After 48 h, 5 μ Ci of U-¹⁴C-labelled amino acids was added to each bottle. After a further 18 h incubation (or 9 h for galactosyltransferase), the cell sheets were washed twice with 25 ml of unlabelled medium and the cells harvested by trypsin treatment (see Warburton & Wynn, 1976b). The cell pellets were washed with 3 $\times$ 10 ml of 0.25 M-sucrose, and then homogenized by a Teflon/glass Potter-Elvehjem homogenizer in 2 ml of 5 mM-Tris/HCl buffer, pH 7.5. After the addition of Triton X-100 to a final concentration of 0.1% (v/v) and the removal of a portion for the determination of total cell protein radioactivity, cellular debris was removed by centrifugation at 100 000g for 30 min. Various enzymes were purified as described below and the specific radioactivity of the purified enzyme was determined. The experiment was performed with paired bottles, one of which contained 0.08 M-sucrose.

(b) *Rate of enzyme degradation.* About 5 $\times$ 10⁵ cells were inoculated into 1-litre Roux bottles containing 50 ml of medium and 5 μ Ci of U-¹⁴C-labelled amino acids and the cells were grown to confluency. On reaching confluency, the cell sheet was washed twice with 35 ml of unlabelled medium and further incubated in the presence of unlabelled medium. The medium was replaced with fresh medium after 6 h, 12 h, 24 h and then every 36 h. The cells from individual bottles were harvested at various time-intervals and homogenized in 5 mM-Tris/HCl buffer, pH 7.5, and treated as before. Various enzymes were purified as described below and the specific radioactivity of the purified enzyme was determined. To investigate the effects of sucrose, cells were grown in the presence of 0.08 M-sucrose throughout the labelling period and subsequent 'chase'.

Enzyme purification

All enzyme purifications were carried out at 4°C and all solutions contained 0.1% (v/v) Triton X-100.

(a) *Arylsulphatase B* (EC 3.1.6.1). Arylsulphatase B was purified by the affinity-chromatographic procedure of Agogbua & Wynn (1975). The adsorbent was prepared by coupling 4-hydroxy-2-nitrophenyl

sulphate (Smith, 1951) to *p*-aminobenzamidoethyl-Sepharose (Cuatrecasas, 1970). The adsorbent contained 11 μ mol of 4-hydroxy-2-nitrophenyl sulphate/ml of Sepharose.

The cell homogenate was adjusted to pH 6.2 by the addition of 20 mM-sodium acetate buffer, pH 6.2, and applied to a column (7 cm $\times$ 1 cm) of the adsorbent. The column was washed free of protein with 0.2 M-sodium acetate buffer, pH 6.2, and the enzyme eluted with 0.2 M-sodium acetate buffer, pH 6.2, containing 1.0 M-NaCl and 0.05% bovine serum albumin.

(b) *UDP-galactose-N-acetylglucosamine galactosyltransferase* (EC 2.4.1.22). Galactosyltransferase was purified by the affinity-chromatographic procedure described by Trayer & Hill (1971), by using α -lactalbumin-Sepharose as adsorbent. The adsorbent contained 0.78 mg of α -lactalbumin/ml of Sepharose.

The cell homogenate was adjusted to pH 8.5 with 0.1 M-Tris/HCl buffer, pH 8.5, containing 0.2 M-glucose, and applied to a column (5 cm $\times$ 0.5 cm) of the adsorbent. The column was washed with the glucose/Tris buffer until the eluate was free from protein. The enzyme was eluted by omitting glucose from the washing buffer, i.e. with 0.1 M-Tris/HCl, pH 8.5, to which 0.1% bovine serum albumin had been added to aid enzyme stabilization.

(c) *Alkaline ribonuclease* (EC 3.1.4.22). Alkaline ribonuclease was purified by the affinity-chromatographic procedure of Wilchek & Gorecki (1969) by using agarose-5'-(4-aminophenylphosphoryl)uridine 2':3'-phosphate as adsorbent.

The cell homogenate was adjusted to pH 5.0 with 20 mM-sodium acetate buffer, pH 5.0, applied to a column (3 cm $\times$ 0.5 cm) of the adsorbent, and the column was washed free of unadsorbed protein with 0.1 M-sodium acetate, pH 5.0. The enzyme was eluted with 0.2 M-Tris/HCl, pH 8.0, containing 0.1% bovine serum albumin.

Assay procedure

Arylsulphatase B was assayed as described previously (Warburton & Wynn, 1976b). Galactosyltransferase was determined by the method of Fleischer *et al.* (1969) and alkaline ribonuclease was assayed at pH 7.5 in 0.01 M-Tris/HCl buffer by measuring the release of HClO_4 /uranyl acetate-soluble ^3H -labelled oligonucleotides from [^3H]RNA. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. The radioactivity of samples was counted in a liquid-scintillation spectrometer (Packard, model 3003) with a xylene/Triton X-100 (2:1, v/v)-based scintillator containing 0.4% (w/v) 2,5-diphenyloxazole and 0.04% (w/v) 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)-benzene. Counting was to 5% error or less. The efficiency of counting was determined by the channels-

ratio method and counts were corrected for quenching.

Results

Effect of sucrose uptake on total protein degradation

The effect of sucrose uptake on total intracellular protein degradation was investigated by measuring the decay in radioactivity of prelabelled protein.

Over the initial 6 h, protein degradation was more rapid in cells grown in the presence of sucrose, but after this time, the half-life of total protein became similar in the absence or presence of sucrose (Fig. 1). Over 72 h the rate of protein degradation was about 0.8%/h. Initially, cells grown in the presence of sucrose had a higher proportion of the total radioactivity in the intracellular amino acid pool. The intra-

Fig. 1. *Effect of sucrose uptake on the rate of degradation of total intracellular protein*

Cells were grown to confluency in the presence of 1 μ Ci of ^{14}C -labelled amino acids. The medium was then replaced with unlabelled medium and cells were harvested at various times. The cells were processed for the determination of intracellular protein and amino acid radioactivity as described in the text. (a) Rate of degradation of total intracellular protein; (b) rate of decay of the intracellular ^{14}C -labelled amino acid pool. Cells were grown in the absence (○) or presence (●) of 0.08 M-sucrose.

cellular ^{14}C -labelled amino acid pool decayed rapidly over the initial 6 h of the 'chase', after which time the rate of decay gradually decreased until the radioactivity of the intracellular amino acid pool accounted for only 1 % of the total radioactivity after 72 h.

Enzyme purification

To investigate the effects of sucrose uptake on the turnover of specific enzymes, the rate of incorporation of ^{14}C -labelled amino acids into these enzymes and the rate of loss of radioactivity from prelabelled enzymes was investigated. The proteins investigated, arylsulphatase B (a lysosomal enzyme), galactosyltransferase (from the Golgi apparatus) and alkaline ribonuclease (a soluble cytoplasmic enzyme), were purified by affinity-chromatographic procedures. The purification summary for each enzyme is shown in Table 1. In each case, a high degree of purification was achieved. Re-chromatography of ^{14}C -labelled enzymes on DEAE-cellulose showed that more than 80 % of the radioactivity corresponded to the purified enzymes (M. J. Warburton, unpublished work).

Effect of sucrose uptake on the rate of incorporation of ^{14}C -labelled amino acids into purified enzymes

The effect of sucrose uptake on the rates of synthesis of the three enzymes investigated was determined by pulse-labelling the cells with ^{14}C -labelled amino acids followed by isolation of the purified labelled enzymes. The results of these experiments are shown in Table 2. When hamster fibroblasts are grown in the presence of sucrose, the rate of growth as evidenced by cell numbers and cellular division time is slower (M. J. Warburton, unpublished work), and, during the pulse-labelling period, the radioactivity incorporated into total protein is consequently decreased by about 40 %. The radioactivity of enzymes purified from cells grown in the presence of sucrose has therefore been increased by 40 % to obtain a true comparison with the enzymes purified from cells cultured in the absence of sucrose. The lysosomal enzyme arylsulphatase B showed an increased rate of ^{14}C -labelled amino acid incorporation of 270 %, whereas the Golgi-apparatus enzyme galactosyltransferase showed an increase of 90 %. No significant change in the rate of incorporation of

Table 1. Summary of the purifications of arylsulphatase B, galactosyltransferase and alkaline ribonuclease achieved by the affinity-chromatographic procedures described in the text

Arylsulphatase B units are expressed as μmol of methylumbelliferone formed/min, galactosyltransferase units as nmol of *N*-acetyl-lactosamine produced/min and alkaline ribonuclease units as 1000 d.p.m. of HClO_4 -soluble ^3H -labelled oligosaccharides liberated/min.

Enzyme		Protein (μg)	Activity (units)	Specific activity (units/mg)	Purification	Yield (%)
Arylsulphatase B	Homogenate	23 400	0.573	0.025	1	100
	Affinity chromatography	29.6	0.419	14.1	576	73
Galactosyltransferase	Homogenate	28 200	0.026	0.00093	1	100
	Affinity chromatography	20.8	0.023	1.12	1205	89
Alkaline ribonuclease	Homogenate	24 700	3.56	0.144	1	100
	Affinity chromatography	18.0	2.63	146.0	1014	74

Table 2. Effect of sucrose uptake on the rates of incorporation of ^{14}C -labelled amino acids into arylsulphatase B, galactosyltransferase and alkaline ribonuclease

Hamster fibroblasts were pulse-labelled with $5 \mu\text{Ci}$ of ^{14}C -labelled amino acids for 18 h (9 h for galactosyltransferase) in the presence or absence of 0.08 M-sucrose. The cells were harvested, homogenized and the enzymes purified and counted for radioactivity as described in the text. Results (means $\pm$ s.d., for the number of experiments in parentheses) were subjected to Student's *t* test. N.S., not significant ($P > 0.05$).

Enzyme	Radioactivity (c.p.m./unit of enzyme)		Increase (%)	Significance level
	Control cells	Cells after uptake of sucrose		
Arylsulphatase B	2250 $\pm$ 450 (4)	8400 $\pm$ 1750 (6)	270	$P < 0.001$
Galactosyltransferase	3250 $\pm$ 450 (4)	6250 $\pm$ 1000 (3)	90	$P < 0.01$
Alkaline ribonuclease	1150 $\pm$ 150 (3)	1150 $\pm$ 160 (3)	0.4	N.S.

¹⁴C-labelled amino acids into alkaline ribonuclease was observed.

Effect of sucrose uptake on the rates of enzyme degradation

The effect of sucrose uptake on the rates of degradation of the three enzymes was investigated by measuring the decay of radioactivity of prelabelled enzymes with time. The results of these experiments are shown in Table 3 and Fig. 2. In the absence of sucrose, aryl-

sulphatase B had a half-life of 53 days over the initial 6 days of the chase. After this time, the half-life decreased to about 30 days. In the presence of sucrose, the half-life was about 10 days throughout the period of the experiment. The half-life of galactosyltransferase, in the absence of sucrose, was 58 h over the initial 24 h of the chase and decreased to 29 h during the next 24 h. In the presence of sucrose, galactosyltransferase was degraded more rapidly, with a half-life of 10–13 h. The half-life of alkaline ribonuclease, about 4 days, was unaffected by the

Table 3. *Effect of sucrose uptake on the rates of degradation of arylsulphatase B, galactosyltransferase and alkaline ribonuclease*

Hamster fibroblasts were grown to confluency in the presence of 5 μ Ci of ¹⁴C-labelled amino acids. The medium was then replaced with unlabelled medium and the cells were harvested at various time-intervals. To investigate the effects of sucrose, 0.08 M-sucrose was included in the culture medium throughout the period of the experiment. The different enzymes were purified as described in the text and counted for radioactivity. Results (means $\pm$ S.D. for the numbers of experiments in parentheses) were subjected to Student's *t* test. N.S., not significant (*P* > 0.05).

	Time (days)	Control cells		Cells after uptake of sucrose		Significance level
		Decay constant [10 ² <i>k</i> (days ⁻¹)]	Half-life (days)	Decay constant [10 ² <i>k</i> (days ⁻¹)]	Half-life (days)	
Arylsulphatase B	6	1.31 $\pm$ 0.29 (4)	52.9	6.20 $\pm$ 1.72 (4)	11.2	<i>P</i> < 0.002
	8	2.34 $\pm$ 0.57 (3)	29.6	7.31 $\pm$ 1.28 (3)	9.5	
	11	2.02 $\pm$ 0.95 (3)	34.3	8.35 $\pm$ 0.76 (3)	8.3	<i>P</i> < 0.001
	(h)	[10 ² <i>k</i> (h ⁻¹)]	(h)	[10 ² <i>k</i> (h ⁻¹)]	(h)	
Galactosyltransferase	7	—	—	5.25 $\pm$ 1.48 (3)	12.7	—
	24	1.19 $\pm$ 0.45 (3)	58	6.99 $\pm$ 1.86 (3)	9.9	<i>P</i> < 0.01
	36	1.43 $\pm$ 0.27 (3)	48	—	—	—
	48	2.39 $\pm$ 0.18 (3)	29	6.24 $\pm$ 0.18 (3)	11.1	<i>P</i> < 0.001
	(days)	[<i>k</i> (days ⁻¹)]	(days)	[<i>k</i> (days ⁻¹)]	(days)	
Alkaline ribonuclease	4	0.177 $\pm$ 0.023 (3)	3.9	0.183 $\pm$ 0.022 (3)	3.8	N.S.
	6	0.166 $\pm$ 0.014 (3)	4.2	0.186 $\pm$ 0.017 (3)	3.7	N.S.
	8	0.164 $\pm$ 0.010 (3)	4.2	0.193 $\pm$ 0.011 (3)	3.6	N.S.

Fig. 2. *Time-course of the decay in radioactivity of purified enzymes after labelling with ¹⁴C-labelled amino acids*

For further details, see the text and Table 3. Cells were grown in the absence (○) or presence (●) of 0.08 M-sucrose. (a) arylsulphatase B; (b) galactosyltransferase; (c) alkaline ribonuclease.

uptake of sucrose. In each case, semilogarithmic plots of the decay of enzyme radioactivity against time were approximately linear (Fig. 2), indicating that the degradative processes have first-order kinetics.

Discussion

The present investigation has sought to determine the effects of the endocytosis of a non-degradable substance (sucrose) on the turnover of lysosomal and Golgi-apparatus enzymes. To test the specificity of the response to sucrose storage, the turnover of total intracellular protein and a specific cytoplasmic enzyme, alkaline ribonuclease, have also been measured.

The methodology that we have used to study this problem deserves some comment. Chinese-hamster CH-23 fibroblasts have previously been subjected to analytical subcellular-fractionation experiments (Warburton & Wynn, 1976b). Hamster fibroblast lysosomes were shown to contain arylsulphatase B and to be the site of intracellular sucrose storage. The various enzymes in the turnover studies have been purified by affinity chromatography rather than immunoprecipitation, for the reasons discussed by Don & Masters (1975). The purifications obtained are comparable with those obtained by other workers (Agogbua & Wynn, 1976; Trayer & Hill, 1971). A major problem in protein-degradation studies is the re-utilization of labelled amino acids (Poole, 1971). This problem is minimized in cultured cells because of the rapid exchange of intracellular amino acids with those in the medium; indeed, in our system the intracellular amino acid radioactivity has fallen to 1% of the total radioactivity after 72h. Frequent changing of the medium would be expected to decrease this even further.

Total rat liver lysosomal protein appears to be degraded with a half-life of 1.5–3.5 days (Segal & Dunaway, 1975; Wang & Touster, 1975). The half-lives of individual acid hydrolases appear to be considerably longer. Rat kidney acid hydrolases turn over with half-lives of 3–19 days (Goldstone & Koenig, 1974). Wang & Touster (1975) estimated the half-life of rat liver β -glucuronidase to be about 30 days, whereas the mouse liver enzyme is degraded more rapidly, with a half-life of 4–6 days (Smith & Ganschow, 1975). Using a non-isotopic method, Weismann (1975) found the half-life of human fibroblast arylsulphatase A to be about 14 days.

Hamster fibroblast arylsulphatase B has an apparent half-life of 30–60 days. Although re-utilization of labelled amino acid may contribute somewhat to this value, the results of previous workers and the low endocytic activity of hamster CH-23 fibroblasts suggest that lysosomal enzymes in

this cell line would turn over very slowly. The half-life of galactosyltransferase is about 30–50h, in agreement with the value of 37h for total Golgi-membrane protein in rat liver (Franke *et al.*, 1970).

The hyperactivity of acid hydrolases after overloading of the lysosomes with non-degradable material has been shown, on the basis of experiments with inhibitors of protein synthesis, to be dependent on an increase in the rate of enzyme synthesis (Axline & Cohn, 1970). Van Hoof & Hers (1972) have suggested that the binding of acid hydrolases to the storage products of lysosomal storage diseases may afford some protection against denaturation and degradation.

The uptake of sucrose by hamster fibroblasts results in a 270% increase in the rate of synthesis of arylsulphatase B and a decrease in the half-life from about 30 days to 10 days. However, during growth in the presence of sucrose, the specific enzyme activity of hamster fibroblast arylsulphatase B increases by 50% (Warburton & Wynn, 1976a). It appears that the increase in the rate of degradation is insufficient to permit complete degradation of the excess of enzyme synthesized and the specific enzyme activity therefore increases. If, as suggested by Van Hoof & Hers (1972), the accumulated material in lysosomal storage diseases protects the acid hydrolases from degradation, then larger increases in lysosomal enzyme activities will occur in these diseases than during the storage of sucrose. Similar results were obtained with the Golgi-apparatus enzyme, galactosyltransferase. The uptake of sucrose resulted in a 90% increase in the rate of synthesis and a decrease in the half-life from 40h to 10h, suggesting an increase in the rate of membrane flow through the Golgi apparatus. Sucrose has little effect on the degradation of total intracellular protein or on the turnover of the cytoplasmic enzyme, alkaline ribonuclease. This reinforces the suggestion that the effects of sucrose uptake in hamster fibroblasts are restricted to the components of the vacuolar apparatus (Warburton & Wynn, 1976a).

Dingle (1968) has postulated that the increased size of the lysosomes after overloading with sucrose results in the instability of the lysosomal membrane, favouring fusion with primary lysosomes. The enlarged sucrose-filled lysosomes act as a 'sink' for primary lysosomes and the need to maintain a constant cellular concentration of primary lysosomes would necessitate an increased rate of synthesis. The hypertrophy of the Golgi apparatus under such conditions (Dingle *et al.*, 1969) would be a consequence of the increased production of primary lysosomes. Although our results are in agreement with this hypothesis, they offer little insight into the mechanisms involved. Similarly, the reason for the increased rate of degradation of arylsulphatase B after the uptake of sucrose is unclear. Little is known of the

internal structure of the lysosome, although disruption of any internal structure might result in a more rapid rate of denaturation and degradation of acid hydrolases by lysosomal proteinases.

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