Defective catabolism of low-density lipoprotein by fibroblasts from patients with I-cell disease

Julian C. WILLIAMS,* David B. WEINSTEIN, Arnold L. MILLER and Daniel STEINBERG
*Department of Medicine and Department of Neurosciences, University of California, San Diego,
La Jolla, CA 92093, U.S.A.*

(Received 24 August 1981/Accepted 27 August 1981)

Skin fibroblast cultures from patients with I-cell disease (mucopolipidosis II) are characterized by multiple lysosomal enzyme deficiencies. The present studies deal with the consequences of these deficiencies with respect to the metabolism of plasma low-density lipoproteins. Degradation of the protein moiety was defective in I-cells compared with control cells, but the binding and internalization of low density lipoprotein were much less affected. Measurements of low-density lipoprotein degradation in homogenates demonstrated directly for the first time a deficiency of acid proteinase activity in I-cell fibroblasts. Comparison of results in 6-h incubations with those in 24-h incubations showed accumulation of intracellular low-density lipoprotein in I-cell fibroblasts and an accelerating rate of degradation, possibly attributable to intracellular accumulation of low-density lipoprotein substrate. The significance of these findings with respect to low-density lipoprotein metabolism *in vivo* is discussed.

Skin fibroblasts from patients with I-cell disease (or mucopolipidosis II), an autosomally inherited fatal childhood disorder, are characterized by a range of deficiencies in the activities of multiple enzymes (Leroy *et al.*, 1972; Leroy & O'Brien, 1976; Wenger *et al.*, 1976; Poiter *et al.*, 1977). Culture medium from I-cell skin fibroblasts and extracellular fluids from I-cell-disease patients show elevated activities of several lysosomal hydrolase activities (Wiesmann *et al.*, 1971; Hickman & Neufeld, 1972; Kress & Miller, 1979).

Biochemical evidence suggests that I-cell disease is the result of an alteration in a post-translational process involving glycosylation, which is common to the final expression of the lysosomal enzymes (Kress *et al.*, 1980; Hasilik & Neufeld, 1980*a,b*; Tabas & Kornfeld, 1980; Miller *et al.*, 1981). Results further indicate an absence of the phosphomannosyl recognition marker on lysosomal enzymes in I-cell disease (Bach *et al.*, 1979; Hasilik & Neufeld, 1980*b*; Tabas & Kornfeld, 1980). Recent studies suggest that the absence of the phosphomannosyl marker is due to the deficiency of a UDP-*N*-acetylglucosamine lyso-

somal enzyme, *N*-acetylglucosamine 1-phosphate transferase (Hasilik *et al.*, 1981; Reitman *et al.*, 1981).

The work of Goldstein & Brown (1977) has delineated the pathway for binding, uptake and catabolism of LD lipoprotein by normal fibroblasts and the regulation of HMG-CoA reductase that follows on the uptake of LD lipoprotein. They have shown that in fibroblasts from patients with Wolman's disease and with cholesteryl ester storage disease the uptake and degradation of LD-lipoprotein protein occurs normally but the cholesteryl esters are hydrolysed poorly and thus cholesterol synthesis is poorly regulated. Studies in our laboratory (Pittman *et al.*, 1979) have demonstrated a partial reduction of acid cholesteryl ester hydrolase activity in skin fibroblasts and post-mortem liver specimens from several I-cell-disease patients. In the present studies we have examined the binding, internalization and degradation of ¹²⁵I-labelled LD lipoprotein by I-cell fibroblasts, their content of acid proteinase activity and also the regulation of HMG-CoA reductase by LD lipoprotein in these cells.

Materials and methods

Materials

Tissue culture media, foetal calf serum, Dulbecco's phosphate-buffered saline and trypsin were

Abbreviations used: LD lipoprotein, low-density lipoprotein; DME medium, Dulbecco's modified-Eagle's medium; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase.

* Present address: University of Texas Medical School, Houston, P.O. Box 20708, Houston, TX, U.S.A.

obtained from Grand Island Biological Laboratories (Grand Island, NY, U.S.A.); [$1\text{-}^{14}\text{C}$]acetate (sodium salt) and carrier-free ^{125}I were from Amer-sham-Searle Corp. (Arlington Heights, IL, U.S.A.); dithiothreitol and *o*-phthalaldehyde were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); 7-oxo-cholesterol was from Steroids (Wilton, NH, U.S.A.); and cholesterol oxidase and cholesteryl ester hydro-lase were obtained from Boehringer-Mannheim (Germany). Serum samples from patients with I-cell disease were shipped to us either fresh (on ice) or frozen.

Fibroblast cultures

Human skin fibroblasts from a preputial biopsy of a normal infant and fibroblasts from a patient with homozygous familial hypercholesterolaemia were grown in monolayer culture. Metabolism of lipo-proteins by these cell lines has been described previously (Stein *et al.*, 1976; Miller *et al.*, 1977). The cells were maintained in DME medium containing 10% (v/v) foetal calf serum (Stein *et al.*, 1976). Skin fibroblasts from two patients with I-cell disease (lines K.Z. and L.T.) were maintained in Ham's F-12 medium containing 15% (v/v) foetal calf serum. Analysis of these patients' fibroblasts confirmed that at least eight lysosomal hydrolase activities were deficient compared with the enzyme activities of the normal fibroblast control cultures. Cells were all transferred to DME medium 24 h before study and used at or near confluency.

Lipoprotein preparation

LD lipoprotein was isolated from fresh human serum by preparative ultracentrifugation and purity was demonstrated as described previously (Miller *et al.*, 1977). Lipoprotein-deficient foetal calf serum was prepared as described previously (Weinstein *et al.*, 1976). ^{125}I -labelled LD lipoprotein was prepared by a modification of the iodine mono-chloride method of McFarlane (1958) as described previously (Weinstein *et al.*, 1976). Less than 2% of the radioactivity in the final preparations was trichloroacetic acid-soluble or extractable into chloroform/methanol (2:1, v/v). All lipoprotein preparations were sterilized by passage through 0.22 μm Millipore filters (Millipore Corp., Bedford, MA, U.S.A.) and stored at 4°C. Protein content was determined by the method of Lowry *et al.* (1951) after extracting the coloured reaction product with one-half the volume of chloroform to eliminate lipid opalescence.

Lipoprotein catabolism

The growth medium was removed from the culture dishes 18–24 h before the beginning of each experiment and was replaced with 2 ml of fresh

DME medium containing 5% (v/v) lipoprotein-deficient serum. For lipoprotein-binding experi-ments at 4°C, the cells were placed on crushed ice for 10 min before the medium containing ^{125}I -labelled LD lipoprotein was added. The cells were kept on ice in a 4°C cold box for 60 min and then the medium was removed and a portion was assayed for total radioactivity. The cell monolayers were washed three times with 2 ml of Dulbecco's phosphate-buffered saline at 4°C, followed by two washes with cold Dulbecco's phosphate-buffered saline contain-ing 2 mg of bovine serum albumin/ml and finally with the buffered saline alone. The cell monolayers were removed from the dishes by treatment with 1 ml of 0.2 M-KOH for 1 h at room temperature. A portion of the cell digest was removed for protein analysis (Lowry *et al.*, 1951) and for measurement of total radioactivity, which is a measure of the cell-surface-bound lipoprotein.

Binding, internalization and degradation of ^{125}I -labelled LD lipoprotein was measured after pre-incubation of the cells in DME medium containing 5% (w/v) lipoprotein-deficient serum for 24 h and in some experiments for 48 h. After incubation with ^{125}I -labelled LD lipoprotein at 37°C the medium was removed and lipoprotein degradation was measured as trichloroacetic acid-soluble ^{125}I radio-activity in the medium after removal of [^{125}I]iodide as described previously (Weinstein *et al.*, 1976). Pre-incubation of I-cell fibroblasts in lipoprotein-deficient medium for 36–48 h increased the degrada-tion rate of LD lipoprotein by only 10–15% compared with the 24 h period. All the data in this paper are based on the 24 h induction period. Net degradation was calculated as the difference between values obtained from identical incubations in the presence and absence of cells.

Since lysosomal enzymes may leak into the culture medium and contribute to the apparent rate of degradation of ^{125}I -labelled LD lipoprotein, control incubations were performed using 'condi-tioned medium', i.e. medium that had been exposed to I-cell fibroblasts for 24 h. Proteolysis of ^{125}I -labelled LD lipoprotein by 'conditioned' culture medium was negligible at all LD-lipoprotein con-centrations tested.

After removal of the medium, the cells were washed as described above for binding studies at 4°C and were harvested by trypsin treatment as described previously (Stein *et al.*, 1976). The radioactivity released by trypsin treatment was considered to represent lipoprotein bound to the cell surface. The cell pellet was dissolved in 1 ml of 0.2 M-KOH and a portion was removed for measure-ment of lipid-soluble radioactivity [extractable into chloroform/methanol (2:1, v/v)]. The non-lipid trichloroacetic acid-precipitable radioactivity re-maining associated with the cells after trypsin

treatment was considered to represent internalized lipoprotein.

Lipoprotein concentrations are expressed in terms of protein content unless otherwise stated.

Cell cholesterol synthesis

The effects of LD lipoprotein on sterol and fatty acid synthesis were measured by using the [$1\text{-}^{14}\text{C}$]acetate incorporation described previously (Weinstein *et al.*, 1976). The activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, was measured as described by Goldstein & Brown (1974).

Cholesterol content

Serum samples were extracted with 20 vol. of chloroform/methanol (1:1, v/v) at 4°C for 18 h. The extract was washed with water to remove non-lipid contaminants and portions were removed for total cholesterol analysis by the *o*-phthalaldehyde method of Rudel & Morris (1973). The cholesterol and cholesteryl ester content of the cells was measured by the enzymic method of Gamble *et al.* (1978).

Proteolytic degradation of ^{125}I -labelled LD lipoprotein by cell-free extracts

Cells from 10 dishes (60 mm $\times$ 15 mm) were washed free of growth medium with Dulbecco's phosphate-buffered saline and were scraped off the dish with a rubber policeman into the same buffer at 4°C. Cells were collected by centrifugation (2500 g for 5 min at 4°C) and the cell pellet was suspended in 1 ml of water and sonicated for 15 s at maximum power using a micro-ultrasonic cell disruptor (Kontes, Vineland, NJ, U.S.A.). Each assay contained 0.1 M-acetate or 0.1 M-Tris/HCl buffer at the indicated pH, 10 μg of ^{125}I -labelled LD lipoprotein (190 c.p.m./ng)/ml, 5 mM-dithiothreitol, 0.1 mM-EDTA and 80 μg of cell-extract protein. The incubation and assay were performed as described by Goldstein *et al.* (1975a).

Results

Degradation of ^{125}I -labelled LD lipoprotein was first studied over a 6-h period. As shown in Fig. 1, the degradation at all LD-lipoprotein concentrations was abnormal in the two lines studied. In one cell line (L.T.) the degradation was almost as low as it was in a receptor-negative line of cells from a patient with homozygous familial hypercholesterolaemia. In the other cell line (K.Z.), degradation was reduced to less than 50% of that in the control fibroblasts. This clear deficiency in rate of LD-lipoprotein degradation is not attributable to deficient binding, as shown by the results in Fig. 2. Cell line L.T., in which degradation was almost com-

Fig. 1. Comparison of the degradation of ^{125}I -labelled LD lipoprotein by normal fibroblasts (●), fibroblasts from a patient with homozygous familial hypercholesterolaemia (▲) and fibroblasts from two patients with I-cell disease (lines L.T. (○) and K.Z. (Δ)) as a function of lipoprotein concentration

Cell monolayers were incubated with lipoprotein-deficient medium for 24 h. This was replaced with fresh medium containing 5% (v/v) lipoprotein-deficient serum and ^{125}I -labelled LD lipoprotein (specific radioactivity 190 c.p.m./ng). After a 6 h incubation at 37°C the culture medium was harvested and the degradation was determined as described in the Materials and methods section. Each point represents the mean of triplicate determinations.

Fig. 2. Surface-binding of ^{125}I -labelled LD lipoprotein by normal fibroblasts (●), fibroblasts from a patient with homozygous familial hypercholesterolaemia (▲) and fibroblasts from two I-cell-disease patients L.T. (○) and K.Z. (Δ) as a function of lipoprotein concentration

Results were obtained from the same experiments as that illustrated in Fig. 1. After a 6 h incubation at 37°C with ^{125}I -labelled LD lipoprotein, the cells were removed from the dishes by trypsin treatment and LD lipoprotein binding was determined as trypsin-releasable ^{125}I radioactivity.

pletely absent, showed binding that was indistinguishable from the binding to normal fibroblasts at all LD lipoprotein concentrations. Cell line K.Z. showed binding that was perfectly normal at low LD

lipoprotein concentrations but slightly less at higher concentrations of LD lipoprotein both at 37°C (Fig. 2) and at 4°C (results not shown). Still the differences were much smaller than the differences in observed degradation rates (Fig. 1). As shown in Fig. 3, the amount of ^{125}I -labelled LD lipoprotein found intracellularly in I-cell cultured fibroblasts at the end of the incubation ('internalized') was greater than that in normal control cells, indicating the accumulation of undegraded LD lipoprotein. When the amount of LD lipoprotein internalized and the amount degraded were summed, providing an index of total uptake over the 6 h, the differences between I-cell fibroblasts and control fibroblasts (Fig. 4) were much smaller than the differences in degradation (Fig. 1). Thus degradation by L.T. cells was less than 10% of control values, and total uptake was 75% of control values.

As shown in Fig. 5, neither I-cell line showed any significant inhibition of HMG-CoA reductase activity in 6 h even at concentrations of LD lipoprotein cholesterol up to 40 $\mu\text{g}/\text{ml}$, in contrast with the greater than 80% inhibition observed in normal fibroblasts.

The effects of LD lipoprotein (20 $\mu\text{g}/\text{ml}$) were also tested with regard to rates of acetate incorporation into cell sterols, as shown in Table 1. In normal cells, as expected, there was a significant inhibition, approx. 40% in the presence of 20 μg of LD lipoprotein/ml. On the other hand, there was little or no effect in the two I-cell lines. These cells lines did, however, show a marked inhibition of acetate incorporation into sterols in the presence of 7-oxocholesterol, indicating that the systems regu-

Fig. 3. Internalization of ^{125}I -labelled LD lipoprotein by normal fibroblasts (●), fibroblasts from a patient with homozygous familial hypercholesterolaemia (▲) and fibroblasts from two I-cell patients L.T. (○) and K.Z. (△) as a function of lipoprotein concentration

Results were obtained from the same experiment as that illustrated in Fig. 1. Lipoprotein internalization was determined as cell-associated radioactivity that was trichloroacetic acid-precipitable and not extracted by chloroform/methanol.

Fig. 4. Effects of increasing LD lipoprotein concentration on the total uptake of LD lipoprotein (internalization and degradation) by normal fibroblasts (●), fibroblasts from a patient with homozygous familial hypercholesterolaemia (▲) and fibroblasts from two I-cell patients [L.T. (○) and K.Z. (△)]

The data are derived from Figs. 1 and 3.

Fig. 5. Effects of LD lipoprotein on HMG-CoA reductase activity in cultured skin fibroblasts

Fibroblasts from normal (●), homozygous-familial-hypercholesterolaemic (▲) and I-cell-disease [L.T. (○) and K.Z. (△)] subjects were incubated with DME medium containing 5% (v/v) lipoprotein-deficient serum for 24 h. Increasing concentrations of LD-lipoprotein cholesterol were added in fresh medium and after a 6-h incubation cell-free extracts were prepared and HMG-CoA reductase activity was measured as described in the Materials and methods section. Results are means of triplicate determinations.

lating endogenous cholesterol biosynthesis are responsive and that the deficiency relates specifically to the metabolism of LD lipoprotein.

As shown in Fig. 3, the pool of internalized undegraded LD lipoprotein in the I-cell fibroblasts at 6 h was increased, particularly in the L.T. cells. A

Table 1. *Effects of LD lipoprotein and 7-oxocholesterol on the incorporation of [$1\text{-}^{14}\text{C}$]acetate into sterols and fatty acids*

Cells were incubated in medium containing 5% (v/v) lipoprotein-deficient serum for 24 h. The medium was replaced with 2 ml of the same medium containing either no additions, 20 μg of LD-lipoprotein cholesterol/ml or 5 μg of 7-oxocholesterol/ml. After a 6 h incubation at 37°C, 2 μCi of [$1\text{-}^{14}\text{C}$]acetate (sp. radioactivity 58 Ci/mol)/ml and unlabelled carrier acetate (final concentration, 0.1 μmol /ml) were added and the incubation was continued for 60 min. The cells were harvested for measurement of sterol and fatty acid synthesis as described in the Materials and methods section. Results are means of triplicate determinations.

Cell line	Incorporation (c.p.m./mg of cell protein)					
	No additions		LD lipoprotein (20 μg /ml)		7-Oxocholesterol (5 μg /ml)	
	Sterols	Fatty acids	Sterols	Fatty acids	Sterols	Fatty acids
Normal	20 100	99 700	12 100	73 300	5060	124 000
I-cell (K.Z.)	9140	44 200	8100	39 600	2600	73 800
I-cell (L.T.)	19 100	103 000	18 500	79 200	3630	151 000

Fig. 6. *Comparison of the degradation of ^{125}I -labelled LD lipoprotein in 24 h by normal fibroblasts (●), fibroblasts from a patient with homozygous familial hypercholesterolaemia (▲) and fibroblasts from two I-cell-disease patients (L.T. (○) and K.Z. (△))*

The experimental conditions were identical with those described in Fig. 1, except that degradation was measured after a 24 h incubation with ^{125}I -labelled LD lipoprotein (sp. radioactivity 180 c.p.m./ng). Results are means of duplicate determinations.

progressive increase in the concentration of LD lipoprotein available inside the cell might enhance the rate of its degradation and so in longer incubations the rate in I-cells might more nearly approach control values. For this reason we re-studied the process of LD lipoprotein uptake and degradation in 24-h incubations. As shown in Fig. 6, the LD lipoprotein degradation by the I-cell lines in 24 h was still less than that in the control fibroblasts but the difference was less striking than in the 6-h experiments (Fig. 1).

This might reflect a progressive increase in substrate concentration in the lysosome with con-

sequent increases in the net rate of LD lipoprotein degradation, approaching the rate in control cells. Indeed, the total amount of ^{125}I accumulating in the I-cells over a 24 h interval was 8 to 12 times that accumulating in the normal cells. This contrasts with the intracellular accumulation at 6 h, which was 1.6 to 3 times that in control cells.

Fig. 7 shows the time course for ^{125}I -labelled LD lipoprotein degradation in normal cells, the I-cell line K.Z. and the familial-hypercholesterolaemia line. There was a longer initial lag period in the case of the K.Z. line compared with the control cells. Between 12 h and 24 h the degradation rate in the K.Z. line was only about 30% lower than that in the control cells. The results are compatible with the conclusion that at steady state the difference in LD lipoprotein degradation between the I-cells and normal fibroblasts may be small or may not even be observed, probably due to the progressive accumulation of LD lipoprotein in the lysosomes of I-cell fibroblasts thus increasing the available concentration of protein substrate in these lysosomes.

The effects of a 24 h incubation with LD lipoprotein on HMG-CoA reductase activity were examined. As shown in Fig. 8, reductase activity in the control cells was inhibited by more than 80% and there was little if any effect in the familial-hypercholesterolaemia cells. The I-cell line L.T. now showed some inhibition, about 37%, but the inhibition was much less than that seen in the normal cells.

Cell cholesterol content was determined after a 24-h incubation in the presence of lipoprotein-deficient serum with or without 100 μg of LD lipoprotein/ml. As shown in Table 2, the two I-cell lines contained more cholesterol, both unesterified and in the ester form, than control cells whether incubated overnight in lipoprotein-deficient serum or with LD lipoprotein. The ester cholesterol content of I-cells

Fig. 7. Time course of degradation of ¹²⁵I-labelled LD lipoprotein by normal fibroblasts (●), fibroblasts from a patient with homozygous familial hypercholesterolaemia (▲) and fibroblasts from a patient with I-cell disease (K.Z.; △)

After a 24 h pre-incubation in lipoprotein-deficient medium the cells were incubated with ¹²⁵I-labelled LD lipoprotein (10 μg/ml; 180 c.p.m./ng) for the indicated time periods. Results are means of duplicate determinations.

incubated in lipoprotein-deficient serum was three times that of the control cells. After incubation with LD lipoprotein all cell lines showed an increase in cholesterol content but the increment was smaller in the I-cell lines.

Homogenates of fibroblasts were prepared by

Fig. 8. The effects of 24 h incubations with LD lipoprotein on HMG-CoA reductase activity in normal fibroblasts (●), I-cell fibroblasts (L.T.) (○) and fibroblasts from a patient with homozygous familial hypercholesterolaemia (▲)

The experimental conditions were identical with those described in the legend to Fig. 5, except that incubation with LD lipoprotein was for 24 h. Results are means of duplicate determinations.

Table 2. Increase in cell cholesterol content after 24-h incubation with lipoprotein-deficient serum or with LD lipoprotein (100 μg/ml)

Cell monolayers were incubated in lipoprotein-free medium as described in the legend to Table 1. Fresh medium containing 5% (v/v) lipoprotein-deficient serum in the presence or absence of 100 μg of LD-lipoprotein protein/ml (164 μg of LD-lipoprotein cholesterol)/ml was added. After 24 h the cells were washed four times with Dulbecco's phosphate-buffered saline and harvested by scraping with a rubber policeman into the buffered saline at 4°C. The cell pellet was extracted with 10 ml of chloroform/methanol (1:1, v/v) and the cholesterol and cholesteryl ester mass was measured by the enzymic methods of Gamble *et al.* (1978).

Cell line	Addition to lipoprotein-deficient medium	Cell cholesterol content (μg/mg)		
		Unesterified	Ester	Total
Control	None	58	7	65
	LD lipoprotein	78	23	101
I-cell line K.Z.	None	77	21	98
	LD lipoprotein	85	32	117
I-cell line L.T.	None	82	26	104
	LD lipoprotein	88	42	130

Table 3. *Degradation of ^{125}I -labelled LD lipoprotein by cell-free homogenates*

Cell homogenates were prepared by sonication as described in the Materials and methods section. Portions of homogenates containing 80 μg of cell protein were incubated in 0.1 M-acetate or Tris buffer at the indicated pH in the presence of 5 mM-dithiothreitol, 0.1 mM-EDTA and 10 μg of ^{125}I -labelled LD lipoprotein (190 c.p.m./ng)/ml. After a 1-h incubation at 37°C the reaction was stopped with trichloroacetic acid and the products were analysed as described by Goldstein *et al.* (1975a).

Cell line	Degradation of ^{125}I -labelled LD lipoprotein (ng/h)			
	pH 3.0	pH 4.0	pH 5.0	pH 6.0
Normal	78.2	681.4	170.6	14.3
Homozygous familial hypercholesterolaemia	86.1	701.3	180.3	21.7
I-cell (L.T.)	16.0	133.8	34.2	3.1
I-cell (K.Z.)	22.7	189.1	46.6	3.6

sonication and assayed for their ability to degrade ^{125}I -labelled LD lipoprotein as a function of pH. As shown in Table 3, the rates of degradation in normal cells and cells from a patient with homozygous familial hypercholesterolaemia were approximately the same, and both showed optimum activity at pH 4. Degradation by homogenates from the I-cell lines was also optimal at pH 4 but was only 20–30% of the control values. Thus the deficiency in degradation of LD lipoprotein may be attributable to a deficiency of lysosomal cathepsin activity.

Discussion

The deficiency of multiple lysosomal glycohydrolases, acid lipase and aryl sulphatase in fibroblasts from patients with I-cell disease is well recognized (Leroy *et al.*, 1972; Leroy & O'Brien, 1976; Wenger *et al.*, 1976; Pittman *et al.*, 1979). The present studies are the first to directly demonstrate an acid proteinase deficiency in I-cell fibroblasts. These latter results are consonant with the findings of Hasilik & Neufeld (1980b) that the mannose moiety of cathepsin D is not phosphorylated in I-cells and with the evidence for a reduced degradation by intact I-cells of albumin-mannose 6-phosphate conjugates (Karson *et al.*, 1980). In one of the I-cell lines studied, degradation at 6 h was less than 10% of that in control cells and in the other line about one-third that in control cells. In contrast, binding of LD lipoprotein to the I-cells was essentially normal and the overall uptake into them was within 25% of normal values. Thus the demonstrated defect in degradation is attributable primarily to a defect in proteolytic degradation.

This conclusion is supported by the greater accumulation of intracellular LD lipoprotein in I-cells than in control cells. This was evident at 6 h but more striking at 24 h, when the increase was 8–12 times that seen in control cells. The lesser defect in degradation rate seen at 24 h probably

reflects this accumulation and a consequent increase in the availability of LD lipoprotein substrate in the lysosomes.

The consequences of the demonstrated defect in cell culture with regard to LD lipoprotein metabolism *in vivo* are difficult to predict. *In vivo* it is possible that the intracellular pools might go on increasing until the absolute degradation rate matched the rates of LD lipoprotein internalization. In that case, one might not see abnormalities in the rate of degradation of plasma LD lipoprotein at steady state. On the other hand, if there were defective degradation, it might be reflected in an increased steady-state concentration of plasma LD lipoprotein. Data on LD lipoprotein concentrations in I-cell disease are not available. However, we have been able to obtain serum samples for total cholesterol determination in seven patients. Total serum cholesterol exceeded the 95th percentile for age-matched controls in four of these cases (Lipid Research Clinics Population Studies Data Book, 1980). In two of them, the value was more than 50% above the 95th percentile value and in two others just above the 95th percentile value. The significance of this limited and uncontrolled sampling is difficult to assess. It may indicate a primary defect in LD lipoprotein removal *in vivo* or it may reflect an indirect end result of other metabolic consequences of the disease.

In normal fibroblasts, incubation with LD lipoprotein decreases HMG-CoA reductase activity and this is believed to be dependent on prior hydrolysis of the cholesteryl ester moiety (Goldstein & Brown, 1977). The failure of LD lipoprotein to affect reductase activity in I-cells could reflect the proteinase deficiency, the previously demonstrated deficiency in acid cholesteryl esterase (Pittman *et al.*, 1979) or both. The acid cholesteryl esterase activity in the L.T. line studied here was about 15% of normal values (Pittman *et al.*, 1979), which is less of a deficit than that found in the cells of patients with

cholesteryl ester storage disease (Goldstein *et al.*, 1975b). Yet reductase activity in the L.T. line showed only slight inhibition even after 24 h of incubation with 100 μ g of LD lipoprotein/ml, whereas Goldstein *et al.* (1975a,b) found that under the same conditions reductase activity in cholesteryl ester storage disease cells was almost completely inhibited. This difference suggests that the proteinase deficiency in I-cells plays a role in their lack of response to LD lipoprotein.

We are grateful to Judith Gawarecki, Lorna Joy, Erika Sandford and Joellen Barnett for their excellent technical assistance and to Dr. Ray C. Pittman and Dr. Alan Attie for valuable discussions. We thank Judith Cherrington for her skilful preparation of this manuscript. This work was supported by National Institutes of Health Research Grants HL-14197 and NS 12138. A. L. M. is the recipient of Research Career Development Award NS-00050 from the National Institutes of Health and Easter Seal Research Foundation Grant N-7910. J. C. W. was supported by an American Diabetes Association Research and Development Award.

References

- Bach, G., Bargal, L. & Cantz, M. (1979) *Biochem. Biophys. Res. Commun.* **91**, 976–981
- Gamble, W., Vaughan, M., Kruth, H. S. & Avigan, J. (1978) *J. Lipid Res.* **19**, 1068–1070
- Goldstein, J. L. & Brown, M. S. (1974) *J. Biol. Chem.* **249**, 5153–5162
- Goldstein, J. L. & Brown, M. S. (1977) *Annu. Rev. Biochem.* **46**, 897–930
- Goldstein, J. L., Brunschede, G. Y. & Brown, M. S. (1975a) *J. Biol. Chem.* **250**, 7854–7862
- Goldstein, J. L., Dana, S. E., Faust, J. R., Beaudet, A. L. & Brown, M. S. (1975b) *J. Biol. Chem.* **250**, 8487–8495
- Hasilik, A. & Neufeld, E. F. (1980a) *J. Biol. Chem.* **255**, 4937–4945
- Hasilik, A. & Neufeld, E. F. (1980b) *J. Biol. Chem.* **255**, 4946–4950
- Hasilik, A., Waheed, A. & von Figura, K. (1981) *Biochem. Biophys. Res. Commun.* **98**, 761–767
- Hickman, S. & Neufeld, E. F. (1972) *Biochem. Biophys. Res. Commun.* **49**, 992–999
- Karson, E. M., Neufeld, E. F. & Sando, G. N. (1980) *Biochemistry* **19**, 3856–3860
- Kress, B. C. & Miller, A. L. (1979) *Biochem. J.* **177**, 409–415
- Kress, B. C., Freeze, H. H., Herd, J. K., Alhadeff, J. A. & Miller, A. L. (1980) *J. Biol. Chem.* **255**, 955–961
- Leroy, J. G. & O'Brien, J. S. (1976) *Clin. Genet.* **9**, 533–539
- Leroy, J. G., Ho, M. W., MacBrinn, M. C., Zielke, K., Jacob, J. & O'Brien, J. S. (1972) *Pediatr. Res.* **6**, 752–757
- The Lipid Research Clinics Population Studies Data Book (1980) *The Prevalence Study*, vol. 1, pp. 28–34, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- McFarlane, A. S. (1958) *Nature (London)* **182**, 53
- Miller, N. E., Weinstein, D. B., Carew, T. E., Koschinsky, T. & Steinberg, D. (1977) *J. Clin. Invest.* **60**, 78–88
- Miller, A. L., Freeze, H. H. & Kress, B. C. (1981) in *Lysosomes and Lysosomal Storage Disorders* (Lowden, J. A. & Callahan, J., eds.), p. 271, Raven Press, New York
- Poiter, M., Mameli, L., Dallaire, L. & Melancon, S. B. (1977) *Pediatr. Res.* **11**, 462
- Pittman, R. C., Williams, J. C., Miller, A. L. & Steinberg, D. (1979) *Biochim. Biophys. Acta* **575**, 399–409
- Reitman, M. L., Varki, A. & Kornfeld, S. (1981) *J. Clin. Invest.* **67**, 1574–1579
- Rudel, L. L. & Morris, M. D. (1973) *J. Lipid Res.* **14**, 364–366
- Stein, O., Weinstein, D. B., Stein, Y. & Steinberg, D. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 14–18
- Tabas, I. & Kornfeld, S. (1980) *J. Biol. Chem.* **255**, 6633–6639
- Weinstein, D. B., Carew, T. E. & Steinberg, D. (1976) *Biochim. Biophys. Acta* **424**, 404–421
- Wenger, D. A., Sattler, M., Clark, C. & Wharton, C. (1976) *Life Sci.* **19**, 413–420
- Wiesmann, U. N., Vassella, F. & Herschkowitz, N. (1971) *N. Eng. J. Med.* **285**, 1090–1091