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Erythrocyte Spectrin Alteration Induced by Low-Density Lipoprotein

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Addition of human plasma low-density lipoproteins (LDL) to intact human erythrocytes induces the erythrocytes to undergo morphologic transition from biconcave disks to echinocytes and spherocytes. The transformation is time-dependent. Two hours are required before echinocytes are detected by scanning electron microscopy. After two hours, LDL also decrease the phosphate content of spectrin by 40% relative to the control, suggesting that these lipoproteins modulate cell shape by influencing phosphorylation-dephosphorylation of a membrane-associated cytoskeletal protein. LDL do not induce depletion of intracellular adenosine triphosphate (ATP), nor do they inhibit cyclic adenosine monophosphate-independent protein kinases which phosphorylate spectrin. LDL stimulate membrane-bound phosphatases by a factor of two, thereby reducing the amount of phosphate covalently bound to membrane proteins. The observed effects are specific for LDL. High-density lipoproteins (HDL) do not stimulate dephosphorylation of spectrin or alter erythrocyte morphology. However, HDL protect the erythrocytes against LDL-induced alterations. These data suggest that the circulating lipoproteins have a role in maintaining erythrocyte morphology by regulating the extent of phosphorylation of spectrin.

Key words: **plasma lipoproteins, erythrocyte morphology, erythrocyte phosphatase, spectrin phosphorylation**

Recent reports [1–3] suggest that the extent of phosphorylation of spectrin determines erythrocyte morphology. According to this concept, spectrin phosphorylation regulates the interaction of actin with spectrin [3, 4], which in turn specifies the structural state of the spectrin-actin complex on the cytoplasmic surface of the membrane [3]. Since patients with certain types of lipoprotein disorders have erythrocytes of altered morphology [5], it is of interest to determine the effects of individual lipoprotein classes on cell morphology and spectrin phosphorylation. In the present communication, we report the influence of human plasma low-density lipoproteins (LDL) and high-density lipoproteins

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(HDL) on erythrocyte morphology and physiology. Results of these studies suggest that LDL but not HDL alter erythrocyte morphology by stimulating dephosphorylation of spectrin.

MATERIALS AND METHODS

Erythrocytes were isolated from normal human plasma by centrifugation. After the plasma and buffy coat were removed by aspiration, the erythrocytes were further fractionated by the method of Poon and Simon [6]. Isolated cells were washed three times in a buffer containing 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 20 mM KCl. Human lipoproteins were isolated from the freshly collected plasma of normolipemic fasted donors by sequential ultracentrifugal flotation in KBr [7]. LDL were isolated between densities 1.019 and 1.063 and HDL, between 1.063 and 1.21; densities were measured with a hydrometer. Lipoproteins were dialyzed extensively against 150 mM NaCl (pH 7.4) and used immediately. The isolated LDL did not react at any concentration with antisera prepared against HDL or against the major HDL proteins apoAI and apoAII. However, when high concentrations of HDL were analyzed, a very faint precipitin line was detected between the HDL and antiLDL. The lipid compositions of both LDL and HDL were within the normal range of values [8].

Lipoproteins or albumin were added to erythrocytes which were resuspended to 10–20% hematocrit in a buffer containing 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 10 mM glucose, 1 μM adenine, and 1 mM $CaCl_2$. LDL were added at a final concentration of 2.5 mg/ml; HDL, at 0.25 mg/ml or 4.5 mg/ml. The concentration of lipoprotein in each case was calculated from the protein concentration assuming that LDL and HDL are 21 wt % and 50 wt % protein, respectively [8]. Bovine serum albumin or defatted human serum albumin was included at a final concentration of 50 mg/ml in the incubation mixture as indicated. In all experiments, the incubation time was 4 h at 37°C.

Protein concentration was determined by a modified method of Lowry et al [9] using 1% sodium dodecylsulfate (SDS); bovine serum albumin was the standard. Cholesterol (free and esterified) was determined by the method of Roeschlau et al [10]. Triglyceride was assayed enzymatically [11]. Phospholipids were separated by thin-layer chromatography on Silica Gel 1B2 (J.T. Baker Chemical Co.) in a solvent system of chloroform:acetone: methanol:acetic acid:water (10:4:2:2:1, v/v). The method of Bartlett [12] was used to quantitate phospholipids; an average molecular weight of 750 was employed to determine phospholipid concentration. Lipids were extracted from erythrocyte ghosts by chloroform-methanol as described by Waku and Lands [13]. The amount of intracellular adenosine triphosphate (ATP) was determined using a phosphoglycerate kinase assay procedure [14]. Phosphatase activity was measured spectrophotometrically employing the exogenous substrate p-nitrophenyl phosphate [15, 16]. Protein kinase activity was determined by measuring the phosphorylation of casein as described by Hosey and Tao [17].

RESULTS

When LDL are added to erythrocytes, cell morphology is significantly altered. As shown in Figure 1, LDL induce a transformation of erythrocytes from normal biconcave disks (Fig. 1A) to echinocytes (Fig. 1B) and cells of poorly defined morphology (Fig. 1C); 2 h at 37° are required to detect the alterations (Table I). A fraction of the poorly defined spherical cells shown in Figure 1C appear to be fused, although microscopic evidence alone

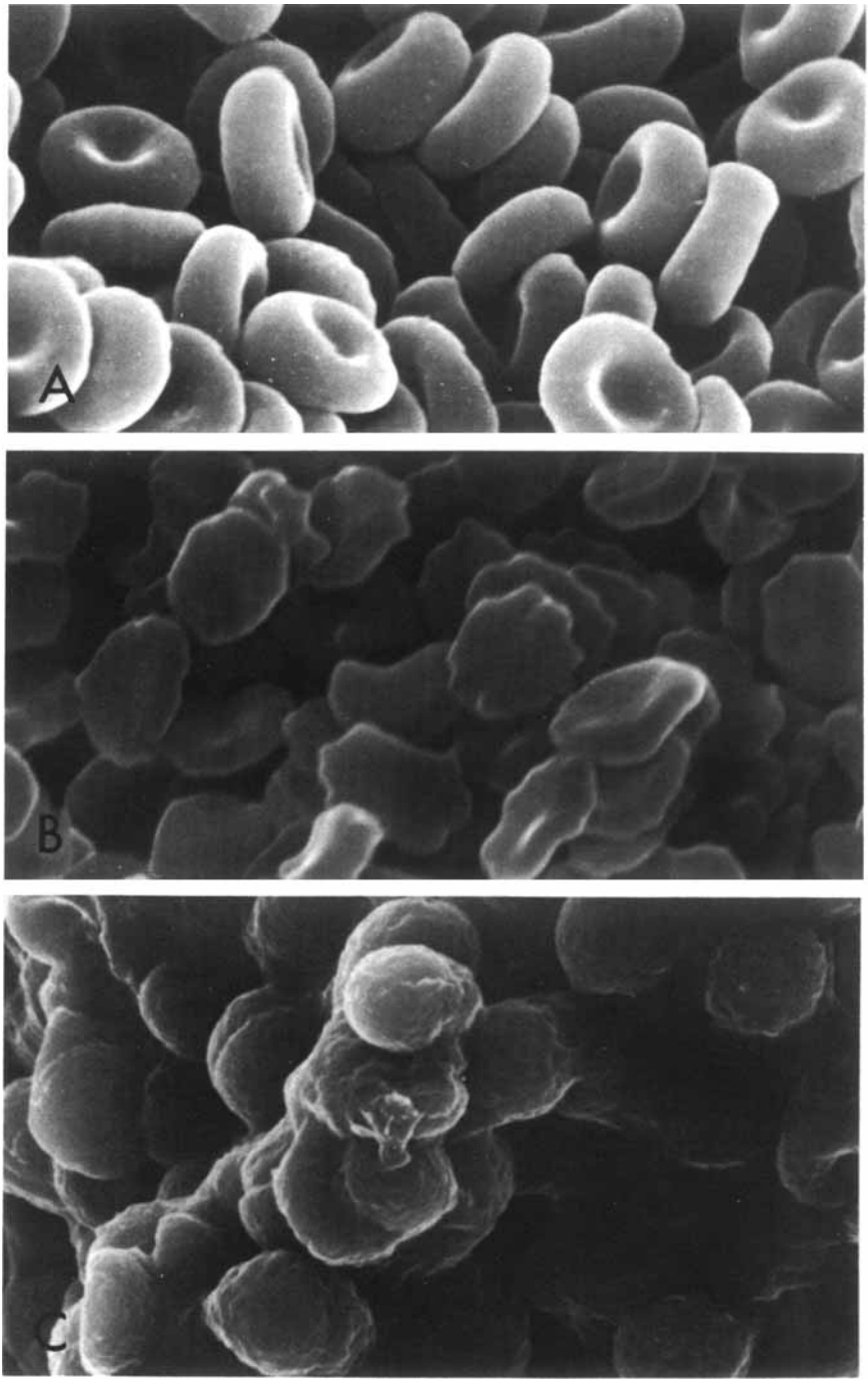

Fig. 1. Effect of LDL on erythrocyte morphology. Erythrocytes were incubated for 4 h at 37°C without LDL (A), and for 3 h (B) and 4 h (C) with LDL (2.5 mg/ml) as described in Materials and Methods. The cells were subsequently fixed at 4° for 1 h by the addition of an equal volume of 1% glutaraldehyde in 150 mM NaCl, 20 mM Tris-HCl (pH 7.4). After extensive washing with distilled water, the packed erythrocytes were dehydrated with acetone and were vacuum-evaporated (1×10^{-5} torr). The specimens were then coated with light carbon and gold-palladium (60:40), and the cells were examined with an ETEC-Autoscann electron microscope.

TABLE I. Erythrocyte Morphology

Experiment	Time (h)	Discocyte	Echinocyte	Spherocyte
Erythrocytes, control	1	++++	–	–
	4	++++	Trace	–
Erythrocytes plus LDL	1	++++	–	–
	2	+++	+	–
	3	+	+++	–
	4	+	++	+
Erythrocytes plus HDL	1	++++	–	–
	4	++++	–	–
Erythrocytes plus LDL and HDL	1	++++	–	–
	4	++++	Trace	–
Erythrocytes plus LDL followed by HDL[a]	4	+	+++	–
Erythrocytes plus LDL followed by heparin[a]	4	$++^{+}/2$	$+^{+}/2$	–
Erythrocytes plus albumin	1	++++	–	–
	4	++++	–	–
Erythrocytes plus albumin and LDL	1	++++	–	–
	4	+	++	+

Erythrocytes were incubated at 37°C and prepared for microscopy as described in Methods and in legend to Fig. 1. Morphology was assessed by scanning electron microscopy and by light microscopy. Morphologic type was scored as follows: ++++) 100%; +++) 75%; ++) 50%; +) 25%; –) 0%.
[a]Erythrocytes were incubated for 3 h with LDL prior to addition of HDL or heparin; incubation was continued for 1 h with HDL or heparin. Concentrations: LDL, 2.5 mg/ml; HDL, 0.25 mg/ml; albumin, 50 mg/ml; heparin, 10 units/ml.

is not conclusive. Once the erythrocytes are transformed to echinocytes by exposure to LDL for 3 h, removal of the LDL with heparin [20] allows the reverse echinocyte-discocyte transformation to occur. The effects of LDL are specific for this class of lipoproteins. HDL do not alter erythrocyte morphology (Table I). However, when added simultaneously with LDL, HDL inhibit the LDL-induced alterations. Inhibition by HDL occurs at a ratio LDL:HDL of 10:1 (wt/wt), a ratio about 10 times normal [8]. HDL are also "protective" when LDL and HDL are added in equal proportions (D.Y. Hui and J.A.K. Harmony, data submitted for publication). If HDL are added to cells which have been exposed to LDL for 3 h (echinocytes), they do not reverse the LDL-induced morphologic alteration; HDL do, however, prevent further alteration which results in formation of spherocytes. Albumin, which is known to reverse lysin-induced sphering [21], does not inhibit the morphologic transformation elicited by LDL (Table I).

To investigate the possible biochemical mechanisms which might account for the morphologic alterations elicited by LDL, we have quantitatively determined intracellular and membrane constituents, the perturbation of which has been associated with changes of erythrocyte morphology. As indicated in Table II, there are no significant differences in the cellular concentration of glucose, inorganic phosphate, or ATP determined for control cells and for cells incubated with LDL, HDL, or LDL plus HDL. Furthermore, no change in the ratios of the major erythrocyte membrane lipids is detected (Table II):

TABLE II. Effect of Lipoproteins on Intracellular and Membrane Constituents of Intact Erythrocytes*

Cell constituent	Erythrocyte control	Erythrocytes plus LDL (2.5 mg/ml)	Erythrocytes plus HDL (0.25 mg/ml)	Erythrocytes plus LDL (2.5 mg/ml) and HDL (0.25 mg/ml)
[^{14}C]-glucose (cpm/mg Hb)	880	880	880	ND
[^{32}P]-phosphate (cpm/mg Hb)	7.0×10^7	6.9×10^7	6.9×10^7	7.1×10^7
ATP (micromoles per liter of packed cells)	400	390	400	395
Membrane lipids[a]				
Phospholipid:cholesterol	3.26 (3.00)	3.28	3.16	3.26
Sphingomyelin: phosphatidylcholine	0.94 (0.84)	0.94	0.89	0.89
Phosphatidylethanolamine: phosphatidylcholine	1.06 (1.00)	1.06	1.00	1.00
Monoacylphosphatidylcholine	Trace	Trace	Trace	Trace

Human erythrocytes were incubated with lipoproteins at the concentration indicated for 4 h at 37°C. At the end of incubation, cells were washed three times with 150 mM NaCl and lysed by the method of Dodge et al [18]. Hemoglobin in the lysate was determined by measuring the absorbance at 576 nm of the supernatant following centrifugation [19]. Erythrocytes were labeled with [^{14}C]-glucose and [^{32}P]-phosphate prior to addition of lipoproteins.

*Abbreviations: Hb) hemoglobin; ND) not determined.

[a]The lipid composition was determined as milligrams lipid per milligram membrane protein; the ratios are subject to an approximate error of 5%. The numbers in parenthesis are from Pennell [39].

cholesterol and the major phospholipids phosphatidylcholine, monoacylphosphatidylcholine, sphingomyelin, and phosphatidylethanolamine are unaltered during incubation of erythrocytes with lipoproteins. Correspondingly, the lipid composition of the lipoproteins is unaltered by interaction with erythrocytes (data not shown).

Subsequently we attempted to determine if the addition of LDL to erythrocytes alters the membrane-associated proteins and, in particular, alters the covalent incorporation of [^{32}P]-phosphate into membrane constituents. Typical polyacrylamide gel electrophoretis patterns of membrane-derived proteins from control cells and from erythrocytes exposed for 4 h to LDL are illustrated in Figure 2. The gel patterns are identical. The amount of protein associated with each stained band as determined spectrophotometrically at 550 nm is not altered by incubation of erythrocytes with LDL, suggesting that membrane proteins are not released. Moreover, exposure of the cells to LDL does not result in the appearance of additional membrane-associated proteins. In agreement with reports of other investigators [22, 23], spectrin band II and band III are the major membrane proteins which are phosphorylated in intact cells in the absence of exogenous cyclic adenosine monophosphate (cAMP) (Fig. 2). When erythrocytes are incubated with LDL there is a 40% (± 5% determined from four separate experiments) reduction of [^{32}P]-phosphate incorporation into spectrin component II; the extent of phosphorylation of band III is not altered by LDL. Furthermore, addition of HDL to erythrocytes does not influence spectrin phosphorylation (data not shown), which is consistent with the fact that HDL do not alter erythrocyte morphology.

Since LDL reduce the level of spectrin phosphate in intact cells, we utilized membrane ghosts and exogenous enzyme substrates to investigate the influence of LDL on phosphorylation and dephosphorylation independently. As is evident in Table III, when ghosts are incubated with [γ-^{32}P]-ATP, membrane-associated protein kinases catalyze the

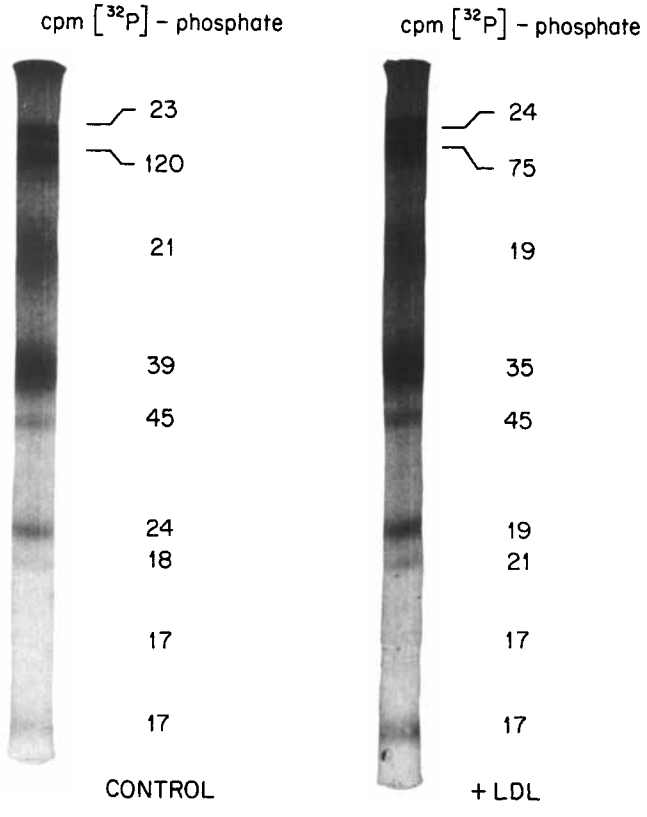

Fig. 2. SDS gel electrophoresis of erythrocyte membrane proteins: A) from control cells; B) from erythrocytes incubated in the presence of LDL (2.5 mg/ml). Human erythrocytes (10% hematocrit) were incubated for 4 h at 37°C. The volume of a typical reaction mixture was 10 ml with final concentrations as follows: 20 mM sodium phosphate (pH 7.4), 150 mM NaCl, 20 mM KCl, 5 mM glucose, 1 μM adenine. The buffer also contained 600 μCi of [^{32}P]-phosphate as $Na_2H^{32}PO_4$. Following incubation, the cells were washed and subsequently lysed according to the method described by Dodge et al [18]. Polyacrylamide gel electrophoresis of membrane ghosts was performed in 6% acrylamide containing 0.16% N,N'-methylene-bis-acrylamide and 0.2% sodium dodecyl sulfate as described by Fairbanks et al [24]. To each gel 50 μg membrane protein was applied. Gels were sliced into 1-mm fractions and each slice was dissolved in 1 ml 30% H_2O_2. The radioactivity of each fraction was determined by liquid scintillation in Aquasol (New England Nuclear).

transfer of [^{32}P]-phosphate to the soluble protein fraction which contains casein. Phosphorylation of casein is diagnostic of the cAMP-independent protein kinase(s) which phosphorylate spectrin [17]. The amount of [^{32}P]-phosphate incorporated into casein is not influenced by preincubating the erythrocyte ghosts for 3 h at 37°C with either LDL or HDL: 1.37×10^4 (± 2%) cpm of [^{32}P]-phosphate are incorporated per milligram casein in the presence or absence of lipoproteins (Table III). Thus, cAMP-independent protein kinase activities are not affected by the LDL-erythrocyte interaction. On the other hand, addition of LDL to the ghost suspension increases twofold the rate of hydrolysis of p-nitrophenyl phosphate catalyzed by membrane-associated phosphatases. Phosphatase activation increases with increasing concentrations of LDL (data not shown): Activation is not maximal at 4 mg/ml LDL. LDL do not catalyze p-nitrophenyl phosphate hydrolysis in the absence of

TABLE III. Effect of Lipoproteins on Phosphorylation-Dephosphorylation

Experiment	Erythrocyte control	Erythrocytes plus LDL (2.5 mg/ml)	Erythrocytes plus HDL (4.5 mg/ml)	Erythrocytes plus LDL (2.5 mg/ml) and HDL (4.5 mg/ml)
Protein kinase activity (^{32}P cpm/mg casein)[a]	1.38×10^4	1.35×10^4	1.34×10^4	1.40×10^4
p-Nitrophenyl phosphatase activity (nmole/mg·h)				
– ATP	162.5	254.2	166.7	165.0
+ ATP	105.0	240.0	101.7	106.3

A volume of freshly prepared membrane ghosts equivalent to 0.8 ml original packed cells was incubated in 20 mM Tris-HCl (pH 7.4) at 37°C. To determine protein kinase activity, erythrocyte ghosts were preincubated for 3 h in the presence and absence of lipoproteins. Lipoproteins suspended in 150 mM NaCl were added to the final concentrations indicated; an equivalent volume of 150 mM NaCl was added to the control experiment. Subsequently, 0.1 ml of the membrane suspension was added to an equal volume of a solution containing 100 μM [γ-^{32}P] ATP and 4 mg/ml casein in 20 mM Tris-HCl (pH 7.4). The final [NaCl] was 50 mM. After incubation for an additional 30 min, the reaction was terminated by the addition of 15 μl of 12 mg/ml bovine serum albumin and 2 ml 33% trichloroacetic acid. The protein precipitate was collected on glass fiber disks (Whatman GF/C, 0.24-mm diameter) and washed exhaustively with 5% trichloroacetic acid; protein-bound ^{32}P was measured by liquid scintillation in Aquasol. To measure phosphatase activity, ghosts were incubated directly with 5 mM p-nitrophenyl phosphate, 0.5 mM $MgCl_2$, and 10 μM ATP, as specified. At the end of incubation, a 1-ml aliquot was removed and the reaction was stopped by the addition of 0.2 ml 33% trichloroacetic acid. Following centrifugation to remove precipitated membranes and lipoproteins, 1 ml of the supernatant was added to 2 ml 0.2 N NaOH and the concentration of p-nitrophenol was determined spectrophotometrically at 418 nm. The maximum hydrolysis of p-nitrophenyl phosphate after a 3-h incubation was less than 20% of the initial amount of substrate present in the assay medium.

[a]Control experiments were performed in the absence of casein; the amount of ^{32}P incorporated into the precipitable fraction was 33% of that in the presence of casein, indicating that the method measures casein phosphorylation and phosphorylation of membrane proteins. The amount of ^{32}P incorporated in the absence of casein was not altered by the addition of LDL or HDL.

erythrocyte ghosts. Although membrane-associated phosphatase activation is inhibited by the addition of ATP, the activity which exists in the presence of LDL is insensitive to ATP. Interestingly, HDL do not enhance phosphatase activity. The lipoproteins of the high-density class do, however, prevent LDL-induced activation of p-nitrophenyl phosphate phosphatase (Table III).

DISCUSSION

Recent reports suggest that a membrane-cytoskeleton complex regulates erythrocyte morphology [1–4]; the cytoskeletal system comprises spectrin, a high-molecular-weight tetrameric protein, and actin – both of which are situated on the cytoplasmic surface of the membrane. One key factor which determines the morphology of the cell is the extent of phosphorylation of spectrin. When spectrin is phosphorylated, erythrocytes are biconcave disks, whereas when spectrin is dephosphorylated by 40–50% [1], the cells are crenated or spherical. Virtually nothing is known concerning the existence of plasma constituents which may modulate erythrocyte morphologic alterations. Since erythrocytes are constantly exposed to the circulating lipoproteins and since the erythrocytes of individuals

with abnormal lipoproteins often are spiculated [5], it is of considerable interest to determine if the plasma lipoproteins are the physiologic factors which specify erythrocyte morphology. The results reported herein demonstrate that low-density lipoproteins isolated from normolipemic individuals induce erythrocytes to undergo a transformation from biconcave disks to echinocytes and to poorly defined spherical cells.

The morphologic transformation which occurs when LDL are added to erythrocytes is a time-dependent process requiring at least 2 h at 37°. It is important to note that the ratio of LDL to erythrocytes in the incubation mixture is approximately that found in the blood of normolipemic humans. Two results of the cell morphology studies are significant. First, the morphologic alteration specifically requires LDL; the high-density lipoproteins do not affect cell shape. Second, HDL inhibit the LDL-promoted morphologic transformation. The HDL were present at subphysiologic concentrations; HDL are equally effective when LDL and HDL are present in the physiologic ratio of 1:1 (wt/wt). Thus, normal erythrocyte morphology may depend not only on the concentration of an individual lipoprotein class but also on the ratio of lipoprotein classes in the plasma. It is premature to assess the physiologic significance of these findings, since the structural features of LDL responsible for the LDL-erythrocyte alteration which results in morphologic alteration are unknown. Moreover, our data certainly cannot yet explain why individuals who lack LDL (abetalipoproteinemia) have spiculated erythrocytes and individuals who lack HDL (Tangier disease) have erythrocytes of normal morphology [5]. It is important to note that erythrocyte morphology is altered by numerous chemical agents which act by different mechanisms [21]; thus, a particular morphology is not diagnostic of one specific cellular alteration. Furthermore, the lipoproteins isolated from the plasma of individuals with inherited diseases of lipid metabolism such as Tangier disease [25] frequently have abnormal composition and structure, and these lipoproteins may not behave normally in the lipoprotein-erythrocyte interaction.

The simplest mechanism which would account for the morphologic alteration induced by LDL is a change in the lipid composition of the erythrocyte membrane [21, 26, 27] as a consequence of lipid exchange/transfer between erythrocytes and lipoproteins. While this mechanism may account for results of experiments in which the incubation time is longer than 4 h, no variation in the concentration of membrane cholesterol or phospholipids is detected within the typical four-hour incubation period (Table II). This result rules out the occurrence of net transfer of lipid constituents, although lipid exchange is probable [27].

Since spectrin phosphorylation is critical to erythrocyte morphology, we subsequently investigated the influence of LDL on this cytoskeletal protein. LDL cause a specific reduction of 40% in the amount of exchangeable phosphate associated with spectrin after 4 h incubation (Fig. 2) relative to the control. On the basis of these data alone, it is not evident whether a correlation exists between the extent of spectrin phosphorylation and the morphologic alterations induced by LDL, or whether LDL elicits dephosphorylation of specific spectrin residues. It is of interest that Harris et al [28] reported four phosphorylated sites for spectrin, two of which have significant turnover rates, and it is possible that LDL influence dephosphorylation of the latter two sites. It is of additional interest that our findings are qualitatively similar to those of Loyter et al [29], who reported that Sendai virus which induce cell lysis and fusion also cause dephosphorylation of membrane proteins. In this context, the indication of cell fusion induced by LDL in Figure 1C is particularly intriguing, since it suggests that lipoproteins and certain viruses may interact with erythrocytes by similar mechanisms. The mechanism for lipoprotein-induced dephosphoryla-

tion of spectrin differs from that for lipid vesicles, which apparently decrease the phosphate content by increasing the cholesterol:phospholipid ratio of the cell membrane [30].

The phosphate content of spectrin is determined by the intracellular concentration of ATP and by the balance between protein kinase-dependent phosphorylation and phosphatase-catalyzed dephosphorylation. Shore and Shore [31] report that LDL activate a Mg^{+2}-ATPase situated in the erythrocyte membrane, suggesting that LDL may deplete ATP in intact cells. However, the concentration of erythrocyte ATP is not influenced by the presence of the plasma lipoproteins LDL or HDL (Table II); the ATP value 400 μM per liter of packed cells agrees with that reported by Nakao et al [32]. Two possibilities remain to be considered: LDL decrease the amount of phosphate associated with spectrin by 1) inactivating a specific membrane-associated protein kinase and/or 2) stimulating a phosphoprotein phosphatase. As indicated in Table III, cAMP-independent protein kinases are not inhibited by LDL. LDL do, however, stimulate membrane-associated phosphatases twofold. Hydrolysis of p-nitrophenyl phosphate reflects contributions from phosphoprotein phosphatases and from the various ATPases. The fact that LDL also accelerate dephosphorylation of [^{32}P]-phosphorylated erythrocyte membrane proteins (D.Y. Hui and J.A.K. Harmony, data submitted for publication) suggests, not unequivocally, that the target of LDL activation is the phosphoprotein phosphatase. Significantly, HDL do not accelerate dephosphorylation of p-nitrophenyl phosphate (Table III) nor do they induce spectrin dephosphorylation or alteration of erythrocyte morphology (data not shown). HDL prevent both LDL-induced activation of membrane-bound phosphatases and alteration of cell morphology.

The mechanism by which LDL enhance phosphatase activity is not immediately evident. Since erythrocytes do not internalize lipoproteins; regulation of this enzyme does not involve endocytosis and lipoprotein degradation, processes required for LDL to regulate the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase in nucleated cells [20]. The initial event resulting in phosphatase activation is attachment of LDL to the erythrocyte membrane: About 10^6 [^{125}I]-LDL particles bind to the surface of each erythrocyte, and the binding event requires just 10–15 min at 37° (D.Y. Hui and J.A.K. Harmony, data submitted for publication). Moreover, HDL inhibit the LDL-induced alterations by preventing the attachment of LDL to the cell surface (D.Y. Hui and J.A.K. Harmony, data submitted for publication). There is some evidence to suggest that phosphatase activity may be regulated by a cyclic AMP-dependent conversion of an inactive enzyme-inhibitor complex to an active enzyme [33]. This possibility is particularly attractive, since LDL activate the membrane adenylate cyclase of human adipocytes [34]. However, the mature erythrocyte lacks a functional adenylate cyclase system [35], and analogous activation of the erythrocyte phosphatase must be controlled by other signals. One possible alternative signal is the perturbation by LDL of the concentration of intracellular ions. LDL do not induce a permeability defect for sodium ion in the cell membrane such as that introduced by the addition of sonicated phosphatidycholine vesicles to intact erythrocytes [36]. The concentration of sodium ion in intact cells incubated for 4 h with LDL is identical to the value obtained for cells incubated without lipoproteins (D.Y. Hui and J.A.K. Harmony, data submitted for publication.) Studies are currently under way to determine whether LDL influence the intracellular concentration of other ions, particularly calcium and potassium.

This is the first report that plasma lipoproteins, in particular the LDL, induce dephosphorylation of membrane-associated proteins. The significance of this finding is augmented by the fact that the target for dephosphorylation is spectrin, a cytoskeletal

protein which specifies cell morphology [1–4] and the distribution of receptors at the cell surface [37]. Spectrin has so far been identified in the erythrocyte only, although the existence of high-molecular-weight cytoskeletal proteins which interact with actin is postulated in other cell types. [38]. Our observations suggest a new focus for investigators attempting to elucidate the role of lipoproteins in normal and pathologic states.

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REFERENCES

1. Greenquist AC, Mohandas N, Shohet SB: Fed Proc 37:1507, 1978.
2. Sheetz MP, Singer SJ: J Cell Biol 73:638, 1977.
3. Birchmeier W, Singer SJ: J Cell Biol 73:647, 1977.
4. Pinder JC, Bray D, Gratzer WB: Nature 270:752, 1977.
5. Stanbury JB, Wyngaarden JB, Fredrickson DS: "The Metabolic Basis of Inherited Disease." 4th Ed. New York: McGraw-Hill, 1978, part 4.
6. Poon RWM, Simon JB: Biochim Biophys Acta 384:138, 1975.
7. Camejo G: Biochim Biophys Acta 175:290, 1969.
8. Nelson GJ: "Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism." New York: Wiley Interscience, 1972.
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265, 1951.
10. Roeschlau P, Bernt E, Gruber W: Klin Chem Klin Biochem 12:403, 1974.
11. Schmidt FH, von Dahl K: Klin Chem Klin Biochem 6:156, 1968.
12. Bartlett GR: J Biol Chem 234:466, 1959.
13. Waku K, Lands WEM: J Lipid Res 9:12, 1968.
14. Adam H: In Bergmeyer HV (ed): "Methods of Enzymatic Analysis." New York: Academic, 1963, pp 543–551.
15. Heller M, Hanahan DJ: Biochim Biophys Acta 255:239, 1972.
16. Ellory JC, Lew VL: Biochim Biophys Acta 332:215, 1974.
17. Hosey MM, Tao M: Biochim Biophys Acta 482:348, 1977.
18. Dodge JT, Mitchell C, Hanahan DJ: Arch Biochem Biophys 110:119, 1963.
19. Lake W, Rasmussen H, Goodman DBP: J Membr Biol 32:93, 1977.
20. Goldstein JL, Brown MS: Curr Topics Cell Reg 11:147, 1976.
21. Ponder E: "Hemolysis and Related Phenomena." New York: Grune and Stratton, 1948, pp 10–49.
22. Shapiro DL, Marchesi VT: J Biol Chem 252:508, 1977.
23. Plut DA, Hosey MM, Tao M: Fed Proc 36:320, 1977.
24. Fairbanks G, Steck TL, Wallach DFH: Biochemistry 10:2606, 1971.
25. Heinen RJ, Herbert PN, Fredrickson DS, Forte T, Lindgren FT: J Clin Invest 61:120, 1978.
26. Deuticke B: Biochim Biophys Acta 163:494, 1968.
27. Bruckdorfer KR, Graham JM: In Chapman D, Wallach DFH (eds): "Biological Membranes." New York: Academic, vol 3, 1976, pp 103–151.
28. Harris HW, Wolfe LC, Lux SE: J Supramol Struct 8(Suppl 2):204, 1978.
29. Loyter A, Ben-Zaquen R, Marash R, Milner Y: Biochemistry 16:3903, 1977.
30. Semenuk M, Vickers J, Rathbone M, Brain MC: Blood 50(Suppl 1): 86, 1978.
31. Shore V, Shore B: Biochem Biophys Res Commun 65:1250, 1975.
32. Nakao M, Nakao T, Yamazoe S, Yoshikawa H: J Biochem 49:487, 1961.
33. Killilea SD, Brandt H, Lee EYC: Trends Biochem Sci 1:30, 1976.
34. Pairault J, Levillier J, Chapman MJ: Nature 269:607, 1977.

35. Rasmussen H, Lake W, Gasic G, Allen J: In Brewer GJ (ed): "Erythrocyte Structure and Functions, Progress in Clinical and Biological Research." New York: Alan R Liss, 1975, vol 1, pp 467–486.
36. Huetis WH: J Biol Chem 252:6764, 1977.
37. Fowler V, Bennett V: J Supramol Struct 8(Suppl 2):207, 1978.
38. Hitchock SE: J Cell Biol 74:1, 1977.
39. Pennell RB: In Surgenor DM (ed): "The Red Blood Cell." New York: Academic Press, 1974, vol 1, pp 93–146.