Ali, S. Y., Sajdera, S. W., & Anderson, H. C. (1970) *Proc. Natl. Acad. Sci. U.S.A. 67*, 1513.

Anderson, H. C. (1967) *J. Cell Biol. 35*, 81.

Anderson, H. C. (1969) *J. Cell Biol. 41*, 59.

Bonucci, E. (1967) *J. Ultrastruct. Res. 20*, 33.

Bonucci, E. (1970) *Z. Zellforsch. Mikrosk. Anat. 103*, 192.

Brighton, C. T., & Heppenstall, R. B. (1971) *J. Bone Jt. Surg., Am. Vol. 53*, 719.

Cotmore, J. M., Nichols, G., Jr., & Wuthier, R. E. (1971) *Science 172*, 1339.

Eisenberg, E., Wuthier, R. E., Frank, R. B., & Irving, J. T. (1970) *Calcif. Tissue Res. 6*, 32.

Fleisch, H., Russell, R. G. G., & Straumann, F. (1966) *Nature (London) 212*, 901.

Folch, J., Lees, M., & Sloane-Stanley, G. H. (1957) *J. Biol. Chem. 226*, 497.

Hill, E. E., & Lands, W. E. M. (1970) in *Lipid Metabolism* (Wakil, S. J., Ed.) p 613, Academic Press, New York, N.Y.

Holtrop, M. E. (1972) *Calcif. Tissue Res. 9*, 140.

Hübscher, G. (1962) *Biochim. Biophys. Acta 57*, 555.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.

Majeska, R. J., & Wuthier, R. E. (1975) *Biochim. Biophys. Acta 391*, 51.

Nash, H. A., & Tobias, J. M. (1964) *Proc. Natl. Acad. Sci. U.S.A. 51*, 476.

Peress, N. S., Anderson, H. C., & Sajdera, S. W. (1974), *Calcif. Tissue Res. 14*, 275.

Rouser, G., Siakotos, A. N., & Fleischer, S. (1966) *Lipids 1*, 85.

Wolinsky, I., & Guggenheim, K. (1970) *Calcif. Tissue Res. 6*, 113.

Wong, P. Y.-K., Majeska, R. J., & Wuthier, R. E. (1977) *Prostaglandins 14*, 839.

Wuthier, R. E. (1966) *J. Lipid Res. 7*, 558.

Wuthier, R. E. (1968) *J. Lipid Res. 9*, 68.

Wuthier, R. E. (1973) *Clin. Orthop. Relat. Res. 90*, 191.

Wuthier, R. E. (1975) *Biochim. Biophys. Acta 409*, 128.

Wuthier, R. E. (1976) in *Lipid Chromatographic Analysis* (Marinetti, G. V., Ed.) p 59, Marcel Dekker, New York, N.Y.

Wuthier, R. E. & Cummins, J. W. (1974) *Biochim. Biophys. Acta 337*, 50.

Wuthier, R. E., & Eanes, E. D. (1975) *Calcif. Tissue Res. 19*, 197.

Wuthier, R. E., & Gore, S. T. (1977) *Calcif. Tissue Res. 24*, 163.

Wuthier, R. E., Majeska, R. J., & Collins, G. M. (1977) *Calcif. Tissue Res. 23*, 135.

Small-Angle X-Ray Scattering and Differential Scanning Calorimetry Studies on Reversibly Modified Human-Serum Low Density Lipoproteins[†]

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ABSTRACT: Small-angle x-ray scattering diagrams of human serum low density lipoprotein (LDL) were recorded at several temperatures in solutions of different freezing points. It was found that modifications of the x-ray patterns observed on cooling the lipoprotein samples below 0 °C are due to reversible alterations of the LDL surface structure induced by the freezing process (independent of temperature). With both intact and partially dehydrated LDL, differential scanning calorimetry (DSC) carried out in the body temperature range revealed a heat absorption characteristic of the transition from a liquid crystal to an isotropic liquid phase of cholesteryl esters within the lipoproteins (Deckelbaum, R. J., Shipley, R. J., Small, P. M., Lees, R. S., & George, P. K. (1975) *Science 190*, 392). However, small-angle x-ray scattering diagrams recorded with the same LDL sample before and after the partial removal of water were found to be very different: the scattering curve for intact LDL showed a strong band centered at $(36 \text{ Å})^{-1}$ which disappeared upon drying and reappeared upon restoring the water. Our results suggest that the presence of this signal strongly depends on the molecular structure of the lipoprotein surface.

The precise location and organization of the molecular constituents within the low density lipoprotein (LDL) is not yet definitely established. The current concept is that the proteins and polar groups of phospholipids are exposed to the aqueous environment, covering the cholesteryl esters and triglycerides which are located toward the center of the particle (Pollard et al., 1969; Mateu et al., 1972; Luzzati et al., 1976; Tardieu et al., 1976).

The organization of the apolar constituents still remains one of the incompletely resolved problems. Deckelbaum et al. (1975) first observed a close resemblance between the thermal behavior of LDL solutions and that of cholesteryl esters derived from these particles. In the same angular region $((36 \text{ Å})^{-1})$ of the small-angle x-ray diagrams recorded at 10 °C, both systems display a diffraction signal whose intensity vanishes near body temperature. In addition, by differential scanning calorimetry (DSC) techniques, they also detected a thermal transition in the same temperature range of the disappearance

TABLE I: Chemical Composition of the LDL Samples Studied.[a]

	Weight %		
	LDL-A	LDL-B	LDL-C
Protein	25.95	25.76	25.87
Phospholipids	22.51	22.22	25.26
Free cholesterol	8.55	8.91	8.51
Cholesteryl esters	34.20	35.64	34.32
Triglycerides	8.79	7.47	6.04
CE/T[b]	3.89	4.77	5.68

[a] The analyses were carried out using procedures described in previous works (Camejo et al., 1976a,b). Samples from three different male donors. [b] CE/T is the cholesterol esters to triglyceride ratio in the three single LDL.

of the $(36 \text{ Å})^{-1}$ line. On the basis of these results, this group proposed that at 10 °C the organization of cholesteryl esters in LDL is similar to that of the smectic phase of pure cholesteryl esters. At body temperature these molecules would be arranged in a more disordered isotropic–liquid state. These observations aroused considerable interest in this type of phenomenon and its possible biological implications (Camejo et al., 1976a,b). In previous work (Camejo et al., 1976a,b), we have shown that triglycerides have a strong influence on the thermal transition in intact LDL and in neutral lipids derived from these particles. Moreover, we have recently reported (Mateu et al., 1977) that the disappearance of the $(36 \text{ Å})^{-1}$ band observed near body temperature also takes place on cooling LDL solutions below 0 °C.

This paper presents a small-angle x-ray scattering study of LDL[1] solutions of different freezing points and x-ray and DSC experiments on intact and partially dehydrated samples. The purpose of this work is to determine whether the disappearance of the $(36 \text{ Å})^{-1}$ band at low temperature is due to a temperature-induced structural transition in the particle itself or if it is due to an alteration of the LDL structure induced by the freezing of the solvent.

Materials and Methods

Preparation of the Samples. Three human serum LDL samples of density 1.019 to 1.063 were individually isolated from three different apparently healthy adult male donors by preparative centrifugation (Camejo et al., 1976a,b). The chemical composition of these lipoproteins is shown in Table I. Following isolation, each LDL from one single donor was divided in four aliquots which were independently prepared in one of the following solutions: (1) H_2O normal buffer (nb) (fp −1 °C); (2) D_2O normal buffer (fp 3.5 °C); (3) 1.85 M NaBr in H_2O normal buffer (fp −7.5 °C); (4) 3.74 M glycerol in H_2O normal buffer (fp −10 °C). After preparation, the lipoproteins were concentrated by centrifugation at 45 000 rpm in a swinging-bucket rotor (Beckman SW-65) during 48 h. The final samples used in this study were recovered as orange-colored transparent pellets either, at the bottom (aliquot 1) or at the top (aliquots 2, 3, and 4) of the tubes. All the steps were performed at 4 °C.

Small-Angle X-Ray Scattering Experiments. Linearly collimated Cu Kα radiation from an Elliott GX6 rotating anode x-ray generator with a nickel-coated bent glass mirror was used in the x-ray experiments. The scattered intensity was
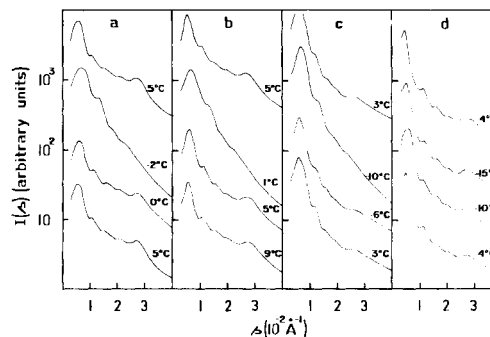
FIGURE 1: Arbitrarily scaled logarithmic plots of some x-ray scattering intensities, corrected for collimation distortions (Lake, 1967). The experiments were performed at several temperatures with LDL prepared in solutions of different freezing points. (a) H_2O normal buffer; (b) D_2O normal buffer; (c) 1.85 M NaBr in H_2O normal buffer; (d) 3.74 M glycerol in H_2O normal buffer. Note that the disappearance of the $2.8 \times 10^{-2} \text{ Å}^{-1}$ $((36 \text{ Å})^{-1})$ band occurs at temperatures which closely correlates with the freezing of the samples. See also in d that the band is present at −15 °C, although the sample has frozen at −11 °C. $s = 2 (\sin \theta)/\lambda$, $(2\theta$, is the scattering angle; λ is the wavelength).

recorded with a linear position-sensitive detector and stored on-line in a computer. When required, the intensities were corrected for collimation distortions (Lake, 1967).

LDL pellets were sealed between two mica windows and placed in a temperature and humidity-controlled sample holder. Temperature control was to ±1 °C as measured with a thermistor probe attached to the holder. The amount of water in LDL could be varied by subjecting the sample to a stream of nitrogen of known relative humidity. In one group of experiments, the temperature dependence of the x-ray scattering intensities for intact LDL prepared in the four solutions of different freezing point (see above) was studied. In the other group, the effect of dehydration on the x-ray diagrams was investigated at 2 °C.

Differential Scanning Calorimetry (DSC) Experiments. Calorimetric studies were performed either on intact or partially dehydrated LDL pellets with a Du Pont 990 thermal analyzer provided with a DSC cell at scale sensitivity of 0.1 mcal s^{-1} in.$^{-1}$ with a heating rate of 5 °C/min. About 15 mg of sample (252 mg of LDL/mL) was hermetically sealed in aluminium sample pans. The reference pan contained an equivalent amount of buffer.

Results

The results reported here are concerned with LDL sample A (Table I). Identical results were obtained with the two other lipoproteins studied (samples B and C, Table I), despite the differences in chemical composition.

X-Ray Scattering Studies of LDL Prepared in Solutions of Different Freezing Point. Small-angle x-ray experiments were performed at different temperatures between 10 and −15 °C on four aliquots of a single LDL in solutions of different freezing points. Figure 1a shows the results obtained with LDL prepared in H_2O normal buffer (fp −1 °C). The 5 and 0 °C intensities were similar to those previously reported (Mateu et al., 1977, 1978) and display four small-angle fringes and a band centered at $(36 \text{ Å})^{-1}$. In agreement with previous observations (Mateu et al., 1977), the −2 °C intensity curve strongly differed from those at higher temperatures: the $(36 \text{ Å})^{-1}$ band was absent and a new fringe—not as intense but sharper than the band—appeared around $(45 \text{ Å})^{-1}$; in addition, the positions of the small-angle fringes were slightly shifted toward higher angles. These changes were completely reversible. This is shown in the upper intensity curve of Figure
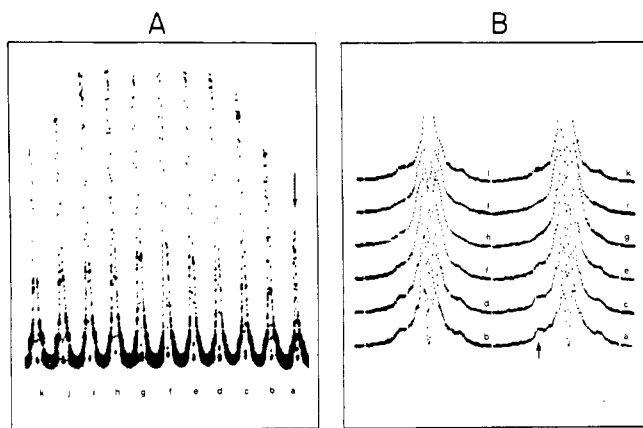
FIGURE 2: Changes in the LDL small-angle x-ray scattering patterns produced by drying and subsequent rehydration. (A) Overall view of a series of experimental x-ray intensities sequentially recorded with the position sensitive detector during 15 min each, such as displayed in the CRT of the computer. Curve a corresponds to intact LDL. The arrow points to the strong correlation peak centered at $s \simeq (150 \text{ Å})^{-1}$ whose intensity increases on drying (curves b-i). Other maxima are not distinguishable because of the compression of the picture. Curves j and k show the reversibility of this phenomenon; on flowing wet N_2 through the sample the peak progressively decreases to its original intensity. (B) Rescaling and higher horizontal magnification of the experimental intensities showed in A. The $(36 \text{ Å})^{-1}$ band (indicated by an arrow) progressively disappears on drying and reappears on rehydration. See text for details.
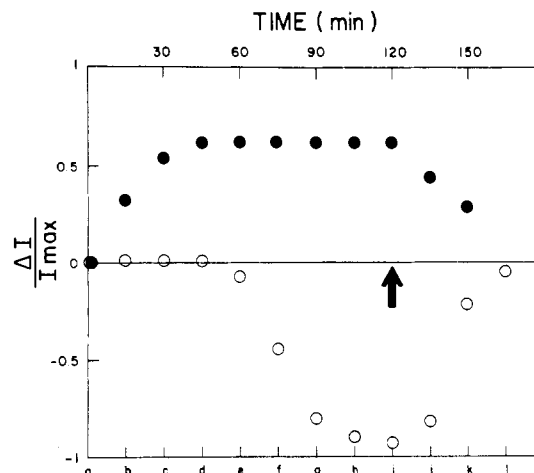

FIGURE 3: Time course of the relative changes in the small-angle x-ray scattering patterns showed in Figure 2. (●) $(150 \text{ Å})^{-1}$ correlation peak; (O) $(36 \text{ Å})^{-1}$ band. $\Delta I/I_{max}$ refers to the intensity measured in each spectrum minus the intensity measured in intact LDL (fully hydrated conditions, curve a) related to its maximum value (I_{max}). The time course of the experiment is indicated at the top of the figure. The letters at the bottom correspond to the spectra of Figure 2 from which the measurements of $\Delta I/I_{max}$ were obtained. Arrow indicates the onset of rehydration. Note that on drying, the changes in the band (O) are delayed with respect to those of the correlation peak (●).

1a which was recorded after reheating the sample to 5 °C. It has to be stressed that, when the temperature decreased below −1 °C, the sample became whitish and turbid as a consequence of freezing and that it recovered its original color and transparency when remelting above 0 °C. Figure 1b shows the results obtained with the sample prepared in D_2O normal buffer (fp 3.5 °C). Changes identical with those observed at −2 °C with the previous sample occurred at higher temperatures (1 °C) when H_2O was replaced by D_2O. Figure 1c shows a similar experiment with LDL prepared in 1.85 M $NaBr$-H_2O normal buffer (fp −7.5 °C). In this case, the changes occurred at a lower temperature (−10 °C). The fact that the $(36 \text{ Å})^{-1}$ band disappears when the sample freezes indicates that the phenomenon is temperature independent and suggests that it is interfacial in nature.

The protection against freezing damage afforded by glycerol in biological systems is well known and thought to be due to the fact that surface damage is prevented (Polge et al., 1949; Brander, 1967; Mazur, 1970). Figure 1d shows x-ray intensities recorded at several temperatures with LDL prepared in 3.74 M glycerol-H_2O normal buffer (fp −10.5 °C). With this preparation, no modifications in the intensities were detected between 4 and −15 °C, although the lipoprotein sample was already frozen (whitish and turbid) at −11 °C. Thus, this result indicates that the disappearance of the $(36 \text{ Å})^{-1}$ band, which is correlated with the freezing of the samples, is a consequence of an alteration of the LDL surface structure since it does not occur when the molecule is protected with adequate glycerol concentrations.

X-Ray Scattering Studies of Partially Dehydrated LDL. Another way to perturb the surface structure of LDL is by gently removing the water from the samples. A dry nitrogen stream was continuously flowed through an LDL pellet. Spectra were recorded during 15-min intervals, one after the other, during drying. Several phenomena took place as the water was removed. In describing these phenomena we will distinguish two regions: one corresponding to the strong maximum centered at around $(150 \text{ Å})^{-1}$, whose intensity is concentration dependent and has been ascribed to the inter-

particle interference (Mateu et al., 1972); the second region is that of the $(36 \text{ Å})^{-1}$.

With regard to the first region, Figure 2A shows several experimental x-ray spectra sequentially recorded for LDL in H_2O normal buffer. Details of the spectra are not resolved because of the low horizontal magnification of the picture, but the very intense $(150 \text{ Å})^{-1}$ correlation peak is clearly evident in all the spectra (indicated by an arrow). Curve "a" corresponds to intact LDL used as a control. As the drying process started, the intensity of the correlation peak was enhanced (curve b) indicating a tighter packing of the particles; this signal continued to increase (curve c) and after 45 min (curve d) its intensity was twice that of the intact LDL. The drying was continued over another 75 min and no further modifications of the peak were detected (curves d to i). At this point, drying was stopped and the sample rehydrated by flowing wet nitrogen (100% relative humidity) through it. After the first 15 min of rehydration (curve j), the intensity of the peak had decreased and continued to do so (curves j and k). Finally its original value was recovered (not shown in the figure).

With regard to the second region, Figure 2B shows a higher magnification and rescaling of the experimental intensities showed in Figure 2A. Curve "a" corresponds to intact LDL and the arrow indicates the $(36 \text{ Å})^{-1}$ band whose intensity remained constant over the first 45 min of drying (curves b-d). At 60 min this signal started to decrease continually (curves e-h) until at 120 min it had completely disappeared (curve i). Rehydration of the sample led to the progressive recovery of the original intensity (curves j-l). During the experiment the sample was transparent, with the same macroscopical appearance as when intact. If the drying was continued beyond 150 min, the changes were no longer reversible, even after rehydrating the sample during several hours.

In Figure 3 we have plotted the relative changes in the $(150 \text{ Å})^{-1}$ correlation peak and the $(36 \text{ Å})^{-1}$ band intensities as a function of time. On drying, the intensity of the correlation peak began to increase immediately and at 45 min it reached a plateau; once these changes were complete the modifications in the pattern in the band started; at 60 min its intensity began to continuously decrease finally disappearing at 120 min. The

recovery of the original intensities were similar for both signals. The strong dependence of the $(36 \text{ Å})^{-1}$ band with the lipoprotein hydration is a clear indication that the presence of this reflection is not exclusively dependent on the physical state of the neutral lipids within the particle (Deckelbaum et al., 1975).

X-Ray and DSC Experiments on Native and Partially Dehydrated LDL. We have shown in a recent paper (Mateu et al., 1978) that, in LDL, the intensity of the $(36 \text{ Å})^{-1}$ band decreases continously as the temperature increases and that the relative magnitude of this signal can be used to estimate the occurrence of the thermal transition. A similar experiment performed with intact LDL is shown in Figure 4a where we have plotted the relative intensity of the band as a function of temperature. Also shown in the figure is a DSC record performed on the same sample. In agreement with Deckelbaum et al. (1975), the transition detected by this technique occurred in the same temperature range as the disappearance of the band. Figure 4b shows an identical experiment performed on the same lipoprotein sample after partial dehydration as indicated above. Under these conditions the $(36 \text{ Å})^{-1}$ band was absent between 0 and 40 °C. However, despite the absence of the band, the DSC record continued to show a heat absorption centered at the same temperature and of the same magnitude as in the experiment performed with intact LDL (Figure 4a).

Discussion

The experiments described here were independently performed on three different LDL samples each derived from a different donor (see Materials and Methods). As previously established (Camejo et al., 1976a,b; Mateu et al., 1978), the increase in triglyceride relative to cholesteryl ester content shifts the thermal transition observed near body temperature toward the lower temperatures. In fact, the disappearance of the $(36 \text{ Å})^{-1}$ band occurred at 25, 29, and 32 °C for LDL samples A, B, and C (Table I). However, the low temperature phenomenon was independent of the chemical composition of the samples: the band disappeared at the same low temperature for each separate LDL when immersed in identical solutions.

In this paper we have shown that, besides the previously known effect of temperature on the $(36 \text{ Å})^{-1}$ band intensity described above, the reversible disappearance of this diffraction signal can also be induced by treatments such as freezing and dehydration, which modify the surface structure of LDL.

The fact that the disappearance of the band occurs at various temperatures which closely follow the freezing point of the different solutions in which LDL was prepared, and since glycerol prevents this disappearance (Figure 1d), suggests that this phenomenon is related to changes in the surface region of the particle.

This point of view is in accord with the concept that the role of glycerol in protecting biological systems from freezing damage is related to the protection of the surface of these systems (Brander, 1967).

Further evidence that implicates surface interactions with the disappearance of the $(36 \text{ Å})^{-1}$ band is provided by the dehydration experiments. We have shown that the band disappears after the $(150 \text{ Å})^{-1}$ correlation peak indicates close packing of the particles. The most straightforward interpretation is that, as the particles pack, then surfaces interact, changing the surface architecture in such a way that the $(36 \text{ Å})^{-1}$ band disappears. Further dehydration would be expected to lead to stronger surface interactions which could permanently alter the LDL structure such that the $(36 \text{ Å})^{-1}$ band
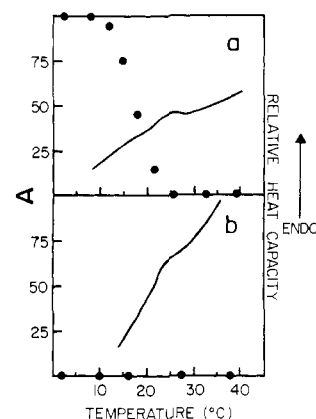

FIGURE 4: Relative intensity of the $(36 \text{ Å})^{-1}$ band (●) and differential scanning calorimetry (DSC) curves (—) of (a) intact and (b) partially dehydrated LDL. Experiments were performed on the same sample, first under normal conditions (time zero, Figure 3) and then after 120 min of dehydration. A is the area of the $2.8 \times 10^{-2} \text{ Å}^{-1}$ ($(36 \text{ Å})^{-1}$) band normalized to the maximum intensity (Mateu et al., 1978). $A = [\int_{s_1}^{s_2} I(s) ds] / |\int_{s_1}^{s_2} I(s) ds|_{\max} \times 100$. s_1 and s_2 correspond to values of s in the reciprocal space where the integration was done ($s_1 = 2.4 \times 10^{-2} \text{ Å}^{-1}$; $s_2 = 3.6 \times 10^{-2} \text{ Å}^{-1}$). As usual, the intensity of this signal progressively decreases on heating until it completely disappears at 25 °C (in the case of this LDL). On the contrary, note that the band is absent between 2 and 40 °C when the sample was dehydrated.

In the two (a and b) DSC records there is an absorption of heat centered at around 24 °C, which is independent of the hydration of the sample, indicating that in both conditions an endothermic transition has occurred.[2]

would not reappear upon hydrating the sample. This is in accord with our experimental observations.

The DSC thermal transition is (a) similar to a liquid crystal to isotropic liquid transition in cholesteryl esters and (b) occurs in a range of temperature that corresponds to the disappearance of the $(36 \text{ Å})^{-1}$ band. Thus, it was reasonable to associate physical events a and b above (Deckelbaum et al., 1975). However, we observe a DSC thermal transition in both intact and partially dehydrated LDL while the disappearance of the $(36 \text{ Å})^{-1}$ band is associated only with the former. Indeed this band is *absent* in the latter. This behavior is different from that observed in artificial cholesteryl ester model systems in which the presence or absence of the strong reflection—characteristic of the smectic phase—is independent of the water content (Loomis et al., 1974; Janiak et al., 1974).

In conclusion, the results here presented suggest that the $(36 \text{ Å})^{-1}$ band observed in the small angle x-ray scattering diagrams of LDL is sensitive to alterations in the surface architecture of the lipoprotein molecule. On the other hand, it seems clear that the ordering or disordering of the internal structure depends both on the physical state of at least a fraction of the neutral lipids and on the integrity of the LDL at the aqueous interface.

Acknowledgment

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[2]The thermal analyzer used for the DSC experiments was not entirely adequate for this kind of measurement, but it was the only one available to us. In order to quantify this phenomenon better, further experiments need to be done with an improved analyzer.

analyzer. T. Kirchhausen was supported by FGMA, Venezuela.

References

Brandes, J. F. (1967) in *Thermobiology* (Rosa, A. H., Ed.) Academic Press, New York, N.Y.

Camejo, G., Mateu, L., Lalaguna, F., Padrón, R., Waich, S., Acquatella, H., & Vegas, H. (1976a) *Artery* 2, 79.

Camejo, G., Waich, S., Mateu, L., Acquatella, H., Lalaguna, F., Quintero, G., & Berrizbeitia, M. L. (1976b) *Ann. N.Y. Acad. Sci. 275*, 153.

Deckelbaum, R. J., Shipley, G. G., Small, P. M., Lees, R. S., & George, P. K. (1975) *Science 190*, 392.

Janiak, M. J., Loomis, C. R., Shipley, G. G., & Small, D. M. (1974) *J. Mol. Biol. 86*, 325.

Lake, J. A. (1967) *Acta Crystallogr. 23*, 191.

Loomis, L. H., Janiak, M. J., Small, D. M., & Shipley, G. G.

(1974) *J. Mol. Biol. 86*, 309.

Luzzati, V., Tardieu, A., Mateu, L., & Stuhrmann, H. G. (1976) *J. Mol. Biol. 101*, 115.

Mateu, L., Tardieu, A., Luzzati, V., Aggerbeck, L., & Scanu, A. M. (1972) *J. Mol. Biol. 70*, 105.

Mateu, L., Kirchhausen, T., & Camejo, G. (1977) *Biochim. Biophys. Acta 487*, 243.

Mateu, L., Kirchhausen, T., Padrón, R., & Camejo, G. (1978) *J. Supramol. Struct.* (in press).

Mazur, P. (1970) *Science 168*, 939.

Polge, C., Smith, A. U., & Parkes, A. S. (1949) *Nature (London) 164*, 666.

Pollard, H., Scanu, A. M., & Taylor, E. W. (1969) *Proc. Natl. Acad. Sci. U.S.A. 64*, 304.

Tardieu, A., Mateu, L., Sardet, C., Weiss, B., Luzzati, V., Aggerbeck, L., & Scanu, A. M. (1976) *J. Mol. Biol. 101*, 129.

Enhanced Binding by Cultured Human Fibroblasts of Apo-E-Containing Lipoproteins as Compared with Low Density Lipoproteins[†]

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ABSTRACT: Specificity for lipoprotein binding to the high affinity cell surface receptors of fibroblasts has been shown to be mediated by the B or arginine-rich (apo-E) apoproteins. The occurrence of a lipoprotein (the HDL$_c$) in cholesterol-fed dogs, distinguished by the presence of the apo-E as the only detectable protein constituent but with other characteristics similar to those of the apo-B-containing low density lipoproteins (LDL), allowed for a direct comparison of the binding activity of the B vs. E apoproteins. The apo-E HDL$_c$ were found to possess an enhanced binding activity which ranged from 10- to 100-fold greater for the HDL$_c$ by comparison with human or canine LDL as determined by competitive binding assays performed at 37 and 4 °C, respectively. Furthermore, the enhanced binding activity of the apo-E HDL$_c$ resulted in an increased rate of cholesterol esterification as measured by [^{14}C]oleate incorporation into intracellular cholesteryl esters. The increased potency of the HDL$_c$ was apparent regardless of whether the data were compared on the basis of lipoprotein protein, cholesterol, or molar ratios and appeared to be related directly to inherent differences in the reactivity of the B vs. E apoproteins. To establish that the differences in the binding activities were not due to differences in the lipid composition or particle size, the human LDL and canine HDL$_c$ were partially delipidated and their reactivities compared before and after such treatment. Partial delipidation, which removed more than three-fourths of the total lipid and converted the spherical particles to disks, did not alter the reactivity of the LDL or HDL$_c$ with the high affinity receptor sites and, furthermore, the inherent differences between the LDL and HDL$_c$ remained apparent. In addition, the increased potency of the E apoprotein appeared to account for all or most of the high affinity receptor binding by the typical HDL ($d = 1.09$–1.21) of the swine. A subfraction of the HDL, representing 15% of the total protein in the $d = 1.09$–1.21 fraction, was isolated by heparin precipitation and was found to possess all of the binding activity. This active subfraction was distinguished from the remaining 85% of the HDL by the increased content of the E apoprotein. The enhanced binding activity of the E apoprotein could modulate HDL binding and competitive displacement of LDL from the cell receptors with relatively minor changes in the E apoprotein content of these plasma lipoproteins.

The binding of specific plasma lipoproteins to high affinity receptors on the cell surfaces of cultured fibroblasts initiates a series of intracellular events which regulate cellular cholesterol metabolism. The bound lipoproteins are internalized by endocytosis and degraded by lysosomal hydrolysis of the protein and cholesteryl esters. Free cholesterol released from the lipoproteins regulates endogenous cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and by stimulating acyl-CoA:cholesterol acyltransferase (ACAT)[1] activity, causing a reesterification of the lipoprotein cholesterol (Goldstein & Brown, 1976). The cell surface receptors, initially shown to bind human low density lipoproteins (LDL), also bind a special class of lipoproteins

[1] Abbreviations used: LDL, low density lipoproteins; HDL, high density lipoproteins; apo-E, arginine-rich apoprotein; ACAT, acyl-CoA:cholesterol acyltransferase; NaDodSO$_4$, sodium dodecyl sulfate; DME media, Dulbecco's modified Eagle's media; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.