Evidence for the Separation of Albumin- and Apo A-I-dependent Mechanisms of Cholesterol Efflux from Cultured Fibroblasts into Human Plasma*

Christopher J. Fielding and Katherine Moser
From the Cardiovascular Research Institute and Department of Physiology, University of California Medical Center, San Francisco, California 94143

The role of albumin has been studied in the plasma-mediated efflux of cholesterol from cultured fibroblasts. Immunoaffinity chromatography of plasma on immobilized anti-albumin antibody decreased by 25-50% total efflux catalyzed by plasma. The remainder of the efflux-promoting capacity of plasma was deleted by immunoaffinity chromatography on antibody to apolipoprotein A-I, the major apoprotein of high density lipoprotein. Both components of efflux were saturable with half-saturation at 0.5-1.0% (v/v) plasma. However, the net transport of sterol from cells to medium, catalyzed by lecithin:cholesterol acyltransferase, was not reduced by the deletion of the albumin-catalyzed component of efflux. This finding was confirmed with congenitally analbuminic plasma. These results indicate that efflux to albumin and to high density lipoprotein in plasma represent independent mechanisms; only the latter is coupled to net transport.

Cholesterol efflux from cultured cells is catalyzed by plasma (1-3), as well as by a number of components isolated from plasma, such as high and low density lipoproteins (1, 2, 4, 5) and albumin (1, 6). Efflux promoted by plasma or isolated lipoproteins furthermore is saturable, and on such grounds it has been suggested (7, 8) that efflux might involve binding of sterol acceptors to cell surface sites. Binding sites for lipoprotein apoproteins have indeed been demonstrated on the surfaces of many cells (7-12). On the other hand, biophysical evidence (13, 14) has suggested rather that efflux was limited by diffusion across an unstirred water layer, without any necessity of cell acceptor interaction.

In studies with whole plasma, it was recently shown that at least the bulk of efflux was closely coupled to the activities of lecithin:cholesterol acyltransferase and the cholesteryl ester transfer factor (3, 15, 16) leading to sterol net transport in normal plasma from cells to medium. In whole plasma, the largest part of efflux was dependent upon unassociated apo A-I; however, a smaller rate of efflux was catalyzed by recrystallized human serum albumin (containing no apo A-I) at its physiological concentration (3). However, albumin has been shown to form no association with cholesterol in purified form (17). Several major unanswered questions therefore remain about the potential role of albumin in the promotion of cholesterol efflux and net transport. Does albumin in whole plasma have the same activity in the promotion of efflux as does the isolated protein? Does either apo A-I- or albumin-dependent efflux involve cell-surface binding? And if there is in plasma an albumin-dependent component of efflux, is it, as efflux-linked to apo A-I, coupled to lecithin:cholesterol acyltransferase activity and sterol ester transfer? The present experiments were designed to determine, in plasma, the significance of albumin-dependent efflux of cholesterol.

EXPERIMENTAL PROCEDURES

Cell Culture—Infant preputial skin fibroblasts were maintained at 37°C in the presence of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (3). For individual experiments, cells were plated at a density of 1-10 × 10^9 cells/6-cm Falcon dish. Three days before reaching the required density, the dishes were changed into medium containing calf serum labeled with [3H]cholesterol (3, 15). After incubation (72 h), the labeled medium was removed; the cells were washed three times with PBS containing recrystallized human serum albumin (Sigma Chemical Co.) (4 mg/ml; pH 7.4) and then three times with PBS alone. The cells were then incubated (15-60 min, 37°C) with PBS containing fractions of human plasma prepared by immunoaffinity chromatography (3, 15).

Preparation of Plasma Fractions—Blood from normocholesteremic laboratory donors was collected into one-twentieth volume of 0.2 M sodium citrate maintained in ice, and, also at ice temperatures, plasma was obtained by centrifugation (2000 X g, 30 min). Cosolubilization of plasma during later incubation was inhibited by removal of fibrinogen by chromatography on columns of antibody (raised in rabbits) to human fibrinogen, coupled to Sepharose 2B. These columns removed no detectable amount of cholesterol from plasma, plasma apoproteins, or albumin, as determined by the corresponding quantitative radial immunoassays (3, 15, 16).

Plasma apo A-I, apo B, apo E, and albumin were selectively removed from plasma by immunoaffinity chromatography with the corresponding immobilized antibodies. Recrystallized human serum albumin had been first purified from any apo A-I by passing it through immobilized anti-apo A-I before injection into rabbits of the albumin in complete Freund's adjuvant.

The complete removal of antigen, after specific immunoaffinity column chromatography, was confirmed in each experiment by immunoassay, if necessary, after concentration by reverse dialysis, under conditions such that a 2% contaminant of the original plasma antigen concentration would have been detected.

Assay of Sterol Efflux—Efflux was determined as the rate of appearance of [3H]cholesterol radioactivity in the medium from cells preincubated with the labeled sterol. As previously described (3), the specific activity of effluxed sterol was not significantly different from that of cell-free or ester sterol. The dishes of cells were washed three times with phosphate-buffered saline containing 0.05% albumin (pH 7.4) at ice temperature, then three times with PBS alone, to remove fetal calf serum. Three ml of medium containing plasma (free of fibrinogen) at the indicated dilution were then added, 1 ml was withdrawn for determination of initial radioactivity and five and ester sterol mass, and the plate containing the remaining 2 ml of medium was then incubated for 60 min at 37°C. To determine sterol net transport from cells to medium, empty dishes containing the same

* This research was supported by National Institutes of Health Grant HL-23738 and through Arteriosclerosis SCOR HL-14237. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby indicated as 'advertisement' in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

† The abbreviations used are: apo A-I, apolipoprotein A-I; PBS, phosphate-buffered saline.
Mechanisms of Cholesterol Efflux and Transport into Plasma

plasma medium were incubated simultaneously (3, 15). Dishes containing cells and empty dishes were prepared and incubated in pentuplicate. At the end of the incubation period, a further 1-ml sample was taken for determination of radioactivity and free and ester sterol mass, and the remaining medium was washed from the dishes with PBS-albumin and PBS (three changes each). The cells were solubilized with 0.1 NaOH, and cell-specific radioactivity was determined. The rate of appearance of radioactivity was linear under the conditions described in these studies. Efflux into medium containing no plasma was 5–6% of plasma values under the same conditions.

Assay of Sterol Net Transport—Pentuplicate empty dishes, and the same number of dishes containing fibroblasts, were incubated with medium containing plasma, as described for efflux. A 1-ml initial sample was taken from each dish for determination of free and ester cholesterol mass (18). After a 60-min incubation at 37 °C, a second 1-ml sample was taken from each dish for a further free and ester sterol determination. Sterol net transport was determined as the difference in the decrease of medium-free sterol (during incubation at 37 °C) between empty dishes and dishes containing cultured fibroblasts (3).

As previously demonstrated (3), the increase in lecithin:cholesterol acyltransferase-derived sterol ester is the same in the presence or absence of fibroblasts and, hence, total sterol demand for esterification is also equivalent. Hence, the change in medium-free sterol decrease in the presence and absence of cells represents net sterol mass entering the medium during the incubation period. The concept has been further validated by cell-medium balance studies under different incubation conditions, as detailed below.

Preparation of Immobilized Lecithin Cholesterol Liposomes—Liposomes were prepared from egg lecithin, dioleoyl phosphatidylethanolamine (Sigma) (5%, w/w), and cholesterol (40%, w/w) in distilled water in the French pressure cell (19). The single walled vesicles so obtained (20) were then covalently coupled to CNBr-activated Sepharose via the free amino group of the basic phospholipid. Any unreacted Sepharose active sites were deactivated with ethanolamine, and the prepared gel was finally washed with distilled water and ethylene glycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid before use in the incubation experiments.

Analysis of Antibody-Associated Lipids—Columns of immobilized antibody, through which plasma had been passed, were then washed with 0.9% NaCl-EDTA (1 mM) for 40 column volumes. Under these conditions, no further protein was detectable in the eluate. Lipids and proteins bound to the antibody gel were then released with 10 volumes of a solution of 3 M NaCNS (pH 7.0). The protein content of the eluate was determined after dialysis, and its composition by quantitative radial immunodiffusion. To extract lipids, the thiocyanate solution was mixed with an equal volume of CH₃OH and CHCl₃. Internal standards of 1,2-[H]cholesterol, 1-[14C]oleic acid, 1-[14C]lysolecithin, and [1H]lecithin (all from New England Nuclear) were added. The chloroform phase was washed (three times) with twice its volume of 0.15 M NaCl (pH 5.5). Sterol was determined enzymatically in portions of the chloroform phase; unesterified fatty acids were determined by titration (pH 5.5). Lecithin and lysolecithin were separated by thin layer chromatography on silica gel layers on glass plates developed in chloroform/methanol/water (65:35:3, v/v) and extracted with chloroform/methanol (1:2, v/v), and lipid phosphorus was determined (22). Recovery through the purification procedure was determined from the radioactivity of the internal lipid standards. Protein was assayed with the Folin phenol reagent (23).

RESULTS

Albumin- and Apo A-I-dependent Efflux—When albumin was removed from plasma by immunoaffinity chromatography on immobilized anti-albumin antibody, there was a significant decrease in the ability of the plasma to promote cholesterol efflux from labeled fibroblasts. This decrease was evident at all concentrations of plasma (Fig. 1). An analogous decrease in efflux was obtained when lipoproteins containing apo A-I were removed from the plasma with the corresponding immunoaffinity column. In eight experiments, the sum of the efflux-promoting activities removed by antibodies to apo A-I and albumin was 95 ± 5% of the activity of unfractionated plasma under the same conditions (range, 92–102%). The efflux components associated with the presence of apo A-I and of albumin therefore represented essentially the whole of efflux into plasma. As further shown in Fig. 1, both components of efflux showed a considerable degree of saturation when plasma was present at increasing concentrations. The proportion of apo A-I-dependent efflux was reproducible (±10%) with different plasma samples from the same source, but varied between 40 and 75% of total efflux in plasma samples from six different normocholesterolemic donors.

A possible relationship between the two components of plasma efflux was further investigated by serial affinity chromatography on antibodies to apo A-I and albumin. If the pathways in fact represented independent mechanisms of efflux, the effect of the removal of albumin should be independent of the previous removal of apo A-I. As shown in Fig. 2, this was found to be the case. In the experiment shown, the...
reduction in efflux caused by the removal of albumin from whole plasma was 0.58 ng µg⁻¹ of cell sterol min⁻¹, while the further reduction by the same antibody, in plasma from which individual antigens had been removed, was 0.52 ng µg⁻¹ of cell sterol min⁻¹. This finding indicates that only not are the apo A-I-dependent and albumin-dependent components of efflux additive, but also that they are independent.

Effect of Cell Density on Efflux Rates—As shown in Fig. 3, the efflux rates promoted by unfractionated plasma were strongly dependent upon cell density. At low density (<1 µg of cell sterol/6-cm culture dish), total efflux was 10-fold greater than in confluent cells. Minimal efflux rates were reached when the dishes were ~50% confluent. There was no difference in the sterol/protein ratio of the cells between the highest and lowest densities used (mean, 36.8 ± 0.9 µg of sterol/mg of protein; range, 36.0–37.4 µg of sterol/mg of protein). A similar effect of cell density has also been observed for cultured vascular smooth muscle and microvesSEL endothelial cells. There was no difference in efflux rates at any cell density when normal fibroblasts were compared to fibroblasts lacking lipoprotein high affinity receptors. This confirms and extends the earlier finding of Wu and Bailey (4).

The property of the density dependence of efflux was used to determine how the proportions of albumin-dependent and apo A-I-dependent efflux would depend on absolute efflux rates. As shown in Table I, there was no significant difference in the proportion of each component of efflux when efflux rates varied over a 12-fold range. Apolipoprotein E, a component of cholesterol-rich lipoproteins in plasma (24), has been implicated in sterol transport from cells. There was no effect of the removal of apo E from plasma on sterol efflux from fibroblasts at any cell density. These findings indicated that the rates of apo A-I- and albumin-dependent components of efflux are proportionate rather than absolute.

Sterol Efflux from Cholesterol-Lecithin Liposomes—Apo A-I in apoprotein or lipoprotein form binds to cultured cells, including fibroblasts, by a high affinity saturable mechanism.

## Table I

| Incorporation medium | Efflux rate \(\text{ng} \cdot \text{min}^{-1} \cdot \text{cell} \cdot \text{mg}^{-1} \) |
|---------------------|--------------------------------------------------|
| Sparse cells        | Medium cells                                     |
| Dense cells         |                                                  |
| Unfractionated plasma | 4.50 ± 0.10, 1.06 ± 0.09, 0.31 ± 0.01 |
| Plasma-apo A-I      | 2.50 ± 0.04, 0.47 ± 0.03, 0.17 ± 0.00 |
| Plasma-apo E        | 4.50 ± 0.25, 1.11 ± 0.07, 0.37 ± 0.01 |
| Plasma-albumin      | 2.92 ± 0.07, 0.60 ± 0.03, 0.21 ± 0.00 |

## Table II

| Incorporation medium | Efflux rate \(\text{ng} \cdot \text{min}^{-1} \) | Net transport rate |
|---------------------|-----------------------------------------------|--------------------|
| Unfractionated plasma | 7.95 ± 0.66 | 3.38 ± 0.38 |
| Plasma-apo A-I      | 3.94 ± 0.20 | 0.04 ± 0.15 |
| Plasma-albumin      | 3.42 ± 0.22 | 2.90 ± 0.20 |
Mechanisms of Cholesterol Efflux and Transport into Plasma

dependent efflux were unchanged, and net transport increased 15–20%.

The effect of temperature was determined over the range of 0–37 °C. Activation energies of efflux were calculated from the Arrhenius plots obtained with unfractionated or albumin-or apo A-I-depleted plasma (Fig. 4). The activation energy of total efflux was 10.7 ± 1.0 kcal/mol (three determinations, range of 9.9–11.4 kcal/mol). Although slightly different plots were obtained for fractionated plasma, such differences did not reach significance and the energy of efflux was in large part independent of the nature of the sterol acceptor in plasma.

Sterol Efflux and Sterol Net Transport in Plasma—As reported earlier (3), efflux may or may not result in sterol net transport, depending in large part on whether efflux is coupled to sterol esterification catalyzed by the lecithin:cholesterol acyltransferase reaction. When efflux was assayed isotopically, a significant portion of total efflux was dependent upon the presence of albumin but when albumin was removed from plasma by immunoaffinity chromatography, there was no reduction in either lecithin:cholesterol acyltransferase activity or the rate of sterol net transport from the fibroblasts to the culture medium (Fig. 5). This finding indicates that only that efflux mediated by apo A-I is significant in promoting net transport of sterol from cells to plasma-containing medium.

This result was further validated by balance studies in which both cell and medium cholesterol mass was measured during net transport. In a representative study (of three), initial cell cholesterol mass was 11.8 ± 0.1 μg/dish and final cell cholesterol mass was 11.1 ± 0.1 μg/dish, a difference of 0.7 μg. Using the difference assay described under “Experimental Procedures,” cholesterol net transport, in terms of medium cholesterol mass, was 0.65 μg/dish under the same conditions. When apo A-I was removed from the plasma medium by immunoaffinity chromatography, cholesterol net transport was reduced to 0.04 μg, while after removal of albumin it was 0.65 μg (compared to the (0.65 – 0.04)) or 0.61 μg expected by difference. The specific activity of the cells in this experiment was 9875 cpm/μg. Initial cell radioactivity was 1.05 ± 0.03 x 10^5 cpm and final radioactivity was 0.94 ± 0.01 x 10^5 cpm, for a total efflux of 0.11 x 10^5 cpm or a calculated total efflux of 1.1 μg of cholesterol. This was reduced by anti-apo A-I affinity chromatography to 0.44 x 10^5 cpm or a calculated 0.67 μg of sterol mass. This can be compared with the reduction of 0.61 μg of sterol determined by direct mass measurement. Finally, the efflux in the absence of albumin in the same experiment was 0.58 x 10^5 cpm or a decrease of 5200 cpm, or 0.53 μg. The sum of apo A-I- and albumin-dependent efflux in this case is therefore 1.14 μg of sterol versus a measured total efflux of 1.10 μg. The efflux to albumin (0.53 μg) can be compared with the difference between total efflux and net transport (1.1 – 0.65 = 0.46 μg of cholesterol).

These results therefore support the concept that the indirect measures of efflux and net transport from medium values reflect the expected changes within the cells, and that only efflux coupled to apo A-I mediates sterol mass transport. In the three studies, differences calculated and found between initial and final cell cholesterol levels were 6–16%.

To confirm that fractionation by affinity chromatography was not a factor in this finding, human analbuminemic plasma was used in place of normal plasma in the cell incubation studies. As shown in Table IV, in this plasma, essentially the whole of efflux is dependent upon apo A-I, and sterol net transport was as high as in normal plasma. This finding

![Fig. 4. Activation energies of efflux of plasma and plasma fractions.](http://www.jbc.org/)

**Fig. 4.** Activation energies of efflux of plasma and plasma fractions. Incubation of fibroblasts prelabeled with ^[3]H|cholesterol was carried out with medium containing unfractionated plasma, plasma-A-I, or plasma-albumin (1.2%, v/v, in PBS) at temperature intervals in the range of 0–37 °C. Incubation was for 60 min. k is the rate constant and T the absolute temperature. Open circles, unfractionated plasma; open triangles, plasma-apo A-I; open squares, plasma-albumin.

**TABLE III**

| Incubation medium | Efflux rate | Per cent |
|-------------------|-------------|----------|
|                   | ng μg^-1 cell sterol min^-1 |          |
| Plasma            |             |          |
| +EGTA (1 mM)      | 0.62 ± 0.04 | 93.2     |
| +Colchicine (2 x 10^-5 M) | 1.18 ± 0.03 | 134.1    |
| +Cytochalasin B (5 x 10^-5 M) | 0.83 ± 0.10 | 95.6     |
| +Azide (0.5 mM)   | 0.90 ± 0.03 | 95.6     |
| +Cyanide (0.5 mM) | 0.93 ± 0.05 | 102.3    |
| +Fluoride (10 mM) | 1.00 ± 0.10 | 113.6    |

**Fig. 5.** Effects of removal of plasma albumin on sterol net transport. Cultured fibroblasts in 6-cm dishes, or empty dishes, were incubated in pentaplicate with unfractionated plasma or plasma from which albumin (ALB) had been removed by affinity chromatography. Incubation was for 60 min at 37 °C. Initial and final 1-ml samples were taken for analysis of free and ester sterol (18). Esterification (EST) was determined from the decrease in medium-free sterol during incubation in the absence of cells. Sterol net transport (TRANS) was determined from the difference between the decrease in medium-free sterol in the presence and absence of fibroblasts, as described under “Experimental Procedures.”
supports the concept that albumin plays no essential role in sterol transport and esterification in plasma.

**Lipids Associated with Albumin in Plasma**—The results reported above indicate that albumin promotes sterol efflux but that this efflux represents exchange and is not coupled to net transport. This requires the presence on albumin of an exchangeable pool of free sterol. No interaction could earlier be detected between highly purified albumin and cholesterol in terms of binding constants (17). This should not exclude, however, interaction between cholesterol and albumin in the presence of other lipids. The possible existence of an albumin-associated sterol pool was investigated by immunoaffinity chromatography of plasma from which lipoproteins had been previously removed with the appropriate immobilized antibodies. As shown in Fig. 6, elution of the bound albumin after extensive washing indicated the presence of free sterol together with phospholipid and large amounts of unesterified fatty acid. The molar ratio between free sterol and phospholipid was ~0.5 and total albumin-associated cholesterol represented about 1% of plasma free cholesterol. This finding indicates that, in human plasma, a small proportion of total free sterol is bound to albumin and may function there as a pool exchangeable with sterol from cell membranes.

**DISCUSSION**

There has been considerable disagreement in the recent literature on the nature of the physiological acceptors of cell sterol efflux. Studies using radiolabeled cells have found that a wide range of plasma fractions in purified form can promote sterol efflux (1-6). These fractions include each of the major plasma lipoprotein classes, lipoprotein-deficient plasma, and albumin either alone or in the presence of phospholipids. On the other hand, in studies where mass transport of sterol between cells and their medium was measured, either directly (3) or indirectly via the effects of cell sterol on a receptor assay (26), efflux was almost wholly dependent upon the presence of high density lipoproteins (27) and, in particular, apoprotein A-I in a lipoprotein form unassociated with other apolipoproteins. The solution to this paradox is shown in the present study to lie in the finding that plasma contains two distinct and independent pathways, both of which promote the efflux of sterol from cultured cells. However, only one of these pathways, that dependent upon apo A-I, mediates sterol net transport from cells. The other, dependent upon the presence of albumin, takes part in an exchange reaction that is not coupled to net transport. While the function of this latter pathway is presently not evident, it clearly represents a considerable potential artifact in studies of sterol transport, since as much as half of total efflux from labeled cells may be uninvolved in the regulation of cell cholesterol content. It was previously shown that, in unfraccionated plasma, the apo A-I-dependent component of efflux is coupled to the activity of lecithin:cholesterol acyltransferase. In the present study, this reaction was not decreased in the absence of plasma albumin, whether in fractionated normal or in analbuminemic plasma. The contributions of the two pathways to efflux were independent of total efflux when the latter was varied by temperature or cell density. This finding suggests that these acceptors act at a point distal to the regulatory step of efflux. This conclusion is reinforced by the observation that the contributions of the albumin- and apo A-I-dependent routes of efflux were essentially the same whether cultured cells or cholesterol-lecithin vesicles immobilized on Sepharose provided the labeled substrate, when the concentration of sterol was the same. Furthermore, the energy of activation for efflux of cholesterol from cells to plasma was also highly similar to that found for cholesterol efflux from vesicles to a variety of media (13, 14, 28). Two types of theory are currently held on the mechanism of sterol efflux. One involves binding of acceptors to the cell surface and subsequent dissociation of the carrier, enriched with cholesterol, into the medium. The second envisages a rate-limiting diffusion step from the cell surface across an unstirred water layer, after which cholesterol would be incorporated with plasma acceptors. The results of the present study appear most in accord with the second concept, since the kinetics of efflux from cell surfaces and from liposomes were very similar, although an interaction with the membrane surface that is not rate limiting would not be ruled out. The present study, in agreement with an earlier report (4), found no difference in efflux rates from fibroblasts homozygous for deficiency of low density lipoprotein receptors (FH cells) and control cells. However, leukocytes heterozygous for the same genetic factor have been reported to show an increased efflux rate (30). Further studies with a variety of cultured cell types from this disorder will be required to

- **Table IV**

  | Plasma       | Total efflux | Sterol demand | Sterol net transport |
  |--------------|--------------|---------------|----------------------|
  | Control      | 0.67 ± 0.03  | 1.32 ± 0.05   | 0.54 ± 0.12          |
  | Analbuminmic | 0.64 ± 0.06  | 1.29 ± 0.06   | 0.65 ± 0.10          |

  a Micrograms of sterol efflux/dish at 37°C during the 60-min incubation period.
  b Total sterol esterified in the medium in the same period (micrograms of sterol esterified/dish).
  c Sterol contributed from cells to medium in the same period (micrograms/dish). APO A-I levels in control and analbuminemic plasma were 1.32 and 1.71 µg/mL, respectively. The absence of albumin from the plasma of the analbuminemic donor has been documented elsewhere (29).

**Fig. 6**

Elution of albumin and albumin-associated lipids from anti-albumin affinity chromatography. Plasma (1.0 ml) was passed successively through affinity columns of antibodies to apo A-I and apo B to remove bulk plasma lipoproteins, and then through immobilized antibodies to albumin in a column (2 X 10 cm) of covalently linked Sepharose 2B equilibrated in 0.15 M NaCl, 1 mM disodium EDTA (pH 7.4). The column was washed with 100 volumes of saline-EDTA and albumin eluted with 3 M NaCNS (pH 7.0). Protein and individual lipids were determined as described under "Experimental Procedures." Values are expressed as nanomoles/fraction.

---

Downloaded from http://www.jbc.org/ on March 24, 2020
Mechanisms of Cholesterol Efflux and Transport into Plasma
determine the basis of this difference.

It should be emphasized that only a small proportion of the
total apo A-I or albumin in plasma appears to be involved in
the sterol efflux reaction. As previously described (3), only
about 5% of total apo A-I is in unassociated form and it is this
fraction which is coupled to esterification via the
lecithin:cholesterol acyltransferase reaction. While the recov-
ery of cholesterol with albumin using affinity chromatography
is probably incomplete, only a small proportion of the albumin
in plasma (0.6 μmol/ml) could contain bound cholesterol;
even the binding of sterol to a single site per molecule would
involve the major part of plasma free sterol, a finding incom-
patible with the affinity chromatography of plasma lipopro-
teins (3), as well as extensive ultracentrifugal data. Whether
this portion is distinguished by physical or chemical properties
(for example, association with specific phospholipids) is pres-
ently unknown. Taken together, the findings of this study
indicate that in unfractionated plasma there is considerable
heterogeneity in the associations of free sterol molecules de-
rived from cells with plasma factors. These associations have
significance because they determine, at least in part, the
subsequent metabolic fate of the sterol.

Acknowledgment—We are very grateful to Dr. E. J. Cormode for
supplying analbuminemic plasma for this study.

REFERENCES
1. Bailey, J. M. (1965) Exp. Cell Res. 37, 175–182
2. Bates, S. F., and Rothblat, G. H. (1974) Biochim. Biophys. Acta 360, 38–55
3. Fielding, C. J., and Fielding, P. E. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3911–3914
4. Wu, J.-D., and Bailey, J. M. (1979) J. Lipid Res. 20, 472–480
5. Miller, N. E., Weinstein, D. B., and Steinberg, D. (1977) J. Lipid Res. 18, 438–450
6. Goldstein, J. L., and Brown, M. S. (1974) J. Biol. Chem. 249, 5153–5162
7. Fielding, P. E., Vlodavsky, I., Gospodarowics, D., and Fielding, C. J. (1979) J. Biol. Chem. 254, 749–755
8. Fielding, P. E., Vlodavsky, I., Gospodarowics, D., and Fielding, C. J. (1979) J. Biol. Chem. 254, 749–755
9. Mahley, R. W., Hui, D. Y., Innerarity, T. L., and Weisgraber, K. H. (1981) J. Clin. Invest. 68, 1197–1206
10. Chen, Y.-D. I., Kraemer, F. B., and Reaven, G. M. (1980) J. Biol. Chem. 255, 9162–9167
11. Phillips, M. C., McLean, L. R., Stoudt, G. W., and Rothblat, G. H. (1980) Atherosclerosis 36, 409–422
12. McLean, L. R., and Phillips, M. C. (1981) Biochemistry 20, 2893–2900
13. Fielding, P. E., and Fielding, C. J. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 3327–3330
14. Fielding, C. J., and Fielding, P. E. (1981) J. Biol. Chem. 256, 2192–2194
15. Haberland, M. E., and Reynolds, J. A. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2313–2316
16. Heider, J. G., and Boyett, R. L. (1978) J. Lipid Res. 19, 514–518
17. Aron, L., Jones, S., and Fielding, C. J. (1978) J. Biol. Chem. 253, 7220–7226
18. Hamilton, R. L., Goerke, J., Guo, L. S. S., Williams, M. C., and Havel, R. J. (1980) J. Lipid Res. 21, 981–992
19. Fielding, C. J., and Fielding, P. E. (1976) J. Lipid Res. 17, 248–256
20. Bartlett, G. R. (1969) J. Biol. Chem. 334, 465–468
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
22. Pitas, R. E., Innerarity, T. L., Arnold, K. S., and Mahley, R. W. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2311–2315
23. Riordan, J. R., Alon, N., and Buchwald, M. (1979) Biochim. Biophys. Acta 574, 39–47
24. Opar, P. F., Albers, J. J., Cheung, M. C., and Bierman, E. L. (1981) J. Biol. Chem. 256, 8348–8356
25. Ho, Y. K., Brown, M. S., and Goldstein, J. L. (1980) J. Lipid Res. 21, 391–396
26. Werb, Z., and Cohn, Z. A. (1971) J. Exp. Med. 134, 1545–1569
27. Frohlich, J., Pudek, M. R., Cormode, E. J., Sellers, E. M., and Abel, J. G. (1981) Clin. Chem. 27, 1213–1216
28. Fogelman, A. M., Seager, J., Edwards, P. A., and Popjak, G. (1977) J. Biol. Chem. 252, 644–651
Evidence for the separation of albumin- and apo A-I-dependent mechanisms of cholesterol efflux from cultured fibroblasts into human plasma.
C J Fielding and K Moser

J. Biol. Chem. 1982, 257:10955-10960.