Comparison of cholesterol transport in pulmonary, peritoneal, and blood-derived macrophages from normo- and hypercholesterolemic rabbits

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Abstract The influx and efflux components of cholesterol transport were separately determined in pulmonary, peritoneal, and monocyte-derived macrophages from rabbits fed a diet containing either 4.5% fat or 4.5% fat plus 2% cholesterol. Both influx and efflux in pulmonary macrophages increased with increasing concentration of either normal or hypercholesterolemic serum in the medium. The mass of cholesterol entering the macrophages continued to increase beyond the mass of cholesterol effluxed, leading to an increase in cholesterol mass. Similar results were obtained with peritoneal macrophages. Cholesterol-enriched peritoneal macrophages in most cases had a net efflux of sterol when incubated with normocholesterolemic serum. Pulmonary and peritoneal macrophages from cholesterol-fed rabbits tended to have slower sterol influx and a slightly faster sterol efflux than pulmonary and peritoneal macrophages from control rabbits, but the combined effect of these mechanisms did not prevent these macrophages from accumulating sterol ester from hypercholesterolemic serum. Hypercholesterolemic rabbit serum was fractionated by heparin-Sepharose affinity chromatography into a beta-VLDL-deficient nonadsorbed fraction, which had very little effect on pulmonary macrophage sterol content, and an adsorbed beta-VLDL-containing fraction which promoted a large increase in macrophage sterol. As with unfractionated hypercholesterolemic serum, macrophages incubated with the adsorbed beta-VLDL-containing fraction accumulated large amounts of cellular sterol. Monocyte-macrophages cultured in vitro for 21 hr, in contrast to extravascular macrophages, closely regulated their cellular sterol, primarily by limiting the rate of sterol influx. —**Fontaine, R. N., and C. J. Fielding.** Comparison of cholesterol transport in pulmonary, peritoneal, and blood-derived macrophages from normo- and hypercholesterolemic rabbits. *J. Lipid Res.* 1985. **26:** 915–923.

Supplementary key words β-VLDL • mononuclear phagocytes • cholesterol influx • cholesterol efflux • scanning electron microscopy

Macrophages accumulate little sterol when incubated with lipoproteins isolated from normocholesterolemic plasma (1, 2). Peritoneal macrophages and mature monocyte-derived macrophages will interiorize massive amounts of sterol when incubated with chemically altered lipoprotein or with beta-migrating VLDL isolated from hypercholesterolemic animals (1–4). These interiorization processes occur through specific and saturable pathways. These pathways have been made the basis of a theory of atherogenesis in which the accumulation of macrophages at vascular lesions and the accumulation of cellular sterol within these cells play a key pathological role (2, 4). However, the same cells also have effective pathways by means of which interiorized sterol is returned to the medium (4, 5).

In the rabbit, dietary cholesterol readily leads to hypercholesterolemia and the accumulation of cholesterol-rich macrophages at vascular fatty lesions (6). Herein, we describe firstly the properties of the influx and efflux pathways that determine whether mononuclear phagocytes from three tissue sources will accumulate sterol when incubated with autologous serum or its isolated beta-VLDL; and secondly, whether macrophages isolated from a hypercholesterolemic rabbit have different properties of cholesterol transport than do macrophages from normocholesterolemic rabbits.

METHODS AND MATERIALS

Male New Zealand white rabbits (1.6 ± 0.1 kg) were fed either a control or a cholesterol-rich diet for 6–8 weeks. The control diet was Purina rabbit chow supplemented with Wesson oil to 4.5% fat (w/w) as glyceride, while the cholesterol-rich diet contained 4.5% fat + 2% (w/w) cholesterol (USP grade; Sigma Chemical Co., St. Louis, MO). There were no significant differences in daily food intake or body weight in the two groups. Food intake in the control group was 54 ± 18 g/kg per day and in the cholesterol-fed group it was 55 ± 23 g/kg per day. Increase in body weight was 15.2 ± 6.3 g/day in the control group and 13.7 ± 8.1 g/day for the cholesterol-fed group (n = 7).

Abbreviations: PBS, phosphate-buffered saline; DME-H21, Dulbecco's Eagle's medium with 4.5 g/l glucose; FC, free cholesterol; CE, cholesteryl ester.

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Initial serum cholesterol in animals entering the study was 66 ± 13 mg/dl; after 6–8 weeks on the control diet the serum cholesterol was 77 ± 33 mg/dl; the animals on the cholesterol-rich diet had a final serum cholesterol concentration of 2007 ± 297 mg/dl. The proportion of total cholesterol as ester (mol/mol) was 60 ± 2% for the control animals and 71 ± 5% for the cholesterol-fed group.

For the experiments on cholesterol flux, pairs of cholesterol-fed rabbits were chosen that had serum cholesterol concentrations that varied by less than 10%; the same was done with control rabbits. The blood cells and plasma of one animal of each pair were then radiolabeled in vivo by the repeated injection of tritiated cholesterol ([1,2-^{3}H]-cholesterol, 45–60 Ci/mmol; New England Nuclear, Boston, MA). A stock solution of labeled cholesterol was prepared by injection of 1–1.5 mCi of the label in 0.25 ml of ethanol from a 27-gauge needle into 5 ml of stirred autologous rabbit serum. The solution was sterilized through a 0.45-μm Gelman Acrodisc filter (Gelman, Ann Arbor, MI). Serial injection of the labeled serum was made via a marginal ear vein over 13 days. Twenty-four hours was allowed to elapse before collecting macrophages and serum. In both control and cholesterol-fed animals, plasma cholesterol specific activity had reached a plateau by the end of this period, and at this time the specific activity of cholesterol in the macrophages (isolated as described below) was also equivalent (± 9%) to that of the plasma of the corresponding cell donor.

Preparation of blood serum and macrophages

The rabbits were first sedated with Rompun (Haver-Lockhart, Shawnee, KA), 4 mg/kg subcutaneously. Ketaset (ketamine HCl, Bristol Laboratories, Syracuse, NY), 80 mg/kg, was given subcutaneously. The femoral artery was dissected, utilizing Lidocaine as a local anesthesia, and cannulated with PE-60 tubing (Clay Adams, Parsippany, NY). About 90–120 ml of blood could be obtained from a 2.5-kg rabbit. The blood (20 ml) to be used in the experiments as serum was then promptly dispensed into 15-ml plastic tubes (Falcon, Oxnard, CA) and cooled in ice-water. Another 10 ml of blood, from which serum was obtained to culture monocytes, was allowed to clot at room temperature for 30 min. The cooled blood was centrifuged (1500 *g*, 4°C, for 20 min) and the supernatant plasma was allowed to clot overnight at 4°C in glass tubes. The serum was then obtained by one or two further centrifugations at 1500 *g*, 4°C, and by squeezing the clot with wooden applicators. A small portion of the serum was incubated at 37°C to ascertain that the clotting process had terminated.

Blood monocytes were obtained by centrifugation on a Ficoll-Hypaque gradient (7, 8). The remaining blood (60–90 ml) was collected with 50 U/ml heparin (Elkins-Sinn, Inc., Cherry Hill, NJ; 10,000 U/ml), into an equal volume of Ca^{2+}, Mg^{2+}-free Hanks' buffer at 4°C. Twelve ml of Ficoll-Hypaque (20°C) was carefully layered under 33 ml of the blood–buffer, now at 20°C, in a 50-ml plastic centrifuge tube (Falcon). The red blood cells were centrifuged through the Ficoll-Hypaque layer at 400 *g* for 30–40 min at room temperature. The white blood cell band was diluted at least 1:2 (v/v) with Hanks' buffer (Ca^{2+}, Mg^{2+}-free) and then centrifuged (200 *g* for exactly 9 min) to sediment the cells. This procedure was repeated twice more to remove Ficoll-Hypaque and platelets. The monocytes were finally resuspended at 10 × 10^6 cells/ml in medium RPMI-1640 containing 25% (v/v) autologous serum plus 50 μg/ml gentamycin, 1.2 mg/ml fungizone, 2 mM glutamine, and 8 μg/ml insulin (Regular Iletin I, Eli Lilly, Indianapolis, IN) (8).

Pulmonary alveolar macrophages were isolated by intubating the trachea and lavaging the lungs 8–10 times with 35 ml of sterile 0.9% (w/v) NaCl at room temperature (9). The macrophages in the lavage were then pelleted by centrifugation (200 *g*, 8 min) and resusupended at 1 × 10^6 cells/ml in Dulbecco's modified Eagle's medium supplemented with 4.5 g glucose/liter and containing 50 μg/ml gentamycin, 1.2 mg/ml fungizone, and 2 mM glutamine.

Peritoneal macrophages were isolated by lavaging the peritoneal cavity of the same animals with saline–0.05% (w/v) sodium citrate. The cells were pelleted as described for pulmonary macrophages, then resuspended at 0.6 × 10^6 cells/ml in modified Eagle's medium as above.

Pulmonary and peritoneal macrophages were dispensed in 2-ml portions into 35-mm Costar dishes (Cambridge, MA) and incubated in humidified 7% CO_2 in air at 37°C. After 30–45 min, the plates were rinsed to remove debris and nonadherent cells. The macrophages were then incubated overnight under the same conditions before use in the sterol flux experiments described below. The white blood cells were dispensed in 0.5-ml aliquots into 24-well cluster dishes (Costar, Cambridge, MA) and the monocytes were allowed to adhere in ambient atmosphere for 1 hr at 37°C. Unattached cells were then removed by suction, the attached cells were carefully washed three times with 1 ml of RPMI-1640 medium, and then incubated for 21 hr in 0.5 ml of the 25% autologous serum–RPMI medium described above. Greater than 99% of the cells phagocytized carbon black and stained for nonspecific esterase with naphthyl AS acetate (10).

Determination of efflux and influx rates

Plates of labeled or unlabeled pulmonary and peritoneal macrophages were washed three times with 1 ml of phosphate-buffered saline (37°C). The plates of monocyte-derived macrophages were washed once with PBS-albumin (0.2%) and then three times with PBS. For determination of efflux, labeled cells from normo- or hypercholesterol-

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emic rabbits were incubated at 37°C with unlabeled serum from either normo- or hypercholesterolemic animals. Each experiment consisted of four groups of incubations: cells from normocholesterolemic rabbits + normocholesterolemic plasma; the same cells + hypercholesterolemic serum; cells from the hypercholesterolemic rabbits + normocholesterolemic serum; and the same cells + hypercholesterolemic serum. Within each series, determination was made of the effects of incubation period and medium serum concentration, as described in the details of individual experiments. After the incubations, a portion of medium was taken for determination of radioactivity, the cells that may have been loose were pelleted at 500 *g* for 15 min, and a 400-μl aliquot was transferred to glass vials containing 10 ml of Aquasol liquid scintillation cocktail (New England Nuclear, Boston, MA). Radioactivity was determined in a Beckman LS-8000 scintillation counting photometer. Differential quenching of fluorescence due to varying serum dilutions was corrected, if necessary, by the H-number method. Similar samples were analyzed as a function of time during incubation at 37°C. Macrophage cholesterol specific activity was determined from unincubated plates of cells. The cells were solubilized with two 0.5-ml portions of 0.1 N NaOH, and a 0.15-ml portion was taken for determination of protein by the Folin-phenol method (11). Sterol was extracted from the remaining portion of the cell extract with chloroform–methanol 1:1 (12). A portion of the chloroform phase was taken for the determination of cell free and ester cholesterol mass by an enzymatic-fluorimetric assay (13) using a Farrand 150730 fluorimeter. A final portion of the chloroform extract was taken for determination of radioactivity, and cell cholesterol specific radioactivity was determined. Efflux was calculated as the rate of transfer of cell cholesterol label to the unlabeled medium as a function of time.

Influx was determined analogously, as the rate of transfer of radioactivity from labeled plasma to unlabeled macrophages. Plates of unlabeled cells were washed as described above. The cells were then incubated at 37°C for the indicated time period with 0.7 ml of medium containing labeled serum diluted with DME medium. At the end of the incubation, the cells were washed once with phosphate-buffered saline containing 0.2% (w/v) human serum albumin (pH 7.4) and allowed to remain with the wash at 4°C for 5 min. The wash was then removed by aspiration, and the cells were washed a further three to six times with 2.2 ml of phosphate-buffered saline (no albumin). The cells were then solubilized with 0.1 N NaOH and their content of protein, cholesterol, and radioactivity was determined as described above.

Isolation of beta-VLDL from rabbit plasma

Hypercholesterolemic plasma from rabbits contains beta-migrating VLDL, a characteristic lipoprotein shown to react with specific macrophage receptors, and to mediate cholesterol loading into macrophages (1). Blood was collected in plastic tubes and allowed to clot at 4°C as described above. The serum was then applied in 0.3-ml portions to 1.2-cm columns containing 35 ml of heparin-Sepharose (Pharmacia, Piscataway, NJ) (14, 15). A non-retained fraction was collected with 2 mM phosphate buffer (pH 7.5) containing 50 mM NaCl as the eluant. The adsorbed fraction was eluted quantitatively with 2 mM phosphate–3 M NaCl. Both adsorbed and nonadsorbed fractions were dialyzed against 0.15 M NaCl–0.3% EDTA, and then against DME medium before incorporation into the macrophage media. Agarose strip gel electrophoresis of the adsorbent fraction revealed one band and it migrated with beta mobility.

Chemical analysis of macrophages

Cellular content of DNA was determined fluorometrically and protein was determined colorimetrically on the same aliquot (16).

Monocyte-derived macrophages were scraped off the plates with a rubber policeman and three 300-μl aliquots of 0.1 N NaOH–2% Na_2CO_3–0.01% $CuSo_4$–0.02% sodium tartrate and the amount of protein was determined (11). The sample was then extracted for sterol determination, after which the sample was transferred quantitatively to scintillation vials and radioactivity was determined as described above. There was no detectable quenching due to the cholesterol assay reagents.

Scanning electron microscopy

The three sources of rabbit macrophages were cultured for 20 hr on discs made from the bottom of tissue culture plates (Costar, Cambridge, MA). The monocyte-derived macrophages were rinsed twice with PBS-albumin (0.2%) and four times with PBS. Then the pulmonary, peritoneal, and monocyte-derived macrophages were fixed for 30 min in 2% glutaraldehyde–1% paraformaldehyde in Sorenson's phosphate buffer, and 15 min in 1.5% OsO_4–veronal acetate buffer, pH 7.4. The discs were then dehydrated with increasing concentrations of ethanol, and then critical-point dried with carbon dioxide. The discs were then mounted onto specimen stubs with silver paste, coated with 200 Å of gold, and observed in a scanning electron microscope (Cambridge S 150) with an aperture of 100 nm and 20 Kev accelerating voltage.

Statistical methods

The differences between the means were analyzed by the Student's *t*-test, and considered to be statistically significant at $P < 0.05$. The differences in influx or efflux rates for macrophages incubated with various dilutions were analyzed by two-way analysis of variance and considered to be significant at $P < 0.05$ (17).

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RESULTS

Characterization of plasma lipids

Initial serum cholesterol in animals entering the study was 66 ± 13 mg/dl. After 6–8 weeks on the control diet, the serum cholesterol was 77 ± 33 mg/ml (not significantly different), while animals on the cholesterol-rich diet had a final serum cholesterol concentration of 2007 ± 297 mg/dl. The proportion of total cholesterol as ester (mol/mol) was 60 ± 2% for the control animals and 71 ± 5% for the cholesterol-fed group. Control rabbits had serum triglycerides of 160 ± 72 mg/dl, while the value for the cholesterol-fed group was 245 ± 150 mg/dl. The specific activity of serum cholesterol from control rabbits in this study ranged from 140 to 260 cpm/μg, while that in cholesterol-fed animals ranged from 26 to 58 cpm/μg.

Characteristics of macrophages from control and cholesterol-fed rabbits

The yield of pulmonary macrophages in this study varied between 20 and 80 × 10^6 cells/rabbit, while the yield of peritoneal macrophages (6–10 × 10^6 cells/rabbit) was much lower. The recovery of monocyte-derived macrophages at the start of the efflux experiment varied between 23,000 and 56,000 cells/ml of blood; the recovery of monocyte-derived macrophages at the end of the influx experiment varied from 16,000 to 23,000 cells/ml of blood. **Fig. 1** illustrates scanning electron micrographs of each of the three sources of macrophages 21 hr after isolation from control and cholesterol-fed rabbits. Peritoneal macrophages from both control and cholesterol-fed rabbits spread during culture to a much larger extent than either pulmonary or monocyte-derived macrophages. However, there were no major surface morphological differences between the three sources of macrophages isolated from control rabbits and those taken from cholesterol-fed rabbits. The lipid and protein content of macrophages from these sources is shown in **Table 1**. Relative to DNA content, macrophages from cholesterol-fed rabbits contained a significantly increased amount of free and esterified cholesterol. About 65% (wt %) of the increase in cellular sterol in pulmonary and peritoneal macrophages was attributable to an increase in esterified cholesterol,

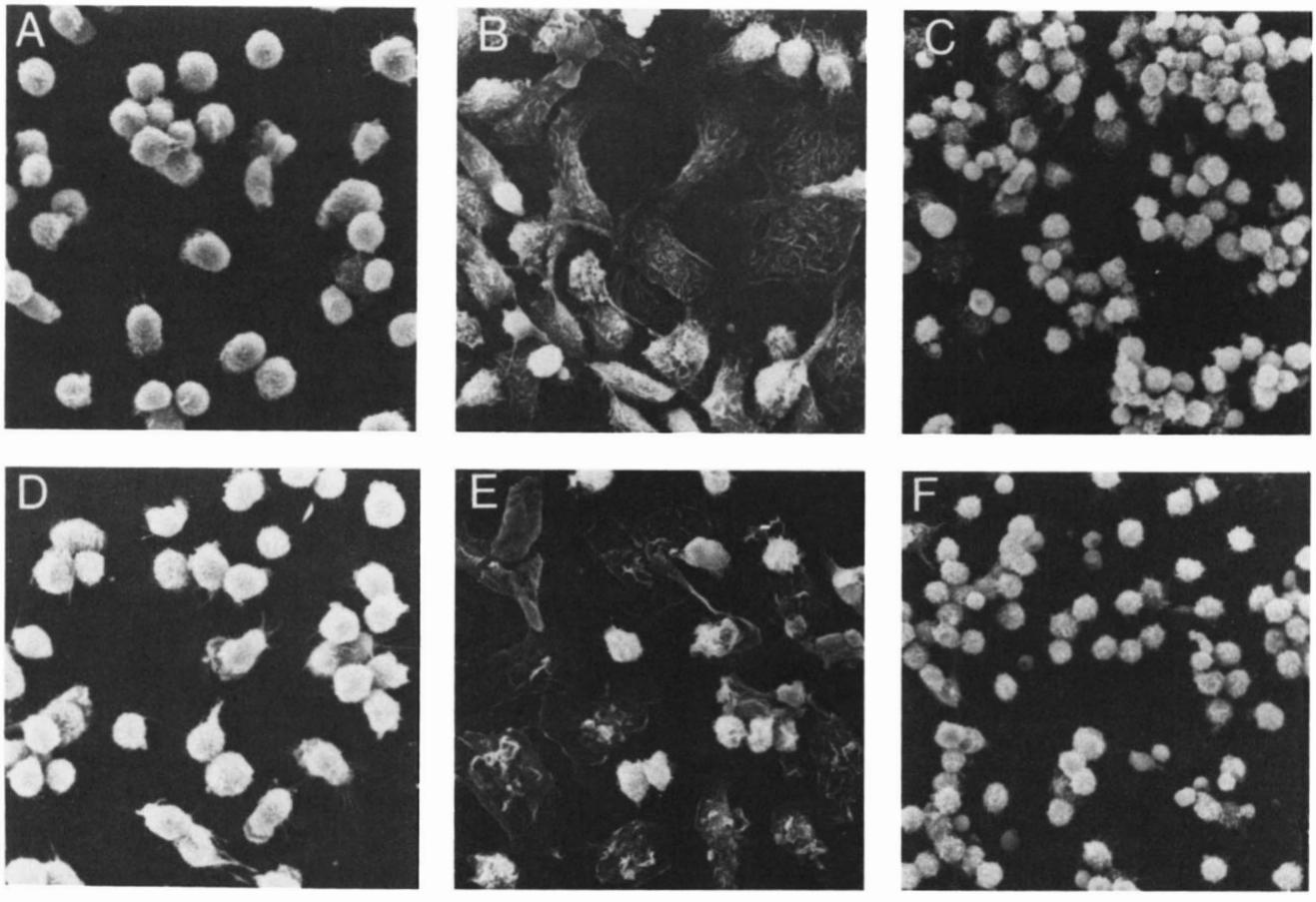

Fig. 1. Scanning electron micrographs of pulmonary, peritoneal, and monocyte-derived macrophages. First row, panels A, B, and C are pulmonary, peritoneal, and monocyte-derived macrophages, respectively, isolated from control rabbits. Second row, panels D, E, and F are pulmonary, peritoneal, and monocyte-derived macrophages, respectively, isolated from rabbits fed a hypercholesterolemic diet for 8–12 weeks, as described in Methods. All photographs are at 650 × magnification.

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TABLE 1. Composition of pulmonary, peritoneal and blood-derived macrophages

	Pulmonary		Peritoneal		Blood-derived	
	Control	Chol-fed	Control	Chol-fed	Control	Chol-fed
	μg per μg DNA					
Protein	32.8 ± 1.8	33.9 ± 6.6	23.5 ± 2.4	18.6 ± 3.3	9.81 ± 0.99	13.9 ± 2.6
Unesterified cholesterol	0.660 ± 0.010	0.708[a] ± 0.027	0.765 ± 0.028	0.989[c] ± 0.0167	0.183 ± 0.016	0.320[c] ± 0.015 (4)
Cholesteryl ester	0.003 ± 0.005	0.106[b] ± 0.032	0.120 ± 0.030	0.526[a] ± 0.011	0.00 ± 0.006	0.070[a] ± 0.005 (4)

Assays for DNA, protein, and free esterified cholesteryl were conducted as described in Methods. Values represent the mean ± SD. Number of observations equals 3 unless otherwise indicated in parentheses.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

whereas in monocyte-derived macrophages the increase of sterol as ester was about one-third.

Cholesterol efflux from pulmonary macrophages

Efflux of radioactivity from ^{3}H-labeled macrophages to unlabeled medium was linear for at least 9 hr. The dependence of efflux on medium serum concentration is shown in **Fig. 2**. Efflux of sterol from macrophages from both normo- and hypercholesterolemic rabbits increased rapidly as serum concentrations were increased to 20% (v/v), and increased more gradually thereafter. Very similar efflux rates were obtained with normo- or hypercholesterolemic

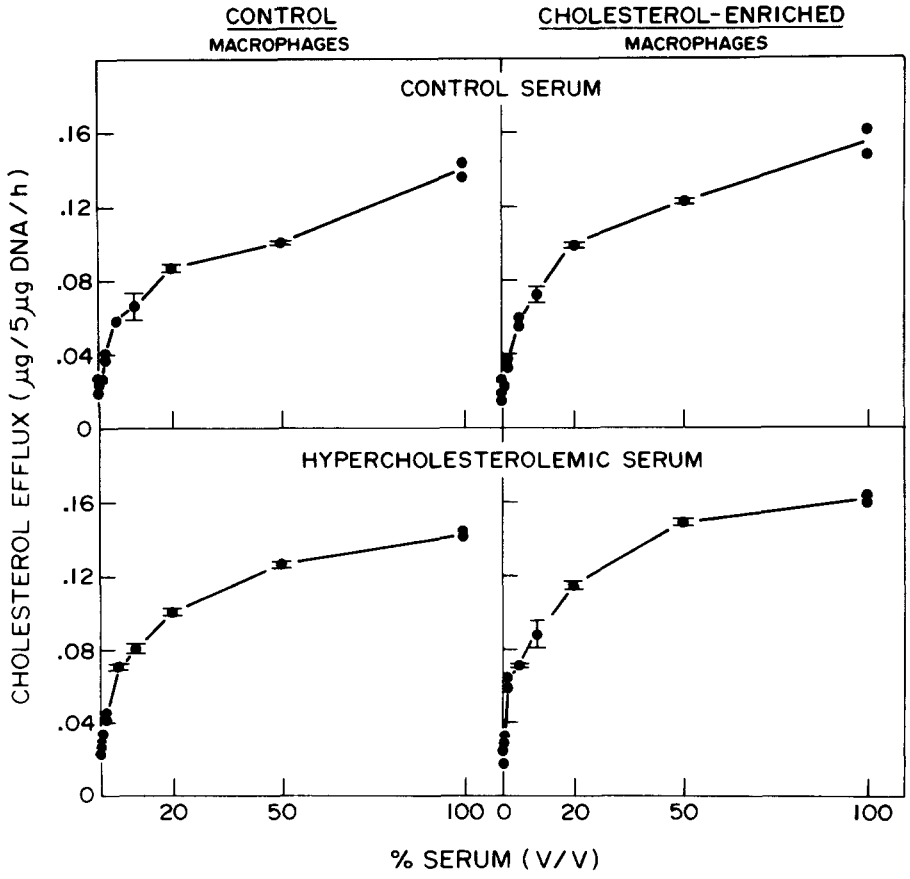

Fig. 2. The efflux of [^{3}H]cholesterol from control or cholesterol-enriched pulmonary macrophages as a function of serum concentration. Control and hypercholesterolemic rabbits were labeled in vivo with [^{3}H]cholesterol. Pulmonary macrophages were isolated from these two rabbits and each set of macrophages was incubated for 5 hr with control and hypercholesterolemic serum obtained from non-labeled rabbits. Efflux of sterol was determined as described in Methods.

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peritoneal macrophages incubated with normo- or hypercholesterolemic serum.

Cholesterol influx into pulmonary macrophages

Influx of cholesterol from 20% hypercholesterolemic serum was proportional to time over a 5-hr incubation period. As shown in **Fig. 3**, influx, like efflux, was highly dependent upon medium serum concentration. However, two differences were apparent. Firstly, influx was several-fold greater from hypercholesterolemic serum than from the same serum concentration of normocholesterolemic serum over the whole concentration range ($P < 0.001$). Secondly, macrophages from cholesterol-fed rabbits also had lower rates of influx of cholesterol from hypercholesterolemic serum when compared to control macrophages, as determined by two-way analysis of variance ($P < 0.05$) (Fig. 3) (17).

The consequences of the integration of influx and efflux processes in terms of cholesterol balance are shown in **Fig. 4**. Hypercholesterolemic serum at all concentrations mediated cholesterol net transport from the medium to the cells. Normocholesterolemic serum had much less effect on the sterol content of macrophages. Finally, the net transport of cholesterol from hypercholesterolemic serum into macrophages from cholesterol-fed rabbits was much less than that into macrophages from control rabbits. This finding indicates that macrophages from rabbits made hypercholesterolemic by cholesterol feeding are resistant to a significant extent to the cholesterol loading mediated by lipoproteins of hypercholesterolemic serum ($P < 0.025$). On the other hand, influx from normocholesterolemic serum into macrophages from either group of animals showed no apparent difference.

The effects of plasma lipoproteins on cholesterol balance

The lipoprotein basis of the increased influx observed with hypercholesterolemic serum was investigated by using serum labeled in vivo with [^{3}H]cholesterol and then fractionated by heparin-Sepharose affinity chromatography, as described under Methods. After fractionation of the serum, the distribution of radiolabeled cholesterol was

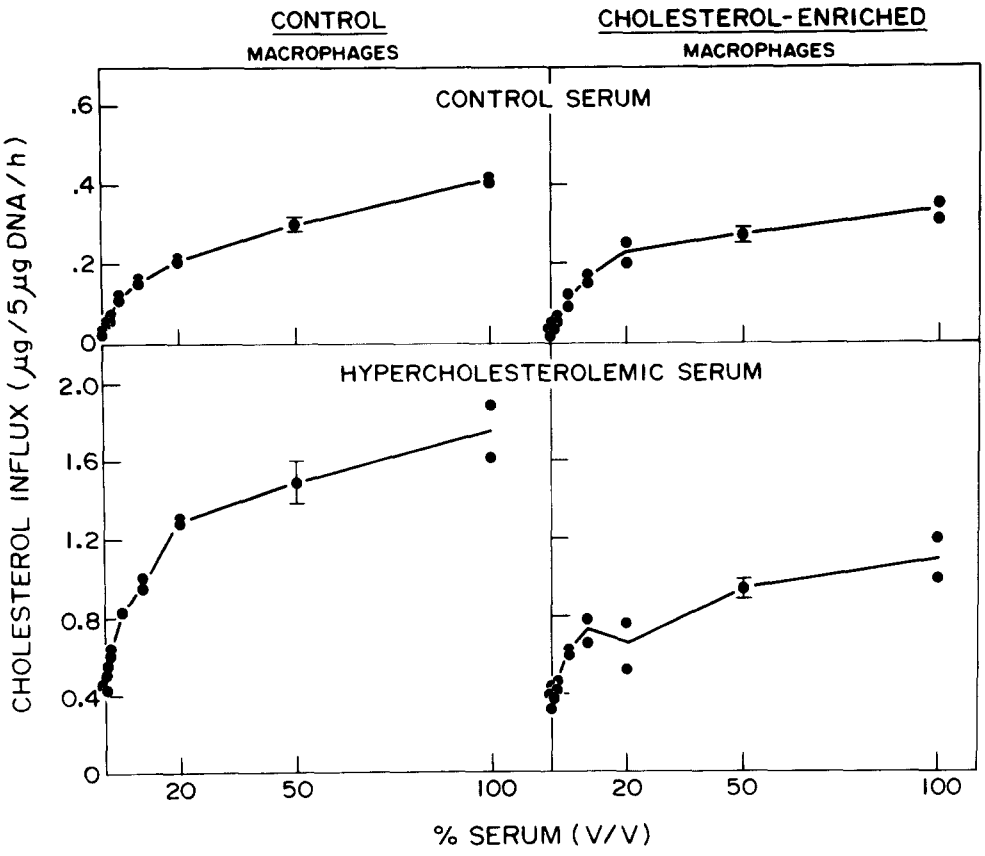

Fig. 3. Influx of [^{3}H]cholesterol from control or hypercholesterolemic serum into control or cholesterol-enriched pulmonary macrophages. The sera from the rabbits of the previous experiment (Fig. 2) were incubated for 5 hr with control or cholesterol-enriched pulmonary macrophages. Influx of sterol was determined as described in Methods.

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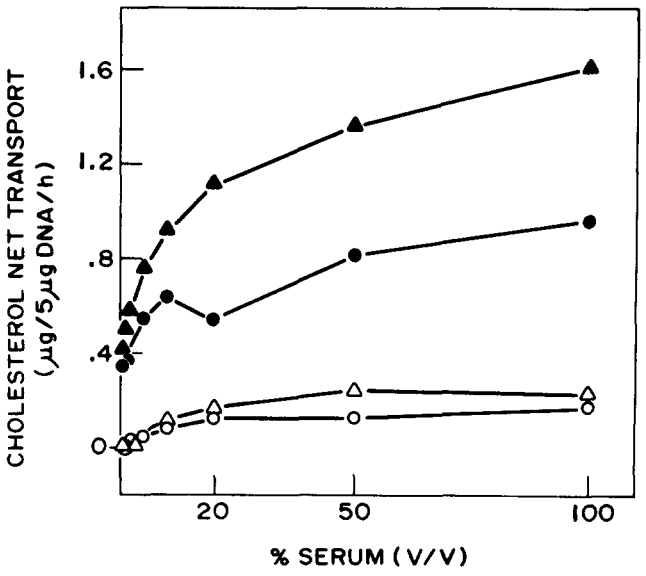

Fig. 4. Net cellular accumulation of sterol in control (triangles) or cholesterol-enriched (circles) pulmonary macrophages incubated with control (open symbols) or hypercholesterolemic (closed symbols) serum. The difference between influx and efflux was calculated from Figs. 2 and 3. A positive value indicates an influx that is greater than efflux when the macrophages are incubated in rabbit serum closely matched as to serum cholesterol.

96% in the retained fraction and 3.7% in the nonretained fraction. The volumes of the two pooled fractions were adjusted to reflect this distribution. As shown in **Fig. 5**, essentially the whole of cholesterol influx in serum was mediated by the retained (beta-VLDL) fraction of serum.

The cellular accumulation of sterol was also determined directly (Fig. 5). Practically all (86–104%) of the influx of radiolabeled sterol from whole plasma was reflected as an equivalent cellular accumulation of sterol.

Comparison of the effects of pulmonary, peritoneal, and blood-derived macrophages

Qualitatively, pulmonary and peritoneal macrophages from the different dietary groups showed generally comparable reactions during incubation with 50% serum (**Fig. 6**). Both had a much greater rate of influx when incubated with hypercholesterolemic than with control serum (both $P < 0.001$). There were no statistically significant differences in efflux when comparing efflux into control or hypercholesterolemic serum.

Quantitatively, control and cholesterol-enriched peritoneal macrophages were as active in taking up cholesterol from hypercholesterolemic serum as pulmonary macrophages for the same rabbits, but the peritoneal macrophages were more active in releasing cholesterol than pulmonary macrophages ($P < 0.05$ in all cases). These same trends in efflux between peritoneal and pulmonary macrophages from either control or cholesterol-fed rabbits were also observed when the cells were incubated in control serum ($P < 0.005$ in all cases). There was always a net inward flux of cellular sterol (mean 0.378 ± 0.150 μg/5 mg of DNA per hr; range 0.181–0.610, n = 9) when cholesterol-enriched peritoneal macrophages were incubated in hypercholesterolemic serum. The same macrophages showed a slight net efflux of cholesterol when incubated with control serum (0.078 ± 0.064 μg/5 mg of DNA per hr; n = 8).

Pulmonary and peritoneal macrophages from cholesterol-fed rabbits generally showed lower rates of influx of cholesterol from 50% hypercholesterolemic serum when compared to control pulmonary and peritoneal macro-

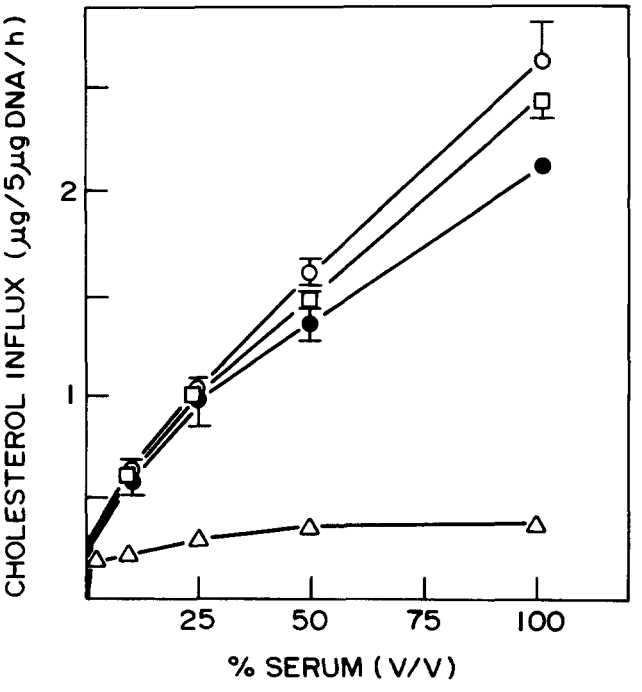

Fig. 5. The influx of radiolabeled cholesterol and the accumulation of cholesterol mass in pulmonary macrophages. Pulmonary macrophages from control rabbits were incubated for 5 hr with various dilutions of hypercholesterolemic serum or with hypercholesterolemic serum fractionated by heparin-Sepharose affinity chromatography. The hypercholesterolemic serum was obtained from rabbits labeled in vivo with [^{3}H]cholesterol. The influx of tritiated cholesterol was then determined as described in Methods. ○—○, Unfractionated serum; △—△, the fraction not retained on the affinity column at 50 mM NaCl; □—□, the fraction adsorbed to the column at 50 mM NaCl but released with 3 M NaCl (beta-VLDL); ●—●, influx from whole plasma determined chemically. Values represent the mean ± SD, n = 3, except at 100% serum where n = 2, variation about the mean was ± 10%. Undiluted serum contained 1750 mg % total serum cholesterol. The beta-fraction represented 93% of total recovered plasma cholesterol; its composition (w/w %) was: protein, 4.4 ± 1.1%; phospholipid, 13.4 ± 2.2%; free cholesterol, 8.9 ± 1.9%; esterified cholesterol, 60.6 ± 5.7%; and triglyceride, 12.7 ± 2.0% (n = 6).

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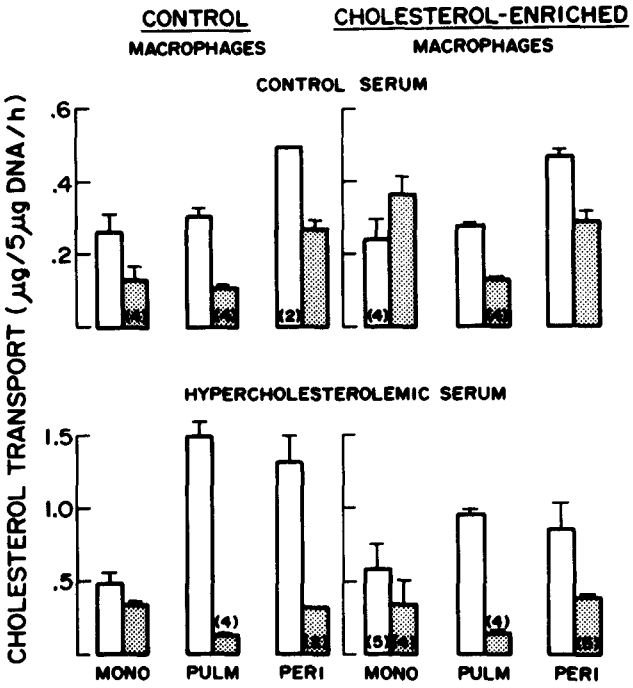

Fig. 6. The influx and efflux of cholesterol in pulmonary, peritoneal, and monocyte-derived macrophages. Mononuclear phagocytes from three tissue sources (lung, peritoneal cavity, blood) were isolated from control or hypercholesterolemic rabbits and incubated for 2–3 hr with 50% control or hypercholesterolemic serum. Solid bars indicate influx; dotted bars indicate efflux; mono, monocyte-derived macrophages; pulm, pulmonary alveolar macrophages; peri, peritoneal macrophages.

phages (1.49 ± 0.11 for control vs. 0.95 ± 0.04 for cholesterol-fed pulmonary cells ($P < 0.005$); 1.32 ± 0.18 for control vs. 0.86 ± 0.18 for peritoneal cells ($P < 0.05$)) (Fig. 6). Nevertheless, despite the resistance to influx and the increase in efflux in cholesterol-enriched pulmonary and peritoneal macrophages, these cells still had a net influx of cholesterol when incubated with hypercholesterolemic serum.

A quite different response was seen in the case of the monocyte-derived macrophages. Monocyte-derived macrophages had an influx of cholesterol that was more similar to their efflux of cholesterol, even when incubated with 50% hypercholesterolemic serum. Consequently, these monocyte-derived macrophages did not accumulate major amounts of cellular sterol, as did pulmonary and peritoneal macrophages when incubated with 50% hypercholesterolemic serum, and the differences in flux rates between control and hypercholesterolemic monocytes in hypercholesterolemic plasma did not reach significance. As with pulmonary and peritoneal macrophages, monocyte-derived macrophages from cholesterol-fed rabbits had a significantly greater rate of cholesterol efflux than did monocyte-derived macrophages from control rabbits when both of the sources of macrophages were incubated with normocholesterolemic serum ($P < 0.005$).

DISCUSSION

All tissue macrophages are believed to be derived from a homogeneous precursor pool of blood-borne monocytes (18). In vivo, the monocytes differentiate into macrophages after their passage through the blood vessel walls, and in vitro, after culturing in 20–30% serum (2, 19). The properties of macrophages are quite distinct in many respects from those of monocytes. Also, not only are the environments of the macrophages very different from that of the monocytes, but the macrophages of different tissues also have a varied set of environments. Pulmonary macrophages are in the alveolar fluid, which is high in surfactant and which is exposed to the aqueous–air interface of the alveolus. Surfactant is low in cholesterol/phospholipid ratio, relative to plasma (20). The peritoneal macrophages are bathed in the modified intercellular fluid of the relatively hypoxic peritoneal cavity (18). Resident unactivated and unstimulated pulmonary macrophages appear as roundish cells in cell culture. Peritoneal macrophages, on the other hand, have a much greater propensity to spread upon the culture plate; their cell shape becomes very irregular. Nevertheless, the results of this study indicate that pulmonary and peritoneal macrophages, as a whole, have qualitatively similar cholesterol transport properties. Both have a large increase in the rate of sterol influx when incubated in hypercholesterolemic serum, while their rate of sterol efflux is relatively stable. In view of the widespread use of the rabbit as a model for studies of cholesterol deposition, the relative difficulty of isolating and culturing monocytes (21), and the relatively low numbers of peritoneal macrophages in this species (22), the rabbit pulmonary macrophage appears to represent a useful model for studies of cellular cholesterol balance.

This is not the case for monocytes cultured for 21 hr in the presence of autologous serum. The 21-hr monocyte-derived macrophages exhibit properties of cellular metabolism that are similar in some respects to those in mature macrophages (19, 23). However, in the former cells, the ability to accumulate lipoprotein cholesterol when incubated with unfractionated hypercholesterolemic serum is much less than in the case of pulmonary and peritoneal macrophages. These cells minimize cholesterol accumulation primarily by limiting the influx of cholesterol. The cholesterol storage capacity of the macrophage is therefore, presumably, a function acquired in later stages of differentiation. Resident pulmonary and peritoneal macrophages are mature macrophages and, as we have shown herein, do accumulate large amounts of sterol. That monocytes in the blood do not accumulate sterol may perhaps represent a protective function in hypercholesterolemia, preventing the transport of sterol into tissues when monocytes cross the vascular bed.

Pulmonary and peritoneal macrophages from hypercholesterolemic rabbits are less active in the rate of influx

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of sterol than are macrophages from normocholesterolemic rabbits under the same conditions. This resistance may be due to the interplay of several factors. Two of these are: the potential down-regulation of lipoprotein receptors (1), and the encumbrance of plasma membrane activities by a high cholesterol to phospholipid ratio, which decreases the fluidity of the plasma membrane (24).

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