METABOLISM OF $^{125}$I-LABELED LIPOPROTEINS
BY THE ISOLATED RAT LUNG

GIUSEPPE G. PIETRA, LUIGI G. SPAGNOLI, DAVID M. CAPUZZI,
CHARLES E. SPARKS, ALFRED P. FISHMAN, and JULIAN B. MARSH

From the Cardiovascular-Pulmonary and Pathologic Anatomy Divisions,
Departments of Medicine and Pathology, School of Medicine, and
the Department of Biochemistry, School of Dental Medicine,
University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT
The capacity of the isolated perfused rat lung to metabolize the protein moieties of
serum lipoproteins was assessed using homologous (rat) and heterologous (hu-
man) plasma lipoproteins. The protein and lipid moieties of the plasma lipopro-
teins were labeled in vitro with Na$[^{125}$I]. In selected cases the lipoprotein peptides
were labeled in vivo with $^{14}$C- or $^{3}$H-labeled amino acids. Uptake of lipoprotein
label during perfusion was monitored by measure of losses in perfusate label and
by rises in pulmonary tissue labeling as shown by radioassay and by light and
electron microscope radioautography. Lipoprotein degradation was assessed by
fractionation of perfusate and lung tissue radioactive material into trichloroacetic
acid (TCA)-insoluble, TCA-soluble, and ether-ethanol-soluble fractions.

When heparin was included in the perfusion medium, there was selective
degradation of the protein portion of very low density lipoprotein (VLDL) in the
perfusate and concomitant uptake of radioactive label by the lungs. Low density
lipoprotein (LDL) was neither taken up nor catabolized by the isolated rat lung in
the absence or presence of heparin. By light and electron microscopy, the label
was localized over the interalveolar septa, predominantly the capillary endothe-
lium. Disappearance of TCA-insoluble radioactivity from the perfusate was asso-
ciated with the generation of both TCA-soluble iodide and noniodide radioactiv-
ity. Greater than 50% of the radioactive label taken up by the lungs was found in
the delipidated TCA-insoluble fraction. This study provides in vitro evidence for
pulmonary catabolism of VLDL apolipoproteins and uptake of peptide catabolic
products of VLDL by the lung.

The endothelium of capillaries (8, 41, 45) and of
large arteries (38, 51, 52) of the systemic circula-
tion contains a lipoprotein lipase (LPL)$^1$ that par-
ticipates in the hydrolysis and conversion of chy-
lomicrons (CM) and very low density lipoproteins
(VLDL) to cholesterol-rich remnants (3, 7, 12-

$^1$ Abbreviations used in this paper: BSA, bovine serum albumin; CM, chylomicrons; EDTA, ethylenediamine-
tetraacetate; FA, fatty acids; KRB, Krebs-Ringer bicar-
bonate buffer; LDL, low density lipoproteins; LPL, lipoprotein lipase; TCA, trichloroacetic acid; VLDL, very
low density lipoproteins.
The pulmonary circulation also contains a vast capillary and arterial endothelial bed, and a pulmonary LPL active against labeled chylomicrons has been described (22, 23). However, very little data are available on the metabolism by the lung of the circulating lipoproteins in this low pressure vascular system which is much less susceptible to arteriosclerosis (25). Previous studies have strongly suggested that the lung can hydrolyze endogenous and exogenous triglycerides (15, 24, 25) perhaps by virtue of its LPL, possibly for the continuous generation of free fatty acids for surfactant phospholipid production. But the data available on lipoprotein-lipid degradation by the lungs are meager, and the possible role of the lungs in metabolizing the protein moieties of the circulating lipoproteins remains virtually unexplored (33). In the present study with an isolated perfused rat lung preparation (2, 19), we have investigated this problem, and our data provide the first documentation that the rat lung in vitro can catabolize the proteins of either rat or human VLDL.

EXPERIMENTAL PROCEDURE

Experimental Methods

Isolated Perfused Lung: Male rats (200-250 g) were anesthetized by an injection of sodium pentobarbital (50 mg/kg, body weight) intraperitoneally. The animals were artificially ventilated through a tracheostomy by placing a small magnetic stirrer at the bottom of the pulmonary vein to the bottom of the chamber and was recirculated to the lungs after being aerated with 95% O$_2$ and 5% CO$_2$. The lungs were ventilated with room air at a tidal vol of 2.5 ml and an end-expiratory pressure 1-2 mm Hg. Complete mixing of the perfusate was insured by placing a small magnetic stirrer at the bottom of the incubation chamber. Perfusion fluid pH, PO$_2$, and PCO$_2$, were continuously measured with an in-flow glass cuvette with appropriate electrodes. Pulmonary artery and intracheal pressures were continuously monitored by means of pressure transducers connected to an oscilloscopic recorder (2). The perfusion pressure was maintained between 8 and 15 mm Hg at a perfusion rate of 10 ml/min. Duplicate samples of the perfusate for radioassay were taken immediately before connecting the lung to the perfusion apparatus and after 10 min of perfusion for equilibration (0 time). Subsequent duplicate samples were taken 20, 40, and 60 min after connecting the lung. In the initial series of experiments, at the end of the perfusion the lungs were fixed for anatomic studies by intravascular perfusion with 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4, 450 mosmol). In a second series of experiments, the lung was homogenized for biochemical studies (see below).

Materials

Male rats (200-250 g) were supplied by Charles River Breeding Laboratories, Wilmington, Mass. Rodent respiratory and peristaltic pumps were obtained from Harvard Apparatus Co., Millis, Mass. and Po$_2$, Pco$_2$, pH electrodes from Radiometer, London Co., Cleveland, Ohio. Photographic emulsions NTB-2 and Ilford L-4 were purchased from Eastman Kodak, Rochester, N. Y. and Ilford Ltd., Ilford, Essex, U. K., respectively. Commercial sources of chemicals were as follows: Miles Laboratories, Kankakee, Ill., bovine serum albumin, Fraction V; Polysciences, Inc., Warrington, Pa., paraformaldehyde, glutaraldehyde, uranyl acetate, and other chemicals for electron microscopy; New England Nuclear, Boston, Mass., Na$^{[35]I}$ (17 Ci/mg) carrier-free, [1-$^4$C]glycine (47 mCi/mmol), [2-$^3$H]glycerol (200 mCi/mmol), and [$^3$H]-l-amin acid mixture. All other chemicals were of analytical grade.

Lipoproteins

Isolation of VLDL: Serum was obtained by plasmapheresis of a human donor or by bleeding rats via the abdominal aorta. Plasma lipoproteins were separated by ultracen trifugal flotation in the Spinco model L2-65B ultracentrifuge according to Havel et al. (21). Plasma (d = 1.006) containing 1 mg/ml ethylenediaminetetraacetate (EDTA) was centrifuged at 4°C for 16 h at 350,000 g in the 60 Ti rotor. The supernatant fraction was carefully removed from the top of the tubes with a needle and syringe. The VLDL obtained was purified by two subsequent floatations through a layer of saline (0.15 M)-EDTA (2 mM) in the 65 rotor. The VLDL migrated as a single band with pre-$eta$-mobility during agarose gel electrophoresis (32).

Isolation of LDL: The infranatant fraction after removal of VLDL was adjusted to a density of 1.063 with a solution of KBr-NaCl (1.35 g/ml) for isolation of human LDL and to a density of 1.050 for isola-
ion of rat LDL. Ultracentrifugation of the density-adjusted fractions was then carried out for 18 h at 350,000 g in the 60 Ti rotor. The top fraction was twice recentrifuged in the 65 rotor through a layer of KBr-NaCl solution (d = 1.063). The purified LDL migrated as a single band with \( \beta \)-mobility during gel electrophoresis.

**Iodination:** Human or rat VLDL and LDL were each iodinated with carrier-free Na\(^{125}\)I (17 Ci/mg) by a modification (7) of the iodine monochloride method of McFarlane (34). Rat albumin was also radioiodinated by the McFarlane method. The labeled lipoproteins were then dialyzed against three-four changes of saline-EDTA over a 24-h period. Labeling efficiency of the lipoproteins ranged from 10 to 40%. The labeled lipoproteins contained less than 1 g-atom iodine per \( 2 \times 10^8 \) g protein. At least 92% of the labeled material was TCA-precipitable, and no change in electrophoretic mobility occurred after lipoprotein iodination. The proportion of label in human lipoprotein lipids separated by extraction with CHCl\(_3\)-CH\(_2\)OH (2:1) by the method of Folch et al. (17) ranged from 2.6 to 11.0% for \([^{125}\text{I}]\text{VLDL}\) and 0.6 to 0.8% for \([^{125}\text{I}]\text{LDL}\), while the proportion of label in lipids of the corresponding rat plasma lipoproteins was consistently higher. The percentage distribution of extracted labeled lipids of human \([^{125}\text{I}]\text{VLDL}\) separated by thin-layer chromatography as previously described (10) was as follows: phospholipids (33-42%), unesterified cholesterol (18-35%), triglycerides (7-16%), and esterified cholesterol (2-6%).

**Anatomic Studies**

For light microscope autoradiography, lung slices, fixed as described earlier, were embedded in methacrylate (42); 1-\( \mu \)m thick sections were coated with Kodak NTB-2 emulsion diluted in equal parts of distilled water. The sections were exposed for 6 wk at 4\( ^\circ \)C in light-tight boxes containing drierite, developed in D-19 (Eastman Kodak) full strength, cleared in Hypo, washed in water, and stained with hematoxylin and eosin.

For electron microscope autoradiography, small cubes of lung tissue were postfixed in 2% OsO\(_4\) in 0.1 M sodium cacodylate buffer, stained en bloc with uranyl acetate (27), dehydrated in ascending grades of acetone, and embedded in Epoxy resin. Sections with silver interference color were coated with Ilford L-4 emulsion with a Kopriwa coating apparatus (30), exposed for 9-12 wk, developed by gold latensification method (43), and stained with lead citrate or lead hydroxide.

**Biochemical Studies**

Decline of radioactivity was measured in duplicate aliquots (2 ml) of perfusate containing labeled human lipoproteins. The perfusate samples were precipitated in cold 10% trichloroacetic acid (TCA) and the precipitates were washed once with 5% TCA. The lipids of the TCA precipitates were removed by extraction with ether-ethanol (3:1, vol/vol) by the method of de Jong and Marsh (11). The delipidated TCA-insoluble precipitates were dried with ether, dissolved in 88% formic acid, and counted in a Nuclear-Chicago gamma counter with an efficiency of 73%. The TCA-soluble fractions were similarly counted. Thus, both TCA-insoluble and TCA-soluble fractions were lipid-free in all our experiments. Results were subjected to Student's \( t \) test for statistical significance. Differences between mean values were considered to be statistically significant at the \( P < 0.05 \) level.

In experiments with rat \([^{125}\text{I}]\text{VLDL}\) duplicate 2-ml aliquots of perfusate were precipitated in 5% TCA. The aliquots were separated into precipitate and supernatant fractions. The precipitate lipid was extracted as before with ether-ethanol, resulting in lipid and protein fractions. The supernatant iodide was removed with H\(_2\)O\(_2\) and CH\(_3\)\(_4\) (4), resulting in iodide and aqueous fractions (presumably amino acids and small peptides). All fractions were counted for \( ^{125} \)I label in a Beckman Biogamma 1 (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) with an efficiency of 81%. After correcting for chloroform absorption of iodine counts, there was greater than 90% recovery of the initial counts in the iodine, aqueous, protein, and lipid fractions.

**Lung Homogenate:** After perfusion with \([^{125}\text{I}]\text{VLDL}\) the rat lungs were washed with cold perfusion medium until the effluent contained less than 0.5% of initial radioactivity. The lung parenchyma was removed from supporting structures, frozen in liquid N\(_2\), and mechanically pulverized. Weighed aliquots of lung powder were suspended in saline, and precipitated in 5% TCA. Radioactivity in the TCA-insoluble and TCA-soluble fractions was assessed as described in the preceding section.

**Analysis of Radioautograms**

Light microscope autoradiographic studies were carried out on 11 lungs perfused with \([^{125}\text{I}]\text{VLDL}\), five perfused with \([^{125}\text{I}]\text{LDL}\), and three perfused with \([^{125}\text{I}]\text{albumin}\). Tissue samples were taken from randomly selected areas of three different lobes of each lung. In each section, grain density over lung structures was obtained by determining the number of grains per surface area in five randomly selected high-power fields (\( > 1,000 \)). Surface area was measured with the aid of an ocular micrometer containing evenly spaced grid lines, excluding the lumens of the blood vessels. Grain density over lung structures was corrected for background radiation which was measured in a similar manner over parts of the slides devoid of lung tissue.

For electron microscope radioautography, low power (\( > 4,000 \)) micrographs were taken to include every developed grain seen over lung tissue, excluding the lumens of the capillaries and areas containing faults in the sections or emulsions, such as folds, holes, etc. 72 electron micrographs so obtained were magnified to a final magnification of 10,000, and silver grains were
assigned to the tissue compartments over which their geometric center fell (18, 44). The following compartments were identified: endothelium, interstitium, type I and type II pneumocytes, and an area of alveolar space 1–2 μm in width adjacent to the epithelium.

The surface area of each tissue compartment was measured by superimposing over the radiographs a lattice with uniformly spaced points (18). Grain density was obtained by the division of the number of grains by the number of points falling within the various tissue compartments. Areas that did not contain lung tissue were used to determine the background density for each grid. Grain density over tissue compartments was then corrected for background radiation. Differences between mean values in grain density over various compartments were subjected to Student's t test for significance.

RESULTS

Criteria of Anatomic and Functional Integrity of the Isolated Perfused Rat Lungs

The preservation of the normal permeability characteristics of the pulmonary circulation in our preparations was judged by continuous recording of perfusion- and end-expiratory pressures and by examination of ultra-thin sections by electron microscopy. With these criteria, preparations showing persistent elevation of end-expiratory pressure or ultrastructural evidence of widespread cell damage or interstitial edema were discarded. Previous work with isolated perfused rat lungs (D. J. P. Basset, personal communication) has shown that elevation of end-expiratory pressure is a very sensitive gauge of interstitial edema that presages alveolar edema. It is known that normal pulmonary capillaries in vivo are virtually impermeable to macromolecules of the size of albumin or larger (39). In preliminary experiments, the integrity of the pulmonary capillary endothelium of isolated perfused rat lungs was tested by adding, to the perfusate, probes for large endothelial leaks (colloidal carbon, Pelikan Biological Ink, Guenther Wagner Pelikan Co., New York), or [125I]albumin. For this purpose, isolated rat lungs were perfused for 60–180 min with the complete medium plus heparin (2 U/ml). At the beginning of the perfusion, colloidal carbon (0.1 ml) or [125I]albumin (1.5 mg) was added. In the case of albumin, perfusate samples were taken and treated as described earlier for lipoproteins. In both groups of experiments, the lungs were examined either by conventional transmission electron microscopy or by light and electron microscope radioautography. No extravascular accumulation of either tracer was detected, indicating that, under our experimental conditions, the permeability of the capillary endothelium of the isolated perfused lung was not distinguishable from that of the lung in vivo (39).

Anatomic Observations

Light Microscope Radioautography: 19 lungs perfused with labeled lipoproteins or albumin were utilized for light and electron microscope autoradiographic observations. Developed silver grains in numbers exceeding that of background were present only over the interalveolar septa of lungs perfused with [125I]VLDL and heparin at dose levels of 2 U/ml or higher. Silver grains were distributed locally near the capillary walls and over alveolar septal cells located predominantly at the confluence of two or more alveolar septa (Fig. 1); however, precise identification of cell types incorporating radioactive label could not be made with confidence with the light microscope. No uptake of radioactive label was seen over extra-alveolar structures (large blood vessels, conducting airways, or visceral pleura). In areas of uptake, the density of developed silver grains over the interalveolar septa after correction for background averaged 7.1 ± 1.9 SEM per 100 μm² of lung tissue (P < 0.001).

Electron Microscope Radioautography: The sites of uptake of radioactive label were more clearly identified by electron microscopy. Silver grains over lung tissue were present only in micrographs of lungs perfused with VLDL and heparin at concentrations which resulted in biochemical evidence of uptake of radioactive label. The label was most frequently located over capillary endothelial cytoplasmic processes (Fig. 2) or attached to the luminal surface of capillary endothelial cells (Fig. 3). Several grains were found over interstitial cells, the ground substance of the interstitial space, the cytoplasmic processes of type I pneumocytes, or cytoplasmic organelles of type II pneumocytes (Fig. 4). Since the overall number of grains was small, no attempt was made to establish any preferential distribution of the label over intracellular structures. Nevertheless, in endothelial and epithelial cells, most grains were located over areas of the cytoplasm containing plasmalemmal vesicles (Fig. 2) or profiles of endoplasmic reticulum. Only few grains were distributed over mitochondria or nuclei. Only rarely was label found over the lamellae bodies of type II
Figure 1 Light microscope autoradiogram of 1-μm thick methacrylate-embedded section of a rat lung perfused with [125I]VLDL and heparin (2 U/ml). Silver grains (thick arrows), representing the isotope, are seen over two cells near the confluence of three interalveolar septa. Two endothelial cells (fine arrow) contain silver grains, although not so clearly seen as in the other two cells. The lumens of the alveolar capillaries contain only rare grains representing background radiation. ALV, alveolar space. × 750.

As indicated in the histogram (Fig. 5), all compartments of the alveolar septa had significantly higher density of label than did the neighboring alveolar spaces, but the highest concentration of label was over the endothelium. The difference in mean grain density between the endothelium and the other tissue compartments was statistically significant (P < 0.02). Since the silver grains are sometimes so large that they may overlie adjacent tissue compartments, either compartment could be the source of radioactivity responsible for the developed grains. Accordingly, the concentration of grains over the various compartments of the alveolar capillary membrane should be taken to indicate a trend rather than the absolute distribution of label throughout interalveolar septa.

Loss of radioactive label during fixation and dehydration from lungs that had been perfused with [125I]VLDL and heparin was determined in duplicate experiments. As shown in Table I, the label in the acetone extracts of the lungs was negligible even in absence of osmium tetroxide fixation and uranyl acetate en bloc staining. Hence, the radioactivity in lung lipids was probably quite low.

Biochemical Observations

Uptake and Degradation of Human [125I]VLDL: Lungs were perfused with [125I]VLDL in the absence or presence of heparin, an activator of LPL of various tissues (8, 31, 49). It was presupposed that this enzyme might play some initiating role in the pulmonary metabolic handling of circulating lipoproteins. For studies of human lipoprotein catabolism, heparin concentrations of 0.1 and 2 U/ml were tested in our system which contained 80–100 ml of perfusate. In the absence of heparin, there was no loss of labeled protein from the lung perfusate during a 60-min perfusion of the rat lung with [125I]VLDL (Fig. 6). In contrast, the inclusion of heparin (2 U/ml) in the perfusing medium produced a significant, progressive decline in levels of labeled protein in the perfusate. As shown in Fig. 7, the decline in total radioactivity of each unfractionated aliquot was accompanied by a fall in delipidated TCA-insoluble radioactivity in the perfusate and by a rise in TCA-soluble radioactivity. These changes occurred most rapidly during the initial 20 min of perfusion. At this level of heparin (2 U/ml), there was net loss of [125I]VLDL from the perfusate and a progressive conversion of labeled apolipoproteins to catabolic end products measured in the aqueous TCA-soluble phases. However, when lower concentrations of heparin (0.1 U/ml) were present in the perfusate, there was no removal or catabolism of [125I]VLDL (Table II). Thus, somewhat surprisingly, the presence of heparin (2 U/ml) in the medium appeared to stimulate the uptake and degradation of the protein moieties of human [125I]VLDL.

In contrast to the results with human [125I]VLDL, there was no uptake or degradation of labeled proteins when the lungs were perfused with either human or rat [125I]LDL or with [125I]albumin, at levels of heparin that had been shown to promote pulmonary metabolism of [125I]VLDL (Fig. 8, Table II). With [125I]LDL, there were no significant changes in radioactivity found in perfusate aliquots, in TCA-insoluble, or in TCA-soluble fractions over the course of perfu-
UFURGE 4 Type II pneumocyte with several silver grains over lamellar bodies and mitochondria. Experimental conditions as in preceding figures. LB, lamellar body; M, mitochondria. x 33,000.

sion (Fig. 8). Perfusion of the apparatus, per se, with perfusing solution containing $^{125}$I-VLDL and heparin (2 U/ml) but without the lung did not produce any change in perfusate radioactivity (Table II).

**Uptake and Catabolism of Rat $^{125}$I-VLDL:** In two experiments, rat lungs were perfused with medium containing rat $^{125}$I-VLDL and heparin to determine whether the homologous radioiodinated VLDL were metabolized in a fashion comparable to that of human $^{125}$I-VLDL. The results of the two experiments were very similar, and the data from one of the two are depicted in Fig. 9. As shown in the figure, while the total perfusate radioactivity declined by only 2% (Fig. 9a) over 60 min of perfusion, there were more striking changes in the chemical nature of the radioactive perfusate. Fractionation of the perfusate aliquots revealed a 15% decline in the TCA-insoluble precipitates of perfusate over 60 min of perfusion. Delipidation of these precipitates demonstrated that this decline represented a

![Figure 2](image2.png) Rat lung perfused with $^{125}$I-VLDL and heparin. The electron microscope radioautogram illustrates the localization of silver grains over a capillary endothelial cell. The grains are in a region of the cytoplasm containing numerous plasmalemmal vesicles (PV) and few profiles of smooth and rough endoplasmic reticulum (arrows). CAP, capillary; END, endothelium; BL, basal lamina; IC, interstitial cell. x 100,000.

![Figure 3](image3.png) Another view of a capillary endothelial cell after perfusion of the lung with $^{125}$I-VLDL and heparin, (2 U/ml). Two silver grains are seen attached to the luminal surface of an endothelial cell. IS, interstitial space. x 50,000.

PIETRA ET AL. Metabolism of $^{125}$I-Labeled Lipoproteins by Rat Lung 39
fall in labeled protein while the lipid radioactivity showed no significant changes with time (Fig. 9 b). Moreover, the TCA-soluble radioactivity tripled over the 60 min, and this rise was due almost equally to rises in inorganic 125I label and in non-iodide label probably representing amino acids and small peptides (Fig. 9 c).

When expressed in terms of labeled protein

The differences between mean values at 20 min are significant at P < 0.01.

**Table 1**

| Treatment          | Sample | Perfusate* | Lung† | Aldehydes§ | Buffer wash| Acetone§ |
|--------------------|--------|------------|-------|------------|-----------|----------|
| [125I]VLDL and heparin | 1      | 50,800     | 1,160 | 244        | 89        | 9        |
|                    | 2      | 31,000     | 280   | 40         | 13        | 4        |
|                    | 3      | 31,000     | 300   | 14         | 6         | 8        |

In each of two experiments, a rat lung was perfused for 60 min with heparin (2 U/ml) and human [125I]VLDL in perfusion medium. At the end of the experiment, the lungs were washed free of intravascular label by a postperfusion of 100 ml of unlabeled perfusion medium. Aliquots of perfusate and fractions of homogenized lung as obtained in Methods were isolated and counted in a gamma counter. Samples no. 2 and 3 were obtained from two different areas of the same lung.

* Cpm in 2-ml aliquots at 60 min.
† Cpm per 100 mg wet weight.
§ Cpm in 3 ml total volume.

In this experiment, osmication and uranyl acetate en bloc staining of lung tissue were omitted.
FIGURE 7 Decline in total perfusate radioactivity, decline in TCA-insoluble, delipidated protein radioactivity, and rise in TCA-soluble material in five experiments in which isolated rat lungs were perfused with 1.5-6.0 mg human \( ^{131} \text{I} \) VLDL and heparin (2 U/ml). Results are expressed as mean percent change in perfusate radioactivity from baseline values; bars show SEM. The experiments are the same as in Fig. 5 (x-x). The other experimental details are outlined under Experimental Methods. The differences between mean values at 0 and 60 min are significant at \( P < 0.01 \).

**TABLE II**

**Effect of Perfusion Isolated Rat Lungs with Heparinized Solution Containing \( ^{125} \text{I} \)-Labeled Human Lipoproteins or Albumin**

| No. of lungs | Labeled protein | Heparin | Uptake |
|--------------|-----------------|---------|--------|
|              | U/ml of perfusate | \( \mu \text{g} \) lung/h |        |
| 2            | VLDL            | 0.1     | 0      |
| 5            | VLDL            | 2       | 44.4 ± 10.7\$ |
| 3            | LDL             | -       | 0      |
| 3            | Albumin*        | 2       | 0      |
| 0*           | VLDL            | 2       | 0      |

* Lungs were omitted from perfusion system.

\$ \text{SEM.}

**FIGURE 8** Lungs were isolated from three rats and perfused with 1.0-15.0 mg \( ^{125} \text{I} \) VLDL and heparin (2 U/ml) for 60 min as in Experimental Methods. Total perfusate, delipidated TCA-insoluble and TCA-soluble radioactivity are depicted.

**FIGURE 9** In one experiment, lungs from one rat were perfused with medium containing 595 \( \mu \text{g} \) of rat \( ^{125} \text{I} \) VLDL (1,680 cpm/\( \mu \text{g} \) protein) and heparin (10 U/ml). The label in lipids in the rat VLDL amounted to 12%, with the remainder on the protein. The perfusate was fractionated at time 0, 30, and 60 min as described in Methods. (a) Decline in concentration of radioactive label in 2-ml aliquots of perfusate during 60-min perfusion; (b) Decreasing radioactivity in the perfusate is a result of loss of delipidated protein precipitate from the medium; (c) The increase in TCA-soluble radioactivity corresponds to both an increase in iodide and aqueous radioactivity.
taken up and degraded in the two experiments, 139 μg and 70 μg of the starting labeled VLDL protein were removed from the medium by 60 min. Of this total, 105 μg and 41 μg, respectively, of labeled VLDL protein were converted to TCA-soluble material. Surprisingly, in neither experiment was there an observed decrease in the lipid extractable radioactivity in the medium (Fig. 9 b). Homogenates of lung postperfused with unlabeled medium in each of the two experiments revealed a retention of, respectively, 1.5 μg and 3.8 μg of delipidated TCA-insoluble protein, and 0.6 and 1.7 μg of noniodide TCA-soluble label. The difference in lipoprotein uptake as determined by disappearance of radioactivity from the perfusate and by radioassay of the postperfused lung homogenate was probably due to removal of loosely bound or trapped lipoproteins by the unlabeled wash medium. Minimal amounts of ether-ethanol-soluble radioactivity were detected in the lung homogenates after perfusion. Of the total lipid label initially present in the perfusate, only 0.5% and 1.6%, respectively, were found in the lungs after 60 min. Thus, these two experiments provide convincing evidence for the uptake of rat [125I]VLDL protein and for conversion of a significant proportion of the labeled apolipoprotein material into catabolic products.

PULMONARY UPTAKE AND CATABOLISM OF BIOLOGICALLY-LABELED RAT VLDL:
In order to eliminate the possibility that iodination of lipoproteins in vitro altered these molecules and changed their biological behavior, experiments were done in which lungs were perfused with VLDL labeled biologically either in vivo or in the isolated perfused rat liver (36). As shown in Fig. 10, [3H]-labeled rat VLDL obtained by in vivo labeled amino acid injection was rapidly taken up by the isolated rat lung. Since only a negligible amount of this batch of VLDL was extractable with either CHCl₃:CH₃OH or ether:ethanol, the disappearance of label from the perfusion medium clearly indicates an uptake of VLDL protein. As further shown in the figure, the decline in perfusate radioactivity occurred only when the lung was present in the system. In a second experiment with rat VLDL, protein-labeled by perfusing an isolated rat liver with [14C]glycine (Fig. 11), there was a rapid significant decline in delipidated TCA-insoluble protein in the lung perfusate, hence confirming the uptake of the protein portion of rat VLDL by the rat lung. Thus, both biologically labeled VLDL which circulated in vivo in the whole animal before its isolation, and biologically labeled VLDL released de novo from the liver were similarly removed by the rat lung during an in vitro perfusion.

DISCUSSION
Ample evidence now exists to indicate that the lung plays an active role in lipid metabolism. Previous reports have suggested that this organ catabolizes the lipid moieties of chylomicra and that this lipid cargo may supply precursors for the synthesis of surfactant and cell membrane phospholipids (9, 22, 23, 25). The lung also is reported to be a source of lipoprotein lipase activity that is partially releasable by heparin but is unaffected by fasting (22). Felts has demonstrated that lung slices hydrolyze VLDL triglyceride and incorporate TG-FA (15). However, it is possible that VLDL hydrolysis found in the latter study may be due to release of tissue lipases, since, with organ slices, leakage of protein and enzymes into the medium can occur (26, 28, 35). However, with the isolated, perfused rat lung, we could find no evidence

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure10}
\caption{[3H]Protein-labeled rat VLDL was prepared by injecting two rats, which had been fed a high (60%) carbohydrate diet for 5 days, with 1 mCi of a [PH] labeled amino acid mixture. Plasma obtained by exsanguination 3 h after the intravenous injection was twice spun in the ultracentrifuge at d = 1.006 as described in Methods. Only 1.5% of the label was localized in VLDL lipids, with the remainder in the protein moiety. Each point represents total radioactivity in a 2-ml aliquot of perfusate of intervals up to 90 min of perfusion of labeled VLDL through an isolated rat lung preparation. The lung was present in the perfusion system between time 0 and 60 min.}
\end{figure}
In this experiment, the rat lung was perfused as previously described. However, the VLDL was biologically prepared in vitro by a 3-h perfusion of an isolated rat liver with KRB containing 4% BSA, [14C]glycine, [3H]glycerol, and unlabeled palmitate (36). The perfusate was spun twice in the ultracentrifuge as described in Methods, and the supernatant-labeled VLDL was used for a rat lung perfusion. Aliquots of perfusate were precipitated with TCA and extracted with ether-ethanol as in Methods. Each point represents a mean of duplicate TCA-insoluble protein samples of perfusate obtained at various intervals.

For significant removal of radioiodinated lipid from [125I]VLDL. Similarly, Hamosh and Hamosh (22, 23) found that uptake of the triglyceride moiety of chylomicrons in a similar in vitro lung preparation was of very low magnitude.

On the other hand, our data strongly indicate that the isolated rat lung possesses an unexpectedly great capacity for catabolism of the protein moiety of VLDL. Pulmonary degradation of labeled VLDL apolipoproteins in the rat was demonstrated with both rat and human [125I]VLDL and with two batches of biologically labeled rat VLDL. Radioautographic examination of the perfused lung corroborated this finding. Interestingly, the proteolytic action of the rat lung on labeled VLDL appeared to be specific for this lipoprotein class and was not found in perfusions with [125I]LDL or [125I]albumin. The physiological significance of our findings is unclear. However, the lung is known to be the source of other proteases with specific metabolic functions (16, 46, 50).

Though a number of tissues are considered to have a capacity for the catabolism of lipoprotein peptides (4–6, 20, 47, 48), the present study provides the first evidence that the lung may participate in the proteolytic process. The prospect that this catabolic role of the lung may be physiologically important is suggested by the anatomical position of the lungs at the outlet of the liver where VLDL is synthesized and released. The pulmonary microcirculation is the initial capillary bed traversed by newly formed VLDL, and therefore the lung may perform the first metabolic alterations of these molecules. In view of the rapidity of the process and the predominant ultrastructural localization of developed silver grains over the alveolar endothelium, it is likely that this endothelium which possesses protease and dipeptidase activities (46, 50) is largely responsible for the catabolic activity observed.

Since only a negligible amount of radioactivity was removed by the lipid solvents used to extract the samples of postperfused lung, and since [125I]VLDL lipids did not decline in the perfusion medium with time, the uptake of radioactive label by the lung probably represented predominantly labeled peptides. Clearly, a portion of the TCA-soluble radioactivity generated in experiments with rat [125I]VLDL represented deiodination, but an equivalent amount was aqueous noniodide radioactivity. The relatively large quantity of TCA-soluble, noniodide label that accumulated in the perfusate may indicate proteolysis of VLDL occurring at the cell surface and subsequent partial uptake of the catabolized products. Alternatively, it may result from release of small peptides and/or amino acids into the medium after intracellular hydrolysis of interiorized VLDL. Thus, the lung appears to behave differently than cultured arterial smooth muscle cells (4) or fibroblasts (1) which take up lipid in disproportionately greater quantities than protein.

The mechanism of the stimulatory effect of heparin on [125I]VLDL apoprotein catabolism by the lung is uncertain. Since the effect on [125I]VLDL catabolism occurred in the presence of a large excess (3 g/100 ml) of BSA, and was not observed with rat [125I]LDL or [125I]albumin, it is clear that there is a high degree of specificity for this phenomenon. Heparin may bind [125I]VLDL to the endothelium in the presence of calcium so that peptides associated with the surface endothelium can digest the labeled protein. Alternatively, heparin activation of a pulmonary lipoprotein lipase may partially hydrolyze or destabilize the VLDL molecule, thereby rendering it readily susceptible to proteolytic digestion. The possibility...
also exists that heparin may actually activate an endothelial protease which has a substrate specificity for VLDL apoproteins. Finally, it is possible that heparin releases proteases into the perfusate that digest [125I]VLDL. The last possibility is most improbable because of the specificity of the proteolysis for labeled VLDL even in the presence of a large excess of BSA. Nonetheless, the presence of heparin is essential to VLDL catabolism in the in vitro preparation we used, and may also have physiological relevance since the pulmonary mast cells under in vivo conditions store large quantities of this sulfated polysaccharide (16). Thus, the present experiments strongly suggest that the lung provides an initial, perhaps critical step in the remodelling of VLDL apoproteins which may influence the subsequent fate of these lipoproteins as they travel to the heart and other extrahepatic tissues.

We thank Melanie Minda, Patricia L. Rostron, Janet DeHoff, Martha A. Reed, and Lewis W. Johns for their excellent technical assistance and Kathleen Blewitt for secretarial assistance.

This work was supported by National Heart and Lung Institute grants HL-15061 (Specialized Center of Research, Lung), with additional support by HL-05285 and HL-16520.

Preliminary reports of this work (Pietra, G. G., et al., 1975. Am. J. Pathol. 78:10; Pietra, G. G. et al., 1975. Am. Rev. Respir. Dis. 111:929) were presented at the 72nd Meeting of the American Association of Pathologists and Bacteriologists, New Orleans, La., 1975 and at the International Conference on Lung Diseases, Montreal, Quebec, Canada, 1975.

Dr. Capuzzi is the recipient of Research Career Development Award KO 4 HL-00089. Dr. Spagnoli is on leave of absence from the Institute of Pathologic Anatomy II, Faculty of Medicine and Surgery, University of Rome, Italy. Dr. Marsh’s present address is the Department of Biochemistry and Physiology, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19104.

Received for publication 18 August 1975, and in revised form 26 January 1976.

REFERENCES

1. Bailey, J. M., and J. Butler. 1973. Cholesterol uptake from doubly labelled α-lipoproteins by cells in tissue culture. Arch. Biochem. Biophys. 159:580-581.

2. Bassett, D. J. P., A. B. Fisher, and J. L. Rabinowitz. 1974. Effect of hypoxia on incorporation of glucose carbons into lipids by isolated rat lung. Am. J. Physiol. 227:1103-1108.

3. Bergman, E. N., R. J. Havel, B. M. Wolfe, and T. Bohmer. 1971. Quantitative studies of the metabolism of chylomicron triglyceride and cholesterol by liver and extrahepatic tissues of sheep and dogs. J. Clin. Invest. 50:1831-1839.

4. Bierman, E. L., S. Eisenberg, O. Stein, and Y. Stein. 1973. Very low density lipoprotein “remnant” particles: uptake by aortic smooth muscle cells in culture. Biochim. Biophys. Acta. 329:163-169.

5. Bierman, E. L., O. Stein, and Y. Stein. 1974. Lipoprotein uptake and metabolism by rat aortic smooth muscle cells in tissue culture. Circ. Res. 35:156-150.

6. Bierman, E. L., and J. J. Albers. 1975. Lipoprotein uptake by cultured human arterial smooth muscle cells. Biochim. Biophys. Acta. 338:198-202.

7. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein particles. I. Preliminary in vitro and in vivo observations. Biochim. Biophys. Acta. 260:212-221.

8. Borensztain, J., and D. S. Robinson. 1970. The effect of fasting on the utilization of chylomicron triglyceride fatty acids in relation to clearing factor lipase (lipoprotein lipase) releasable by heparin in the perfused rat heart. J. Lipid Res. 11:111-117.

9. Bradham, J. H., and R. S. Gordon. 1958. Tissue distribution of C 14 after the intravenous injection of labelled chylomicrons and unesterified fatty acids in the rat. J. Clin. Invest. 37:574-578.

10. Capuzzi, D. M., V. Rothman, and S. Margolis. 1974. The regulation of lipogenesis by cyclic nucleotides in intact hepatocytes prepared by a simplified technique. J. Biol. Chem. 249:1286-1294.

11. de Jong, J. J. B., and J. B. Marsh. 1968. Biosynthesis of plasma lipoproteins by rat liver ribosomes. J. Biol. Chem. 243:192-199.

12. Eisenberg, S., D. W. Bilheimer, R. I. Levy, and F. T. Lindgren. 1973. On the metabolic conversion of human plasma very low density lipoprotein to low density lipoprotein. Biochim. Biophys. Acta. 326:361-377.

13. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein. I. Fate in circulation of the whole lipoprotein. Biochim. Biophys. Acta. 326:378-390.

14. Eisenberg, S., and D. Rachmilewitz. 1973. Metabolism of rat plasma very low density lipoprotein. II. Fate in circulation of apoprotein subunits. Biochim. Biophys. Acta. 326:391-405.

15. Felts, J. M. 1964. Biochemistry of the lung. Health Phys. 10:973-979.

16. Fishman, A. P., and G. G. Pietra. 1974. Handling of bioactive materials by the lung. New Engl. J. Med. 291:884-890, 953-959.

17. Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. Simple method for the isolation and purifica-
18. Gambetti, P., L. A. Autillo-Gambetti, N. K. Gonatas, and B. Saffer. 1972. Protein synthesis in synaptosomal fractions. Ultrastructural radioautographic study. J. Cell Biol. 52:526-535.

19. Godinez, J. R., and W. J. Longmore. 1973. Use of the isolated perfused rat lung in studies on lung lipid metabolism. J. Lipid Res. 14:138-144.

20. Goldstein, J. L., and M. S. Brown. 1974. Binding and degradation of low density lipoproteins by cultured human fibroblasts. J. Biol. Chem. 249:5153-5162.

21. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J. Clin. Invest. 34:1345-1353.

22. Hamosh, M., and P. Hamosh. 1975. Lipoprotein lipase in rat lung. The effect of fasting. Biochim. Biophys. Acta. 380:132-140.

23. Hamosh, M., and P. Hamosh. 1974. Lipoprotein lipase in rat lung. Fed. Proc. 33:346.

24. Heinemann, H. O. 1961. Free fatty acid production by rabbit lung tissue in vitro. Am. J. Physiol. 201:607-610.

25. Heinemann, H. O., and A. P. Fishman. 1969. Non-respiratory functions of mammalian lung. Physiol. Rev. 49:1-47.

26. Kalant, H., R. K. Murray, and W. Mons. 1964. Effect of EDTA on leakage of proteins from slices of normal rat liver and DAB-induced hepatoma. Cancer Res. 24:570-576.

27. Karnovsky, M. J. 1967. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. J. Cell Biol. 35:213-236.

28. Kaufman, E., and E. Wertheimer. 1957. Effect of fasting on protein release by liver slices. Am. J. Physiol. 190:133-138.

29. Koga, S., L. Bolls, and A. Scanu. 1971. Isolation and characterization of subunit polypeptides from apoproteins of rat serum lipoprotein. Biochim. Biophys. Acta. 236:416-430.

30. Kopriwa, B. M. 1973. A reliable standardized method for ultrastructural electron microscopic radioautography. Histochemistry. 37:11-17.

31. LaRosa, J. C., R. I. Levy, H. G. Windmueller, and D. S. Fredrickson. 1972. Comparison of the triglyceride lipase of liver, adipose tissue and post-heparin plasma. J. Lipid Res. 13:356-363.

32. Levy, R. I., R. S. Lees, and D. S. Fredrickson. 1966. The nature of prebeta (very low density) lipoproteins. J. Clin. Invest. 45:63-77.

33. Margolis, S., and D. M. Capuzzi. 1972. Serum lipoprotein synthesis and metabolism. In Blood Lipids and Lipoproteins, Quantitation, Composition and Metabolism. G. J. Nelson, editor. John Wiley & Sons, Inc., New York. 825-880.

34. McFarlane, A. S. 1963. In vivo behavior of 131I-labeled fibrinogen. J. Clin. Invest. 42:346-361.

35. Mellamby, J., and D. H. Williamson. 1963. Effect of calcium ions on ketone-body production by rat liver slices. Biochem. J. 88:440-444.

36. Noel, S. P., and D. Rubinstein. 1974. Secretion of apolipoproteins in very low density and high density lipoproteins by perfused rat liver. J. Lipid Res. 15:301-308.

37. Oliverona, T., and P. Belfrage. 1965. Mechanisms for removal of chyle triglyceride from the circulating blood as studied with [14C]glycerol and [3H]palmitic acid-labelled chyle. Biochim. Biophys. Acta. 90:81-93.

38. Patelski, J., Z. Waligóra, and S. Szulc. 1967. Demonstration and some properties of the phospholipase A, lipase and cholesterol esterase from the aortic wall. J. Atheroscler. Res. 7:453-461.

39. Pietra, G. G., J. P. Szidon, and A. P. Fishman. 1973. Leaky pulmonary vessels. Proc. Assoc. Amer. Physiol. 85:369-376.

40. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. J. Clin. Invest. 49:465-471.

41. Robinson, D. S. 1970. Function of the plasma triglycerides in fatty acid transport. In Comprehensive Biochemistry. M. Florkin and E. H. Stotz, editors. Elsevier Publishing Co., Amsterdam. 18:51-116.

42. Ruddel, C. L. 1967. Embedding media for 1–2 micron sectioning. II. Hydroxy ethyl methacrylate combined with 2-butoxy ethanol. Stain Tech. 42:253-255.

43. Salpeter, M. M., and M. Szabo. 1972. Sensitivity in electron microscope autoradiography. I. The effect of radiation dose. J. Histochem. Cytochem. 20:425-434.

44. Salpeter, M. M., L. Bachman, and E. E. Salpeter. 1969. Resolution in electron microscope radioautography. J. Cell Biol. 41:1-20.

45. Scow, R. O., M. Hamosh, J. E. Blanche-Mackie, and A. J. Evans. 1972. Uptake of blood triglyceride by various tissues. Lipids. 7:497-505.

46. Smith, U., and J. W. Ryan. 1973. Electron microscopy of endothelial and epithelial components of the lung: correlations of structure and function. Fed. Proc. 32:1957-1966.

47. Stein, O., and Y. Stein. 1975. Comparative uptake of rat and human serum low density and high density lipoproteins by rat aortic smooth muscle cells in culture. Circ. Res. 36:436-443.

48. Stein, O., D. Rachmilewitz, L. Sanger, E. Eisenberg, and Y. Stein. 1974. Metabolism of iodinated very low density lipoprotein in the rat. Autoradiographic localization in the liver. Biochim. Biophys. Acta. 360:205-216.

49. Stewart, J. E., and M. C. Schott. 1974. Release

PIETRA ET AL. Metabolism of 131I-Labeled Lipoproteins by Rat Lung 45
of lipoprotein lipase activity from isolated fat cells. 

50. WARREN, B. A. 1965. Fibrinolytic properties of vascular endothelium. Br. J. Exp. Pathol. 44:365-372.

51. ZEMPLÉNYI, T. 1962. Enzymes of the arterial wall. J. Atheroscler. Res. 2:2-24.

52. ZSOLDOS, S. J., and H. O. HEINEMANN. 1964. Lipolytic activity of rabbit aorta in vitro. Am. J. Physiol. 206:615-617.