Uptake and Subcellular Localization of Bacterial Lipopolysaccharide in the Adrenal Gland

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For determination of the kinetics of uptake and subcellular localization of lipopolysaccharide (LPS) from LPS–high density lipoprotein (LPS–HDL) complexes in the adrenal gland, LPS–HDL complexes were isolated by immunoaffinity chromatography of ^{125}I-*Salmonella minnesota* Re595 LPS that had been incubated with 20 mM EDTA–rabbit plasma. After intravenous injection of LPS–HDL complexes in rabbits, preferential uptake of the LPS was observed in the adrenal, so that by 5 hours, adrenal-tissue–bound LPS concentrations (determined by use of ^{131}I-BSA blood marker) exceeded all other tissues examined, including liver and spleen, by at least threefold. For determination of the subcellular localization of LPS, cholesterol-rich (lipid droplet) fractions and cholesterol-depleted fractions were obtained by ultracentrifugation of homogenates of adrenal tissue from rabbits killed at various times after injection of LPS–HDL complexes. As much as 40% of the adrenal-tissue–bound LPS was recovered in the cholesterol-rich fraction 2.5–24 hours after injection of LPS–HDL complexes. Electron-microscopic autoradiographic and immunocytochemical analysis of adrenal cortex of animals killed 5 hours after injection of LPS–HDL complexes demonstrated specific localization of LPS in lipid droplets. These data thus provide direct evidence for the uptake of LPS into the adrenal cortex of animals with intravascular LPS–HDL complexes and indicate that further study of the effect of LPS on adrenocortical function is warranted. (Am J Pathol 1985, 120:79–86)

SEVERAL EXPERIMENTAL observations suggest that the functional integrity of the adrenal cortex is an important factor in host survival in shock and disseminated intravascular coagulation (DIC) arising from gram-negative bacillary infection. These include the following: 1) Adrenalectomy renders experimental animals several hundred times to 1000 times more sensitive to the lethal effects of endotoxin.[1] 2) Berry and Smythe[2] presented data suggesting that intravenous injection of endotoxin in mice resulted in a diminished adrenal response to adrenocorticotropic hormone (ACTH). 3) In clinical studies, Sibbald et al[3] reported that 5 of 26 septic patients were found to be hyporesponsive to ACTH stimulation, suggesting that adrenocortical insufficiency could be a component of endotoxic shock. Finally, 4) administration of pharmacologic doses of glucocorticoids is believed to have a beneficial effect in patients with endotoxic shock and experimental animals given lethal doses of endotoxin.[4,5] Most recently, Keri et al[6] showed that plasma removed from rabbits infused with live *Escherichia coli* bacteria suppressed ACTH-induced steroid production by explanted rat adrenocortical cells.

The mechanisms by which adrenal cortical function may be altered during endotoxemia are not known. Previously, the adrenal cortex was not considered to be a site of lipopolysaccharide (LPS) localization. However, recent findings of Mathison and Ulevitch[7] and Munford et al[8] have provided evidence that the adrenal gland is indeed a site of LPS uptake when the LPS is complexed with high-density lipoprotein (HDL). In order to further characterize the interaction of LPS–HDL complexes in the adrenal, we have examined the kinetics of uptake of LPS–HDL complexes by the adrenal and have employed biochemical and ultrastructural analyses to determine the subcellular localization of LPS in the adrenal. These studies show that at least a portion of the LPS from LPS–HDL complexes is found in the lipid droplets of the cells of the adrenal cortex and suggest

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that further study of the effect of LPS on adrenocortical cell function is warranted.

Methods

Experimental Animals

New Zealand white rabbits (2–2.5 kg) were obtained from Rancho de Conejo, Vista, California, and were maintained on a standard pelleted rabbit diet and water.

Lipopolysaccharide

LPS was extracted from *Salmonella minnesota* Re595 bacteria by the phenol–chloroform–petroleum ether method of Galanos[9] with modifications that have been described previously.[10] Stock solutions of the LPS (5 mg/ml) were prepared in distilled water containing 20 mM EDTA (pH 7.5) by sonication (Model W-375, Heat Systems-Ultrasonics, Inc., Plainview, NY). Radioiodinated LPS (1 μCi ^{125}I/μg LPS) was prepared as described by Ulevitch[11] with the use of insolubilized lactoperoxidase–glucose oxidase (Enzymobeads, BioRad Laboratories, Richmond, Calif).

LPS–HDL complexes were prepared in 20 mM EDTA–normal rabbit plasma (250 μg LPS/ml) and isolated by affinity chromatography on anti-Re595 LPS-Sepharose.[10] The protein and lipid composition of LPS–HDL complexes prepared by this method has been described previously.[10] Lipoproteins ($d < 1.2$ g/cu cm) for injection in control animals were prepared from EDTA plasma by sequential flotation in KBr and CsCl.[12]

LPS Injections

Rabbits were restrained in the supine position, and a cannula was introduced (under local anesthesia with procaine) through the femoral artery for collection of blood samples.[13] To measure the kinetics of LPS uptake into tissues, LPS–HDL complexes (formed with ^{125}I-LPS) were injected through a marginal ear vein for a total amount of 250, 50, or 5 μg LPS; and the animals were sacrificed at various times (10 minutes to 24 hours) by an intravenous injection of 120 mg sodium pentobarbital. Doses of LPS–HDL complexes containing greater than 100 μg Re595 LPS produce lethality in at least 50% of the rabbits within 24 hours (R. J. Ulevitch, unpublished observation). Radioiodinated bovine serum albumin (^{131}I-BSA) was injected 5 minutes prior to sacrifice, and tissue blood volumes and tissue-bound LPS concentrations were determined as described previously.[7] The tissue-bound LPS concentrations were normalized with respect to the injected dose: normalized tissue-bound LPS concentration = μg LPS per gram tissue × 1000/μg LPS injected.

Preparation and Fractionation of Adrenal Homogenates

The capsules were removed from two adrenals, and the tissue was homogenized as described by Moses et al[14] in a final volume of 0.9 ml of 17 mM phosphate-buffered saline (PBS) with 1 mM $MgCl_2$ with the use of a glass tube with a cylindrical Teflon pestle (3431-E04, Arthur H. Thomas Company, Philadelphia, Pa). A 100-μl aliquot of the homogenate was removed for measurement of radioactivity and cholesterol determination. The remaining homogenate was diluted to 5 ml with PBS, transferred to a cellulose nitrate tube, and centrifuged at 32,000 rpm (100,000*g*) in an SW 50.1 rotor for 20 minutes at 20 C (Beckman Instruments, Palo Alto, Calif). As a result, 3 distinct layers were produced: 1) a white lipid layer (2–3 mm thick) at the top of the tube; 2) a clear intermediate layer (3.5–4 ml); and 3) a pellet (≅ 100 μl). The radioactivity in each layer was measured with a gamma counter, and the total amount of LPS and plasma marker in each layer was determined. On the basis of the ^{125}I/^{131}I ratio in plasma and the ^{131}I-BSA present in each fraction, the amount of plasma LPS in each adrenal fraction was subtracted from the total to yield tissue-bound LPS.

The adrenal fractions were extracted with chloroform methanol (2:1) according to the method of Folch et al,[15] and the resultant lipid residues were dissolved in isopropyl alcohol. The total cholesterol content in each fraction was measured with the use of an enzymatic assay (Cholesterol SVR reagent, Calbiochem, La Jolla, Calif). When ^{14}C-cholesterol or cholesteryl ^{14}C-oleate were added to adrenal tissue prior to homogenization, greater than 90% of the radioactivity was recovered in the isopropanol solution after extraction. As shown in Table 1, 90% of the recovered cholesterol was present in the top layer.

Ultrastructural Localization

Two rabbits were killed 5 hours after injection of 250 μg ^{125}I-LPS–HDL (150 μCi), and electron-microscopic autoradiography was performed on lead-citrate–stained sections that were coated with Ilford L4 emulsion.[7,16,17]

Electron-microscopic immunocytochemical localization of LPS was performed on adrenal cortex from two rabbits that were killed 5 hours after injection with 250 μg LPS–HDL complexes. The adrenals were fixed by perfusion with 0.1 M phosphate-buffered (pH 7.5) 1.5% depolymerized paraformaldehyde followed by 0.2% glutaraldehyde (30 minutes each). Blocks (≤ 1 cu mm)

Table 1—Percent Recovery of Cholesterol in Fractions of Adrenal Homogenates

	Distribution of total adrenal cholesterol* (%)
Top	72 ± 6.8
Middle	0.66 ± 0.13
Pellet	7.1 ± 0.78
Sum of fractions	80 ± 7

* The amount of total cholesterol (measured by enzymatic assay) present in the homogenate prior to fractionation was 3.8 ± 0.32 mg (SE, n = 6), and the cholesterol content of the adrenal glands was found to be 50 ± 6.0 mg/g.

of the fixed adrenal cortex were incubated overnight at 4 C in 0.25M Tris–0.25M sodium cyanoborohydride (pH 7.5) for blockage of free aldehyde groups. After infiltration with 0.8 M sucrose in 30 mM Tris, pH 7.5, thin sections were prepared with a Sorvall-Christensen TC-2 cryosectioning unit as described by Tokuyasu and Singer.[18] The sections were exposed to immunopurified anti-Re595 LPS (50 μg/ml) antibody[10] followed by 5 nM colloidal gold-protein A conjugate (CG-pA)[19,20]; and after staining with 2% uranyl acetate–0.15 M oxalic acid, the grids were coated with 15cp-methyl cellulose, dried, and examined.[21]

Results

Uptake of LPS-HDL Complexes by Tissues

To determine the kinetics and specificity of tissue uptake of LPS which is complexed to HDL, we prepared ^{125}I-Re595 LPS-HDL complexes *in vitro* as described in Materials and Methods. After intravenous injection of these isolated complexes, the tissue concentrations of LPS were determined at 10 minutes and at 1, 2.5, and 5 hours after injection. The kinetic parameters of the disappearance of the immunopurified LPS-HDL complexes from blood ($t_{1/2} \simeq 15$ hours) were identical

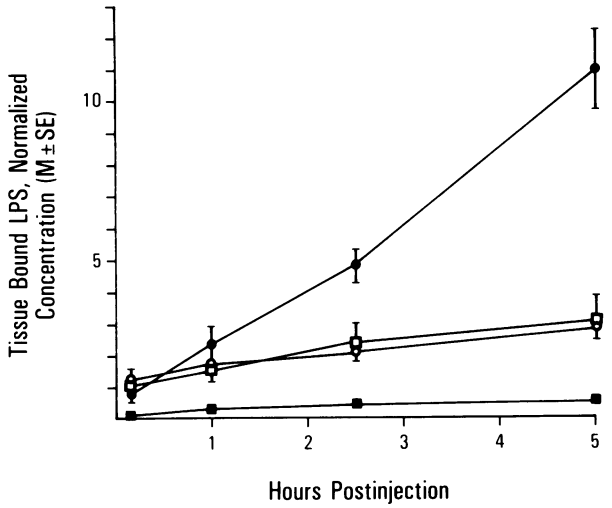

Figure 1—Kinetics of uptake of ^{125}I-LPS by adrenal (●), liver (○), spleen (□), and kidney (■) after injection of 250 μg LPS–HDL complexes. ^{131}I-BSA blood marker was injected 5 minutes before sacrifice for determination of the amount of tissue-bound LPS. The number of animals in each group was as follows: 10 minutes to 2.5 hours, 3; 5 hours, 4.

to those we have observed previously for LPS-HDL complexes prepared by sequential flotation in KBr and CsCl.[22]

The kinetics of LPS uptake into various tissues following injection of 250 μg of Re595 LPS as an LPS-HDL complex are shown in Figure 1. The adrenal tissue-bound LPS concentration 10 minutes after injection was comparable to that observed in liver and spleen. However, by 2.5 hours adrenal LPS concentrations were significantly higher than those in the other tissues and at 5 hours the LPS concentrations in adrenal were three to four times greater than those observed in liver and spleen. During this 5-hour period the percentage of the LPS dose accounted for in blood decreased from 89% to 35%, whereas the LPS specifically bound in liver increased from 9% to 22%. The remaining tissues examined, including skeletal muscle, kidney,

Table 2—Concentration of Tissue-Bound ^{125}I-LPS After Intravenous Injection of LPS–HDL Complexes

	Normalized LPS concentration						
Time of sacrifice	Adrenal	Liver	Spleen	Kidney	Lung	Skeletal muscle	Blood
250 μg LPS							
5 hours (4)*	11 ± 2.5	3.1 ± 0.60	3.0 ± 1.5	0.56 ± 0.20	1.4 ± 0.52	0.040 ± 0.01	3.8 ± 0.88
50 μg LPS							
5 hours (4)	7.6 ± 1.9	2.6 ± 0.26	2.6 ± 0.10	0.32 ± 0.096	0.70 ± 0.072	0.030 ± 0.012	3.4 ± 0.40
24 hours (4)	14 ± 2.2	3.2 ± 0.52	3.6 ± 0.58	0.48 ± 0.058	0.94 ± 0.20	0.090 ± 0.034	1.5 ± 0.30
5 μg LPS							
5 hours (2)	9.2	2.4	2.2	0.24	0.48	0.022	2.4
24 hours (2)	15	1.8	1.9	0.74	0.48	0.060	0.9

Mean ± SD.
* Number of animals in each group.

spleen, lung, and adrenal, contained less than 5% of the injected LPS.

After injection of 50 μg or 5 μg of LPS-HDL complexes, the normalized concentrations of tissue-bound LPS at 5 hours and 24 hours were determined to be similar to those observed after injection of 250 μg LPS (Table 2).

Fractionation of Adrenal Homogenates

In order to determine the subcellular localization of LPS in the adrenal, we killed the rabbits at various times after injection of ^{125}I-LPS-HDL complexes, and fractions of adrenal homogenates were prepared and examined for the presence of LPS as described in Materials and Methods. Specifically, we sought to determine whether the LPS was associated with the cholesterol-rich lipid droplets found in the cytoplasm of adrenocortical cells.[14] After centrifugation of the adrenal homogenates and separation of the cholesterol-rich and cholesterol-depleted fractions, the amount of LPS contained in these fractions was quantitated after correcting for tissue-bound versus plasma LPS.

As was shown in Figure 1, at 10 minutes and 1 hour after injection of 250 μg LPS-HDL complexes, the concentration of tissue-bound LPS in adrenal was low, and as much as half of the total adrenal LPS was contained in tissue blood (Table 3). When correction was made for the amount of blood LPS contained in the adrenal homogenate fractions, less than 10% of the tissue-bound LPS was recovered in the cholesterol-rich fraction, and almost 90% was recovered in the pellet (Figure 2). However, at 2.5 hours and 5 hours, approximately 90% of the total adrenal LPS was tissue-bound, and 30% of the tissue-bound LPS was recovered in the cholesterol-rich fraction (Table 3, Figure 2). When the cholesterol-rich fractions from the 5-hour animals were resuspended in PBS and centrifuged (20 minutes, 100,000*g*), greater than 95% of the radioactivity was recovered in the lipid droplets, which again formed a compact layer at the top of the tube. This washing procedure resulted in the formation of a small pellet, which contained less than 5% of the total radioactivity present. When rabbits were killed 24 hours after injection of 50 μg LPS-HDL complexes, 98% of the adrenal LPS was determined to be tissue-bound, and 42% of the tissue-bound LPS was present in the cholesterol-rich fraction.

Table 3—Proportion of LPS in Adrenal That is Tissue-Bound

Time after injection of LPS-HDL complexes	Percent of total adrenal LPS
10 minutes	47 ± 21
1 hour	72 ± 12
2.5 hours	89 ± 1.7
5 hours	86 ± 1.3
24 hours	98 ± 0.82

The dose of LPS injected was 250 μg for animals killed at 10 minutes to 5 hours and 50 μg for animals killed at 24 hours.

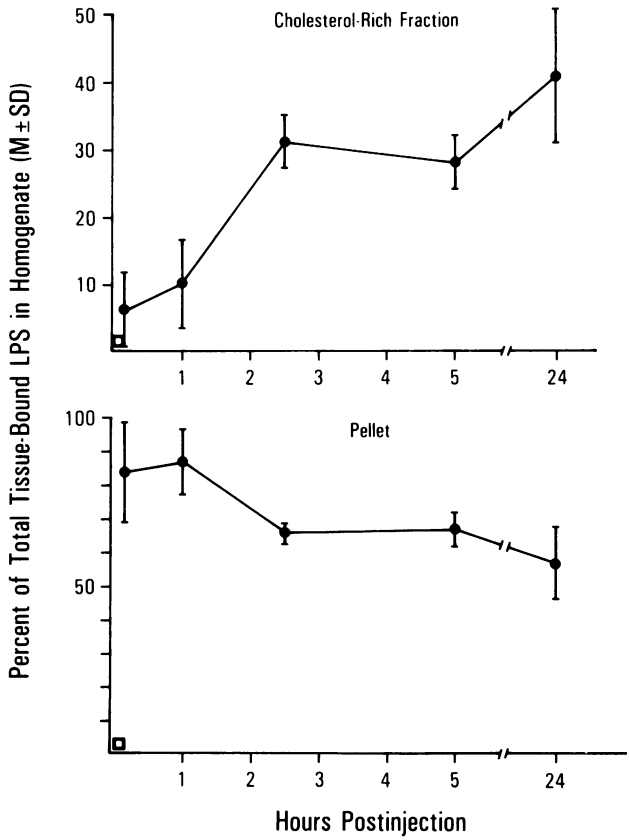

Figure 2—Recovery of ^{125}I-LPS in the cholesterol-rich (lipid droplet) fraction and pellet of adrenal homogenates from rabbits that received an intravenous injection of LPS-HDL complexes. The dose of LPS administered was 250 μg except for the animals sacrificed at 24 hours, which received 50 μg LPS-HDL. The number of animals in each group was as follows: 10 minutes to 2.5 hours, 3; 5 hours and 24 hours, 4.

To examine the possibility that free LPS-HDL complexes (in blood or in the cytoplasm of cells) might bind to the cholesterol-rich fraction during homogenization, adrenals were collected from control animals 5 minutes after injection of ^{131}I-BSA, homogenized in the presence of ^{125}I-LPS-HDL complexes, and centrifuged. On the basis of ^{131}I/^{125}I ratios of the homogenate and fractions, less than 2% of the LPS was bound to either the pellet or lipid droplet fraction. As an additional control, plasma was obtained from rabbits 5 hours after injection of ^{125}I-LPS-HDL complexes, diluted fivefold in PBS, and centrifuged under the same conditions used for tissue homogenates. The ratio of ^{131}I-BSA/^{125}I-LPS in each fraction was found to be essentially identical to that in plasma before centrifugation, demonstrat-

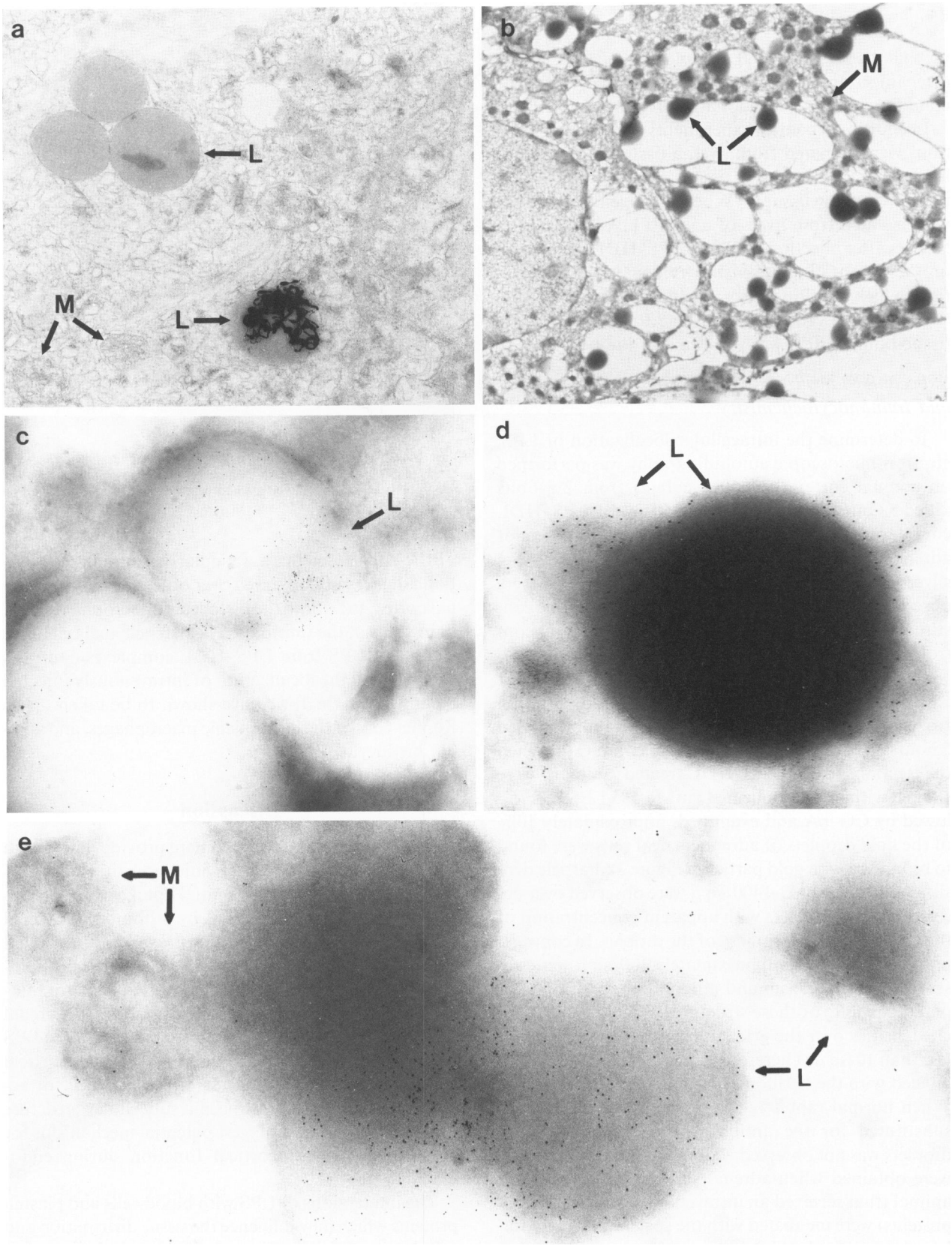

Figure 3—Electron-microscopic localization of LPS in adrenal cortex of rabbits that were killed 5 hours after intravenous injection of ^{125}I-LPS–HDL (**a**) or unlabeled LPS–HDL complexes (**b–e**). **a**—Autoradiographic localization of LPS was observed over lipid droplets (*L*) in cells of the zona fasciculata. **b–e**—Immunocytochemical localization of LPS in frozen sections with the use of anti-Re595 IgG and CG–pA (5 nm). **b**—Low magnification micrograph of a zona fasciculata cell (frozen section). The space surrounding the lipid droplets is an artifact which is produced when the sections are thawed and applied to grids. **c–e**—High-magnification micrographs showing labeling of lipid droplets. *M*, mitochondria. (**a**, ×17,700; **b**, ×4000; **c**, ×38,000; **d**, ×58,000; **e**, ×61,000)

ing that the ^{125}I-LPS–HDL complexes in plasma 5 hours after injection neither pelleted nor floated under the conditions used to fractionate adrenal homogenates.

In additional control experiments homogenate fractions were prepared from liver, a tissue with low cholesterol content (2–3 mg/g tissue), compared with adrenal (20–60 mg/g tissue).[23] When homogenate fractions were isolated from livers of animals killed 1, 2.5, and 5 hours after injection of 250 μg LPS–HDL complexes, only a trace of lipid was visibly evident in the top fraction, which was found to contain less than 5% of the ^{125}I-LPS. The remainder of the LPS (>95% of the tissue-bound LPS) was recovered in the pellet.

Subcellular Localization of LPS by Autoradiography and Immunocytochemistry

To determine the intracellular localization of LPS, electron-microscopic autoradiography was performed on adrenal cortex (zona fasciculata) from 2 rabbits killed 5 hours after injection of 250 μg ^{125}I-LPS–HDL complexes (150 μCi ^{125}I). Occasional lipid droplets of adrenocortical cells were found to be labeled with 2–4 silver grains, whereas only isolated single grains were observed over mitochondria and other cytoplasmic constituents (Figure 3). Background grain counts on control sections were low (≤1 grain/1 × 10^4 sq μ).

To further examine the intracellular localization of LPS, we used immunocytochemical studies of ultrathin frozen sections of adrenal cortex from rabbits killed 5 hours after injection of LPS–HDL complexes. When the sections from LPS–HDL-treated animals were stained with immunopurified anti-Re595 LPS IgG followed by CG–pA and examined, approximately 10% of the lipid droplets of adrenocortical cells were found to be labeled with gold particles (Figure 3). Particle densities as high as 1000–1400/sq μ were observed over occasional lipid droplets with apparent concentration of the staining at the periphery of the droplet. In contrast, particle densities of approximately 100/sq μ were observed over cytoplasm and mitochondria with no apparent labeling of these structures. Background particle densities over the grid support film were generally less than 10/sq μ. Therefore, the only structures clearly labeled with the antibody CG–pA were lipid droplets. When normal rabbit IgG or Tris-buffered saline was substituted for the antibody, labeling of the lipid droplets was not observed. Comparable negative results were obtained when adrenal sections from a control animal (that received an intravenous injection of lipoproteins) were incubated with the specific antibody followed by CG–pA (Figure 4).

Thus, with autoradiography and immunocytochemistry, localization of LPS from LPS–HDL complexes was demonstrated in lipid droplets of adrenocortical cells

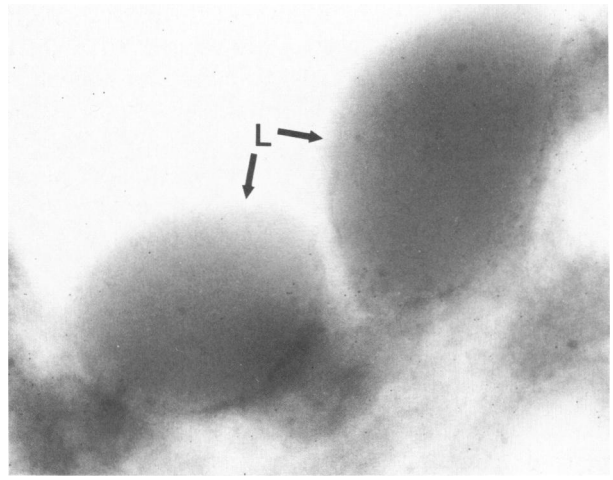

Figure 4—Electron micrograph of a frozen section of adrenal cortex (zona fasiculata) from a control rabbit that received an intravenous injection of lipoproteins. The section was stained with anti-Re595 LPS followed by CG–pA. (×40,000)

of the zona fasciculata, reticularis, and glomerulosa. Labeling was not observed over other cells of the adrenal cortex, including the histocytes (macrophages) and lining cells of the sinusoids. The parenchymal cell localization of LPS from LPS–HDL complexes contrasts sharply with the localization of intravenously injected parent LPS, which we have shown to be taken up by Kupffer cells of the liver, splenic macrophages, and adrenal histiocytes.[7]

Discussion

The experiments described here provide data which show that after intravenous injection of LPS–HDL complexes in rabbits, preferential uptake of LPS is observed in the adrenal, so that by 5 hours the adrenal tissue-bound LPS concentration exceeds that of all other tissues by at least 3 and as much as 20 times. Biochemical as well as electron-microscopic autoradiographic and immunocytochemical analyses of adrenal tissue demonstrate that a significant portion of the LPS is localized within lipid droplets of the adrenocortical cells. These data thus provide direct evidence for accumulation of LPS in a specific subcellular structure of adrenal cortex and suggest potential mechanisms for alteration of adrenocortical function during endotoxemia.

The interactions of LPS with blood cells and plasma proteins which may influence the tissue distribution and cellular localization of the LPS are not fully understood. Recently we and others have shown that exposure of LPS to plasma results in the formation of LPS–HDL complexes.[7,8,10,12,24] Although a number of the biochem-

ical and endotoxic properties of the LPS–HDL complex differ markedly from those of the parent LPS,[12] the LPS–HDL complex retains the capacity to produce DIC, hypotension, and death in experimental animals.[22] Therefore, knowledge of the specificity of tissue distribution and cellular localization of LPS after injection of LPS–HDL complexes might provide important information about the mechanisms of endotoxic shock.

In a previous study, Ulevitch et al[10] demonstrated that LPS–HDL complexes isolated from mixtures of LPS and rabbit plasma contained polypeptides and lipids characteristic of HDL. LPS–HDL complexes were found to be stable *in vitro* under a variety of conditions, including exposure to 2.5 M KBr or KSCN.[8,10] In addition, when preformed LPS–HDL complexes were injected intravenously into animals, >96% of the plasma LPS could be recovered in HDL fractions that were isolated from plasma samples collected 0.5–14 hours after injection.[8] Munford et al[8] demonstrated also that adrenal binding of ^{125}I-HDL and ^{3}H-LPS–HDL was increased in ACTH-pretreated animals. In contrast, decreased binding of both ligands was observed in animals that were pretreated with dexamethasone or HDL infusions sufficient to raise the plasma HDL cholesterol levels approximately fourfold. The results of the present studies provide additional evidence that LPS from plasma LPS–HDL is internalized by a mechanism which regulates adrenal uptake of cholesterol, because a substantial amount of cell-associated LPS was localized in the cholesterol-rich lipid droplets of the adrenocortical cells. Thus, these data are consistent with the hypothesis that LPS uptake by adrenocortical cells is coupled to the uptake of HDL cholesterol.

Despite the detailed information available concerning the mechanism of uptake of LDL by receptor-mediated endocytosis,[25] much remains to be learned about the process by which HDL cholesterol is internalized by cells. A number of experimental observations suggest that there is an *in vivo* transport system in endocrine tissues which allows specific uptake of HDL cholesterol.[26–28] Gwynne and Hess[29] provided evidence that HDL uptake by rat adrenocortical cells is a saturable process, the uptake mechanism differs from that for LDL, and lysosomal degradation of HDL may not be required for cholesterol utilization. They also suggested that newly internalized cholesterol is partitioned between at least two pools, a storage pool and a steroidogenic substrate pool. Thus, with respect to interactions between LPS–HDL and adrenocortical cells, it will be important to determine the stoichiometry for the internalization of LPS/HDL-cholesterol/apoprotein.

A number of questions remain unanswered concerning the fate of LPS that is taken up by cells. In a previous study employing SDS-PAGE, we did not observe degradation of LPS that was recovered from liver 3 hours after intravenous injection of ^{125}I-LPS.[7] Furthermore, with this same approach, we have not observed degradation of LPS that was recovered from adrenal 5 hours after intravenous injection of ^{125}I-LPS–HDL complexes (J. C. Mathison, unpublished observations). Using gas chromatography/mass spectroscopy to detect the β-hydroxymyristic acid of LPS, Maitra et al[30] obtained evidence that a majority of the LPS taken up by liver remained structurally intact 48 hours after injection of *E coli* 0127:B8 endotoxin in rats. On the other hand, Hall and Munford reported that the granule fraction of human neutrophils contained enzyme that partially deacylated *Salmonella typhimurium* lipid A by removing the nonhydroxylated fatty acids. However, because almost all of the 3-hydroxytetradecanoic acid residues remained linked to the lipid A glucosamine backbone, the authors suggested that the partial deacylation might actually enhance the biologic properties of the LPS.[31] Recently, Duncan and Morrison observed that biosynthetically labeled LPS (^{3}H-galactose) which was recovered from murine macrophages 48 hours after phagocytosis of labeled bacteria was unchanged with respect to reactivity in the Limulus amebocyte lysate assay and lethality in actinomycin-D–treated mice. Together, these findings suggest that at least some of the interactions of LPS with cells may result in amplification of the biologic activity of the LPS, rather than detoxification.

The pathogenic significance of LPS uptake by adrenocortical cells remains unclear. As we reported previously, minimal histologic changes were observed in adrenals of rabbits that died 8–20 hours after intravenous injection of 250 μg LPS–HDL complexes.[22] Nevertheless, it will be important to determine whether localization of LPS in adrenocortical cells leads to suppression of the response to ACTH.

Progress has been made in defining possible mechanisms for adrenal insufficiency arising during septic shock. In recent studies we have demonstrated that macrophages can be induced by LPS to release a product that suppresses the response of adrenocortical cells to ACTH.[34,35] Results from control experiments indicate that the suppressive activity is not due to carry-over LPS from the macrophage cultures, and the suppression is not caused by decreased viability of the adrenocortical cells.[34] Studies are in progress to isolate and identify the macrophage-derived suppressive activity. In other studies Keri et al demonstrated that shock plasma which was obtained from rabbits 3–4 hours after infusion of *E coli* inhibited the response of explanted rat adrenocortical cells to ACTH.[6] These investigators provided evidence that the shock-plasma–treated adrenocortical

cells were unable to convert cholesterol to pregnenolone.[33] However, the shock plasma factor(s) responsible for the suppression of steroidogenesis were not identified. Ultimately, to determine the pathogenic significance of LPS localization in adrenocortical cells, it will be necessary to differentiate between the suppressive activity of the LPS-induced macrophage product and the potential direct effect of LPS on the adrenocortical cell.

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