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Research Article

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Sites of Tissue Binding and Uptake In Vivo of Bacterial Lipopolysaccharide-High Density Lipoprotein Complexes

STUDIES IN THE RAT AND SQUIRREL MONKEY

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ABSTRACT When gram-negative bacterial lipopolysaccharides (LPS) are injected intravenously into the rabbit or rat, they bind to plasma lipoproteins, particularly high density lipoproteins (HDL). The present studies were performed to examine the mechanisms by which LPS-HDL complexes are removed from the circulation and taken up by various tissues. Our approach was to compare the sites of specific tissue binding and uptake of HDL and of LPS-HDL complexes in the rat and squirrel monkey. In the rat, binding of homologous ^{125}I-HDL was demonstrated principally in the adrenal gland, ovary, liver, and spleen. [^{3}H]LPS-HDL complexes (produced in vitro by incubating *Salmonella typhimurium* [^{3}H]LPS with rat HDL and lipoprotein-free plasma) bound to the same tissues, but with apparently lower affinities. The specificity of binding of both ^{125}I-HDL and [^{3}H]LPS-HDL to these organs was demonstrated in two ways. First, tissue binding of both radiolabeled preparations was swamped out by raising the circulating levels of HDL-cholesterol from 32 to 140 mg/dl. Second, treatment of the animals with dexamethasone abolished specific binding of both HDL preparations to the adrenal gland while administration of adrenocorticotropin increased the specific adrenal binding of the two preparations. The steady-state plasma clearance rate for ^{125}I-HDL equaled 774±29 μl/h and was significantly lower (557±39 μl/h) for the LPS-HDL complex, a finding that presumably reflected the lesser ability of the various tissues to bind the LPS-HDL complex. Binding studies were also done in the squirrel monkey, an animal that has the same level of circulating HDL cholesterol as the rat, but nearly three times more cholesterol in low density lipoproteins. Specific binding of homologous ^{125}I-HDL and [^{3}H]LPS-HDL was again found principally in the adrenal gland and liver. The results indicate that the sites of tissue uptake of bacterial LPS are strongly influenced by binding of LPS to HDL. In particular, LPS-HDL binding may be an important determinant of the extent to which LPS are taken up by the adrenal gland during bacterial sepsis.

INTRODUCTION

Although much is known about the toxic activities of gram-negative bacterial lipopolysaccharides (LPS; endotoxins),[1] an understanding of the mechanisms by which the animal host transports, modifies, and degrades these important molecules is only beginning to emerge. Recent evidence suggests that, after an intravenous injection of LPS into the rabbit or rat, a significant fraction of the LPS binds to plasma lipoproteins, particularly to high density lipoproteins (HDL) (1, 2). LPS that have bound to HDL undergo a decrease in density (to $\rho < 1.21$ g/ml) and have a longer half-life in the plasma space; indeed, most of the LPS that remain in the plasma after intravenous bolus injection appear to be bound to HDL (3). LPS that do not bind to HDL in plasma are apparently rapidly trapped in tissues such as the spleen, liver, and lung (3).

In studies done in rabbits, Mathison and Ulevitch (3) found that LPS that are bound to HDL ("low-

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[1] *Abbreviations used in this paper*: BSA, bovine serum albumin; HDL, high density lipoproteins; LPS, lipopolysaccharides.

density" LPS) are taken up preferentially by the adrenal gland. There are several mechanisms that might account for the uptake of LPS-HDL complexes by different tissues. First, LPS might follow HDL to those tissues that ordinarily take up HDL. Second, LPS might change the properties of HDL so that the normal binding of HDL to tissue receptors is altered. Third, tissue uptake of LPS-HDL complexes might be dependent primarily upon LPS uptake; by rendering the LPS more bulky or by altering their net charge, bound HDL might protect LPS from phagocytosis or other mechanisms that account for the trapping of LPS in the liver and spleen.

To examine these various possibilities, we have compared the sites of tissue binding and uptake of HDL and LPS-HDL complexes. Most of the experiments were performed in the rat, an animal in which the majority of the plasma cholesterol is carried in HDL. To a limited degree, similar comparisons were also carried out in the squirrel monkey, an animal in which low density lipoproteins (LDL) are quantitatively a more important carrier of plasma cholesterol. Our results indicate that LPS-HDL complexes are taken up by the same tissues that take up HDL, though the LPS-HDL complexes are bound to these tissues with slightly less affinity than HDL. Moreover, hormonal changes which modify the uptake of HDL by certain tissues also influence the uptake of LPS-HDL complexes. The ultimate disposition of "circulating" endotoxin thus may depend critically upon the factors that influence the binding of LPS to HDL and the uptake of HDL by target tissues.

METHODS

Animal preparations. Female, Sprague-Dawley derived rats weighing 125–150 g were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass. They were housed and fed as previously described (4) for at least 2 wk before being used in specific experiments. By that time they weighed 190–220 g. The squirrel monkeys (*Saimiri sciureus*) were shipped to the laboratory from South American Primates, Inc., Miami, Fla. and were placed in isolation for 6–8 wk before use. During this time they were allowed free access to water and monkey chow (Wayne Monkey Diet, Allied Mills, Inc., Chicago, Ill.) and were given orange juice once each week. They were screened for tuberculosis and parasites. By the time they were used for experiments, the monkeys had achieved stable weights in the range of 680 to 790 g.

Lipoprotein and albumin preparations. HDL from both rat and monkey plasma were prepared in a narrow density range to avoid contamination with lower density lipoproteins. Using fed animals maintained on a low cholesterol chow diet, blood was aspirated from the abdominal aorta (rats) or from the femoral vein (monkeys) and anticoagulated with EDTA (1 mg/ml). HDL were then harvested from the plasma between the densities of 1.095 and 1.21 g/ml. The lipoproteins were washed and concentrated by centrifuging an additional time at ρ = 1.21 g/ml. These HDL preparations ran as a single band with alpha mobility on agarose gel electrophoresis (5). On 12.5% polyacrylamide-SDS gels (6) the rat HDL showed a major apoprotein band corresponding to apo AI and a consistent, but more minor band, corresponding to apo E as has been reported (7, 8). The constituents of plasma that sedimented at a density of 1.21 g/ml ("1.21 bottom") were dialyzed against 0.9% NaCl and kept at 4°C prior to use.

The rat and monkey lipoproteins were then either labeled with ^{125}I or bound to bacterial [^{3}H]LPS. HDL and bovine serum albumin (BSA) were radioiodinated by a modification of the method of McFarlane and others (9, 10) and exhaustively dialyzed against 0.9% NaCl at 4°C prior to use. It should be emphasized that the preparations of ^{125}I-HDL, ^{125}I-BSA, ^{131}I-BSA, and [^{3}H]LPS-HDL were always used within 2 d of preparation. Preliminary studies demonstrated that the patterns of tissue binding and the albumin spaces changed significantly if materials that had been stored for long periods were utilized.

LPS preparations. *Salmonella typhimurium* strain G-30, a mutant that lacks the enzyme UDPglucose-4-epimerase (E.C. 5.1.3.2.) and consequently incorporates exogenous D-galactose almost exclusively into LPS (11), was intrinsically labeled with D-1[^{3}H]galactose (New England Nuclear, Boston, Mass.) as described (12). LPS were extracted from cell envelopes (12) by the phenol-water method of Westphal and Jann (13) and treated with diethyl ether, RNAase, DNAase, and pronase to remove contaminants (14). LPS were then sedimented by ultracentrifugation in distilled water (100,000 *g*, 4 h, 4°C) and lyophilized. For each experiment, small amounts of LPS were suspended in saline and treated for 30–60 s in a water bath sonicator to achieve a uniform suspension. The preparations used in these experiments contained ~10,000–16,000 cpm/μg of LPS.

In vitro binding of LPS and HDL. Greater than 98% of the purified LPS used in these experiments sedimented to the bottom of tubes at a density of 1.21 g/ml when centrifuged at 225,000 *g* for 24 h. Because LPS that had bound to HDL assumed the density of the HDL (i.e., $\rho < 1.21$ g/ml), binding of [^{3}H]LPS to HDL could be monitored by the changes in LPS density that occurred when binding took place. HDL (1.0–2.0 mg of protein), 1.21 bottom (30–60 mg of protein) and [^{3}H]LPS (200–300 μg) were incubated together in a total volume of 2.1 ml of 0.9% NaCl for 60 min at 37°C. The mixture was then chilled and its density was adjusted to 1.21 g/ml with cold KBr solution. The [^{3}H]LPS-HDL complexes were isolated by centrifugation, washed by centrifuging an additional time at ρ = 1.21 g/ml, and exhaustively dialyzed against 0.9% NaCl at 4°C. Experiments, described in detail elsewhere (15), indicated that under these conditions ~80% of the [^{3}H]LPS floated to the top of the tube at a density of 1.21 g/ml, whereas <20% of the [^{3}H]LPS floated if either HDL or 1.21 bottom were not present in the incubation mixture.

Determination of tissue spaces. Radiolabeled compounds were administered intravenously to the experimental animals as a bolus over ~20 s. This was accomplished through a tail-vein catheter in the rat and by injecting directly into the femoral vein in the monkey. Approximately 10×10^6 cpm of ^{125}I-HDL, ^{125}I-BSA, or ^{131}I-BSA were given to individual rats, whereas the doses of [^{3}H]LPS and [^{3}H]LPS-HDL were $1–2 \times 10^6$ cpm per rat. At the desired time point after intravenous injection, the animals were anesthetized with diethyl ether, the abdomen was quickly opened, and blood was aspirated from the abdominal aorta. Various organs were then removed as quickly as possible: these were cut into pieces ~2–3 mm thick which were then rinsed briefly in cold 0.9% NaCl, blotted on filter paper, and weighed on an electric recording balance. For the larger organs these tissue aliquots weighed 150–250 mg; for the smaller organs, e.g., adrenal

gland and ovary, the whole tissue was used to determine the content of radioactivity. In the case of the ^{125}I- or ^{131}I-labeled compounds, triplicate tissue samples, along with triplicate 100-μl samples of plasma, were counted directly in a gamma counter (Packard Instrument Co., Inc., Downers Grove, Ill.). The plasma and tissue samples containing ^{3}H were placed directly into counting vials. 300 μl of 1 N NaOH was added, the vials were capped, and the tissues were allowed to dissolve slowly over 48–72 h with occasional vigorous agitation at room temperature. After the tissues had disintegrated, the contents of the vials were neutralized with 1 N HCl, solubilized in Aquassure (New England Nuclear) and assayed in a liquid scintillation counter (Packard Instrument Co., Inc.). Quenching in each individual sample was corrected by using an external standardization technique.

In many experiments the tissue spaces for ^{125}I-HDL or [^{3}H]LPD-HDL and for ^{125}I-BSA were measured in parallel in separate groups of rats. In some experiments with rats and in all of the studies using monkeys, the ^{131}I-BSA and ^{125}I-HDL spaces were measured simultaneously in the same animals. Because the tissue spaces for BSA were the same when determined with ^{131}I-BSA or ^{125}I-BSA, these data were combined in the various figures. Data from these experiments are presented as the equivalent plasma space attained by a particular molecule in each organ and were calculated by the following formula: Tissue space = (counts per minute of HDL, LPS, LPS-HDL or BSA)/(tissue weight, grams) (counts per minute of HDL, LPS, LPS-HDL or BSA/microliter of plasma). The values for the tissue spaces have the units of microliters of plasma per gram of tissue (microliter/gram). The specific HDL, LPS, and LPS-HDL tissue spaces were calculated by subtracting the tissue space of BSA in each organ from the tissue spaces obtained in the same organ with the two HDL preparations or LPS.

Determination of HDL turnover. Whereas classical turnover studies with multicompartmental analysis of lipoprotein degradation have been undertaken in small animals such as the rat, we have found that this method is relatively imprecise and subject to large animal-to-animal variations. We have therefore chosen to use the constant infusion method for measuring lipoprotein clearance rates. Rats were fitted with femoral vein catheters and placed in individual restraining cages. Each animal was infused with a solution containing NaCl (0.9 g/dl), glucose (0.2 g/dl), and BSA (0.1 g/dl) at a rate of 1.2 ml/h. Trace quantities of [^{3}H]-LPS-HDL or ^{125}I-HDL were added to the infusates of different groups of animals to give ~50,000–100,000 cpm/ml of radioactivity. The infusions were then continued at a constant rate for 14 h. At that time the animals were anesthetized and blood was aspirated from the abdominal aorta as quickly as possible. The delivery rate of each pump was calibrated by allowing it to continue to deliver the infusate directly into counting vials for two consecutive 30-min periods. These samples, along with triplicate 100-μl aliquots of plasma, were then assayed for radioactivity. Aliquots of the infusate and plasma also were subjected to lipid extraction and to precipitation with trichloroacetic acid. The clearance rate from the plasma of each labeled compound was then calculated as follows: Clearance rate = (protein-bound counts per minute infused into the animal per hour)/(protein-bound counts per minute per microliter of plasma). The values are expressed as the microliters of plasma cleared of a particular lipoprotein or LPS preparation per hour (microliters per hour), normalized to 100 g of body wt.

Chemical methods. Plasma cholesterol levels were determined colorimetrically (16). Protein was measured by the method of Lowry et al. (17).

Analysis of data. In all cases the data are presented as mean values±1 SEM. Where appropriate, the difference between the mean values for two groups of data were tested by the unpaired Student's t test. In the various figures, those values that are significantly different from 0 at the $P < 0.05$ level are indicated by an asterisk.

RESULTS

Stability of LPS-HDL complexes. Two types of preliminary experiments were carried out. First, [^{3}H]LPS-HDL complexes were prepared, suspended in KBr ρ = 1.21 g/ml), and centrifuged at 225,000 g for 36 h at 4°C: 98% of the ^{3}H was found in the top 1 cm of the tube. The complexes were resuspended and recentrifuged two additional times; again, 97.5 and 99% of the radioactivity floated to the tops of the tubes. Because unbound LPS have a density > 1.21 g/ml, this experiment indicated that the complexes were stable under these rather extreme conditions of centrifugation.

In the second preliminary experiment the [^{3}H]LPS-HDL complexes were injected intravenously into 20 rats and groups of these animals were killed at intervals from 0.5 to 14 h later. Aliquots of plasma from each rat were adjusted to the densities of 1.095 and 1.21 g/ml and centrifuged. At every time point tested, from 96 to 99% of the ^{3}H floated in the density range of 1.095–1.21 g/ml. We concluded from this experiment that there was no demonstrable transfer of the [^{3}H]LPS from HDL to other lipoprotein fractions of lower density under these in vivo conditions.

^{125}I-HDL and [^{3}H]LPS-HDL binding in the rat. The rats used in these studies had been maintained on a low cholesterol intake; the total plasma cholesterol averaged 64±4 mg/dl and, as reported (18), the majority of this was carried in HDL. As seen in Fig. 1, ~58% of the plasma cholesterol floated in the ρ = 1.095–1.21 g/ml density range (HDL) and only 14% floated in the ρ = 1.020–1.055 g/ml fraction (mostly LDL); 21% was in the ρ = 1.055–1.095 g/ml fraction (LDL and HDL), and lesser amounts were recovered in the other density classes. When such animals were administered [^{3}H]LPS-HDL intravenously and killed at various time intervals, there were markedly different amounts of ^{3}H found in the various tissues (Fig. 2). From the amount of [^{3}H]LPS-HDL injected and the concentration of ^{3}H in the plasma 5 min after the injections, it was calculated that the LPS-HDL complex had been distributed into a volume equal to 4.25±0.12 ml/100 g body wt: this volume of distribution essentially equals the plasma volume in the rat [4.04 ml/100 g (19)]. The plasma concentration of [^{3}H]LPS-HDL then declined over the subsequent 2 h as an apparent first-order decay curve. As is also shown in Fig. 2, very high concentrations of ^{3}H were achieved in the adrenal gland, ovary, and liver only 5 min after the injection, after which there was a progressive, but much slower,

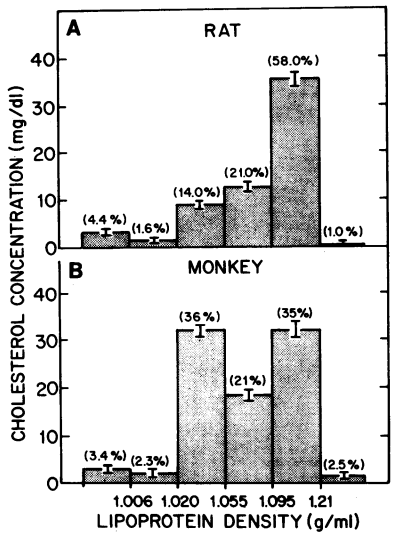

FIGURE 1 Distribution of plasma cholesterol in the rat and squirrel monkey. The lipoproteins from rats and monkeys were separated into six different density classes by centrifuging the plasma sequentially at ρ = 1.006, 1.020, 1.055, 1.095, and 1.21 g/ml. The final column represents the cholesterol recovered from the bottom of the centrifuge tube after harvesting the ρ = 1.095–1.21 g/ml fraction. The diagram shows both the concentration of cholesterol in each fraction (mg/dl) and the percent of the total plasma cholesterol in each density range. Each column shows the mean±SEM for three animals.

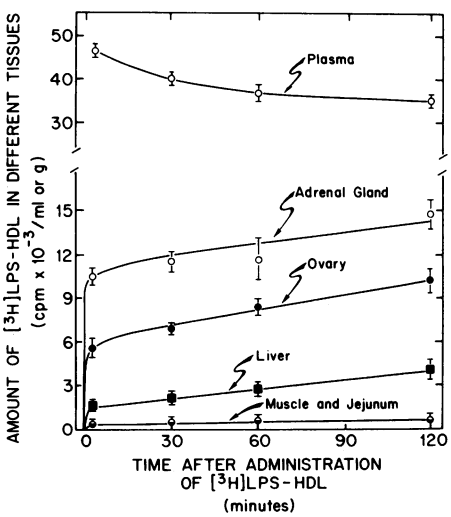

FIGURE 2 The content of [^{3}H]LPS-HDL in various tissues of the rat. Animals were administered [^{3}H]LPS-HDL intravenously at time 0 and groups were killed 5, 30, 60, and 120 min later. Plasma and samples of various tissues were obtained and the content of ^{3}H was measured. The vertical axis gives the amount of [^{3}H]LPS-HDL in a milliliter of plasma or 1.0 g of tissue at each time interval. Each point represents the mean±1 SEM for four animals.

increase in the tissue concentration of the isotope over the next 2 h.

To further characterize the early, putative binding of [^{3}H]LPS-HDL to tissues, additional groups of animals were administered either ^{125}I-HDL, [^{3}H]LPS-HDL, or ^{125}I-BSA and killed 5, 10, or 15 min later. The amount of each of these molecules found in 1.0 g of tissue was divided by the concentration of the same molecule found in plasma; this calculation yielded the tissue spaces shown in Fig. 3. In the adrenal gland (Panel A), the tissue space for BSA achieved a constant value of about 95 μl/g. The tissue spaces for ^{125}I-HDL and [^{3}H]-LPS-HDL were much higher and equalled ~650 and 340 μl/g, respectively. Thus, both of the HDL preparations had been concentrated by the adrenal gland relative to BSA and so had achieved much greater volumes of distribution. The liver also concentrated the two HDL preparations relative to BSA (Panel B) while tissues such as the pancreas (Panel C) had identical tissue spaces for the three compounds.

Because such studies appeared to identify those tissues capable of binding HDL, spaces of distribution were measured in 17 different tissues of the rat 10 min after injection of the 3 radiolabeled materials. As shown in Fig. 4 (Panel A) the ^{125}I-HDL tissue space was highest in the adrenal gland, ovary, liver, spleen, lung, and heart. The tissue spaces obtained after injection of [^{3}H]LPS-HDL (Panel B) showed essentially the same profile; however, the spaces obtained in the adrenal gland, ovary, and liver were only about one half as large as were found for ^{125}I-HDL. The spaces of distribution of ^{125}I-BSA (Panel C) also varied con-

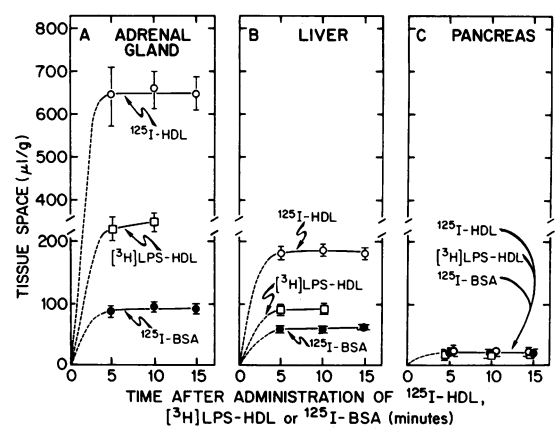

FIGURE 3 Tissue spaces determined at various times after the administration of ^{125}I-HDL, [^{3}H]LPS-HDL, and ^{125}I-BSA. Different groups of rats were administered one of these three compounds at time 0 and then killed 5, 10, or 15 min later. The tissue space achieved by each compound was calculated and is shown on the vertical axis expressed as microliters per gram of tissue. Each point represents the mean±1 SEM for three to eight animals.

siderably from tissue to tissue; relatively large values were found in ovary, adrenal gland, liver, spleen, lung, and heart.

Because the tissue spaces measured for ^{125}I-HDL and [^{3}H]LPS-HDL were determined both by the amount of these molecules in the plasma in each tissue and by the amount of these substances presumably bound to the cell surfaces, it was necessary to calculate that portion of the space of distribution that was due to specific tissue binding. This was done by subtracting the tissue space obtained for ^{125}I-BSA in each tissue (Panel C) from the respective tissue space obtained in the same organ with ^{125}I-HDL or [^{3}H]LPS-HDL. This calculation yielded the specific tissue spaces shown in Panels A and B of Fig. 5. In six organs the specific tissue space for ^{125}I-HDL was significantly different from 0: the adrenal gland (565±60 μl/g), ovary (335±55 μl/g), liver (95±8 μl/g), jejunum (10±2 μl/g), ileum (6±1 μl/g), and spleen (39±2 μl/g). The specific [^{3}H]LPS-HDL tissue spaces (Panel B) again showed a similar distribution; the highest values were achieved in the adrenal gland, ovary, and liver.

To further support the contention that these specific tissue spaces reflected the binding of HDL to specific tissue receptors, two other experiments were carried out. In the first, a group of animals was administered intravenously an amount of HDL that raised the cholesterol concentration in the ρ = 1.095–1.21 g/ml fraction from the normal value of 35 to 139 mg/dl. 20 min later the animals were given either [^{3}H]LPS-HDL or ^{125}I-BSA, and tissue spaces were measured. As shown in Panel C of Fig. 5, when the plasma HDL-cholesterol

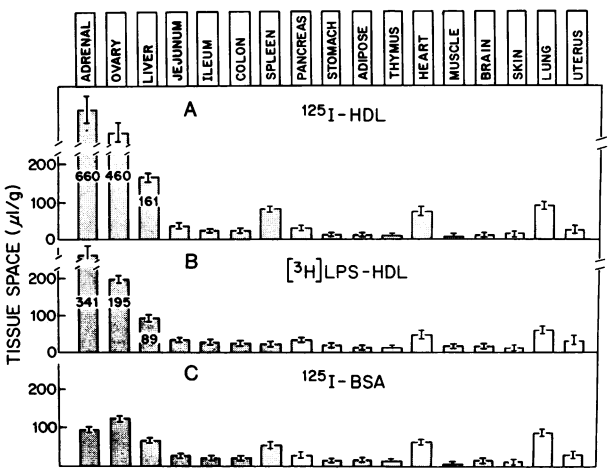

FIGURE 4 Tissue spaces of ^{125}I-HDL, [^{3}H]LPS-HDL, and ^{125}I-BSA in rats at 10 min. These three compounds were administered intravenously at time 0 and the animals were killed 10 min later and the spaces of distribution were determined in 17 different tissues. Each point represents the mean±1 SEM for six to eight animals.

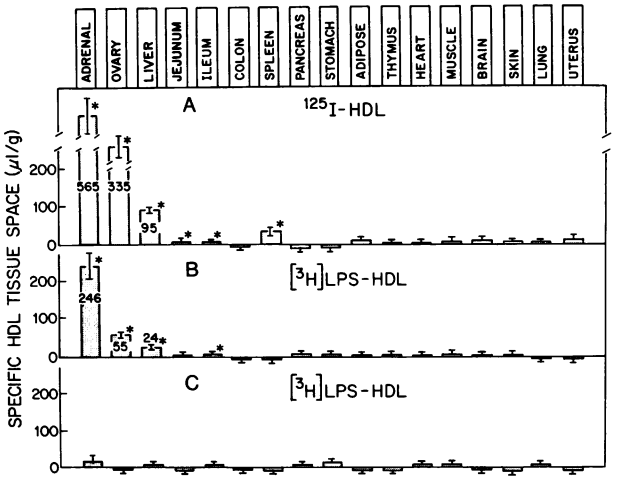

FIGURE 5 Specific ^{125}I-HDL and [^{3}H]LPS-HDL tissue spaces in the rat at 10 min. The values in Panels A and B were calculated by subtracting the tissue space of ^{125}I-BSA found in each organ from the tissue space found in the same organ for ^{125}I-HDL and [^{3}H]LPS-HDL, respectively (Fig. 4). Panel C shows the results of a separate experiment in which the animals were administered ~12 mg of rat HDL-cholesterol (ρ = 1.095–1.21 fraction) intravenously 20 min before they were given [^{3}H]LPS-HDL or ^{125}I-BSA. This panel shows the specific [^{3}H]LPS-HDL tissue spaces in these animals calculated from these two sets of data. Each point represents the mean±1 SEM for four to eight animals.

was elevated to this level, the specific [^{3}H]LPS-HDL tissue spaces decreased essentially to zero in the adrenal gland, ovary, liver, and ileum. This ability to swamp-out specific HDL tissue binding in vivo by elevating circulating levels of HDL was examined in greater detail in studies illustrated by the data in Fig. 6. Individual rats were administered intravenously from 0 to 15 mg of HDL-cholesterol, after which the tissue spaces (Panels A and C) and specific tissue spaces (Panels B and D) were measured at 10 min for both ^{125}I-HDL and [^{3}H]LPS-HDL. As the plasma HDL-cholesterol concentration was raised, the tissue spaces for both compounds decreased significantly in the adrenal gland, ovary, and liver and the specific binding (Panels B and D) dropped essentially to zero.

In a second group of studies, the effect of manipulating the circulating levels of ACTH on tissue binding of HDL was explored. As summarized by the data in Fig. 7, groups of rats were administered either a buffer solution (control), dexamethasone, or ACTH, and tissue spaces for ^{125}I-BSA, ^{125}I-HDL, and [^{3}H]LPS-HDL were measured at 10 min in all three groups. As seen in panels A and D, specific tissue binding was again seen in the adrenal gland, ovary, and liver for both ^{125}I-HDL and [^{3}H]LPS-HDL. After suppression of endogenous ACTH secretion by the administration of dexamethasone, the specific binding of both HDL

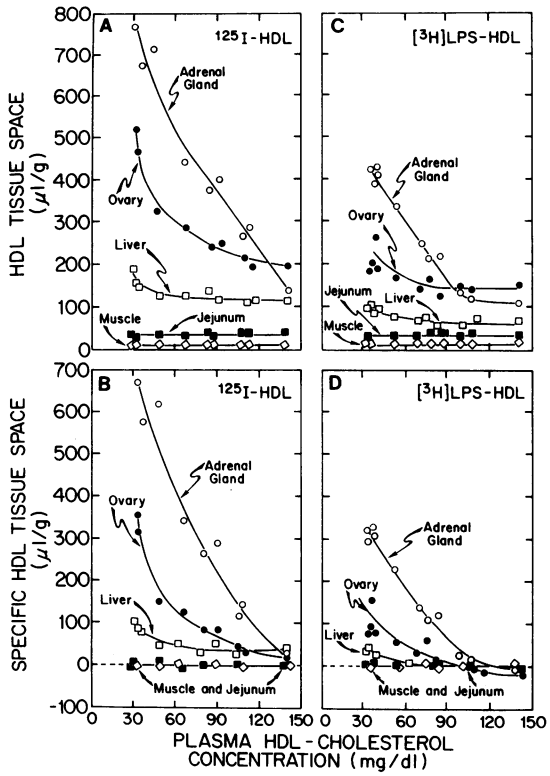

FIGURE 6 Specific ^{125}I-HDL and [^{3}H]LPS-HDL tissue spaces at 10 min in rats with different concentrations of circulating HDL-cholesterol. Individual animals were administered intravenously varying amounts of rat HDL containing from 0 to 15 mg of cholesterol. 20 min later they were administered ^{125}I-HDL, [^{3}H]LPS-HDL, or ^{125}I-BSA. 10 min later they were killed and the tissue spaces were determined. The tissue spaces (Panels A and C) and the specific HDL tissue spaces (Panels B and D) for ^{125}I-HDL and [^{3}H]LPS-HDL are shown as a function of the plasma HDL-cholesterol concentration. Each point represents the result obtained in an individual animal.

preparations completely disappeared in the adrenal gland while binding in the ovary and liver was largely unaffected. Conversely, after treatment with exogenous ACTH specific adrenal gland binding of both HDL preparations significantly increased. Again, ACTH had relatively little effect on HDL binding by the ovary and liver.

Taken together, these three lines of evidence indicated that specific binding of ^{125}I-HDL and [^{3}H]LPS-HDL to tissues could be measured in vivo by this technique, and that the values found using the two preparations were similar.

[^{3}H]LPS binding to HDL in vivo in the rat. The previous studies indicated that the bacterial LPS, once attached to HDL, was bound to and presumably taken up by the same group of tissues that normally bind and take up HDL. The next group of studies was designed to determine how rapidly purified LPS bind to HDL and other plasma lipoproteins under in vivo conditions and to determine the sites of tissue uptake of LPS at 10 min. Purified [^{3}H]LPS were administered intravenously as a bolus to rats at time 0 and animals were killed at intervals up to 2 h later. The initial plasma concentration of [^{3}H]LPS, estimated by assuming a plasma volume of 4.04 ml/100 g body wt (19), averaged 6.2 μg/ml. The measured plasma concentration of [^{3}H]LPS fell over the first 10 min after injection to a level of 3.5 μg/ml and then decreased gradually to 2.9 μg/ml at 120 min. Binding of the [^{3}H]LPS to plasma lipoproteins was determined by measuring the percentage of the [^{3}H]LPS in plasma that floated at ρ = 1.21 g/ml. As shown in Fig. 8, <2% of the plasma LPS floated at this density when added to rat plasma at 0°C (time 0 point). However, 0.5 min after administration of the [^{3}H]LPS to the animals, 37% had acquired

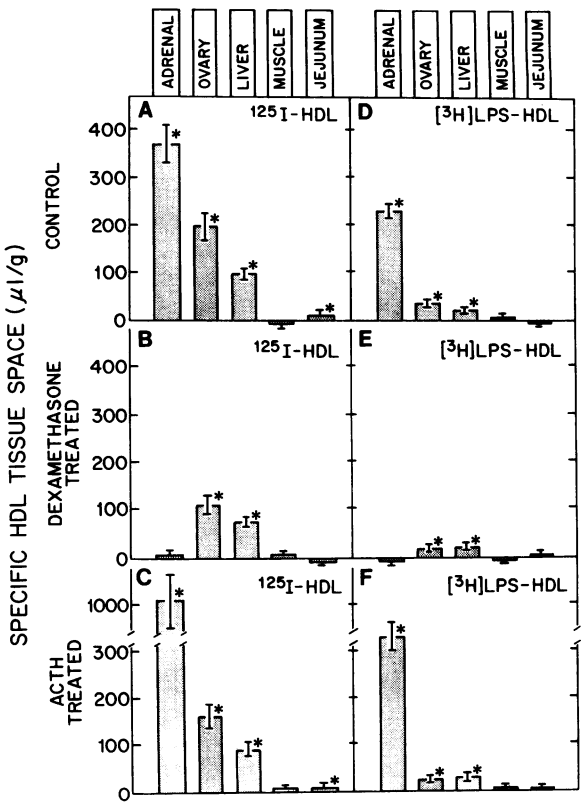

FIGURE 7 Effect of ACTH and dexamethasone administration on the specific ^{125}I-HDL and [^{3}H]LPS-HDL tissue spaces in the rat. Groups of rats were administered either ACTH (10 U/d), dexamethasone (1 mg/d) or 0.9% NaCl solution subcutaneously for 4 d. On the morning of the fifth day four animals from each treatment group were administered either ^{125}I-HDL, [^{3}H]LPS-HDL, or ^{125}I-BSA intravenously and killed 10 min later. The tissue spaces for the three substances were determined in each experimental group and these values were used to calculate the specific HDL tissue spaces shown. Each value represents the mean±1 SEM for four animals.

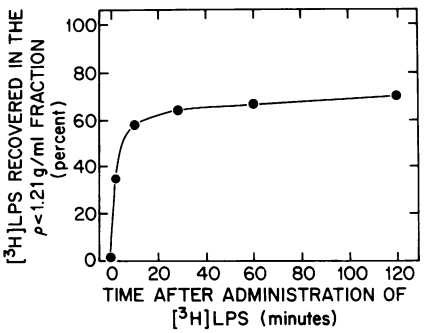

FIGURE 8 Binding of [3H]LPS to lipoproteins under in vivo conditions in the rat. Animals were administered [3H]LPS intravenously at time 0. At intervals from 0.5 to 120 min later, groups of rats were anesthetized and blood was quickly aspirated from the abdominal aorta and chilled in an ice bath. The plasma was then adjusted to a density of 1.21 g/ml with KBr and centrifuged. This figure shows the percent of the [3H]LPS in the plasma that became associated with lipoproteins and so floated at this density. Each point represents the mean±1 SEM for two to three rats.

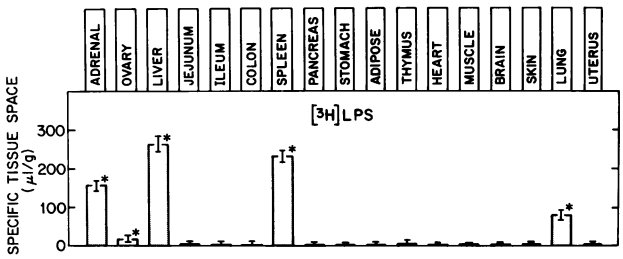

FIGURE 9 Specific [3H]LPS tissue spaces in the rat at 10 min. The animals were administered [3H]LPS intravenously at time 0 and killed 10 min later. The [3H]LPS tissue spaces were determined in the various organs and, after subtracting the appropriate ^{125}I-BSA spaces, yielded the specific [3H]LPS tissue spaces shown in this figure. Each value equals the mean ±1 SEM for five animals.

a density of <1.21 g/ml and this figure rose to 60% at 10 min. Over the next 110 min there was a further, but small, increase to 70% in the percentage of plasma LPS that floated at ρ = 1.21 g/ml. The absolute amount of [3H]LPS that was in the ρ < 1.21 fraction remained rather constant over the interval 10–120 min after injection, falling from 2.10 to 2.03 μg/ml. The [3H]LPS that was in the plasma at the 60- and 120-min time points was further fractionated into the major lipoprotein density classes: the ρ < 1.006 g/ml fraction had <1% of the radioactivity; ρ = 1.006–1.020 g/ml (%); ρ = 1.020–1.055 g/ml (3%); ρ = 1.055–1.095 g/ml (12%); and ρ = 1.095–1.21 g/ml (84%). Thus, under these in vivo conditions the great majority of the [3H]LPS in plasma were bound to lipoproteins in the high density range.

Fig. 9 shows the distribution of specific [3H]LPS tissue binding 10 min after the intravenous administration of the labeled unbound LPS. When compared with the data on specific binding of injected [3H]LPS-HDL (Fig. 5) there were several notable differences. Binding to the adrenal gland (160 vs. 246 μl/g) and ovary (25 vs. 55 μl/g) was reduced while there were marked increases in the tissue spaces observed in the liver (262 vs. 24 μl/g), spleen (235 vs. 0 μl/g), and lung (80 vs. 5 μl/g). Presumably the very high tissue spaces found in the liver, spleen, and lung reflect binding or uptake of the free [3H]LPS in the plasma by organs that are rich in phagocytic activity. Because at 10 min only 60% of the [3H]LPS was associated with lipoproteins, the tissue spaces in the adrenal gland and ovary could be recalculated to equal 275 and 43 μl/g, respectively, when expressed in terms of the lipoprotein-associated [3H]LPS. These values are not significantly different from those obtained when preformed [3H]LPS-HDL complexes were administered (Fig. 5). The striking differences in the tissue uptake profiles obtained for [3H]LPS and [3H]LPS-HDL provide additional evidence that the preformed [3H]LPS-HDL complexes remain tightly bound in vivo.

Clearance of ^{125}I-HDL and [3H]LPS-HDL in the rat. Whereas the preceding studies indicated that [3H]-LPS-HDL were bound by the same tissues that specifically bound ^{125}I-HDL, in every experimental setting there were consistently lower specific tissue spaces found with the LPS-HDL complexes than with HDL. This finding suggested that the binding and/or transport sites were less able to interact with HDL that had been complexed to LPS. To test the physiological significance of this finding, clearance rates for the two preparations were measured in groups of four animals over a 14-h period. The clearance of ^{125}I-HDL averaged 774±29 μl/h per 100 g body wt. The clearance of [3H]LPS-HDL was significantly lower and equalled 557±39 μl/h per 100 g body wt. These values were thus consistent with the previous results and confirmed that the uptake of LPS-HDL complexes was reduced relative to the uptake of HDL.

Tissue accumulation of [3H]LPS-HDL at 14 h. In a final group of studies in the rat, the content of ^{3}H in the various tissues was determined 14 h after the intravenous administration of the [3H]LPS-HDL complex. As shown in Fig. 10, the specific [3H]LPS-HDL tissue spaces were >10-fold higher at 14 h than the values found at 10 min. It is noteworthy that the same group of tissues that manifested specific HDL binding at 10 min, i.e., the adrenal gland, ovary, gastrointestinal tract, and spleen (Fig. 5) were the same tissues that accumulated large amounts of ^{3}H in this study.

^{125}I-HDL and [3H]LPS-HDL binding in the squirrel monkey. To determine whether these results would also be seen in a species with higher plasma LDL levels, similar binding studies were carried out in the squirrel monkey. The total plasma cholesterol in these

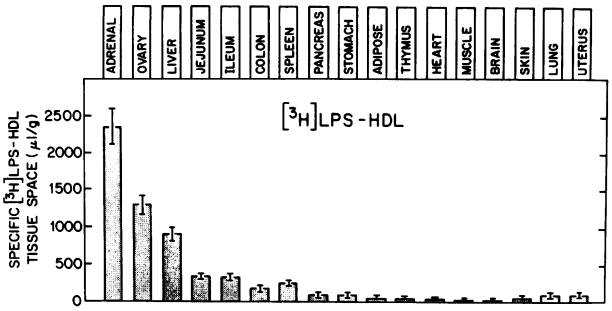

FIGURE 10 Specific [3H]LPS-HDL tissue spaces in the rat at 14 h. These studies were undertaken as described in the legend to Fig. 5, except that the animals were killed at 14 h, rather than 10 min, after administration of the [3H]LPS-HDL. Each value represents the mean±1 SEM for four animals.

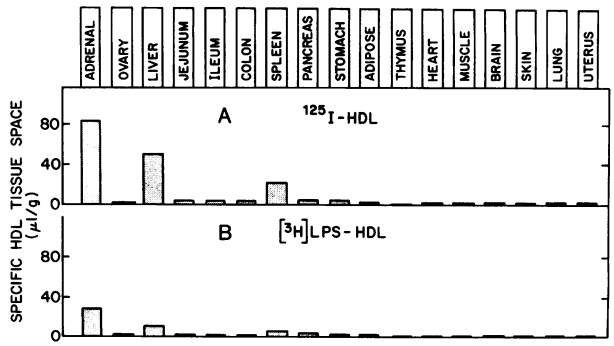

FIGURE 11 Specific ^{125}I-HDL and [3H]LPS-HDL tissue spaces in the squirrel monkey at 10 min. Groups of monkeys were administered intravenously ^{131}I-BSA and ^{125}I-HDL or [3H]LPS-HDL and killed 10 min later. After subtracting the appropriate tissue space for ^{131}I-BSA in each organ, the specific ^{125}I-HDL and [3H]LPS-HDL tissue spaces shown in this figure were obtained. There were two animals in each group.

animals averaged 88±4 mg/dl and, as seen in Fig. 1, the distribution of the cholesterol in the various density classes of lipoproteins was similar to that of the rat except that the amount of cholesterol in LDL was about three times higher. HDL was harvested from monkey plasma, labeled with ^{125}I or bound to [3H]LPS, and injected intravenously into the same groups of animals. After subtracting the tissue spaces obtained with ^{131}I-BSA, the specific ^{125}I-HDL and [3H]LPS-HDL spaces were determined at 10 min. As seen in Fig. 11, significant specific ^{125}I-HDL binding was demonstrated in the adrenal gland, liver, and spleen, (Panel A). The [3H]LPS-HDL complex also appeared to bind to these three tissues (Panel B). The major difference between these data and those found in the rat was that specific binding of HDL was not observed in the ovary. However, because the estrus cycle was not controlled in these experiments, it is possible that this finding was a result of inadvertently using animals at a point in the cycle where HDL-cholesterol uptake was minimal.

DISCUSSION

One of the major functions of plasma lipoproteins is to deliver cholesterol to various tissues where it is used for the normal turnover of membrane sterols and as a substrate for the synthesis of substances such as bile acids and steroid hormones. In the rat, most of the plasma cholesterol is carried in HDL (18). It is thus likely that in this species much of the cholesterol taken up by the various organs is acquired by the tissue uptake of HDL. It follows that the uptake of other compounds that are tightly bound to HDL, such as gram-negative bacterial LPS, may largely occur in those tissues that normally bind and transport large amounts of HDL. Alternatively, LPS could modify (either quantitatively or qualitatively) the tissue uptake of the HDL molecules to which they are bound. It is also possible that uptake of LPS-HDL complexes might be mediated by the binding of LPS, and not HDL, to tissues, or by some other process that totally bypasses the usual mechanisms for the uptake of either HDL or unbound LPS.

To evaluate these possibilities, we compared the tissue uptake of radiolabeled HDL and LPS-HDL complexes. Because the sites of tissue binding and uptake of HDL in the rat have not been previously reported, the first group of experiments dealt with this problem. Within minutes after their administration, the HDL attained a space of distribution in many tissues that was significantly greater than the space of distribution of albumin. This implied that such tissues had binding sites accessible to the plasma space where they were able to concentrate the ^{125}I-HDL relative to albumin (Figs. 3 and 4). Furthermore, this putative binding could be swamped out if the circulating levels of HDL were acutely elevated (Panel C of Fig. 5 and Fig. 6) and, in the case of the adrenal gland, could be increased or totally abolished by altering the degree of stimulation of the adrenal cells by ACTH (Fig. 7). Taken together, these three sets of observations strongly suggest that the specific HDL tissue spaces measured in these studies and shown in Fig. 5 do indeed reflect specific HDL binding by certain organ systems. When expressed per gram of tissue, the greatest amount of binding was seen in the adrenal gland and ovary: the liver, spleen, and various parts of the intestinal tract showed lower, but definite, amounts of binding.

Such specific binding presumably reflects the presence of lipoprotein receptors on the cells of these various tissues. The first cell surface lipoprotein receptor was described on human fibroblasts and was thought to recognize primarily the B apoprotein of LDL; it is commonly referred to as the LDL receptor (20). However, it was soon demonstrated that lipoproteins that

contain E apoprotein also are recognized by, and bind to, this receptor. Indeed, the presence of E apoprotein in certain subclasses of HDL preparations appears to explain the high-affinity binding that is observed in vitro between these lipoproteins and human fibroblasts (8, 21). Similar findings recently have been reported using the rat fibroblast; these cells manifest high affinity binding of rat LDL, which contains only B apoprotein, and of a subfraction of rat HDL (HDL_1), which contains essentially only E apoprotein (8). In contrast, a higher density rat HDL fraction (ρ = 1.125–1.21 g/ml) that contains predominantly A-I apoprotein and little or no E apoprotein, is not bound by the LDL receptors on these fibroblasts. However, cells derived from the adrenal gland of the rat and maintained in culture do show high affinity binding for both rat (which contains E apoprotein) and human (which contains little or no E apoprotein) HDL (22). This latter finding raises the possibility that at least some cells of the rat studied in vitro manifest a second type of receptor-mediated lipoprotein uptake that is different from the LDL receptor (23).

The presence of these lipoprotein transport systems in various organs of the rat can be readily inferred from the results of several types of in vivo experiments. Administration of the drug 4-aminopyrazolo[3,4-D]-pyrimidine to the rat over 3–4 d markedly lowers the circulating levels of lipoproteins and results in a significant increase in the rates of cholesterol synthesis in a number of tissues including the adrenal gland (35-fold), ovary (3.8-fold), lung (3.7-fold), spleen (2.6-fold), small intestine (3.3-fold), and colon (2.8-fold) (24). There is little change in the rates of synthesis in other tissues. When a mixture of lipoproteins obtained from whole rat plasma is infused back into such treated animals, rates of cholesterol synthesis in the responsive tissues return to near normal values (24, 25). Such findings imply that many tissues of the rat normally obtain much of the cholesterol they require through the uptake of lipoprotein-cholesterol from the plasma and so respond to changes in circulating levels of these lipoproteins by reciprocal changes in the rates of tissue sterol synthesis.

In the rat, most of the plasma cholesterol is carried in HDL fractions and most of these appear to contain at least some E apoprotein in addition to the A apoproteins (7, 18). Hence, it is likely that in this species much of the cholesterol taken up by the various organs is acquired by the tissue uptake of HDL, and that this uptake presumably is mediated through the LDL receptor mechanism or, possibly, in tissues like the endocrine glands, through another transport system (23–25).

The second major point to be derived from these experiments concerns the sites of tissue binding and uptake of [^{3}H]LPS-HDL complexes. When the complexes were performed in vitro and then injected intravenously into the animals, they were specifically bound to the same tissues that bound ^{125}I-HDL. This was true in the squirrel monkey (Fig. 11) as well as in the rat (Fig. 5). Furthermore, the specific [^{3}H]LPS-HDL binding was also eliminated by raising the circulating levels of HDL (Figs. 5, 6) and, in the adrenal gland, was altered by the administration of either dexamethasone or ACTH (Fig. 7). Thus, it appears that the [^{3}H]LPS-HDL complexes bound to essentially the same receptor sites as the ^{125}I-HDL. Quantitatively, however, in all experimental circumstances the magnitude of the specific binding was less for LPS-HDL than for HDL alone. If the magnitude of such specific binding is directly related to the rate of tissue uptake of the lipoproteins, then this difference in specific binding presumably accounts for the further observation that the [^{3}H]LPS-HDL complexes were cleared from the circulation more slowly (557 μl/h per 100 g body wt) than ^{125}I-HDL (774 μl/h per 100 g body wt).

Whereas ^{125}I-HDL can be used to examine rapid binding phenomena in vivo, this isotope cannot be used to follow the uptake of lipoproteins into tissues over a more prolonged period because the ^{125}I-HDL is presumably internalized and degraded and the ^{125}I is released back into the blood. The [^{3}H]LPS used in these studies, in contrast, were intrinsically labeled with [^{3}H]galactose using a mutant strain of *S. typhimurium* that inserts galactose into the O-antigen and R-core regions of LPS. Whereas there is little information concerning the rates of tissue degradation of LPS in the rat, experiments using ^{125}I-labeled LPS in the rabbit indicated that there was little or no degradation of LPS by the liver for periods of up to 180 min (3). Our own preliminary studies found only insignificant amounts of free ^{3}H in urine, bile, or plasma during the 12–24 h period following the administration of [^{3}H]LPS-HDL. We thus measured the accumulation of ^{3}H in the tissues of the rat for several hours after the bolus injection of [^{3}H]LPS-HDL. After the initial rapid binding phase there was a progressive, apparently linear, accumulation of label in tissues such as the adrenal gland, ovary, and liver (Fig. 2). Furthermore, the same tissues that showed specific ^{125}I-HDL binding in 10 min (Fig. 5) also demonstrated the greatest amounts of tissue accumulation of ^{3}H 14 h after administration of [^{3}H]LPS-HDL (Fig. 10). Thus, regardless of the isotope used or the time period allowed for accumulation of label, the highest rates of HDL binding/uptake were found in the adrenal gland, ovary, liver, gastrointestinal tract, and spleen. When these values (expressed per gram of tissue) are multiplied by the organ weights, the liver becomes the single most important site of uptake and, for example, accounts for about 56% of the ^{3}H that was recovered from the tissues of the rat 14 h after the administration of [^{3}H]LPS-HDL. These results are in

general agreement with those obtained using ^{125}I-LPS in the rabbit (3).

The third set of studies concerned the magnitude of the binding that takes place in vivo between LPS and HDL and the sites of tissue uptake of the LPS. When [^{3}H]LPS were administered intravenously, only 30% were associated with lipoproteins at 10 min and even at 2 h only 70% of the LPS in plasma were lipoprotein-bound. At 10 min, the amount of specific binding of [^{3}H]LPS to the adrenal gland and ovary was only about half (Fig. 9) of that observed when preformed [^{3}H]LPS-HDL were administered (Fig. 5). This reduction in specific binding by the endocrine glands reflected very closely the observation that only about half of the [^{3}H]LPS in the plasma at this time point was associated with HDL. Thus it appears that if the association of the [^{3}H]LPS with HDL could have been totally blocked, the LPS would have been cleared entirely by the liver, spleen, and lung: three tissues that are rich in phagocytic cells. On the other hand, once the LPS becomes associated with HDL, uptake by the phagocytic cells decreases and binding to the parenchymal cells of the adrenal gland, ovary, liver, etc., is promoted through receptor-mediated lipoprotein binding. These conclusions are entirely consistent with results recently reported by Mathison and Ulevitch (3) in the rabbit. These authors found that intravenously administered ^{125}I-LPS disappeared from the plasma as a two-component process. There was an initial rapid phase of disappearance that was associated with the accumulation of large amounts of radioactivity in the phagocytes of the liver, spleen, and lung (presumably, this rapid uptake represented the clearance of LPS not bound to lipoproteins in vivo). The second, and slower, phase of disappearance was associated with a reduction in the density of LPS in plasma (to <1.2 g/ml); radioactivity accumulated preferentially in the adrenal gland (presumably, the lipoprotein-mediated uptake of LPS-HDL complexes). Thus, once LPS become bound to HDL they are apparently targeted to the adrenal gland in the rat, rabbit, and squirrel monkey.

The biological importance of HDL-mediated uptake of LPS by the adrenal gland is unknown, but it is at least possible that this mechanism may account for the occurrence of adrenal hemorrhage in some patients with gram-negative bacterial sepsis (26). Evidence supporting this hypothesis was described by Levin and Cluff (27), who found that the incidence of adrenal hemorrhage in rabbits given bolus doses of LPS could be increased by the prior administration of ACTH and decreased by the administration of dexamethasone. Our results suggest that these hormonal treatments should increase (ACTH) or decrease (dexamethasone) the uptake of LPS-HDL complexes by the adrenal gland. One may thus speculate that in some individuals with gram-negative bacteremia, LPS that have dissociated from the bacteria bind to HDL. The stress of sepsis is often associated with elevations in ACTH levels, which would augment adrenal gland uptake of the LPS-HDL complexes. Although essentially nothing is known about the cellular uptake of these complexes, it is conceivable that in some patients the accumulation of LPS in this manner may provoke adrenal cortical insufficiency (28, 29) or hemorrhage.

These and previous studies (3) demonstrate that factors that influence the binding of LPS to HDL in vivo may dramatically influence the ultimate sites of tissue uptake of the LPS. Although little is known about the binding reaction, the available evidence is consistent with the hypothesis (30) that LPS are initially disaggregated by some nonlipoprotein component(s) of plasma; after disaggregation, LPS may bind to HDL. Calcium appears to inhibit LPS-HDL binding by blocking LPS disaggregation (15). Factors that might influence the binding reaction thus include the physical state of the LPS, the ionic composition of plasma, the types and amounts of lipoproteins present, and the plasma factors that disaggregate LPS. As the studies presented in this paper demonstrate, the tissue uptake of LPS-HDL complexes is further influenced by the prevailing levels of HDL in plasma and, in the case of the adrenal gland, by hormonal influences that regulate the uptake of HDL. Further study of these various components of the LPS-HDL interaction should yield important insights into the pathogenetic effects of endotoxin in clinical states.

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