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Effects of early endotoxemia and dextran-induced anaphylaxis on the size selectivity of the glomerular filtration barrier in rats

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Axelsson J, Rippe A, Venturoli D, Swärd P, Rippe B. Effects of early endotoxemia and dextran-induced anaphylaxis on the size selectivity of the glomerular filtration barrier in rats. *Am J Physiol Renal Physiol* 296: F242–F248, 2009. First published November 12, 2008; doi:10.1152/ajprenal.90263.2008.—This study was performed to investigate the glomerular permeability alterations responsible for the microalbuminuria occurring in endotoxemia and during anaphylactic shock. In anesthetized Wistar rats, the left ureter was catheterized for urine collection while, simultaneously, blood access was achieved. Endotoxemia was induced by lipopolysaccharide (LPS) from *Escherichia coli*, and glomerular permeability was assessed at 60 and 90 ($n = 7$) and 120 ($n = 7$) min. Anaphylaxis was induced by a bolus dose of Dextran-70, and glomerular permeability assessed at 5 min ($n = 8$) and 40 min ($n = 9$). Sham animals were followed for either 5 or 120 min. The glomerular sieving coefficients (θ) to fluorescein isothiocyanate-Ficoll (70/400) were determined from plasma and urine samples and assessed using size-exclusion chromatography (HPLC). After start of the LPS infusion (2 h), but not at 60 or 90 min, θ for Ficoll_{70A} had increased markedly [from $2.91 \times 10^{-5} \pm 6.33 \times 10^{-6}$ to $7.78 \times 10^{-5} \pm 6.21 \times 10^{-6}$ ($P < 0.001$)]. In anaphylaxis, there was a large increase in θ for Ficolls $>60 \text{ \AA}$ in molecular radius already at 5 min, but the glomerular permeability was completely restored at 40 min. In conclusion, there was a transient, immediate increment of glomerular permeability in dextran-induced anaphylaxis, which was completely reversible within 40 min. By contrast, endotoxemia caused an increase in glomerular permeability that was manifest first after 2 h. In both cases, θ to large Ficoll molecules were markedly increased, reflecting an increase in the number of large pores in the glomerular filter.

endotoxin; systemic inflammatory response syndrome

MICROALBUMINURIA, I.E., JUST moderately increased urinary albumin excretion rates (20–200 $\mu\text{g}/\text{min}$ in humans), is an early predictor of many glomerular diseases, such as various glomerulonephritides and diabetic nephropathy and is an established marker of endothelial dysfunction. It is also a feature of acute systemic inflammation or stress, such as following trauma (18), operations (6, 16), thermal injury (30), or in the systemic inflammatory response syndrome (SIRS) (4). The pathophysiological alterations resulting in microalbuminuria have only rarely been investigated. For example, it is not precisely known whether (proximal) tubular dysfunction is the major cause of microalbuminuria or whether charge- or size-selective alterations of the glomerular filtration barrier, or both, are affected. In a recent study, we found that early diabetic

microalbuminuria in rats was mainly related to size-selective changes in the glomerular barrier (25) and that charge alterations were just secondary. Also, the glomerular alterations following pronounced ischemia-reperfusion (I/R) injury could be mainly ascribed to size-selective changes, although after less severe I/R challenge, charge-selective alterations were also implicated (24).

The purpose of the present study was to investigate how systemic inflammation induced by bacterial lipopolysaccharide (LPS) affects the glomerular filtration barrier and to compare these changes with those occurring in acute anaphylaxis. It should be noted that not only cells of the innate immune system, but also podocytes (5), have LPS receptors, such as toll-like receptor-4 (TLR-4) and CD14 (2). Exposure of mammals to relatively small quantities of LPS leads to an acute inflammatory response, mediated in part by the release of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α . SIRS, such as induced by LPS, involves vasodilatation and increases in vascular permeability, leading to an increased vascular leakage of macromolecules in many organs. The renal responses to early septicemia with respect to tubular function, changes in glomerular filtration rate (GFR), and in renal blood flow, particularly that in the renal medulla, have been thoroughly investigated earlier (17). However, the effects of systemic inflammation on glomerular permeability in vivo have not been assessed in depth. Even though SIRS, or postoperative or posttraumatic conditions (16, 18), often leads to microalbuminuria, it is actually not known to what extent the increased urinary albumin excretion rate may just be due to a tubular defect, or really reflects an increased glomerular permeability.

Dextran causes anaphylaxis in rats by inducing degranulation of mast cells, with massive release of serotonin (5-HT) and histamine, a marked fall in mean arterial blood pressure (MAP), and peripheral edema (19, 31). Both 5-HT and histamine cause vascular protein leakage via gaps that form between endothelial cells, mostly in postcapillary venules (11, 15). These changes are transient and occur within 10–15 min. In this study, we wanted to study whether such rapid alterations also occur within the glomerular filtration barrier and to compare such glomerular permeability changes with those occurring in endotoxemia-induced systemic inflammation.

To study the functional glomerular alterations occurring in early septicemia and in anaphylactic shock in rats, we inves-

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tigated the urine excretion of infused fluorescein isothiocyanate (FITC)-Ficoll (70/400), a neutral, polydisperse polysaccharide that is not reabsorbed by the proximal tubules, allowing the assessment of glomerular permeability by the simultaneous measurement of fractional glomerular clearances (θ ; sieving coefficients) of a broad spectrum of molecular sizes. The experiments were specially designed so as to detect changes in the sieving pattern of Ficolls of high molecular weight (MW \sim 400,000). Furthermore, using a tissue uptake technique, we also assessed the θ for native (negatively charged) ^{125}I -labeled human serum albumin (RISA). Although we noted rapid, transient, and reversible changes in glomerular permeability to Ficoll after dextran-induced anaphylaxis, increases in glomerular permeability during endotoxemia-induced inflammation occurred first after 2 h of LPS exposure.

MATERIALS AND METHODS

Animals. Experiments were performed in 46 male Wistar rats (Møllegaard, Lille Stensved, Denmark) with an average body weight of 279.5 ± 6.3 g. The rats were kept on standard chow and had free access to water until the day of the experiment. The animal Ethics Committee at Lund University approved the animal experiments.

Surgery. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium, 60 mg/kg, and animals were placed on a thermostatically controlled heating pad to keep body temperature at 37°C . A tracheotomy was performed to facilitate breathing. The tail artery was cannulated (PE-50 cannula) for blood pressure monitoring and registration of heart rate (HR) on a polygraph (model 7B; Grass Instruments, Quincy, MA) and for repeated injections of maintenance anesthesia (pentobarbital sodium). The left carotid artery and the left and right external jugular veins were cannulated (PE-50 cannulas) for blood sampling and infusion purposes, respectively. After a small flank incision, the left ureter was cannulated (PE-10 cannula) for urine sampling, and the incision was sealed with Histoacryl (Melsungen, Germany).

Experimental procedures. Depending on exposure schedule and time to glomerular sieving measurements, rats were divided into four experimental groups and two sham groups described below. Drug (or saline) infusion started after an initial resting period (20 min), during which a control GFR measurement was performed.

Endotoxemia. Endotoxemia was induced by an intravenous bolus dose of 3 mg/kg of LPS (*Escherichia coli* 0111:B4; Sigma, St. Louis, MO), followed by a constant intravenous infusion of 6 mg/kg LPS for 30 min. To find out the response time to LPS, measurements were continued for an additional 60 min in one group of rats, in which Ficoll sieving measurements were performed at 60 and 90 min [ENDO-(60)/90; $n = 7$] after start of the infusion, or for 120 min in another group (ENDO-120; $n = 7$). For the last 33 min in the ENDO-120 group and the last 63 min in the ENDO-(60)/90 group, FITC-Ficoll (70/400) was infused intravenously, of which the first 20 min (in the ENDO-60 and ENDO-120 groups) or 50 min (in the ENDO-90 group) served as a "Ficoll saturation period" and the following 5 min as a period of glomerular Ficoll sieving assessment. RISA was given intravenously for the last 8 min of the experiment, as described below.

Anaphylaxis. Rats immediately develop anaphylaxis in response to dextran (31). Thus, to induce anaphylaxis, Dextran-70, 250 μl Macrodex (60 mg/kg; Meda, Solna, Sweden), was given intravenously as a bolus. It was possible to obtain urine and to measure Ficoll sieving and GFR during the first 5 min following dextran administration (ANA-5; $n = 8$), but after 10 min urinary flow was markedly reduced. In this group, FITC-Ficoll (70/400) was infused (for equilibration) for 25 min before the dextran challenge, with a Ficoll measurement period starting at 2 min after the dextran bolus and ending at 7–8 min after the bolus. In a second group of rats followed for 40 min after the

dextran injection, we noted a large fall in urine flow and MAP after \sim 10 min following the dextran challenge, and, because of that, 200 μl of horse serum (SVA, Uppsala, Sweden) were given one time or several times to try to normalize urine flow and MAP to preanaphylactic levels. The rationale for performing this "volume resuscitation," except for keeping the animals alive during the anaphylactic shock, was to obtain urine for measurement of GFR or θ in the time period following the hyperacute reaction to anaphylaxis. Thanks to the volume replenishment, measurements could be performed at 40 min (ANA-40; $n = 9$), but, because of subsequent reductions in urine flow and GFR, we were unable to follow the animals adequately for time periods essentially longer than 40 min during anaphylaxis.

Sham animals. The θ to high-MW Ficolls (mol. radius >50 Å) in sham animals in vivo in this experimental setting are extremely low and comparable to the fractional clearance of proteins of equivalent size (24, 25). Sham animals for the ENDO groups (SHAM-120; $n = 7$) received 120 min of constant NaCl infusion, 10 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, and, matching (in time) the infusion of LPS in the ENDO groups, a sham infusion of an extra 200 μl NaCl during 30 min. Sham animals for the ANA-5 group (SHAM-5; $n = 8$) obtained a bolus of NaCl (250 μl) 5 min before the Ficoll sieving assessment. Because there were no indications of changes in glomerular permeability between 5 and 120 min in the sham animals in the present experiments, nor in control rats in previous studies (24, 25), we employed the glomerular sieving curves for the SHAM-120 animals as control curves for the ENDO-(60)/90 groups and those for SHAM-5 as controls for the ANA-40 group. In all sham groups, FITC-Ficoll (70/400) was infused intravenously using a protocol identical to those in the experimental groups.

Sieving of FITC-Ficoll. A mixture of FITC-labeled Ficoll-400 (10 mg/ml) (TdB Consultancy, Uppsala, Sweden) and FITC-Ficoll-70 (10 mg/ml) (TdB Consultancy) in a 24:1 relationship, was administered as a bolus together with FITC-inulin (TdB Consultancy). The bolus dose (FITC-Ficoll-70, 40 μg ; FITC-Ficoll-400, 960 μg and FITC-Inulin, 500 μg) was followed by a constant infusion of 10 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (FITC-Ficoll-70, 20 $\mu\text{g}/\text{ml}$; FITC-Ficoll-400, 0.48 mg/ml ; FITC-inulin, 0.5 mg/ml ; Cr-EDTA, 0.3 MBq/ml) for 20 min, after which urine was collected for a 5-min period, with a midpoint (2.5 min) plasma sample (80 μl) also collected. For animals treated with dextran, the intravenous infusion rate was increased to 15 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ throughout the experiments to avoid dehydration and to try to preserve MAP in the animals. A high-performance size exclusion chromatography (HPLC) system (Waters, Milford, MA) was used to determine size and concentration of the Ficoll samples. Size exclusion was achieved using an Ultrahydrogel-500 column (Waters). The mobile phase was driven by a pump (Waters 1525), and fluorescence was detected with a fluorescence detector (Waters 2475) with an excitation wavelength at 492 nm and an emission wavelength at 518 nm. The system was controlled by Breeze Software 3.3 (Waters). The column was calibrated with Ficoll standards and protein standards described at some length in a previous paper (1). The θ of FITC-Ficoll 70/400 was determined as the fractional clearance from:

$$\theta = (C_{\text{FU}} \times C_{\text{IP}}) / (C_{\text{FP}} \times C_{\text{IU}}) \quad (1)$$

where C_{FU} represents the Ficoll urine concentration, C_{IP} represents the inulin concentration in plasma, C_{FP} the Ficoll concentration in plasma, and C_{IU} the inulin concentration in urine.

GFR. GFR was determined by measuring renal clearance of ^{51}Cr -EDTA (Amersham Bioscience, Buckinghamshire, UK) and FITC-inulin. ^{51}Cr -EDTA was given as a bolus dose at the start of the experiments (0.3 MBq in 0.2 ml iv) followed by a constant infusion of 10 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ (0.3 MBq/ml) (together with NaCl) throughout the experiments. For repeated measurements of the plasma to urine ^{51}Cr -EDTA clearance during the study, blood sampling was performed approximately every 20 min using microcapillaries (10 μl). Urine was sampled every 10–30 min of the experiment. Radioactivity in blood samples and urine samples was measured in a gamma counter (Wizard 1480; LKP Wallac, Turku, Finland). Hematocrit (50 μl) was

assessed two or three times throughout the experiment, to be able to convert blood radioactivity into plasma radioactivity.

The urinary excretion of $^{51}\text{Cr-EDTA}$ (and FITC-inulin) per minute ($U_t \times V_u$) divided by the concentration of tracer in plasma (P_t) was used to calculate GFR according to:

$$\text{GFR} = (U_t \times V_u) / P_t \quad (2)$$

where U_t represents the tracer concentration in urine, and V_u the flow of urine per minute.

Tissue uptake technique for assessing θ for $^{125}\text{I-HSA}$. After collection of urine and plasma for Ficoll determinations, $^{125}\text{I-HSA}$ (0.2 MBq; Institute for Energy Technique, Kjeller, Norway) was administered via the tail artery as a bolus. During an 8-min period, six blood samples (25 μl) and one urine sample were collected for estimation of the sieving coefficient of $^{125}\text{I-HSA}$ (RISA). Thereafter, a whole body washout was performed via the left external jugular vein (25 ml/min) for 8 min. The washout fluid mixture contained equal amounts of 0.9% saline and heparinized horse serum (SVA). After a laparotomy, the inferior vena cava was freed (within 1 min after start of the washout) and cut open for collection of the rinse fluid. Following complete washout, the kidneys were removed, and the cortex was dissected out and assessed with respect to radioactivity. To reduce the influence of free ^{125}I , urine samples were precipitated with 10% trichloroacetic acid (TCA) and spun down, and the supernatant (free ^{125}I) was discarded. All samples were measured for radioactivity in the gamma counter mentioned above.

The sieving coefficients for RISA were calculated from the amount of tracer radioactivity accumulated in the left kidney cortex plus the TCA-precipitable urine tracer activity (collected during the tracer infusion period and amounting to a maximum of 5–10% of total cortical radioactivity) divided by the average plasma tracer concentration, by the tracer circulation time, and by GFR.

Pore analysis. The two-pore model (10, 22, 29) was used to analyze the θ data for Ficoll (mol. radius 15–80 \AA). A nonlinear least-squares regression analysis was used to obtain the best curve fit, using scaling multipliers as described at some length previously (10).

Statistics. Values are expressed as means \pm SE. Differences between groups were tested using nonparametric analysis of variance with the Kruskal-Wallis test and post hoc tested using the Mann Whitney U -test. Significance levels were set at $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. All statistical calculations were made using SPSS 11.0.3 for Macintosh OSX (SPSS, Chicago, IL).

RESULTS

MAP and HR. The SHAM group showed a stable, or only moderately reduced, MAP during the course of the experiment (Fig. 1), and also a moderate increase in HR. By contrast, endotoxin caused a rapid (within 5 min) fall in MAP, from 130 ± 2.4 to 54.3 ± 4.6 mmHg ($P < 0.01$) in the ENDO-(60)/90 group and from 116 ± 3.9 to 53.3 ± 4.8 mmHg ($P < 0.01$) in the ENDO-120 group. After a spontaneous recovery of the MAP, which usually occurred within the next 20–30 min, there was again a slow reduction of MAP with time. Thus there appeared to be a bimodal MAP response to endotoxin. Furthermore, endotoxemia caused an increased HR, from 383 ± 13.8 beats/min at the start to 447 ± 17.1 beats/min ($P < 0.05$) after 120 min, which was not seen in the 120 sham group. Similar to the LPS group, the anaphylactic group (Fig. 2) showed an initial, but less rapid, decrease in MAP. Thus, during the first 5 min after the dextran infusion, MAP was rather stable, starting at 116 ± 5.7 and being reduced to 104 ± 6.0 mmHg [not significant (NS); ANA-5 group]. In the ANA-40 group, MAP fell from 99.2 ± 3 to 47.1 ± 1.5 mmHg ($P < 0.01$) 15 min after the dextran administration. The

ANA-40 group nearly recovered their MAP toward the end of the experiment, conceivably mainly because of the volume resuscitation performed (Fig. 2). There was also a slight tendency of increase in HR from 320 ± 18.1 to 332 ± 8.9 (NS).

GFR. In the SHAM group, GFR showed a slightly higher value at the end of the experiments than at the start (Fig. 3), whereas, in the ENDO groups, there was a decrease in GFR, ~ 20 min after start of the LPS infusion, from 0.70 ± 0.05 to 0.35 ± 0.04 $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ($P < 0.01$) in the ENDO-(60)/90 group and from 0.73 ± 0.05 $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ to 0.49 ± 0.09 $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ ($P < 0.05$) in the ENDO-120 group. In the following period (at ~ 50 min), GFR had recovered, but then again tended to fall as part of the septic condition. In the anaphylaxis groups, GFR could not be measured during the period from 10 to 20 min after the dextran bolus because of the marked reduction in urine flow. However, early in the experiment (0–10 min), urine flow and GFR were remarkably well preserved (Fig. 4) in the ANA-5 and ANA-40 group. By the end of the experiment (at 40 min), conceivably thanks to the volume resuscitation, GFR seemed to have partly recovered vs. preanaphylactic value(s) in the ANA-40 group (0.65 ± 0.06 vs. 0.72 ± 0.04 $\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$; NS).

θ for FITC-Ficoll. Figure 5 demonstrates the sieving coefficient (θ) vs. Stokes-Einstein radius (a_e) curves for Ficoll molecules ranging in radius from 15 to 80 \AA for the ENDO-120 group vs. the SHAM-120 group. In the a_e range between 55 and 80 \AA , the ENDO-120 group showed a clearly increased θ compared with SHAM ($P < 0.001$). This increased permeability to high-mol. Ficolls was, however, not seen for either the ENDO-60 or the ENDO-90 measurements, as shown in Fig. 6, demonstrating a time dependency of the changes in glomerular permeability following endotoxin.

In the acute anaphylaxis (ANA-5) group, there were significantly higher sieving coefficients for Ficoll 55–80 \AA ($P < 0.001$) than in the SHAM-5 group (Fig. 7). By contrast, there was no significant difference between the ANA-40 group and either of the SHAM groups.

Two-pore modeling. The best curve fits of θ vs. a_e for Ficoll according to the two-pore model were obtained using the parameters listed in Table 1 (ENDO groups) and Table 2 (ANA

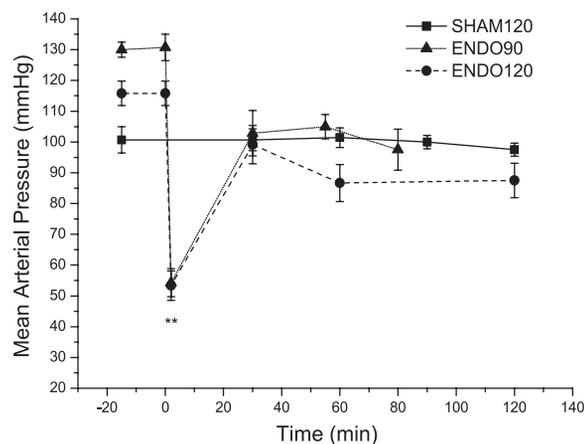


Fig. 1. Mean arterial pressure (MAP) vs. time in the endotoxin (ENDO) groups vs. SHAM. \blacktriangle , Ficoll sieving measurements performed at 60 and 90 min after start of the lipopolysaccharide (LPS) infusion [ENDO-(60)/90]; \bullet , Ficoll sieving measurements performed 120 min after start of LPS infusion (ENDO-120); \blacksquare , SHAM group. Note the marked, immediate fall in MAP by endotoxin.

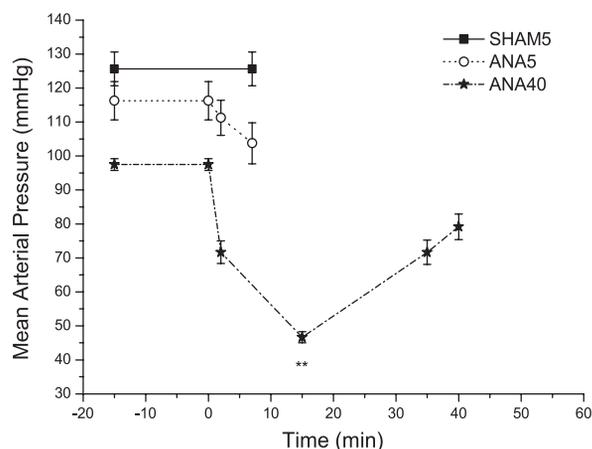


Fig. 2. MAP vs. time in the two anaphylactic groups investigated. ○, 5-min anaphylactic group (ANA-5); ★, 40-min anaphylactic group (ANA-40). Symbols for the SHAM-5 group are the same as for the SHAM-120 group.

groups). The fractional ultrafiltration coefficient accounted for by large pores, mainly reflecting the number of large pores, and the fractional fluid flow through large pores were more than doubled in the ENDO-120 group and the acute anaphylactic (ANA-5) group, but remained unchanged vs. control in ENDO-(60)/90 groups and in the ANA-40 group. In acute anaphylaxis, not only the large pore number, but also the large pore radius, was acutely increased (188 ± 10.1 vs. 115 ± 3.0 ; $P < 0.01$). The effective area over unit diffusion path length seems to have been reduced in the ANA-5 group.

θ for albumin. Figure 8 shows θ for albumin in the ENDO groups. Because tissue uptake experiments could only be performed at the termination of the experiments, data could only be obtained at 90 min for the ENDO-(60)/90 group. θ for albumin was significantly increased in the ENDO-120 group vs. the SHAM-120 group [$4.86 \times 10^{-4} \pm 5.1 \times 10^{-5}$ vs. $3.06 \times 10^{-4} \pm 3.22 \times 10^{-5}$ ($P < 0.05$)] but not in the ENDO-90 group ($4.0 \times 10^{-4} \pm 4.89 \times 10^{-5}$ vs. $3.06 \times 10^{-4} \pm 3.22 \times 10^{-5}$; NS). Figure 9 demonstrates the increase in the albumin sieving coefficient in the ANA-5 and ANA-40 groups. In the ANA-40 group, contrary to the unchanging θ for large Ficolls, θ for albumin was significantly increased vs. the

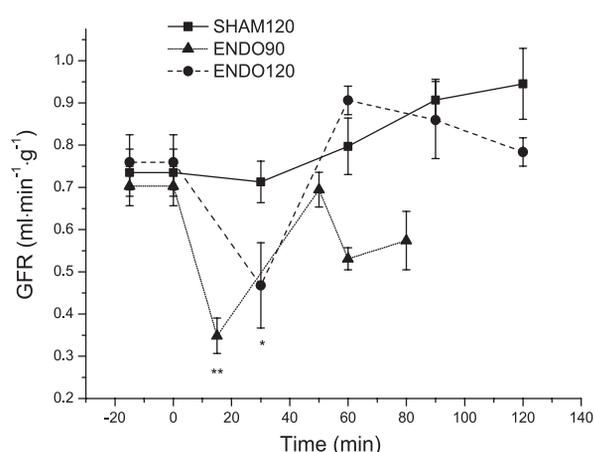


Fig. 3. Glomerular filtration rate (GFR) per gram kidney, measured as a function of time for the two endotoxin groups and for the SHAM group. Note the fall in GFR during the first 10–30 min of the dwell in the ENDO group.

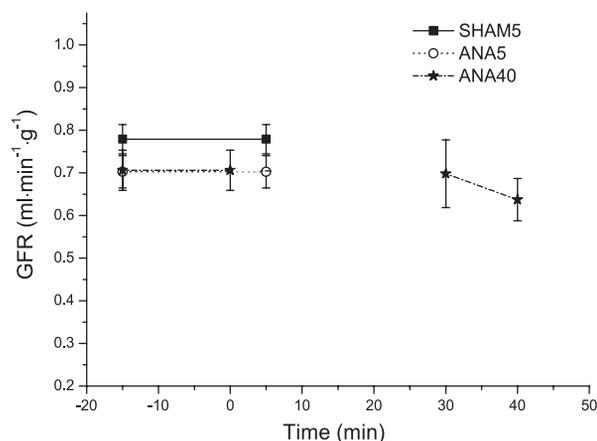


Fig. 4. GFR per gram kidney, measured as a function of time in anaphylaxis. Symbols as in Fig. 2. Note the absence of measurements in the ANA-40 group in the time period between 5 and 30 min because of oliguria.

SHAM-5 group, i.e., $6.68 \times 10^{-4} \pm 3.34 \times 10^{-5}$ vs. $3.56 \times 10^{-4} \pm 4.75 \times 10^{-5}$ ($P < 0.01$). The fractional clearance of RISA reaching the final urine (and not being reabsorbed) did not differ significantly between groups and was on average $\sim 3\%$ ($2.83 \pm 0.55\%$) of filtered load, except in the ANA-40 group, where it was only $0.54 \pm 0.20\%$ ($P < 0.05$ vs. SHAM-5) of filtered load.

DISCUSSION

This is, to our knowledge, the first detailed in vivo study of the alterations in the glomerular permeability that occur during systemic inflammation induced by endotoxemia and in anaphylactic shock. The major result of the study is that early endotoxemia-induced systemic inflammation and anaphylactic shock both reduce the size-selectivity of the glomerular filtration barrier by increasing the number of large pores in the glomerular filter, rather than affecting the glomerular charge-selectivity. Acute anaphylaxis caused a rapid, transient change in glomerular permeability, completely reversible within 40

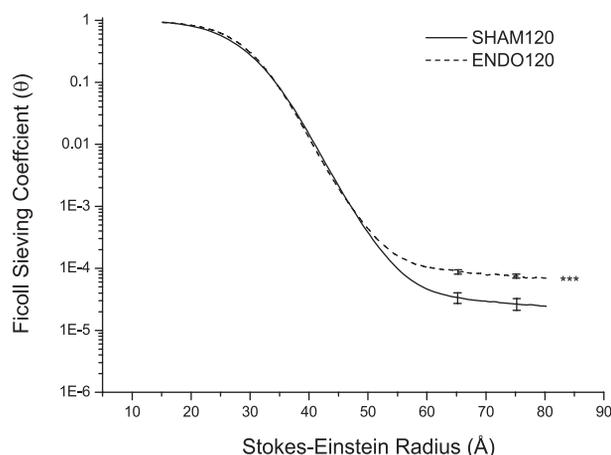


Fig. 5. Sieving coefficients (θ) vs. Stokes-Einstein radius (a_e) for Ficoll in the left kidney for the ENDO-120 group (dashed line) vs. the SHAM group (solid line). 400 data points are sampled between 15 and 80 Å. The sieving coefficients for large Ficoll molecules (mol. radius 50–80 Å) were significantly increased in the ENDO-120 group compared with SHAM or the ENDO-60/90 measurements ($P < 0.001$).

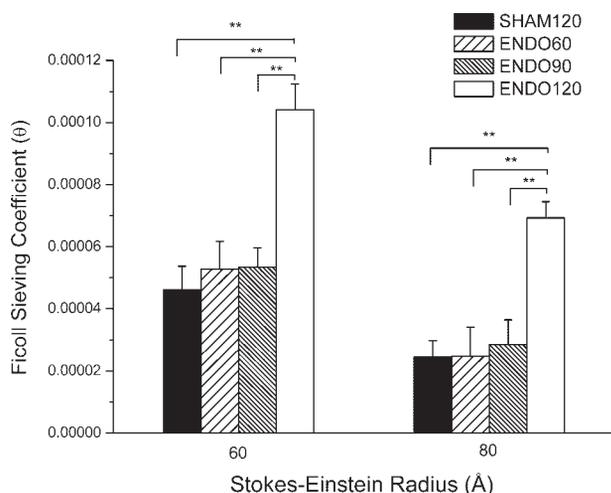


Fig. 6. Time dependency of Ficoll sieving coefficients for Ficoll molecules of radius 60 and 80 Å. Significant increases in θ for large Ficolls occurred only for the 120-min ENDO group compared with either SHAM or the ENDO-60 and ENDO-90 measurements.

min, whereas LPS induced an increase in the θ to large Ficoll molecules, which was manifest only after 2 h. These two completely different response patterns apparently reflect two different mechanisms of action of the mediators involved in endotoxin-induced shock and in anaphylactic shock.

It is well accepted that LPS administration leads to activation of TLR4 on cells in the innate immune system (2). TLR4 is present on monocytes and other cell types, including podocytes, and mediates a series of inflammatory events. Exposure to endotoxin thus initiates a complex cascade, involving various proinflammatory and anti-inflammatory cytokines, such as IL-6, TNF- α , IL-10, and IL-18, activating white cells and other inflammatory response mediators, including adhesion molecules, complement factors, oxygen-derived free radicals, and procoagulant factors. Especially, TNF- α is considered a crucial mediator of both acute and chronic inflammatory responses, including microalbuminuria (9). The slow and gradual change in glomerular permeability in response to endotoxin thus seems

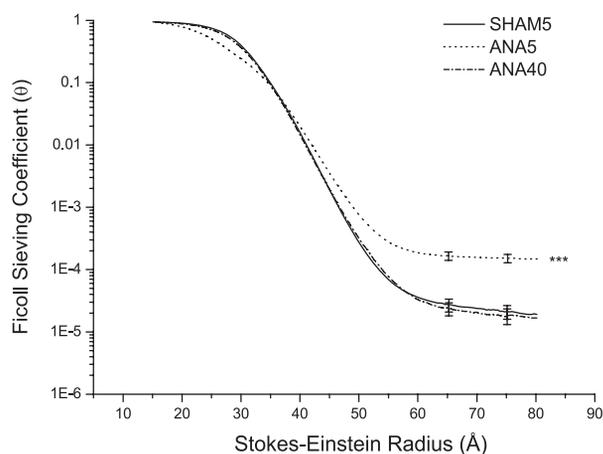


Fig. 7. The θ vs. a_e for Ficoll in the left kidney for the ANA-5 group (dotted line) and the ANA-40 group (dashed/dotted line) vs. the acute SHAM group (SHAM-5; solid line). Note the marked increase in the sieving coefficient to large Ficoll molecules ($a_e > 50$ Å; $P < 0.001$) in the ANA-5 group! There was also a moderate increase in large pore radius.

Table 1. Two-pore parameters in the ENDO assessment groups vs. SHAM

Two-Pore Parameters	SHAM-120	ENDO-60	ENDO-90	ENDO-120
n	7	7	7	7
r_s , Å	45.7 \pm 0.20	46.3 \pm 0.26	46.4 \pm 0.09	45.4 \pm 0.20
r_L , Å	138.1 \pm 3.76	132.4 \pm 9.32	146.7 \pm 20.97	143.2 \pm 5.96
$\alpha_L \times 10^5$	4.08 \pm 0.76	3.44 \pm 0.79	3.25 \pm 0.31	10.20 \pm 1.43†
$J_v/L/GFR \times 10^5$	12.32 \pm 2.56	9.97 \pm 2.40	9.06 \pm 0.78	21.60 \pm 2.82*
$A_0/\Delta X$, $\text{cm} \cdot \text{g}^{-1} \times 10^{-5}$	3.76 \pm 0.59	1.47 \pm 0.13	1.31 \pm 0.12*	4.56 \pm 0.81

Values are given as means \pm SE; n , no. of rats. SHAM-120, 120-min sham group; ENDO-60, ENDO-90, and ENDO-120, Ficoll sieving measurements performed at 60, 90, and 120 min, respectively, after start of endotoxin (ENDO) infusion; r_s , small pore radius; r_L , large pore radius; α_L , fractional UF coefficient accounted for by large pores; J_v/L , fractional fluid flow through large pores; GFR, glomerular filtration rate; $A_0/\Delta X$, effective area over unit diffusion path length. Statistical difference between SHAM and endotoxin groups are as follows: * $P < 0.05$ and † $P < 0.01$.

to reflect the gradual activation of multiple and integrated inflammatory response systems over time.

Perhaps the most startling finding of the present study was the immediate and prominent increase in glomerular permeability in anaphylaxis, a response completely reversible within 40 min. In rats, dextran induces anaphylaxis by causing degranulation of mast cells, with massive release of 5-HT and histamine. In turn, these biogenic amines cause precapillary vasodilation (histamine) and/or postcapillary vasoconstriction (5-HT), raising capillary pressure, and an increased microvascular leakage of proteins (12, 22). This leakage occurs via gaps that transiently form between endothelial cells, mostly in postcapillary venules (11, 19). Despite the fall in MAP, there was thus a marked, transient increase in glomerular permeability occurring in parallel with an increased transvascular leakage of RISA in skin, lung, and muscle (presented in a subsequent study) in anaphylaxis.

Overall, it should be noted, however, that the glomerular barrier apparently is rather unresponsive to 5-HT and histamine following the immediate insult, because glomerular permeability was completely restored within 40 min. This is actually in line with previous studies using dextran molecules (18–44 Å in radius) as markers of glomerular permselectivity (8) or using a technique for assessing vascular labeling of endothelial gaps with carbon (27). The mentioned studies thus failed to demonstrate any renal permeability effects of histamine or 5-HT, except for showing some occasional leaky sites in peritubular

Table 2. Two-pore parameters in ANA-5 and ANA-40 groups vs. SHAM

Two-Pore Parameters	SHAM-5	ANA-5	ANA-40
n	8	8	9
r_s , Å	46.0 \pm 0.05	46.9 \pm 0.16	45.8 \pm 0.10
r_L , Å	114.6 \pm 2.95	188.3 \pm 10.13*	140.3 \pm 10.20
$\alpha_L \times 10^5$	3.90 \pm 0.83	8.15 \pm 1.21*	2.94 \pm 0.50
$J_v/L/GFR \times 10^5$	10.1 \pm 2.21	27.30 \pm 4.07†	6.58 \pm 1.35
$A_0/\Delta X$, $\text{cm} \cdot \text{g}^{-1} \times 10^{-5}$	6.82 \pm 0.75	1.10 \pm 0.08*	4.41 \pm 0.75

Values are given as means \pm SE; n , no. of rats. SHAM-5, 5-min sham group; ANA-5 and ANA-40, respective 5 and 40 min anaphylactic groups. † $P < 0.01$ and * $P < 0.05$, statistical difference between SHAM and anaphylaxis groups.

capillaries (27). Another factor behind the reversibility of the glomerular filter to histamine may be the acute catecholamine release that is usually associated with anaphylaxis, which can abrogate the actions of histamine on vascular permeability (12, 21). Anyway, the present study strongly indicates that, except for the immediate, very marked changes in glomerular permeability, the kidneys are largely protected against the actions of biogenic amines, such as histamine and 5-HT, under conditions of anaphylaxis. In other words, microalbuminuria should just be a very transient and initial event in this condition.

Also, a number of previous *in vitro* studies on isolated glomeruli indicate that glomerular permeability can indeed increase very rapidly and transiently (within 5–15 min) in response to various challenges, such as puromycin aminonucleoside (PAN), TNF- α , vascular endothelial growth factor, or transforming growth factor (3, 13, 14, 26, 28). In isolated glomeruli *in vitro*, the acute permeability effects of TNF- α and PAN were found to be mediated in part by reactive oxygen species, since inhibition of these, or their generation, apparently reduced the changes in glomerular permeability (13). The exact mechanisms of the described rapid, dynamic changes of the glomerular permeability are at present unknown, leaving room for speculation. Previous results from our group strongly indicate that the major barrier to protein permeability of the glomerular filter is not at the podocyte slit diaphragm (10, 23). Thus it seems that all three sequential barriers, i.e., the glomerular basement membrane (GBM) and the two cellular layers, need to interact to create the nearly perfect glomerular permeability barrier normally present (7). Especially, the way that the podocytes interact with the GBM may be crucial. Biogenic amines may interact with the podocytes to produce changes in their actin cytoskeleton that may alter the shape of the podocyte and the tension that they exert on the GBM. Also, endothelial cells may be involved, in a way similar to how they react to biogenic amines in nonfenestrated endothelium. In addition, the molecular complexes in the slit diaphragm and/or the intergrins linking the podocyte foot process to the GBM may be acutely involved in these changes (3).

The Ficoll molecules investigated in this study are largely uncharged, whereas albumin is negatively charged at physiologic pH. Despite the unchanging θ for large Ficoll molecules in the ANA-40 group, there was an increase in θ for albumin in this group. One way of explaining this discrepancy is to

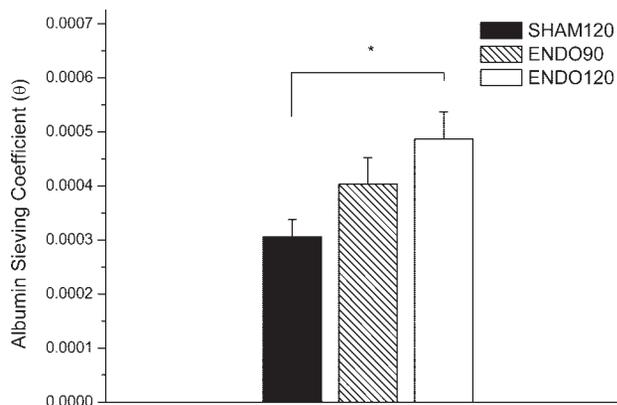


Fig. 8. Albumin sieving coefficient as a function of time in the ENDO groups (ENDO-90: hatched bar; ENDO-120: open bar) vs. SHAM-120 (filled bar). Only the increase in θ for albumin after 2 h was statistically significant.

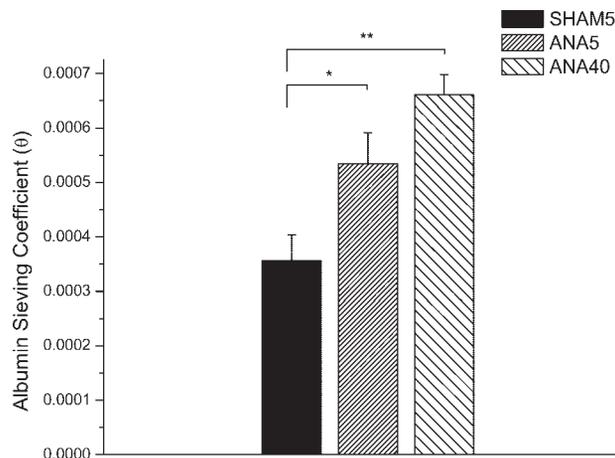


Fig. 9. Sieving coefficient for albumin in the acute anaphylactic (ANA-5) group (densely hatched bar) and the ANA-40 group (loosely hatched bar) vs. SHAM (filled bar). Similar to the large increase in θ for high-molecular-weight Ficoll, there was an increase in the albumin sieving coefficient in the ANA-5 group. However, in stark contrast to the unchanging sieving coefficient to high-molecular-weight Ficoll, θ for albumin was increased also in the ANA-40 group.

imply that a reduction in the negative charge selectivity of the glomerular barrier had occurred by dextran. Indeed, high concentrations of dextran have been shown increase the permeability to albumin in muscle capillaries without markedly affecting the capillary filtration coefficient (20). However, given the low circulating dextran concentrations in the present study, this effect is not the most likely. An alternative explanation may instead be that the increased θ for albumin may have resulted from an early, transient period of leakage of macromolecules from the peritubular capillaries in the cortical interstitium, causing interstitial edema and more space for subsequent (tracer) albumin accumulation. This may have resulted in a “falsely” high accumulation of RISA, assessed by the tissue uptake procedure at 40 min, in excess of that occurring by the albumin reabsorption by the proximal tubular cells. Thus the biogenic amines released in anaphylaxis, through their immediate effects, actually may affect the permeation of proteins across the peritubular capillaries (27) while largely leaving the glomerular permeability unaffected in the long term.

In conclusion, the present study, specially designed to assess glomerular sieving coefficients for high-molecular-weight Ficoll molecules, demonstrated an early, transient increase in glomerular permeability to Ficoll in anaphylactic shock, completely reversible within 40 min. By contrast, endotoxemia increased glomerular permeability first after 2 h. In both endotoxemic and anaphylactic shock, the increased glomerular barrier permeability appeared to occur by an increase in the number of large pores in the glomerular filter, rather than affecting the glomerular charge selectivity.

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