Leakage responses to L-NAME differ with the fluorescent dye used to label albumin

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Rumbaut, Rolando E., Norman R. Harris, Arshad J. Sial, Virginia H. Huxley, and D. Neil Granger. Leakage responses to L-NAME differ with the fluorescent dye used to label albumin. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H333–H339, 1999.—Nitric oxide synthase (NOS) inhibitors have been reported to increase as well as to decrease microvascular transport of macromolecules in a variety of models. This study was performed to determine whether the influence of NOS inhibition on albumin leakage was dependent on the fluorescent dyes used to label albumin. Albumin leakage was assessed in rat mesenteric venules during control conditions and after exposure to the NOS inhibitor N\(^{G}\)-nitro-L-arginine methyl ester (L-NAME). Albumin was labeled with any one of four dyes: FITC, sulforhodamine 101 [Texas Red (TR)], dichlorotriazinyl aminofluorescein (DTAF), or Oregon Green 514 (OG). Superfusion with L-NAME (10\(^{-5}\) M) was accompanied by an increase in leakage of FITC-labeled albumin (n = 12) but not of albumin labeled with DTAF (n = 10), TR (n = 10), or OG (n = 4). In vessels perfused with both FITC- and TR-labeled albumin (n = 12), superfusion with L-NAME increased leakage of FITC- but not TR-labeled albumin. In conclusion, albumin leakage responses to L-NAME differ among various fluorescent dyes. Therefore, caution is advised in comparison of albumin leakage results that utilize different fluorescent dyes.

macromolecular leakage; nitric oxide; photohemolysis

THE MAJORITY of macromolecules used to probe the integrity of the microvascular barrier are colorless; therefore dyes are attached to render them visible. With improvements in fluorescence chemistry and microscopy, fluorescent dyes are used widely to quantify microvascular leakage under a variety of conditions. FITC is a popular dye for microvascular research because it is soluble in aqueous media, has a high fluorescence quantum yield, and is relatively inexpensive (5). One of the limitations of FITC is photobleaching, which has been described both in vitro (8) and in vivo (15). Other investigators have quantified macromolecular leakage by the use of alternate dyes that are more resistant to photobleaching, such as Texas Red (28) and Oregon Green (9).

Nitric oxide (NO) released by the vascular endothelium plays an important role in regulation of local blood flow (20). The influence of NO on microvascular permeability is unclear. NO synthase (NOS) inhibitors have been reported to elevate as well as to reduce the permeability of exchange microvessels in several tissues from a variety of species, both under basal conditions and on exposure to agonists that increase permeability (4, 6, 10–14, 24, 29).

Several dyes have been used by different investigators to examine the influence of NO on microvascular permeability. For example, whereas the NOS inhibitors \(N^{G}\)-nitro-L-arginine methyl ester (L-NAME) and \(N^{G}\)monomethyl-L-arginine (L-NMMA) were reported to increase leakage of FITC-labeled albumin in rat mesenteric venules (13, 14), L-NMMA was without effect on leakage of dichlorotriazinyl aminofluorescein (DTAF)-labeled albumin in frog mesenteric venules (22). In the quest to determine the basis for dissimilar responses, we performed the present study to determine whether the use of different dyes to label albumin for assessment of macromolecular leakage altered the observed response to NOS inhibition.

MATERIALS AND METHODS

The albumin leakage experiments described in this paper were duplicated by investigators at each of the two participating centers, using a similar protocol. The apparent permeability and photohemolysis experiments were performed at Columbia.

Solutions. Bicarbonate-buffered saline (BBS) was prepared in each center with similar composition: in Columbia, BBS contained (in mM) 132 NaCl, 4.7 KCl, 2.0 CaCl\(_{2}\), 1.2 MgSO\(_{4}\), 20 NaHCO\(_{3}\), and 5.0 glucose, bubbled with 95% N\(_{2}\)-5% CO\(_{2}\); in Shreveport, BBS contained 132 NaCl, 4.7 KCl, 2.0 CaCl\(_{2}\), 1.2 MgSO\(_{4}\), and 20 NaHCO\(_{3}\), bubbled with 95% N\(_{2}\)-5% CO\(_{2}\). Phosphate-buffered saline (PBS) consisted of (in mM) 130.0 NaCl, 1.9 NaH\(_{2}\)PO\(_{4}\)-H\(_{2}\)O, 9.0 Na\(_{2}\)HPO\(_{4}\)-7H\(_{2}\)O, and 22.2 glucose.

FITC-labeled BSA (FITC-BSA) was purchased from Sigma Chemical (St. Louis, MO), and Texas Red-labeled BSA (TR-BSA) was purchased from Molecular Probes (Eugene, OR).
and Sigma Chemical. Oregon Green 514 (OG) was purchased from Molecular Probes, DTAF from Research Organics (Cleveland, OH), L-NAME from Bachem (Torrance, CA) and Calbiochem (La Jolla, CA). All other chemicals were purchased from Sigma Chemical. Labeling of albumin with DTAF and OG was performed by the methods outlined by Huxley et al. (8).

Briefly, bovine serum albumin (catalog no. A4378, Sigma Chemical) was dissolved in 0.05 M borate buffer and 0.04 M NaCl (9 mg protein/ml) and placed in 12,000 molecular weight (MW) cutoff tubing (Spectra/por, Spectrum Medical Industries, Laguna Hills, CA). The fluorescent dye was dissolved in 0.05 M borate buffer (initial dye-to-protein molar concentration ratio of 3 to 1), and the protein solution was dialyzed against the dye solution for 12 h at room temperature. The labeled protein was then dialyzed against three 2-liter changes of Krebs solution over a 72-h period (4°C).

The labeled protein was centrifuged for 90 min at 4,500 rpm in 5,000 MW cutoff centrifuge tubes (Millipore, Tokyo, Japan), aliquotted, and stored at 15°C until use. Animal preparation. Male Sprague-Dawley rats (150–250 g) were anesthetized with an intraperitoneal injection (130 mg/kg body wt) of thiobutabarbital (Inactin, Research Biochemicals, Natick, MA). A tracheotomy was performed to facilitate breathing, and the right carotid artery and left internal jugular vein were cannulated for arterial blood pressure monitoring and intravenous drug administration, respectively. A midline abdominal incision was made, and a loop of small intestine was exteriorized to allow visualization of a small section of mesentery. The rats were placed in a lateral decubitus position on a custom Plexiglas animal tray, which allowed observation of the section of mesentery. The mesentery was draped over a nonfluorescent coverslip (Shreveport) or a polished quartz pillar (Columbia), and the intestine was covered with saline-moistened gauze. The preparation was superfused continuously with BBS at 37°C, bubbled with a gas mixture containing 5% CO2 (pH 7.4 ± 0.5).

Intravital microscopy. An inverted microscope (Diaphot, Nikon) was used to observe the mesenteric circulation with either a ×32 or a ×40 objective. A video camera mounted on the microscope projected the transilluminated image onto a monitor, and the image was recorded using a videotocassette recorder. A video timer provided time functions. The preparation was epi-illuminated with an intensified 75-W xenon arc lamp, and fluorescence was assessed with an intensified charge-coupled device (CCD) camera, using dichroic filter cubes matched to the dyes (FITC, DTAF, and OG: excitation 460–500 nm, emission 535 nm (Chroma Technology), Brattleboro, VT); TR: excitation 560, emission 630 nm (Omega Optical, Brattleboro, VT)). Neutral density filters were placed in the epifluorescence light path in the in vivo experiments.

Microcirculatory rig components. The following components were used in Columbia: Nikon Diaphot 200 microscope (Nikon) with Leitz ×32 UM objective (NA 0.30), 75-W xenon lamp (Leitz), Panasonic AG-6300 video recorder (Matsushita Electric Industries), Pulnix TM-7CN video camera (Pulnix America, Sunnyvale, CA), PTI IC-100 intensified CCD camera (ChromTech International, Monmouth Junction, New Jersey), VTG-33 video timer (FOR-A, Tokyo, Japan), and Buchler instruments peristaltic pump. The following components were used in Shreveport: Nikon Diaphot 300 microscope with a Nikon ×40 Fluor objective (NA 0.85), 75-W xenon lamp (Nikon), JVC BR-S601MU S-VHS video recorder, VK-C150 color video camera (Hitachi, Tokyo, Japan), Hamamatsu C2400–60 CCD camera and C2400–68 intensifier (Hamamatsu Photonics), Minipuls 3 peristaltic pump (Gilson, Middleton, WI).

Assessment of albumin leakage and leukocyte adherence. Albumin leakage was determined by a modification of the methods described by Kurose et al. (13). Briefly, single, nonbranched venules with diameters ranging from 25 to 40 µm were selected; one venule was used per animal. Fluorescently labeled albumin (20–25 mg/kg) was injected intravenously 30 min before the initial determination of albumin leakage. Albumin leakage was quantified by measuring mean fluorescence intensity (with video analysis software) of two 10 × 50 µm windows, one placed within the venule (Iv) and the other in the perivenular interstitium (Ii). 10 µm away from the venule wall. Albumin leak index (AL index) was determined by (Ii − background)/(Iv − background) × 100%.

To minimize light exposure to the tissues, each measure of albumin leakage was performed during a brief (~5–15 s) exposure to epi-illumination, controlled with a manual shutter. In experiments involving FITC- and TR-BSA, assessment of AL index with each fluorescence filter cube was determined sequentially. Occasionally, a selected venule had a high baseline AL index, those experiments with venules with an initial AL index >20% were not included in the final analysis.

Leukocyte adherence was determined off-line during playback of videotaped images for 5-min periods at specified intervals. A leukocyte was considered adherent if it remained stationary for at least 30 s. Adherence was expressed as the number of adherent leukocytes per 100 µm of vessel length.

Assessment of venular permeability to albumin. Venular apparent permeability (P) to the various dye-labeled albumins was determined using microscope photometry, as described previously (8, 22). Briefly, a venule was cannulated with a double-lumen (theta) micropipette, connected to a double water manometer for control of hydrostatic pressure. The experiments were conducted as paired experiments. For each vessel, apparent permeability to TR-BSA and one of the other dyes was assessed. One side of the pipette contained heat-treated, dialyzed rat serum with TR-BSA (final concentration 2.5 mg/ml) and DTAF-, OG-, or FITC-BSA (2.5 mg/ml). The other side of the pipette contained unlabeled BSA in serum ("washout" solution). Measures of P to BSA (PBSA) with each dye pair were obtained simultaneously. This was performed with a dual-excitation filter (Chroma Technology) and a dual-microscope photometer (Photon Technology International) with a dual-emission filter (Chroma). The dual photometer system allowed ~1% overlap in fluorescence between the dye pairs. Fluorescence intensity was monitored downstream from the cannulation site over an area, defined with an adjustable rectangular diaphragm, that included a portion of the vessel and perivascular interstitium (4 diameters wide, 8 diameters long). The output of the dual photometer was directed to a Gateway 2000 computer (Pentium II, 333 MHz) using analog-to-digital hardware and software (Felix, Photon Technology International). The experiments were imaged with an intensified CCD camera (PTI IC-100) to assess flow conditions, cannulation site, and the presence of visible leaks. Solute flux (Js) per surface area (S) and concentration gradient (∆C) between the plasma (Cp) and interstitium (Ci) was determined from the fluorescence intensity (∆Ij) as the solute filled the vessel of radius r and from the rate of change in intensity after entry of solute into the vessel (∆I/∆t)Pt = J_0S/C_0(1/A)J_0(1/A)(r/2).

In vitro erythrocyte photohemolysis. The phototoxicity of the four labeled albumin compounds was assessed in vitro, using an erythrocyte photohemolysis assay as described by Miller et al. (19). Blood was collected from anesthetized rats through a carotid arterial line and placed in heparinized blood collection tubes. The blood was centrifuged for 10 min, the plasma and buffy coat were removed, and the erythro-
cytes were suspended in PBS (see MATERIALS AND METHODS) with albumin (5 g/l). The erythrocytes were diluted with the buffer-fluorescent dye solution to a final hematocrit of 4% (vol/vol). The suspensions were stored overnight in the dark at 4°C. Thereafter, the erythrocyte-dye solution was placed in a Neubauer hemacytometer and covered with a glass slide. After a 10-min equilibration period on the microscope stage, a 150-µm-diameter area of the sample was epi-illuminated (75-W xenon lamp, ×20 Nikon Fluor objective, NA 0.75) for 10 min. Erythrocyte photohemolysis was quantified by measuring the intensity of light transmission from a 150-W halogen lamp with a fiber-optic bundle (Navitar, Rochester, NY) with a 405-nm narrow band-pass filter (Chroma Technology), using the microscope photometer. Light transmission was measured by centering a 80 × 80 µm square window within the area of activation. Responses were quantified as a percentage of the maximal transmission readings (defined as <5 erythrocytes in the measuring window). In the case of albumin labeled with TR, OG, or DTAF (1.5 mg/ml), because maximal hemolysis did not occur, maximal light transmission was determined with longer durations of exposure and 5 mg/ml of labeled albumin.

Experimental protocols. The mesentery was superfused continuously (5 ml/min) with BBS alone, and initial (control) assessment of albumin leakage was performed after 30 min. Subsequently, the superfusate was changed to 10^-4 M L-NAME in BBS and measurements were repeated 15 and 30 min later. In a separate set of experiments, platelet-activating factor (PAF; 10^-6 M) was the test agent in BBS. Venular apparent permeabilities were determined during superfusion of BBS alone.

Statistical analysis. Albumin leakage index and leukocyte adherence values are presented as means ± SE, and apparent solute permeability (P_a) data (which are not normally distributed) are presented as medians. Comparison of AL_index and adherence values were performed with one-way analysis of variance, and comparison of P_a values was performed by Wilcoxon’s signed-rank test. A P < 0.05 was considered significant.

RESULTS

The influence of 10^-4 M L-NAME on albumin leakage with each of the four fluorescent dyes is shown in Fig. 1A. During superfusion with L-NAME, a significant increase in FITC-BSA (n = 12) leakage was noted after both 15 and 30 min. However, albumin leakage of TR-BSA (n = 10) and OG-BSA (n = 4) remained unchanged during the study period, and leakage of DTAF-BSA (n = 10) decreased from basal levels over 30 min. Superfusion with L-NAME led to increases in venular leukocyte adherence independent of the fluorescent dye used to label albumin (Fig. 1B).

To control for changes in AL_index as a function of time, AL_index measurements were performed for 60 min during superfusion with BBS alone (Fig. 2A). TR-BSA (n = 5) or FITC-BSA (n = 5) leak indexes were not changed from control conditions, whereas DTAF-BSA (n = 4) tended to decrease (not significantly). Leukocyte adherence did not increase during superfusion with BBS alone (Fig. 2B).

In an additional 12 experiments, both FITC- and TR-BSA were present in the circulation. This allowed assessment of the influence of L-NAME on leakage of both FITC- and TR-BSA in the same vessels and under the same conditions. AL_index of FITC-BSA increased during L-NAME treatment, whereas TR-BSA leakage did not differ statistically from control values (Fig. 3A).

To determine whether leakage of TR-BSA could be elicited with an agonist known to increase microvascular permeability, the influence of PAF on TR-BSA leakage was assessed in five experiments. Superfusion with 10^-7 M PAF caused a marked increase in TR-BSA AL_index at 15 and 30 min (Fig. 3B).

To determine whether a higher venular permeability to FITC-BSA could account for the differences in albumin leakage (Figs. 1 and 3), apparent permeabilities to albumin labeled with TR (P_{TR-BSA}^s) and FITC (P_{FITC-BSA}^s), OG (P_{OG-BSA}^s), or DTAF (P_{DTAF-BSA}^s) were performed. In 11 venules, apparent P_{TR-BSA}^s (median 6.7 × 10^-7 cm/s) was greater than P_{FITC-BSA}^s (P_{TR-BSA}^s/P_{FITC-BSA}^s = 1.4 ± 0.2, P < 0.05). In another group of six venules, apparent P_{TR-BSA}^s (median 4.9 × 10^-7 cm/s) was not different from P_{DTAF-BSA}^s (P_{TR-BSA}^s/P_{DTAF-BSA}^s = 0.9 ± 0.1, P > 0.05). Similarly, in six additional venules, apparent P_{OG-BSA}^s (median 10.9 × 10^-7 cm/s) was not different from P_{DTAF-BSA}^s (P_{OG-BSA}^s/P_{DTAF-BSA}^s = 0.9 ± 0.2).

To determine whether the phototoxicity of FITC-BSA was greater than that of the other labeled BSA compounds, an in vitro photohemolysis assay was performed with rat erythrocytes incubated with any one of the four fluorescent albumin compounds. Epi-illumination led to hemolysis, which was limited to the area of...
activation (Fig. 4, inset). Exposure to epi-illumination for 10 min caused marked photohemolysis of erythrocytes incubated with 1.5 mg/ml FITC-BSA (50% hemolysis in 19 min) and minimal hemolysis to cells incubated with 1.5 mg/ml TR-BSA (20% hemolysis in 60 min), DTAF-BSA (14% hemolysis in 60 min), or OG-BSA (8% hemolysis in 60 min) (Fig. 4). Photohemolysis was dependent on excitation of the fluorophore and not on heat generation from the light exposure, because epi-illumination of cells incubated with TR-BSA with the FITC filter did not lead to hemolysis. Similarly, epi-illumination of cells incubated with unlabeled BSA did not lead to hemolysis (data not shown). The differences in phototoxicity among the dye-labeled conjugates were not caused by differences in light dose, because the excitation light intensity was comparable between the two filter cubes (~0.95 W/cm², measured with a model IL1400A Radiometer/Photometer with a model SEL033 detector (International Light, Newburyport, MA)). Differences in fluorescence intensity did not account for the results, because the intensity of fluorescence of erythrocytes incubated with FITC-BSA (determined with the microscope photometer) was less than that of cells incubated with BSA labeled with one of the other three fluorescent dyes (data not shown).

DISCUSSION

The main finding of this study is that the increases in albumin leakage after exposure to L-NAME were detected only when the vessel barrier properties were probed with FITC-labeled albumin (FITC-albumin). When albumin was labeled with one of the three other fluorescent dyes, two of which had spectral properties similar to FITC, no increase in albumin leakage during NOS inhibition was observed. The increases in leukocyte adherence by L-NAME were similar with the probed with FITC-labeled albumin (FITC-albumin). When albumin was labeled with one of the three other fluorescent dyes, two of which had spectral properties similar to FITC, no increase in albumin leakage during NOS inhibition was observed. The increases in leukocyte adherence by L-NAME were similar with the
fluorescent dyes, suggesting that the differences in albumin leakage were independent of leukocyte adhesion.

One possible explanation of our findings is that excitation of FITC-albumin, per se, influences venular permeability. Exposure of fluorescent dyes to excitation light may induce phototoxic cell damage (19, 21, 25), presumably via generation of reactive O₂ intermediates (16). Various reports of light-dye-induced microvascular dysfunction exist in the literature. For example, Vink and Duling (26) reported that a 1- to 5-min exposure to epifluorescence light in the presence of FITC-dextran increased functional capillary diameter in the hamster cremaster in a process that appeared to involve reactive O₂ intermediates. Furthermore, light-dye-induced microvascular dysfunction and leakage has been reported in the rat cremaster with FITC-albumin (21) and in the hamster cheek pouch with FITC-dextran (3). The responses were dependent on excitation light intensity (21); albumin leakage was evident with exposure to excitation light for 30 min, but not with intermittent exposure (<15 s at 10-min intervals). In addition, Zhang et al. (30) have reported that photoactivation of FITC-dextran inhibits lymphatic pump activity in the rat mesentery. The in vitro photohemolysis assay demonstrated that FITC-albumin was considerably more phototoxic than albumin labeled with any of the other three fluorescent dyes. The differences in phototoxicity may be related to a different molar dye content of the albumin compounds. Based on the product labels, the dye-to-albumin ratio of FITC-BSA (12 moles dye/mole of albumin) was 3.5-fold higher than that of TR-BSA (3.2 moles dye/mole of albumin) and 4-fold higher than the initial dye-to-protein molar concentration ratio used for labeling with DTAF and OG.

Under control conditions, however, albumin leakage did not develop over time during perfusion with FITC-BSA. Conceivably, basal NO release is protective against light-dye-induced oxidant damage. In this paradigm, inhibition of NO production would exacerbate light-dye-mediated microvascular dysfunction, leading to increases in FITC-BSA leakage. This is consistent with the report by Kurose et al. (14) that increases in FITC-BSA leakage by L-NAME were inhibited by agents that decrease oxidant generation. Whether light-dye-induced oxidant damage participates in the L-NAME-induced increases in FITC-BSA leakage remains to be determined.

A greater venular permeability to FITC-BSA during control conditions does not appear to account for the results, because the control levels of ALindex were similar among the various fluorophores (Fig. 1A). However, the ALindex experiments performed with both FITC- and TR-BSA in the circulation suggest differential mobility of the two molecules after exposure to L-NAME (see Fig. 3A), favoring FITC over TR-BSA leakage. In apparent contrast, in a separate set of venules, PBFITC-BSA was 30% lower than PSTR-BSA, with both dyes present in the vessel. Contamination of the TR signal with FITC did not account for the greater apparent permeability coefficients (PBFITC) to TR-BSA. The dichroic filter sets used for these experiments allowed ~1% of the fluorescein signal to be detected with the TR filter (determined with the dual-microscope photometer). Correction of TR-BSA permeabilities for this overlap did not alter the venular permeability results (data not shown). Note that the photomultiplier tube used to assess PBFITC is considerably more sensitive to fluorescence (by >2 orders of magnitude as reported by the manufacturer) than the intensified CCD camera used to measure ALindex. Therefore, differences in PBFITC to the labeled solutes during control conditions may exist and not be detected by the ALindex method. In addition to the differences in sensitivity of the two measures of albumin flux (PBFITC and ALindex), the experimental design of the methods also differs. Each determination of PBFITC is obtained by measuring solute flux for ~60 s after the labeled protein is perfused into the venules, whereas the labeled protein is present in the circulation 30 min before the initial measure of ALindex. Vink and Duling (27) reported that the endothelial glycocalyx of hamster cremaster capillaries excludes anionic dyes (such as FITC) with half-times between 15 and 80 min, whereas no exclusion was detected for neutral dyes (such as TR). If the endothelial glycocalyx of rat mesenteric venules, like that of hamster cremaster capillaries, excludes FITC-BSA, then measures of PBFITC performed with FITC-BSA may reflect permeation into the glycocalyx over short time periods rather than flux across the endothelium. This mechanism might then account for the 30% lower permeability coefficients to FITC-BSA than to TR-BSA. These mechanisms and the role of the glycocalyx in macromolecular flux warrant further study.

Differences in charge of the dyes themselves do not account for the albumin leakage results (FITC, DTAF, and OG are anionic, TR is neutral). A change in charge or conformation of the dye-albumin complex cannot be excluded. FITC (1) and DTAF (2) bind primarily to lysine residues on proteins, and FITC has been reported to decrease slightly the net charge of BSA (17). TR binds primarily to lysine, although it can also form unstable bonds with tyrosine, histidine, cysteine, and other residues (5, 7). To our knowledge the specific amino acids on BSA bound by OG have not been reported. However, as a fluorescein derivative (5), OG, like FITC and DTAF, probably binds to lysine residues. Modifications of arginine (but not lysine) sites on BSA were reported by Michel et al. (18) to modify capillary permeability. Vink and Duling (27) reported differential rate of passage of fluorescent dyes and fluorescently labeled macromolecules across the endothelial surface coat (glycocalyx), implying that changes in charge, size, and protein conformation influence microvascular solute flux. Thus it is conceivable that a change in charge or conformation of albumin by binding of the various fluorescent dyes modifies interactions between the protein and the vascular wall structures responsible for macromolecule exclusion. Amino acid analyses and two-dimensional electrophoresis of the various labeled
albumin compounds would be of interest in evaluating these possibilities. Another possibility to account for our results is that dyes other than FITC-BSA are not adequate probes to evaluate macromolecular leakage in rat mesenteric venules. However, the sevenfold increase in TR-BSA leakage induced by PAF argues against this possibility.

Differences in the results of studies of macromolecular leakage using various fluorescent dyes can account for some, but not all, of the inconsistencies in the literature regarding the influence of NOS inhibition on microvascular permeability (11). In a model devoid of fluorescent dyes, Kubés and Granger (12) reported that vascular protein clearance in the feline intestine increased after NOS inhibition. Similarly, Harris (4) reported that L-NAME led to an increase in rat mesenteric capillary fluid filtration during control conditions but to a decrease in filtration in the absence of neutrophils as well as in the presence of antibodies against the leukocyte adhesion molecule CD18. However, NOS inhibitors have also been reported to decrease hydraulic conductivity in rat mesenteric venules (23) and to decrease (24) as well as to increase (6) transiently hydraulic conductivity in frog mesenteric capillaries in the absence of adherent leukocytes. NOS inhibition may influence microvascular permeability by direct changes in the exchange barrier as well as by indirect effects via leukocytes, mast cells, macrophages, platelets, and changes in oxidant potential. The interaction of these processes in determining the actions of NO on permeability remains to be determined.

In summary, we have shown that the influence of L-NAME on albumin leakage of rat mesenteric venules is dependent on the individual fluorescent dyes used to label the protein. On the basis of the studies reported in this paper, it is premature to select one of the fluorescent probes as a preferred dye for albumin leakage experiments. However, the differences in phototoxicity and albumin leakage reported with FITC-labeled albumin as well as the higher photobleaching rate of FITC (5) do not favor the use of this dye over the other fluorophores. The light-dye-induced toxicity reported herein and by others emphasizes the need for careful attention to excitation light intensity and dye concentration in microvascular experiments involving fluorescent dyes.

We thank Dr. Frederick N. Miller (University of Louisville, Louisville, KY) for insightful suggestions regarding the erythrocyte photolysis assay and Dr. Steve Alexander (Louisiana State University Medical Center) for meaningful input. Similarly, we acknowledge Christina Samples, Dr. Li Ping Ji, Susan Bingaman, Kimberly Langlois, and Craig Nicholson for technical assistance.

This work was supported by National Heart, Lung, and Blood Institute Grants K08-HL-03738 (R. E. Rumbaut), R29-HL-55255 (N. R. Harris), R37-HL-42528 (V. H. Huxley), and R01-HL-26441 (D. N. Granger).

This work represents a portion of the research by R. E. Rumbaut in partial fulfillment of the requirements for the PhD in Physiology, University of Missouri-Columbia.


Received 9 February 1998; accepted in final form 24 September 1998.

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