

Analyses of microvascular permeability in response to mediators of immediate hypersensitivity

Thomas M. Keahey, MD, Jeff Indrisano, Martha V. White, MD, and Michael A. Kaliner, MD Bethesda, Md.

Mediator-induced changes in microvascular permeability were studied in rodent skin. To monitor these changes, Evan's blue dye, iodinated rat albumin and IgG, and tritiated neutral dextran molecules having different molecular radii were used. After intradermal injections of serotonin, histamine, and bradykinin, the degree of radiolabeled tracer efflux was determined by measuring the extent of blueing and the level of radioactivity in skin biopsy specimens. Although the mediators varied in potency on a molar basis (serotonin > bradykinin > histamine), the efflux resulting from each was similar. Furthermore, kinetic studies revealed that each of these vasoactive agents caused a marked but transient increase in macromolecular permeability that lasted less than 30 minutes. To determine the pore size created and the effect of charge on the transudation of macromolecules into the interstitium, fractions of neutral [³H]dextran with a known molecular size (average radii, 12 and 4.4 nm, respectively) were infused either singly or in combination with ¹²⁵I-labeled rat albumin and/or IgG before intradermal injection of serotonin, histamine, bradykinin, or compound 48/80. Regardless of the mediator used, no differences could be demonstrated in macromolecular efflux, as indicated by either the molecular size or the charge of the tracer used. Thus, all mediators of immediate hypersensitivity reactions that were studied produce venular gaps >12 nm in addition to displaying similar vasopermeable characteristics. (J ALLERGY CLIN IMMUNOL 1991;87:586-94.)

It is well established that intradermal administration of several mediators of immediate hypersensitivity reactions induce permeability in the cutaneous vasculature of many species, including man.¹ It has been demonstrated that these mediators act directly on the microvascular membrane and cause a marked increase in the number of venular gaps or pores, which then allows the rapid escape of serum proteins.²⁻⁴ The efflux of proteins into the interstitium decreases the effective transmural colloid osmotic pressure gradient, promoting marked increases in net fluid filtration. Our current understanding of the process is that intercellular gaps are formed exclusively in the postcapillary venules. This mechanism is secondary to a dose-related, mediator-induced contraction of the actomyosin filaments within the endothelial cells.⁵ Although these gaps have been demonstrated through

Abbreviations used

DPM:	Disintegrations per minute
HMW-D:	High molecular weight dextran
LMW-D:	Low molecular weight dextran
PBS:	Phosphate-buffered saline

direct intravital microscopy of various organ systems^{6,7} little information exists on the effective size of the gaps created by mediators in the cutaneous microvasculature.

The increase in macromolecular permeability produced by vasoactive mediators may be demonstrated through the efflux of intravital dye (i.e., Evan's blue dye). Furthermore, the degree of efflux can be determined by quantifying the escape of radiolabeled proteins or other macromolecules from the plasma into the skin or by measuring the increase in the concentration of proteins in lymphatic drainage. In many permeability studies, the molecular probes used have been treated as rigid structures, and the electrical charge of the probes and their relative molecular size have been considered unimportant. Recent advances in microvascular research have demonstrated that there are inherent physiologic barriers to the passage of macromolecules, demonstrating the importance of

From the Allergic Diseases Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

Received for publication Oct. 17, 1989.

Revised Sept. 24, 1990.

Accepted for publication Sept. 25, 1990.

Reprint requests: Thomas M. Keahey, MD, Suite 202, 1145 19th St., NW, Washington, DC 20036.

1/1/25752

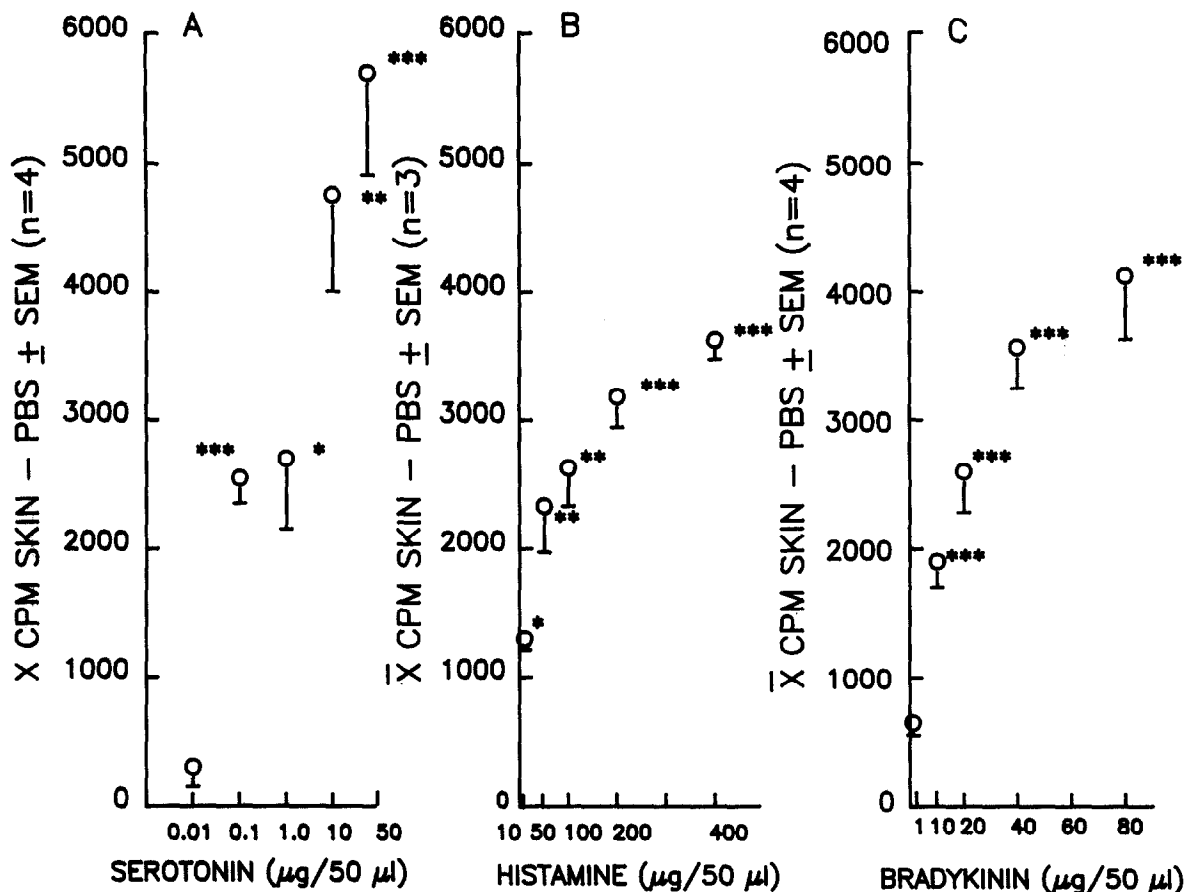


FIG. 1. The effects of serotonin, histamine, and bradykinin on cutaneous vascular permeability. Rats were injected intravenously with 0.5 ml of 2.5% Evan's blue dye containing 1 μ Ci of ^{125} I-labeled IgG. Immediately thereafter, intradermal injections of A, 50 μ l of serotonin (0.01 to 50 μ g), B, histamine (10 to 400 μ g), and C, bradykinin (1 to 80 μ g) were made. Animals were killed 30 minutes later, and biopsy specimens of injected sites were counted for ^{125} I-labeled IgG. Results are presented as the mean counts per minute in the skin biopsy specimens $\pm$ SEM corrected for counts per minute after injection with PBS (* p < 0.01, ** p < 0.01, and *** p < 0.001 compared with control sites). *N*, number of animals used; plasma samples (1 ml), obtained from each animal at the time of death, are presented as the mean counts per minute $\pm$ SEM (A, 26,673 $\pm$ 2348; B, 23,265 $\pm$ 1865; C, 28,463 $\pm$ 1264).

molecular size, molecular charge, and molecular composition in the determination of which molecular species will successfully traverse the microvascular membrane into the extravascular space.⁸⁻¹⁰

In the present study, intradermal injections of serotonin, histamine, bradykinin, and compound 48/80 were used to measure permeability in the cutaneous microvasculature of rodents. Neutral dextran tracers of varying Stokes-Einstein radii were used to measure the venular gap size created by these inflammatory mediators. Furthermore, we examined the ability of anionically charged protein tracers, as compared to neutral dextran tracers, to traverse the cutaneous microvascular membrane.

MATERIAL AND METHODS

Chemicals

Histamine dihydrochloride, bradykinin triacetate, 5-hydroxytryptamine hydrochloride (i.e., serotonin), compound 48/80, sodium barbital, protein A Sepharose CL-48 (Sigma Chemical Co., St. Louis, Mo.), dextran T2000, Sepharose 6B, Sepharose 4B, Sephadex G150, PD-10 (Sephadex G-25) columns (Pharmacia Fine Chemicals, Piscataway, N.J.), [^{125} I]sodium iodide (10 μ Ci/0.1 mol/L NaOH) (New England Nuclear, Boston, Mass.), Iodobeads (Pierce Chemical Co., Rockford, Ill.), rat albumin, goat antirat IgG, goat antirat albumin (Cappell Laboratories, West Chester, Pa.), ultrafiltration membranes (Diaflo; Amicon Corp., Lexington, Mass.), tissue solubilizer 450, Ready

TABLE I. Characteristics of dextran fractions

Dextran fraction (DPM/ μ l)	Average Stokes-Einstein radius (nm)	Concentration (μ mol/mol)
A		
31,600	12	150
B		
73,054	4.4	42

Solve NA scintillation cocktail, and Ready Solve MP scintillation cocktail (Beckman Instruments, Inc., Irvine, Calif.) were purchased directly from the manufacturers.

Preparation of purified rat IgG

Rat IgG was prepared by ammonium sulfate fractionation of serum (fraction precipitating between 20% and 50%), followed by dialysis against deionized water for 2 hours and affinity chromatography over protein A Sepharose 4B. IgG was applied to the column in buffer and eluted with 60 to 100 ml of 1 mol/L of acetic acid, and the elute was then dialyzed against PBS. The retentate was concentrated by ultrafiltration (filter UM10, Amicon Corp.) under nitrogen pressure. Ouchterlony analysis revealed a single precipitin band against goat antirat IgG.

Molecular sizing on a Sepharose 4B gel filtration column revealed a single peak at a molecular size of 160,000 daltons. One hundred microliter aliquots were frozen at -70°C for subsequent radiolabeling.

Iodination of rat IgG and rat albumin

Radioiodination of rat IgG and albumin was performed with the use of Iodobeads. Four Iodobeads were added to 100 μ g of either IgG or albumin in 1 ml of PBS and allowed to incubate with [^{125}I]sodium iodide for 30 minutes at room temperature. The reaction volume was separated from the Iodobeads to terminate iodination and separated on a PD-10 column. HMW fractions were pooled, dialyzed against PBS (24 hours), and frozen at -70°C in aliquots. This procedure resulted in a 50% incorporation of the sodium iodide (resulting in a specific activity of 4 to 5 $\mu\text{Ci}/\mu\text{g}$ of protein) and an 80% protein recovery.

Preparation of tritiated neutral T2000 dextran fractions

One gram of neutral dextran T2000 was radiolabeled by catalytic ion exchange in solution with 25 Ci of tritium gas (Amersham Corp., Arlington Heights, Ill.). Aliquots of [^3H]T2000 were chromatographed on a Sepharose 6B column (60 by 1.6 cm) equilibrated with 1 mol/L of PBS (pH 7.2). Distribution coefficients (determined with the formula $V_e - V_o/V_t - V_o$, in which V_e = the elution volume for the test substance, V_o = void volume, and V_t = total bed volume) for the molecules that served as standards were calculated and plotted against the known molecular radii.

The molecular radii for [^3H]dextran fractions were calculated from this curve as previously reported.¹⁰

Column fractions were pooled, dialyzed against pyrogen free water (2 hours), lyophilized, and frozen at -70°C . Samples were subsequently resuspended in PBS and rechromatographed on Sepharose 6B, and the average molecular radius was then determined for each group.

Measurement of cutaneous vascular permeability in rat skin

All animal experiments were in accordance with Health and Human Services/Public Health Services/National Institutes of Health publication 86-23, "The Guide for the Care and Use of laboratory Animals." The dorsal aspect of normally hydrated male Sprague-Dawley rats (250 to 300 gm) were shaved 18 to 24 hours before experimentation. On the day of experimentation, the animals were lightly anesthetized with ether and injected intraperitoneally with sodium pentobarbital (40 mg/kg; total injection volume, 1.0 to 1.5 ml). Once anesthetized, the animals were injected intravenously with 2.5% Evan's blue dye and radiolabeled protein or dextran tracers, either singly or in combination (total injection volume, 500 to 600 μ l) before intradermal injection of the test mediators and PBS. The intradermally injected volume was 50 μ l, and the skin injections were made in a standardized manner in six to eight symmetrically located sites on the back. In the control sites, diluent alone was used. After predetermined time intervals, the animals were killed in a CO_2 inhalation chamber. At postmortem, 3 ml of blood was collected by intracardiac puncture and stored in tubes with ethylenediaminetetraacetic acid.

In experiments with Evan's blue dye, the blue-stained areas were measured with calipers, and radioactivity of all plasma samples were determined from use of a 1 ml aliquot of plasma. The microvascular permeability was expressed as the mean area (square millimeter) of blueing. In instances in which [^{125}I]labeled protein tracers were used, the entire reaction site was excised with a punch (14.25 mm in diameter) for counting in a gamma spectrophotometer (Searle model 1185, Searle Analytic, Inc., Des Plaines, Ill.). The amount of radioactivity in postmortem plasma was used for determining the adequacy of intravenous injections. Skin biopsy specimens and plasma containing [^3H]dextran tracers were added to 3 ml of tissue solubilizer in glass scintillation vials with plastic cone-lined caps and incubated overnight at 55°C until they were completely digested. The samples were neutralized to a final pH of 7.0. The radioactivity in a 1 ml aliquot placed in scintillation cocktail was then determined with an LS 9000 scintillation counter (Beckman Instruments, Irvine, Calif.). When two radionuclides were present in the same sample (i.e., [^{125}I] and [^3H]), skin biopsy specimens and plasma were initially counted in a gamma spectrometer before tissue digestion and subsequent scintillation counting. The activity of the [^{125}I]labeled proteins was determined with a Beckman T9000 with Horrock's [^{125}I] efficiency program. These samples were then used to develop a dual label [^{125}I]/ ^3H quench curve. Tritium counts were

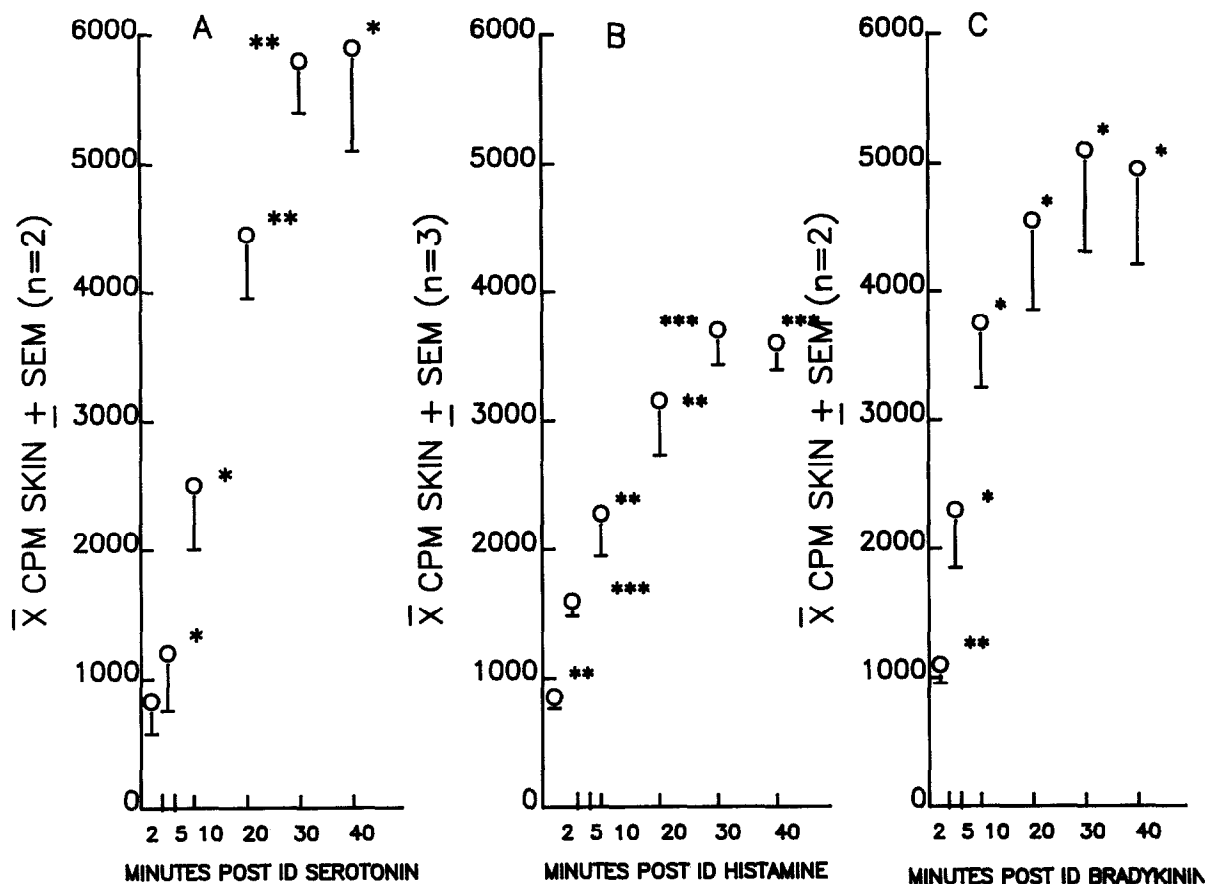


FIG. 2. Time course of microvascular permeability in response to vasoactive agonists as measured by radiotracer efflux. Animals were injected intradermally immediately after receiving ^{125}I -labeled IgG and Evan's blue dye and at varying time intervals up to 2 minutes before being killed. All animals were killed 40 minutes after tracer injection. **A**, The response to serotonin (10 µg). **B**, Response to histamine (200 µg). **C**, Response to bradykinin (40 µg) (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with control sites). *N*, the number of animals used; plasma samples, obtained from each animal at the time of death, are presented as the mean counts per minute $\pm$ SEM (**A**, 25,192 $\pm$ 2802; **B**, 21,571 $\pm$ 598; **C**, 24,832 $\pm$ 724).

discriminated from the ^{125}I in the scintillation counting by a correction based on the established quench curves.

Microvascular permeability was calculated by expressing the relative amount of radiolabeled tracer in skin corrected for that in PBS control sites (usually <350 cpm). The amount of radioactivity in postmortem plasma was used to indicate the adequacy of intravenous injections. In all experiments in this article, each animal had the same plasma radioactivity $\pm$ 12%.

Statistics

Mean values were compared by application of Student's two-tailed *t* test. Linear regression analysis was used to compare the magnitude of extravasation of radiolabeled markers after treatment with the various agonists. The distribution of the deviations from the fitted values of data illustrated in Fig. 2, A and C (six time points, each panel)

was checked with a Shapiro-Wilk test, and in both cases the deviations were found to be normally distributed.

RESULTS

Determination of Stokes-Einstein radii and radiolabeled tracers

To monitor the increase in size of the pores between endothelial cell walls and the resulting increase in microvascular permeability, radiolabeled tracers of widely ranging size were required. ^3H -neutral dextrans were prepared and fractionated on a calibrated Sepharose 6B column.¹¹ The average Stokes-Einstein radii were calculated from diffusion coefficients of two pooled dextran fractions and are presented in Table I. For purposes of reference, dextran fraction

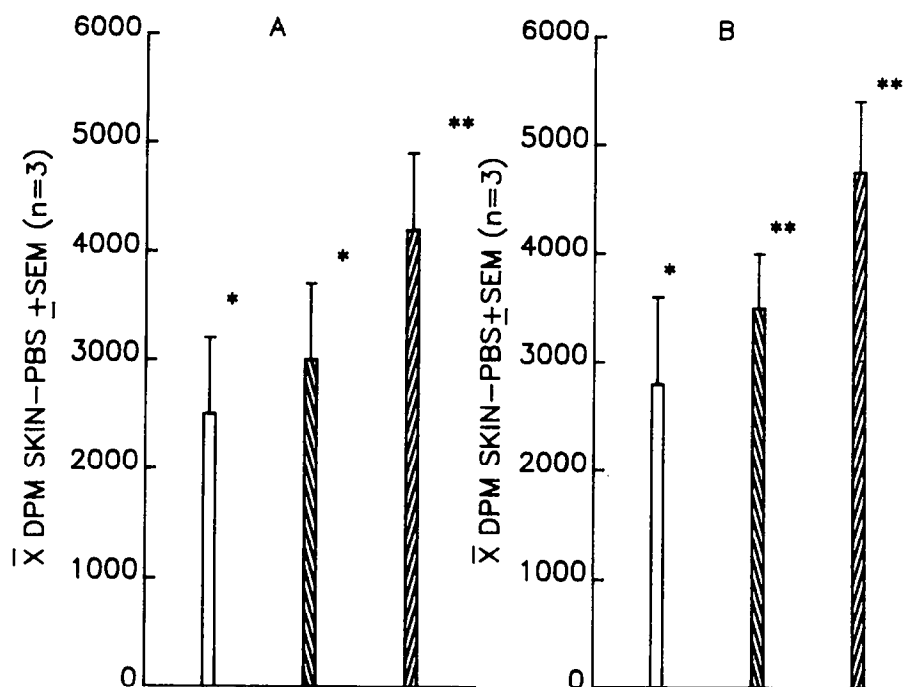


FIG. 3. Comparison of the vasopermeable characteristics of $[^3\text{H}]$ HMW-D and ^{125}I -labeled IgG in response to intradermal injections of vasoactive agonists. In these experiments, 2 μCi of $[^3\text{H}]$ HMW-D and 0.5 μCi of ^{125}I -labeled IgG were intravenously injected simultaneously before intradermal injection of serotonin (10 μg) (■), histamine (100 μg) (////), and bradykinin (40 μg) (||||). Animals were killed at 30 minutes. Results are presented for A, $[^3\text{H}]$ HMW-D and B, ^{125}I -labeled IgG as the mean DPM in the skin biopsy specimens $\pm$ SEM corrected for DPM after injection with PBS (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the control sites). N , the number of animals used; plasma samples are also expressed as the mean DPM $\pm$ SEM (A, 39,122 $\pm$ 2128; B, 34,190 $\pm$ 622).

A (radius, 12 nm) will be referred to as HMW-D and fraction B (radius, 4.4 nm) as LMW-D. The Stokes-Einstein radii of rat albumin and IgE are 3.55 and 4.8 nm, respectively.

The radius of each radiotracer was initially determined in buffer but was confirmed after exposure to rat plasma in vivo and in vitro. The radiotracers recovered in postmortem plasma fractionated at the same size as equivalent markers in buffer. Thus, the radii determined in vitro held true under in vivo conditions.

Measurement of cutaneous microvascular permeability

In initial studies, we used 1.0 μCi of $[^{125}\text{I}]$ IgG in association with Evan's blue dye to outline the area of microvascular permeability. The results of $[^{125}\text{I}]$ IgG leakage into sites injected with vasoactive agonists are illustrated in Fig. 1, A to C. Intracutaneous serotonin (0.01 to 50 μg per 50 μl injection, Fig. 1, A), histamine (10 to 400 μg , Fig. 1, B), and bradykinin (1 to 80 μg , Fig. 1, C) each caused a dose-related increase in cutaneous radioactivity in the pres-

ence of equivalent circulating levels of $[^{125}\text{I}]$ IgG (mean $\pm$ SEM, 26,133 $\pm$ 1524 cpm); on a molar basis, serotonin > bradykinin > histamine in terms of their ability to cause vascular permeability. The median effective dose for serotonin was 1 μg ; for histamine, $\sim$ 50 μg ; and for bradykinin, 10 μg .

Before biopsy specimens were obtained from skin sites and the radioactivity was quantitated, the area of microvascular permeability was simultaneously measured as a function of Evan's blue dye leakage. Linear regression analysis was used to compare the area of blueing with IgG leakage for each of the three agonists. In each instance, the correlation coefficient (r) was highly significant (≥ 0.98). When Evan's blue dye circulates, it is complexed primarily with albumin, which has a Stokes-Einstein radius of 3.55 nm, whereas IgG has a radius of 4.8 nm. Thus, the close concordance noted between these monitoring systems were not unexpected and further substantiates the validity of both for measuring microvascular permeability.

The time course of leakage of IgG and Evan's blue

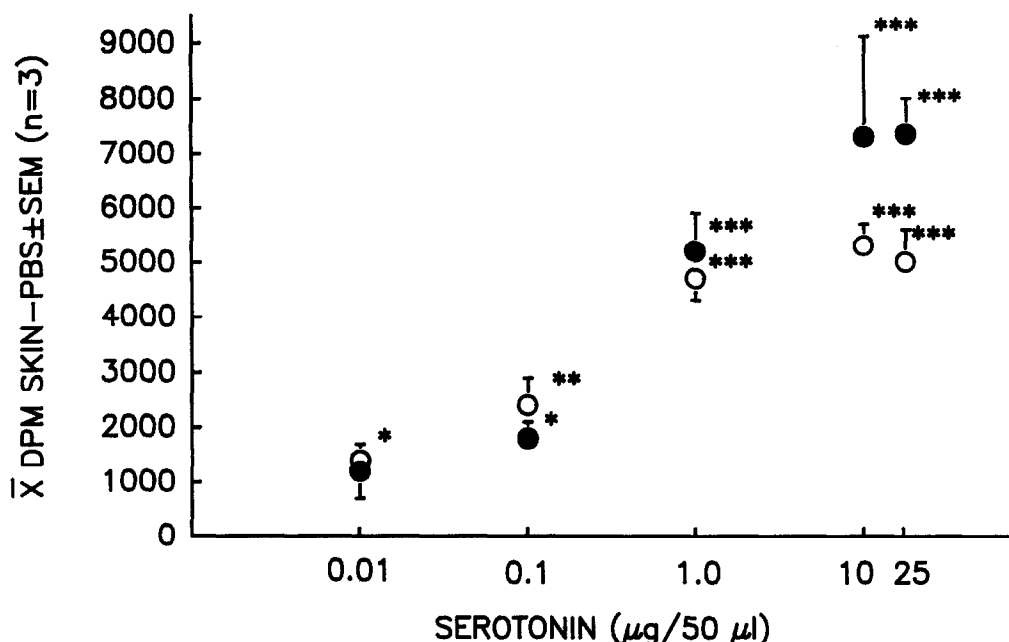


FIG. 4. Comparison of the ability to use HMW-D and LMW-D to monitor vascular permeability in response to vasoactive agonist. In these experiments, animals were intravenously injected with radiolabeled dextran in parallel with serotonin (0.01 to 25 μ g). Animals were killed 30 minutes after injection. Results are presented as the mean DPM in skin biopsy specimens $\pm$ SEM: HMW-D ($\bullet$); LMW-D ($\circ$); (* p < 0.05, ** p < 0.01, and *** p < 0.001 compared with control sites). N , the number of animals used; plasma samples, obtained from each animal at the time of death, are presented as the mean DPM $\pm$ SEM (HMW-D, 46,557 $\pm$ 2481; LMW-D, 25,510 $\pm$ 1846).

dye into skin test sites was examined next. The anesthetized animals were injected intravenously with both tracers. Subsequently, the animals were skin tested with serotonin (10 μ g), histamine (200 μ g), and bradykinin (40 μ g) at various times thereafter. After 40 minutes, the animals were killed. Each experiment was done two or more times. In these experiments, the amount of plasma [^{125} I]IgG at the time animals were killed ranged from 21,571 to 25,192 cpm (mean $\pm$ SEM, 23865 $\pm$ 1151). With all three agonists, the time course of increased permeability lasted 20 to 30 minutes, although the bulk of the leakage was completed within 10 to 15 minutes (Fig. 2, A to C). Moreover, vascular leakage was apparent in all instances within 2 minutes of skin testing. In parallel, we used Evan's blue dye leakage in the same animals to assess the vascular permeability. No dye leakage was noted within 2 minutes of skin testing, although the results of this means of assessment were roughly equivalent to results from the assessment of radiolabeled leakage into skin at later time points. Thus, IgG was a marginally more sensitive monitor of early vascular leakage than visual detection of Evan's blue dye in these experiments.

Because IgG is negatively charged and of inter-

mediate size (4.8 nm), it was of interest to determine whether larger neutral molecules behaved in a similar fashion (Fig. 3). In this experiment, 1 μ Ci of neutral HMW-D and 0.5 μ Ci of [^{125} I]IgG were administered intravenously, after which, the animals were skin tested with serotonin (10 μ g), histamine (100 μ g), and bradykinin (40 μ g). Biopsy specimens were obtained 30 minutes later. At plateau concentrations of vasoactive agonists, both HMW-D (Fig. 3, A) and IgG (Fig. 3, B) leaked into the skin, suggesting that microvascular leakage resulted from the development of endothelial pores >12 nm. Moreover, the anionic charge of IgG versus neutral HMW-D appeared to have no effect on permeability. This experiment was extended by comparing the leakage of HMW-D to LMW-D in relation to a dose response of serotonin (0.01-25 μ g) (Fig. 4). In these experiments, animals were injected with either HMW-D or LMW-D and immediately skin tested with serotonin; vascular permeability was determined 30 minutes later. Although slightly higher levels of radioactivity were noted in skin specimens from animals injected with HMW-D, this was attributable to the relatively higher circulating plasma levels of this tracer as compared to LMW-D (HMW-D, 46,557 $\pm$ 2481; LMW-D,

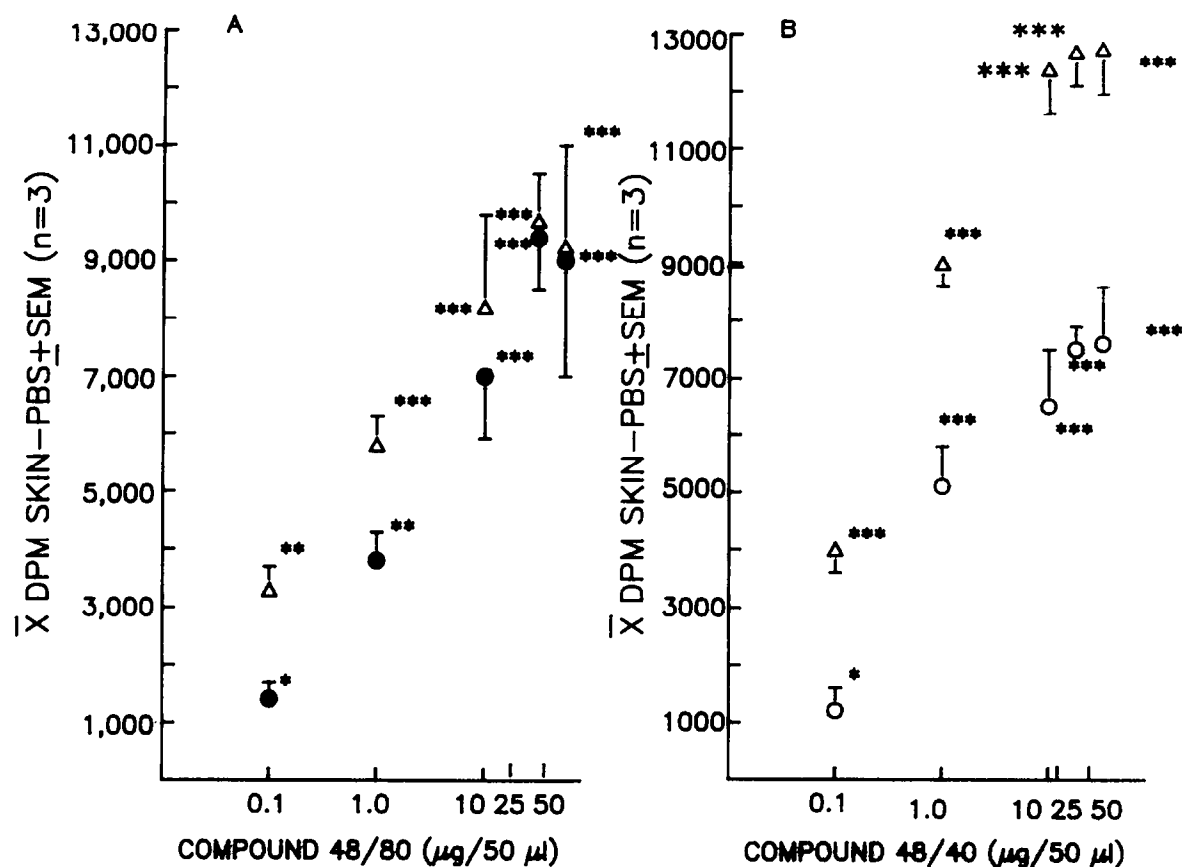


FIG. 5. Comparison of the relative ability of HMW-D, LMW-D, and albumin to monitor vascular permeability in response to compound 48/80. Groups of animals were intravenously injected with either 2 µCi of HMW-D, 2 µCi of LMW-D, or 1 µCi of ^{125} I-labeled rat albumin. Compound 48/80 was subcutaneously injected (0.1 to 50 µg), and the animals were killed 30 minutes after injection. Results are presented for both ^{125} I-labeled albumin and ^{3} H-dextran tracers as the mean DPM in skin biopsy specimens $\pm$ SEM: HMW-D (●); LMW-D (○); albumin (△) (* p < 0.05, ** p < 0.01, and *** p < 0.001 compared with control sites). N , the number of animals used; plasma samples are also expressed as the mean DPM $\pm$ SEM (HMW-D, 34,909 $\pm$ 2495; LMW-D, 11,578 $\pm$ 617; albumin, 24,412 $\pm$ 856).

25,510 $\pm$ 1846). Thus, no significant difference between the cutaneous permeability as measured by either tracer could be demonstrated.

The activity of compound 48/80 causes mast cells to release vasoactive mediators, increasing vascular permeability and causing edema. The objective for performing the following experiments was to analyze endothelial "pore" size after mast cell degranulation. Thus, groups of animals were injected with HMW-D, LMW-D, and [^{125} I]albumin. The animals were subsequently skin tested with compound 48/80 (0.1 to 50 µg). These data are presented in Fig. 5 and indicate that mast cell-induced vascular leakage can be assessed equally well with any of these agents. As a corollary conclusion, compound 48/80 induces leakage in the microvascular membrane by producing pores >12 nm.

It may be noted that plasma radioactivity was higher for HMW-D than for either albumin or LMW-D (Figs.

3 to 5). These observations are consistent with previous studies and suggest that small molecules (~4.4 nm) are cleared from the circulation rapidly, particularly via renal filtration, neutral molecules of comparable size being filtered more easily than anionically charged molecules.¹⁰ To determine the time course of the various radiotracers used in these experiments, groups of rats were injected intravenously with radiolabeled HMW-D, LMW-D, and albumin. HMW-D remained relatively constant in the circulation throughout the 30-minute increase in permeability, whereas both LMW-D and [^{125} I]albumin decreased progressively over this time (Fig. 6).

DISCUSSION

Vasoactive mediators produce an increase in the macromolecular permeability of the microvascular membrane as a consequence of venular endothelial cell separation.^{12, 13} As a result of endothelial gap for-

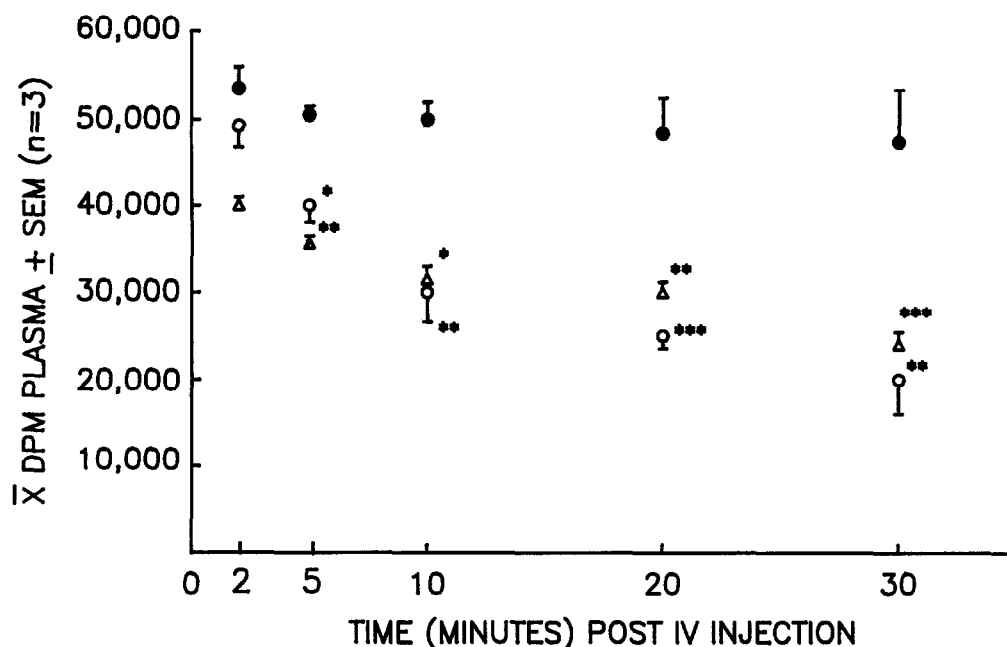


FIG. 6. Time course of macromolecular tracer clearance after intravenous injection. In these experiments, groups of rats were injected intravenously with 2 μ Ci of either ^{125} I-labeled albumin, [^{3} H]HWM-D, or [^{3} H]LMW-D. The animals were killed at time intervals of 2, 5, 10, 20, and 30 minutes after injections. At postmortem, 3 ml of blood was collected by intracardiac puncture and stored with ethylenediaminetetraacetic acid. The amount of radioactivity in 1 ml of plasma was measured, and the results were expressed as the mean DPM $\pm$ SEM ($n = 3$) for each radiotracer at each time interval: HWM-D (●); LMW-D (○); albumin (△) (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with 2-minute values); N , the number of animals used.

mation, macromolecules rapidly exit from the circulation, decreasing the transmural colloid osmotic pressure gradient, which results in the production of tissue edema. The passage of macromolecules across microvascular walls should be limited to the size of the venular cap created as well as by natural physiologic barriers, including the negatively charged basement membrane separating the endothelium from the interstitium. Pericytes may also be capable of affecting the effective "pore" size. Thus, factors important in determining which molecular species will cross any or all of these barriers include molecular size, molecular charge, and perhaps molecular composition.¹⁴

To compare the vasoactive characteristics of serotonin, histamine, bradykinin, and compound 48/80 in the rodent, the cutaneous efflux of various exogenously administered macromolecular tracers was studied. The tracers used were Evan's blue dye, anionically charged proteins (i.e., IgG and albumin), and neutral dextran polymers with average radii of 12 and 4.4 nm. The question addressed was, "Could specific mediators be distinguished by their relative ability to induce macromolecular leakage on the basis of either tracer size (i.e., Stokes-Einstein radii) or charge?" These data clearly demonstrate that with each of the mediators studied, venular gaps >12 nm were created, and similar kinetic characteristics were displayed.

Studies with Evan's blue dye and radiolabeled rat IgG confirmed differences in the potency of vasoactive mediators on a molar basis (serotonin $>$ bradykinin $>$ histamine), although each respective mediator induced similar vascular leakage at plateau doses. Furthermore, since Evan's blue dye circulates in a complex primarily with albumin and both albumin and IgG are anionically charged proteins of similar molecular size, the concordant dose relationship noted between these systems might be expected. These findings further underscore the established usefulness of both systems in measuring increases in vascular permeability.

Kinetic studies with the same tracers revealed that macromolecular leakage could be demonstrated by radiotracer efflux, but not by visual detection of blueing, within 2 minutes of intradermal mediator injection. With all three vasoactive agonists, vascular permeability reached its highest level within 10 to 15 minutes of injection and was complete within 20 to 30 minutes. Thus, the increase in macromolecular permeability is marked but transient, lasting less than 30 minutes regardless of the vasoactive mediator used. Previous studies in which intravital microscopy of fluorescein isothiocyanates-dextran leakage was used as a marker for vascular permeability have demonstrated similar kinetics for histamine-, bradykinin-, and

leukotriene-induced vascular leakage in the hamster cheek pouch.^{15, 16} These findings may be interpreted to reflect the temporal development of an increase in "pore" size and density that accompany mediator activation of postcapillary venules.¹⁷

To study potential differences in the venular gap size created by the various mediators, molecular tracers of various radii were used. Neutral dextran molecules were isolated for this purpose because they could be demonstrated to retain their state of dispersion and size distribution in plasma. Studies with single and multiple tracers failed to demonstrate any differences between the respective vasoactive agents based on the efflux of 3.55, 4.4 or 12 nm molecules. Thus, after mediator activation, the cutaneous microvascular membrane develops venular gaps in excess of 12 nm and does not selectively impede the efflux of anionic proteins into the interstitium as compared to neutral dextran molecules of similar radii. Degranulation of skin mast cells with compound 48/80 was also examined and found to be associated with endothelial pores >12 nm. Furthermore, it should be noted that both albumin and LMW-D were progressively cleared from the plasma but that HMW-D has a longer and predictable plasma half-life.

REFERENCES

1. Haddy FJ, Scott JB, Grega GJ. Peripheral circulation: fluid transfer across the microvascular membrane. In: Guyton AC, Cowley AW, eds. Cardiovascular physiology (International review of physiology), vol 9. Baltimore: University Park, 1976:63-109.
2. Majno G, Gilmore V, Leventhal M. On the mechanism of vascular leakage caused by histamine-type mediators. *Circ Res* 1967;21:833-47.
3. Majno G, Palade GE, Schoeffl GI. Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree: a topographic study. *J Biophys Biochem Cytol* 1961;11:607-26.
4. Majno G, Shea SM, Leventhal M. Endothelial contraction induced by histamine-type mediators. *J Cell Biol* 1969;42:647-72.
5. Becker CG, Nachman RL. Contractile proteins of endothelial cells, platelets and smooth muscle. *Am J Pathol* 1973;71:1-22.
6. Hulstrom D, Svensjö E. Intravital and electron microscopic study of bradykinin-induced vascular permeability changes using FITC-dextran as a tracer. *J Pathol* 1980;129:125-33.
7. Fox J, Galey F, Wayland H. Action of histamine on the mesenteric microvasculature. *Microvasc Res* 1980;19:108-16.
8. Deen WM, Veki IF, Brenner BM. Permeability of renal peritubular capillaries to neutral dextran and endogenous albumin. *Am J Physiol* 1976;231:283-91.
9. Vehaskari VM, Chang CTC, Stevens JK, Robson AM. The effects of polycations on vascular permeability in the rat: a proposed role for charge sites. *J Clin Invest* 1984;73:1053-61.
10. Chang RLS, Veki IF, Troy JL, Deen WM, Robertson CR, Brenner BM. Permselectivity of the glomerular capillary wall to macromolecules. II. Experimental studies in rats using neutral dextran. *Biophys J* 1975;15:887-906.
11. Keahey TM, Indrisano J, Kaliner MA. Dissociation of cutaneous vascular permeability and the development of cutaneous late-phase allergic reactions. *J ALLERGY CLIN IMMUNOL* 1989;83:669-77.
12. Majno G, Palade G. Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J Biophys Biochem Cytol* 1961;11:571-605.
13. Joris I, Majno G, Ryan GB. Endothelial contraction in vivo: a study of the rat mesentery. *Virchows Arch [Zellpathol]* 1972;12:73-83.
14. Wayland H. Permeability characteristics of microvascular walls. *Biorheology* 1984;21:104-14.
15. Björk J, Arfors KE, Dahlen SE, Hedqvist P. Effects of leukotrienes on vascular permeability and leukocyte adhesion. In: Venge P, Lindbom A, eds. The inflammatory process. Stockholm: Almqvist & Wiksell International, 1981:103.
16. Svensjö E, Joyner WL. The effects of intermittent and continuous stimulation of microvessels in the cheek pouch of hamsters with histamine and bradykinin on the development of venular leaky sites. *Microcirc Endothelium Lymphatics* 1984;1:381-96.
17. Grega GJ, Svensjö E, Haddy FJ. Macromolecular permeability of the microvascular membrane: physiological and pharmacological regulation. *Microcirc* 1981;1:325-41.