Possible Mechanistic Roles of Anatomical and Functional Vascular Changes in Rat Urinary Bladder Carcinogenesis Induced by N-Butyl-N-(4-hydroxybutyl)nitrosamine

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ABSTRACT

Anatomical and functional vascular changes during rat urinary bladder carcinogenesis were studied by scanning electron microscopy of vascular casts, transmission electron microscopy of bladder capillaries, and fractional distributions of $^{51}$Cr-erythrocytes, $^{125}$I-human serum albumin, and $^{86}$RbCl which were used to determine vascular volume, permeability, and perfusion. Histopathological changes and focal capillary changes in vascular casts were measured quantitatively by an image analyzer. Male Wistar rats received 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in their drinking water for 8 weeks and were then maintained on tap water without BBN for an additional 32 weeks. Simple hyperplasia was first seen at Week 2. The percentage of the area of hyperplastic epithelium increased to about 95% by Week 8 and then decreased to 4 to 6% at Weeks 20 or 40. Papillary or nodular hyperplasia was first seen at Week 6. The percentage of the area of papillary or nodular hyperplasia increased with time to 31.0% at Week 40. Papillary transitional-cell carcinomas were found from Week 20, increasing with time, and their incidence was 100% after Week 35. Vascular cast diameters of normal-looking capillaries were larger during than after BBN treatment. Type 3 vascular proliferations were found beneath papillary or nodular hyperplasia and cancer. Capillaries beneath simple hyperplasia and type 3 capillaries beneath papillary or nodular hyperplasia were fenestrated and dilated. Changes in vascular volume were independent of changes in permeability and perfusion. Best-fit curve analyses showed the maximum vascular volume at 8 weeks and minimum at 25 weeks, and the permeability maxima at 4 and 25 weeks with minima at 15 and 32 weeks. While $^{86}$Rb values correlated $^{125}$I values ($r = 0.58$), they were unstable in intermediate time periods. Changes of vascular volume were coincident initially with increased areas of dilated capillaries beneath simple hyperplasia and later with areas of type 3 capillary proliferation beneath papillary or nodular hyperplasia and cancer. Changes of vascular permeability were related to inflammation indices throughout the study. Increases in permeability were coincident with fenestrated capillaries beneath simple hyperplasia in early stages, and subsequently with fenestrated type 3 capillaries beneath papillary or nodular hyperplasia and cancer. BBN appears to cause alterations in vascular volume via induction of capillary dilation and also possibly by enhancing the responsiveness of host endothelium to angiogenic stimulation from neoplastic or preneoplastic tissues.

INTRODUCTION

There appear to be important relationships between malignant tumors and host vasculature mediated by a chemical angiogenic factor (4). Premalignant breast nodules secrete an angiogenesis factor while benign nodules do not (2). These findings have suggested parenchymal effects on host vasculature during progressive tissue changes to neoplasia. Studies on chemical carcinogenesis in hamster cheek pouch (23) and rat urinary bladder (13, 22) provided morphological and functional data consistent with neovascularization and hemodynamic changes in these tissues during chemical applications. These studies suggested that such changes may be induced by action of the carcinogen and/or altered parenchymal cells and thus may be an important part of the mechanism in epithelial chemical carcinogenesis.

The objectives of the present study were to explore possible interrelated mechanistic roles of functional and anatomical vascular changes during rat urinary bladder carcinogenesis by BBN. These objectives were approached by: defining progressive functional vascular changes in rat urinary bladder during and following BBN carcinogenesis via simultaneous multiple fractional distributions of radiolabeled indicators; temporally correlating these changes with histopathological changes in the same bladders; and correlating, at comparable times, functional vascular changes with anatomical vascular changes as studied by scanning electron microscopic analysis of vascular casts of bladders from BBN-treated rats (22) and by transmission electron-microscopic analysis of BBN-induced changes in the fine structures of the capillaries.

MATERIALS AND METHODS

Animals and Animal Housing. Rats for morphological studies were male Wistar rats (Nihon Rat Co., Saitama, Japan), weighing approximately 180 g. They were fed rat food (Oriental MF; Oriental Yeast Co., Tokyo, Japan) and tap water ad libitum and were housed 3 to 4/cage in a temperature- and humidity-controlled room.

Rats for functional vascular studies were male Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.), weighing approximately 200 g at purchase. They were housed 2 to 3/cage in a temperature- and humidity-controlled room and fed Purina rat chow (Ralston-
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Purina Co., St. Louis, Mo.) and tap water ad libitum. These animals were housed in the University of Connecticut Health Center for 2 weeks prior to initiating BBN treatments. Five animals were sacrificed from the original shipment for bladder parasite and serum microbiological testing. These studies were negative.

BBN Treatment and Sacrifice Schedule. All animals received 0.05% BBN in their drinking water for 8 weeks and were then maintained on tap water without BBN for an additional 32 weeks.

At 2, 4, 6, 8, 10, 20, 30, 35, and 40 weeks after the start of BBN treatments, 3 to 4 rats were sacrificed for preparations of vascular casts and 40 weeks as controls.

At 2, 4, 6, 8, 10, 20, 30, 35, and 40 weeks after the start of BBN treatments, groups of 4 to 5 rats were sacrificed for functional vascular studies. Additional groups of untreated animals were sacrificed at 0, 20, and 40 weeks as controls.

Vascular Casts. The preparations of vascular casts of rat urinary bladders were detailed previously (22) and are summarized only briefly here. Bladders were perfused with warm 0.9% NaCl solution followed by Mercox CL-2B (Dainippon Ink and Chemicals, Inc., Tokyo, Japan). The bladders were digested with 25% NaOH until only the vascular casts remained. The vascular casts were coated with gold and examined with a Hitachi HHS-2R scanning electron microscope. Vascular proliferations observed in the vascular casts were classified as follows: type 1 vascular foci with numerous long terminal branches projecting toward the lumen; and type 3 vascular foci, with highly tortuous capillary loops (3, 22).

Histopathological and Ultrastructural Studies. Bladders were inflated by a transurethral injection of 0.4 to 0.5 ml of 2% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, through a 25-gauge needle under constant pressure of 50 mm of Hg. The neck of the urinary bladder was then ligated, and the bladder was rapidly excised in toto and placed in the same fixative. After 1 hr, the inflated bladders were bisected, and 6 to 8 slices were cut from the right and left sides. Each bladder studied for light microscopy was placed in 10% buffered formalin and processed routinely by paraffin embedding, sectioning, and staining with hematoxylin and eosin. Small pieces of the bladders of transmission electron microscopy were rinsed briefly in 0.05 M cacodylate buffer, pH 7.4, postfixed in 1.0% OsO4 in 0.05 M cacodylate buffer, and dehydrated rapidly by passage through a series of increasing concentrations of alcohol (9). Specimens were then immersed in propylene oxide, embedded in Epon 812, and examined with a Hitachi HU-12 electron microscope.

Histological appearances of the urinary bladders were classified as follows (7, 14–16, 22): simple hyperplasia showing a diffuse proliferation of bladder epithelial cells increasing the mucosa up to 8 cell layers; papillary or nodular hyperplasia with an obvious fibrovascular core by light microscopy; and papillary transitional-cell carcinoma with cellular atypia.

Quantitative Analysis of Anatomical Changes. A general-purpose color image processor, Olympus Model VIP-21C (Olympus Co., Tokyo, Japan), was used. This machine processes microscopic or macroscopic images through a color television camera with either fully automatic or semiautomatic analysis using a light pen aid (15). Histopathological changes (simple hyperplasia, papillary or nodular hyperplasia, and cancer) were quantitated by determining the PLBM beneath each change per unit length of the basement membrane on that observed bladder specimen. Types 1 and 3 vascular proliferations were quantitated by determining the percentage of the area of each change in 2 dimensions on the scanning electron micrograph of the vascular cast per unit area of vascular bed on the same scanning electron micrograph.

Preparation of Radionuclides. 51Cr-labeled erythrocytes were prepared by incubating heparinized whole blood from a normal rat with sodium chromate (51Cr) (Amersham/Searle Corp., Arlington Heights, Ill.) for 30 min at room temperature. A few drops of ascorbic acid were then added to reduce unbound hexavalent 51Cr, and the cells were washed 3 times with cold 0.9% sodium chloride solution. 125I-HSA (Malinnrodt, Inc., St. Louis, Mo.) was added to the 51Cr-labeled erythrocytes, and the suspension was brought to original volume by addition of cold 0.9% sodium chloride solution. The resulting suspension had approximate activities of 100 μCi of 51Cr and 7 μCi of 125I per ml. Centrifugation of a sample of the suspension and scintillation spectrometry of the pellet and supernatant showed over 98% of the 51Cr activity to be in the pellet and over 95% of the 125I activity to be in the supernatant. A solution of 86RbCI in 0.9% sodium chloride solution (200 μCi/ml) was used as received from the supplier (Amersham).

Functional Vascular Studies. Hemodynamic parameters were studied by simultaneous fractional distributions of radionuclide labeled indicators (11, 13, 21). Vascular volumes were determined using 51Cr-RBC, vascular permeabilities were determined using 125I-HSA, and vascular perfusions were determined using 86RbCl.

Each rat was anesthetized with ethyl ether, and the left saphenous vein was exposed by blunt dissection for isotopic injections. The erythrocyte solution was injected i.v. using a glass tube in a syringe with a 27-gauge needle (0.5 to 1.0 ml; approximately 50 μCi of 51Cr plus 4 μCi of 125I), after which the incision was closed with autocolips. A 1-hr period followed to allow for mixing of blood components and leakage of 125I-HSA. Rats were then reanesthetized with ether, the saphenous vein was reexposed, and 50 μCi of 86RbCl were injected i.v. Ninety sec after 86RbCl injection, the bladder was exposed and clamped at its base to prevent further exchanges between bladder and systemic vascular beds. The bladder was quickly excised in toto and cut open, and any tumours were excised and placed in 10% neutral phosphate-buffered formalin for future histopathological study. The bladder was rinsed, blotted dry, weighed, and placed in a glass γ scintillation counting vial containing buffering formalin.

Following the bladder excision, a whole-blood sample was taken from the abdominal aorta in a heparinized syringe, and the hematocrit was determined in quadruplicate. Triplicate 100-μl whole-blood and plasma samples from each rat were placed in a γ scintillation vials for radioactivity determination.

Radioactivities in the tissue, blood, and plasma samples were determined by scintillation spectrometry using a γ well scintillation counter with a thallium-doped NaI crystal (Nuclear Chicago Searle Radiographies, Inc., Chicago, Ill.). Spectral overlaps among 125I, 51Cr, and 86RbCl were corrected by inverted matrix solutions of 3 simultaneous equations with 3 unknown. The equations were prepared for each sacrifice using γ scintillation spectrometry data from standard solutions of the isotopes used for that sacrifice.

Vascular volume, permeability, and perfusion were calculated for each bladder as follows. For vascular volume (21)

\[ ml \text{ blood/g tissue} = \frac{51Cr \text{ activity/g tissue}}{51Cr \text{ activity/ml blood}} \]

For vascular permeability (21)

\[ ml \text{ plasma extravasated in 1 hr/g tissue} = \frac{\text{total plasma/g tissue} - \text{intravascular plasma/g tissue}}{hr} \]

\[ \text{Total plasma} = \frac{125I \text{ activity/g tissue}}{125I \text{ activity/ml plasma}} \]

\[ \text{Intravascular plasma} = \text{vascular volume} \times \left(1 - \frac{\text{hematocrit}}{100}\right) \]

\[ \text{Vascular perfusion} = \% \text{ of injected dose} \times \frac{86Rb/g \text{ of tissue}}{11} \]

Following determinations of radioactivities, all bladders and tumors...
were embedded in paraffin, sectioned through their entire thicknesses, mounted, and stained with hematoxylin and eosin. Slides were treated as unknowns and were examined light microscopically for histopathological changes. The criteria were essentially the same as those described in the preceding section on histopathological and ultrastructural changes (7, 14–16, 22).

**Statistical Treatments of Data.** Multivariate analysis, univariate analyses, and step-down F testing were used in a manner similar to that of earlier studies (12, 13) to define possible interactions among vascular volume, perfusion, and permeability changes with respect to time as well as significant changes in the individual parameters with time. The significance level (α) was set at 0.05. Graphs were prepared by using best-fit analysis of sequential higher-order polynomial equations (17) as well as by consideration of significant higher-degree univariate effects.

Analysis of vascular, epithelial, and inflammatory changes and of their possible relationships was carried out after grouping histopathological changes as follows: epithelial changes = none, hyperplasia, carcinoma; inflammatory changes = none, focal, diffuse. Attempts at more detailed classification were not effective for statistical analyses due to the resulting small numbers of animals within individual groups. Changes in individual functional hemodynamic parameters were analyzed in both raw and logarithmically transformed states versus epithelial and inflammatory changes with univariate and multivariate analysis of variance (1) as well as with correlation statistics.

**RESULTS**

**Histopathological Findings.** Histopathological results are shown on Table 1. There was excellent agreement among results in the 2 laboratories. In Nihon Wistar rats, simple hyperplasia was first seen 2 weeks after BBN administration, and the PLBM beneath simple hyperplasia per total length of the basement membrane beneath the bladder epithelium increased from 13.4% at 2 weeks to about 95% at 8 weeks. BBN was discontinued after 8 weeks, and the PLBM decreased to about 4 to 6% at Weeks 20, 30, 35, and 40. Papillary and nodular hyperplasias were found from Week 6 of BBN administration, and their PLBM increased with time to a maximum of 31% at 40 weeks. Papillary transitional-cell carcinomas were found from Week 20 in increasing numbers with time. Submucosal inflammatory cell infiltrations were found in all rats during BBN treatment. After BBN treatment, inflammatory changes first decreased and later increased in the late-experimental period with tumor onset (Table 1). Changes observed in the Charles River Wistar rats were essentially the same as those described for Nihon Wistar rats. The greater carcinoma incidence at 30 weeks in Charles River rats was matched at 35 weeks by Nihon Wistar rats and was probably due to the smaller group size in the Charles River rats.

**Vascular Cast Studies.** Sequential changes in percentages of areas with types 1 and 3 vascular proliferative foci are shown in Table 2. Small foci of type 1 vascular proliferations were found from Week 2 of the experiment. The numbers of foci appeared to increase until Week 8 and then decrease after BBN administration; however, the percentage areas of type 1 foci per unit capillary bed never exceeded 1%. Diameters of normal-looking capillaries appeared larger during BBN treatment than after BBN treatment. Small type 3 foci (Fig. 1) were first found at Week 6, and the percentage of the foci per unit capillary bed increased gradually for the remainder of the study. From 20 weeks on, the

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**Table 1**

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<th>Wk after beginning of BBN treatment</th>
<th>No. of animals</th>
<th>No. of rats</th>
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<th>Simple hyperplasia</th>
<th>Papillary or nodular hyperplasia</th>
<th>Cancer</th>
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* Degree of infiltration by inflammatory cells in submucosa shown according to the following symbols: −, none; ±, very few and spotty; +, few and diffuse.
* Beneath area of pathology per total length of basement membrane beneath the bladder epithelium.
* Changes observed in the Wistar rats from Nihon Rat Co.
* Numbers and symbols in parentheses, changes observed in the Wistar rats from Charles River Breeding Laboratories, Inc.
* Mean ± S.D.
* Areas of type 3 excised tumors not calculated.
diameters of capillary casts of type 3 varied from area to area
and were occasionally irregular (Fig. 2). Sequential changes of
type 3 foci correlated with the sequential changes of papillary or
nodular hyperplasia and cancer. Type 2 foci reported previously
(22) were not found in this study.

Ultrastructural Findings. The subepithelial layer of normal
bladder epithelium contains small numbers of capillary vessels
and fibrocytes. The capillary endothelial cells have many cyto-
plasmic pinocytotic vesicles but few or no apparent fenestrations
(Fig. 3). Under areas of simple hyperplasia, the capillary endo-
theelial cytoplasm had developed fenestrations with diaphragms. In
areas under papillary or nodular hyperplasia, fenestrations of the
capillary endothelial cells were also found (Fig. 4). In areas
beneath transitional-cell carcinoma, capillary endothelium
showed bizarre contours with fenestration. Fenestration of cap-
illaries in each lesion was found in almost all capillary endothelial
cells. The ratio of capillary diameter under normal bladder epithe-
lium, simple hyperplasia, papillary or nodular hyperplasia, and
cancer is about 1:1.2:1.5:2.

Hemodynamic Changes versus Time. The results of the
functional hemodynamic studies are shown in Charts 1 to 3.
There were significant and complex changes in all 3 functional
hemodynamic parameters with time. Changes in vascular volume
were independent of multivariate interactions with changes in
permeability and perfusions and showed significant cubic (p <
0.0001) and seventh-degree (p < 0.001) trends versus time.
Borderline significance (p < 0.06) was also observed for linear
and quartic trends versus time for vascular volume. While there
were significant multivariate correlations among changes in vas-
cular permeability and perfusion (r = 0.58) both during and
following treatment with BBN, most multivariate significance was
associated with changes in permeability (p < 0.0001 for linear,
cubic, and quintic effects). Best-fit analyses indicated that the
volume maximum was at 8 weeks, and the volume minimum
was at 25 weeks; and that the permeability maxima were at 4
and 25 weeks, and the permeability minima were at 15 and 32
weeks (Charts 1 and 2). The peak value of the volume at 8
weeks was verified by repeating this time point with an additional
group of 6 animals. The dotted line in Chart 1 suggests a best-
fit curve utilizing this datum point. While 86Rb values correlated
125I values, they were quite unstable in intermediate time periods,
and the polynomial best fit was much poorer than for either 51Cr
or 125I.

Values for 51Cr, 125I, and 86Rb were extremely variable and
peculiar at 40 weeks. These results were probably due to an
uncontrollable technical problem: the extremely large tumors
present in all of the bladders. Since bladders had to be clamped,
excised, and opened prior to excision of these tumors, there
was substantial time period between bladder clamping and tumor
incision for vascular compartments contents to be uncontrollably
exchanged between tumor tissue and bladder epithelium. This
was probably true to a lesser degree for 35-week animals as
well. Thus, it was felt that the bladder epithelium measurements
at 40 weeks were quite inaccurate, and they were omitted from
the statistical analyses.
Hemodynamic Changes versus Epithelial and Inflammatory Changes. No multivariate interactions were detected among hemodynamic parameters when bladders were reordered into groups on the basis of inflammatory and epithelial changes rather than time of sacrifice. Vascular volume changes, while strongly time dependent, did not show multivariate or univariate significance in these groupings. There was an approach to significance for vascular volume versus tissue changes ($p < 0.11$), perhaps due to a strong $^{51}$Cr increase in animals with carcinomas; however, animal variability within groups with hyperplastic and neoplastic changes probably obscured the significance of this relationship. Significant univariate effects of inflammation on vascular permeability ($p < 0.0002$) and epithelial changes on vascular permeability ($p < 0.0003$) were detected. Variability of $^{86}$Rb measurements in these groupings precluded showing statistically significant effects regarding vascular perfusion.

**DISCUSSION**

By duplicating experimental designs, our laboratories have been able to correlate a variety of parenchymal and vascular changes in rat urinary bladder during carcinogenesis by BBN. The similarities in incidences and degrees of epithelial and inflammatory changes observed in the 2 laboratories allow correlations to be made between histopathology, qualitative and quantitative microvascular architectural changes, vascular volume, vascular permeability, and vascular perfusion.

The only appreciable difference between the laboratories was the slightly earlier appearance of high transitional-cell carcinoma incidence in the Charles River rats (30 versus 35 weeks). Incidences were equal at 35 weeks, and this slightly earlier higher tumor incidence may have been due to a variety of factors, including strain differences, housing, specific dietary content, and/or minute differences in carcinogen intake.

In earlier functional vascular studies of vascular volume and perfusion following DMBA carcinogenesis in hamster cheek pouch epithelium, increases in vascular volume were observed in tumor-bearing pouches (11, 12). It was postulated that these increases were due to (a) proliferation of new blood vessels, (b) dilation of existing blood vessels, (c) leakage of erythrocytes through damaged vessel walls secondary to inflammation, or (d) combinations of the above factors.

The cubic response of vascular volume versus time was significantly correlated with the presence of BBN, increases in capillary dilation, the early appearance of some type 3 capillary foci, and the later appearance and development of tumors and type 3 capillary foci. The maximal $^{51}$Cr values at 8 weeks coincided with the period of maximal accumulated administered BBN, with the maximal incidence and severity of type 1 vascular proliferative foci, with associated widespread capillary dilation, and with the initial appearance of type 3 vascular foci. Since type 1 capillary proliferation represents less than 1% of the capillary bed, and since changes in $^{51}$Cr values were independent of changes in $^{125}$I values, the early increases in vascular volume appear to be due to widespread dilation of the capillary bed. The alternate graph in Chart 1 (---), using the 8 week maximal value, might be explained by the initial appearance of type 3 vascular proliferation superimposed on the time of maximal BBN accumulation and induced capillary bed dilation. The subsequent sharp decrease in volume correlates with the diminution of dilated capillaries under simple hyperplasia following removal of BBN.

The later rise in vascular volume (25 to 35 weeks) coincided with increasing severity of papillary or nodular hyperplasia and of type 3 capillary proliferative foci. $^{125}$I-HSA has been established as an effective measure of capillary permeability (21), and the extremely high multivariate interaction between the inflammatory indices and $^{125}$I-HSA measurements tends to confirm the effectiveness of this tracer as a measure of permeability and inflammation in rat urinary bladder epithelium (Chart 2). The same relationship between $^{125}$I-HSA activity and inflammatory tissue changes has also been made with multiple simultaneous tracer studies in DMBA-treated hamster cheek pouch epithelium. In addition, changes in vascular permeability were also related to changes of PLBM beneath simple hyperplasia, papillary or nodular hyperplasia, and cancer. Changes of PLBM beneath each lesion were interpreted to represent corresponding changes in the total area of fenestrated capillaries in the bladder. The initial peak $^{125}$I values coincided with the maximal area of fenestrated capillaries beneath simple hyperplasia. Subsequent falls and rises also correlated with decreases and increases in total areas of fenestrated capillaries beneath simple hyperplasia, papillary or nodular hyperplasia, and carcinoma. Areas of fenestrated capillaries in Weeks 20 and 30 were smaller than those in the other experimental periods. Morphological data suggested a cubic curve (---) for the $^{125}$I data (Chart 2). Polynomial best-fit analysis of the $^{125}$I data, however, showed the quintic best-fit curve. While there is no clear explanation for the quintic best-fit curve of the $^{125}$I data and the cubic curve suggested by morphological data, the rise and fall of $^{125}$I values from 15 to 30 weeks may represent a transient inflammatory response to early dysplastic or carcinomatous changes which preceded the ultimate increases in $^{125}$I accompanying the establishment and growth of frank carcinomas and their associated type 3 fenestrated capillaries.

$^{86}$Rb/Cl measurements were subject to considerably more variability than the $^{51}$Cr and $^{125}$I measurements. Theoretically, $^{86}$Rb+$\kappa$ is extracted as a bolus in a single circulatory passage by paranchymal cells, as $\kappa$ would be, and this extraction, in many normal tissues, is directly proportional to blood flow (19). Recent evidence has introduced several other possible factors which, in a pathological tissue, could substantially alter $^{86}$Rb+$\kappa$ uptake. These include countercurrent flow systems (5, 6, 12), altered endothelial permeability to $^{86}$Rb+$\kappa$, and increased $K^+$, $Na^+$ pumping in membranes of proliferating (10, 20) and transformed (24) cells. The lack of control of these variables and their unknown contributions to $^{86}$Rb+$\kappa$ uptake make conclusions on the relationships between vascular perfusion and specific events in the progression of chemical carcinogenesis inappropriate.

Early changes in microvascular functions [(a) increased vascular volume secondary to capillary dilation and (b) increased vascular permeability related to capillary fenestration and to inflammation] may be either the direct result of BBN action on the vessel wall or microvascular response secondary to inflammation in the bladder tissue during BBN administration. BBN induces the initial simple epithelial hyperplasia through a toxic reaction in the bladder epithelium (8, 18). While the exact sequence of capillary and epithelial changes cannot be gleaned...
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from the present data, it could be postulated that either (a) increased nutrient need from the greater number of cells in the hyperplastic epithelium stimulates capillary dilation and fenestration or (b) dilation and fenestration are an immediate response to BBN injury and allow subsequent BBN-induced epithelial proliferation.

Inflammation induced by freeze ulceration and cyclophosphamide has been shown to induce reversible types 1 and 3 vascular proliferation in rat bladder (22). These changes appeared to have been less widespread and of shorter duration than similar changes induced by BBN. Although the morphology of early BBN- and inflammation-induced types 1 and 3 vascular changes appears to be indistinguishable, the carcinogen-induced proliferation and capillary dilation may involve a fundamental and irreversible alteration of control of endothelial response to proliferative stimuli from host or tumor angiogenic factors, while the inflammation-induced changes do not. Reaction of chemical carcinogens with nonparenchymal elements presumably at the level of the endothelial cells could be significant with respect to the mechanisms of the later appearance and growth of the transitional-cell carcinomas by making these cells more responsive to the angiogenic stimulus from premalignant and/or malignant foci of adjacent parenchymal cells.

Later changes in microvascular form and function may be responses to the further development of preneoplastic and cancerous foci. These changes may be accompanied by a second inflammatory response and by additional secretion of angiogenesis factor. Angiogenesis factor is likely to stimulate additional increases in vascular volume and permeability via type 3 fenestrated vascular proliferation and widespread capillary bed dilation in bladders bearing large malignant tumors. The initial development of type 3 fenestrated vascular beds, perhaps resulting from BBN-induced changes in capillary endothelial responsiveness to angiogenic stimulation, could theoretically create a favorable environment for growth and development of the premalignant foci into clinically manifest carcinomas.

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Fig. 1. Early stage of type 3 vascular proliferation arising from an area of normal bladder capillaries in a rat after 8 weeks of BBN administration. × 160.

Fig. 2. Advanced type 3 vascular proliferation in a papillary hyperplasia at 20 weeks after the start of BBN treatment. × 120.

Fig. 3. Capillary vessel under the normal bladder epithelium has many pinocytic vesicles but no apparent fenestration. × 13,000.

Fig. 4. Capillary vessel under the papillary hyperplasia at 10 weeks after the start of BBN treatment has developed fenestrations (arrows) in its cytoplasm. × 12,000.
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