A Study of the Ultraviolet Microscopy of Renal Vascular Diseases

By Sheldon C. Sommers, M.D., Ruth Crozier, M.A., and Shields Warren, M.D.

Newer methods of microscopy promise to provide a better insight into the physicochemical aspects of tissue structure than has been attained by conventional techniques. An exploratory investigation is reported of the ultraviolet absorptive behavior of renal arterioles and arteries, employing the Polaroid color- translating ultraviolet microscope. In the arteriolar necrosis accompanying malignant hypertension and renal periarteritis nodosa changes of particular interest were observed.

In recent years pathologists have applied many new tools to the analysis of tissue changes in kidney disease. Histochemistry,1-6 microspectrography,7 phase contrast microscopy,8 ultraviolet microscopy9 and electron microscopy3,10 have yielded new evidence to support or refute traditional ideas of the morphologic bases of various kidney lesions. Another new technic, which permits color photomicrography in ultraviolet light, has recently become available. The Polaroid color- translating ultraviolet microscope allows the operator to choose any three wave lengths in the range of 2330 to 4000 Angstrom units, and to obtain accurately focused photomicrographs on 35 mm. film of tissues and other material, with each of the three wave lengths translated into a different color; blue, green and red. In the machine are a rapid film processor and a projector which superimposes the three images into a single colored picture.11,12 As many as 40 or more fields can be photographed in one work day. The range of wave lengths studied includes those at which proteins and nucleoproteins demonstrate peak absorptions, such as at approximately 2630 Angstrom units for nucleoproteins.

To investigate whether stromal tissues had any peculiarities of ultraviolet absorption, an inquiry into the properties of normal and abnormal human and animal kidney tissues was undertaken. Kidney was chosen because it proved easy to identify its histologic components with the optical telescope and green-black contrasting appearances of unstained tissue used in the Polaroid instrument, which has green light for preliminary visual survey of the slide.

A rather surprising variation in the ultraviolet absorptions of glomeruli from different human kidney diseases was observed and is being reported elsewhere.13 The absorption behavior was uniform between different slides, different kidneys and different persons with the same pathologic condition. In the case of persons with diabetes mellitus, the changes of glomerular stroma were considered distinctive enough to be diagnostic by ultraviolet photomicrography, in the presence or absence of identifiable pathologic alterations by ordinary pathologic criteria.

In the present communication the ultraviolet absorptive properties of arteries and arterioles in the same kidneys are considered.

Methods and Materials

As described previously, sections of paraffin-embedded kidney tissues were cut 4 μ or less thick, dry-mounted on Vycor slides, covered with glycerin after deparaffination with xylol, and Vycor cover glasses were then employed with a rim of paraffin. The tissues had either been Zenker-fixed and Autotechnicon-processed through to paraffin blocks, or had been frozen-dried and paraffin-embedded in vacuo. The design and operation of the Polaroid CTUV microscope are described elsewhere.11,12

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In each unstained slide studied, at ×2000 magnification on the projector screen, sufficient fields were examined to exclude possible local variations in tissue absorptions as a significant factor. More than one slide and case of each disease were used, up to the limitations of available time and adequate materials.

Human kidney diseases studied included cases of benign arterionephrosclerosis, malignant arteriolar nephrosclerosis, intercapillary glomerulosclerosis (Kimmelstiel-Wilson) in diabetes mellitus, chronic pyelonephritis, chronic pyelonephritis, proliferative glomerulonephritis, amyloidosis, lupus erythematosus and periarteritis nodosa.

Each slide was studied at three sets of wavelengths:

<table>
<thead>
<tr>
<th>Set 1</th>
<th>280, 263, 240 μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>250, 263, 248</td>
</tr>
<tr>
<td>3</td>
<td>248, 240, 235</td>
</tr>
</tbody>
</table>

In each instance blue was used for the longest, green for the intermediate, and red for the shortest wavelength. The photographs taken would permit densitometry studies and plotting of absorption curves in the range 2600 to 2350 Angstrom units.

Following ultraviolet photomicrography, the slides were stained with hematoxylin and eosin, Masson-Goldner trichrome, Mallory aniline blue, or other special stains. The pathologic diagnosis was again confirmed using ordinary criteria. Often the same areas studied in ultraviolet light were scrutinized and compared with the ultraviolet photomicrographs. Aside from color projection of the 35 mm. film strips, black-and-white photographic prints and water-color paintings of some fields were available for review.

RESULTS

The normal collagenous stromal components of a variety of tissues in humans and animals have a quite uniform ultraviolet absorptive behavior. The same ultraviolet absorptive properties characterized normal glomerular stroma. Zenker fixation had not altered relative color values, but produced hard bright colors instead of the soft pastels seen in frozen-dried tissue. Some nuclei were rendered opaque by chromation. In set 1 colors translated as pink to red violet, in set 2 violet, and in set 3 from red-orange to brown. In contrast to the wide variations in ultraviolet absorptions of diseased glomeruli observed, the color translated appearance of normal or diseased arteries and arterioles proved quite uniform (table 1).

Both arteries and arterioles in the set 1 gave red-pink to red-violet colors, in set 2 changing to violet or blue-gray. Sets 3 ranged from yellow-tan through orange-tan, olive-tan to brown. No distinctive color differences comparable to those observed in glomerular diseases were found in the arterioles or arteries studied. The elastica of arteries had absorptions differing from other elements: bright red in set 1, rose violet in set 2 and orange in set 3, contrasting respectively with red-violet, blue-violet and yellow-tan colors of muscle and stroma. This corresponded to greater absorption of elastica at shorter wavelengths.

The major disease processes showing distinctive arteriolar ultraviolet absorptions were those attended by arteriolar necrosis. Thus in malignant arteriolar nephrosclerosis and periarteritis nodosa, an unusual opacity to ultraviolet light was noted in all wavelengths tested. With color-translated projection the opaque areas appeared white or gray, indicative of greatly increased ultraviolet absorptions.

TABLE 1—Color-Translated Ultraviolet Absorptions of Arteries and Arterioles

<table>
<thead>
<tr>
<th></th>
<th>Case Fields</th>
<th>Set 1 (280, 263, 240 μm)</th>
<th>Set 2 (280, 263, 248 μm)</th>
<th>Set 3 (248, 240, 235 μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3</td>
<td>red-violet</td>
<td>gray-violet</td>
<td>yellow-brown orange-tan</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>3</td>
<td>red-violet</td>
<td>gray-violet</td>
<td>tan-brown</td>
</tr>
<tr>
<td>Chronic pyelonephritis</td>
<td>2</td>
<td>red-violet</td>
<td>gray-violet</td>
<td>tan-brown</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>1</td>
<td>red-violet</td>
<td>gray-violet</td>
<td>pink-tan</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>3</td>
<td>red-violet</td>
<td>blue-violet</td>
<td>yellow-tan orange</td>
</tr>
<tr>
<td>elastica</td>
<td>2</td>
<td>red</td>
<td>rose-violet</td>
<td>gray-orange</td>
</tr>
<tr>
<td>Lupus erythematosus</td>
<td>2</td>
<td>pink-violet</td>
<td>red-violet</td>
<td>gray-tan</td>
</tr>
<tr>
<td>Malignant arteriolar nephrosclerosis</td>
<td>2</td>
<td>carmine</td>
<td>violet</td>
<td>yellow-gray</td>
</tr>
<tr>
<td>Arteriolar necrosis</td>
<td>1</td>
<td>white</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4X exposure</td>
<td>3</td>
<td>white &amp; carmine</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>8X exposure</td>
<td>1</td>
<td>carmine</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Periarteritis nodosa</td>
<td>2</td>
<td>pink-violet</td>
<td>red-violet</td>
<td>gray-tan</td>
</tr>
<tr>
<td>elastica</td>
<td>1</td>
<td>red</td>
<td>violet</td>
<td>tan</td>
</tr>
<tr>
<td>arterial necrosis</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>gray</td>
</tr>
<tr>
<td>4X exposure</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>olive-tan</td>
</tr>
<tr>
<td>8X exposure</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>olive-brown</td>
</tr>
</tbody>
</table>
only other components of tissues reacting similarly that have been studied included deposits of calcium or hemosiderin, and some nuclei which had been chromated by Zenker’s solution. Photographic exposures of eight times the usual time interval resulted in the restoration of the colors usual for the arterioles investigated (figs. 1 and 2). This indicated that while ultraviolet absorption was markedly increased, absorptive properties were not apparently qualitatively altered.

Similar observations by microspectrography of increased ultraviolet absorptions at shorter wavelengths have been made in a study of arteriolar sclerosis and necrosis in human and animal tissues.7

As already mentioned, benign nephrosclerosis, chronic pyelonephritis and amyloidosis failed to demonstrate distinctly abnormal ultraviolet absorptions of arterioles and arteries (fig. 3).
Fig. 3. Positive photographic prints of original 35 mm. film strip. Top horizontal row, set 1: left to right 280, 263 and 240 mμ; middle horizontal row, set 2: left to right 280, 263 and 248 mμ; bottom horizontal row, set 3: left to right 248, 240 and 235 mμ. Left-hand vertical rows are translated into blue, middle vertical rows into green, and right-hand vertical rows into red. Darker shades represent increased ultraviolet absorption. A sclerotic arteriole from a kidney with chronic pyelonephritis, frozen-dried, is shown. Absorptions are not significantly abnormal. (×1000.)
Discussion

Studies of tissue pathology using ultraviolet light over a considerable range of wavelengths are quite new. Many findings have been and will probably be unexpected, such as the distinctive differences observed in glomerular stromal absorptions in various clinically and pathologically identifiable glomerular diseases. Aside from concluding from the study of different blocks of kidney tissues from various patients that the observations were not due to localized peculiarities of certain areas of tissue, and were uniform for individual disease entities, no complete explanation was available to explain the ultraviolet absorptive behavior of diseased glomeruli. Biochemical observations of the ultraviolet absorption spectra of collagen during successive purification procedures which removed ground substances have shown similar changes in the same wavelength range as investigated in the present study. This suggests that mixtures of partly denatured glomerular stromal proteins surrounded by abnormal amounts of normal or abnormal ground substances could be responsible for the observations.

Arteries and arterioles of diseased kidneys studied have proved less labile than glomeruli in demonstrating abnormal ultraviolet absorptions. In fact, short of necrosis of their walls, no significant alterations from normal ultraviolet properties were observed in various important kidney diseases. Despite definite morphologic changes in arterial walls in these conditions, ultraviolet absorptions were considered within normal limits.

Possible explanations suggested for the negative findings are (1) that the material was chosen particularly to study glomeruli and did not illustrate the most outspoken arterial and arteriolar lesions. However, there were striking vascular changes at least in the diabetic, amyloidosis and periarteritis nodosa material. (2) Despite histologic changes, the preponderance of normal smooth muscle and collagen still unaltered in most of the blood vessel walls perhaps obscured abnormalities in ultraviolet absorption. In glomeruli a significantly greater proportion of stroma was likely damaged, with resulting visibly altered ultraviolet properties.

The positive findings of greatly increased ultraviolet absorption, up to eight times normal, in the vascular necroses of malignant arteriolar nephrosclerosis and periarteritis nodosa were unexpected, since no such increased density is found with visible light. The normal ultraviolet absorptions of adjacent kidney tissues testified that this change was not attributable to increased thickness of sections or other technical factors. Apparently protein denaturation and coagulation with precipitation of colloids may be partly responsible. Whether some inorganic materials like calcium or iron also are attached to protein and add to the ultraviolet opacity remains to be determined.

Further studies with ultraviolet photomicrography of renal vascular diseases would appear promising. It would be of interest to investigate the effects of enzymes, hydrolyzing agents and salts upon the ultraviolet absorptive properties of vessel walls. Since the slides are examined unstained, later histochemical studies of the identical sections are feasible.

Summary

An exploratory study of the ultraviolet absorption properties of renal arteries and arterioles from cases of diabetic nephropathy, chronic pyelonephritis, arteriosclerosis, malignant arteriolar nephrosclerosis, glomerulonephritis, amyloidosis, lupus erythematosus, and periarteritis nodosa has been carried out with the Polaroid color-translating ultraviolet microscope. Unlike the distinctive alterations of ultraviolet absorptions found in diseased glomeruli, the arterial and arteriolar ultraviolet properties generally were unaltered. In the vascular necroses of arteriolar nephrosclerosis and periarteritis nodosa the ultraviolet absorptions of vessel walls were increased up to eight times normal. The basis of the findings is discussed.

Summario in Interlingua

Esseva executate per medio del polaroido microscepio ultraviolette coloritrudecente un studio exploratori del qualitates de absorption
ultravioletas de arterias e arteriolas en casos de nefropatía diabética, pielonefritis crónica, arteriosclerosis, maligne nephrosclerosis arteriolar, glomerulonefritis, amyloidosis, lupus eritematoso e periarteritis nodose. En contraste con las alteraciones características de la absorción ultravioleta observada en glomérulos moribundos, las cualidades ultravioletas de las arterias e arteriolas estaban generalmente intactas. En casos de necrosis vascular de nefrosclerosis arteriolar e de periarteritis nodosa, la absorción ultravioleta del parietes vascular se aumentaba hasta 8 veces el normal. Le base de estes constatações es discutite.

REFERENCES

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