# ULTRAVIOLET FLUORESCENCE OF BLADDER TUMORS FOLLOWING ORAL ADMINISTRATION OF TETRACYCLINE COMPOUNDS

## *A Macroscopic, Microscopic and Fluorescence Spectrophotometric Study*

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**Ultraviolet fluorescence was studied in specimens of bladder tumor tissue removed from 29 patients who had received tetracycline or methacycline, and from 8 patients who had received no tetracycline compound. Fluorescence was observed by macroscopic and microscopic examination and by spectrophotometry of tumor extracts. Tetracycline compounds imparted a bright yellow fluorescence to calcium salts encrusted upon the tumor but in only 3 cases was any similar degree of fluorescence observed in tumor tissue itself. Spectrophotometry has shown that the auto-fluorescence of bladder tumor tissue occurs at a similar wavelength to tetracycline fluorescence. Lesser degrees of yellow-green fluorescence of bladder tumors cannot be regarded as specific to tetracycline, therefore. Visual qualitative assessment of ultraviolet fluorescence in bladder tumors after tetracycline administration does not appear to provide a satisfactory basis on which to establish a reliable diagnostic method.**

TETRACYCLINE FLUORESCENCE IN ULTRAVIOLET light first was reported in a breast tumor by Rall et al.[14] in 1957. Since then various workers have demonstrated fluorescence in a variety of animal and human tissues after the administration of tetracycline compounds. McLeay et al.[11] noted that fluorescence was greater in bone tumors, but that carcinomas also fluoresced especially those of rapid growth. Machado et al.[10] studied transplantable mouse tumors and found tetracycline fluorescence only in the presence of necrosis. Tetracycline can chelate calcium ions[1, 12] and necrotic areas of tumor have been found to have a higher calcium content than viable areas.[15, 16] It might be expected, therefore, that calcium deposits in sites other than bone could give rise to tetracycline fluorescence and this has proved to be true of calcium-containing urinary calculi.[13]

Many sites have been suggested for tetracycline localization—inflammatory tissue,[4] macrophages,[18] mitochondria,[2] peptides,[9] protein bound complexes,[20] depolymerized mucopolysaccharides and chondroitin sulphate[4] and serum lipoproteins, especially the β fraction in the presence of calcium ions.[8] Whitmore et al.[19] recently have described fluorescence in bladder carcinomas when viewed with an ultraviolet quartz rod endoscope; 13 of 17 grossly evident carcinomas thus examined exhibited a yellow-green fluorescence considered to be specific for tetracycline; also, areas of in situ carcinoma were identified in 6 bladders by this technique. The present observations concern ultraviolet fluorescence of bladder tumor tissue by macroscopic and microscopic examination and by fluorescence spectrophotometry of tumor tissue extracts.

### MATERIALS AND METHODS

All patients presented at the urological clinic and, on examination and investigation, were found to have one or more bladder tu-

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TABLE 1. Ultraviolet Fluorescence in Bladder Tumors Not Receiving Drug

| Case no. | Histological findings | Histological evidence of invasion | Direct U.V. examination* | Calcium salts | Necrosis | Fluorescence spectrophotometry |
|---|---|---|---|---|---|---|
| 1 | Well-differentiated papillary transitional cell carcinoma | None | − | + | ++ | |
| 2 | Well-differentiated papillary transitional cell carcinoma | None | + | − | − | |
| 3 | Well-differentiated papillary transitional cell carcinoma | None | − | − | − | Performed |
| 4 | Well-differentiated papillary transitional cell carcinoma | None | − | − | − | Performed |
| 5 | Well-differentiated papillary transitional cell carcinoma | Present | − | + | + | |
| 6 | Moderately differentiated papillary transitional cell carcinoma | Present | + | − | − | |
| 7 | Moderately differentiated papillary transitional cell carcinoma | Present | − | − | − | Performed |
| 8 | Moderately differentiated solid transitional cell carcinoma | Present | − | − | − | Performed |

* All tumors were negative on microscopic U.V. examination.

mors. Tetracycline,* or its analogue methacycline† was administered orally. Tetracycline was used at a dosage of 500 mg every 6 hours for 2 days followed by 250 mg every 6 hours for a further 3 days, methacycline was administered at a dosage of 150 mg every 6 hours for 5 days. A lapse of 24 to 36 hours was allowed between the last dose of tetracycline and biopsy, and of 60 to 72 hours in the case of methacycline since this compound has a slower rate of excretion. During this period the tetracycline compounds are cleared from the body fluids and nonretaining tissues.

Tumor tissue was obtained either (1) by endoscopic biopsy with Lowsley's or Buerger's forceps or with a McCarthy loop resectotome or (2) at open suprapubic cystotomy during elective surgery. By these methods, with the exception of 4 specimens taken with Buerger's forceps, a large amount of tumor tissue was made available for examination.

The biopsy specimen first was examined macroscopically and with the aid of a hand lens in a darkened room under an Hanovia mercury vapor lamp fitted with a Wood's filter having a peak emission at 365 mμ.

A portion of the tissue then was frozen with solid carbon dioxide and cryostat sections were cut at 5 and 20 μ. Unstained sections were mounted in a nonaqueous ultraviolet inert medium‡ and adjacent sections were stained with hematoxylin and eosin and with von Kossa's silver impregnation method for calcium salts. The unstained sections were examined by phase-contrast and ultraviolet microscopy using a Reichert-Zetopan microscope with a mercury vapor lamp. The peak emission spectrum was 360 mμ when using the exciter filter combination UG 1/2.5 mm and BG 12/3 mm (according to the Schott u. Gen. Catalogue). The absorption filter GG 9/1 mm was used with a contrast-fluorescence condenser.

A second portion of the original tissue was fixed in 10% formol-saline, wax embedded, sectioned and stained with hematoxylin and eosin for critical histological evaluation. Bladder tumors were assessed according to the classification of Dukes.[3] This system permits a histological diagnosis of malignancy in papilliferous tumors on cytological grounds, even though infiltration or invasion are not demonstrable in the biopsy material. Such a policy brings pathological nomenclature into line with clinical experience of the behavior of these bladder tumors.

Tissue from 13 of the cases was homogenized

* Achromycin, Lederle Laboratories, Pearl River, N.Y.

† Rondomycin, Pfizer Laboratory, New York, N.Y.

‡ Uvinert Mountant (non-aqueous), G. T. Gurr Ltd., London, England.

by cryostat sectioning[17] and extracted with N/100 hydrocholoric acid; the cell debris was removed by ultracentrifugation. Immediately prior to fluorescence spectrophotometry aliquots of the supernatant fluid were corrected to pH 5.6 and 7.4 by the addition of acetate and tris buffers. Such pH conditions would be within the range expected of the urine and tissues.

A Zeiss recording spectrophotometer RPQ 20A was used, with fluorescent attachment ZFM 20. The exciting wavelength of 365 mμ

TABLE 2. Ultraviolet Fluorescence in Bladder Tumors Receiving Tetracycline

| Case no. | Histological findings | Histological evidence of invasion | U.V. examination | | Calcium salts | Necrosis | Fluorescence spectrophotometry |
|---|---|---|---|---|---|---|---|
| | | | Direct | Microscopic | | | |
| 9 | Well-differentiated papillary transitional cell carcinoma | None | − | − | − | + | |
| 10 | Well-differentiated papillary transitional cell carcinoma | None | + | − | − | − | |
| 11 | Well-differentiated papillary transitional cell carcinoma | None | +++ | Fluorescence of calcium salts and areas of fibrinoid necrosis | + | + | |
| 12 | Well-differentiated papillary transitional cell carcinoma | None | − | − | − | − | |
| 13 | Well-differentiated papillary transitional cell carcinoma | None | − | − | − | − | |
| 14 | Well-differentiated papillary transitional cell carcinoma | None | + | − | − | − | |
| 15 | Well-differentiated papillary transitional cell carcinoma | None | − | − | − | − | |
| 16 | Well-differentiated papillary transitional cell carcinoma | None | ++ | Fluorescence of superficial area of necrosis | − | + | |
| 17 | Well-differentiated papillary transitional cell carcinoma | None | − | − | − | − | Performed |
| 18 | Well-differentiated papillary transitional cell carcinoma | None | − | − | − | − | Performed |
| 19 | Well-differentiated papillary transitional cell carcinoma | None | − | − | − | − | Performed |
| 20 | Well-differentiated papillary transitional cell carcinoma | Present | − | − | − | − | |
| 21 | Well-differentiated papillary transitional cell carcinoma | Present | + | − | − | − | |
| 22 | Carcinoma-in-situ with central area invasive squamous cell carcinoma | Present | + | − | − | − | |
| 23 | Moderately differentiated papillary transitional cell carcinoma | Present | − | − | − | − | |
| 24 | Moderately differentiated papillary transitional cell carcinoma | Present | + | − | − | + | Performed |
| 25 | Moderately differentiated papillary transitional cell carcinoma | Present | +++ | Fluorescence of calcium salts | + | + | |
| 26 | Poorly differentiated solid transitional cell carcinoma | Present | +++ | Fluorescence of calcium salts | + | − | |
| 27 | Anaplastic carcinoma | Present | +++ | Fluorescence of calcium salts | + | + | Performed |
| 28 | Anaplastic carcinoma | Present | + | − | − | + | |

TABLE 3. Ultraviolet Fluorescence in Bladder Tumors Receiving Methacycline

| Case no. | Histological findings | Histological evidence of invasion | U.V. examination | | Calcium salts | Necrosis | Fluorescence spectrophotometry |
|---|---|---|---|---|---|---|---|
| | | | Direct | Microscopic | | | |
| 29 | Well-differentiated papillary transitional cell carcinoma | None | – | – | – | – | |
| 30 | Well-differentiated papillary transitional cell carcinoma | None | – | – | – | – | Performed |
| 31 | Well-differentiated papillary transitional cell carcinoma | None | + | – | – | – | Performed |
| 32 | Well-differentiated papillary transitional cell carcinoma | Present | – | – | – | – | |
| 33 | Well-differentiated papillary transitional cell carcinoma | Present | – | – | – | – | |
| 34 | Well-differentiated papillary transitional cell carcinoma | Present | +++ | Fluorescence of calcium salts | + | + | Performed |
| 35 | Moderately differentiated papillary transitional cell carcinoma | Present | – | – | – | – | |
| 36 | Moderately differentiated papillary transitional cell carcinoma | Present | +++ | Superficial tumor fluorescence | – | – | Performed |
| 37 | Poorly differentiated solid transitional cell carcinoma | Present | – | – | – | – | |

was selected from a mercury vapor lamp with a Zeiss filter M 365. The emission spectrum was examined on a purely qualitative basis without measurement of intensity.

The extraction procedure was designed to demonstrate the fluorescent characteristics of tumor tissue and not in any way intended for the determination of tetracycline itself. Indeed, in-vitro experiments[7] have shown that simple extraction of tissues into weakly acid solution does not permit tetracycline estimation because of the interfering fluorescence of tissue components. Proteins, phosphates and oxalates, in particular, have inhibitory effects on the fluorescence of tetracycline complexes, probably by the competitive binding of calcium ions. The presence of divalent metal ions and pH conditions vitally influence not only the intensity of tetracycline fluorescence but also the stability of the fluorescent complex and the maximum wavelengths of excitation and emission.

On the basis of these findings methods have been devised for the quantitative estimation of tetracycline after removal of interfering tissue components, by spectrophotometric[5] or fluorometric[6, 7] measurement.

Thirty-seven bladder tumors were observed by macroscopic and microscopic examination in ultraviolet light and fluorescence spectrophotometry was performed on extracts of 13 of these tumors. In 5 instances biopsy material was obtained from patients both before and after administration of a tetracycline compound (cases 2 and 31, 3 and 16, 4 and 17, 5 and 34, and 7 and 25, Tables 1–3). All specimens except two (cases 23 and 25) were obtained by endoscopic biopsy, these latter being obtained at autopsy. Eight tumors were obtained from patients not receiving any tetracycline compound; 20 were examined after administration of tetracycline and 9 after methacycline.

## RESULTS

*Macroscopic examination:* Six of 20 tumors from patients who had received tetracycline and one of 9 tumors from patients who had received methacycline, exhibited a weak general yellow-green fluorescence (Table 3); however, a similar fluorescence was emitted by 2 of 8 tumors from patients who had not received any tetracycline derivative. This fluorescence was unrelated to the presence of necrosis or to the degree of differentiation of the tumor.

A bright yellow fluorescence (denoted as ++ or +++ in Tables 2 and 3) was noted at focal areas of 5 tumors from tetracycline-treated patients and of 2 tumors from methacycline-treated patients. In 5 of these tumors with

obvious fluorescence the fluorescent areas appeared related to calcium salt encrustation. One of these latter tumors (case 26) was removed at autopsy; the area of carcinoma, measuring 3 cm in diameter, previously had been treated with radioactive gold grain implantation and was heavily encrusted with intensely fluorescing calcium salts. Histology revealed that only microscopic foci of carcinoma remained within the bladder wall.

Two of the tumors from patients who had not received tetracycline showed macroscopic evidence of calcium salt encrustation (case 1 and 5) but no fluorescence was observed in these areas. In one of these (case 5) biopsy was repeated after methacycline administration (case 34) and the calcium salts then emitted a brilliant yellow fluorescence.

Since alkalinity and divalent metal ions may enhance tetracycline fluorescence, solutions of dilute ammonia and zinc acetate were added on occasions to the surface of pieces of tumor tissue but no enhancement of fluorescence was noted.

*Microscopic examination:* The bright yellow fluorescence observed macroscopically in 7 tumors also was located by ultraviolet microscopy. In 4 instances (cases 25, 26, 27 and 34) this was proved to be due to the presence of calcium salt crystals after the same microscopic fields were examined in polarized light when the crystals were shown to be birefringent. Adjacent sections stained with hematoxylin and by von Kossa's silver impregnation method also confirmed the nature of these deposits.

In contrast, the fluorescence in two cases (cases 16 and 36) was situated in tumor tissue itself. Although in case 36 there was extensive fluorescence, it was only present in the superficial part of the tumor; in case 16 the fluorescence was located in an area of superficial tumor necrosis from which tissue had been removed at a previous biopsy (case 3). In the remaining case (case 11) both calcium salt and tumor fluorescence were demonstrated in a few small areas of fibrinoid necrosis around small vessels.

Microscopic studies of all other tumors showed a weak blue fluorescence in the epithelial element and a weak green fluorescence in the stromal element. This fluorescence was the same regardless of whether or not the patient had received a tetracycline compound. No significant fluorescence was observed microscopically in those tumors which had given rise to a weak yellow-green fluorescence on macroscopic examination.

TABLE 4. Wavelengths of the Maximum Emissions of Tetracycline Compounds and Bladder Tissue Extracts on Excitation at 365 mμ

| Drug | Emission (mμ) | | | |
|---|---|---|---|---|
| | Major pH 5.6 | Lesser pH 5.6 | pH 7.4 | Addition of zinc acetate |
| Tetracycline | 550 | — | 460 | 525 |
| Methacycline | 475 | — | 460 | 540 |
| Bladder tumor tissue | 525 | 460 | 460 | No effect |
| Normal bladder mucosa | 525 | 460 | 460 | No effect |

Washing the cryostat sections in a saturated solution of zinc acetate in methanol did not enhance fluorescence; calcium salts were shown not to fluoresce in the absence of tetracycline administration (cases 1 and 5).

*Fluorescence spectrophotometry:* Extracts of 13 bladder tumors and of 2 specimens of normal bladder mucosa were examined by fluorescence spectrophotometry. Table 4 shows that the qualitative characteristics of the fluorescence of normal bladder mucosa, tumor tissue, tetracycline and methacycline are very similar under these in vitro conditions. The intensity of fluorescence was greater at pH 7.4 than at pH 5.6 for both the tetracycline compounds and for the tissue extracts.

The characteristics of the emission spectra of all the tumor extracts were remarkably similar. The ratio of the emission at 500 mμ to that at 460 mμ at pH 5.6, however, was greater for tumor tissue than for normal bladder mucosa. Addition of zinc acetate only enhanced the fluorescence of the tetracycline compounds and also induced a bathochromic shift in their emission spectra. The extraction technique, as expected, did not demonstrate the presence of tetracycline in the tumor tissue extracts.

These in vitro studies suggest that, within the pH range which would be expected in-vivo of the urine and tissues, autogenous tumor fluorescence mainly occurs at the same wavelength as that of tetracycline fluorescence when an exciting wavelength of 365 mμ is used.

## DISCUSSION

The results indicate that only the presence of bright yellow fluorescence definitely indi-

cates tetracycline fluorescence. Slight yellow-green fluorescence cannot be considered as specific to tetracycline retention since it also is found in tumors from patients who have not received tetracycline compounds. This view is supported by in vitro studies of tumor extracts which indicate that auto-fluorescence of tumor tissues is qualitatively similar to that of tetracycline compounds.

Visual observations, therefore, have provided definite proof of tetracycline retention in only 7 of 29 tumors; in only 3 of these, however, has the fluorescence been due to tumor tissue itself; in the other 4 it was due solely to calcium salt fluorescence.

These results differ from those of Whitmore et al.,[19] who described fluorescence, which they attributed to tetracycline, in 13 of 17 grossly evident bladder carcinomas. These authors, however, were unable to demonstrate this fluorescence on ultraviolet microscopy nor did they define the intensity of the observed fluorescence or distinguish between calcium salt and tissue fluorescence. Our cases certainly include a high proportion of well-differentiated carcinomas but the results do not suggest any definite relationship between fluorescence and tumor differentiation or necrosis. Our findings do not necessarily apply to in situ carcinoma of the bladder since we have studied only one such case.

Although ultraviolet endoscopy may provide better visualization of areas of bladder carcinoma than does incandescent light, our findings suggest that this may be at least in part attributable to the different fluorescent characteristics of normal and malignant bladder tissue and need not necessarily indicate tetracycline retention. It must be emphasized that these results only apply to bladder carcinomas and do not necessarily relate to the problems of tetracycline retention by tumors at other sites.

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