The modal chromosome number in the 50 papillary cancers did not differ materially from the original one, although a slight modification in the chromosome number may occur. On the other hand, invasive transitional cell cancer of the bladder was accompanied by a large number of chromosomes and a relatively large number of marker chromosomes with many complicated karyotypic pictures. The presence of marker chromosomes in papillary cancers may be indicative of the likelihood of recurrence and/or progression of such tumors and should attract the attention of those involved in the care of patients with these cancers.

Materials and Methods

Specimens of tissues were obtained by means of cystoscopic procedures or following cystectomy. In some cases, specimens from different areas of the tumor were processed for analysis; in others, samples from multiple lesions present in the bladder were examined, and in still others specimens were obtained on more than 1 occasion. Included in the present study are those patients who had been followed for a period of at most 3 years and for no less than 6 months.

The specimens were processed immediately after being obtained, and chromosome preparations were performed by 2 approaches. In one, the tissue was prepared by a direct technique previously described (5–8, 12), and in the other the minced tissue was incubated overnight in the presence of Colcemid. A few details will be given. The tissue was minced on a watch glass into small segments with a small scalpel, and the material was mixed with a hypotonic glucose solution [0.6% glucose in 0.7% sodium chloride:0.44% sodium citrate (1:1)], pipetted into a centrifuge tube, and allowed to stand for 2 to 5 min. The supernatant was removed and was incubated for 20 to 30 min at 37°. These procedures were performed in the presence of Colcemid, 10 ppm, in order to obtain well-contracted metaphases. After incubation, the cells were collected by centrifugation and fixed with 50% cold acetic acid (stored at 4°), and chromosome preparations were made. In the 2nd procedure, utilizing short-term culture (about 12 hr), the cells were incubated in Roswell Park Memorial Institute Tissue Culture Medium 1640 and then were harvested. Such a procedure yields a high number of metaphases suitable for analysis that do not appear to differ significantly from the karyotypes obtained in the tissues by a direct technique. The methods used in our laboratory for the preparation of chromosomal materials, photography, the presentation of karyotypic data, and the performance of G, Q, and C banding have been described previously (5–8, 12).
Results

Chromosomes of Normal Bladder Tissues. Examination of the chromosome counts of 75 specimens (52 male, 23 female) of normal bladder mucosa revealed the preponderant number (more than 90%) of the 757 metaphases observed to have 46 chromosomes with a diploid karyotype. The number of metaphases available for analysis varied from as few as 2 to as many as 50 following short-term incubation (6 to 8 hr) in the presence of Colcemid. The cells with less than 46 chromosomes, constituting no more than 5 to 8% of the metaphases of any single bladder specimen, were due to random loss of chromosomes.

Metaphases with a polyploid number of chromosomes, e.g., tetraploid cells with 92 chromosomes, were not observed in any of the specimens examined. Since it is possible that polyploid cells may not undergo mitosis and, hence, that metaphases with 92 or more chromosomes may not be available for observation, we examined interphase cells in the bladder specimens of the male patients using a quinacrine fluorescent technique (5). This method leads to high fluorescence of the Y chromosome, which can be readily identified as a single Y body in diploid interphase cells. No cells with 2 such fluorescent bodies were observed, which would be expected if polyploid cells were present in the male bladder specimens. Thus, these observations lend further support to the lack of polyploid cells in human bladder mucosa.

In contrast to the observations on elderly males, of whom a high percentage have a missing Y chromosome in their marrow cells (11), we could not find any significant number of cells with a missing Y chromosome in the bladder, including 22 specimens from males over the age of 65.

Noninvasive Papillary Tumors of the Bladder. A total of 62 noninvasive papillary tumors of the bladder has been examined. Of these, 12 were thought to be benign lesions (Chart 1). The number of metaphases available for examination in these 12 tumors was rather small (2 to 19 metaphases per tumor), a characteristic previously observed in other benign tumors (11); in the 107 metaphases examined, a preponderantly diploid chromosome constitution was found except for 2 cases in which some abnormal metaphases were present. In 1 case, 3 metaphases of 12 examined had a chromosomal number of 45. This tumor recurred and became invasive 11 months after the original observation, at which time all the cells had a chromosomal picture similar to that observed in the aneuploid cells of the original papillary tumor. In the 2nd case, 2 metaphases out of 14 were aneuploid, i.e., contained 47 chromosomes; these tumors recurred about 1.5 years later, was noninvasive, and was completely resectable. No recurrences have been seen in the other 10 benign tumors, with the time of follow-up ranging from 8 months to 3 years.

The 50 noninvasive papillary cancers had modal chromosome numbers in the diploid range (p = 44 to 49), but all were abnormal cytogenetically, either containing numerical deviations from the diploid number of 46 chromosomes, i.e., aneuploid, and/or morphologically abnormal chromosomes (markers) (Figs. 1 to 4; Chart 1). In contrast to the benign papillary tumors, the cancerous lesions usually yielded from a sufficient (18 metaphases) to a large number (more than 300 metaphases) of dividing cells for reliable karyotypic examination, with the average number of metaphases being about 50. Six papillary cancers were found to have a chromosome mode of 44 (3 with markers), 13 tumors had a mode of 45 (8 with markers), 16 had a mode of 46 chromosomes but with a pseudodiploid karyotypic picture (11 with markers), 9 had a mode of 47 chromosomes (6 with markers), 4 had a mode of 48 chromosomes (2 with markers), and 2 had a mode of 49 chromosomes (2 with markers). Thus, of the 50 papillary tumors examined, 32 had markers in a few (5%) to a large proportion (nearly 100%) of the cells examined. It appears, then, that marker chromosomes may appear frequently in papillary bladder cancers regardless of the chromosome count.

Although most of the papillary tumors had a definite modal number of chromosomes, the distribution of the number of chromosomes about the mode was decidedly different from that observed in benign tumors or normal tissues, in which the cells with 46 chromosomes constitute more than 90% of the metaphases (Chart 2) (11). Thus, in papillary tumors the metaphases with the modal number did not exceed 35% of the total cells and were as low as 19% in 1 of the tumors. Although the papillary tumors had modal numbers around the diploid range, no similarity in the chromosomal picture among the various papillary cancers was observed, with the karyotype varying from one case to another regardless of the chromosome count. Six of the papillary cancers (3 with markers) had multiple foci in the bladder; the karyotypic picture was very similar in the foci of...
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The application of various banding techniques in the present study has afforded a rigorous identification of not only the normal chromosomes in the tumors but also the genesis of markers. As stated above, the latter were present in 32 of the 50 papillary cancers examined, such markers being seen in 5% to nearly 100% of the metaphases analyzed in the tumors. In no case were more than 2 markers observed in a cell. This differs from the usual picture of a large number of markers seen in the bulk of the more advanced forms of cancer of the bladder (2, 4) (see below). Banding analysis revealed that the most common markers originated from chromosome 1 or involved a translocation of chromosomes 14, 17, and some of those in the C group.

Each tumor.

Of the papillary cancers recurred; 10 of these had markers, and 1 did not. The latter tumor had a chromosome mode of 48, and, when it recurred, the chromosome constitution differed only slightly from the original one, i.e., the chromosome mode that was 48 persisted but some cells with a near-tetraploid number of chromosomes were present. The recurrence of the tumor without markers occurred locally, whereas all the recurrent tumors with markers became invasive. In the 11 cases with markers and in whom the papillary cancers recurred, the karyotypic picture did not change materially in 6 cases; whereas in 5 cases near-tetraploid cells with more than 2 markers appeared, although the original chromosome constitution was observed in most of the cells. When deviations from the original picture in these tumors were seen, the chromosomal picture that emerged appeared to be a variation on the chromosomal picture of the original examination. Recurrence of the tumors took place within 6 months of the initial chromosome examination in 8 of the 11 cases with markers.

Transitional Cell Cancer of the Bladder. For comparison with the findings in papillary tumors, data are presented on 75 patients with invasive bladder cancer studied cytogenetically (Figs. 5 and 6). Karyotypic examination revealed, generally, a picture similar to that already published in the literature; i.e., relatively well-differentiated tumors with only local invasiveness were shown to have a near-diploid range of chromosomes, with the more advanced cancers having a near-triploid or tetraploid number of chromosomes (Chart 3) (2, 9). Of more importance, perhaps, was the appearance of an increased number of marker chromosomes with progression of the cancer, with 2 large markers of metacentric morphology being most common. These large markers have been shown with banding techniques to be probably of common origin (chromosome 1?) in most of the tumors investigated (Fig. 4). Thus, it appears that the evolution and progression of marker chromosomes may be a more important cytogenetic parameter to evaluate than either the chromosomal number or the numerical progression of the tumor. In a preliminary evaluation, the impression was gained that the response to radiation therapy was more readily obtained in tumors that had a relatively large number of marker chromosomes, usually 4 to 9 markers, than in those...
in which 0 to 2 markers were present. A correlation with chemo- and/or radiation therapy is somewhat premature at this time, since many of the patients are still on various courses of such therapy. We hope to have such a correlation within 2 to 3 years of this writing.

In 9 cases, a recurrence and spread of the disease took place, and in each case it was shown that the chromosomal picture did not deviate materially from the previous one and essentially represented a variation on the karyotypic theme of the original chromosomal pattern. All 9 tumors had 5 to 7 marker chromosomes. However, all 9 of these cancers of the bladder were very poorly differentiated and had already shown rather advanced disease when studied initially.

**Discussion**

Although cytogenetic studies in cancer of the bladder have not revealed a characteristic or specific chromosomal change, akin to the Ph' chromosome in chronic myelocytic leukemia (10, 11), the present findings, as well as those published in the recent literature (1–4), may prove to be of considerable value in the evaluation of cancer of the bladder, particularly of papillary lesions. Thus, the presence of marker chromosomes appears to endow papillary tumors with more likelihood of recurrence and/or invasiveness than those tumors that do not contain such markers. In the present study it was shown that only 1 of the 18 papillary cancers without markers recurred, whereas 11 of the 32 papillary cancers with markers recurred and became invasive.

Similar findings and conclusions have been reached in another study (1, 4). In a recent publication the cytogenetic data are presented on 27 well-differentiated noninvasive cancers of the bladder (4). Almost all the patients who had markers in their tumors (14 of 15) developed recurrences of the cancer, whereas only 1 of the 12 patients without markers in their tumors developed such recurrence. Eleven of the latter 12 patients have been recurrence free for 8 years. The authors believed that in early lesions of the bladder the presence of markers is "a highly accurate prognostic aid" (4).

It is possible during the genesis and progression of papillary cancer of the bladder that the initial chromosomal change is merely a deviation from the diploid number of chromosomes, followed by the appearance of abnormal marker chromosomes and, ultimately, by the development of a totally abnormal karyotypic picture consisting of chromosome numbers above the diploid range and the presence of a large number of marker chromosomes. It is possible that the genesis of this latter picture may be related to endoreduplication and/or nondisjunction in the original tumor cells or to some other processes leading to the complicated cytogenetic pictures. In any case, it would appear that the presence of marker chromosomes points to a definitely more serious lesion than their absence. Thus, it behooves clinicians to pay particular attention to the cytogenetic picture of papillary cancers, since the presence of marker chromosomes may point to a more complicated lesion requiring a more careful follow-up, with particular attention being paid to recurrence of such lesions. Recently, others have also pointed out that marker chromosomes have potential value as a prognostic aid in early cancer of the bladder and, furthermore, that the triad of tetraploidy, markers, and submucosal, invasive, moderately well-differentiated carcinoma appears to carry such a lethal prognosis as to mitigate for early radical resection (3). Thus, it appears that the chromosomal findings in cancer of the bladder, whether early or late, may prove to be of considerable value to those engaged in the evaluation, diagnosis, and therapy of these complicated lesions.

**References**

Chromosome Markers and Progression in Bladder Cancer

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