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Urinary Cytology in Workmen Engaged in the Petrochemical Industry with Reference to Non-Industrial Risk Factors

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Summary. Urine cytology was evaluated in 8,406 male workers of 8 petrochemical factories in western and northern Germany during the routine medical check-up performed by the department of industrial medicine of the respective factory. All relevant data referring to possible private and occupational risk factors were registered and evaluated. Four percent ($n = 358$) of the 8,406 workers examined exhibited Pap 3/4 cytology. Urological examination did not reveal any bladder tumor in those workers with either a single Pap 4 or a repeated Pap 3 finding on cytology. Our study showed that deterioration of cell differentiation correlated significantly with age and cigarette smoking. Furthermore, a risk group (males above 40 years of age exposed to occupational chemicals, smokers, and coffee drinkers) differed from a non-risk group. Age and cigarette smoking seemed to be the determinant factors. No correlation could be adduced between any kind of industrial exposure and urine cytology.

Key words: Urine cytology, Bladder cancer, Occupational chemicals, Age, Smoking, coffee.

Introduction

During the past decades, the incidence of urinary bladder tumor has increased worldwide [1, 2, 5, 24, 28, 35]. In the Federal Republic of Germany, the Federal Bureau for Statistics (Wiesbaden) registered an increase of 15% mortality in men with this tumor from 1970–1980. In the German Democratic Republic the incidence of urinary bladder cancer in men increased from 5/100,000 inhabitants in 1956 to 13/100,000 in 1976 [2].

Exogenous factors seem to be responsible for this increase [27]. Occupational exposure to carcinogenic substances has been suggested in the dyestuff, petrochemical, gasoline, and plastic industries [4, 8, 9, 11, 29, 31]. Although the significance of cigarette smoking in the development of bladder cancer has been established in epidemiologic studies [3, 8, 16, 22, 28, 38, 39, 43, 44], there is no study which relates the effect of cigarette smoking in combination with other industrial substances which may exhibit only weak carcinogenic activity. Hoffmann and Wynder [20] postulated that smoking acts as a co-factor in the carcinogenesis of bladder tumors.

Currently several case-control studies are being conducted to examine the influence of certain parameters in the development of bladder cancer [30]. These, however, are retrospective investigations comparing patients with existing tumors with matched controls. Although the value of urinary cytology as a screening method is considered to be high [12, 13], to our knowledge, there is no report using this method in epidemiological studies to examine occupational and other risk factors.

Our investigation was designed to answer the question whether workers employed in the petrochemical industry are at risk from bladder cancer. By examining occupational and known private risk factors, we tried to define a risk group in which screening would appear to be worthwhile.

Material and Methods

Our investigation[1] comprised a total of 9,248 male employees of the following 8 petrochemical factories located in western and northern Germany: 1. Rheinische Olefinwerke, Wesseling, 2. Degussa, Wesseling, 3. Hoechst, Knapsack, 4. Veba Oel, Gelsenkirchen, 5. Deutsche Shell, Godorf, 6. Chemische Werke Hüls, Marl, 7. Deutsche Shell, Hamburg, 8. Oelwerke Julius Schindler, Hamburg[2].

In the first two firms (No. 1 und 2), 4,475 male workers were submitted to an initial urine cytology test and to a control examination 12 months later. Final analysis (see below) consisted of 3,813

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Table 1. Distribution of urothelial cell differentiation of 3,1813 workers of factories No. 1 and 2 at the first and second cytological examination

Pap	first examination	second examination
1	2,026	2,046
2	1,678	1,662
3	109	105
4	–	–
total	3,813	3,813

cases. On the basis of these results it was considered that for the 4,773 employees of the remaining companies No. 3–8 one urine examination was sufficient. Final analysis (see below) of the second part of this study comprised 4,593 workers. All examinations were performed from March 1980 to March 1982 (No. 1 and 2) and from January 1982 to March 1983 (No. 3–8).

Urine specimens were collected during a routine medical examination performed by the physician in charge of the department of industrial medicine of each firm. Personal and epidemiologically relevant data were obtained from a questionnaire. Information relating to urological and non-urological diseases, drugs, cigarette smoking, coffee consumption, and different occupational exposure, were documented.

Processing of Urine Specimens

Approximately 20 ml of fresh voided urine was fixed with the same volume of Esposti fixative (10% acetic acid, 48% methanol, 42% dest. water) and stored at +4 °C. The specimens were further processed within one week. Preliminary studies in our laboratory revealed that using this method of fixation no impairment of the cytological diagnosis could be demonstrated. Two cytological smears were made from each urine aliquot using a cytocentrifuge. Staining was performed according to a modified Papanicolau technique [37].

Documentation and Evaluation

The data obtained by the physician in charge of each firm as well as the results of the cytological urinalysis were documented on forms for computer evaluation. All data were checked for errors and plausibility.

Only the results from workers who did not exhibit any anamnestical evidence of benign urological disease and/or leucocyturia due to urinary tract infection were considered. This selection was necessary since many benign urological diseases are associated with an atypia of urothelial cells. In accordance with the results of epidemiological investigations we defined a risk group as follows: male workers above 40 years who where exposed to one or more chemical substances, who smoked cigarettes and drank more than 4 cups of coffee daily. Conversely, a non-risk group consisted of workers showing none of these criteria.

For descriptive evaluation relative frequencies and cross table analysis were computed by use of the statistical programms SPSS and BMDP. In addition, relative frequencies were determined by the 95% confidence intervals. Non-overlapping confidence intervals were considered as evidence for relevant statistical differences. Cross tables were tested by X^2 analysis. Few parameters, i.e. the influence of the quantity of cigarette smoking and coffee consumption, could only be evaluated for the factories No. 1 and 2 because of the modified questionnaire for the factories 3–8. The other data which were obtained exactly in the same way for the factories 1–8 were evaluated together.

Results

For final analysis, 8,406 cytological reports were available for evaluation. In 95.8% of the examinations normal cytological findings (Pap 1/2) were obtained, in 4.2% (n = 358) we encountered atypical (n = for Pap 3) or pathological (n = 6 for Pap 4) results.

Data of the firms No. 1 and 2 revealed a very low correlation of urine cytology when repeated after one year. In one of 109 cases a Pap 3 reading was confirmed and in 54 cases the initial Pap 3 finding had changed either to Pap 1 or to a Pap 2.

Influence of Age

With increasing age, particularly beyond 60 years, a tendency towards Pap 3/4 findings was evident (Table 2). Comprising all age categories in two groups, workers below 45 years (n = 4,128) exhibited a statistically better urothelial cell differentiation with 3.1% Pap 3/4 readings compared to 6% in the group beyond 45 years (n = 4,278).

Influence of Cigarette Smoking

There is a statistically significant relation ($p < 0.05$) between cigarette smoking and urothelial cell differentiation. Of

Table 2. Urothelial cell differentiation in relation to the age of the workers (n = 8,406)

age (years)	–20	21–30	31–40	41–45	46–50	51–55	56–60	60
n	198	706	1,407	1,817	1,713	1,623	824	118
Pap 3/4	n = 5 (2.5%)	n = 13 (1.8%)	n = 40 (2.8%)	n = 70 (3.9%)	n = 69 (4.0%)	n = 99 (6.1%)	n = 71 (8.6%)	n = 18 (15.2%)

Table 3. Urothelial cell differentiation in relation to the smoking habits, all age groups (n = 8,406). Differences between both groups are statistically significant ($p < 0.05$)

	smokers	non-smokers
n	4,016	4,390
Pap 3/4	n = 162 (4.0%)	n = 132 (3.0%)

Table 4. Urothelial cell differentiation in relation to the age of smokers (n = 4,016). Differences between both groups are statistically significant ($p < 0.05$)

smokers	< 45 years	> 45 years
n	2,171	1,845
Pap 3/4	n = 63 (2.9%)	n = 98 (5.3%)

Table 5. Urothelial cell differentiation in relation to the duration of exposure to chemicals (n = 8,279)

Exposure (years)	n	Pap 3/4
none	1,685	n = 51 (3.0%)
1– 2	275	n = 10 (3.6%)
3– 5	1,316	n = 61 (4.6%)
6–10	1,469	n = 46 (3.1%)
> 10	3,534	n = 144 (4.1%)

4,390 non-smokers 3.0% showed an atypical cytology whereas this percentage was slightly but significantly higher in 4% of 4,016 smokers examined (Table 3). Separating the group of smokers according to their age below and above 45 years the older workers showed significantly worse cytological findings (Table 4). Evaluating only the duration of the smoking habit without consideration of the age, a trend towards a positive correlation with an increasing Pap 3/4 rate was noted. Quantitative evaluation of nicotine consumption according to Vutuc (no. of cigarettes/day x duration of smoking in years) [38, 39] which was only possible in the factories 1 and 2, did not reveal any relation to urinary cytology.

Influence of Coffee Consumption

Total examination failed to show any correlation between coffee drinking and urothelial cell differentiation. The same applies for the duration and/or quantity of coffee consumption (only factory 1 and 2).

Influence of the Residental Area

The place of residence, i.e. within or outside the industrial zone, revealed no significant differences: of 3,112 workers living in the industrial area 4.7% showed Pap 3/4 cytology whereas 4,117 men living outside in more rural environment only 3.8% showed Pap 3/4 findings. In 1,177 cases no clear answer was given with respect to the residence.

Influence of Occupational Exposure

The relation between exposure, duration and number of chemicals potentially harmful to the urothelium was examined. All the substances were grouped in the following 6 categories: heavy metals, aromatic hydrocarbons, aliphatic hydrocarbons, organic substances, highly condensed hydrocarbons, amines, and others. No correlation between exposure to any of these substances and the cytological differentiation could be corroborated with respect to either the duration (Table 5) or the kind (Table 6) of exposure. Even with respect to each of the documented 37 single chemicals to which workers were exposed no effect on urine cytology could be established.

Risk Group

720 men fulfilled the defined criteria of a risk group (above 40 years, smoker, coffee drinker, exposure of one or more occupational chemicals). This risk group exhibited a statistically significant higher rate of Pap 3/4 findings (4.7%) than those 1,204 workers without these risks (2.2%) (Table 7).

Table 6. Urothelial cell differentiation in relation to different groups of chemical exposure (single and predominant mixed exposures) (n = 7,859)

exposure	heavy metals	aromatic hydrocarbons	aliphatic hydrocarbons	anorg. substances	highly condens. hydrocarbons	amines	others
n	529	3,946	194	2,281	536	72	301
Pap 3/4	3.6%	4.8%	3.0%	3.3%	1.8%	2.8%	6.3%

Table 7. Distribution of urothelial cell differentiation in relation to groups of different risks (n = 8,406). Differences between the risk group and non-risk group are statistically significant ($p < 0.05$)

groups	all workers	risk group	non-risk group
n	8,406	720	1,204
Pap 3/4	n = 293 (3.5%)	n = 34 (4.7%)	n = 26 (2.2%)

Discussion

Evidence of multifactorial carcinogenesis has been accumulated on the basis of animal experiments. Generally, malignant transformation takes place in two steps, i.e. tumor initiation and promotion. These facts have already been confirmed for tumors of the skin [21, 36], the stomach [15], and the urinary bladder [7, 19, 25, 26, 27]. Furthermore, there are clear indications that weak or questionable carcinogenic substances can induce an urothelial carcinoma of the bladder in combination with a low dose of a strong carcinogen. Using this experimental design the following substances with weak carcinogenic potential on the urothelium were identified: chronic bladder inflammation [33], vitamin B_6 and A deficiency [6], saccharin and cyclamate [7, 18, 19, 32], tryptophane [7], cyclophosphamide [18], leupeptin [23], allopurinol [41], and indol [34]. Conversely, it was shown that neither caffeine acts as Co-carcinogen [32] nor do cigarette smoke condensates exhibit full carcinogenic properties in the rat [40]. Accumulated experimental data may lead to the conclusion that in man the development of bladder cancer is a multifactorial process.

Despite the convenience of urinary cytology for screening purpose and the high diagnostic sensitivity [12], reproducibility of our cytological screening in factory 1 and 2 was problematic. The extent of reversibility of the various grades of dysplasia or atypical hyperplasia is still controversial and poorly documented [10]. All workers with Pap 4 cytology and those with confirmed Pap 3 findings were submitted to an urological examination. In no case was a tumor diagnosed at that time.

Interpreting our results one has to acknowledge that without any Pap 5 finding only two groups of cytological categories were compared: the normal (Pap 1/2) and the abnormal Pap 3/4 readings. For that reason it is difficult to draw conclusions as to the premalignant potential of the urothelium exhibiting Pap 3/4 findings, because we did not perform long-term controls. The repeated cytological examination after one year (factory 1 and 2) did not indicate any tendency towards urothelial cell atypia. Benign and even asymptomatic urological disease can significantly alter the urothelial differentiation. Therefore, we have excluded those workers (n = 324) with a proven history of benign disease and/or significant leucocyturia.

The main question of our study was concerned with the risk of bladder cancer to workers employed in the petrochemical industry. Our results indicate that no risk exists: no urothelial tumor was diagnosed by combined cytological and urological examinations. Considering all the different chemicals, the number of substances exposed, the duration of exposure, and the area of residence, no statistically significant correlation between those factors and the urothelial cell differentiation could be established. Thus, our results do not conform to those of other investigators [8, 9, 11, 14, 31], who retrospectively examined patients with bladder tumors for possible risk factors during the past decades. Except for the different design of our study it should be noted that many known carcinogenic substances have been eliminated from industrial processes and awareness of industrial carcinogens has increased.

Other factors were found to correlate significantly with urothelial abnormalities indicated by Pap 3/4 readings.

With increasing age, the incidence of urothelial tumors becomes higher and reaches its peak between 60–70 years [35]. In our study, urothelial cell differentiation deteriorates with increasing age. We demonstrated a clear effect of cigarette smoking on urine cytology. This finding confirms reports on case-control-studies indicating a definite correlation between tabacco consumption and bladder cancer [3, 8, 16, 23, 28, 38, 39, 44]. Controversy, however, exists as to the question of a dose-effect relationship in this regard. Whereas Wynder and Goldsmith [44] found such a correlation, Hartege et al. [17] and our own study failed to confirm this. The fact that smokers over 45 years showed a higher incidence of urothelial abnormalities relates to the known age dependency of these changes rather than to the duration of smoking.

Coffee consumption did not show any effect on urinary cytology in our study. Thus, our results confirm the findings presented by Najem et al. [31] whereas other investigators came to opposite conclusions concerning coffee drinking [42].

By combining certain risk factors known from the literature, we defined a group of workers who exhibited a significantly worse cytology compared to a non-risk group. From our study design one cannot exclude that within this risk group age and tabacco use are the most predominant factors and the other parameters contribute only insignificantly to Pap 3/4 findings.

Therefore, the only risk group of note from an epidemiological point of view are the smokers over 45 years of age. Considering a screening of this particular group one should emphasize that such a project is technically feasible in cooperation with physicians engaged in industrial medicine. However, interpretation of urinary cytology with respect to the existence of precancerous lesions is difficult since definite results on the reversibility of Pap 3 findings (dysplasia) are missing. Long-term studies are necessary to answer this question.

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References

1. Adolphs H-D, Thiele J, Vahlensieck W (1980) Epidemiologie und Pathogenese der Harnblasenkarzinome. Onkologie 5:214
2. Berndt H, Berndt R (1982) Inzidenz und Mortalität an bösartigen Neubildungen der Harnorgane und der männlichen Geschlechtsorgane in der DDR. Urologe (A) 21:185
3. Cartwright RA, Adib R, Glashan R, Gray BK (1981) The epidemiology of bladder cancer in West Yorkshire. A preliminary report on nonoccupational aetiologies. Carcinogenesis 2:343
4. Case RAM, Hosker ME, McDonald DB, Pearson JT (1954) Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the british chemical industry. Br J Industr Med 11:75
5. Clemmesen J, Nielsen A (1956) Cancer incidence in Denmark 1943 to 1953. II. Tumors of the urinary system and prostate. Dan Med Bull 3:36
6. Cohen SM, Wittenberg JF, Bryan GT (1976) Effect of avitaminosis A and hypervitaminosis A on urinary bladder carcinogenicity of N-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide. Cancer Res 36:2334
7. Cohen SM, Arai M, Jacobs JB, Friedell GH (1979) Promoting effect of saccharin and DL-Tryptophan in urinary bladder carcinogenesis. Cancer Res 39:1207
8. Cole P, Monson RR, Haning H, Friedel GH (1971) Smoking and cancer of the lower urinary tract. N Engl J Med 284:129
9. Cole P (1973) Population based study of bladder cancer. In: Doll R, Vodopija I (eds) Host environment interactions in the etiology of cancer in man. 83. Lyon, France: International Agency for Research on Cancer
10. Coon JS, Pauli BU, Weinstein RS (1983) Precancer of the urinary bladder. Cancer Surveys 2:479
11. Davies JM, Sommerville M, Wallace DM (1976) Occupational bladder tumor cases identified during ten years interviewing of patients. Br J Urol 48:561
12. El-Bokainy MN (1980) Cytology of bladder carcinoma. J Urol 124:20
13. Glashan RW, Wijesinghe DP, Riley A (1981) The early changes in the development of bladder cancer in patients exposed to know industrial carcinogens. Br J Urol 53:571
14. Glashan RW, Cartwright RA (1981) Occupational bladder cancer and cigarette smoking in West Yorshire. Br J Urol 53:602
15. Goerttler K, Loehrke H, Schweizer J, Hesse B (1979) Systemic twostage carcinogenesis in the epithelium of the forestomach of mice using 7,12-dimentylbenz (a) anthracence as initiator and the phorbolester 12-0-tetradecanoyl-phorbol-13-acetate as promoter. Cancer Res 39:1293
16. Hammond EC, Horn D (1958) Smoking and death rates. Report on forty-four months of follow-up of 187,183 men. II. Death rates by cause. J Am Med Ass 166:1294
17. Hartege P, Hoover R, West DW, Lyon JL (1983) Coffee drinking and the risk of bladder cancer. J Natl Cancer Inst 70:1021
18. Hicks RM, Wakefield JSt, Chowaniec J (1975) Evaluation of a new model to detect bladder carcinogens of co-carcinogens; results obtained with saccharin, cyclamate and cyclophosamide. Chem Biol Interact 11:225
19. Hicks RM (1980) Multistage carcinogenesis in the rat urinary bladder-further investigation of the promotion phase. Tox Res Proj Direc 5:8801
20. Hoffmann D, Wynder EL (1976) Smoking and occupational cancers. Prev Med 5:245
21. Inoue T, Yoshimoto T, Ogura T, Hirao F (1981) Effect of nocardia rubra cell-wall skeleton treatment on tumor in two-stage chemical carcinogenesis of mouse skin. Cancer Immun 11:207
22. Kahn HA (1966) The Dorn study of smoking and mortality among United Staates veterans. Report on $8^1/_2$ years of observation. In: Epidemiological approaches to the study of cancer and other chronic diseases. Nat Cancer Inst Monogr 19:1 (Government Printing Office, Washington DC)
23. Kakizoe T, Esumi H, Kawachi T, Sugimura T, Takeuchi T, Umezawa H (1977) Further studies on the effect of leupeptin, a protease inhibitor, on induction of bladder tumors in rats by N-Butyl-N-(4-hydroxybutyl) nitrosamine. J Nat Cancer Inst 59:1503
24. Lopez-Abente G (1983) Bladder cancer in spain. Cancer 51:2367
25. Lower GM (1982) Concepts in causality: chemically induced human urinary bladder cancer. Cancer 49:1056
26. Matsushima M (1977) The role of L-tryptophan's promoting factor on tumorgenesis in the urinary bladder. II: Urinary bladder carcinogenicity of FANFT (initiating factor) and L-tryptophan (promoting factor) in mice. Nippon Hinyokika Gakkai Zasshi 68:731
27. Miyata Y, Bagaya K, Oyasu R (1981) Carcinogenesis in heterotopically-transplanted urinary bladder N-methyl-N-nitrosourea: a model for initiation and promotion. Carcinogeneses 2:495
28. Morrison AS, Cole P (1976) Epidemiology of bladder cancer. Urol Clin North Am 3:13
29. Morrison AS (1978) Cancer of the urinary bladder, epidemiology and aetiology. Urol Res 6:183
30. Muir CS, Wagner G (1980) Directory of on-going researchs in cancer epidemiology 1980. IARC (International agency for research on cancer), Lyon
31. Najem GR, Louria DB, Seebode JJ, Thind IS, Prusakowski JM, Ambrose RB, Fernicola AR (1985) Life time occupation, smoking, caffeine, saccharine, hair dey and bladder carcinogenesis. J Urol (in press)
32. Nakanishi K, Hirose M, Ogiso T, Hasegawa R, Arai MM, Ito N (1980) Effects of sodium saccharin and caffeine on the urinary bladder of rats treated with n-butyl-n-(4-hydroxybutyl) nitrosamine. Gann 71:490
33. Okajima E, Hiramatsu T, Motomiya Y, Kondo T, Hirao J (1973) Effects of foreign bodies on development of urinary bladder tumors in tats treated with n-tutyl-n-(3-hydroxybutyl) nitrosamine. Urol Res 1:77
34. Oyasu R, Kitajima T, Hopp LM, Sumie H (1972) Enhancement of urinary bladder tumorgenesis in hamsters by coadministration of o-acetylaminofluorene and indole. Cancer Res 32:2027
35. Schubert GE, Steinert M (1978) Das Harnblasenkarzinom im Biopsiegut der vergangenen 50 Jahre. Urologe A 17:361
36. Stenbaeck F (1980) Characteristics of neoplastic progression and tumor behaviour in skin carcinogenesis by different methods: an experimental study. Oncology 37:163
37. Voogt PJ de, Rahert P, Beyer-Boon MB (1979) Praxis der Urinzytologie. Springer, Berlin Heidelberg New York
38. Vutuc C, Kunze M (1979) Rauchgewohnheiten von Blasenkrebspatienten, Versuch zur Quantifizierung der Schadstoffexposition. Aktuel Urol 10:159
39. Vutuc C, Kunze M (1981) Quantitative Bestimmung der Zigarettenrauch-Kondensat-Exposition bei männlichen Blasenkarzinompatienten. Aktuel Urol 12:114
40. Wagle DG, Lee L (1973) Experimental bladder carcinogenesis using cigarette smoke condensate in the rate bladder. J Surg Oncol 5:197
41. Wang CY, Hayashida S, Pamukcu AM, Bryan GT (1976) Enhancing effect of allopurinol on the induction of bladder cancer in rats by N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide. Cancer Res 36:1551
42. Weinberg DM, Ross RK, Mack TM, Paganini-Hill A, Henderson BE (1983) Bladder cancer etiology, a different perspective. Cancer 51:675
43. Weir JM, Dunn JE Jr (1970) Smoking and mortality. A prospective study. Cancer 25:105
44. Wynder EL, Glodsmith R (1977) The epidemiology of bladder cancer. A second look. Cancer 40:1246

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