Erythrocyturia, smoking, and occupation

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SUMMARY In a population of 432 apparently healthy men aged 50 years and over 19.2% had one or more erythrocytes per high-power field in the urine while 8.1% had more than 10. Erythrocyturia seemed to be strongly correlated with tobacco consumption. No significant association with occupation could be demonstrated but the data were insufficient to refute the possibility of such an association. Raised levels of orthoaminophenols as a result of abnormal tryptophan metabolism induced by smoking might cause microhaematuria in smokers. As these metabolites are carcinogens microhaematuria might be a valuable early sign of cancer of the urinary bladder.

Microhaematuria occurs in widely differing diseases ranging from lesions of the kidney to inflammation and tumour of the lower urinary tract. Cancer of the bladder has attracted our special interest because its incidence in industrialised countries is increasing rapidly (Clemmesen, 1974).

According to Leader and Stell (1963) at least 20% of patients presenting with painless haematuria are likely to have a neoplasm somewhere in the urinary tract. Wallace and Harris (1965) emphasised that microscopical or gross haematuria was the earliest and commonest sign of bladder cancer. The clinical records of 110 consecutive patients with urothelial tumours in the St. Lucas Hospital, Amsterdam, noted both gross and microscopical haematuria in all but three cases (unpublished data).

We therefore studied the incidence of erythrocyturia during a population survey for bladder cancer among the apparently healthy male population of two villages in an industrialised area north of Amsterdam. Since a causal relationship has been noted between smoking and occupation and cancer of the bladder we also investigated the men's smoking habits and their occupation. This paper reports the results of the investigation.

Materials and methods

The investigations carried out in the survey included electrocardiogram, basic blood chemistry, analysis of urine, and cytological examination of the urinary sediment. X-ray examination of the lungs had been

performed in an earlier survey. Participation in the cytology project was voluntary, free of charge, and limited to men aged 50 and over.

Early morning specimens of urine were centrifuged in 40-ml pointed tubes for nine minutes at 1230 *g*. The supernatant was carefully aspirated with a vacuum-connected Pasteur pipette, inserted just beneath the surface, until about 0.5 ml fluid remained. After resuspending the sediment the content was carefully transferred on to two albumen-coated slides per sample. The slides were stained by the Papanicolaou method and then screened microscopically for cytological abnormalities. The presence of erythrocytes and leucocytes was recorded in numbers per high power field (RBC/hpf and WBC/hpf). Current routine methods of preparing urinary sediments were adapted to meet the requirements of mass screening for cancer of the bladder (Freni *et al.*, 1977; Freni and Freni-Titulaer, 1977).

The amount of microhaematuria was classified within arbitrary limits—occasional (< 1 RBC/hpf), slight (1–10 RBC/hpf), moderate (11–50 RBC/hpf), severe (51–100 RBC/hpf), and very severe (> 100 RBC/hpf).

The amount of tobacco consumed was classified as follows: A—7 or more packets of cigarettes/week; B—3 to fewer than 7 packets of cigarettes/week; C—fewer than 3 packets of cigarettes/week; D—pipe or cigar only; E—stopped smoking 1 to 3 years previously; F—stopped smoking for more than 3 years; G—never smoked; H—smoking habit not known. Those who smoked a pipe or cigars and cigarettes were classified in the corresponding cigarette group.

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The occupations were classified as follows: I—workers in chemical industry; II—workers in food industry; III—workers in other industries; IV—printers; V—painters; VI—farmers and fishermen; VII—independent technical workers (for example, welders, engineers, electricians); VIII—workers in the building trade, except painters; IX—non-technical occupations (for example, salesmen, bakers, administrative staff); X—occupation unknown.

Results

Out of a total of 1061 men aged 50 and over, 432 (40.7%) participated in the trial. Erythrocytes were found in all urine samples—occasional RBC in 80.8%, 1–10 RBC/hpf in 11.1%, and more than 10 RBC/hpf in 8.1%. There were 219 cigarette smokers, 50 pipe and/or cigar smokers, 102 ex-smokers, and 22 non-smokers. No information on smoking was obtained from 39 participants.

The degree of erythrocyturia appeared to be unrelated to age (Table 1) but a highly significant difference was found between smokers (groups A, B, C, D) and non-smokers plus ex-smokers (groups E, F, G) in relation to erythrocyturia of ≥ 1 RBC/hpf ($\chi^2 = 13.719$, $P < 0.001$) and > 10 RBC/hpf ($\chi^2 = 8.422$, $P < 0.004$) (Table 2). No gradient in proportion with 1–10 RBC/hpf was found from light to heavy cigarette smokers (Fig. 1). A gradient, however, was present on the level of ≥ 1 RBC/hpf

Table 1 Erythrocyturia in relation to age

Age (yr)	No. of men	No. (%) with erythrocyturia of:	
		≥ 1 RBC/hpf	> 10 RBC/hpf
50–54	141	21 (14.9)	6 (4.3)
55–59	105	23 (21.9)	11 (10.5)
60–64	89	14 (15.7)	6 (6.7)
65–69	47	11 (23.4)	6 (12.8)
70–74	32	11 (34.4)	6 (18.8)
75–79	9	1 (11.1)	—
≥ 80	9	2 (22.2)	—
Total	432	83 (19.2)	35 (8.1)

Table 2 Erythrocyturia in relation to smoking

Smoker group	No. of men	No. (%) with erythrocyturia of:	
		≥ 1 RBC/hpf	> 10 RBC/hpf
A	40	12 (30.0)	5 (12.5)
B	130	34 (26.2)	16 (12.3)
C	49	12 (24.5)	3 (6.1)
D	50	9 (18.0)	6 (12.0)
E	20	2 (10.0)	1 (5.0)
F	82	9 (11.0)	2 (2.4)
G	22	—	—
H	39	5 (12.8)	2 (5.1)
Total	432	83 (19.2)	35 (8.1)

Fig. 1 Smoking and microhaematuria. Letters and figures in left-hand column represent types of smokers (see 'Material and methods') and number of subjects per group. Other figures are percentages per smoker group in relation to degree of erythrocyturia.

Fig. 2 Occupation and microhaematuria. Figures in left-hand column represent various occupations (see 'Material and methods') and number of men per group.

and > 10 RBC/hpf from ex-smokers (groups E, F) via light to heavy cigarette smokers (Fig. 1). This is illustrated by the significant difference found between ex-smokers and cigarette smokers with regard to microhaematuria of either ≥ 1 RBC/hpf ($\chi^2 = 9.164$, $P < 0.001$) or > 10 RBC/hpf ($\chi^2 = 5.805$, $P < 0.02$).

No clear relation was found between occupation and microhaematuria (Fig. 2). However, if the non-exposed subjects (groups II, VII, VIII, IX) could be considered representative for a population with a normally existing risk of non-industrial microhaematuria-inducing factors an adapted χ^2 test (with the frequency distribution within the non-exposed groups as a fixed norm) might be applied. In this case the 'exposed' groups (I, III, IV, V) appeared to differ significantly from the norm in

relation to microhaematuria of 1–10 and > 10 RBC/hpf ($\chi^2(2) = 8.198, P < 0.02$).

Discussion

A participation rate of 40.7% of those eligible is normal for a voluntary population survey, according to records of the Central Bureau for Medical Examinations, The Hague. This bureau organised our survey. None but personal reasons could be found for non-participation. The survey was well publicised by local authorities and family physicians in personal letters to the inhabitants and by repeated announcements in the local newspapers. Therefore we believe that the participants may be considered to be a large and representative sample of the male population of the two villages aged 50 and over.

Erythrocytes were found in all samples, while in 19.2% more than one RBC/hpf was present. This differs largely from the observations of others. Wright (1959) reported no blood at all in 78.4% and an occasional RBC in 12.8%. Sanders (1963) reported no RBC in 25.6% and an occasional RBC in 44.1%. Rofe (1955) found RBC in all his samples, but the maximum daily blood loss was estimated at only 312 000 RBC, roughly corresponding to 1 RBC/hpf if the samples had been examined by our technique (Freni *et al.*, 1977). This low value and the small number of 12 men examined make it difficult to interpret Rofe's results. In our opinion, the frequency with which other investigators failed to detect any RBC in the urine of apparently healthy men should be attributed to less efficient ways of transferring the cellular content of the samples on to the slides and to the examination of wet and unstained slides (Freni *et al.*, 1977).

The unexpectedly large number of 83 men with ≥ 1 RBC/hpf in the urine offered the opportunity to investigate the possible influence of age, occupation, and smoking on microhaematuria. The absence of a detectable age effect might indicate that it occurs much earlier in life. A second survey is therefore planned which will include men of 30 to 50 years.

An occupational influence on microhaematuria was not distinct among the subgroups. This is not due to differences in smoking habits, since the distribution of smoking habits within these subgroups seemed to be fairly comparable. The result of the adapted χ^2 test ($P < 0.02$), however, suggests that industrial workers might be exposed to more microhaematuria-inducing factors than other workers. A more detailed exposure history, further subdividing the occupation groups, and much larger numbers of workers per group would be needed to trace possible occupational high risk groups. The farmers, with their rather high incidence of > 10 RBC/hpf, were

not matched as we were unable to assess their contact with pesticides and other chemicals. The strikingly high incidence of microhaematuria of > 10 RBC/hpf among painters is interesting, although the group is too small for statistical evaluation. This might form a link with the high incidence of bladder cancer among dye workers (Wynder *et al.*, 1963; Anthony and Thomas, 1970).

The most surprising feature in our results is the highly significant relation between smoking and erythrocyturia. Clinicians generally regard the upper limit of 'physiological' microhaematuria to be about 2 to 5 RBC/hpf. Owing to the greater sensitivity of our method of measuring erythrocyturia compared with routine analysis of urine we tentatively set the lower limit for 'abnormal' erythrocyturia at 10 RBC/hpf (Freni *et al.*, 1977). This explains why a gradient in proportion with > 10 RBC/hpf can be seen from ex-smokers via light to heavy cigarette smokers, but not on the level of 1–10 RBC/hpf. It must be clearly understood that in future studies the limit of 10 RBC/hpf is not valid for other methods of preparation. Moreover, in our opinion, based on the preliminary results of the clinical check-up of our patients, that for population studies the lower limit for abnormal erythrocyturia might be set at a higher level. The number of subjects with more than 50 RBC/hpf, however, is still too small for statistical evaluation.

It became obvious from detailed analysis that the group of pipe and/or cigar smokers was heterogeneous. We were unable to measure the amount of tobacco consumed. In many cases the participants inhaled when smoking. Others reported that they had recently changed from cigarettes to pipe or cigar. Clearly such a group cannot be matched with cigarette smokers in respect of the quantity of tobacco consumed.

A causal relation between smoking and occupation and cancer of the bladder has been reported (Wynder *et al.*, 1963; Clemmesen, 1974). Carcinogenic agents, both exogenous and endogenous, may be assumed primarily to cause haemorrhagic cystitis with haematuria, as do certain chemicals such as ortho-toluidine. Continuous exposure to the agents might lead to metaplasia and hyperplasia of the mucosa and ultimately, in extreme cases, to the development of cancer.

Kerr *et al.* (1965) reported raised levels of 3-OH kynurenine and 3-OH anthranilic acid as a result of an abnormal tryptophan metabolism in smokers. These orthoaminophenols, known to be carcinogenic (Kerr *et al.*, 1965), might be responsible for the microhaematuria in apparently healthy men which we found exclusively in smokers and ex-smokers. Raised levels of abnormal tryptophan metabolites

have also been found in cases of pyridoxine deficiency (Karlson, 1972), and borderline pyridoxine deficiency is considered to be fairly common in Western populations. A combination of this deficiency and smoking could have an augmenting effect. Further study of this would seem worthwhile.

Cytological evidence of 'cystitis/pyelitis' (more than 25 WBC/hpf) was present in 26 of our cases and of pyuria (WBC forming a closed layer on the slides) in seven. In five samples abnormal cells were found (strong atypia, tumour cannot be excluded, Papanicolaou class III). All these patients and those with microhaematuria of more than 50 RBC/hpf are now under urological surveillance. The outcome will be reported in due course.

Simple methods for detecting microhaematuria are available, but great care is needed in methods for measuring haematuria which employ centrifugation. Preliminary results of trials of a stick reaction (based on the orthotoluidine-peroxidase reaction) are promising (Freni *et al.*, 1977). The method is simple, quick, and inexpensive and therefore very suitable for use in population studies. Further large-scale investigations with two brands of the stick are now in progress.

References

- Anthony, H. M. and Thomas, G. M. (1970). Tumors of the urinary bladder: an analysis of the occupations of 1,030 patients in Leeds, England. *J. nat. Cancer Inst.*, **45**, 879-895.
- Clemmesen, J. (1974). Statistical studies in the aetiology of malignant neoplasms. IV. *Acta path. microbiol. scand.*, [A], Supplement, 247.
- Freni, S. C. and Freni-Titulaer, L. W. J. (1977). Microhaematuria found by mass screening of apparently healthy males. *Acta Cytol.*, (In press).
- Freni, S. C., Heederik, C. J., and Hol, C. (1977). Centrifugation techniques and reagent strips in the assessment of microhaematuria. *J. clin. Path.*, **30**, 336-340.
- Karlson, P. (1972). *Kurzes Lehrbuch der Biochemie*, 8th edition, p. 360. Georg Thieme, Stuttgart.
- Kerr, W. K., Barkin, M., Levers, P. E., Woo, S. K. C., and Menczyk, Z. (1965). The effect of cigarette smoking on bladder carcinogens in man. *Canad. med. Ass. J.*, **93**, 1-7.
- Leader, A. J. and Stell, R. B. (1963). Urologic diagnosis and the urologic examination. In *Urology*, edited by M. F. Campbell, 2nd ed., vol. I, pp. 1027-1100. Saunders, Philadelphia and London.
- Rofe, P. (1955). The cells of normal human urine. *J. clin. Path.*, **8**, 25-31.
- Sanders, C. (1963). Clinical urine examination and the incidence of microscopic haematuria in apparently normal males. *Practitioner*, **191**, 192-197.
- Wallace, D. M. and Harris, D. L. (1965). Delay in treating bladder tumours. *Lancet*, **2**, 332-334.
- Wright, W. T. (1959). Cell counts in urine. *Arch. intern. Med.*, **103**, 76-78.
- Wynder, E. L., Onderdonk, J., and Mantel, N. (1963). An epidemiological investigation of cancer of the bladder. *Cancer (Philad.)*, **16**, 1388-1407.