Association of 1-hydroxypyrene-glucuronide in human urine with cigarette smoking and broiled or roasted meat consumption

Pornchai Sithisarankul, Paolo Vineis, Daehee Kang, Nathaniel Rothman, Neil Caporaso and Paul Strickland

Humans are exposed to polycyclic aromatic hydrocarbons (PAHs) from various occupational, dietary, environmental and medicinal sources. We measured 1-hydroxypyrene-glucuronide (1-OHP-gluc) concentration in urines from male non-smokers ($n = 50$), smokers of blond tobacco ($n = 31$), smokers of black tobacco ($n = 16$), and pipe smokers ($n = 3$). Immunoaffinity chromatography was used as a preparative step and synchronous fluorescence spectroscopy as the quantitation method. The concentration of 1-OHP-gluc in urine from smokers (mean ± SE: 1.04 ± 0.13 pmol ml^{-1} urine) was significantly higher than in urine from non-smokers (0.55 ± 0.05 pmol ml^{-1} urine) by the Wilcoxon rank sum test (non-smokers versus all smokers, $p = 0.001$; vs black-tobacco smokers, $p = 0.001$; vs blond-tobacco smokers, $p = 0.007$). Urinary 1-OHP-gluc concentration among subjects who had consumed roasted, grilled or broiled meat within the past 24 h was elevated compared with those who had not ($p = 0.025$). Multiple linear regression showed significant associations of urinary 1-OHP-gluc with number of cigarettes smoked ($p = 0.002$) and consumption of roasted, grilled or broiled meat ($p = 0.028$). Systemic CYP1A2 activity estimated by caffeine metabolism was significantly correlated with urinary 1-OHP-gluc concentration. However, this association was probably due to cigarette smoking, since adjusting for cigarette smoking by multiple linear regression made the association between urinary 1-OHP-gluc and CYP1A2 phenotype non-significant. These results further support the use of urinary 1-OHP-gluc as a biomarker of recent pyrene exposure through inhalation or diet.

Keywords: *1-hydroxypyrene-glucuronide, smoking, meat consumption, biomarker.*

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are commonly formed during the combustion of organic materials. Major sources of PAH exposure in much of the population are diet and cigarette smoke (Lijinsky and Shubik 1964, Baum 1978, Sontag 1981, Fazio and Howard 1983, IARC 1983, WHO 1984, Bjorseth and Becher 1986, Lioy *et al.* 1988). The measurement of PAH metabolites in urine is a potential means of assessing recent exposure to these compounds (Jongeneelen *et al.* 1990), and urinary 1-hydroxypyrene concentration is sometimes used as an index biomarker of exposure to mixed PAHs. We have recently shown that the major pyrene metabolite excreted in human urine is 1-hydroxypyrene-glucuronide (1-OHP-gluc) (Strickland *et al.* 1994). This metabolite of pyrene can be measured as free 1-OHP after deconjugation using β-glucuronidase, or directly as 1-OHP-gluc without deconjugation.

Elevated levels of urinary 1-OHP or 1-OHP-gluc have been demonstrated in smokers (vs non-smokers), in patients receiving coal tar treatment (vs pre-treatment), in post-shift road pavers (vs pre-shift or vs controls), in post-shift coke oven workers (vs pre-shift), and in subjects ingesting charbroiled meat (vs pre-ingestion) (Jongeneelen *et al.* 1985, 1986, 1987, 1988, Tolos *et al.* 1990, Buckley and Lioy 1992, Sherson *et al.* 1992, Kang *et al.* 1995a, b). A study of subjects with no occupational exposure to PAHs indicated that smoking and dietary PAH were the major sources of interindividual variability in 1-OHP excretion (Van Rooij *et al.* 1994).

In order to evaluate the association between urinary 1-OHP-gluc and amount of cigarette smoking, we quantitated 1-OHP-gluc in urine samples from male smokers and non-smokers who did not have significant occupational PAH exposure. We also examined the effect of diet-related PAH exposure and CYP1A2 phenotype on urinary 1-OHP-gluc concentration, since CYP1A2 is induced in smokers (Sherson *et al.* 1992) and in subjects who have recently consumed grilled meat (Conney *et al.* 1977).

MATERIALS AND METHODS

Description of study population

The urine samples analysed were provided from a study of smoking-related biomarkers in 100 healthy male blood donors, aged 45–64 years, living in Italy (Bartsch *et al.* 1990, Vineis *et al.* 1990). Relevant demographic variables, type of tobacco smoked, dietary data and CYP1A2 phenotype based on caffeine metabolite ratio (Butler *et al.* 1992) were available for the study subjects. A questionnaire completed by the study subjects provided information on type and amount of cigarettes smoked (number per day, pack-years, total years), consumption of meat in the 24 h prior to sample collection, and methods used to cook the meat consumed (boiled, baked, grilled, fried, roasted, etc.).

Fifty subjects were non-smokers, 31 were blond (flue-cured) tobacco smokers, 16 were black (air-cured) tobacco smokers, and three were pipe smokers. Seventy-four subjects did not consume any roasted, grilled, charbroiled or fried meat in the 24 h prior to urine collection, whereas 24 subjects consumed such meat once, and two subjects consumed such meat twice. The urine samples were stored at –70 °C until analysis of coded aliquots

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Assay for 1-hydroxypyrene-glucuronide

1-OHP-gluc in urine was quantitated using a recently developed assay (Strickland *et al.* 1994). Urine samples (2 ml) were treated with 2N HCl (90°C) to hydrolyse acid-labile metabolites and pre-purified on Sep-Pak C18 cartridges (Waters). Samples were then purified on immunoaffinity chromatography columns filled with CNBr-activated sepharose 4B (Sigma) coupled with monoclonal antibody 8E11 which recognizes several PAH adducts and metabolites (Santella *et al.* 1984, Weston and Bowman 1991). This antibody was originally selected to recognize benzo[*a*]pyrene–DNA adducts, but was subsequently shown to cross-react with 1-OHP-gluc (Strickland *et al.* 1994); it does not recognize unconjugated 1-hydroxypyrene (unpublished). Purified samples were reduced in volume by evaporation and analysed by synchronous fluorescence spectroscopy (Perkin-Elmer LS50), as previously described (Strickland *et al.* 1994). The coefficient of variation of the assay was 8–10% during the period of sample analysis; the limit of detection was 0.04 pmol ml^{-1} with assay recovery of 85–95%. A subset of samples was further purified by HPLC followed by SFS analysis of individual fractions to confirm the identity of the fluorophore, as described by Strickland *et al.* (1994).

Statistical analysis

All statistical analyses were performed using SAS software package version 6.07 (SAS Institute, Cary, NC). Non-detectable samples were assigned a value at the limit of detection (0.04 pmol ml^{-1} urine). Untransformed data were analysed according to type of tobacco smoked by the Wilcoxon rank sum test. Multiple linear regression was used to assess the relationship between urinary 1-OHP-gluc and number of cigarettes smoked daily or dietary PAH exposure estimated from recent consumption of roasted, grilled or broiled meat.

Results

Pyrene-specific fluorescence associated with 1-OHP-gluc was detectable in 97 of 100 study subjects. As in previous studies, samples assayed by SFS gave comparable quantitative results when further purified by HPLC (not shown). The urinary 1-OHP-gluc concentration among non-smokers, all smokers, blond-tobacco smokers, and black-tobacco smokers was (mean ± SE) 0.55 ± 0.05, 1.04 ± 0.13, 1.00 ± 0.15, and 1.12 ± 0.23 pmol ml^{-1} urine, respectively (Table 1, Figure 1). The concentration among smokers was significantly higher than among non-smokers by the Wilcoxon rank sum test: all smokers versus non-smokers, $p = 0.001$; blond-tobacco smokers vs non-smokers, $p = 0.069$; black-tobacco smokers vs non-smokers, $p = 0.0009$. A significant dose–response relationship was observed between number of cigarettes smoked daily (range: 0–90) and log urinary 1-OHP-gluc concentration ($r = 0.32$, $p = 0.0013$ by linear regression) (Figure 2). Subjects who reported possible passive smoke exposure or possible occupational PAH exposure did not show increased urinary 1-OHP-gluc concentration (data not shown).

Subjects who had consumed roasted, grilled, or broiled meat in the 24 h prior to urine collection had higher urinary 1-OHP-gluc concentrations (1.02 ± 0.20 pmol ml^{-1}, n=26) than subjects not consuming meat cooked by those methods (0.70 ± 0.06 pmol ml^{-1}, n = 71) (p = 0.04 by t-test). No significant difference was observed between subjects who consumed meat cooked by other methods and subjects who consumed no meat (category 1 vs category 0 in Figure 3). CYP1A2 phenotype ratios ([1,7-dimethylxanthine+1,7-dimethyluric acid]/caffeine) had been previously determined (Vineis *et al.* 1990) on the urine samples used in this study. When analysed as a continuous variable, phenotype ratio was correlated with urinary 1-OHP-

	n	Mean	SE	Median	Range	*p*-value[a]
Non-smokers	50	0.55	0.05	0.46	0.04–1.81	Reference
All smokers[b]	47	1.04	0.13	0.90	0.04–4.26	0.001
Blond tobacco	31	1.00	0.15	0.87	0.04–3.41	0.007
Black tobacco	16	1.12	0.23	0.96	0.34–4.26	0.001

Table 1. Concentration of 1-hydroxypyrene-glucuronide in urine (pmol ml^{-1}) by smoking status.

[a] Wilcoxon rank-sum test versus non-smokers.

[b] Three pipe smokers excluded.

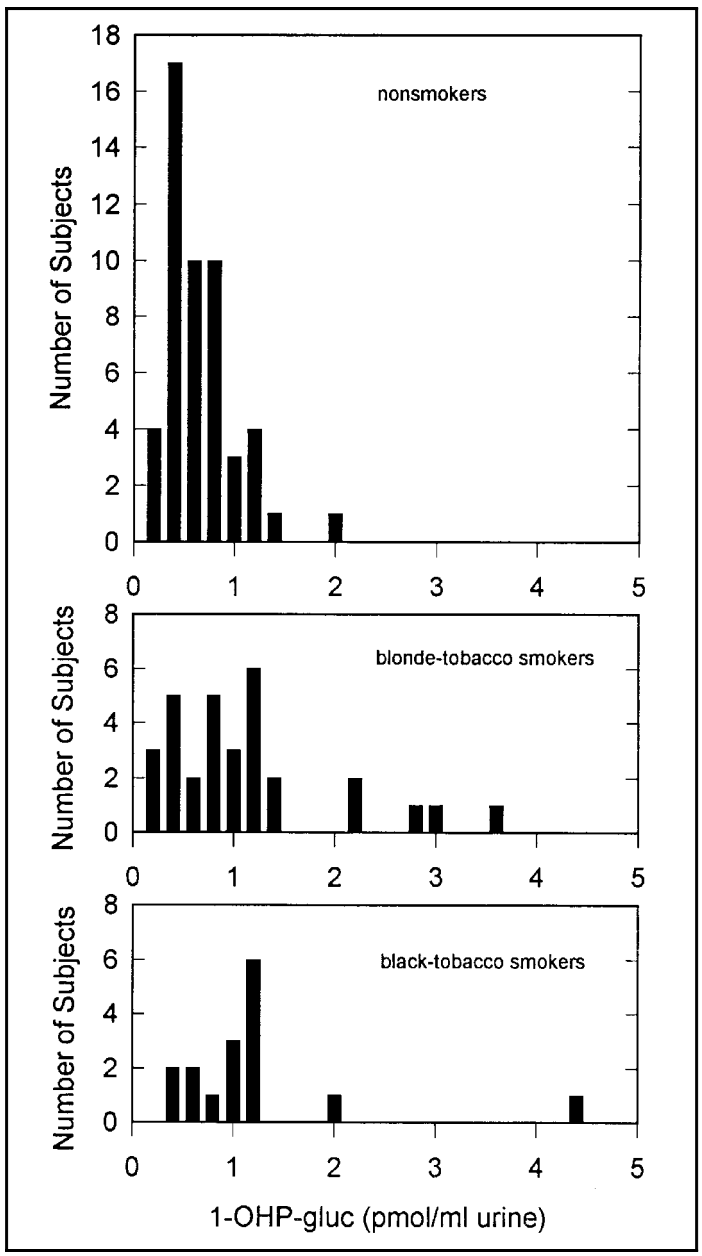

Figure 1. Frequency distribution of urinary 1-OHP-gluc concentration from non-smokers (n = 50), blond-tobacco smokers (n = 31), and black-tobacco smokers (n = 16). Three pipe smokers were excluded fro

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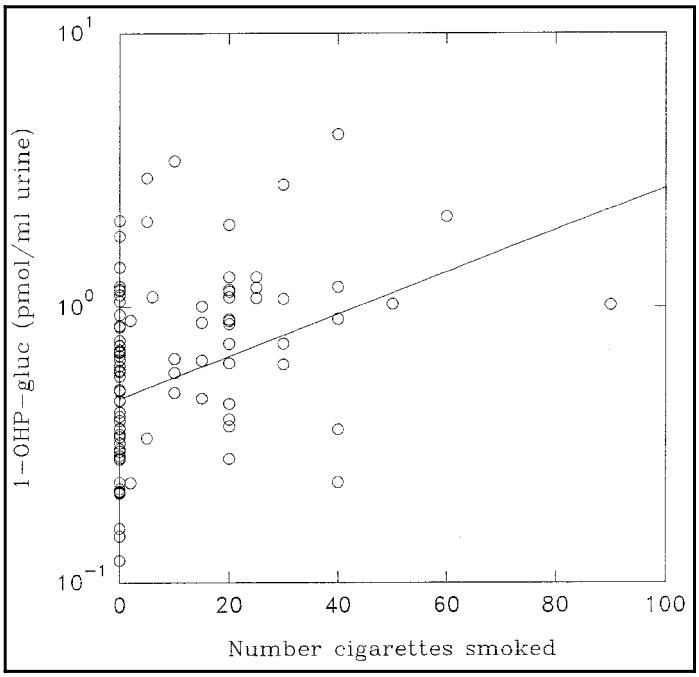

Figure 2. Scatter plot of number of cigarettes smoked daily vs log concentration of 1-OHP-gluc in urine. Regression line is: $y = e^{0.018x-0.77}$ ($r = 0.32$, $p = 0.0013$, $n = 97$).

gluc ($r = 0.23$, $p = 0.023$, $n = 97$); however, the correlation became non-significant after adjustment for number of cigarettes smoked and consumption of roasted, grilled or broiled meat by multiple linear regression (not shown). The best fit multiple linear regression model (Table 2) indicated that number of cigarettes smoked ($p = 0.002$) and consumption of roasted, grilled or broiled meat ($p = 0.028$) were significant and positive predictors of urinary 1-OHP-gluc.

Discussion

The concentration of PAH metabolites in urine is a potential indicator of recent exposure or internal dose of PAHs. The major metabolite of pyrene found in human urine, 1-OHP-gluc (Strickland *et al.* 1994, Singh *et al.* 1995), is increased in subjects exposed to mixtures of PAHs containing pyrene. We found higher levels of 1-OHP-gluc in urine from smokers of blond or black tobacco than from non-smokers (Table 1). There was no significant difference in the urinary concentration of 1-OHP-gluc between smokers of blond versus black tobacco. Previous reports indicate that the arylamine adduct, 4-aminobiphenyl–haemoglobin, is elevated in the black-tobacco smokers relative to the blond-tobacco smokers (Bartsch *et al.* 1990). Although black tobacco contains higher levels of aromatic amines than blond tobacco (IARC 1986), relative levels of PAHs in blond and black tobacco have not been reported.

The number of cigarettes smoked daily by study subjects was correlated with urinary 1-OHP-gluc concentration. Non-smokers had detectable urinary-1-OHP-gluc, suggesting that other sources of PAH exposure were present (e.g. diet, occupation, passive smoke, etc.). Urinary 1-OHP-gluc was found to be elevated in those subjects that had ingested

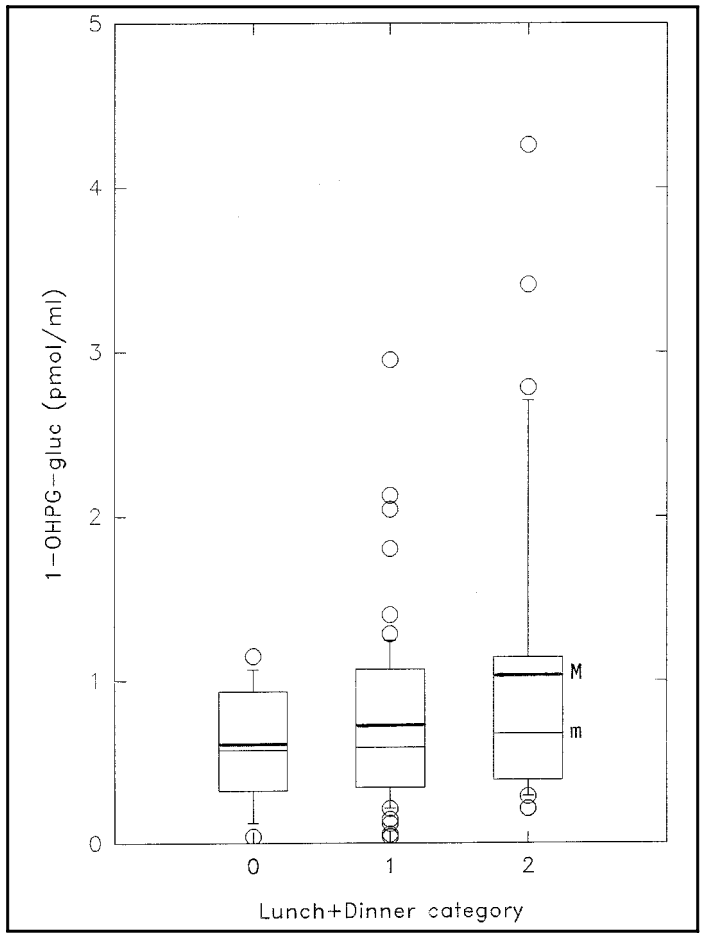

Figure 3. Box and whisker plot of urinary 1-OHP-gluc (pmol ml^{-1}) by cooked meat consumption in previous 24 h. Category 0: consumed no meat ($n = 12$; 4 smokers, 8 non-smokers); category 1: consumed only meat cooked by methods other than roasting, grilling or broiling ($n = 59$; 28 smokers, 31 non-smokers); category 2: consumed roasted, grilled or broiled meat ($n = 26$; 15 smokers, 11 non-smokers). m = median; M = mean.

roasted, broiled or grilled meat in the previous 24 h. Previous reports of controlled feeding studies have shown a strong association between urinary 1-OHP-gluc and recent consumption of charbroiled meat (Buckley and Lioy 1992, Kang *et al.* 1995b). This is the first report of such an association in an observational study with no manipulation of diet. The association was observed in spite of the crude assessment of dietary PAH intake. For example, there are PAHs

Variable	DF	β Estimate	β SE	*p*-value[a]
Intercept	1	0.38	0.14	0.007
Smoking[b]	1	0.01	0.004	0.002
Meat consumption[c]	1	0.23	0.10	0.028

Table 2. Multiple regression analysis of 1-OHP-gluc in urine ($n = 97$).

[a] *p*-value for $\beta = 0$.

[b] Number of cigarettes smoked daily.

[c] Meat consumption in previous 24 h — category 0: consumed no meat; category 1: consumed only meat cooked by methods other than roasting, grilling or broiling; category 2: consumed roasted, grilled or broiled meat once; category 3: consumed roasted, grilled

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in foods other than cooked meats which could not be assessed from the questionnaire. Furthermore, there is substantial variation in PAH levels within the meat categories used to assess exposure. A number of variables influence PAH levels in cooked meats including source and intensity of heat, distance from meat, length of cooking time, surface temperature of meat, thickness of meat, etc. These variables preclude even a rudimentary estimate of actual PAH content of the meats consumed. Thus, considerable non-differential exposure misclassification probably occurred, which would be expected to reduce the strength of the overall association observed.

Another potential source of error in the accuracy of the 1-OHP-gluc concentrations determined is the possibility of metabolite instability and degradation. Although we have found the metabolite to be stable in human urine stored at −70°C for up to 2 years (unpublished), the urine samples used in the current study were collected in 1988 and assayed in 1994. Therefore, it is possible that the concentrations of 1-OHP-gluc determined may underestimate the concentration at the time of urine collection. However, assuming that possible degradation occurs at comparable rates in all samples, the relative concentrations of 1-OHP-gluc among samples would not be expected to change appreciably. Thus, our overall conclusions should not be affected by metabolite degradation, should it occur.

Although we did not measure free 1-hydroxypyrene in the urine samples, previous reports (Jongeneelen *et al.* 1987, Strickland *et al.* 1994, Singh *et al.* 1995) indicate that only 0–15% of 1-hydroxypyrene excreted in urine is unconjugated. We have also chosen not to adjust our 1-OHP-gluc values by urinary creatinine concentration as this had little effect on the results of our analyses or our conclusions. This is consistent with previous studies of urinary 1-OHP-gluc after occupational (Kang *et al.* 1995a) or dietary (Kang *et al.* 1995b) exposure to PAHs, in which adjustment for creatinine had little or no effect on associations between 1-OHP-gluc and PAH exposure.

The ratio of (1,7-dimethyxanthine + 1,7-dimethyluric acid) to caffeine was used as an index of systemic CYP1A2 activity. When analysed as a continuous variable, this ratio was correlated with urinary 1-OHP-gluc concentration. However, this association was probably due to cigarette smoking, since adjusting for cigarette smoking by multiple linear regression made the association between urinary 1-OH-gluc and phenotype ratio non-significant. In the final regression model, number of cigarettes smoked and recent consumption of roasted, grilled, or broiled meat were significant positive predictors of urinary 1-OHP-gluc concentration.

The mean concentration of 1-OHP-gluc in non-smokers (0.55 ± 0.05 pmol ml^{-1}) from the current study was higher than that reported previously (17, 19) by our laboratory in non-smoking subjects without dietary (0.23 ± 0.11 pmol ml^{-1}) or occupational (0.26 ± 0.06 pmol ml^{-1}) exposure. This may be due in part to the limited information available in the current study on other potential sources of PAH exposure. Subjects with potential for exposure to low levels of PAH from their occupation did not exhibit increased urinary 1-OHP-gluc concentration. However, few of the study subjects had jobs expected to have moderate to high PAH exposure. Furthermore, the information on occupation was not sufficiently detailed to estimate recent (previous 24 h) exposure. Previously, the association of 1-OHP-gluc with occupational PAH exposure has been examined by comparing exposed with unexposed workers on the same work site or comparing pre-shift and post-shift levels in the same workers (Jongeneelen *et al.* 1985, 1986, 1988, 1990, Tolos *et al.* 1990, Sherson *et al.* 1992, Kang *et al.* 1995a). These studies have consistently shown increased 1-OHP-gluc in the urine from workers with PAH exposure and in post-shift versus pre-shift measurements. The current study was designed primarily to examine smoking effects and, therefore, was not ideal for assessing occupational PAH exposures. Thus, our power to detect potential occupational contributions to urinary 1-OHP-gluc was reduced.

Subjects who reported possible passive smoke exposure in the current study did not show increased urinary 1-OHP-gluc concentration. This may be due to poor or inaccurate self-reporting of passive smoke exposure, or could be due to poor correlation of urinary 1-OHP-gluc concentration with inhalation of low levels of pyrene in this population. An alternative measure of passive smoking, urine cotinine, was also examined. We had previously reported that urinary cotinine plus nicotine is highly correlated with 1-OHP-gluc in this study population (Vineis *et al.* 1996); however, cotinine/nicotine levels were very low in self-reported non-smokers and were not associated with 1-OHP-gluc in non-smokers.

Overall, the findings reported here further support the use of urinary 1-OHP-gluc as a useful biomarker of recent exposure to PAH from multiple sources. We show that two non-occupational sources of PAHs, active smoking and diet, can contribute significantly to the urinary concentration of this biomarker. Given the high degree of variability of PAH concentration in foods processed by similar methods, questionnaire-based approaches to assessing dietary PAH intake are somewhat limited. This study and previous reports indicate that urinary 1-OHP-gluc is useful for assessing dietary exposure to PAHs as a supplement to questionnaire data.

Acknowledgements

This research was supported in part by DHHS grants P01-ES06052 and P30-ES03819, and Italian National Research Council contract 95.00449.PF39. The authors thank Dr Regina Santella for supplying antibody 8E11.

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Received 20 December 1996, revised form accepted 8 February 1997

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