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Preliminary Studies of Urinary Hydroxyproline Levels in Rodents and in Smokers

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Groups of rats were exposed to four dilutions of cigarette smoke over a period of our weeks. Compared to the control (untreated) group of animals, the 24-hour hydroxyproline output (relative to creatinine) was lower for all dilutions of smoke and showed a negative dose-response relationship. In human smoking studies, in which groups of 20 male subjects and 20 female subjects were switched between various cigarettes for periods of 2 weeks, hydroxyproline output (relative to creatinine) for each sex did not change with increasing nicotine uptake although the outputs for men and women were significantly different.

(Key Words: hydroxyproline, smoking, rats, humans)

INTRODUCTION

Increase in hydroxyproline to creatinine ratios (measured as excreted material in urine) have been proposed as a marker for smoke uptake and for exposure to NO_2 (Kasuga *et al.*, 5, 6).

Within BAT, opportunities to examine urinary hydroxyproline and creatinine levels exist both in animals, as a result of smoke inhalation studies in rats, and in certain human studies involving experimental cigarettes. While the main objectives of such studies are in other areas, the collection in these studies of 24-hour urine samples permits examination of the effects of smoking on hydroxyproline levels.

ANIMAL STUDIES

Five groups of 10 male rats (ex Charles River) were studied over a period of four weeks using the BAT-Mason smoke inhalation machine as described by Beven (1).

Four groups were exposed to cigarette smoke according to the following regime:

Cigarettes:	UK 84 mm KS filter-tipped type, delivering 18 mg total particulate matter (TPM) per cigarette, 9 puffs, when smoked with a puff volume of 35 mℓ, 2 seconds' duration, 1 puff per minute. Butt length filter (15 mm) + 8 mm.
Exposure:	9 minutes, twice a day, 5 days per week.
Chamber Concentrations:	2.9 mg TPM/litre (1:20 whole smoke dilution) 3.5 mg TPM/litre (1:16 whole smoke dilution) 4.8 mg TPM/litre (1:12 whole smoke dilution) 7.2 mg TPM/litre (1:8 whole smoke dilution)

Control animals were exposed to fresh air only, using identical procedures including the smoke inhalation machine.

During the experiment, 24-hour urine samples were collected on two occasions for each animal, corresponding to one sample in weeks 1 and 2 and a subsequent sample in weeks 3 and 4, the animals being placed in metabowls for this purpose. The mean results (± SD) ob-

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tained for each group of animals for excretion of hydroxyproline and creatinine are shown in Figures 1 and 2.

The hydroxyproline and creatinine were determined by methods described by Kasuga *et al.* (5).

Within this rat inhalation study, the daily output of hydroxyproline and creatinine relative to the reported human study were in the range 5–10 and 1–2 respectively, when expressed on a mg/kg body weight basis.

The results for hydroxyproline to creatinine ratios are shown in Figure 3.

At all levels of smoke exposure, the levels of 24-hour creatinine and hydroxyproline excretion were significantly lower than controls ($p < 0.05$). Similarly, the hydroxyproline/creatinine ratios for the smoke exposed animals were lower than the controls.

There was also a statistically significant trend in the decreasing amounts of hydroxyproline excreted with increasing smoke concentrations.

HUMAN STUDIES

Groups of 20 male and 20 female regular cigarette smokers were used in this study. Each group was equally divided between smokers of low-tar and middle-tar cigarettes. The subjects were recruited in an English University City, were aged between 20 and 60 years and were selected to approximate to the UK population of smokers in terms of occupation and other demographic variables.

The experiment lasted for six weeks, during which time each subject smoked their own brand (2 weeks), a common experimental low-tar cigarette (2 weeks) and one of two further experimental cigarettes. The order in which the subjects smoked the experimental cigarettes was randomised. On two occasions each subject provided a 24-hour urine sample which was analysed for nicotine and cotinine as an estimate of nicotine uptake by the subjects. During this brand switching study, the oppotunity was taken to determine the changes in hydroxyproline and creatinine output under these conditions. Hydroxyproline and creatinine were analysed by the methods described by Kasuga *et al.* (5) and nicotine and cotinine were determined by a gas chromatographic method developed from that published by Feyerabend and Russell (3).

A statistically significant trend of increasing urinary output of creatinine and hydroxyproline ($p < 0.005$) with increasing output of urinary nicotine and cotinine was observed for males but not females (Figures 4, 5). Based on the urinary hydroxyproline to creatinine ratio, no statistically significant within-sex differences were observed. However, statistically significant differences were observed between the sexes (Figure 6).

DISCUSSION

The human studies suggest that hydroxyproline/creatinine ratios do not increase with increasing nicotine uptake, while the animal studies actually point to a decrease in this ratio. Furthermore, we note that the mean hydroxyproline/creatinine ratio quoted in the literature (9) for healthy subjects is close to the mean of our figures and, indeed, agrees with our limited values for non-smokers (Figure 6).

Kosmider *et al.* (8) have reported that the level of urinary hydroxyproline excretion in rats is increased following exposure to nitrogen dioxide. Kasuga has reported similar findings in subjects exposed to environmental pollutants, cigarette smoke and passive smoke exposure, and has implicated nitrogen dioxide in this process.

Our observations that smoke exposure has no positive effect on the ratio of hydroxyproline to creatinine are more consistent with the observation by Borland and Higenbottam (2) that the conversion of nitric oxide to nitrogen dioxide in cigarette smoke is negligible.

Likewise, we note that the results of Hugod (7) and Klus (4) suggest the level of nitrogen dioxide in ambient air are vanishingly small.

These preliminary findings indicate that caution should be exercised when using hydroxyproline/creatinine ratios as a measure of lung response in subjects exposed to cigarette smoke.

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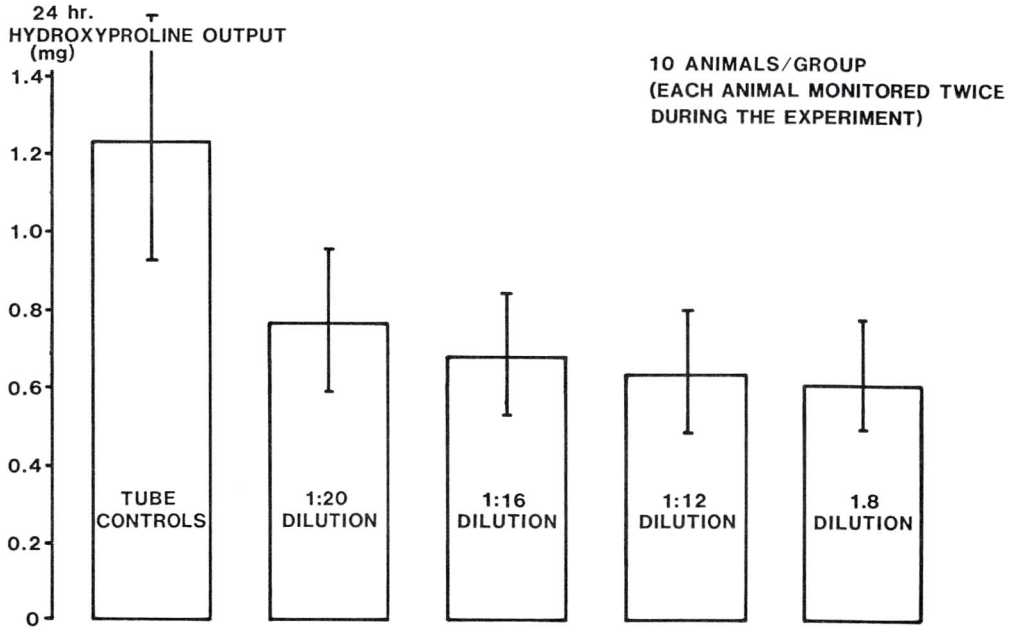

Fig. 1 Preliminary data from animal study

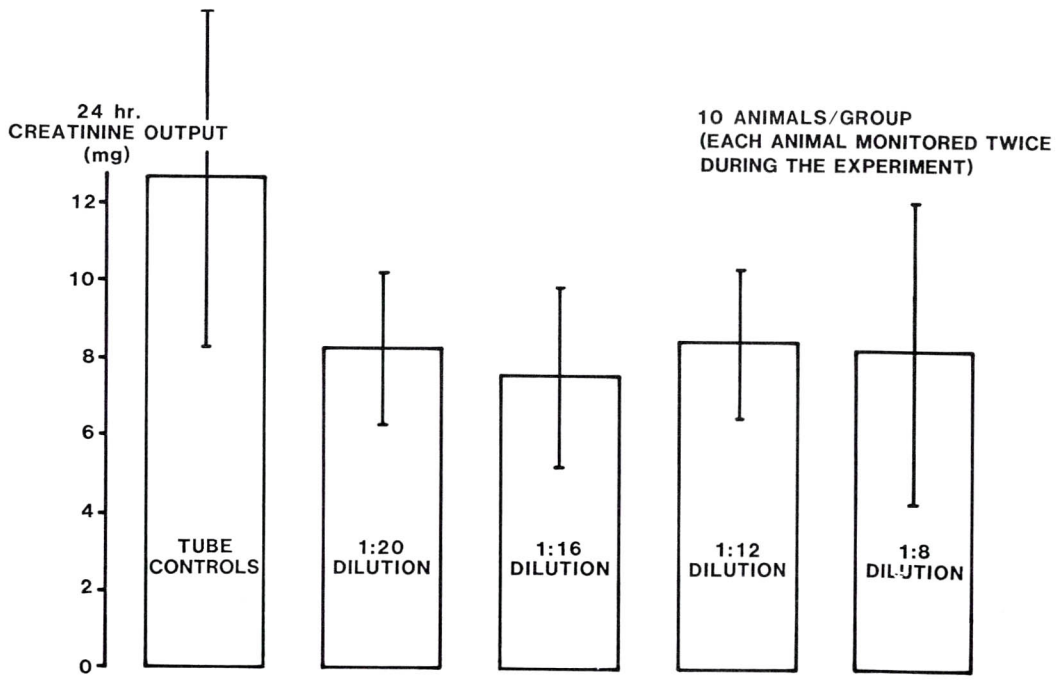

Fig. 2 Preliminary data from animal study

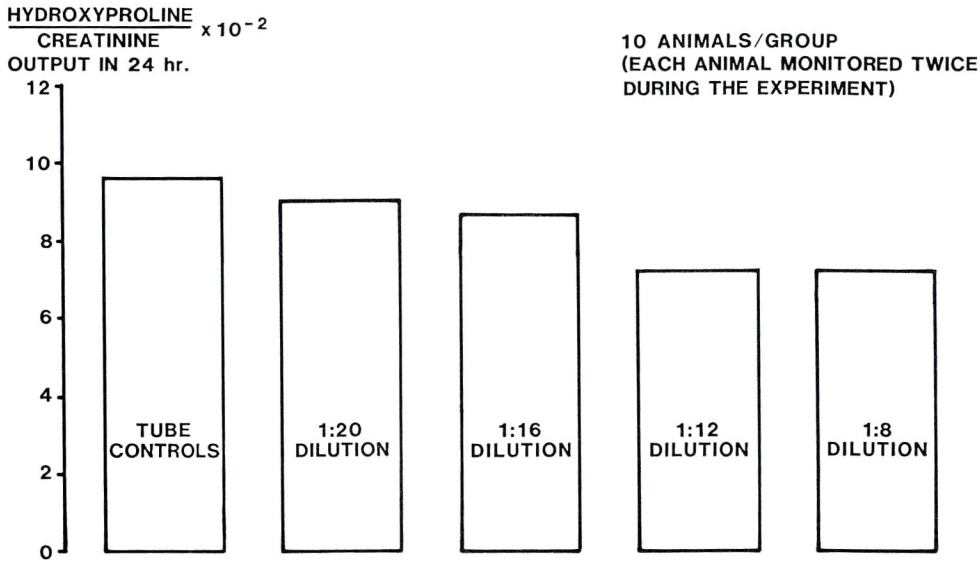

Fig. 3 Preliminary data from animal study

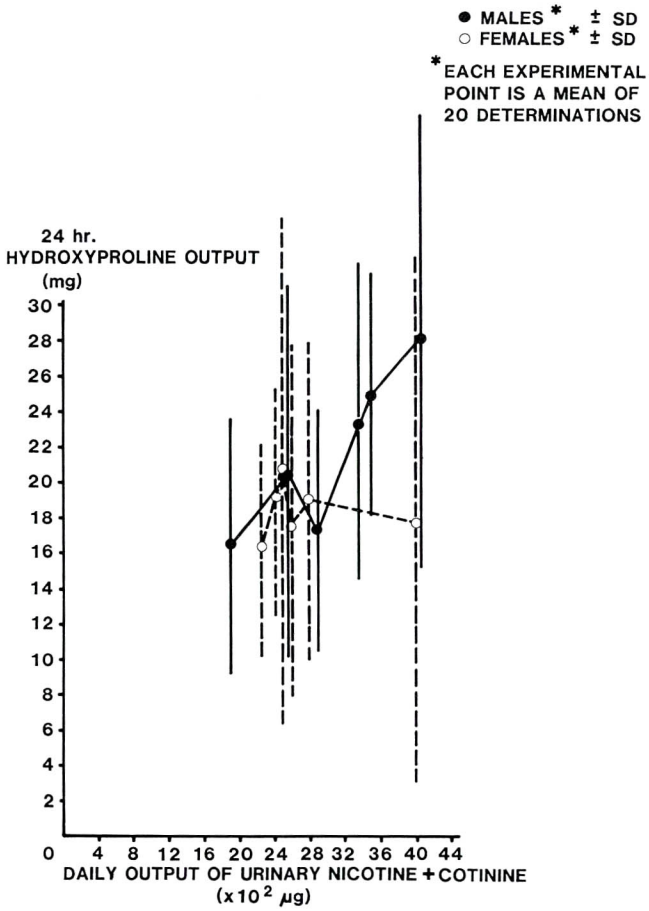

Fig. 4 Preliminary data from human study

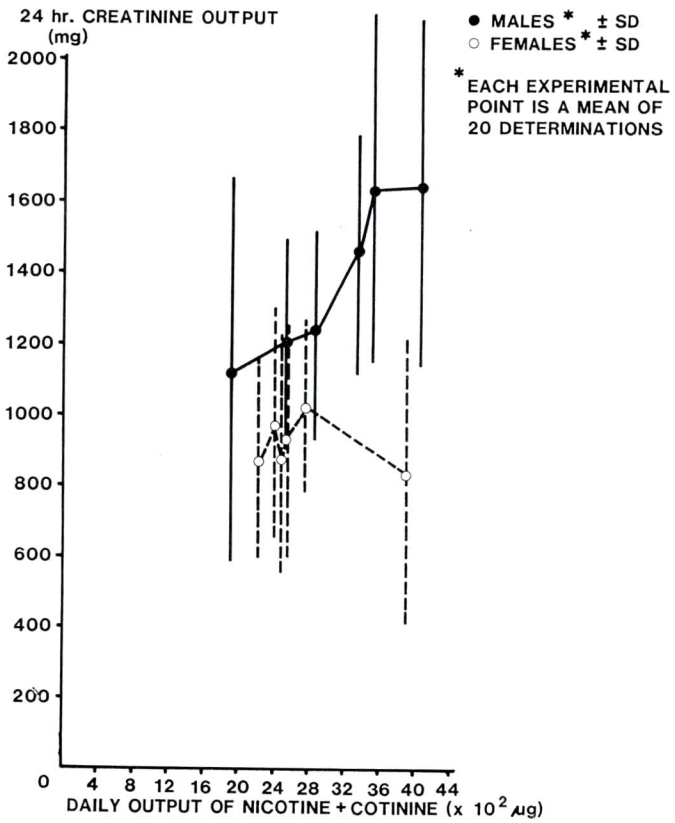

Fig. 5 Preliminary data from human study

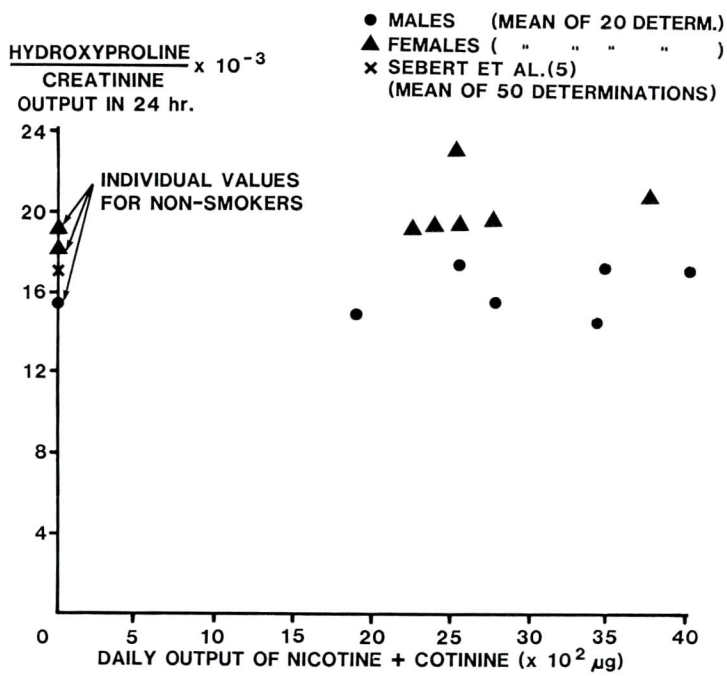

Fig. 6 Preliminary data from human study