We compared cotinine, carboxyhemoglobin, and thiocyanate concentrations in blood sampled from 187 cigarette smokers and 181 non-smokers. All three differed significantly between smokers and non-smokers. Cotinine performed best as a test for assessing smoking status, with a sensitivity of 98% as compared with 94% for carboxyhemoglobin and 80% for thiocyanate, all at a specificity of 95%. These differences were statistically significant. Results by none of these three methods correlated well with number of cigarettes smoked per day.

Additional Keyphrases: validating smoking claims • cutoff value

Several biochemical methods are used to distinguish smokers from non-smokers in health surveys, anti-smoking programs, and heart-disease prevention studies (1–3). These methods—which include plasma cotinine, blood carboxyhemoglobin, plasma thiocyanate, and expired-air carbon monoxide—are a valuable aid to interpretation of the questionnaires used in smoking-cessation programs. Information supplied solely by the volunteers in these programs is often unreliable (5, 6), and to validate results independent measures of cigarette smoking are necessary. Thus it seems important to document and inter-compare the performance of these tests, but there are few such studies adequately comparing the discriminating power of the various biochemical methods (1, 2). Here, we examine the performance of three of these tests: cotinine, carboxyhemoglobin, and thiocyanate, and compare them, both with each other and with the number of cigarettes reportedly smoked per day. We attempted to fulfill the criteria suggested by Zweig and Robertson (7): subject selection, independent classification, performance of all tests on all subjects, and comparison of tests at the same specificities. We have also performed statistical tests to validate impressions of difference in performance.

Subjects and Methods

The study population consisted of 187 smokers and 181 non-smokers, the former being participants in a voluntary smoking-reduction campaign in an suburban general practice, the latter being unselected patients attending the clinic.

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for various reasons, who agreed to take part in the development of counselling methods for smokers. The proportions of men and women were the same in the smoker and non-smoker groups, and the ages in the two groups were comparable. The smokers consumed 22.8 ± 12.5 (mean ± SD) cigarettes per day. Blood was sampled for analysis from the volunteers at the start of the program, when information on smoking habits was obtained from them. Each sample was then analyzed for each of the three analytes without the analysts' knowledge of the smoking habits of the participant. The data were correlated at the conclusion of all analyses by use of the SPSS (9).

Plasma thiocyanate was determined colorimetrically after ion-exchange chromatography on Amberlyst A21 resin (B.D.H. Chemicals Ltd., Poole, England) (9, 10). Carboxyhemoglobin was measured in whole blood by an automated spectrophotometric method, with a CO-Oximeter (Instrumentation Laboratory, Lexington, MA) (11). Cotinine in plasma was determined by gas chromatography on a 12.5 m × 0.3 mm (i.d.) Carbowax capillary column (Hewlett Packard, Avondale, PA) (12, 13).

The analytical precision (CV) for the three methods was 0.17% at a mean concentration of 1.9% for carboxyhemoglobin, 27 nmol/L at 247 nmol/L for cotinine, and 3.6 μmol/L at 52 μmol/L for thiocyanate.

**Results**

Figures 1, 2, and 3 illustrate the frequency distributions for results for carboxyhemoglobin, cotinine, and thiocyanate for the study population, classified according to their responses to the questionnaire. The mean respective values (and SD) for non-smokers and smokers were 0.93 (0.52%) and 4.36 (2.09%), 25 (78) and 1905 (1321) nmol/L, and 33 (15) and 109 (47) μmol/L. For each, the differences between smokers and non-smokers were highly significant (p < 0.001). The value for carboxyhemoglobin that misclassifies the fewest subjects overall is 2.0%, and this figure gives a sensitivity of 87.7% and a specificity of 97.8%. For thiocyanate the best discriminating value is 70 μmol/L, which yields a sensitivity of 75.9% and a specificity of 96.7%. Cotinine, with a cutoff point of 250 nmol/L, has a sensitivity of 96.2% and a specificity of 98.3%. Figure 4 shows the sensitivity and specificity of the three tests at various cutoff values and compares the sensitivities of the three assays at a constant 95% specificity.

Inter-comparison of the sensitivity of the three tests at constant specificity (Table 1) showed the three tests to be significantly different.

Table 2 shows coefficients of correlation for the relation between results of each of the three tests and the number of cigarettes smoked, and of the methods with each other. It
cotinine to be a far more sensitive and specific test than either of them. This agrees with the study by Haley et al. (4), who found cotinine to be a better marker to determine smokers than thiocyanate. This difference is well illustrated by comparing the relative sensitivities of the three tests at a fixed specificity of 95% (Figure 4). We have also been able to establish statistically significant differences in the sensitivities of the three tests, the most likely explanation for which is that factors other than cigarette smoking can affect the concentrations of these three analytes in the blood. For example, carboxyhemoglobin can be affected by atmospheric pollution, particularly from motor exhaust fumes (1), and thiocyanate can be increased after consumption of certain vegetables or after industrial exposure to cyanides (1). Cotinine is not subject to these extraneous influences.

In vivo, nicotine from cigarette smoker is converted to cotinine (14). Gas-chromatographic estimation of nicotine can be very difficult (15), and nicotine is an ubiquitous contaminant of our laboratory air-conditioning system. Thus we elected to assay cotinine as an indirect measure of nicotine intake. Cotinine is not widely distributed in the environment, and falsely increased cotinine concentrations in non-smokers arise only by passive smoking, i.e., by passive inhalation of cigarette smoke—and even this effect reportedly is small (16). We have also found that passive intake of cigarette smoke by a non-smoker rarely results in plasma cotinine concentrations >100 nmol/L (data not shown). These facts may account for the superior performance of cotinine as compared with carboxyhemoglobin and thiocyanate. In the same way as it has previously been suggested that measurement of carboxyhemoglobin is a better test than plasma nicotine (17), cotinine would be expected to be a better discriminating test than a direct assay for nicotine.

We found poor correlation between values for the three analytes and stated daily cigarette consumption. This agrees with previous studies (17,18) and is probably caused by such variables as different patterns of smoke inhalation, differences in the subject's biochemical responses, or inaccurate survey information supplied by the subjects. It would seem from the pattern of correlation between them that factors affecting the relationship between number of cigarettes and test results affect each of the three tests similarly. Partial correlation estimates the strength of this association between the test results, after correcting for a factor that may influence each of them—in this case the number of cigarettes inhaled. Evidently the correlations between results in the three tests in the smokers are only slightly ascribable to differences in number of cigarettes smoked.

In most previous studies, values for either plasma thiocyanate or blood carboxyhemoglobin were used to distinguish smokers from non-smokers, but we find the performance of these two tests to be quite different. Neither test appears to be as good as plasma cotinine. In fact, cotinine would appear to be the best single biochemical test with which to distinguish smokers from non-smokers in clinical and epidemiological studies; the high sensitivity of cotinine and the correlation between results of all three tests suggests that using two tests would not be advantageous.

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Sex- and Age-Related Differences in Bilirubin Concentrations in Serum

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The relation between age and sex and the concentration of bilirubin in serum was evaluated in 6740 men and 11 215 women, ages 13 to 96 years. Mean serum bilirubin concentrations in the men significantly exceeded values in the women over all age groups examined. Further, mean serum bilirubin concentrations were greatest both in males and females in the 19–24 years age group and then declined to former values, which persisted throughout life. Pearson correlation coefficients for bilirubin with liver function indices (albumin and total protein) and with hemoglobin were low in all ages and in both sexes, suggesting that bilirubin concentrations do not correlate with those liver functions not directly concerned with bile pigment processing.

Additional Keyphrases: reference interval · liver function

Aging usually implies a decline in physiological function, but the rate of aging of different organ systems may vary widely. Little information exists regarding the effect of aging on the liver. Indeed, investigators disagree regarding the influence of age and sex on the concentration of bilirubin in serum (1–4), one measure of liver function. We undertook the present study to examine changes in serum bilirubin as a function of sex and age in a large population.

Materials and Methods

Serum samples for bilirubin analysis were obtained from outpatients attending doctors’ private offices and clinics at the Presbyterian Hospital of the Columbia Presbyterian Medical Center during 1982. Patients with possible liver disease or hemolysis were excluded from the study by eliminating all those with above-normal values for one or more of the following: lactate dehydrogenase (EC 1.1.1.27), aspartate and alanine aminotransferases (EC 2.6.1.1 and 2.6.1.2), and alkaline phosphatase (EC 3.1.3.1). We also excluded those with low concentrations of total protein and (or) albumin. We investigated a total of 17 955 samples (6740 from men) from patients 13 to 96 years old. To assess the influence of age and sex, patients were grouped as shown below in Tables 1 and 2.

All sera were assayed for total bilirubin by continuous-flow analysis (SMAC system; Technicon Instruments Corp., Tarrytown, NY 10591) as routinely used in our laboratory, a modification of the Jendrassik–Gröf method (5, 6). We routinely standardized the SMAC by using bilirubin standards with concentrations of 8.8, 23, and 39 mg/L (Beckman Decision Liquid Comprehensive Chemistry Control Serum Levels 1, 2, 3; Beckman Instruments, Fullerton, CA 92634). For quality control, we ran a control sample (bilirubin 26 mg/L; Ortho Diagnostic Systems, Raritan, NJ 08869) after every nine samples. The standard deviations for determination of total serum bilirubin were 1.2 mg/L within-day and 1.4 mg/L day-to-day for the control.

We analyzed the data for the presence of age- and sex-related variations in serum bilirubin concentration, using the SPSS package (McMaster University) on an HP3000