Acetonitrile in Body Fluids Related to Smoking

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WITHIN recent years, tobacco smoking has come to be regarded as a significant public health problem by various health officials. Extensive statistical studies have been made to examine possible relationships between smoking and various diseases. Experimental investigations have also been conducted to study the physiological effects of smoking. In most of these studies, it has been necessary to rely on information supplied by the test subjects concerning whether or not they smoke and their rate of smoking. In many cases, such information is known to be inaccurate. Volunteer subjects may try to conceal the fact that they smoke, or they may not give an accurate estimate of their rate of smoking.

During the course of developing methods for measuring trace constituents in body fluids, acetonitrile was identified in samples of human urine and expired air. This substance was present in the body fluids of smokers, but was essentially absent in nonsmokers. Additional work was then undertaken to see if measurement of acetonitrile could be used to provide an objective, reproducible indication of the smoking habits of test subjects. If this could be done, research workers could be freed from the necessity for relying on subjective self-evaluation by test subjects.

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Method

Morning urine samples were obtained from 60 men, 20 nonsmokers and 40 smokers, after fasting. The daily smoking rate of the smokers ranged from 0.25 to 2.5 packs of cigarettes per day. No consideration was given to the brand of cigarettes or the use of filtered cigarettes.

The samples were analyzed by a modification of the method reported by Rhoades (*1,2*) for the analysis of coffee volatiles. The modification permitted the stripping of urinary volatiles at 37° C. and at reduced pressure. The stripped volatiles were collected in a liquid nitrogen trap, vaporized, and analyzed by gas chromatography, using a Perkin-Elmer model 154–D vapor fractometer equipped with a thermal conductivity detector. The column employed was 3 meters of 15 percent Carbowax No. 1500-silicone oil No. 200 (ratio 2:1) on 40–60 mesh chromosorb P and was operated at 40° C. The carrier gas was helium at a pressure of 4 pounds per square inch.

The various substances measured were identified by comparing their elution times from the gas chromatograph with that of known compounds. The presence of acetonitrile in smokers was confirmed by mass spectrometer analysis of the gas chromatographic fraction obtained from a composite sample of smokers' urine. The very small amount indicated in nonsmokers was not sufficient to permit a mass spectrometer analysis. The values obtained for nonsmokers may be either acetonitrile or a small amount of some other substance that is eluted simultaneously from the chromatographic column.

Average values of trace constituents, determined by gas chromatographic analyses, in urine samples of cigarette smokers and nonsmokers

Compound	Average for 40 smokers (μg per 100 ml. urine)	Average for 20 nonsmokers (μg per 100 ml. urine)	Correlation coefficient
Acetaldehyde	1. 99	1. 89	0. 0019
Propionaldehyde	. 36	. 27	. 045
Acetone	81. 9	84. 8	. 0001
Methyl ethyl ketone	1. 27	1. 34	. 0905
Methanol	52. 8	54. 1	. 0029
Acetonitrile	11. 76	. 29	. 707

Results

The average values obtained for several trace constituents in urine samples of smokers and nonsmokers are shown in the table. Correlation coefficients indicate the degree of correlation between smoking and the concentration of the various compounds determined. Most of the compounds show approximately the same values for smokers and nonsmokers, and the correlation coefficients are sufficiently low to indicate little or no possibility of any relationship. For acetonitrile, however, a definite relationship is indicated. This was further substantiated by the Students t test. The t value for acetonitrile was 15.5 compared with a critical value of 2.000 for a 95 percent confidence level and a critical value of 2.660 for a 99 percent confidence level. Thus there seems to be no doubt that acetonitrile values separate smokers and nonsmokers into two distinct groups.

Discussion

Among those who consistently smoke three cigarettes per day or more, the lowest value determined for acetonitrile was 2.2 micrograms per 100 ml. of urine. The highest single value found in a nonsmoker was 0.74 micrograms per 100 ml. Thus it appears that the urine acetonitrile content can be used to divide a random group of subjects on the basis of smoking, by selecting an arbitrary dividing line of perhaps 1.0 or 1.5 micrograms per 100 ml. Based on the results obtained with 60 subjects, it appears that such a division will be 100 percent accurate in indicating which subjects in a test group are smokers and which subjects are nonsmokers, provided only that the persons who smoke do so consistently at a rate of three cigarettes per day or more.

Heavy smokers showed higher acetonitrile content, ranging up to values in excess of 20 micrograms per 100 ml. However, a perfect quantitative correlation was not found, but rather the data tended to scatter about a regression line indicating the best possible fit. This scatter is thought to be due to variations in individual smoking habits such as depth of inhalation, length of cigarette left unsmoked, and number of puffs per cigarette. Work is now underway to attempt to control some of these variables so that their effect on the quantitative relationships can be determined. The possibility has been suggested that urine acetonitrile content may provide a more accurate measure of the actual degree of exposure to tobacco smoke than the number of cigarettes smoked or other similar measures commonly used.

Although isocyanates have previously been reported as occurring in the body fluids of smokers (*3*), to our knowledge the presence of acetonitrile is reported for the first time.

REFERENCES

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