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ABSTRACT

This is a study of carbon monoxide (CO) in the blood of human subjects participating in the Second National Health and Nutrition Survey (HANES II), a detailed study of health indicators in sample populations of many communities throughout the U.S. The purpose of this aspect of the survey is to evaluate the levels of blood carboxyhemoglobin in normal individuals of all ages in typical U.S. communities, from whom accurate histories and clinical studies are available. This report gives results of the first of three years of analyses. A careful calibration of the analytical method has been completed, and more than 3000 blood samples have been analyzed. Although smoking histories are not yet available to permit evaluation of carboxyhemoglobin in non-smokers, in children under 12 years of age, blood COHb has been found to be consistently low, with less than 3% greater than 1.5% COHb. These preliminary results suggest that urban exposure to carbon monoxide among the general population is not now significant in the U.S., at least during the periods of these early examinations.

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An Evaluation of Carbon Monoxide in Blood Samples from
the Second Health and Nutrition Survey

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Scientific background: Carbon monoxide is quantitatively the most important toxic air pollutant released from fossil fuel combustion. Its toxicity for persons with pre-existing coronary vascular disease, a very widespread disease especially in middle-aged males, is such that a very strict ambient air standard is presently the basis of control strategies for this pollutant. Whether these standards are being met nationwide is therefore of major concern in devising fossil fuel combustion emission standards for the future, especially for automobile exhaust, the chief source of carbon monoxide (CO) in the general environment.

Carbon monoxide is unique among the air pollutants in that there is a readily available technique for assessing exposure to this gas. This is to measure the percent of carboxyhemoglobin (COHb) in the blood of people living, travelling and working in the urban areas. The kinetics of uptake of CO are well understood, and the results can be interpreted in terms of an integrated

exposure. Because of the importance of smoking tobacco products in raising COHb, the assessment of CO from ambient sources can best be done in non-smokers.

Two large studies of COHb in the general population have been published, with conflicting conclusions. Stewart et al (1) obtained blood from 29,000 blood donors in 18 regions of the U.S. They found that 45% of non-smokers had COHb greater than 1.5%, the concentration which one would expect from prolonged exposure to the ambient CO standard of 10 mg/m^3 . This percentage of non-smokers with blood COHb, interpreted as indicating exposure to concentrations of CO above the standard, varied from 18% in rural Vermont and New Hampshire, 35% in New York City and St. Louis, Missouri, 39% in Honolulu, to 76% in Denver and Los Angeles. Thus prolonged exposure to CO above the standard in a substantial part of the population was essentially universal in the U.S. if these results are correct.

The other study (2) was carried out on blood from 16,600 blood donors in St. Louis, Missouri, a city also investigated by Stewart et al. In this second report, Kahn et al found little influence of ambient CO on the COHb values; they observed that among "non-industrial" non-smokers the mean COHb was 0.75%, with only 10% of this group above 1.0% and about 6% above 1.5%. At least part of this small fraction of people with higher values of

COHb can be accounted for by persons stating that they are ex-smokers, but who have in fact continued to smoke cigarettes, or by those who smoke marijuana. The St. Louis group also compared COHb in people living in the rural area around the city to values in city dwellers, and found only 0.18% COHb difference. The blood concentrations observed by Kahn et al were consistent with nearly complete equilibration with the twelve-hour mean CO concentration reported for the urban area during the year of the study.

Because of the difference in results obtained in St. Louis by these two studies, the measurement technique employed by Stewart et al has been questioned (3). In view of the importance of the conclusions in terms of the significance of CO as an urban pollutant from fossil fuel consumption, it is worthwhile to try to resolve the matter by repeating an evaluation of COHb in the general population.

This project is designed to achieve this reevaluation with the aid of blood samples being obtained in normal subjects from the Second Health and Nutrition Survey (HANES II) being carried out by the National Institute of Health. During this past year, Stewart and his colleagues have published (4) a report of an evaluation of blood carboxyhemoglobin in blood donors in Chicago in 1974-75, and compared this later study with their earlier one done in Chicago. The new study finds that whereas in November 1970,

74% of non-smokers had COHb greater than 1.5%, in December and January 1974-75, 42% of non-smokers had COHb above this value. Thus although their results suggest some improvement in ambient CO levels, they also indicate that if they are correct, even in 1974-75, exposure to CO remains a significant urban public health problem.

Chicago was one city included in the HANES II sampling in 1976. Samples were taken in late September and October, and we analyzed 129 samples from this station. We do not yet have smoking histories for these individuals, but of those measured 22 were children under the age of 12. If we assume that these children were all non-smokers (not necessarily a safe assumption in all cases), the mean COHb of these 22 bloods was 0.94% (range 0.34-1.57%). While a final evaluation awaits further analysis, it is apparent from these preliminary data that we do not find evidence of significant exposure above the ambient standard during the sampling period, even though the Chicago data on children were higher than for children sampled at nearly all the other stations. Our early results therefore confirm the findings of Kahn et al in St. Louis. We have some preliminary evidence that seasonal changes may influence the COHb measured, and thus it will be important to continue the analysis, especially where a repeat sampling is taken at a different time of year, for example in Chicago.

Experimental methods: Blood samples obtained by routine venipuncture during the Second Health and Nutrition Examination Survey (HANES II) are being sent to our laboratory. One ml of blood with EDTA as an anticoagulant is sent in a 2 ml capped vial, the vials being held under refrigeration prior to shipment. We have already determined that EDTA does not interfere with our method. Approximately 150 samples a week are being sent in a refrigerated container, and we find that one full-time trained technician is required to carry out this number of COHb measurements.

The method of measurement is that of Small et al (5) developed in our laboratory with the collaboration of Dr. F. Lee Rodkey of the Naval Medical Research Institute of Bethesda. Briefly, the method utilizes the difference in the light absorption spectra between oxyhemoglobin, carboxyhemoglobin and methemoglobin in the Soret band (390-435 nm). Measurements have been shown to be accurate and reliable at all levels of carbon monoxide saturation, including those from 0 to 5% COHb, the range found to be least reliable when monitored with other rapid techniques such as the CO-Oximeter. Since many of the samples to be collected in this study come from children and non-smoking subjects expected to have low levels of COHb, it is imperative that the method of analysis be reliable in this range.

Because of the crucial nature of the accuracy of the results, once a month a series of at least six to seven duplicate samples

are run by our laboratory and Dr. Rodkey's, where a highly accurate gas chromatograph method is available for measuring COHb (6). In addition blood samples from male non-smokers in our laboratory are added to the test samples every week; we have found that such individuals usually show consistent blood COHb, and their inclusion as unknowns in the samples provide an additional quality control. Special attention to these quality control checks is paid to the "zero level" check, that is, the absolute level measured in blood of non-smokers and not occupationally exposed to CO. We find that such individuals usually give COHb values of 0.6-0.8%, indicating routine exposure to less than 3 ppm CO in the ambient air. When ambient air is higher, we have easily been able to detect the rise in blood COHb of our non-smoking controls. Another quality control technique is to determine the precision of duplicate measurements, which should be within the range predicted by the measurement precision already calculated (5). For all individual stations the data meet this criterion, despite differences in the length of time from shipping to analysis.

Recalibration of method. In anticipation of the importance of having very close quality control on the data from this project, and especially on establishing the "zero set" of our method, we began investigations well in advance of the award date to determine any potential problem areas. In the original method (5) the "zero"

level was actually determined on blood obtained from non-smokers and independently checked by Dr. Rodkey's laboratory. A repeat of this procedure indicated that control bloods were now slightly low (by about 0.4-0.5% COHb). Check of the linearity of the photodetection unit indicated that there were slight non-linearities relating absorbance to pigment concentration for both potassium dichromate and hemoglobin solutions. Unfortunately the non-linearity was variable depending on wavelength, and this led to errors which were dependent on the blood concentration in our final dilution. Evidently this change in the photodetector unit had occurred slowly over the last two years.

We therefore programmed corrections for this non-linearity in the computer, and after repeated samples had been run both by our method and by Dr. Rodkey, we were able by late summer to "fine-tune" these corrections so that all samples again showed very close checks independent of blood concentration, as would be expected from the method if Beer's Law held exactly. Note that this non-linearity problem did not involve any change in collecting the raw data for absorbances at the four wave lengths (which of course were being obtained from the bloods being received). The only change was in the computation of the COHb from the observed data. Thus when the final computer program was determined, we recalculated all bloods measured prior to that time.

We now monitor especially the low end of the calibration curve by duplicate analyses by our method and that of Dr. Rodkey to assure that this degree of reliability continues to exist. Figure 1 shows a plot of blood COHb by our method compared with results from Dr. Rodkey (method of Collison et al (6)). These data have been obtained from 57 blood samples analyzed from May 1976 - March, 1977.

The correlation of our results with the gas chromatograph data is excellent. The correlation coefficient is 0.996, the slope of the regression line is very close to 1.00 and the y intercept at the origin is very close to zero. To bring out the reliability at the low end of the calibration curve the points below 2% COHb are plotted in the enlarged insert graph in Fig. 1. For this segment of the graph alone (where the minimum errors of our method, $\pm 0.3\%$ COHb, result in a large percentage error), the regression line even over this limited range of values is insignificantly different from the identity line.

Results of analyses to date. Through May 31, 1977, 3,096 bloods have been analyzed, nearly all in duplicate, from 22 stations. We have submitted the results of these analyses to the HANES II coordinator at the National Bureau for Health Statistics. Because of delay in the contract approval, blood samples drawn in February and March 1976 in Miami, Florida were not analyzed until late April.

Figure 1. Determinations of COHb in duplicate samples of blood by our method compared with that of Rodkey.

Main graph: Comparison of 57 duplicate determinations from May 1976 - Mar. 1977. Twenty of the non-smokers (<2%) samples from laboratory personnel; all others HANES II blood samples. Solid line is identity line, dashed line is regression line. Correlation coefficient 0.996, slope 1.023, y intercept +0.014.

Insert: Expanded graph of samples <2%. Dashed line: regression line for this range only. Correlation coefficient 0.880, slope 1.122, y intercept -0.082.

These samples showed relatively high values of methemoglobin (about 5%), indicating some hemolysis and oxidation of reduced hemoglobin. We therefore consider the results from this station somewhat less reliable than for the later stations. Occasionally blood samples have arrived in our laboratory unrefrigerated, and some analyses indicate that methemoglobin had been formed, but in general since the beginning of the study there has been no indication of significant deterioration of the blood prior to analysis.

Because we do not have smoking histories for these subjects as yet, it is not possible to evaluate the results for non-smokers in order to assess ambient exposure in these cities. To estimate data for non-smokers we have analyzed the results obtained for children under age 12 from each station. Table 1 gives these results.

Several points from Table 1 are worth noting. First, of course, is that generally the values of carboxyhemoglobin in these children are quite low. Of the 621 blood samples only 16, or 2.6%, were above 1.5% COHb. Five samples were over 2.2% and were excluded as smokers; in addition there may be some smokers among the groups included in Table 1.

The second point is that there appears to be a seasonal variation in the expected direction. For cities of comparable size the results in the children are lower when the blood is

TABLE I
CARBOXYHEMOGLOBIN IN CHILDREN UNDER 12 YEARS OF AGE
HANES II SAMPLES FEB. 76 - MAY 77

Station Number and Location	Dates Drawn	Dates Analyzed	Number Analyzed	% COHb Mean S.D.	Number >1.5% COHb
1. Miami, Fla.	Feb.-Mar. 76	Apr. 76	13	0.93 0.21	0
2. San Jose, Calif.	Feb.-Mar. 76	Apr. 76	25	1.01 0.32	1
3. Shreveport, La.	Apr.-May 76	May 76	25	0.75 0.32	1
4. Fresno, Calif.	May -June 76	June 76	53	0.74 0.33	1
5. Newport News, Va.	May -June 76	June 76	28	0.47 0.29	0
6. Tacoma, Wash.	May -June 76	June 76	25	0.68 0.27	0
7. Dexter, Mo.	June-Aug. 76	July-Aug. 76	28	0.57 0.37	1
8. Blackfoot, Ida.	June-July 76	June-July 76	40	0.55 0.24	0
9. Des Moines, Ia.	Aug.-Sep. 76	Aug.-Sep. 76	21	0.29 0.18	0
10. Laramie, Wyo.	July-Aug. 76	Aug. 76	29	0.41 0.23	0
11. Farmington, Minn.	Sep.-Oct. 76	Sep.-Oct. 76	24	0.42 0.32	0
12. Minneapolis, Minn.	Aug.-Sep. 76	Aug.-Sep. 76	22	0.55 0.20	0
13. Waukegan, Ill.	Oct.-Nov. 76	Oct.-Nov. 76	21	0.94 0.35	2
14. Chicago, Ill.	Sep.-Nov. 76	Sep.-Nov. 76	22	0.94 0.33	1
15. Greenville- New Bern, N.C.	Nov.-Dec. 76	Dec. 76	36	0.62 0.27	1
16. Athens-Pikes- ville, Tn.	Nov.-Dec. 76	Nov.-Dec. 76	49	0.75 0.38	1
17. Lafayette, La.	Jan.-Mar. 77	Feb.-Mar. 77	41 [†]	0.84 0.35	2
18. Cocoa, Fla.	Jan.-Feb. 77	Jan.-Feb. 77	16	0.50 0.22	0
19. Gainesville, Ga.	Mar.-Apr. 77	Mar.-Apr. 77	24*	0.69 0.38	1
20. Oneonta, Ala.	Feb.-Mar. 77	Mar.-Apr. 77	30**	0.62 0.33	1
21. Bronx, N.Y.	Apr.-May 77	Apr.-May 77	33	0.89 0.27	0
22. New York, N.Y.	Apr.-May 77	Apr.-May 77	16	1.08 0.28	1

[†] One sample of 5.15% omitted as smoker.

* One sample of 2.56% omitted as smoker. If included mean = 0.76.

** Four samples >2.2% omitted as smokers. If included mean = 0.85.

drawn in the summer vs. winter. We have noted a similar seasonal trend in Baltimore, with a rise of 0.3 to 0.4% COHb in our control non-smokers during December through March. This seasonal trend is observed in the carbon monoxide measured from the city monitoring stations.

Third, the standard deviation of the mean of each station is generally similar to the standard deviation of duplicate determinations on the same blood. For the latter we now find a value of 0.25% COHb, very close to the theoretical value calculated for our method (5). Thus the chief variability is methodological, and there is a relatively small contribution from "true" variability, or actual differences in COHb among the children. Obviously further results are needed to substantiate this point, but it is consistent with the view that COHb in the group of non-smokers is a good index of the community-wide carbon monoxide on the day of sampling.

Finally, the highly urbanized areas such as Chicago-Waukegan and New York City do appear to have higher mean values than the more rural communities, but the difference is not great, and is consistent with the observations of Kahn et al (2) in St. Louis.

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