CHAPTER IV

EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS: IV -- BIOCHEMICAL OBSERVATIONS

Ramon D. Buckley, PhD
Jack D. Hackney, MD
Kenneth Clark
Clara Posin

ABSTRACT

Statistically significant changes ($p \leq 0.05$) were observed in erythrocytes (RBC) and sera of young adult human males following a single acute exposure to 0.50 ppm ozone (O_3) for 2 3/4 hours. RBC membrane fragility, glucose-6-phosphate dehydrogenase (G-6-PDH) and lactate dehydrogenase (LDH) enzymes activities were increased, while RBC acetylcholinesterase (AcChase) activity and reduced glutathione (GSH) levels were decreased. RBC glutathione reductase (GSSRase) activities were not significantly altered. Serum GSSRase activity, however, was significantly decreased while serum vitamin E, and lipid peroxidation levels were significantly increased. These alterations tend to disappear gradually but were still detectable two weeks following exposure.

The possible role of these changes in the adaptation to the toxic effects of inhaled oxidants was discussed.

The toxicity of inhaled ozone (O_3) is well known and the presence of the oxidant in many urban and industrial environments has made the study of the biological effects of inhaled O_3 important.[1] Recent studies have been directed toward the elucidation of the biochemical changes which occur in tissues following O_3 exposure. Radiomimetic changes were described[2] as well as the oxidation of unsaturated fatty acids,[3] and oxidation of biologically active reducing substances such as reduced sulfydryl groups and the co-factors NADH and NADPH.[4] Sufficient knowledge is now available to allow speculation about the significance of some of the observed changes in the

development of acute or chronic pulmonary disease, or in the development of O_3 tolerance. Ozone is known to produce a dose-related reaction in *in vitro* cell cultures extending from mild metabolic suppression at low levels to cell death at high levels.[5] It is natural that the target organ for O_3 toxicity studies is the lung,and much of our knowledge derives from studies of effects of oxidants in this organ. The results point to loss of reduced sulfhydryl groups and reduction of activities of sulfhydryl-containing enzymes, while the pentose and glycolytic pathway activity levels are increased.[6,7,8] Oxidant effects of O_3 inhalation have also been shown to occur in blood of rodents following high, but sublethal levels of the irritant, and changes in erythrocyte (RBC) and sera also suggest that oxidant-induced alterations have taken place beyond the blood-air barrier.[9,10]

It is not known if O_3 at ambient levels crosses the air-blood barrier in humans or if detectable biochemical changes occur. The present study was undertaken to answer that question.

Methods and Materials

Seven healthy young adult human male volunteers were studied. The same chamber was used for sham control exposures and for exposure to O_3. The chamber contents are monitored for O_3, NO_x, CO, hydrocarbons and particles. Pollutant levels were essentially zero during sham exposures, and chamber background particle levels (0.5-5μ) were less than 20,000/ft.^{3} when empty, and less than 120,000/ft.^{3} during subject exercise. Subjects performed identical exercise and pulmonary function tests during a sham control period and while O_3 was being administered. During exposure, a 0.5 ppm atmosphere of

O_3, produced by a silent-arc generator, was added to the chamber air supply. Chamber temperature was maintained at 86°F and relative humidity at 35% to simulate a typical summer day in Los Angeles.

Venous blood samples were collected in heparinized and unheparinized tubes immediately after the sham and O_3 exposure. Heparinized blood was stored on ice until the erythrocyte (RBC) studies could be completed. The unheparinized blood was allowed to remain at 2-4°C for 4-6 hours until the serum could be removed.

Experiments were planned to detect blood tissue oxidation after O_3 inhalation since this has been shown to be the principal effect of high levels of the irritant on lung and blood tissues of experimental animals. The methods are essentially the same as described in the following references with only minor modifications. RBC membrane fragility was measured by determining the degree of hemolysis in the presence of H_2O_2.[11] Activities of RBC enzymes glucose-6-phosphate dehydrogenase (G6PDH),[12] lactate dehydrogenase (LDH),[13] glutathione reductase (GSSRase),[14] and acetylcholinesterase (AcChase),[15] were measured as well as red cell glutathione levels.[16] Serum levels of vitamin E,[17] and lipid peroxides[18] were determined as well as activity levels of the serum enzyme glutathione reductase.[19]

Each experimental subject served as his own control and paired-group analyses were performed. The small students "t" test was used to test the null hypothesis with the critical level at $p \leq 0.05$.

Results

Data in Figures 1-4 are means and standard errors of the means, of paired-group analyses of seven experimental subjects.

The evidence indicating O_3 - induced changes in the RBC membrane is shown in Figure 1. The single O_3 exposure resulted in a significant increase ($p < 0.001$) in RBC fragility to H_2O_2, while the activity of the membrane-bound enzyme AcChase was decreased ($p < 0.001$). Figure 2 shows that the activity of G6PDH was significantly increased ($p < 0.001$) while RBC levels of GSH were decreased ($p < 0.01$). Ozone inhalation also stimulated an increase in LDH activity ($p < 0.001$) although red cell GSSRase activities were not altered. (Figure 3). Serum GSSRase activity levels are shown on Figure 4 to be significantly decreased ($p < 0.05$). Vitamin E levels were also increased ($p < 0.025$) at the same time that increased oxidation of unsaturated fatty acids ($p < 0.025$) were observed.

Discussion

The very high oxidation potential of O_3 has led research workers from the beginning to suspect that the major damage from inhalation of this irritant was due to oxidation of labile components in biological systems to produce structural or biochemical lesions.[20] More recent work has verified that inhaled O_3 causes oxidation of components of rodent lung.[21] Other studies have shown that changes in the rate of tissue metabolism also accompany O_3 - induced changes in the oxidation states of lung tissue components.[22]

This study was necessitated by our need to seek evidence of changes in human tissues due to inhalation of ambient levels of O_3, and was encouraged by evidence from past experiments which showed that significant alterations in the blood tissue of rodents did occur as a result of inhalation of oxidant levels, although the levels were generally much higher than ambient levels anticipated during a smoggy day. Experiments performed by others,[18] and in our laboratories,[23,24] have shown that inhalation of high levels of O_3 by rodents results in oxidation changes in blood similar to those detected in lung. Considerable question arose about possible metabolic changes in blood following low-level O_3 inhalation since it is known that the efficiency of the upper airway in the removal of O_3 is quite high, and that the efficiency increases as the levels of inhaled O_3 decrease.[25] The results indicate that a single exposure to 0.5 ppm for 2 3/4 hours is above the threshold level under the conditions of the experiment.

The observed changes suggest that oxidation is the initial event, and that additional changes occur as a result of the systems attempt to compensate for the changes in blood tissue redox potentials. Reduced glutathione, considered to be an important biological antioxidant, is significantly decreased by O_3 inhalation. The RBC enzyme acetylcholinesterase (AcChase), containing a -SH group essential for its' activity is also depressed. The increase in the presence of perioxidized lipids and increase in vitamin E in the sera also suggest that oxidation is responsible for the primary O_3

response. Glutathione reductase (GSSRase) activities showed no change in RBCs while the serum enzyme activities were decreased. We have no explanation for this observation except to suggest that the RBC enzyme may be protected by its site within the cell.

The increases in glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) activities suggest that RBC metabolism is stimulated by O_3. The increase in G6PDH activity may also be related to the adaptation phenomenon described by others.[22] One of the possible pathways by which tissue levels of GSH are maintained is schematically represented in Figure 5. An increase in G6PDH activity could provide reduced cofactor essential for the function of GSSRase. The amounts of GSH normally present in human tissues is small compared to many other species of higher animals but the levels of GSSGrase were higher in humans (RBCs) than in any specie tested. RBC levels of GSSRase were not decreased by O_3 exposure so the mechanism for the regeneration of NADPH appears to be unaltered. The increase in G6PDH activity has been shown in other experiments (unpublished) to persist for a period of at least two weeks following O_3 stimulation. It will be important in future experiments to follow the time course of the O_3-induced blood changes in order to determine the length of time they persist and if they are related to the adaptation phenomenon observed in laboratory animals.

The increase in serum vitamin E levels probably results from the adapture response of the whole organism resulting in the availability of increased levels of circulating antioxidants. This mobilization of vitamin E again suggests that O_3, or oxidizing free-radical, does pass the air-blood

barrier at the comparatively low levels used in the experiment. Whether vitamin E acts alone to inhibit lipid peroxidation,[27] acts in concert with vitamin A or other substances to protect tissue from oxidation,[28] or functions in some other way, there is now little doubt of its' protective function[23,24,29] in animals exposed to a strong oxidant such as ozone. It would be important in a future experiment to determine if ingestion of relatively large amounts of vitamin E would protect humans against the biochemical effects of O_3.

Future experiments will determine the level of inhaled O_3 below which no observable changes occur in humans. Knowledge of these "threshold" levels will be useful in determining the acceptable and permissible atmospheric standards. It will also be important to establish the length of time required for changes to disappear, as well as to determine if successive exposures produce cumulative effects. A very wide range of subjective reactions to the oxidizing air pollution is expressed by people living in cities where this type of smog is severe. It will be important to seek possible correlations between the degree of subjective irritative response and biochemical change, to help determine if differences exist in peoples' capacity to resist O_3.

Comments on Significance of Biochemistry Studies on Blood of Humans Breathing Air Pollutants

The biochemical changes so far observed in human blood during our current set of experiments are assumed to be due primarily to oxidant effects since that assumption best fits the data. Also, the magnitude of the changes seem to be directly related to ozone levels, and oxidation is thought to be the chief biological effect of this irritant.

We do not know if the biochemical changes can be called "illness" because we do not yet know what effects these changes might have on normal body function. We have only studied "healthy" young adult males and we do not know what the metabolic patterns are for erythrocytes of people who have well-advanced pulmonary disease. We do not yet know if the metabolic pathway for reconstitution of RBC reduced glutathione is intact and functioning in these people.

BIBLIOGRAPHY

1. Stockinger, H.E.: Evaluation of the hazards of ozone and oxides of nitrogen. *Arch. Industrial Health* 15: 181-190, 1957.

2. Menzel, D. B.: Toxicity of ozone, oxygen and radiation. *Ann. Rev. Pharmacol.* 10: 379-384, 1970.

3. Roehm, J. N., J. G. Hadley, D. B. Menzel: Oxidation of unsaturated fatty acids by ozone and nitrogen dioxide: A common mechanism of action. *Arch. Environ. Health,* 23: 142-148, 1971.

4. Menzel, D. B.: Oxidation of biologically active reducing substances by ozone. *Arch. Environ. Health,* 23: 149, August 1971.

5. Pace, D. M., P. A. Landolt, B. T. Aftonomos: Effects of ozone on cells *in vitro*. *Arch. Environ. Health,* 18: 165, Feb. 1969.

6. DeLucia, A. J., P. M. Hoque, M. G. Mustafa, and C. E. Cross: Ozone interaction with rodent lung: Effect on sulfhydryls and sulfhydryl-containing enzyme activities. *J. Lab. Clin. Med.,* 80(4): 559-566, October 1972.

7. Chow, C. K. and A. L. Tappel: Activities of pentose shunt and glycolytic enzymes in lungs of ozone-exposed rats. *Arch. Environ. Health,* 26: 205-208, April 1973.

8. Chow, C. K. and A. L. Tappel: An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. *Lipids,* 7: 518-524, 1972.

9. Goldstein, B. D., R. D. Buckley, R. Cardenas, and O. J. Balchum: Ozone and vitamin E. *Science,* 169: 606-606, 1970.

10. Goldstein, B. D., B. Pearson, C. Lodi, R. D. Buckley, and O. J. Balchum. The effect of ozone on mouse blood *in vivo*. *Arch. Environ. Health,* 16: 648-650, May 1968.

11. Younkin, S., I. A. Oski, and L. A. Barness: Mechanism of the H_2O_2 hemolysis test and its reversal with phenols. *Am. J. Clin. Nutrition* 24: 7-13, 1971.

12. Lohr, G. W. and H. D. Waller: In methods of enzymatic analysis.- Bergmeyer, H. V. *Academic Press*, pages 744-751, 1963, New York and London.

13. Bergmeyer, H. V., E. Bernt, and B. Hess: In methods of enzymatic analysis - Bergmeyer, H. V. *Academic Press*, Pgs. 736-741, 1963, New York and London.

14. Beutler, E.: Effect of flavin compound on glutathione reductase activity. *J. Clin. Invest.* 48: 1957, 1969.

15. Ellman, G. L., K. D. Courtney, V. Andress, and R. M. Featherstone: A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7: 88-95, 1961.

16. Beutler, E., O. Duron, and B. M. Kelly: Improved method for determination of blood glutathione. *J. Lab. Clin. Med.*, 61(5): 882-888, 1963.

17. Bieri, J. G., and E. L. Prival: Serum vitamin E determined by thin layer chromatography. *Proc. Soc. Biol. Med.*, 120: 554-577, 1965 and TSEN, C.C.: An improved spectrophotometric method for the determination of using 4, 7 - diphenyl.-1, 10-phenanthroline. *Anal Chem.* 33(7): 849-851, 1961.

18. Mengel, C. E. and H. E. Kahn, Jr.: Effects of *in vivo* hyperoxia on erythrocytes III. *In vivo* peroxidation of erythrocyte lipid. *J. Clin. Invest.*, 45(7): 1150-1158, 1966.

19. Horn, Hans-Dieter: Methods of enzymatic analysis - Bergmeyer, H.V. *Academic Press*, pgs. 875-879, 1963, New York and London.

20. Stokinger, H. E. and D. L. Coffin: Biologic effects of air pollutants In: Stern, A. C. Ed.: Air Pollution. Ed. 2. New York. *Academic Press*, Vol. 1, 401-546, 1968.

21. DeLucia, A. J., P. M. Hoque, M. G. Mustafa, and C. E. Cross: Ozone interaction with rodent lung: Effect on sulfhydryls and sulfhydryl-containing enzyme activities. *J. Lab. Clin. Med.*, 80(4): 559-566, 1972.

22. Chow, C. K. and A. L. Tappel: Activities of pentose shunt and glycolytic enzymes in lungs of ozone-exposed rats. *Arch. Environ. Health*, 26: 205-208, 1973.

23. Goldstein, B. D., C. Lodi, C. Collinson and O. J. Balchum: Ozone and lipid peroxidation. *Arch. Environ. Health*, 18: 631-635, 1969.

24. Goldstein, B. D. B. Pearson, C. Lodi, R. D. Buckley, and O. J. Balchum: The effect of ozone on mouse blood *in vivo*. *Arch. Environ. Health*, 16: 648-650, 1968.

25. Yokoyama, E., and R. Frank: Respiratory uptake of ozone in dogs. *Arch. Environ. Health,* 25: 132-138, 1972.

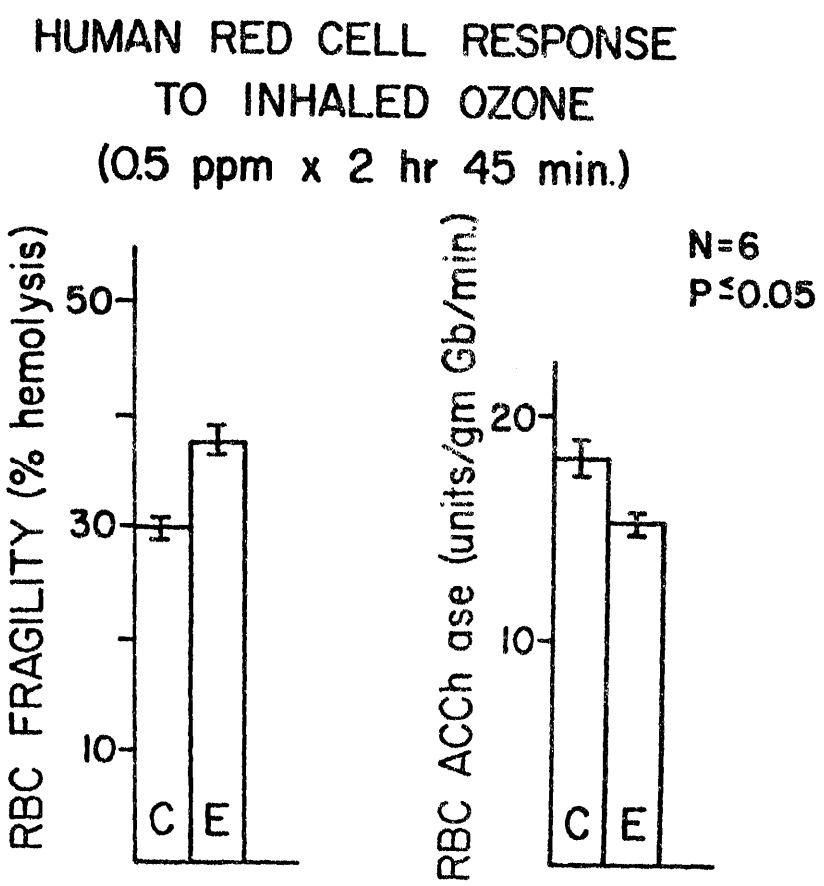
26. Lankish, P. G., R. Schroeter, L. Lege, and W. Vogt: Reduced glutathione and glutathione reductase - a comparative study of erythrocytes from various species. *Comp. Biochem. Physiol.,* 46B: 639-641, 1973.

27. Kann, H. E., Jr., C. E. Mengel W. Smith, and B. Horton: Oxygen toxicity and vitamin E. *Aerospace Medicine,* 35: 840-844, 1964.

28. Menzel, D. B.: Vitamins A and E help maintain lung health. *Chemical and Engineering News,* 48(27): 38-39, 1970.

29. Menzel, D. B., J. N. Roehm, and S. Duk Lee: Vitamin E: The biological and environmental antioxidant. *J. Agr. Food Chem.,* 20(3): 486-490, 1972.

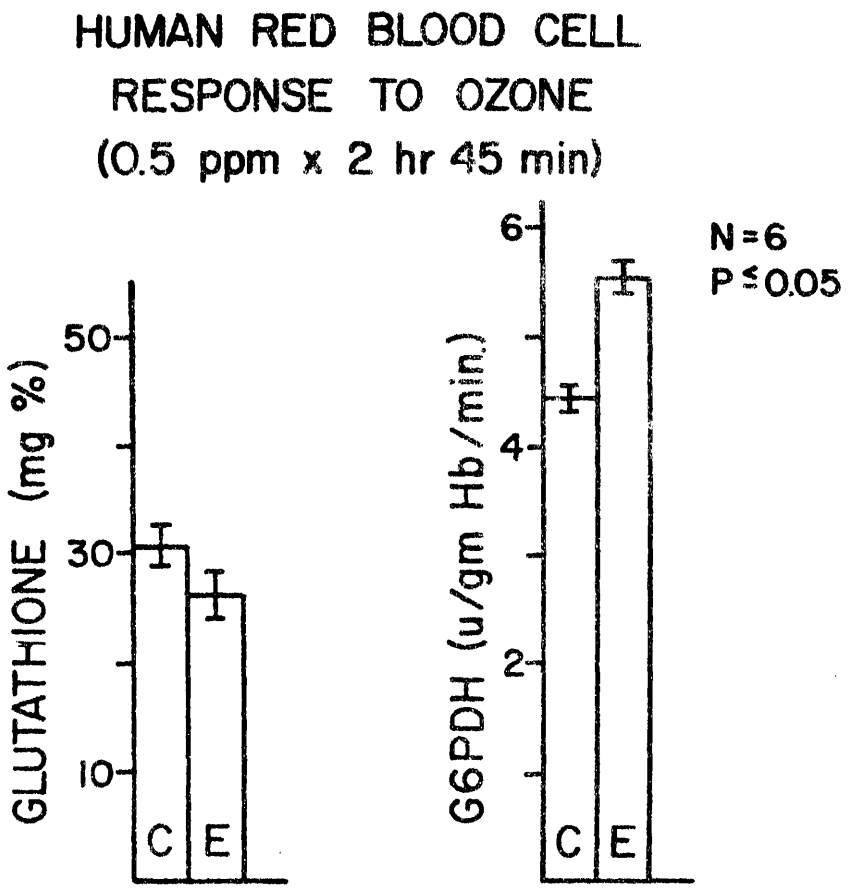
FIGURE 1



Fragility was measured as % hemolysis in 2% hydrogen peroxide and incubated for 1 hour at pH 7.4 in Krebs Ringer bicarbonate buffer.

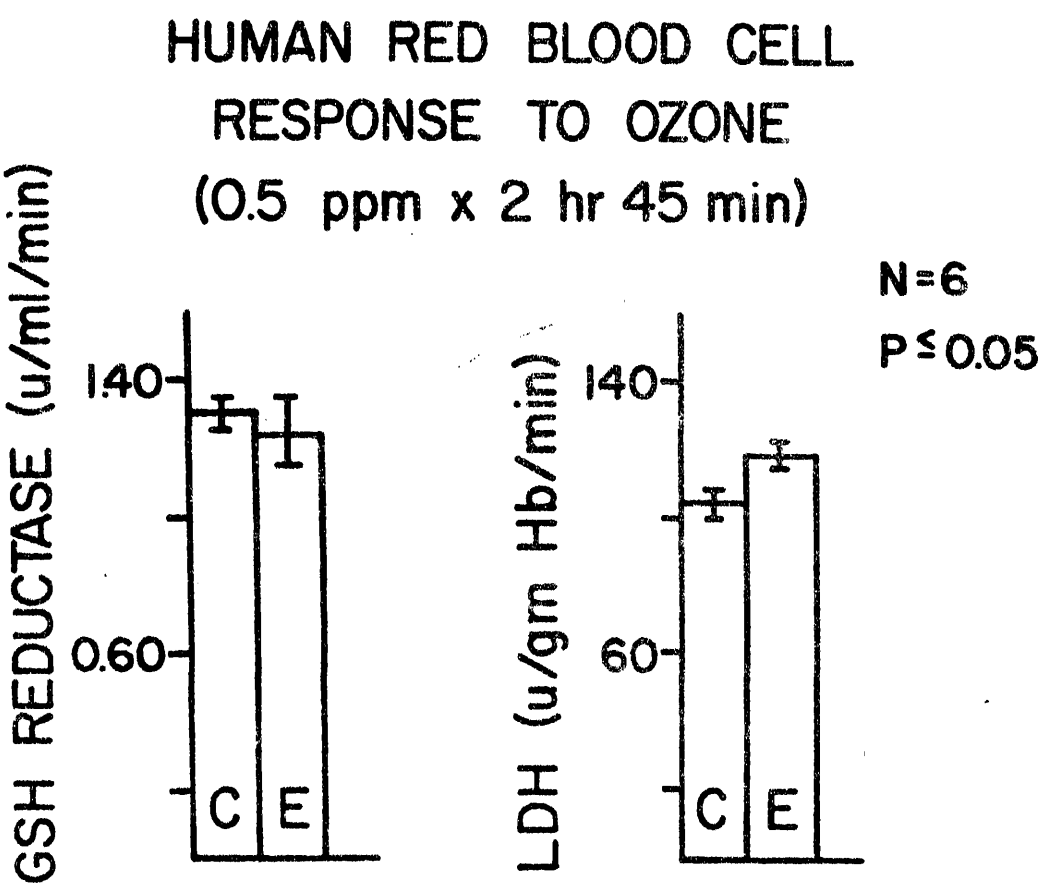
Acetylcholinesterase was measured at pH 8.0 in 0.1 M. phosphate buffer employing acetylthiocholine as substrate. Activity is expressed as mM/ml blood/min.

FIGURE 2



Glutathione assay detects soluble GSH employing 5, 5' dithiobis (2-nitrobenzoic acid) as coupling reagent.

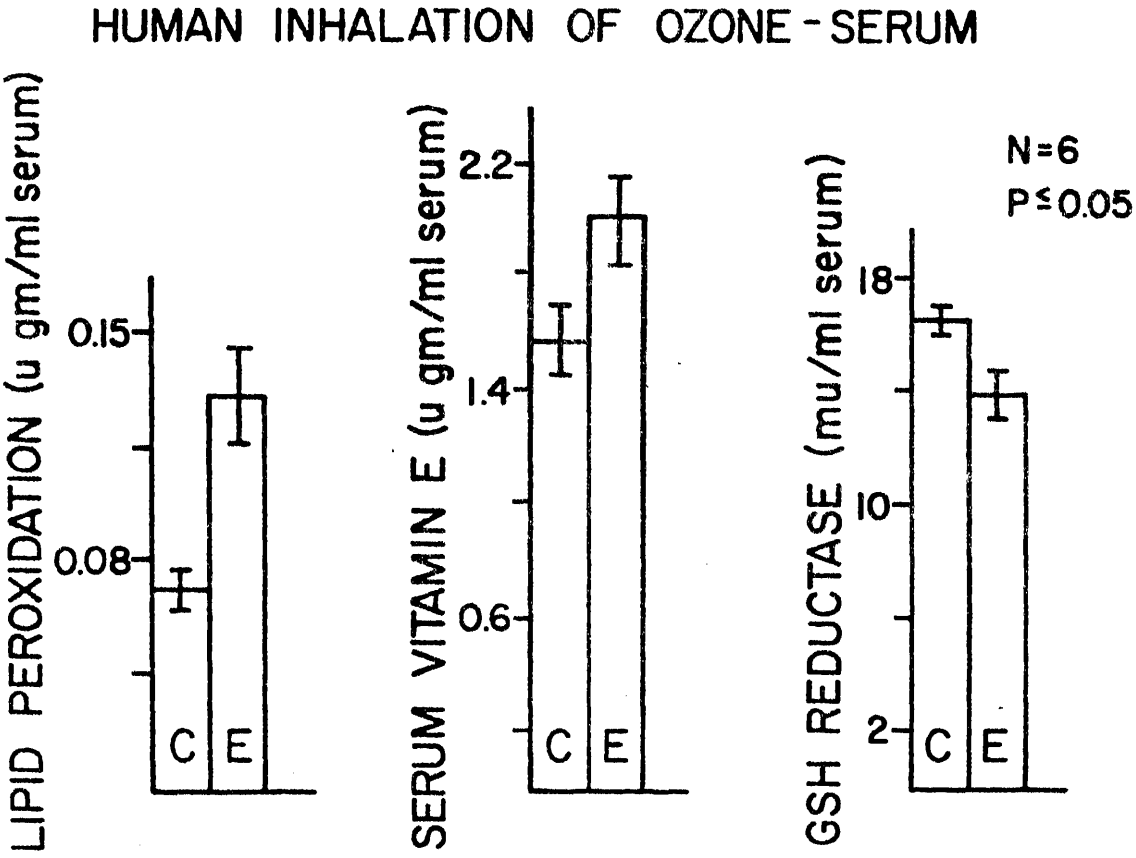
FIGURE 3



Glutathione reductase activity was expressed as international units/ml of blood/min. Oxidized glutathione was used as substrate.

Lactate dehydrogenase activity was measured by following the disappearance of NADH at 340 nm and was expressed as international units/gl hemoglobin/min.

FIGURE 4



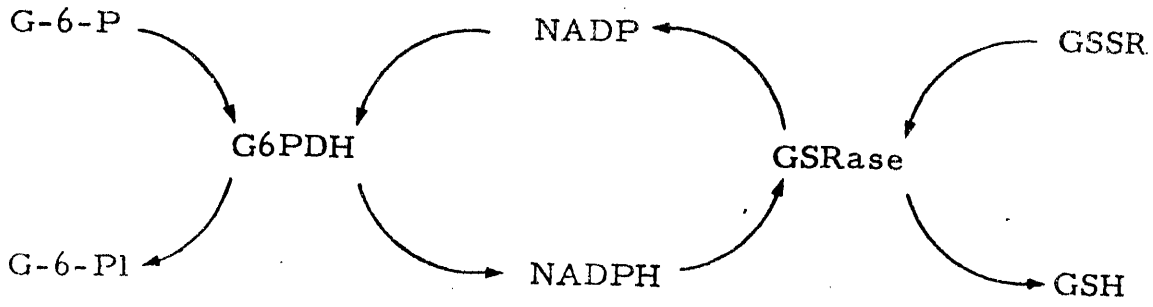
Lipid peroxidation is expressed as micrograms malonaldehyde per ml serum.

Vitamin E is expressed as microgram alpha tocopheral per ml serum.

Glutathione (GSH) reductase activity was expressed as m units/ml serum/min.

FIGURE 5

POSSIBLE METABOLIC PATHWAY BY WHICH NORMAL ERYTHROCYTE REDOX LEVELS COULD BE MAINTAINED



CHAPTER V

EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS:

V -- PSYCHOPHYSIOLOGICAL AND PSYCHOMOTOR ASSESSMENT

E. Eugene Pedersen

Peter Breisacher

Julie Patterson

Jack D. Hackney

ABSTRACT

Human subjects have been tested in an environmental control chamber under conditions simulating a smoggy summer in the Los Angeles Coastal Basin. The pollutants studied were ozone (O_3), nitrogen dioxide (NO_2) and carbon monoxide (CO) together with elevated temperature. A divided attention task was given at the end of the exposure period. The subjects' heart rate variability, a potential psychophysiological measure of attention, was also evaluated. Subjects were run in three different groups. (Groups 1, 2, and 4)

Subjects displayed a significant decrement in peripheral attention associated with elevated ambient temperature. Effects attributable to pollutant gases were variable. Subjects in Group 1 (4-5 hr. exposure to 0.5 ppm O_3 + 0.5 ppm NO_2 + 30 ppm CO) showed some decreased attention when exposed to the mixed pollutants. This occurred in the ability to detect stimuli in the periphery. Subjects in the fourth group (2 hr. exposure to 0.25 ppm O_3, 0.30 ppm NO_2, and 30 ppm CO) displayed a

decreased ability to perform the central attention task when exposed to the mixed pollutants. However, a decrement in peripheral attention was not shown. Subjects in the second group (4-5 hr. exposure to 0.5 ppm O_3) showed only marginal effects. These subjects, however, were not exposed to the mixed pollutants with CO.

EXPERIMENTAL STUDIES ON HUMAN HEALTH EFFECTS OF AIR POLLUTANTS: V -- PSYCHOPHYSIOLOGICAL AND PSYCHOMOTOR ASSESSMENT

A number of authors[1,2,3] have argued that measures of behavioral change are more sensitive to the effects of air pollutants than other parameters such as frank physiological or clinical changes. If this is true such changes should be taken into consideration when establishing air quality standards. Further, it may be argued that behavioral assessment is important in that the measures generally involve familiar activities and skills that people are called upon to perform in their daily lives. Therefore, the study of the performance of such tasks under adverse or less than optimum conditions would be of considerable interest. If the ability to perform routine tasks such as the operation of an automobile or a complex piece of machinery is compromised under pollutant exposure, then standard setting procedures should take such effects into consideration. This becomes particularly important when performance of skilled operations such as driving an automobile on or near freeways or heavily traveled thoroughfares where the pollution

levels have been shown to reach high levels and where detrimental effects are even more likely to occur.

Detrimental effects have been reported from extremely low levels of CO using a time estimation task,[4] and various psychomotor and cognitive tasks.[5] The levels of CO found to show effects were estimated in the former case to produce a concentration of only two percent COHb in the blood while in the latter the levels were around three percent COHb. Others have reported effects using a variety of visual parameters during low level exposures to ozone,[6] and shown decrements similar to CO effects using the visual evoked response with animals.[7]

However, other workers[8,9] have been unable to obtain effects with performance measures at even higher concentrations. The differences in these results are probably due to the type of subjects used, motivation of individuals, and procedural differences, including the length of time during which the subjects were tested. The latter is known to be a key issue in other areas of stress research.[10]

A most important consideration is the control of environmental conditions, such as ambient temperature, humidity, presence of other pollutants, noise, or other potential stressor agents that may be present during the actual test.

This study investigated the effects of atmospheric pollution upon a divided attention task. One reason for selecting this task is that it is relatively more complex than conventional performance tasks and thus may be more vulnerable to the influence of environmental stressors.[11] Similar performance measures have been used with thermal stress.[12,13] It has been shown that performance on a central tracking task will show little or no decrement from heat stress, but with increasing levels of temperature, the subject will begin to display a type of behavioral compensation involving a decreased ability to attend to and consequently detect visual stimuli in the periphery. This effect has been identified as a "funneling of attention" and is believed to be a principal result of heat stress. The applicability of the divided attention task to such skills as the operation of a motor vehicle where peripheral

attention is extremely critical is apparent. Similar divided attention tests have also been studied in relation to the effects of exposure to realistic levels of CO.[14,15,16] The test apparatus used in the current study was patterned after an earlier apparatus.[12]

The study has also included an effort to obtain data using a potential psychophysiological measure of stress. The measure is based upon changes in beat-to-beat heart rates (HR) or sinus arrhythmia. Previous studies have focused upon heart rate variability (HRV) as a measure of attention and conditions resulting in decreased ability to attend to incoming stimuli.[17,18] Others have used measures of HRV as an indicator of fatigue in motor vehicle operation.[19] The arrest of sinus arrhythmia has been used as an index of "mental load" in performance of various tasks including a complex task[20] similar to that used in the current work.

With increased fatigue, a decrease in attention would be expected along with a diminution of arousal level. This should be reflected in an increase in HRV or sinus arrhythmia. Contrarily, an increase in

attention and arousal level while performing a mental task should lead to a decrease of HRV.[20]

The simultaneous evaluation of both physiological and behavioral parameters is important and affords the opportunity to delineate isolated and possibly synergistic effects of both mental and physical decrements that could significantly deteriorate important performance functions.

This study was a part of a larger research program which has as a principal objective the detection of potential changes in a variety of cardio-pulmonary parameters occurring in individuals exposed to realistic levels of mixtures of air pollutants and elevated temperatures. The environmental conditions under which these performance tasks were conducted were therefore controlled by the needs of the physiological studies. The conditions were designed to simulate a smoggy summer in the Los Angeles Basin. This was therefore an environment simulating that in which many people are required to work and perform a variety of complex sensory or judgment tasks on a daily basis. The hypotheses that were tested include:

1. That measures of central and peripheral attention will show effects of exposure to low levels of pollutants and elevated temperature especially evidenced by lengthened reaction times and reduced ability to detect stimuli in the periphery.
2. The effects of heat stress will be reflected in increasing heart rate variability which will also be correlated with the reduction in performance of the attention tasks.
3. Similar effects will be shown with exposure to the pollutants -- particularly CO.

METHODS

Thirteen male volunteer subjects were tested (and two have been retested) in the previously described chamber[21] for either a two-hour exposure period or a four-five hour exposure period. Table 1 presents a summary description of the three groups of subjects that were investigated.

During the exposure or sham runs, the subjects alternately exercised on a bicycle ergometer (or briskly paced) and rested for

15-minute intervals. The detailed protocol was presented earlier.(21) At the completion of the final exercise period the subject rested for ten minutes and then began the performance test, which lasted a total of 15 minutes. The initial three minutes were provided to allow the subject to adjust to the darkened test chamber and the test was carried out during the final twelve minutes. The test was administered under the following conditions:

1. Normal room temperature ($72^{o}F$) and clean environmental conditions.
2. Under elevated temperature ($88\text{-}90^{o}F$) but without pollutants present.
3. Under elevated temperature together with exposure to O_3 (.50 ppm) + NO_2 (.50 ppm) + CO (30 ppm), or elevated temperature together with three levels of O_3 (0.25, 0.37, or 0.50 ppm). This was done in an effort to describe a possible dose-response relationship. A final group was tested under O_3 (.25 ppm) + NO_2 (.30 ppm) + CO (30 ppm).

Central and Peripheral Attention Task

The test apparatus was designed so that performance evaluation could be conducted in the chamber without interfering with other on-going testing activities. This design feature was important for economy of operation, allowing for a number of subjects to be run simultaneously in the chamber. The apparatus was a large "look-in" plexiglass chamber designed to eliminate extraneous visual stimuli. The apparatus was devised so that other subjects would be unaware of the individual's performance, therefore controlling for the operation of certain psycho-social influences such as competition. The subjects wore ear plugs and a Mine Safety Appliance (MSA) soundproofing headset to control for extraneous auditory stimuli in the chamber. Preliminary tests indicated the influence of surrounding auditory stimuli could be effectively controlled in this way.

The subject was placed in front of the apparatus in a "head harness" to maintain the head in a standard position. One hand was positioned on the control handle for the tracking task. At the same time, the

subject was required to press one of six response buttons positioned immediately under his other hand when one of the six peripheral lamps (Type 222 General Electric miniature lamps, 2.25 volts at .25 amperes) was energized. The central attention test consisted of a tracking task in which the subject was instructed to follow a moving target in the center of the apparatus (24 inches in front of subject's eyes) by keeping a light beam in the center of a visible light-sensitive target. The object traveled in a random fashion, making 35 excursions per minute. The amount of time the beam was on target was determined by recording the signal from the light sensitive cell using a strip chart recorder. The peripheral lamps were located 23 inches from the central face plate at angeles of 20^{o}, 50^{o}, and 80^{o} from the center on both sides of the central tracking target. The lamps were programmed to be activated in a random sequence at a rate of six per minute, and stayed lit for two seconds. Lack of response after this time interval was scored as an error of omission. Programming and timing of reaction times and responses were accomplished using an Iconix Logic Cabinet

Set (Models 6171-1600) and Iconix Time Base Controller and Counter (Models 6255-6010). The subject's task was to press the appropriate response button as rapidly as possible and to make every effort not to miss any lamps. The subject's performance was recorded and the programming of events was conducted by the attending technician stationed outside the chamber. The technician provided a signal prior to the onset of each test session.

The following aspects of performance were recorded:

1. Accuracy in tracking performance (i.e., percent of time on target);
2. Average response latency for each peripheral light;
3. Number of errors for each light including both errors of omission and commission.

Subjects were given preliminary instruction in the testing procedures in an effort to ensure their familiarity with the test. During the preliminary sessions an effort was made to determine the subject's ability to detect visual stimuli in the extreme periphery. Subjects were instructed to focus their attention on the central task and to

respond to the peripheral lamps only when they were noticed. This instruction was added so that the tracking would indeed represent a central task for the subject. Such precaution is necessary in order to ensure obtaining the "funneling" effect.(22)

Heart Rate Variability (HRV)

EKG electrodes were attached prior to the test using a two-lead placement configuration with the active electrode at the V_5 chest position and the reference on the manubrium of the sternum. This arrangement was used to optimize the signal and to minimize noise.(23) The skin surfaces under the electrodes were prepared with alcohol washing. EKGs were recorded during the final one-minute segments of each of the three four-minute blocks of the performance task. This was done to relate changes in HRV with fatigue. Also, for comparison, the subjects were requested to rest in a supine position on a hospital gurney in the darkened, quiet chamber at $72^{o}F$ for one-half hour. During the final one-minute portion of this period, the subject's resting HR was also recorded.

A measure of HRV was derived from the moment-to-moment fluctuations in HR. Specifically, HR's were measured for each R-R interval of the EKG record, and the differences between HR's for adjacent intervals were tabulated. The average difference was calculated for each of the recording periods and compared to the resting HRV.

RESULTS AND DISCUSSION

One of the principal effects of elevated ambient temperatures is thought to be a reduced capability to perform tasks requiring close attention. Accordingly, the results from this study will be considered initially from the standpoint of stress effects due to heat followed by an appraisal of the combined effects of air pollution and thermal stress. Data will be pooled for all subjects for the thermal stress analyses. Since three different groups of subjects have been run under different air pollutant challenge conditions, data from each of these groups will be considered separately.

Thermal Stress Effects

Results of analyses done with the performance data under elevated temperature as compared with normal temperature are presented in Table 2 and in Figures I and II. A significant increase in reaction times was evident for the total group at four of six lamp positions. For lamps 1 and 6 (i.e., 80^{o}L and R) the results of the t tests for correlated

measures were 2.16 and 2.92 ($p \leq .05$ and $p \leq .02$, respectively). The t for lamp 2 (50^{o}L) was <1.00 but for lamp 5 (50^{o}R) the t was 2.80 ($p \leq .02$). For the two middle lamp positions (3 and 4, 20^{o}L and R) the results of the t tests were 3.86 ($p \leq .01$) and 1.27 ($p > .05$). The percent of missed lamps showed significance only at the extreme peripheral (80^{o}) positions, confirming earlier findings that "funneling" of attention occurs under thermal stress.[12,13] The t for lamp 1 was 2.87 ($p \leq .02$) and for lamp 6 the t was 2.21 ($p \leq .05$). Consideration of the number of wrong responses revealed no significant differences. HRV during performance as a percent of resting HRV was greater under elevated temperature than under normal temperature but this difference was not significant. Tracking performance was quantitated only for Group 4, and evidence for significant decrement in performance occurred when subjects in this group were exposed to elevated temperatures. The measure of performance was the percent of time on target. Under the normal temperature condition the subjects were found to stay on target

an average of 45.1 percent of the time while under the elevated temperature condition, the subjects were on the target an average of 30.9 percent of the time ($t = 5.78$, $p \leq .01$).

COHb Determinations

As a part of the physiological test protocol, subjects were administered a 20-second breathhold test to estimate COHb concentration both prior to and upon completion of each daily run. Details of the test have been described previously.[21] The estimation of COHb from expired CO concentration is based upon the method of Gaensler and co-workers.[24] Table 3 contains the results of this test for each of the subject groups. Group 1 which was exposed to the mixed pollutant challenge containing CO displayed a significant elevation of the estimated COHb level at the completion of the exposure days (3-4 percent COHb). The Group 2 subjects which were not exposed to CO generally showed an expected drop in COHb levels. Group 4 which received the mixed pollutant exposure displayed a significant increase

in the COHb. These levels are comparable to the concentrations at which behavioral decrements have been reported by some previous workers.[4,5]

Pollution Plus Thermal Stress

Data from Group 1 subjects reveal trends that can be associated with both thermal stress and exposure to the mixed pollutant challenge (i.e., 0.50 ppm O_3 + 0.30 ppm NO_2 + 30 ppm CO). Reaction time or the amount of time required to respond to the peripheral lamps, was increased under exposure to elevated temperature (see Table 4 and Figures III and IV). Results of one-way analyses of variance and Neuman-Kuels[25] tests revealed significant changes in reaction time for lamps 1 and 6 (i.e., 80^{o}L and R) ($F = 4.74$, $df = 2, 94$, $p < .01$ and $F = 14.00$, $df = 2, 86$, $p < .01$) and lamp 5 (50^{o}R) ($F = 11.60$, $df = 2, 84$, $p < .01$), but not for lamp 2 (50^{o}L). The increase in reaction time from normal temperature to elevated temperature was significant for the two extreme lamp positions ($p \leq .05$ and $p \leq .01$,

respectively) and lamp 5 ($p \leq .01$), but was not significant for the two central lamp locations (20^{o} R and L). There were also significant increases in reaction time from normal temperature to exposure to pollutant mixtures for lamps 5 and 6 ($p < .01$) and for lamp 1 ($p < .05$), but reaction time under elevated temperature was not significantly different from reaction time under mixed pollutant exposure. With the two central lamp locations (i.e., lamps 3 and 4, 20^{o}L and R; $F = 6.23$, $df = 2, 94$, $p < .01$ and $F = 13.70$, $df = 2, 90$, $p < .01$, respectively) there was a significant increase in reaction time from elevated temperature to exposure to pollutant mixture ($p \leq .05$ for lamp 3 and $p \leq .01$ for lamp 4), and also significant increases in reaction time from normal temperature to pollutant exposure (both $p < .01$). There was no significant difference, however, between reaction times under normal and elevated temperature exposure for the central lamp positions. The increase in reaction times under thermal stress or thermal plus pollution stress appears to be greatest for the extreme lamp positions. Thus, with respect to the

reaction time data the concept of "funneling of attention" has been borne out. This is of considerable importance since safe operation of an automobile requires close attention and rapid response to stimuli occurring in the peripheral visual fields.

Review of the data dealing with the percent of trials in which no response occurred (i.e., errors of omission) indicates similar trends. There were significant increases in the percent of non-responses at the two extreme positions (80^{o}R and 80^{o}L). For lamp 1 (80^{o}L) ($F = 3.83$, $df = 2, 22$, $p < .05$) the difference in percent of omitted responses between the sham at normal temperature and mixed pollutant exposure was significant ($p \leq .05$) but the increase from normal to elevated temperature was not significant. For lamp 6 (80^{o}R) ($F = 5.89$, $df = 2, 22$, $p < .01$) there was a significant increase in the percent of omitted responses when subjects were exposed to elevated temperature as compared to sham under normal temperature ($p \leq .05$) and a significant increase from normal temperature when subjects were exposed to the mixed pollutants ($p \leq .05$). However,

the performance under mixed pollutants for lamp 6 was not significantly different from performance under elevated temperature.

The HR data showed some significant differences when comparing the resting values with exercise and elevated temperature and pollutant exposure results ($F = 31.10$, $df = 3, 9$, $p < .01$). The trends are in the predicted direction, i.e., significant increases (all $p < .01$) in HR from 74.6 (±6.7) beats per minute at rest to 81.8 (±8.0) at sham under normal temperature to 87.0 (±7.6) when temperature was increased. HR under pollutant exposure (84.5±6.4) was also significantly increased over HR during rest ($p < .01$), and there was a significant increase in HR from normal temperature to elevated temperature ($p < .05$). HRV data show some changes in the expected direction but these were fairly small and non-significant. There was a non-significant decrease in sinus arrhythmia from resting values to testing under normal conditions. In addition, the HRV was found to increase slightly but non-significantly from sham with normal temperature to sham under elevated temperature and to testing

with pollutant exposure (HRV was 2.10 at rest, 1.94 under normal temperature, 2.06 under elevated temperature, and 2.08 under pollutant exposure).

Data for subject Group 2 present some relationships that depart somewhat from the Group 1 data (see Table 5 and Figures V and VI). The results showed only marginal increases in reaction time under thermal stress and exposure to .50, .37, and .25 ppm O_3 (i.e., significance was obtained only for the lamp 2 position or $50^{o}L$ ($F = 5.01$, $df = 4, 44$, $p < .01$) where there were significant increases in reaction time from normal temperature to elevated temperature and to exposure to .50 ppm O_3 (both $p \leq .05$)). However, at the low pollution levels employed only marginal effects would be expected. Also, the "percent of errors" and "no response" data failed to show as clear an effect as before and none of these differences was significant. One possible reason for the difference is the discrepancy in the average age of the two groups. The first group of subjects was older and thus may have been relatively more influenced by

environmental stress. The second group of subjects was selected from younger age groups and, therefore, possibly more able to perform at high levels of efficiency under environmental stress conditions. Moreover, the second group developed severe clinical symptoms during the first week of exposure to O_3 alone and the initial protocol with mixed exposures during subsequent weeks had to be altered for medical considerations (in fact, the subjects were unable to complete the testing in the first week). Therefore, the group was not exposed to a mixture containing 30 ppm CO.

HR and HRV were obtained during the testing of Group 2. Changes in HRV were not found to be significant using a repeated measures analysis of variance. However, there was a non-significant reduction in HRV from resting values to sham at normal temperature. In addition, in 3 of 4 subjects there was an increase in sinus arrhythmia under the stress of both thermal and pollutant exposure.

Data obtained from the fourth group displayed some indication of detrimental behavior effects due to thermal stress and pollution

exposure. This was observed in the central tracking task but not in the peripheral attention task. There was a significant decline in central tracking performance when compared to performance under normal temperature ($F = 3.67$, $df = 4, 12$, $p \leq .05$). Neuman-Kuels tests for mean differences found that percent of time on target was significantly greater under normal temperature conditions than under elevated temperature with pollutant exposure (.25 ppm O_3, .25 ppm O_3 + .3 ppm NO_2, and .25 ppm O_3 + .3 ppm NO_2 + 30 ppm CO). In addition, tracking performance under elevated temperature was significantly better than performance under .25 ppm O_3 + .3 ppm NO_2 + 30 ppm CO, ($t = 2.74$, $df = 5$, $p < .05$). Performance on the peripheral attention task showed no significant decrement (see Table 6 and Figures VII and VIII).

The lack of decrement (except for percent incorrect responses, lamp 5) in the execution of the peripheral task is puzzling with this group. One factor which may account for this is that the subjects may have attended more to performance on the peripheral task

and thus the central tracking task may have in fact functioned as a secondary task. Instructions were given to the subjects stressing the importance of focusing attention on the central task but they still were observed to be overly concerned with optimizing performance on the peripheral detection task. Also, the time of exposure to the pollutants was only two hours in Group 4 whereas Group 1 received a four-hour exposure period. This may account for some of the differences in the results.

HRV values showed the expected trend for some of the subjects during rest and during the psychomotor testing under normal temperature, elevated temperature, and exposure to pollutants, although changes in grouped data were not significant according to the F-test. Thermal effects alone contributed to an increase in HRV when compared to normal temperature in 4 of the 7 subjects. The addition of pollutants contributed to an increase in HRV in 5 of the 7 subjects.

One methodological issue that must be considered is the amount of training that should be given prior to the start of the test. In Group 4, only minimal training was provided prior to the test in an effort to maximize the effects. It is known that an overly practiced task will be most resistant to the influences of environmental stressors.(10) However, the data in Group 4, showed training effects which could have submerged any effects attributable to thermal load and atmospheric pollutants. On the other hand, the second group was given training until a stable performance had been obtained. The results indicated that the task was so well practiced that the subjects' performance was completely resistant to the operation of stressors. Clearly, some compromise in terms of the amount of preliminary training will be sought. The initial group which received some familiarization with the task but not so much so that the task was "over trained" may be closer to the amount that should be used in later studies.

An added approach would be to make the peripheral task more difficult after maximum training has been administered. The aim would be to break through the "training factor" that was instrumental in making the Group 2 results resistant to environmental stress. This could be achieved by either increasing the light activating frequency or by shortening the on-time for the peripheral lights.

In summary the results obtained in the current study relating to the effects of performance under elevated temperature are consistent with previously reported findings.[12,13] There was an apparent "funneling" of attention in that the subjects were less able to attend to and detect simuli that had been presented in the peripheral fields while performing a central tracking task. This was shown both in terms of increased reaction time and an increase in the number of omitted responses. However HRV did not show significant changes from resting to elevated temperature.

With respect to the effects due to exposure to pollutants, the results are somewhat uncertain. Group 1 displayed some increase in reaction time over the increase due to heat alone at some of the lamp positions. However, these results were not shown by the second or fourth groups. Data from Group 4 did display a decrease in tracking ability after a two-hour exposure to the mixed pollutants (O_3 + NO_2 + CO).

In view of the subtle effects that are expected from simultaneous exposures to these environmental stressors, it will be necessary to further refine the methods so that the error variance can be sufficiently reduced to allow more definitive interpretation of the results.

TABLE 1

SUMMARY OF SELECTED BIOGRAPHICAL DATA FROM SUBJECTS

GROUP	SUBJECT NUMBERS	AGE (YRS)	HEIGHT (IN.)	WEIGHT (LBS.)	SMOKING HISTORY
Group I	01	49	70	185	
	06	42	69.5	185	+++
	03	36	70	185	
	04	44	74	195	+
	MEAN ± S.D.	43 ±5.37	71 ±2.09	187.5 ±5.00	
Group II	09	41	74	177	
	10	30	68	172	+++
	07	36	68.5	155	
	08	29	68	157	
	MEAN ± S.D.	34 ±5.59	69.6 ±2.92	165 ±10.90	
Group IV	07	36	68.5	155	
	16	30	72	175	
	09	41	74	177	
	11	30	72	155	++
	15	22	65	128	+++
	10	30	68	172	+++
	17	36	66	142	
	MEAN ± S.D.	32 ±6.12	69 ±3.31	158 ±18.34	

+++ regular smoker
++ occasional smoker
+ occasional pipe smoker

TABLE 2

HEART RATE VARIABILITY AND PERIPHERAL AND CENTRAL TASK PERFORMANCE UNDER NORMAL AND ELEVATED TEMPERATURES

(POOLED FOR ALL SUBJECTS)

	NORMAL TEMPERATURE	SHAM ELEVATED TEMPERATURE	t	df	p
HRV % OF REST	77.12 ± 41.54	84.34 ± 48.69	1.32	10	N.S.
REACTION TIME					
Lamp 1 (80°L)	.928 ± .179	1.058 ± .287	2.16	14	< .05
2 (50°L)	.790 ± .188	.811 ± .182	< 1.00	14	N.S.
3 (20°L)	.719 ± .135	.797 ± .152	3.86	14	< .01
4 (20°R)	.751 ± .170	.794 ± .151	1.27	14	N.S.
5 (50°R)	.779 ± .180	.864 ± .217	2.80	14	< .02
6 (80°R)	.886 ± .193	1.011 ± .263	2.92	14	< .02
% OMITTED RESPONSE					
Lamp 1	4.9 ± 3.6	14.1 ± 16.5	2.87	14	< .02
2	1.7 ± 3.1	1.3 ± 2.2	< 1.00	14	N.S.
3	0.8 ± 1.7	2.5 ± 2.6	2.11	14	N.S.
4	3.9 ± 7.8	1.3 ± 3.3	1.08	14	N.S.
5	1.1 ± 2.5	2.4 ± 4.2	< 1.00	14	N.S.
6	2.8 ± 6.6	12.0 ± 15.2	2.21	14	< .05
% WRONG RESPONSE					
Lamp 1	4.2 ± 5.9	1.8 ± 1.8	1.50	14	N.S.
2	2.2 ± 4.7	2.1 ± 1.8	< 1.00	14	N.S.
3	1.4 ± 2.6	1.0 ± 1.4	< 1.00	14	N.S.
4	2.8 ± 5.4	1.2 ± 1.8	1.06	14	N.S.
5	4.2 ± 6.1	2.6 ± 2.4	1.26	14	N.S.
6	3.9 ± 6.8	3.9 ± 3.5	< 1.00	14	N.S.
TRACKING (data from Group 4 only)	45.1 ± 16.0	30.9 ± 15.9	5.78	23	< .01

NOTE: S.D.'s are based on group data, therefore they will be quite large due to inclusion of intersubject differences whereas the t-test conducted here was a paired t which considers only intrasubject variability.

TABLE 3

RESULTS FROM SINGLE-BREATH
COHb DETERMINATION*

GROUP 1 - (.50 ppm O_3 + .3 ppm NO_2 + 30 ppm CO)

SUBJECT NUMBER	PRE	SHAM 1	PRE	SHAM 2	PRE	EXPOSURE 1	PRE	EXPOSURE 2
01	1.0	-	1.0	-	1.5	3.0	1.5	4.0
03	2.5	-	2.5	-	3.0	-	2.0	4.0
06	1.0	-	1.0	-	1.5	4.0	1.5	4.0
04	1.0	-	1.0	-	1.0	6.0	1.5	4.0

$t = 3.34$ $t = 19.00$

$p \leq .05$ $p \leq .001$

* % COHb prior to entry into chamber (PRE) and post sham or exposure.

NOTE: COHb data from Group 1 were not available during first two weeks of runs.

CO Data from single-breath test were converted to COHb using the empirical data reported by Gaensler and co-workers. (24)

TABLE 3 (cont.)

RESULTS FROM SINGLE-BREATH
COHb DETERMINATION*

GROUP 2 - WEEK 1 (.50 ppm O_3)

SUBJECT NUMBER	PRE	SHAM 1	PRE	SHAM 2	PRE	SHAM 3	PRE	SHAM 4	PRE	EXPOSURE 1	PRE	EXPOSURE 2
07	2.0	-	2.0	1.0	2.5	1.0	3.0	1.5	3.0	1.5	3.0	-
08	2.5	-	2.0	-	2.5	1.5	3.5	1.5	3.5	1.5	3.0	-
09	2.5	-	2.0	1.0	2.0	2.0	3.0	1.0	2.0	0.5	3.5	-
10	9.5	-	10.5	6.5	10.0	6.5	10.5	5.5	13.0	7.5	12.0	-

GROUP 2 - WEEK 2 (.25 ppm O_3)

SUBJECT NUMBER	PRE	SHAM 1	PRE	SHAM 2	PRE	EXPOSURE 1	PRE	EXPOSURE 2
07	1.0	1.0	2.0	2.0	2.0	1.5	2.0	2.0
08	1.5	1.0	2.0	1.5	2.5	1.5	2.0	2.0
09	2.0	1.0	2.5	1.0	3.0	1.5	3.0	1.0
10 **	9.5	7.5	11.0	7.5	12.5	8.0	10.5	8.0

GROUP 2 - WEEK 3 (.37 ppm O_3)

SUBJECT NUMBER	PRE	SHAM 1	PRE	SHAM 2	PRE	EXPOSURE 1	PRE	RECOVERY SHAM
07	2.0	1.0	2.5	1.0	2.0	1.0	1.5	1.0
08	2.0	1.5	3.0	2.0	2.0	1.0	2.0	1.5
09	2.5	1.0	3.0	1.0	2.0	0.5	-	-
10	10.5	9.5	12.5	12.0	9.5	10.5	11.0	12.0

* % COHb prior to entry into chamber (PRE) and post sham or exposure.
** This subject was an extremely heavy smoker and his estimated COHb values will reflect this.

TABLE 3 (cont.)

RESULTS FROM SINGLE-BREATH
COHb DETERMINATION*

GROUP 4 - WEEK 3 (.25 ppm O_3 + .3 ppm NO_2 + 30 ppm CO)

SUBJECT NUMBER	PRE	SHAM 1	PRE	SHAM 2	PRE	EXPOSURE 1	PRE	EXPOSURE 2
07	1.5	1.0	2.0	1.0	2.0	4.5	1.5	4.5
09	-	-	-	-	-	-	-	-
16	2.0	-	2.5	1.5	2.0	5.0	2.0	4.0
11	5.0	1.5	2.0	1.0	1.5	3.5	2.0	4.0
15	3.5	2.0	4.5	2.5	4.0	5.5	4.5	6.0
10	12.0	10.5	12.0	10.5	13.0	14.0	13.0	13.0
17	1.5	1.0	2.0	1.0	1.5	5.0	2.0	5.0
						t = 5.89		t = 4.21
						p ≤.01		p ≤.01

* % COHb prior to entry into chamber (PRE) and post sham or exposure.

TABLE 3 (cont.)

RESULTS FROM SINGLE-BREATH
COHb DETERMINATION*

GROUP 4 - WEEK 1 (.25 ppm O_3)

SUBJECT NUMBER	PRE	SHAM 1	PRE	SHAM 2	PRE	EXPOSURE 1	PRE	EXPOSURE 2
07	1.0	1.5	2.0	1.0	2.0	1.0	2.0	1.5
09	1.0	2.0	2.5	1.0	2.5	1.0	2.5	2.0
16	1.0	1.5	3.0	1.5	2.0	1.0	2.5	2.0
11	2.0	1.0	3.0	1.5	2.0	1.0	3.0	2.0
15	5.0	2.5	6.0	3.5	6.0	3.0	5.5	3.5
10	11.0	10.0	11.5	10.5	12.5	10.0	12.0	10.5
17	2.5	2.0	2.0	1.0	2.0	0.5	2.5	2.0

GROUP 4 - WEEK 2 (.25 ppm O_3 + .3 ppm NO_2)

SUBJECT NUMBER	PRE	SHAM 1	PRE	SHAM 2	PRE	EXPOSURE 1	PRE	EXPOSURE 2
07	1.5	1.0	2.0	1.0	1.5	1.0	2.0	1.0
09	1.5	1.0	2.0	1.5	2.0	1.5	2.0	1.5
16	2.0	1.5	2.0	2.0	2.0	1.5	2.5	1.5
11	-	-	2.5	1.5	-	-	-	-
15	5.0	3.0	6.5	4.0	4.0	2.5	5.5	3.5
10	11.5	10.0	17.0	12.0	13.0	10.0	10.0	8.5
17	2.0	1.0	1.0	1.0	1.5	1.0	2.0	1.0

* % COHb prior to entry into chamber (PRE) and post sham or exposure.

TABLE 4
SUMMARY OF DATA ANALYSES FROM GROUP 1

REACTION TIME TO PERIPHERAL ATTENTION TASK BY LIGHT LOCATION (MSEC)	CONDITION			F	df	p
	SHAM NORMAL TEMP.	SHAM ELEV. TEMP.	$O_3 + NO_2 + CO$			
1(80°L)	1.100±0.274	1.470±0.077	1.600±0.247	4.74	2,94	< .05
2(50°L)	0.995±0.252	0.996±0.197	1.080±0.192	2.90	2,92	N.S.
3(20°L)	0.812±0.161	0.876±0.123	1.000±0.222	6.23	2,94	< .01
4(20°R)	0.853±0.146	0.877±0.165	1.030±0.239	13.70	2,90	< .01
5(50°R)	0.967±0.251	1.120±0.213	1.230±0.212	11.60	2,84	< .01
6(80°R)	1.020±0.268	1.310±0.342	1.440±0.221	14.00	2,86	< .01
PERCENT NO RESPONSE ON PERIPHERAL ATTENTION TASK BY LIGHT LOCATION						
1(80°L)	8.3±9.6	35.9±20.3	49.4±25.3	3.83	2,22	< .05
2(50°L)	6.9±8.3	0.0	7.4±8.7	1.04	2,22	N.S.
3(20°L)	0.0	3.9±3.1	3.2±3.9	< 1.00	2,22	N.S.
4(20°R)	0.0	0.0	2.7±3.6	< 1.00	2,22	N.S.
5(50°R)	0.0	5.6±7.7	7.2±5.0	2.07	2,22	N.S.
6(80°R)	4.2±8.3	32.2±25.0	27.4±10.8	5.89	2,22	< .01

TABLE 4 (continued)
SUMMARY OF DATA ANALYSES FROM GROUP 1

PERCENT INCORRECT RESPONSE TO PERIPHERAL ATTENTION TASK BY LIGHT LOCATION	CONDITION					
	SHAM NORMAL TEMP.	SHAM ELEV. TEMP.	$O_3 + NO_2 + CO$	F	df	p
1(80^oL)	0.0	1.4±1.2	3.1±6.2	3.57	2,4	N.S.
2(50^oL)	4.2±4.8	2.1±0.0	2.1±4.2	<1.00	2,4	N.S.
3(20^oL)	2.1±4.2	0.7±1.2	2.1±2.4	<1.00	2,4	N.S.
4(20^oR)	6.2±8.0	0.7±1.2	3.1±6.2	1.18	2,4	N.S.
5(50^oR)	10.4±8.0	3.6±2.6	3.1±4.0	2.98	2,4	N.S.
6(80^oR)	8.4±9.6	4.9±3.2	1.0±2.1	<1.00	2,4	N.S.
HEART RATE (BEATS/MIN) [REST]	74.6±6.7	81.8±8.0	87.0±7.6	84.5±6.4	31.10	3,9	<.01
HEART RATE VARIABILITY (BEATS/MIN)	2.10±0.68	1.94±0.59	2.06±0.10	2.08±0.26	<1.00	3,9	N.S.

NOTE: Solid Line: P for group difference less than or equal to .05.
Broken Line: P for group difference less than or equal to .01.

TABLE 5
SUMMARY OF DATA ANALYSES FROM GROUP 2

REACTION TIME TO PERIPHERAL ATTENTION TASK BY LIGHT LOCATION (MSEC)	CONDITION					F	df	p
	NORMAL TEMP.	SHAM ELEV. TEMP.	.50 PPM O_3	.25 PPM O_3	.37 PPM O_3			
1(80^{o}L)	0.907±0.130	1.030±0.118	0.939±0.151	1.050±0.231	1.040±0.364	< 1.00	4,44	N.S.
2(50^{o}L)	0.661±0.086	0.742±0.073	0.755±0.115	0.723±0.104	0.638±0.070	5.01	4,44	< .01
3(20^{o}L)	0.634±0.096	0.666±0.120	0.711±0.098	0.682±0.104	0.650±0.063	2.25	4,44	N.S.
4(20^{o}R)	0.611±0.130	0.693±0.104	0.664±0.122	0.681±0.125	0.710±0.130	2.04	4,44	N.S.
5(50^{o}R)	0.709±0.127	0.750±0.046	0.780±0.054	0.744±0.081	0.709±0.127	1.33	4,44	N.S.
6(80^{o}R)	0.790±0.109	0.933±0.172	0.811±0.072	0.853±0.119	0.790±0.109	1.50	4,44	N.S.
PERCENT NO RESPONSE ON PERIPHERAL ATTENTION TASK BY LIGHT LOCATION								
1(80^{o}L)	7.3±7.1	15.5±11.8	8.3±6.8	14.6±14.2	16.6±28.0	< 1.00	4,12	N.S.
2(50^{o}L)	2.1±2.4	3.0±2.6	2.1±4.2	1.0±2.1	0.0±0.0	1.45	4,12	N.S.
3(20^{o}L)	1.0±2.1	0.2±0.5	0.0±0.0	2.1±2.4	0.0±0.0	1.31	4,12	N.S.
4(20^{o}R)	0.0±0.0	1.9±1.7	0.0±0.0	1.0±2.1	4.1±4.8	1.75	4,12	N.S.

TABLE 5 (continued)

SUMMARY OF DATA ANALYSES FROM GROUP 2

	CONDITION							
	NORMAL TEMP.	SHAM ELEV. TEMP.	.50 PPM O_3	.25 PPM O_3	.37 PPM O_3	F	df	p
5(50^{o}R)	5.2±7.9	2.0±1.5	0.0±0.0	0.0±0.0	0.0±0.0	1.57	4,12	N.S.
6(80^{o}R)	1.0±2.1	7.9±10.3	0.0±0.0	2.1±4.2	10.4±2.1	<1.00	4,12	N.S.
PERCENT INCORRECT RESPONSE TO PERIPHERAL ATTENTION TASK BY LIGHT LOCATION								
1(80^{o}L)	3.1±2.1	0.8±0.5	2.1±4.2	1.0±2.1	6.2±8.0	1.32	4,12	N.S.
2(50^{o}L)	0.0±0.0	2.4±1.8	2.1±4.2	3.1±4.0	2.1±4.2	<1.00	4,12	N.S.
3(20^{o}L)	2.1±2.4	0.2±0.5	0.0±0.0	1.0±2.1	4.2±8.4	<1.00	4,12	N.S.
4(20^{o}R)	4.2±5.9	0.8±1.0	2.1±4.2	3.1±2.1	4.2±4.8	<1.00	4,12	N.S.
5(50^{o}R)	1.0±2.1	1.5±0.9	0.0±0.0	5.2±4.0	4.2±4.8	2.68	4,12	N.S.
6(80^{o}R)	1.0±2.1	3.0±3.6	2.1±4.2	1.0±2.1	2.1±4.2	<1.00	4,12	N.S.
HEART RATE VARIABILITY* (BEATS/MIN) [REST] 5.18±2.38	1.44±0.90	1.64±0.94	1.75±0.53	1.62±1.10	2.11±0.31	2.31	5,5	N.S.

NOTE: Solid Line: P for group difference less than or equal to .05.
Broken Line: P for group difference less than or equal to .01.

* Analyses based on 2 subjects

TABLE 6

SUMMARY OF DATA ANALYSES FROM GROUP 4

REACTION TIME TO PERIPHERAL ATTENTION TASK BY LIGHT LOCATION (MSEC)	CONDITION					F	df	p
	NORMAL TEMP.	SHAM ELEV. TEMP.	.25 PPM O_3	.25 O_3 + .3 NO_2	.25 O_3+.3NO_2+30 CO			
1(80°L)	0.836±0.067	0.818±0.144	0.816±0.116	0.744±0.085	0.731±0.093	1.72	4,16	N.S.
2(50°L)	0.737±0.110	0.737±0.179	0.744±0.157	0.669±0.114	0.647±0.164	3.00	4,16	N.S.
3(20°L)	0.727±0.136	0.800±0.158	0.787±0.174	0.695±0.117	0.665±0.122	6.40	4,16	< .01
4(20°R)	0.743±0.200	0.791±0.193	0.757±0.187	0.682±0.134	0.683±0.179	2.16	4,16	N.S.
5(50°R)	0.698±0.082	0.749±0.132	0.746±0.157	0.680±0.049	0.674±0.137	1.98	4,16	N.S.
6(80°R)	0.850±0.193	0.885±0.217	0.874±0.183	0.802±0.159	0.862±0.198	< 1.00	4,16	N.S.
PERCENT NO RESPONSE ON PERIPHERAL ATTENTION TASK BY LIGHT LOCATION								
1(80°L)	0.8±1.9	1.7±1.8	2.5±3.7	2.5±3.7	0.0	1.40	4,16	N.S.
2(50°L)	0.0	1.1±2.5	0.8±1.9	0.0	0.8±1.9	< 1.00	4,16	N.S.
3(20°L)	0.8±1.9	2.8±1.9	1.7±2.3	0.8±1.9	0.8±1.9	< 1.00	4,16	N.S.
4(20°R)	4.2±7.2	2.8±5.5	0.8±1.9	0.8±1.9	1.7±3.7	< 1.00	4,16	N.S.
5(50°R)	0.0	0.3±0.6	0.0	0.0	0.0	< 1.00	4,16	N.S.

* Analyses based on 5 subjects; 2 subjects had incomplete data

TABLE 6 (continued)
SUMMARY OF DATA ANALYSES FROM GROUP 4

	CONDITION							
	NORMAL TEMP.	SHAM ELEV. TEMP.	.25 PPM O_3	.25 O_3 + .3 NO_2	.25 O_3+.3NO_2+30 CO	F	df	p
6(80°R)	5.0±11.2	4.7±2.9	8.3±8.8	5.0±4.6	10.8±6.9	1.00	4,16	N.S.
PERCENT INCORRECT RESPONSE ON PERIPHERAL ATTENTION TASK BY LIGHT LOCATION								
1(80°L)	5.0±7.5	2.5±1.8	0.8±1.9	2.5±2.3	2.5±3.7	<1.00	4,16	N.S.
2(50°L)	4.2±7.2	2.0±1.2	0.0	3.3±3.5	1.7±3.7	1.33	4,16	N.S.
3(20°L)	0.8±1.9	2.0±1.6	1.7±2.3	2.5±3.7	2.5±2.3	<1.00	4,16	N.S.
4(20°R)	0.0	0.8±1.2	1.7±3.7	3.3±5.4	1.7±3.7	<1.00	4,16	N.S.
5(50°R)	0.0	2.2±1.2	1.7±3.7	1.7±2.3	4.2±0.0	3.70	4,16	<.05
6(80°R)	3.3±7.5	2.5±2.5	1.7±2.3	2.5±2.3	2.5±2.3	<1.00	4,16	N.S.
[REST]								
HEART RATE VARIABILITY** (BEATS/MIN)	3.18±2.69	1.27±0.60	1.21±0.39	1.26±0.50	1.22±0.34	1.52±0.44	1.73	5,15	N.S.

Note: Solid Line: P for group difference less than or equal to .05.
Broken Line: P for group difference less than or equal to .01.

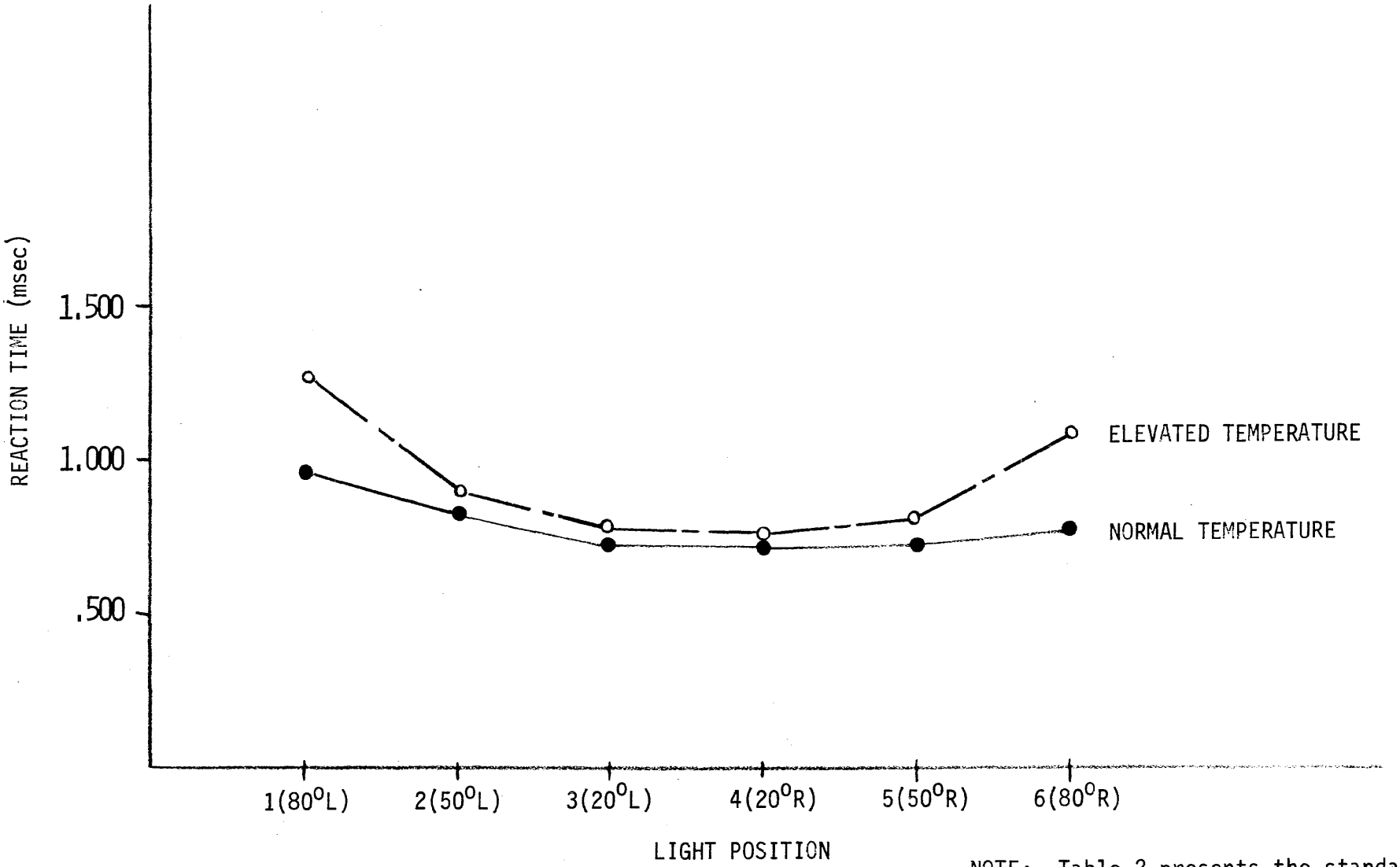
* Analyses based on 5 subjects; 2 subjects had incomplete data ** Analyses based on 4 subjects

TABLE 6 (continued)

PERCENT TIME ON TARGET ON CENTRAL TRACKING TASK

	MEAN ± S.D.	t	df	p
WEEK 1:				
SHAM	26.65±19.22			
.25 ppm O_3	34.54±13.90	-3.24	6	<.05
WEEK 2:				
SHAM	35.08±14.69			
.25 ppm O_3 + .3 ppm NO_2	40.81±13.19	-2.03	5	N.S.
WEEK 3:				
SHAM	41.45±13.57			
.25 ppm O_3 + .3 ppm NO_2 + 30 ppm CO	33.60±14.98	2.74	5	<.05

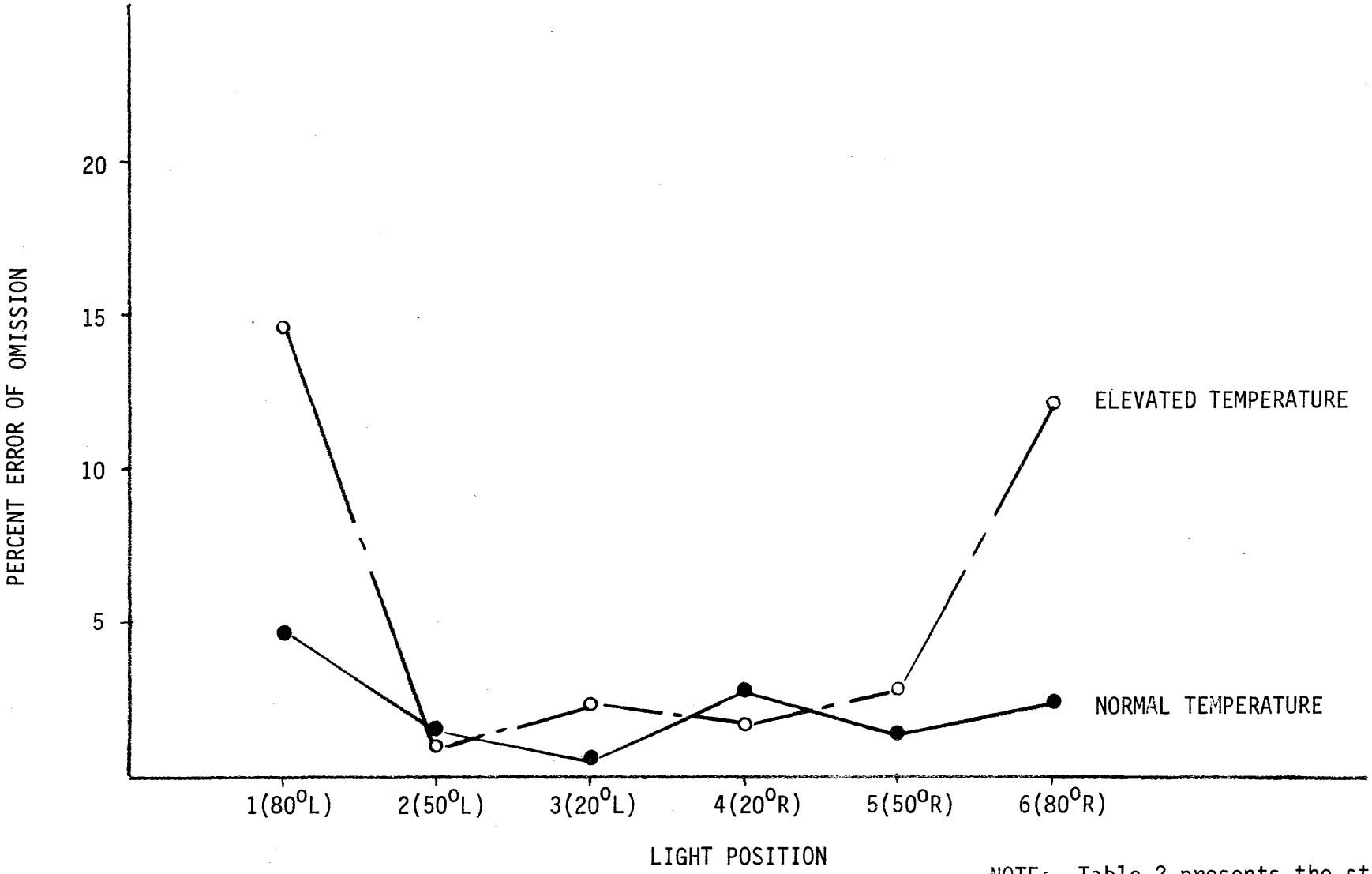
REACTION TIME FOR ALL SUBJECTS AS A FUNCTION OF LIGHT POSITION FOR NORMAL AND ELEVATED AMBIENT TEMPERATURE



NOTE: Table 2 presents the standard deviations for these mean values.

FIGURE I

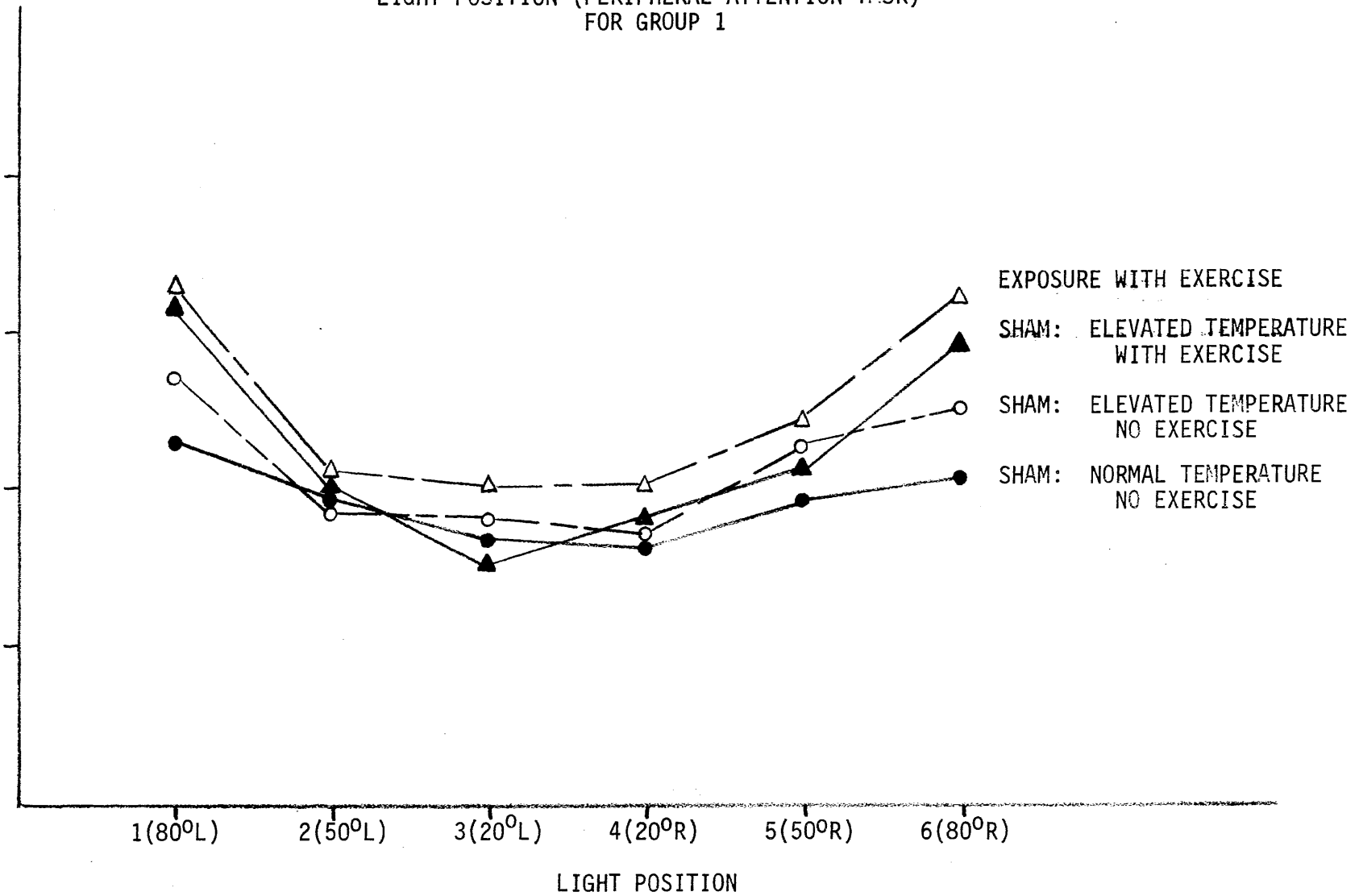
PERCENT ERROR OF OMISSION FOR ALL SUBJECTS AS A FUNCTION OF LIGHT POSITION FOR NORMAL AND ELEVATED AMBIENT TEMPERATURE



NOTE: Table 2 presents the standard deviations for these mean values.

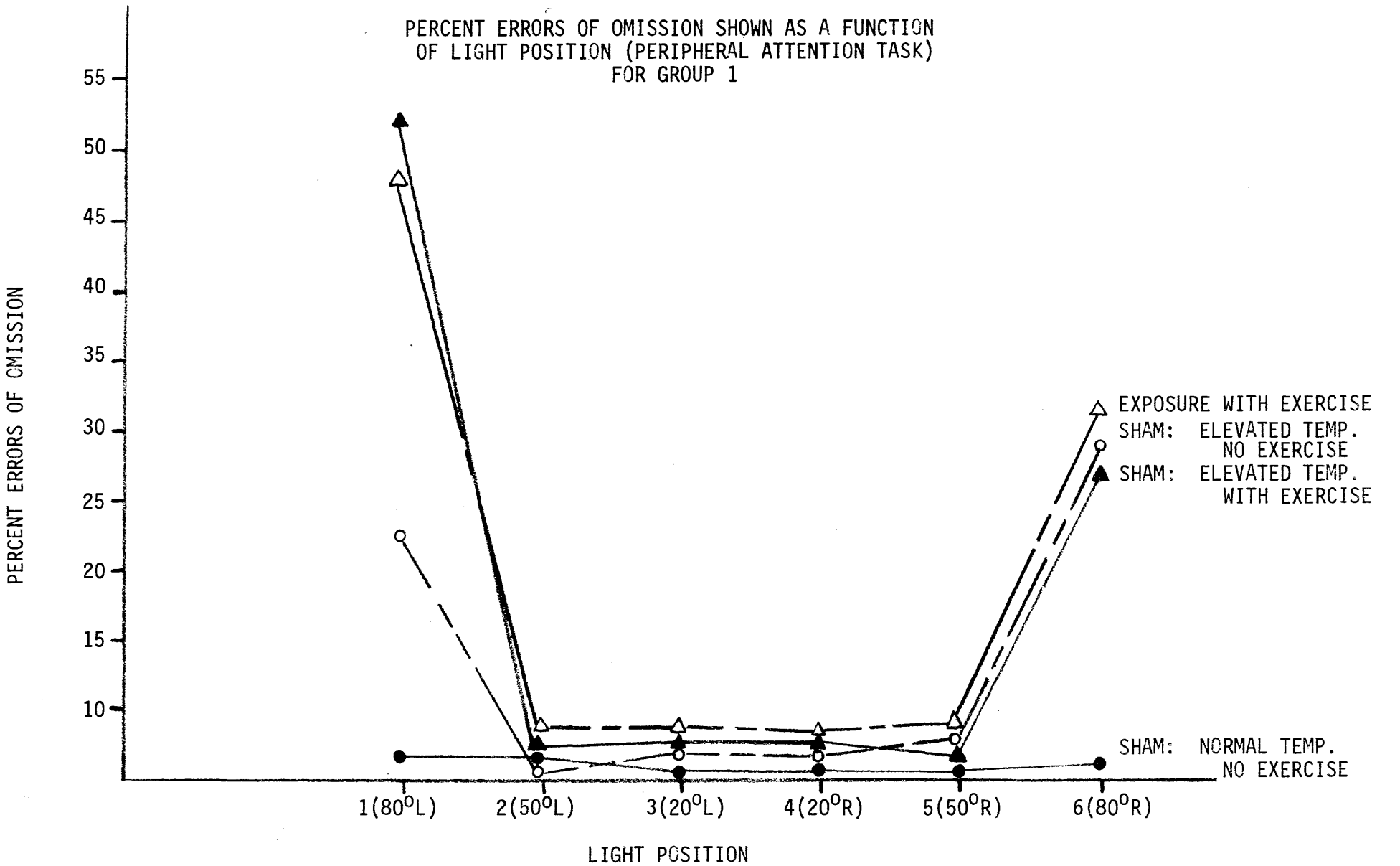
FIGURE II

REACTION TIME SHOWN AS A FUNCTION OF
LIGHT POSITION (PERIPHERAL ATTENTION TASK)
FOR GROUP 1



NOTE: Table 4 presents the standard deviations for these mean values.

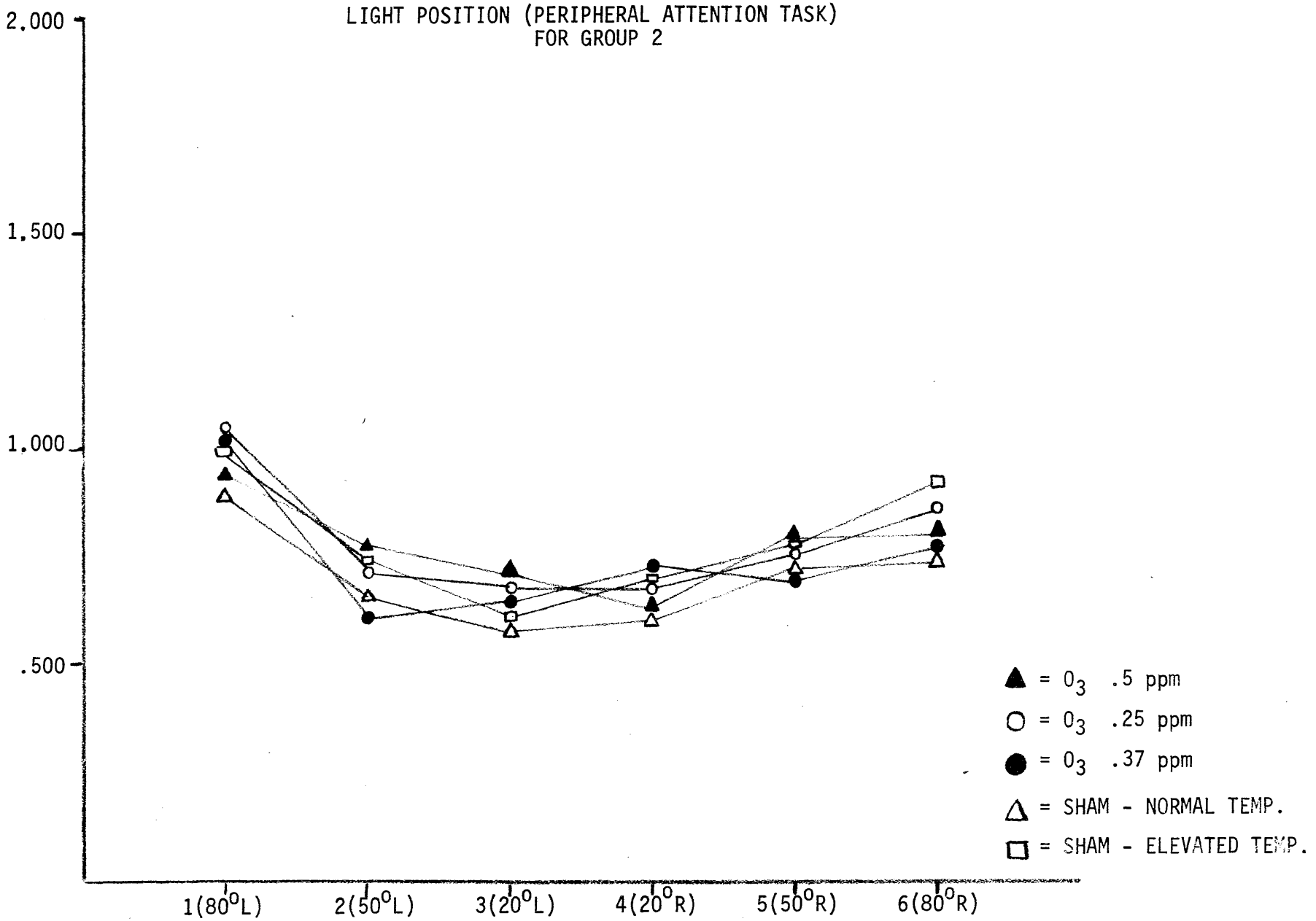
FIGURE III



NOTE: Table 4 presents the standard deviations for these mean values.

FIGURE IV

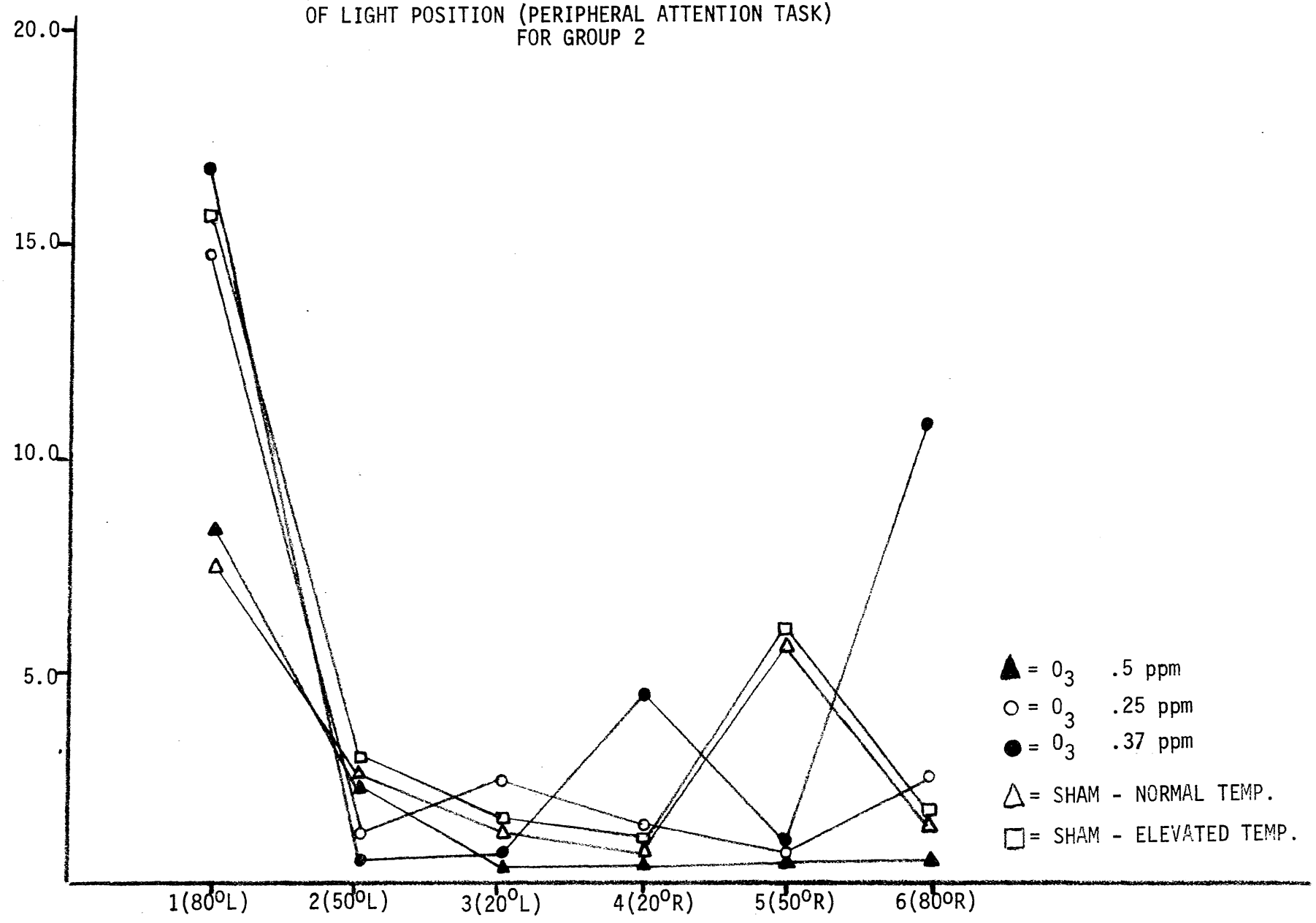
REACTION TIME SHOWN AS A FUNCTION OF
LIGHT POSITION (PERIPHERAL ATTENTION TASK)
FOR GROUP 2



NOTE: Table 5 presents the standard deviations for these mean values.

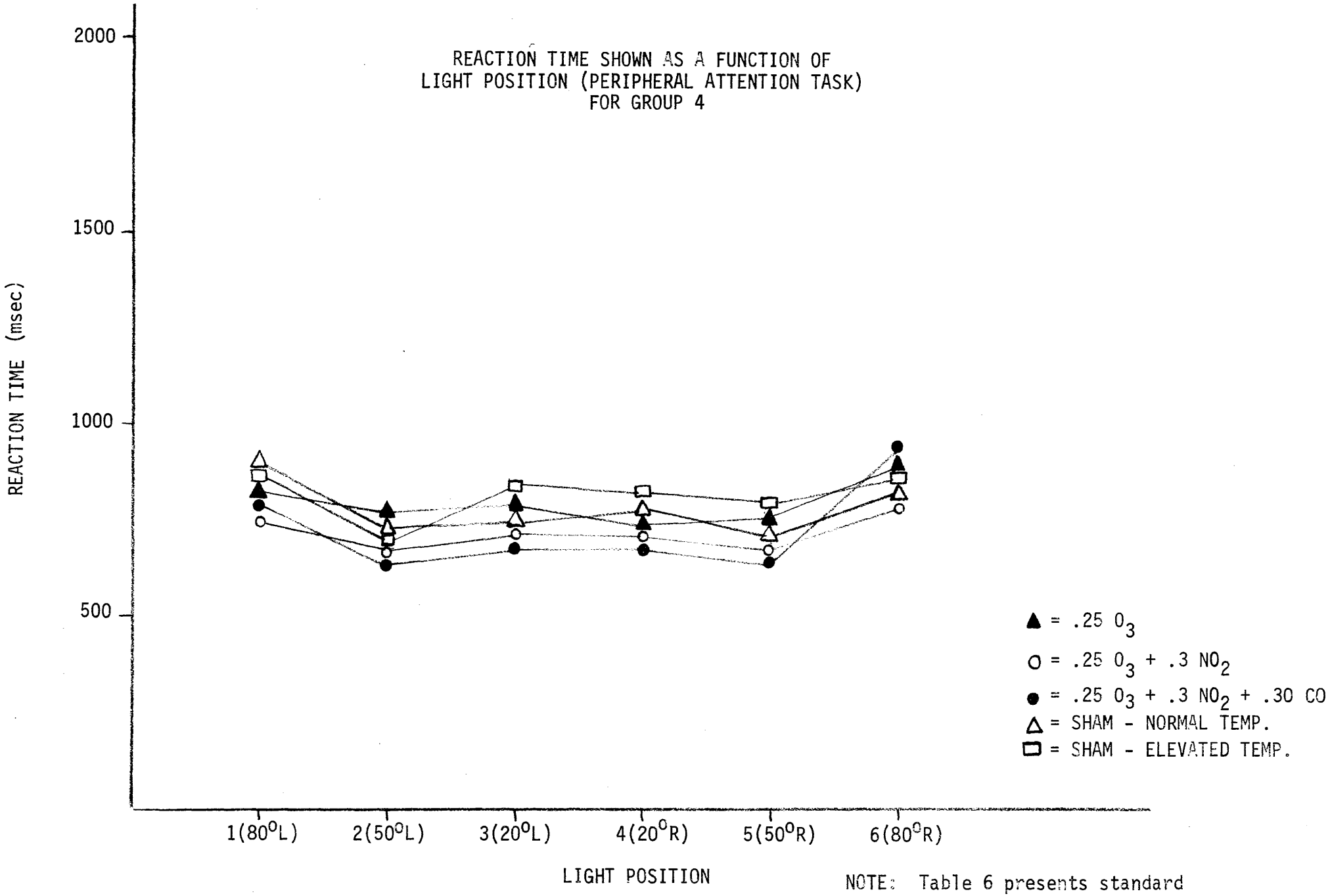
FIGURE V

PERCENT ERRORS OF OMISSION SHOWN AS A FUNCTION OF LIGHT POSITION (PERIPHERAL ATTENTION TASK) FOR GROUP 2



NOTE: Table 5 presents the standard deviations for these mean values.

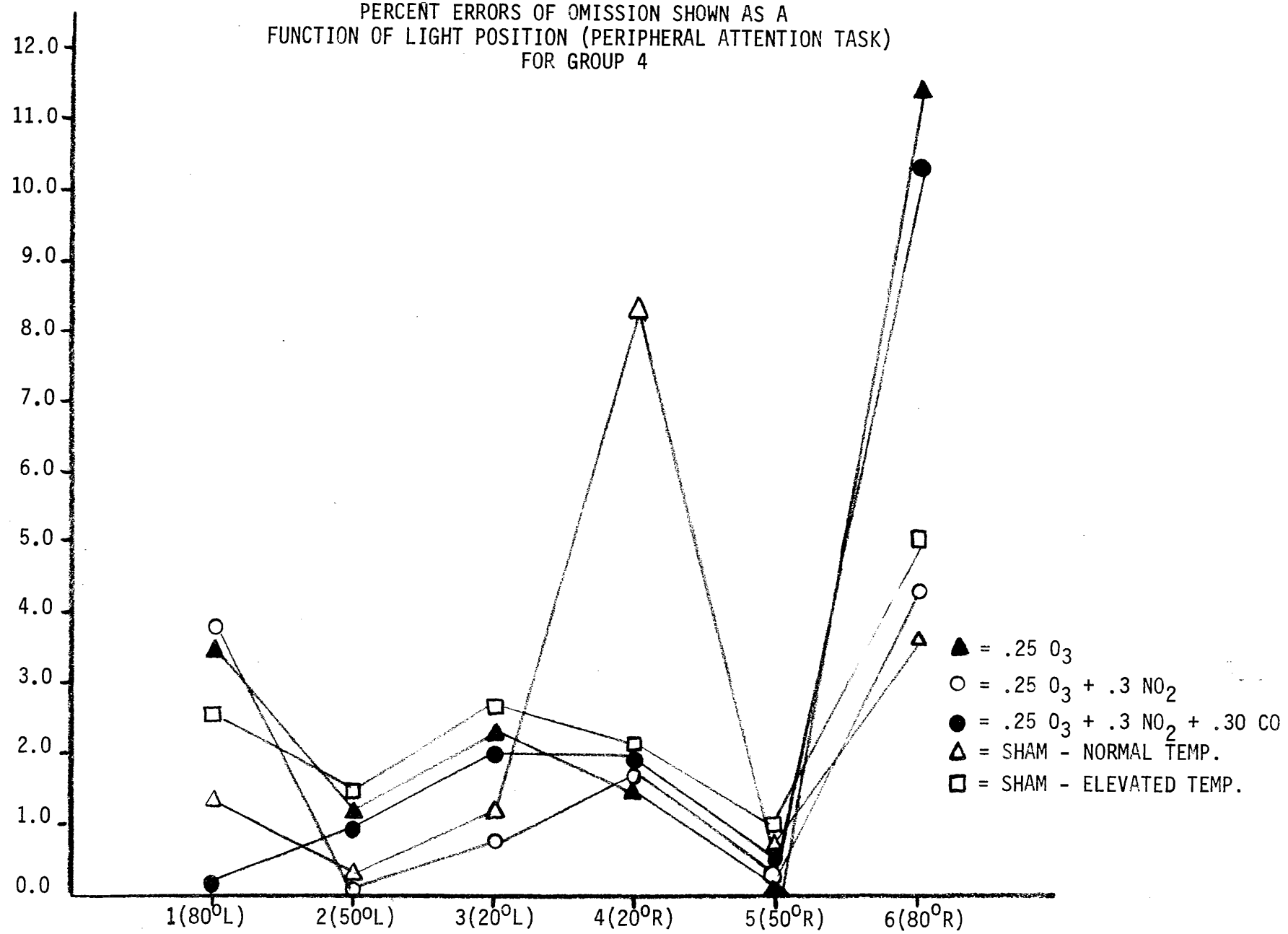
FIGURE VI



NOTE: Table 6 presents standard deviations for these mean values.

FIGURE VII

PERCENT ERRORS OF OMISSION SHOWN AS A
FUNCTION OF LIGHT POSITION (PERIPHERAL ATTENTION TASK)
FOR GROUP 4



NOTE: Table 6 presents standard deviations for mean values.

FIGURE VIII

REFERENCES

1. Ruffin, J. B.: Functional testing for behavioral toxicity: A missing dimension in experimental environmental toxicology. *J Occup Med*, 5: 117-121, 1963.

2. Behavioral toxicology looks at air pollutants: C. Xintaras, interview. *Env Sci Tech*, 2: 731-733, 1968.

3. Reynolds, R. W.: Psychological and behavioral effects. Vol. 3 Project-Clean Air Research Reports, Task Force Assessments, University of California, 1970.

4. Beard, R. R., G. A. Wertheim: Behavioral impairment associated with small doses of carbon monoxide. *Am J Public Health*, 57(11): 2012-2022, 1967.

5. Schultz, J. H.: Effects of mild carbon monoxide intoxication. *Arch Environ Health*, 7: 524-530, 1963.

6. Lagerwerff, J. M.: Prolonged ozone inhalation and its effects on visual parameters. *Aerosp Med*, 34: 479-486, 1963.

7. Xintaras, C., B. L. Johnson, C. E. Ulrich, R. E. Terrill, and M. F. Sobecki: Application of the evoked response technique in air pollution toxicology. *Toxicol Appl Pharmacol*, 8: 77-87, 1966.

8. O'Donnell, R. D., P. Chikos and J. Theodore: Effect of carbon monixide exposure on human sleep and psychomotor performance. *J Appl Physiol*, 31(4): 513-517, 1971.

9. Stewart, R. D., J. E. Peterson, E. D. Baretta, R. T. Bachand, M. J. Hosko and A. S. Hermann: Experimental human exposure to carbon monoxide. *Arch Environ Health*, 21: 154-164, 1970.

10. Wilkinson, R.: Some factors influencing the effect of environmental stressors upon performance. *Psychol Bull*, 72(4): 260-272, 1969.

11. Mc Farland, R. A.: The psycho-physiological effects of reduced oxygen pressure. Research publications of the *Association of Nervous and Mental Disease*, 19: 112-143, 1939.

12. Bursill, A. E.: The restriction of peripheral vision during exposure to hot and humid conditions. *J Exp Psychol*, 10(3): 113-129, 1958.

13. Youngling, E. W.: The effects of thermal environments and sleep deprivation upon concurrent central and peripheral tasks: Univ. microfilms, 65-11, 528, Ann Arbor Michigan, 1965.

14. Beard, R. R., and N. Grandstaff: Decrements in visual vigilance and perceptual tracking associated with small doses of carbon monoxide. *Am Ind Hyg Assoc J*, 1972.

15. Mc Farland, R. A.: Low level exposure to carbon monoxide and driving performance. *Arch Environ Health*, 27: 355-359, 1973.

16. Weir, F. W. and T. H. Rockwell: The investigation of the effects of carbon monoxide on humans in the driving task. CRC-APRAC Project CAPM-9-69. EPA, January, 1973.

17. Lacey, J., and B. C. Lacey: The relationship of resting autonomic activity to motor impulsivity. Research publications of the Association for Research in *Nervous and Mental Disorders*, 36: 144-209, 1958.

18. Obrist, P. A.: Cardiovascular differentiation of sensory stimuli. *Psychosom Med*, 25: 450-459, 1963.

19. O'Hanlon, J.: Heart rate variability: A new index of driver alertness/fatigue. Final Report to Federal Highway Administration. Human Factors Research, Inc., 1971.

20. Kalsbeek, J. W. H.: Sinus arrhythmia and the dual task method in measuring mental load. Measurement of Man at Work, Singleton (Ed.), Van Nostrand Reinhold Co., NY 1971.

21. Hackney, J. D., W. S. Linn, R. D. Buckley, E. E. Pedersen, S. Karuza, D. C. Law, and D. A. Fischer: Experimental studies on health effects of air pollutants: Design consideration. (submitted to *Am Rev Resp Dis*,) 1974.

22. Teichner, W. H.: Concepts for research and evaluation of human performance presented to Human Factors Soc., Anaheim, California, November, 1966.

23. Blackburn, H., (Ed.): Measurement on exercise electrocardiology. Charles C. Thomas, Springfield, Ill., 1969.

24. Jones, R. H., et al.: The relationship between alveolar and CO concentrations during breathholding. *J Lab and Clin Med*, 51(4): 553, April, 1968.

25. Winer, B. J.: Statistical principles in experimental design. McGraw-Hill, New York, 1962.