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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

CHANGE IN PLASMA LACTIC ACID DEHYDROGENASE ISOENZYMES AS AN INDICATOR OF MYOCARDIAL DAMAGE RESULTING FROM EXPOSURE TO CARBON MONOXIDE

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DONALD VICTOR LASSITER

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CHANGE IN PLASMA LACTIC ACID DEHYDROGENASE ISOENZYMES

AS AN INDICATOR OF MYOCARDIAL DAMAGE RESULTING

FROM EXPOSURE TO CARBON MONOXIDE

APPROVED BY

Ronald L. Coleman

Carl A. Nau

John R. Sokoth

Simon H. Wender

Charles Lawrence

DISSERTATION COMMITTEE

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CHANGE IN PLASMA LACTIC ACID DEHYDROGENASE ISOENZYMES AS AN INDICATOR OF MYOCARDIAL DAMAGE RESULTING FROM EXPOSURE TO CARBON MONOXIDE

CHAPTER I

INTRODUCTION

Carbon monoxide (CO) presents as important a world-wide environmental hazard as exists. More persons in the United States succumb each year to acute CO poisoning (> 1000) than to any other single toxic agent except alcohol (1, 2). The CO concentration produced by an automobile engine can reach 600 to 1000 parts per million (ppm) in a garage with the door open (3)!

The acute toxicity associated with tissue hypoxia during CO exposure is quite well understood and involves the preferential binding of CO instead of oxygen to hemoglobin forming carboxyhemoglobin (COHb) (4). Because the affinity of CO for hemoglobin is 200 to 250 times that of oxygen the presence of only small amounts of CO can displace disproportionate amounts of oxygen from hemoglobin (4, 5). It is pos-

sible, therefore, that exposure to concentrations of CO above 0.1 per cent (1000 ppm) can rapidly become fatal to both lower animals and humans (1).

The results of animal exposure to concentrations of CO below 1000 ppm present a picture which is far less clear. On the one hand marked differences of exposure response occur among animals of the same species while on the other hand repeated sub-lethal exposures may result in acclimatization to concentrations of CO which are lethal to unacclimatized animals. Herein lies one of the more pressing questions concerning CO exposure: are present-day ambient air exposure levels of CO harmful only to the extent that COHb renders inactive a portion of the oxygen-carrying capacity of the blood? That is, does the fact that CO can mediate tissue anoxia at a particular level take precedence over the possibility that CO per se may also exert a toxic effect quite apart from such anoxia?

As our society becomes more urbanized, measured by increased concentrations of the combustion-powered engine and industrial combustion processes, the answer to the question of whether we are at the same time slowly and wittingly poisoning ourselves via chronic exposures to noxious agents yet remains largely unknown. At least a portion of the solution

to this question might be uncovered if it could be demonstrated that ambient air concentrations of CO can in fact mediate deleterious cellular/sub-cellular damage to a vital organ such as the heart or brain.

CHAPTER II

LITERATURE REVIEW

The publications concerning CO, its properties, sources, biological effects and environmental fate comprise one of the truly great bodies of scientific literature. Several earnest attempts to evaluate, catalogue, summarize, and update this vast amount of literature on CO have been undertaken by Killiack (6), Grut (7), Lilienthal (2), von Oettingen (8) and Cooper (9). The purpose of this review will be to summarize the more important historical findings, update the present literature, and present the areas where investigation is still necessary.

Beginning with the discovery of fire, CO began an evolutionary association with man as a true environmental hazard. Although natural sources of CO such as forest fires and the catabolism of pyrrole compounds in plants and animals had long existed (10), man's first proximal contact with high concentrations of CO were brought about by his own initiative in attempting to improve his lot by a technological advance. Man

has not significantly deviated from this pattern of technological advancement by means of incomplete combustion even unto the present day. Each year he pollutes the environment with over 200 million tons of CO with some 58 per cent coming from his own private internal combustion machine (11). Thus man is daily exposed to, and dependent upon, the greatest source of CO pollution known - his own automobile. The seriousness of this threat to human and animal health cannot be over emphasized; at least not until the scarcity of information concerning the biological effects of low-level CO exposure over prolonged periods of time is overcome.

However, as tempting as the automobile may be for an anti-pollution target, one other source in the environment may present an even greater threat to a defined group in our society - the effect of inhaled cigarette smoke on the heavy smoker. The concentration of CO in inhaled cigarette smoke has been determined to be between 400 to 500 ppm (1, 12, 13) and cigarette smokers retain approximately 10 mg of CO per king-size cigarette smoked, which results in the saturation of 1 to 1.5 per cent of the total hemoglobin (5, 14). Goldsmith et al. (12) have stated that the cigarette smoker is exposed to 475 ppm of CO for approximately 6 minutes per cigarette. It has been well established that chronic cigarette

smokers have an average COHb level of 5 to 10 per cent (1, 5, 13, 15 through 18) which may increase to 18 per cent (16) following a period of heavy smoking. As a comparison, 30 ppm of CO will produce, at equilibrium, a concentration of 5 per cent COHb (1). Although the COHb content of a smoker will not, of course, increase above 5 per cent until the ambient CO level increases above 30 ppm (15, 19), smokers with conditions which limit the amount of oxygenation of the tissues (e.g. coronary insufficiency, emphysema and myocardial congestion) may be prime targets and on the brink of disaster during a CO episode (15). Indeed, chain smoking and prolonged exposure to a congested traffic situation for such a person might well trigger one of the above conditions of oxygen insufficiency with dire consequences.

Certain of the peculiarities surrounding the actions and mechanisms of CO in exerting biological effects have long captured the imagination of investigators. Questions concerning the acclimatization to continued or subsequent exposures of CO (7, 15, 20 through 24), the course, or even the existence, of chronic CO poisoning (13, 25 through 32), the contribution of factors other than simple asphyxia in the cause of death in acute CO poisoning (33), the mechanisms and relationships of O_2 and CO to myoglobin and other heme proteins

(34) and the inactivation of various subcellular entities by CO (35, 36, 37) have been investigated.

Several studies have focused attention on the effects produced by the presence of both CO and SO_2. One study (38) concluded that the detrimental effects caused by SO_2 are increased in the presence of both SO_2 and CO. Other investigators (39) have postulated that the combined exposure produced a summation effect and, perhaps, even a synergism. In a similar study Melnichenko (40) determined that the combination of CO and H_2S produced more marked effects than did CO alone.

None of the above should be inferred as de-emphasizing the well-understood mechanisms of the interactions of CO with hemoglobin. Since Douglas and the Haldanes (41) formulated their basic laws governing the interactions of CO and O_2 with hemoglobin it has been understood that CO combines with the reduced hemoglobin molecule much more readily than does O_2 when the two are simultaneously present in a breathing mixture.

Douglas and the Haldanes (41) placed the affinity constant (M) for the equation: $\frac{COHb}{O_2Hb} = \frac{M \times P_{CO}}{P_{O_2}}$ within the range, 220 to 290, and later investigations have generally confirmed this figure under conditions of complete hemoglobin

saturation by O_2 and CO (42, 43, 44). Very simply, the affinity constant may be expressed as the number of moles of oxygen which must be present with each mole of CO in order to maintain an equal saturation of hemoglobin. This saturation of hemoglobin by either O_2 or CO occurs in four steps involving intermediates with four equilibrium constants:

$$Hb_4 + X \underset{k_1}{\overset{k_1'}{\rightleftharpoons}} X\ Hb_4 \qquad K_1 = \frac{k_1'}{k_1}$$

$$X\ Hb_4 + X \underset{k_2}{\overset{k_2'}{\rightleftharpoons}} (X)_2\ Hb_4 \qquad K_2 = \frac{k_2'}{k_2}$$

$$(X)_2\ Hb_4 + X \underset{k_3}{\overset{k_3'}{\rightleftharpoons}} (X)_3\ Hb_4 \qquad K_3 = \frac{k_3'}{k_3}$$

$$(X)_3\ Hb_4 + X \underset{k_4}{\overset{k_4'}{\rightleftharpoons}} (X)_4\ Hb_4 \qquad K_4 = \frac{k_4'}{k_4}$$

In the above equations X represents either O_2 or CO with different equilibrium constants for each. It has been determined that in both cases K_4 is much higher (18 to 50 times higher) than K_1, K_2 or K_3 because k_4' is much greater than k_4 (11, 45). Under these circumstances the last ligand to bind to hemoglobin (K_4 equation) dissociates much more readily than it binds (46). The differences between the four constants depend on

intra-molecular forces which occur as a result of the interactions of each ligand with the others, or with other portions of the hemoglobin molecule. Hence, the effect is, in essence, an allosteric one resulting in conformational changes in the hemoglobin molecule. These differences in reaction rates affect the dissociation of O_2 and CO from hemoglobin such that the shape of the dissociation curve is sigmoid rather than a parabola, as is the case with myoglobin. Myoglobin has only one heme group and therefore a single dissociation constant resulting in a dissociation curve in the shape of a parabola (15). The constant may, however, be as high as 245 and as low as 135 depending upon, among other things, the blood pH and amount of reduced hemoglobin (47, 48). The generally accepted figure, 210 (42), is applicable only for human hemoglobin. Each species has its own particular value for the affinity constant because the composition of hemoglobin molecules varies between species (2). The M value for a species remains relatively constant, dependent on the physiological conditions mentioned above (2). Killick (6), however, has reported individual variations in the value of M.

Because the tissue partial pressure of oxygen (P_{O_2}) (approximately 40 mm Hg in mixed venous blood) occurs at a

steep portion of the curve, under normal physiological conditions oxygen is rapidly dissociated from hemoglobin so that tissue saturation is maintained with a large oxyhemoglobin reserve at the lower end of the curve. This reserve is a result of the large value for K_4 which permits only the upper portion of dissociation curve to function under normal physiological conditions (10). The oxygen gradient between freshly oxygenated arterial blood (100 mm Hg) and mixed venous blood (40 mm Hg) is reduced to approximately 5 mm Hg in the tissues (15). Hence a shift of the steep portion of the oxyhemoglobin curve to the left would tend to change this lower gradient appreciably.

Interestingly enough, while the curve shifts to the right allowing for a more efficient dissociation of oxygen to the tissues under conditions of reduced ambient oxygen tension (hypoxic hypoxia) just the opposite occurs during exposure to CO (anemic hypoxia) (2, 10, 11, 15). This shift to the left during CO exposure is caused by the much greater affinity of CO for hemoglobin. As the result, and until equilibrium is reached, once CO is bound to hemoglobin it remains bound such that sites normally available to oxygen become unavailable. This has the effect of shifting the curve for the dissociation of the remaining oxyhemoglobin to the left in that for

any given oxygen saturation of the remaining hemoglobin the P_{O_2} is lower. This situation occurs in spite of the fact that the amount of O_2 physically in solution in the blood remains normal and is due entirely to the greater affinity of CO for hemoglobin (11).

Thus, not only is the oxygen content of the blood lowered during exposure to CO but the leftward shift of the oxyhemoglobin dissociation curve decreases the readiness with which the remaining oxygen is made available to the tissues. Both mechanisms serve to effectively lower the tissue P_{O_2} and hence create a generalized tissue hypoxemia. Superimposed on this picture is the fact that the carotid sinus chemoceptor, which increases pulmonary ventilation when stimulated by the decreased O_2 tension in the blood during hypoxic hypoxia, is unaffected during CO-induced hypoxia since the amount of physically-dissolved oxygen in the blood remains normal (11). With the circulatory system thus embarrassed it is difficult to understand how true acclimatization to CO could be possible under these circumstances. Yet many investigators believe that this can and does occur and the experimental evidence favoring acclimatization is impressive (43, 44) and may well formulate the body's only defense against chronic low-level CO exposure.

It has been repeatedly demonstrated that following an initial exposure to CO, animals become less susceptible to subsequent exposure (11). On the other hand it has also been demonstrated that mice which are tolerant to one exposure may succumb during a subsequent exposure (22). It is very possible that if acclimatization to CO does occur many of the mechanisms would be similar to those which aid in acclimatizing the animal to hypoxia (15, 23, 49). Indeed, such similar acclimatory mechanisms as polycythemia and an increased hematocrit occur during both hypoxic hypoxia and CO-induced hypoxia (49). Whether there is an intrinsic factor involved in acclimatization to CO remains to be seen, although there is evidence to the contrary (22).

Quite apart from acclimatization is the question of the existence and mechanism of chronic CO poisoning (7). Some believe that if this condition does occur it is the result of repeated acute episodes and not merely a continual insult to low levels of CO (24, 25). Regardless of mechanism the considerable amount of clinical evidence does point to a disease entity which could properly be referred to as chronic CO poisoning/toxicity. Numerous case histories have related a CO source with such common effects as headache, dizziness, nausea and anorexia, all of which disappear upon the removal

of the source (1, 7). Of much greater importance is the possibility of chronic CO toxicity at ambient concentrations causing subtle effects which are just within, or beyond, the limits of detectability.

Since the possibility of complete acclimatization to CO is rather remote, the manifestation of biological alterations occurring during exposure to CO become suspect as possible indicators of deleterious effects. This line of reasoning has even led some to believe that the cause of death resulting from acute CO intoxication may be other than simple asphyxia (22, 33, 50, 51, 52).

Among the most subtle of the above alterations are those which involve shifts in compartmental concentrations of trace metals. New techniques (e.g. atomic absorption and anodic stripping voltametry) now allow for the detection of very minute amounts of these important subcellular constituents, and their aberrations during the course of various disease states, including chronic CO exposure, have been documented (53, 54). Difficulties involved in determining the significance of findings and in the extrapolation of findings to the whole organism have so far somewhat undermined the potential values of these techniques. However, the fact that most of the essential trace metals participate, physiologi-

cally, either as co-factors or activators of enzymes underscores the importance of determining their aberrations *in vivo*. In fact, any condition precipitating changes in enzyme activity or concentration merits very close scrutiny, and the activity of several enzymes has been found to be altered during CO exposure (35, 36, 55, 56).

At the other end of the spectrum of alterations occurring in the animal organism as a result of exposure to CO are changes in behavioral patterns. There is evidence to suggest that certain behavioral changes induced in the whole animal via CO exposure are evidence of subtle physiological alterations occurring within the organism which are beyond the detectable range of present techniques. Thus the work of several investigators has suggested that such quantities as time discrimination, responses to learned stimuli (51, 57), choice response tests (58, 59), evoked response techniques (60, 61) and visual discrimination thresholds (35, 50) can be altered at low (50 ppm), and even very low (5 ppm) levels of CO exposure. The results of these tests should be evaluated in the light of the very highly specific test conditions involved which often included distractions that could have easily altered the responses.

Attempts to correlate behavior changes with damage

to the central nervous system (CNS) have produced conflicting results although acute exposure to high concentrations of CO has consistently produced brain lesions (62). The general trend has been the finding of less severe CNS damage as the concentration of CO and length of exposure are decreased (62). At CO concentrations of 100 ppm some investigators have found cortical damage in dogs, especially along the course of blood vessels, while other investigators have detected no direct damage but have discovered that the glial cells were mobilized (63), which usually occurs only during the course of disease. It has been emphasized, however, that CNS damage, when it does occur, is secondary to cardiovascular damage (63).

As mentioned in the beginning of the chapter it has been confirmed that an endogenous source of CO exists as a product of heme catabolism (64, 65). When an α-methylene bridge of the heme portion of hemoglobin is broken during the catabolic process, a molecule of CO is released (64). It has been estimated that this production amounts to approximately 0.3 to 1.0 ml per hour with an additional 0.1 ml per hour resulting from a similar catabolic process involving other heme compounds (e.g. myoglobin and cytochrome and catalase enzymes) (1, 66). This endogenous production of CO would not be expected to be of important consequence since the proposed

mechanism has evolved with man, but its removal would merit due consideration in closed systems (1) (e.g. submarines and space capsules). Dinman (10) has suggested that the great affinity of hemoglobin for CO was, evolutionary-speaking, of a definite survival advantage since this mechanism would permit the expedient removal of endogenously-produced CO.

Although its concentration compared to that of hemoglobin is small, myoglobin has been, nevertheless, implicated by several investigators as an important factor during CO exposure (67, 68) and hypoxia (69). Myoglobin has approximately 16 per cent the oxygen-carrying capacity of an equal quantity of hemoglobin and accounts for only approximately 20 per cent of the body's total CO capacity (15), but its existence as an extravascular carrier of oxygen has prompted Wyman (67) to compare the "translational diffusion" of oxygen into the cell via myoglobin with the pumping of oxyhemoglobin via the heart throughout the body. This investigator has also suggested that a state of oxygen debt would develop in muscle tissue were the myoglobin completely deoxygenated since he estimated that 50 per cent to 90 per cent of the oxygen reaching the muscle cell is carried by myoglobin. If such an oxygen-carrying role could be delegated to myoglobin then certainly whole animal exposure to CO would decrease the oxygen-

carrying capacity of myoglobin with the formation of carboxymyoglobin (COMb), which would be completely analogous to the formation of COHb from hemoglobin. The value of M in the Haldane equation, for myoglobin, however, is only 40, compared with 210 for hemoglobin (70). This mechanism of facilitated oxygen transport within the myocardium under normal conditions could lead to yet another deleterious effect during CO exposure. In fact, Ayers et al. (71) have suggested the possibility of an increased COMb concentration in the myocardium during CO exposure along with a reduced oxygen diffusion between the coronary capillaries and the mitochondria.

It is becoming increasingly evident that cardiac involvement is an important variable in both chronic and acute CO poisoning (1, 26, 33, 72). Only the brain has a higher requirement for oxygen (1). But, in contrast to the cerebral circulation, the coronary circulation must supply an even increased amount of oxygen during periods of generalized tissue anoxia since under these circumstances the heart is forced to increase both its rate and its output in order to meet the oxygen demands of the body (1, 15). This increase in cardiac activity demands an increased oxygen supply to the myocardium which must be met by the coronary circulation. Under hypoxic conditions increased oxygen supply to the tissues

can be accomodated by increased blood flow (via dilatation) and/or increased oxygen extraction by the tissues (11, 71). The myocardium under these circumstances appears only to increase the flow of blood rather than to extract an additional amount of oxygen from the coronary arteries (1, 11, 71). This mechanism has the overall effect of maintaining the myocardial oxygen tension at a higher level than would be present in other muscle tissue and thus insures a continual aerobic metabolism even under hypoxic duress. While the peripheral tissues under normal resting conditions extract only 25 per cent of the oxygen content of the perfusing arterial blood, the myocardium extracts 75 per cent, thus leaving the mixed venous blood only 25 per cent saturated (15, 71). In terms of oxygen tension, the mixed venous blood of the peripheral tissues (and hence the tissues themselves) is approximately 40 mm Hg while the mixed venous blood of the coronary circulation is only 20 mm Hg. In the presence of COHb (with the shift to the left of the oxyhemoglobin dissociation curve), however, the arterio-venus difference can only be maintained by a decrease in the mixed venous oxygen tension, in spite of an increased rate of flow in the coronary circulation. Hence, the P_{O_2} of the most oxygenated portion of the myocardium must drop below 20 mm Hg as a result of the myocardial tissue ex-

traction of required oxygen. This precipitous drop in myocardial mixed venous oxygen tension might very well inactivate certain sub-cellular respiratory functions such as oxidative phosphorylation. This anoxic effect is further enhanced, as mentioned above, by the increase in cardiac rate and output as a general response to peripheral tissue hypoxemia. It would then appear that a person with deminished coronary circulation due to coronary artery disease mat be constantly on the "brink" of myocardial tissue anoxia should a COHb concentration above 10 per cent be present.

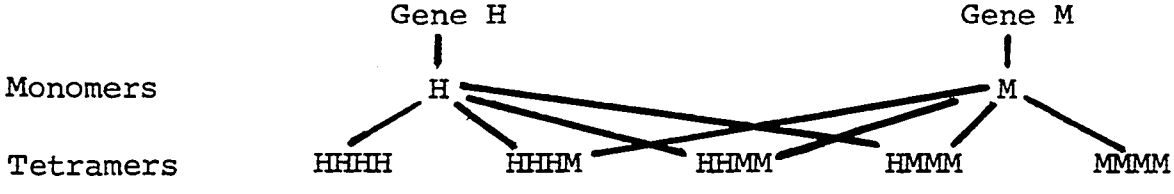
Ayers et al. (71) have attempted to demonstrate the redox state of the myocardium during CO exposure via a biochemical assay procedure. Their technique concerned the determination of the extraction ratios of pyruvate and lactate from the perfusing coronary circulation following exposure to 5000 ppm of CO for 30 to 120 seconds. As glucose is converted to pyruvate under anaerobic conditions via glycolysis, the fate of pyruvate from that point depends largely on the redox state of the cell. With adequate oxygenation pyruvate will be converted to acetyl coenzyme A and from thence it can be oxidized via the citric acid cycle. If there is inadequate oxygenation, however, pyruvate will be converted to lactate, a terminal product in animal anaerobic metabolism, and both

can be transported to the liver for oxidation, should the redox state of the liver be adequate. In Ayers' experiments the concentrations of pyruvate and lactate in the myocardial tissue were compared to the concentrations of these same two metabolites in the coronary arterial blood. Normally the myocardium extracts both of these metabolites from the coronary circulation for purposes of oxidation as mentioned above. When appreciable COHb was present, however, not only did the myocardium fail to extract either of these two substances, it actually produced both. The production of lactic acid in the myocardium at any time indicates the existence of a state of tissue anoxia. A prolonged state of anoxia cannot long exist in any cell without the inevitable consequence of cell death. Even before cell death various characteristics of the cell, including membrane permeability, will change (73). Thus leakage of cellular constituents, including proteins, can occur either through an altered cell membrane or through ruptures in the membrane. Such changes form the basis of various diagnostic techniques for determining the existence of disease states in the whole animal.

For some time it has been possible to diagnose various disease states by means of biochemical assays of materi-

als which are found in greater than normal concentrations in the blood following initiation of a particular disease process. In the diagnosis of myocardial infarct, determination of the serum increase of several enzymes including serum glutamate-oxaloacetate transaminase (SGOT) (74), 2-hydroxybutyrate dehydrogenase (SHBD) (74) and lactate dehydrogenase (LDH) (73, 74, 75) has proven valuable.

A few years ago it was discovered that some enzymes are actually composed of several units which slightly differ from one another (76). In the case of LDH it was determined that two separate genes at different loci on the chromosome each code for a different polypeptide chain. Four of these chains, in any combination, assemble to form the active enzyme according to the diagram:



It can be observed that the only possible combinations of the two monomers to form tetramers are the five diagrammed above. Hence each cell contains each of the five tetramers in mathematical proportions dependent upon the comparable productiveness of each of the two genes. Physiologically the proportions

of the five isoenzymes differ among tissues and organs in that an organ may be "fingerprinted" by its particular LDH isoenzyme pattern. It was also discovered that when cells are damaged, or when the permeability characteristics of their membranes are altered, these isoenzymes are discharged into the interstitial spaces and from thence into the circulation (1, 77). Since there is a constant turnover of body cells in the normal individual a serum sample at any time yields a "normal" enzyme profile of the various proportions of the LDH isoenzymes which tends to be characteristic for a species (73). Similarly this "normal profile" can be altered in various disease states (73). As an example it has been verified that liver and muscle contain a very large proportion of LDH_5 and, therefore, in diseases such as cirrhosis, hepatitis and muscular dystrophy the normal serum pattern is altered by an increase in LDH_5 (78, 79). Similarly it was found that the myocardium is richer, proportionally, in LDH_1 and LDH_2 and a finding of abnormally high serum levels of LDH_1 and LDH_2 (especially LDH_1) are now considered to be indicative, and highly specific for myocardial damage (73). The literature is now replete with references which verify the usefulness of determining the aberration of LDH_1 in cases of myocardial infarction (73, 74, 75, 80 through 91).

Although there have been several attempts at documenting myocardial damage during the course of, or following, CO exposure using various serum enzymes (including total LDH), a search of the literature has failed to reveal any attempt to determine myocardial damage during CO exposure via the aberrations in LDH isoenzymes. It therefore appeared highly probable that instances of minimal myocardial damage occasioned by acute CO exposure to rats could be documented by significant increases in serum LDH_1. The use of the rat for this purpose provides several special benefits. First, because the rat oxyhemoglobin dissociation curve is normally displaced further to the right than the corresponding normal curve for humans, for a given oxygen saturation of rat and human hemoglobin there is a higher P_{O_2} in rat blood and, possibly, even a higher oxygen content per unit volume. Secondly, since the M value for rats is approximately 145 (compared to 210 for humans) the affinity of CO for rat hemoglobin is less than it is for human hemoglobin (92). Finally, the normal LDH isoenzyme pattern of rat serum/plasma is predominantly composed of LDH_5 (the predominant fraction in the rat erythrocyte) which, in addition to being exactly the opposite of the normal serum pattern for humans, means that the concentrations of the predominant myocardial isoenzymes (LDH_1 and LDH_2)

are normally low (93). Thus, while a given concentration of CO would be expected to exert less of an effect on rats than on humans for the reasons given above, any effects which did result in myocardial damage would be more readily identifiable because of the "cleaner" serum LDH isoenzyme pattern in respect to LDH_1 and LDH_2. If myocardial damage, then, could be detected in rats acutely exposed to low levels of CO, the extrapolation of similar results occurring in humans exposed under identical conditions would merit close attention, especially when such humans were heavy cigarette smokers and already afflicted with coronary artery disease.

CHAPTER III

PURPOSE AND SCOPE

This investigation was an attempt to determine if biochemically detectable tissue damage, directly related to acute CO exposure, could be detected in such a vital organ as the heart and to quantitate the time-course of such damage in individual animals. The concentration of CO chosen for the investigation (500 ppm) was the upper limit to which animals could be exposed without the manifestations of acute CO toxicity and also is the ceiling normally encountered by man in his environment (e.g. 400 to 500 ppm of CO inhaled in cigarette smoke and present for brief periods in confined areas of automobile congestion) (1, 3, 12, 13, 94).

Myocardial damage was estimated by changes in the proportional distribution of the lactic acid dehydrogenase (LDH) isoenzymes in plasma and serum.

All animals in the study were treated identically except that the experimental animals were exposed to 500 ppm

of CO while the control animals were exposed to a normal air environment. Analyses of carboxyhemoglobin (COHb) were made to determine the amount of hemoglobin which was present as COHb following an exposure episode.

CHAPTER IV

METHODS AND MATERIALS

Exposure System

The exposure system consisted of a 12-cu ft plexiglass chamber (2-ft x 3-ft x 2-ft) into which CO was introduced after having been diluted with oil-free compressed air in a mixing chamber just prior to entry. The diluted CO entered the chamber through four 0.25-inch ports located at the corners of one end of the chamber. The rate of flow through the chamber was determined to be 0.5 cfm. Exit ports at the other end of the chamber were identical to the entry ports in size and location. Each entry port was connected to the mixing chamber by a 0.25-inch polyvinyl tube.

The mixing chamber was a polyethylene bottle which had been sealed and modified such that the polyvinyl tubing, after being inserted into a bored hole, formed an airtight seal with the bottle. An identical arrangement following the exit ports allowed the chamber's gaseous environment to be ho-

mogenized prior to exit via an exhaust tunnel. Connected to the exit mixing chamber was a 15-ft 0.25-inch polyvinyl gas sampling line which tied the chamber into a Beckman Infrared Analyzer Model 315A(L) (41-inch cell length). Both mixing chambers and the exposure chamber itself were located in a large 90-cu ft (6-ft x 5-ft x 3-ft) hood which was maintained under slight negative pressure utilizing an exhaust fan. This arrangement was necessary since the exposure chamber was under slight positive pressure because of the compressed air and, therefore, any escaping gas would be rapidly evacuated from the hood.

All fittings in the exposure chamber had been caulked with hot glue sealant. At the gas entry end of the exposure chamber was a 1-ft by 1.5-ft access port which could be sealed by an overlapping plexiglass port cover. The seal, in this instance, was facilitated by 0.125-inch bolts on the exposure chamber which ringed the port and matched corresponding holes in the port cover. In this manner when the cover was ringed along the overlapping portion with petroleum jelly, fitted to the access port such that the bolts were inserted through the cover and then pressed against the access port by tightening wing nuts on the bolts the chamber was rendered airtight for

the small positive pressure differential used during its operation.

The CO source tank, which was located outside the hood, was connected to the gas mixing chamber inside the hood by a line (0.25-inch polyvinyl tubing) from a Mine Safety Appliance (MSA) single stage regulator on the tank which entered the hood through a small aperture. The compressed air line was similarly connected from the compressed air source to the gas mixing chamber through a separate aperture. Yet another aperture permitted the gas sampling line (0.25-inch polyvinyl tubing) to exit the hood.

The CO content of the hood atmosphere was analyzed on several occasions by a Mine Safety Appliance (MSA) CO analyzer tube (#47134) while the chamber was in operation and the hood environment was found to contain $<$ 10 ppm CO (except when the port was opened following the termination of an exposure episode as outlined in part III). The CO infrared analyzer could be adjusted to monitor the exposure chamber either continuously or, by manual adjustment, intermittently. Prior to exposure the analyzer was calibrated with 100 per cent nitrogen, in order to adjust the amplifier baseline and then with CO of known concentration (206 ppm) in order to cal-

ibrate the up-scale span. Following calibration of the analyzer the mixture of CO and compressed air within the exposure chamber could be adjusted for proper proportions to meet the desired CO concentration. Once the calibration was completed the desired CO concentration could be maintained within ± 10 ppm using this exposure arrangement.

Animals

Fifty, male Sprague-Dawley rats (150 to 200 g at receipt were obtained from Sprague-Dawley, Madison, Wisconsin. All animals arrived safely and in excellent condition. None of the animals expired from the date of arrival through completion of the investigation. The arrivals were fed *ad libitum* on Purina lab chow and water and were housed in stainless steel cages affording sufficient space and aeration to avoid crowding. Animals were weighed weekly and those few which were discovered to be in negative nitrogen balance (based on weight data) or which exhibited any symptoms of ill health (e.g. blood around the nostrils or diarrhea) were immediately isolated until these symptoms disappeared. Cages were placed on three vertical shelves and were periodically rotated to insure homogeneous exposure of all animals to existant environmental conditions. Earlier attempts to estab-

lish the dose of CO which would prove lethal for 50 per cent of an exposed group of rats was accomplished using locally obtained OKC (Oklahoma City) strain rats.

Exposure Protocol

All experimental animal exposures were conducted at CO concentrations of 500 ± 10 ppm. The atmosphere within the exposure chamber equilibrated at this concentration in approximately 8 minutes following initiation of exposure. The variable in the exposure protocol, then, was the length of exposure to this set concentration of CO. Different groups of animals (325 to 395 g) were exposed to this environment for periods of 1, 4 and 5 hours respectively.

LD_{50} studies attempted earlier with OKC strain rats had indicated that this level of CO was the maximum allowable concentration to which no rats would expire during a 5-hour exposure period. Of course, germane to the study was the fact that this CO concentration represented the maximum normally encountered in the environment (e.g. 400 to 500 ppm during inhalation of cigarette smoke and 500 ppm reported during a peak period of automobile congestion) (1, 12, 13, 94).

Control animals for each exposure period were identically exposed except that no CO was introduced into the sys-

tem. One group of animals, designated animal room controls, were not exposed and were used to determine whether there was a stress connected simply to the exposure protocol per se.

The period of timed exposure commenced when the desired CO concentration was reached and terminated when the CO concentration immediately began to decrease below 500 ppm following the closure of the CO regulator valve. The hood was also opened at this time, the port was unsealed and the port cover was removed to allow for a more rapid elimination of the CO remaining in the exposure chamber. The decrease in the CO concentration was monitored via the analyzer and the time required for elimination of the CO from the chamber to 100 ppm was approximately 5 to 6 minutes. When the level of CO reached 100 ppm the hood was once again opened and the animals were immediately removed from the chamber. This maneuver required less than 30 seconds for 5 to 6 animals.

Preparation Techniques

Withdrawal of blood via cardiac puncture or I.V. withdrawal prior to autopsy (depending on the series) was initiated within 3 to 5 minutes following removal of the first animal of a series with the same procedure accomplished on remaining animals in succession. Total time for blood with-

withdrawal via cardiac puncture (25-gauge needle x 0.75-inch length) for a group of five animals was approximately 15 to 20 minutes while blood withdrawal via the inferior vena cava (20-gauge needle) and complete autopsy required approximately 12 to 15 minutes per animal. Regardless of which procedure was followed, all animals were anesthetized with diethyl ether during withdrawal of blood samples.

Autopsy was accomplished on those animals which were exsanguiated via blood withdrawal from the inferior vena cava. Withdrawn blood was collected using disposable plastic syringes into either dry plastic tubes and allowed to clot or into tubes which contained 0.1 ml of sodium heparin (Organon - 1000 USP units per ml), depending on whether serum or plasma was desired. Clear serum was drawn off with a Pasteur pipette, centrifuged at 1500 x g for 15 minutes and the supernatant fluid placed in a small plastic container and covered with Parafilm. Heparinized blood was centrifuged at 2500 x g for 15 minutes and the clear supernatant was drawn off and likewise placed in a plastic container and covered with Parafilm.

Extreme care was exercised throughout this procedure in order to prevent hemolysis, as this would interfere with the determination of the LDH isoenzyme patterns by in-

creasing the proportion of LDH_5, which is in very high concentration in the rat erythrocyte, over LDH_1 and LDH_2 (93). Such care included slow withdrawal of blood following acurate cardiac puncture, removal of the needle after withdrawal of the blood and release of the blood into the bottom of the plastic receiving tube by slowly withdrawing the plunger completely out of the barrel of the syringe so that the blood flowed into the tube rather than being forced into it by the plunger. Such care was dictated by earlier findings that rapid withdrawal of blood or forcing blood out of the syringe, with or without the needle attached, resulted in hemolysis which was either clearly visible or which was indicated by the finding of an abnormally high LDH_5 concentration upon serum electrophoresis.

Analytical Procedures

Carboxyhemoglobin Determination

Carboxyhemoglobin (COHb) was determined according to Harper (95). In essence the assay relies on the fact that sodium hydrosulfite rapidly reduces oxyhemoglobin and methemoglobin to reduced hemoglobin while carboxyhemoglobin is unaffected. After first measuring (D_1 reading) the total hemoglobins spectrophotometrically (Beckman Spectronic 20) at

635 mu, the sample was placed in the dark and exactly 10 minutes afterward a second reading (D_2) was made. The percentage saturation was then determined from a calibration curve by plotting the ratio: $\frac{(D_2 - D_1)}{D_2}$ against the percentage saturation. The calibration curve was established using rat blood which had been thoroughly saturated by bubbling 100 per cent CO through the blood for a few minutes following the development of a stable, cherry-red color.

LDH

The isoenzyme patterns of lactic acid dehydrogenase were assayed by a Beckman Microzonal electrophoresis apparatus using cellulose acetate (Beckman) strips as the supporting medium. A Beckman Duostat provided the power. Both serum and plasma were assayed using this apparatus according to Barnett (96). Essentially the system used Tris-HCl-Barbital buffer in the microzonal cell, which was connected to the Duostat. A bridge supported the cellulose acetate membrane which was very accurately spotted with the material to be assayed via a mechanized microliter pipette which held 0.25 µl. In all cases 0.75 µl of either plasma or serum was applied on each position on the membrane (each membrane had the capacity for eight different samples per run). Once the membrane had been "loaded", the current was applied and controlled so that

a difference of 150 volts was developed between the anode and cathode ends of the membrane, each of which was slightly submerged in the buffer. The microzonal cell was constructed such that a partition divided the cell into two portions, one of which was connected to the anode and the other to the cathode of the Duostat. In this manner the membrane, which had just previously been dampened with the buffer solution, acted as an electrically conducting bridge between the two oppositely polarized portions of the cell. The sample, which was deposited on the membrane, was thus isolated in an electrical field such that all electrically charged material in the sample migrated toward either the cathode or anode under the influence of approximately 5 to 8 amperes of electrical current. Terminating the completion of a run (40 minutes) the Duostat was disengaged and the membrane was cut to fit a standard Petri dish which contained an agar base (Difco Special Noble Agar) with the following ingredients:

Tris-HCl, 0.2M, pH 8.3

Lactic acid (60 per cent syrup)

Nitroblue tetrazolium solution (1 mg/ml water)

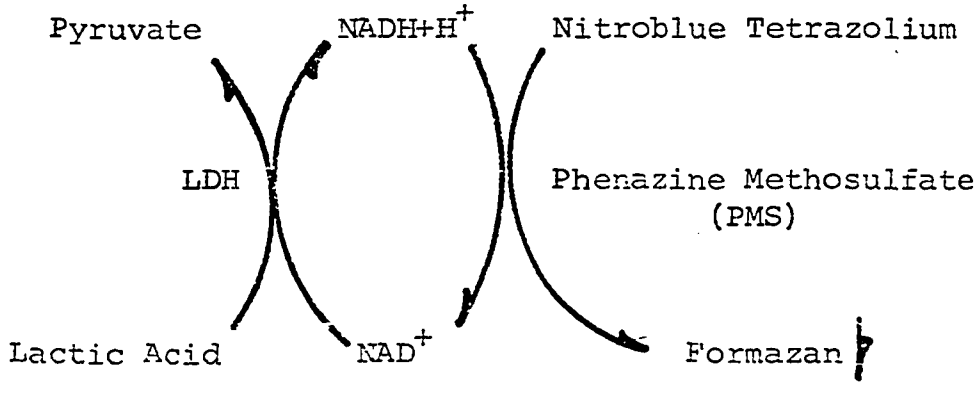
Phenazine methosulfate solution (1 mg/ml water)

β-NAD solution (16.6 mg/ml water)

Being careful to avoid entrapment of bubbles, the modified

membrane was placed on the surface of the agar gel to which it immediately adhered. The dish containing the membrane was then placed in an incubator at 37°C for 30 minutes.

The following reactions occurred between the LDH isoenzymes isolated at various positions on the membrane and the ingredients of the Agar gel:



Following incubation, during which time the membrane was developed as diagrammed above, the plate was removed from the incubator and the membrane was lifted off the agar gel, washed thoroughly in tap water to eliminate contaminating gel (which would add color to the background) and placed into a solution of 5 per cent glacial acetic acid for 5 minutes. The acid rinse served to decolorized the membrane background and to eliminate excess dye. Following a second wash under tap water the membrane was blotted dry, sandwiched between bibilous pa-

per and placed into the incubator at 37°C for 1 hour, or until thoroughly dry. Following the drying period the membrane, now ready for quantitation, was cut in half to fit the holder of a Photovolt Densicord Recording Electrophoresis Densitometer Model 542 fitted with a 545 mu filter and each of the eight specimen patterns was scanned and quantitated via a Photovolt Integraph Automatic Integrator Model 49 using a linear function relationship. The resultant recording was interpreted and the proportion of each, or any, of the five isoenzymes of LDH present was expressed as a percentage of the whole.

CHAPTER V

RESULTS

Attempts to establish an LD_{50} for OKC strain rats (350 to 400 g) exposed to CO failed to provide satisfactory results. At 810 $\pm$ 20 ppm one animal, out of five females, expired after 2 hours of a 4-hour exposure period with a COHb level of 75 per cent. The other four animals had COHb levels of 45 to 67 per cent (Table 1). Four of the animals were observed to be in a comatose state with labored breathing following the first hour of exposure but appeared normal during the last 2 hours of the 4-hour exposure. One animal did not exhibit any abnormal behavioral changes. The animal which expired during exposure exhibited Cheyne-Stokes respiration with several very acute clonic spasms just prior to cessation of respiration. Since the blood sample used for the COHb determination of the expired animal was not taken until after the 4-hour exposure period, (i.e. 2 hours after cessation of breathing) it is possible that clotting, erythrocyte concen-

tration or hemolysis may have been responsible for the higher assay result for this animal. It had been determined by Suzuki (33) that rats which expired during and after exposure to 1000 ppm of CO for 10 minutes had levels of COHb which were almost the same as survivors sacrificed at corresponding times. Hence, a direct correlation between the amount of COHb and physiological effects of sufficient severity to cause death may be questionable.

A similar 5-hour exposure of three animals (female) of the same strain to 500 ± 10 ppm resulted in no observable symptoms manifested in any of the animals during the exposure period. The COHb concentrations in these animals ranged from 35 to 46 per cent (Table 1). All animals in this series were anesthetized with diethyl ether following exposure and blood specimens were taken from the inferior vena cava.

A second series of animals (Sprague-Dawley males 250 to 300 g) were exposed to 500 ± 10 ppm of CO for periods of 1 and 5 hours respectively. None of the animals exhibited any adverse effects during or following exposure. After a specified exposure period the animals were removed from the chamber, anesthetized and a blood specimen was removed via the inferior vena cava. One portion of the blood was placed in plastic tubes containing heparin and the plasma was used for

LDH isoenzyme determinations. The other portion of the blood was used for the determination of COHb. The results of the COHb and LDH isoenzyme determinations are shown in Tables 2 and 3 respectively. There appeared to be no individual correlation between COHb levels and changes in LDH isoenzymes.

A third series of animals (Sprague-Dawley males - 325 to 395 g) were exposed for 1 or 4 hours and blood specimens were taken intracardially. The blood was again collected in heparinized plastic tubes and the plasma was used for LDH isoenzyme determinations (Tables 4, 5 and 6).

In both the second and third series control animals were exposed under conditions which were identical, in as near as possible, to the exposure conditions of the experimentals (Tables 7 and 8). The only difference, of course, was the presence of a known concentration of CO during the exposure of the experimentals. The results in the time-course aberration of the LDH isoenzymes are portrayed in Figures 1, 2 and 3. It will be observed that the LDH_1 values for individual animals exposed for 4 hours followed closely similar patterns during the period of maximum deviation (12 to 24 hours). It will also be observed that the blood sampling during the early stages of the third series was quite frequent and for this

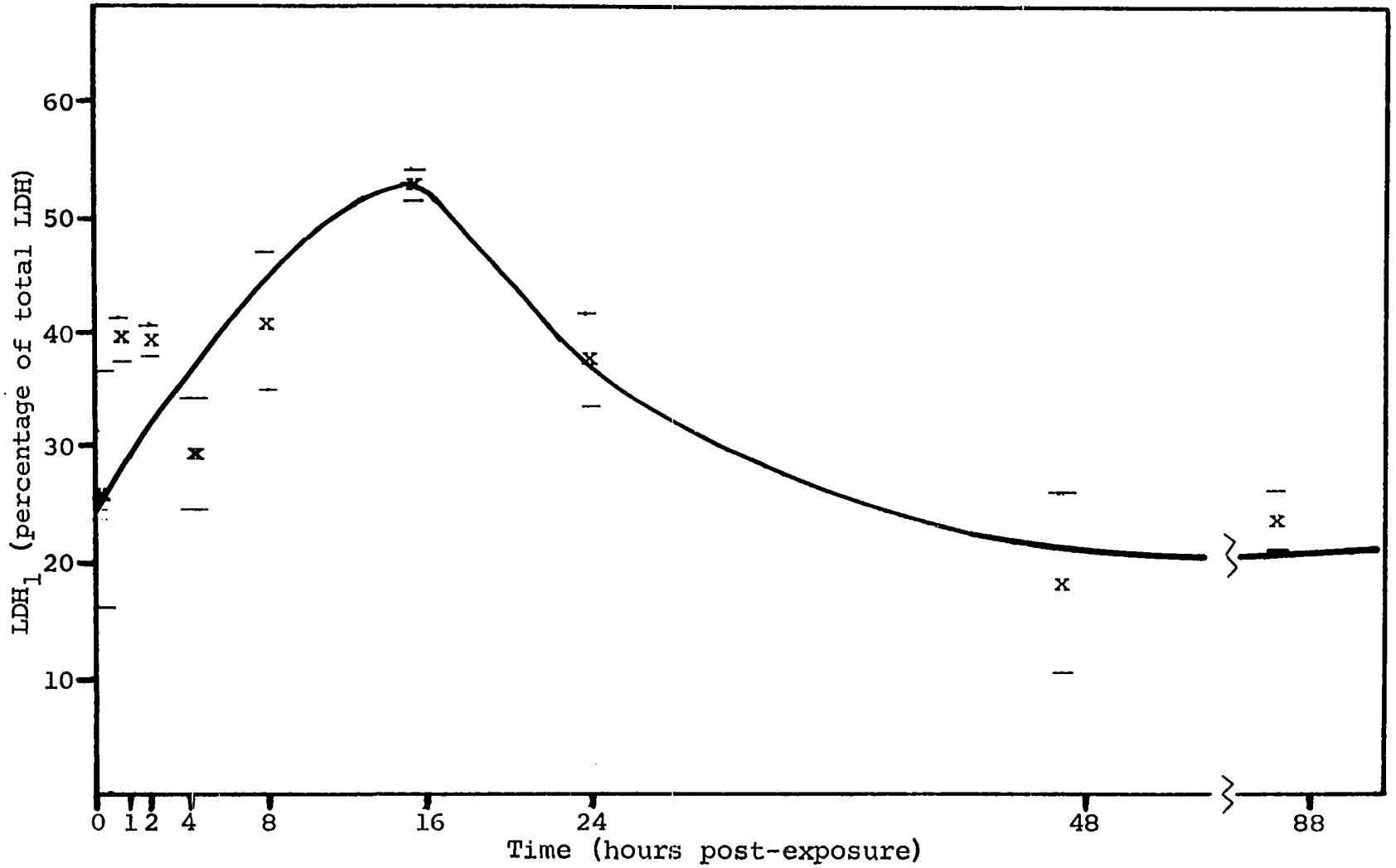


Fig. 1. Plasma lactic acid dehydrogenase isoenzyme #1 (LDH_1) aberration in rats exposed to 500 ± 10 ppm of CO for 4 hours. (Mean and Standard Deviation)

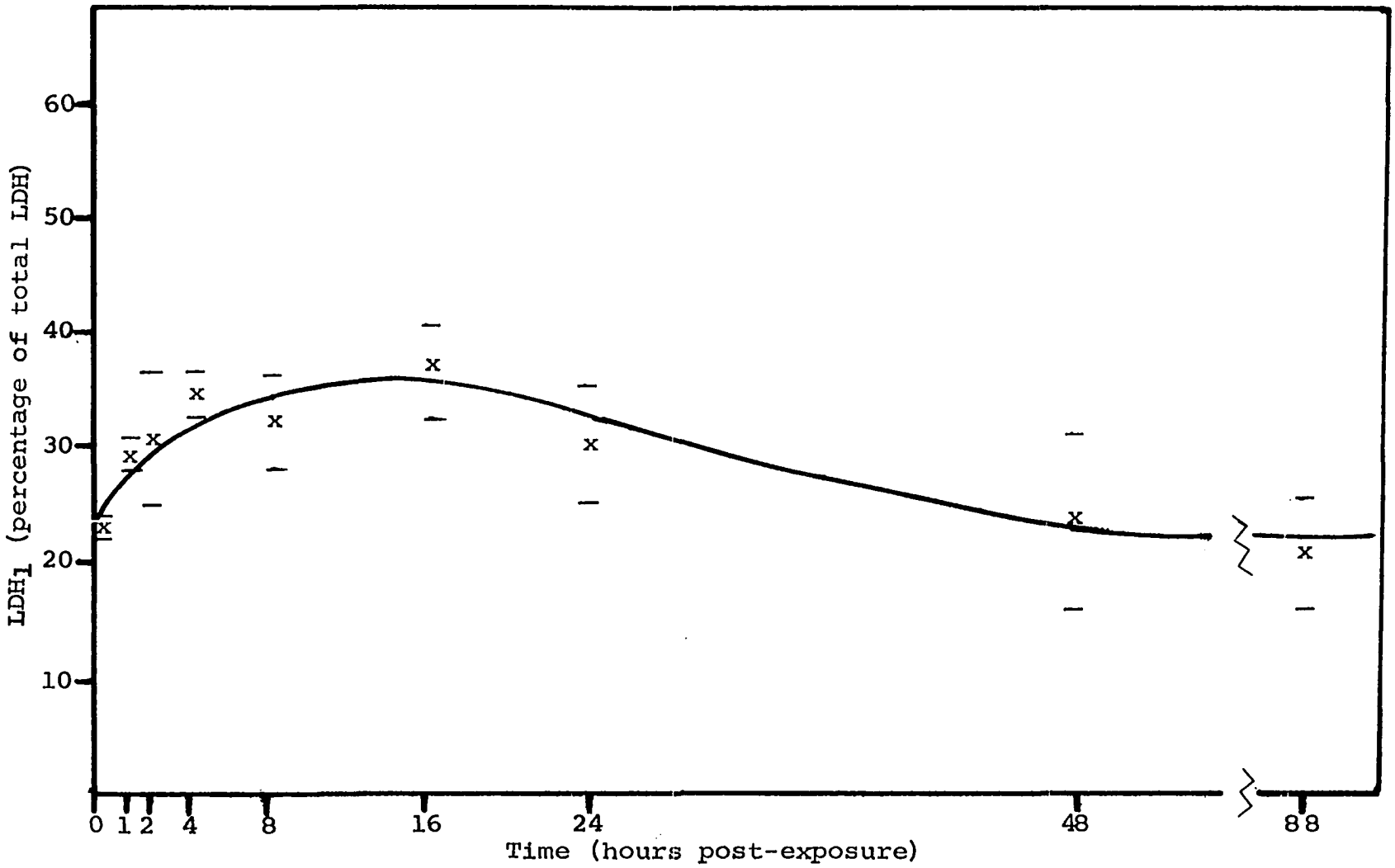


Fig. 2. Plasma lactic acid dehydrogenase isoenzyme #1 (LDH_1) aberration in rats exposed to 500 ± 10 ppm of CO for 1 hour. (Mean and Standard Deviation)

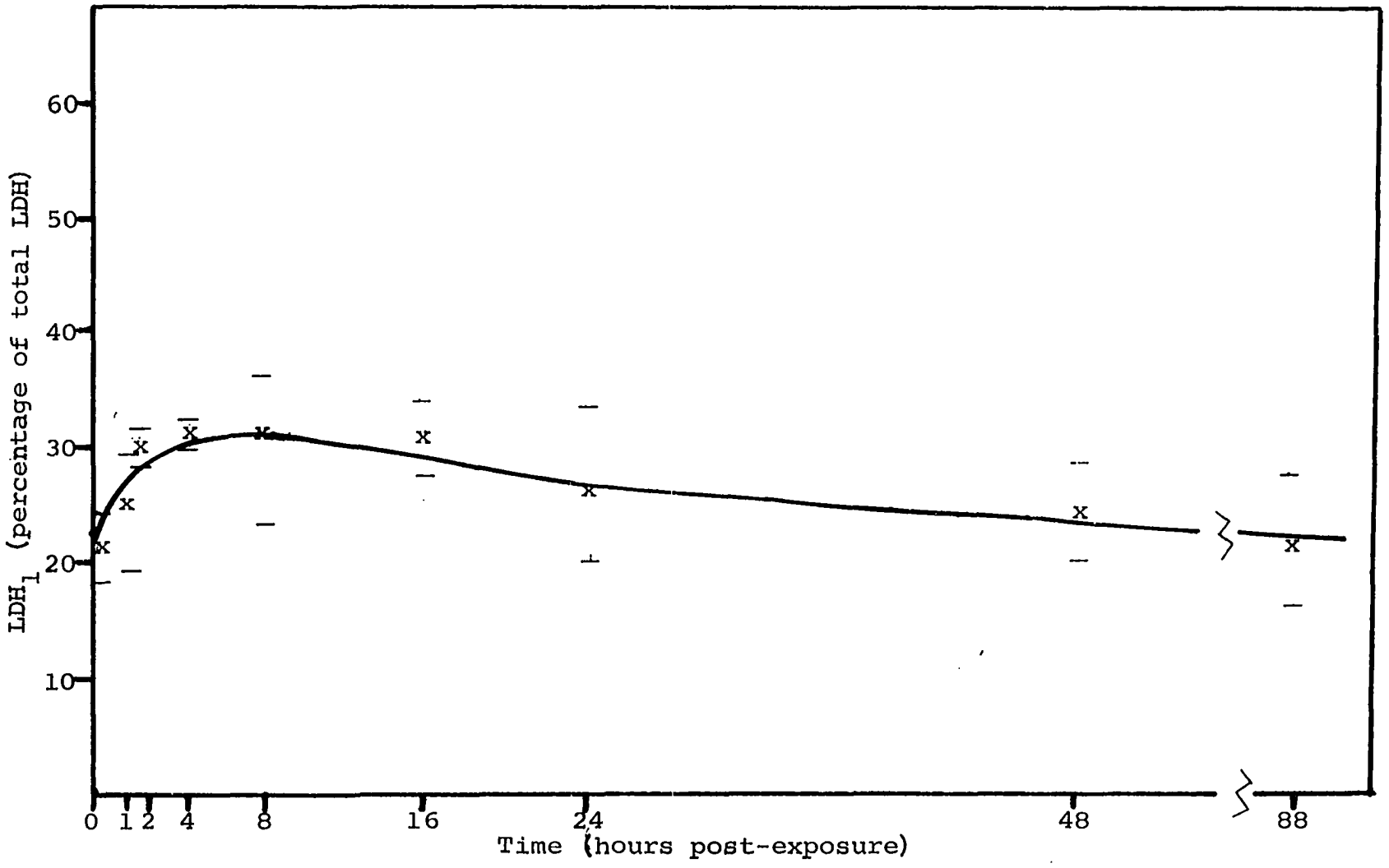


Fig. 3. Plasma lactic acid dehydrogenase isoenzyme #1 (LDH_1) aberration in rats exposed to 500 + 10 ppm of CO for 1 hour. (Mean and Standard Deviation)

reason the sampling intervals were staggered among the animals to avoid undue stress.

The results of several attempts to quantitate the time-course of changes occurring in animals exposed for 1 hour were inconclusive (Figures 2 and 3).

CHAPTER VI

OBSERVATIONS AND DISCUSSION

The results clearly indicated that myocardial damage evidenced by the plasma increase of LDH_1 occurred in animals exposed for 4 and 5 hours to 500 $\pm$ 10 ppm CO. The results following 1-hour exposure were not as revealing and could best be described as inconclusive.

In general the results indicated a threshold which could have depended upon one, or more, of the following parameters:

A) concentration of carbon monoxide (CO)

B) level of blood carboxyhemoglobin (COHb)

C) length of exposure.

It should be realized, of course, that (A) and (B) are responsible for the value of (C) at any given time. It will be observed in Table 2 that while the COHb level was approximately 15 per cent after 1 hour of exposure the level increased to approximately 40 per cent after 5 hours. These amounts of

COHb are greater than the equilibrium value for COHb using the Haldane equation shown on page 7 (41). If one sets the P_{CO} in this equation at 0.05 per cent (500 ppm), the P_{O_2} at 21 per cent and the value of M at 145, then the equilibrium value for COHb should be 25.6 per cent. The value of M, of course, is dependent upon the blood pH (Figure 4). In fact the range of M for the rat can vary from 130 through 195. Haldane placed M at 175 for the rat, but for a normal blood pH of 7.25 this value is 145. There have been several other formulas proposed for the determination of COHb (97 through 100). These have been proposed in order to take advantage of such additional parameters involved with CO exposure as rate of activity (99) (measured as pulse rate), length of exposure (98, 99) and altitude (98, 100). These equations have all been formulated with the view toward human application.

The values in the literature for COHb in rats exposed to similar concentrations of CO vary among investigators so that a precise value is doubtful. The COHb values obtained in this investigation, then, should be limited to Sprague-Dawley males exposed for various lengths of time to 500 ppm CO. Because of the differences previously noted in rats and humans in the affinity of CO for hemoglobin and for the displacement of the oxyhemoglobin dissociation curve (Figure 5), the ex-

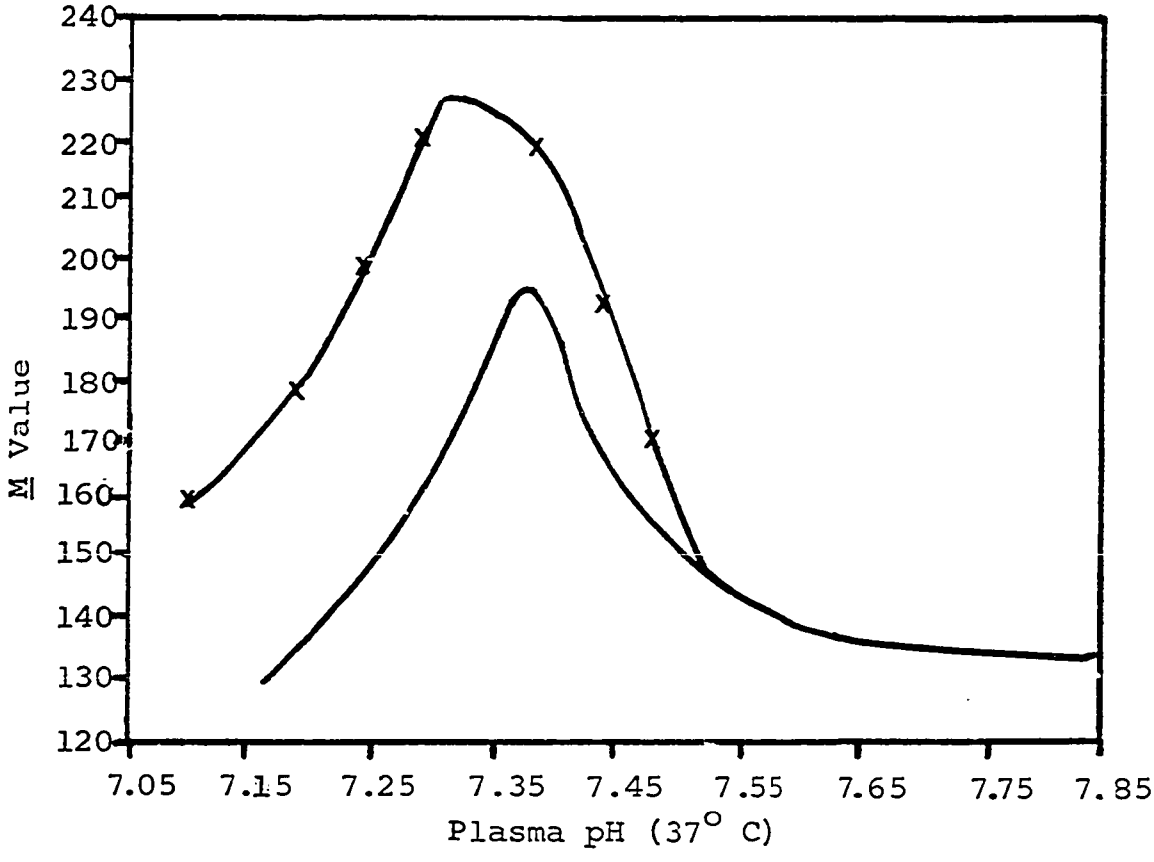


Fig. 4. Effect of plasma pH on the M value for human (—x—) and rat (——). [After Allen and Root (48).]

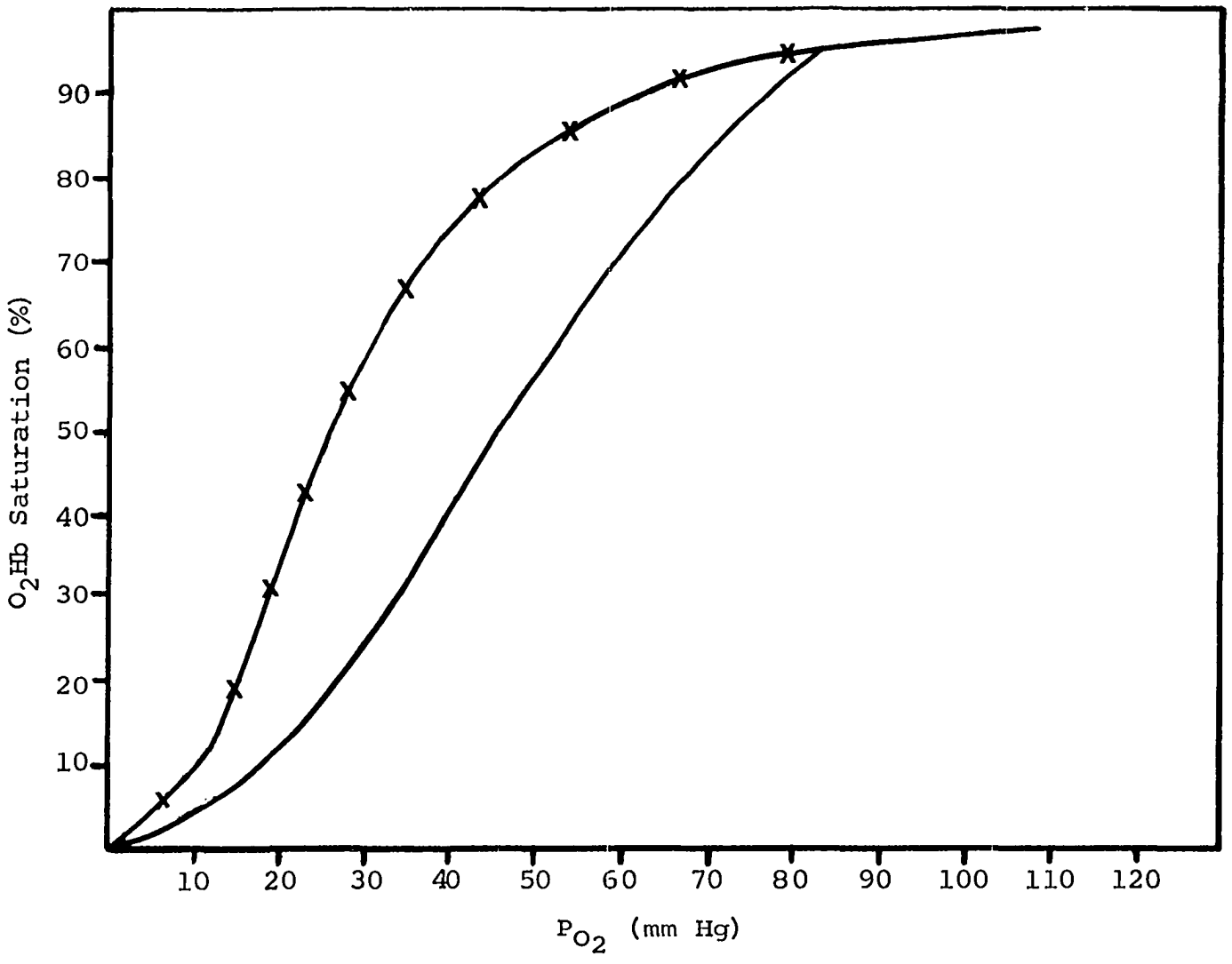


Fig. 5. Oxyhemoglobin dissociation curves for human (—X—) (8) and rat (——) (101).

trapolation of the effects in rats with a given COHb level to similar effects in humans with the same COHb level becomes difficult and more of theoretical than of practical importance. The wide range in COHb content between individuals of the same species exposed to a common concentration of CO further serves to underline this distinction, although del Vecchio (102) has postulated that a so-called "lethal index" of 0.66 exists for COHb $\left(\frac{COHb}{Hb}\right)$.

The results of Suzuki's investigations (33) are of additional interest in this regard. The rats which expired during or following a 10-minute exposure to 1000 ppm of CO had levels of COHb that were almost the same as those for surviving rats which were sacrificed at corresponding times. It would then appear that perhaps a better inter-species common denominator for comparison of effects detected during CO exposure would be simply a time-concentration parameter which would compare exposure episodes to a given concentration of CO for a given length of time. In this manner a 1-hour exposure to 500 ppm of CO, for instance, would be comparable for all species. Although it is readily evident that this regime would negate interspecies differences, such a common base would serve to simplify comparisons of various investigations

without placing the major emphasis on the level of COHb.

It has long been realized that exposure to high concentrations of CO could result in cardiac damage (1, 26, 72). Numerous investigators have reported evidence of cardiac changes ranging from slight alterations in EKG recordings (61, 63, 103 through 106) to visible myocardial necrosis (63, 107). As a rule the EKG recordings have provided the more subtle evidence at low CO concentrations while clearly visible necrotic damage has been observed at the higher concentrations, although Stearns et al. (108) have reported finding myocardial necrosis without concomitant changes in the EKG patterns. However, chronic exposure to lower concentrations (e.g. 100 ppm) have resulted in the discovery of small foci of necrosis in the myocardium by several investigators (63, 107). In general, when paired experiments have been conducted using animals which had been rendered hypoxic either by artificial breathing mixtures containing reduced amounts of oxygen or by an increase in the "altitude" of exposure such that the oxygen deficit was equivalent to that induced by the CO exposure, the overall results have been very similar (109 through 113). This similarity between hypoxia-induced results and CO-induced results has strengthened the case for a common mechanism of

action for the two. In both instances, of course, the oxygen-carrying capacity of the blood is diminished but important differences between the two do exist as emphsized earlier on pages 10 and 18 (32, 110).

A review of the literature has fairly consistently revealed a greater emphasis on the levels of COHb and on the length of time necessary to reach a given level of COHb during exposure to a given level of CO. Much less importance has been attached to the exposure concentration of CO *per se*. As mentioned earlier Suzuki (33) demonstrated that animals with identical levels of COHb, accrued during exposure to identical concentrations of CO, exhibited widely different physiological reactions. These findings indicate that not only are there definite intraspecies differences regarding susceptibility to CO exposure, but also that a given level of COHb present in animals of the same species does not, necessarily, dictate common effects.

There is evidence that CO *per se* may exert a deleterious effect other than simple tissue anoxia resulting from its combination with hemoglobin (33, 36, 37, 50, 51, 61, 92, 114). Pecora *et al*. (34, 54, 115, 116) have postulated that the findings in several of his experiments were caused by CO *per se* and not by tissue hypoxemia. Suzuki (33) determined

the effects of an acute CO exposure (1000 ppm/10 minutes) on the fine structure of the rat heart muscle and concluded that not only was there a definite effect due to CO *per se* but the duration of exposure to CO was more important than the blood COHb level. Fati *et al*. (36) discovered that a progressive reduction in erythrocytic phosphoglucomutase activity in rabbits exposed to CO for 3 days was independent of oxygen deficiency.

Several epidemiological studies have demonstrated a correlation between CO and myocardial infarction (72, 117, 118, 119). Goldsmith (120), in a review of a Japanese investigation, related the discovery (by Komatsu) (119) of a high incidence of cardiac damage in residents of a remote village in Japan. It was found that the construction of the village huts permitted the accumulation of large amounts of CO (2000 to 3000 ppm) from an open charcoal fire during the winter months which resulted in levels of COHb of 20 to 30 per cent and an incidence of abnormal cardiac findings in 35 per cent of the inhabitants. Goldsmith emphasized that Komatsu's investigation was the earliest systematic study of the relation between CO exposure and myocardial disease. Cohen *et al*. (117), in an epidemiological survey of ambient air levels of CO in Los Angeles found correlation between com-

munity exposure to CO and the case fatality rate for myocardial infarction. Hexter and Goldsmith (72) have recently concluded that a significant association exists between community CO concentrations and mortality. They discovered, via statistical analysis, that CO was the only air pollutant studied in which an increase in its concentration could be correlated with excess mortality. Clayton et al. (121) proposed a negative correlation between ambient, street levels of CO and traffic accidents, however.

It will be observed (Figure 6) that the normal serum LDH isoenzyme pattern of rats and humans differs (93). While the normal human pattern includes a large proportion of LDH_1 and LDH_2 with smaller proportions of LDH_3, LDH_4 and LDH_5, the rat pattern is predominantly composed of LDH_5 with much smaller amounts of the other fractions (122, 123). It is presumed that the erythrocytes of the rat make a considerable contribution to the normal pattern since they are composed almost entirely of LDH_5. This fact underscored the importance in avoiding hemolysis throughout the blood sampling procedure.

Earlier attempts to obtain baseline LDH data from OKC strain rats using serum resulted in considerable fluctuations in the resultant isoenzyme patterns. When blood was

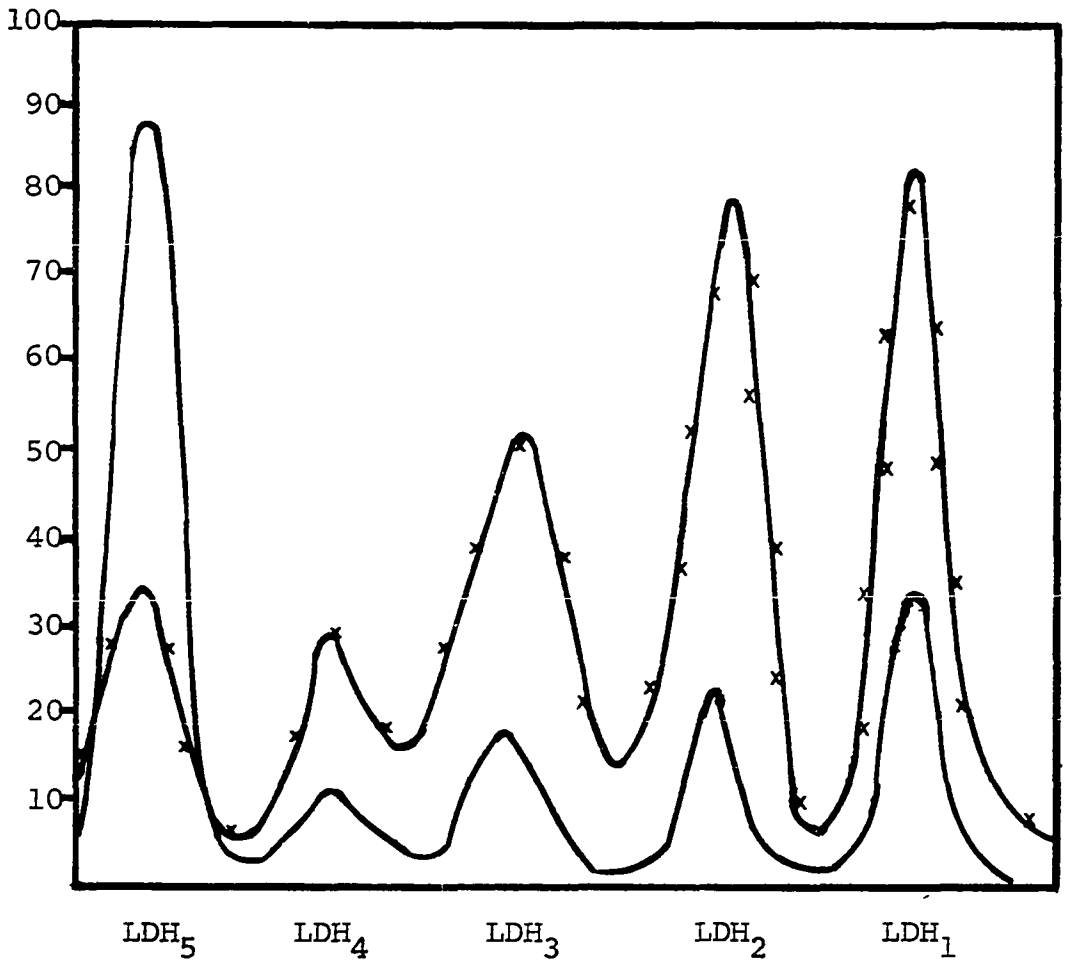


Fig. 6. Normal lactic acid dehydrogenase (LDH) isoenzyme pattern in human (—x—) (80) and in rat (———) plasma.

heparinized and the resultant plasma used for the isoenzyme determinations this problem was eliminated. Apparently the mechanics of clotting and the fact that LDH isoenzymes loosely adhere to the erythrocyte accounted for this discrepancy. A similar discovery was made by Papadopoulos et al. (123) when they found that serum LDH activity increased with clotting time. They postulated that the increase was caused by hemolysis, disruption of platelets (also extremely rich in LDH_5) and myocardial injury due to cardiac puncture. When plasma was used for isoenzyme analysis instead of serum the discrepancies were largely eliminated. As noted by Papadopoulos et al. (123), when blood is collected via cardiac puncture this technique apparently damages the myocardium such that the LDH isoenzyme content of the damaged myocardial cells "leaks" out into the general circulation. Although this fact was known at the time of the experiments concerned with the time-course of the aberrations in the LDH isoenzyme pattern following CO exposure, it was considered that the only practical method of sequential blood sampling (0.5 ml/sample) was via cardiac puncture. This technique was practiced until it was possible to very accurately insert the needle into the heart and withdraw the blood sample with one attempt. Otherwise the value of the control animals would have been dimin-

ished and the results would have been far less conclusive.

The careful handling of the blood at all stages was considered paramount for reasons already discussed. It is evident from the control pattern (Figure 7) that the act of cardiac puncture was sufficient, as emphasized by Papadopoulos, to release a small amount LDH isoenzymes into the circulation. This release is probably responsible for the rise in the control pattern following the "0-time" sample.

It has been verified that LDH_1 is inactivated by small amounts of pyruvate and, for this reason, it is relatively impossible for lactate to be produced in the myocardium under normal circumstances, thus insuring a constant aerobic metabolism (71). During anaerobic conditions, such as would be encountered during exposure to CO and during normal exercise, the coronary circulation increases and thus provides a continuing source of oxygen for the myocardium (1, 11, 75). The danger for persons with impaired coronary circulation due to coronary artery disease under either, or both, of the above anaerobic conditions is self-evident.

The specificity of the observed increase in the LDH_1 fraction of the normal plasma pattern as an indication of myocardial damage is evidenced by both the slight increase in LDH_1 of the control pattern (due to cardiac puncture) and in

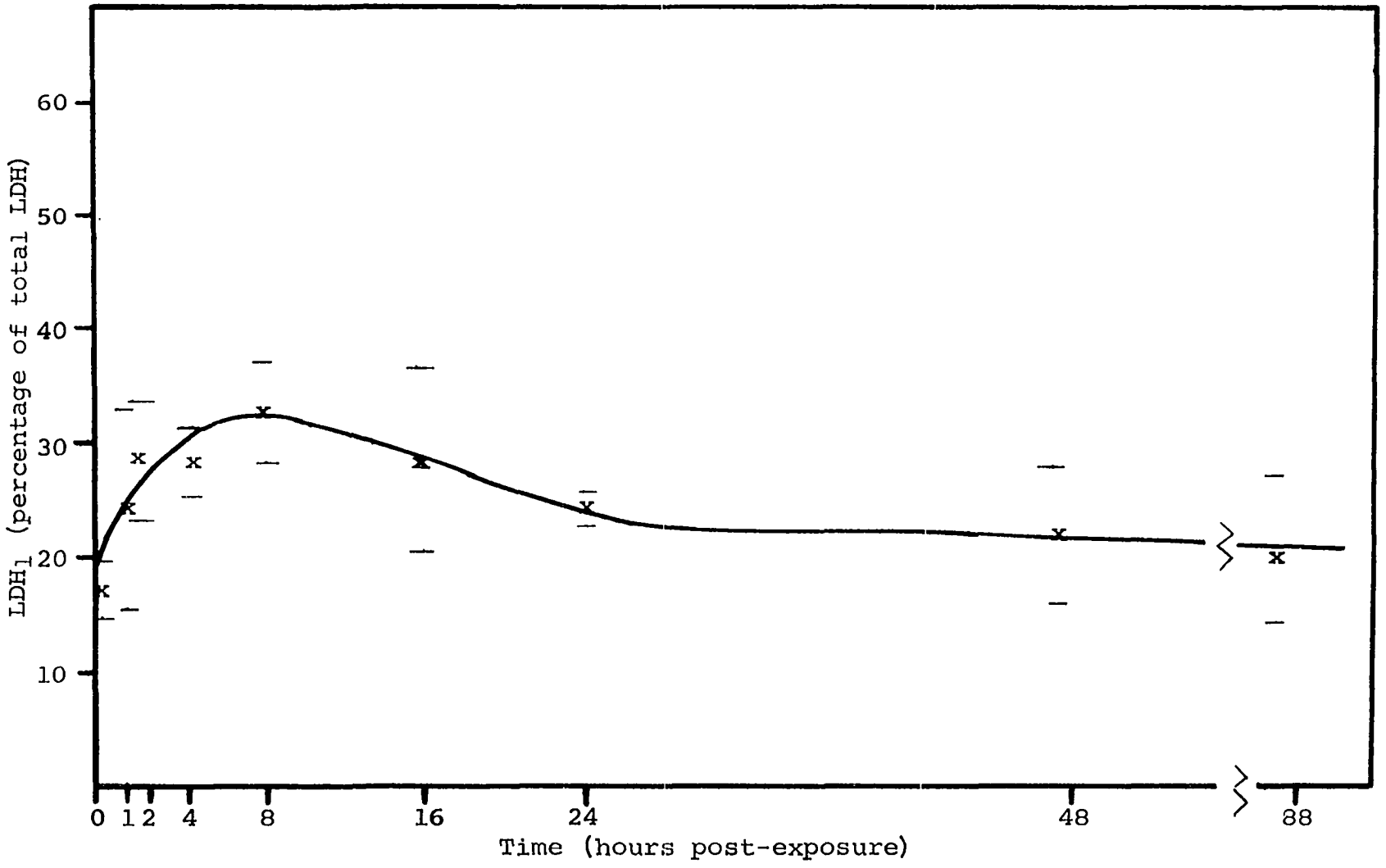


Fig. 7. Plasma lactic acid dehydrogenase isoenzyme #1 (LDH_1) aberration in rats exposed to a normal air environment for 4 hours. (Mean and Standard Deviation)

the LDH isoenzyme concentrations of the various organs, with the heart having the highest proportion of LDH_1 (Table 9). Although the assays of LDH isoenzymes in the same organs vary among investigations it widely accepted that the myocardium is the richest of all tissues in LDH_1 (83, 84, 124). While various serum enzyme parameters have been measured with the view of accurately diagnosing myocardial infarction, it has now been generally accepted that the fractionation of the LDH isoenzymes, with a specific increase in LDH_1, provides the greatest specificity and sensitivity (77). Under the conditions of the investigation, then, it is obvious that the observed increase in the plasma LDH_1 fraction of the experimental animals indicated a release of LDH from the myocardium which could not be explained simply as being due to the somewhat traumatic technique of cardiac puncture. Figure 1 illustrates the close similarity between experimental animals in the course of the LDH_1 aberration following the 4-hour exposure period. This is very significant when compared to the wide range of patterns exhibited by the control animals.

An interesting phenomenon of the time-course investigations was the fact that the maximum deviation in LDH_1 occurred approximately 16 hours after termination of the exposure period. This "lag" effect probably represented the

length of time necessary for any quantity of the isoenzymes to reach the general circulation from the myocardium, but it also may have indicated an active role for CO per se, other than tissue hypoxemia, since the COHb level would have been essentially within normal limits by that time (16 hours). Should the former statement be correct, then the probability of focal necrosis would also be quite high. If the myocardial response to the test conditions had been merely a release of LDH via altered membrane permeability, then there certainly would have been no great "lag" in the increase of plasma LDH_1. If, however, the enzyme came from foci of necrosis within the myocardium, then a "lag" would be expected since the damaged tissue in this area would have altered the normal coronary circulation and would have necessitated extensive diffusion of LDH to reach the circulation. The only other plausible explanation would indicate that CO per se had exerted some deleterious effect resulting in either altered membrane permeability or focal necrosis which became evidenced some 16 hours following exposure. Should this latter explanation be correct then similar changes which would be effected by a loss in the oxygen-carrying capacity of the blood because of increased COHb would have to be relegated to a minor role.

Several investigators (90, 91) have postulated that the increase in LDH isoenzyme activity following myocardial damage cannot be completely accounted for by necrosis.

Fortunately, the necessary "hypoxic control" experiment has been performed using the serum LDH isoenzyme parameter. Selmeci *et al.* (125) exposed rats for 6 hours to a simulated altitude of 18,300 ft in a low-pressure chamber and determined the aberration in the LDH isoenzymes. The altitude of 18,300 ft is equivalent to a total pressure of 384 mm Hg and a P_{O_2} of 80.5 mm Hg. This P_{O_2}, in the rat, is responsible for a hemoglobin saturation of approximately 65 per cent with an arterial P_{O_2} of 56 mm Hg. If the normal oxygen saturation of rat hemoglobin is considered to be 97 per cent, then exposure to this altitude would be equivalent to a COHb content of 32 per cent (98) which, in the case of the rat, would be equivalent to a CO exposure of 500 ppm for approximately 4 hours. Hence, the anoxic hypoxia conditions in the investigation of Selmeci *et al.* are very closely comparable to the anemic hypoxia exposure conditions of the present investigation.

In their study Selmeci *et al.* (125) discovered that while both the total LDH activity and the LDH_1 activity were

elevated in the experimental animals, the proportion of LDH_1 activity remained unchanged. It is evident, therefore, that the experimental animals in the investigation of Selmeci *et al.* did not demonstrate the myocardial damage which characterized the experimental animals exposed for 4 and 5 hours in the present study. One must conclude from this obvious discrepancy that under the conditions of the present investigation CO, and not the indirect effect of tissue anoxia *per se*, was principally responsible for the aberration in LDH_1 in the experimental animals.

Several investigators have reported changes during exposure to CO in various tissues which were more severe than could be relegated to hypoxia alone (33, 34, 52, 54, 115). Niden and Schultz (52) suggested that the severity of ultrastructural lung damage observed in rats exposed to 0.5 per cent to 1.0 per cent CO for 137 to 13 minutes, respectively, was out of proportion to an equivalent degree of hypoxemia alone. They suggested that CO had a direct effect on the pulmonary tissue in addition to the indirect effect of tissue hypoxia. They further hypothesized that an observed occurrence of pulmonary capillary platelet thromboses was initiated by injury to the capillary wall caused by inhalation of CO. Their conclusion supports that of Drinker (126), who

postulated that CO produces cerebral and coronary thromboses as a result of capillary wall damage.

Suzuki (33) exposed rats to a concentration of 1000 ppm of CO for 10 minutes and examined the hearts by both light and electron microscope. His ultrastructure studies were very impressive. While the hearts of animals sacrificed immediately following the exposure period demonstrated no abnormal effects, in rats sacrificed 10 minutes after the exposure period terminated Suzuki discovered extensive, and often quite severe, intracellular edema. In addition, swelling of the sarcoplasmic reticulum occurred in some muscle fibers along with moderate to severe mitochondrial swelling and a decrease in the density of the matrix. The cristae of the mitochondria were sparse in the cells of these fibers, and in some sections the cristae were disorganized and disrupted. Of great importance was the finding that the external membrane of some mitochondria was missing. The most striking changes occurred within 30 minutes to 1 hour following exposure when, in addition to the above alterations, this investigator discovered the presence of what seemed to him to be lysosomes which, when present in the myocardium, are a sure indication of sub-cellular damage. In rats sacrificed after 5 hours following exposure the mitochondria appeared to be returning to normal but

in the cells of some fibers the disappearance of the nuclear membrane was observed. The hearts of rats sacrificed 24 hours following exposure appeared to be essentially normal. Suzuki suggested that CO was responsible for the disruptive effects in the mitochondria and, hence, that the oxidative enzymes of the myocardium were severely inactivated. He also blamed the partial disappearance of the nuclear membrane on the toxic action of CO and summarized his findings by stating "...the functional cardiac disturbances in CO poisoning seem to be due to direct damage to the heart muscle and not due to hypoxia per se."

Based on the evidence obtained in this and related investigations, an area of immediate concern is the comparison of the aberration in the isoenzymes of LDH in heavy, chronic smokers to a cohort population. Although the time intervals used in the study (1 to 5 hours) represent unnatural lengths of exposure under normal conditions, the fact that one king-size cigarette will produce an exposure to approximately 475 ppm of CO for 6 minutes requires the correlation of myocardial alterations (via LDH isoenzymes) between the chain-smoker with the observed aberrations for the longer periods of time in this study.

CHAPTER VII

SUMMARY

Adult Sprague-Dawley male rats were exposed to 500 ± 10 ppm of carbon monoxide (CO) for varying lengths of time including 1, 4 and 5 hours. The concentration of CO was monitored by a Beckman Infrared Analyzer Model IR 315A(L). The animals were continuously monitored during each exposure period for indications of gross, physical abnormalities but none were detected. Control animals were identically exposed except that no CO was administered. The plasma distribution of lactic acid dehydrogenase isoenzymes was determined in blood specimens drawn before, immediately after, and at 1, 2, 4, 8, 16, 24, 48 and 88 hours after exposure.

Immediately following an exposure period the animals were removed from the exposure chamber and a blood specimen was withdrawn either I.V. (inferior vena cava) or intracardially, dependent upon the series. Either plasma or serum was collected and was analyzed both for carboxyhemoglobin

(COHb) content, spectrophotometrically, and for the isoenzymes of lactic acid dehydrogenase (LDH - EC 1.1.1.27), electrophoretically, on a Beckman Microzonal electrophoresis apparatus using cellulose acetate for support.

The ratios of the five LDH isoenzymes were determined and the proportional percentage of each isoenzyme was calculated. When compared to control animals the experimentals exposed to CO for 4 and 5 hours demonstrated a statistically significant increase in LDH_1 (the major isoenzyme of the myocardium).

Attempts to quantitate the time-course of plasma alterations in the LDH isoenzyme pattern following 1 and 4 hours of exposure demonstrated a significant increase in LDH_1 within 1 hour following the 4-hour exposure period. The maximum increase in LDH_1 was observed at 16 hours following exposure from which time the proportion of LDH_1 decreased to within normal limits between 24 to 48 hours. Three attempts to establish the time-course changes following a 1-hour exposure period furnished inconsistent results.

The content of COHb in the 4-hour exposed animals was approximately 47 $\pm$ 11 per cent immediately after exposure and was within control limits by 8 hours.

It was concluded that the delayed response in the

fractional increase of LDH_1 following the 4-hour exposure to 500 ppm of CO was principally attributed to a toxic effect of CO *per se* on the myocardium and not to a tissue hypoxemia occasioned by the decrease in the oxygen-carrying capacity of the blood. The investigation also provided the first evidence of myocardial damage during CO exposure via the plasma aberration of LDH isoenzymes.

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APPENDIX

TABLE 1

CARBOXYHEMOGLOBIN (COHb) VALUES FOR OKC STRAIN FEMALE RATS (Series 1)

Exposure Protocol and Animal Number	COHb (as percentage of total hemoglobin)
810 ± 10 ppm (4-Hour)	
OKC-1	45.6
OKC-2	75.1
OKC-3	52.3
OKC-4	67.9
OKC-5	47.3
Mean	57.6*
Std. Dev.	13.1
810 ± 10 ppm (5-Hour)	
OKC-6	35.1
OKC-7	38.9
OKC-8	46.7
Mean	40.2*
Std. Dev.	5.9

*significant at $P < 0.05$ (4-Hour vs. 5-Hour)
All control animals registered COHb 1.0 per cent.

TABLE 2

CARBOXYHEMOGLOBIN (COHb) VALUES FOR SPRAGUE-DAWLEY MALE RATS (Series 2)

Exposure Protocol and Animal Number	COHb (as percentage of total hemoglobin)
500 ± 10 ppm (1-Hour)	
SD-7	9.2
SD-12	23.5
SD-20	11.7
SD-31	15.8
SD-47	18.1
Mean	14.8*
Std. Dev.	5.5
500 ± 10 ppm (5-Hour)	
SD-14	33.2
SD-17	32.8
SD-25	36.9
SD-30	38.9
SD-49	48.7
Mean	40.0*
Std. Dev.	12.5

*significant at $P < 0.05$ (1-Hour vs. 5-Hour)
All control animals registered COHb 1.0 per cent

TABLE 3

PLASMA LDH ISOENZYME DISTRIBUTION IN RATS EXPOSED TO 500 PPM OF CARBON MONOXIDE FOR 1 AND 5 HOURS (EXPRESSED AS PERCENTAGE OF TOTAL LDH)

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
1-Hour Exposure					
7	16.9	5.5	7.1	2.7	71.7
12	16.4	4.6	5.4	2.3	72.3
20	25.4	3.5	5.3	1.7	64.0
31	22.0	3.7	2.7	0.9	70.7
47	28.9	15.1	8.4	1.8	45.8
Mean	21.9	6.5	5.8	1.9	64.9
Std. Dev.	5.4	4.9	2.1	0.7	11.2
5-Hour Exposure					
14	33.2	23.2	9.1	1.2	33.2
17	48.7	25.0	2.4	-	23.2
25	36.9	23.2	5.7	1.4	32.7
30	38.9	16.9	1.7	-	41.8
49	32.8	22.8	11.2	.1.3	31.8
Mean	38.1	22.2	6.0	1.3	32.5
Std. Dev.	6.5	3.1	4.1	0.1	6.6

Levels of Significance (Student's "t" Test)
(x = P<0.05)

500 ppm (1-Hour) Air Control (5-Hour)	(x)*	-	-	x	-
500 ppm (5-Hour)	x	x	-	x	x

*significant at P 0.10

TABLE 4

PLASMA LDH ISOENZYME DISTRIBUTION IN RATS EXPOSED TO 500 PPM OF CARBON MONOXIDE FOR 1 HOUR:A (EXPRESSED AS PERCENTAGE OF TOTAL LDH)

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
"0"-Time Post-Exposure					
5	18.6	-	-	-	82.4
22	23.7	3.7	2.1	1.1	69.3
28	21.7	-	-	-	78.3
32			(Hemolyzed)		
40	22.8	6.8	3.1	-	78.3
Mean	21.7	5.3	2.6	-	74.3
Std. Dev.	2.2	2.2	0.7	-	7.3
1-Hour Post Exposure					
5	25.7	6.9	4.2	1.7	61.4
28	31.2	11.2	5.8	3.2	48.5
32	28.6	5.3	4.3	-	61.8
Mean	28.5	7.8	0.9	-	57.2
Std. Dev.	2.8	3.1	0.9	-	7.6
2-Hour Post-Exposure					
22	35.4	15.8	7.3	1.2	40.2
40	25.7	11.2	3.1	-	61.0
Mean	30.6	13.5	5.2	-	14.7
Std. Dev.	6.9	3.3	3.0	-	14.7
4-Hour Post-Exposure					
5	37.8	17.8	2.6	-	42.8
28	31.2	5.6	-	-	63.1
32	33.7	11.7	1.1	-	53.4
Mean	34.2	11.7	1.9	-	53.1
Std. Dev.	3.3	6.1	1.1	-	10.2

TABLE 4 Continued

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
8- Hour Post-Exposure					
5	35.3	12.6	3.6	-	48.4
22	25.7	7.8	-	-	67.5
28	38.9	12.8	4.2	2.1	41.4
32	31.6	15.6	1.2	-	51.4
40	30.3	14.3	-	-	55.4
Mean	32.4	12.6	3.2	-	52.8
Std. Dev.	5.0	3.0	1.8	-	9.7
16-Hour Post-Exposure					
5	37.8	12.6	-	-	51.5
22			(Hemolyzed)		
28	41.6	18.1	5.7	3.1	31.4
32	30.5	7.8	-	-	61.6
40	35.7	12.3	1.6	-	50.3
Mean	36.4	12.7	3.7	-	48.7
Std. Dev.	4.6	4.2	2.9	-	12.6
24-Hour Post-Exposure					
5	32.7	5.6	-	-	61.7
22	31.6	8.7	-	-	59.6
28	35.8	12.6	2.3	-	49.3
32	27.6	7.7	1.1	-	63.6
40	19.7	3.2	-	-	78.1
Mean	29.5	7.6	0.8	-	62.5
Std. Dev.	6.2	3.5	0.8	-	10.3
48-Hour Post-Exposure					
5	27.3	3.6	-	-	69.1
22	25.4	-	-	-	74.1
28	30.1	11.2	1.5	-	57.1
32	25.6	1.3	-	-	74.1
40	13.2	-	-	-	86.7
Mean	24.3	5.4	-	-	72.3
Std. Dev.	6.5	5.2	-	-	10.7

TABLE 4 Continued

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
88-Hour Post-Exposure					
5	25.3	6.7	1.3	-	66.7
22	19.4	-	-	-	80.6
28	15.7	-	-	-	84.2
32	24.6	1.2	-	-	74.5
40	15.9	-	-	-	85.0
Mean	20.2	4.0	-	-	78.2
Std. Dev.	4.6	3.9	-	-	7.6
	Levels of Significance (Student's "t" Test) (x = P<0.05)				
Post-Exposure					
"0"-Time	x	-	-	-	-
1-Hour	-	-	-	-	-
2-Hour	-	-	-	-	-
4-Hour	x	-	-	-	-
8-Hour	-	x	-	-	-
16-Hour	(x)*	-	-	-	x
24-Hour	-	-	-	-	-
48-Hour	-	-	-	-	-
88-Hour	-	-	-	-	-

*significant at P<0.10

TABLE 5

PLASMA LDH ISOENZYME DISTRIBUTION IN RATS EXPOSED TO 500 PPM OF CARBON MONOXIDE FOR 1 HOUR:B (EXPRESSED AS PERCENTAGE OF TOTAL LDH)

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
"0"-Time Post-Exposure					
16	23.6	2.1	1.6	-	72.7
27	20.0	-	-	-	79.0
48	19.7	-	2.6	-	81.7
50	15.8	3.1	2.6	-	69.0
44	25.8	5.3	-	-	69.0
Mean	20.9	3.5	1.8	-	74.3
Std. Dev.	4.0	1.6	0.7	-	5.8
1-Hour Post-Exposure					
16	22.3	6.3	4.3	-	67.3
27	21.8	11.4	7.8	-	58.9
48	30.6	2.1	1.3	-	65.8
Mean	24.9	6.6	4.5	-	64.0
Std. Dev.	4.9	4.7	3.2	-	4.4
2-Hour Post-Exposure					
50	31.5	18.6	8.4	1.6	41.6
44	28.3	12.1	7.3	-	52.4
Mean	29.9	15.4	7.8	-	47.0
Std. Dev.	2.3	4.6	0.8	-	7.6
4-Hour Post-Exposure					
16	28.7	10.6	-	-	60.8
27	33.6	13.2	3.2	-	53.9
48	31.2	7.6	6.1	1.2	53.9
Mean	31.2	10.5	4.7	-	56.2
Std. Dev.	2.5	2.8	2.0	-	4.0

TABLE 5 Continued

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
8-Hour Post-Exposure					
16	30.1	11.8	3.1	-	55.1
27	31.6	9.6	-	-	58.7
48	38.9	15.2	4.8	-	41.2
50	24.6	6.3	-	-	55.4
44	21.2	7.6	6.2	3.1	62.0
Mean	29.3	10.1	4.7	-	54.5
Std. Dev.	6.8	3.5	1.6	-	7.9
16-Hour Post-Exposure					
16	36.2	13.6	11.1	3.5	39.1
27	24.3	10.7	-	-	65.1
48	25.7	12.8	3.6	-	57.8
50	31.3	4.3	-	-	66.4
44	29.4	9.6	4.7	-	56.1
Mean	29.4	10.2	4.7	-	56.1
Std. Dev.	4.7	3.7	4.1	-	10.9
24-Hour Post-Exposure					
16	35.2	7.2	2.8	-	54.9
27	19.6	11.7	-	-	68.7
48	22.3	5.8	-	-	71.9
50	29.6	10.1	1.3	-	58.9
44	28.7	7.1	-	-	64.0
Mean	27.1	8.4	2.1	-	63.7
Std. Dev.	6.2	2.4	1.1	-	6.9
48-Hour Post-Exposure					
16	27.6	5.4	2.1	-	64.9
27	34.1	3.7	-	-	62.2
48	21.1	1.2	-	-	77.3
50	19.8	-	-	-	81.2
44	25.4	4.9	-	-	69.7
Mean	25.7	3.8	-	-	71.1
Std. Dev.	5.6	1.9	-	-	8.1

TABLE 5 Continued

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
88-Hour Post-Exposure					
16	25.3	1.2	-	-	73.4
27	30.6	5.4	2.3	-	61.6
48	24.2	-	-	-	75.8
50	17.6	-	-	-	83.6
44	18.1	-	-	-	81.9
Mean	23.2	3.3	-	-	75.3
Std. Dev.	5.4	3.0	-	-	8.7
	Levels of Significance (Student's "t" Test) (x = $P<0.05$)				
Post-Exposure					
"0"-Time	-	-	-	-	-
1-Hour	-	-	-	-	-
2-Hour	-	-	-	-	-
4-Hour	-	-	-	-	-
8-Hour	-	-	-	-	-
16-Hour	-	-	-	-	-
24-Hour	-	-	-	-	-
48-Hour	-	-	-	-	-
88-Hour	-	-	-	-	-

TABLE 6

PLASMA LDH ISOENZYME DISTRIBUTION IN RATS EXPOSED TO 500 PPM OF CARBON MONOXIDE FOR 4 HOURS (EXPRESSED AS PERCENTAGE OF TOTAL LDH)

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Before Exposure					
1	18.1	-	-	-	82.0
8	17.6	9.1	2.3	-	70.9
21	19.3	-	-	-	80.6
36	23.0	-	-	-	77.0
39	15.3	5.5	4.2	-	75.0
Mean	18.7	7.3	3.3	-	77.1
Std. Dev.	2.8	-	-	-	5.6
"0"-Time Post-Exposure					
1	29.4	16.4	10.7	-	43.5
8	21.5	10.2		15.0	53.2
21	23.3	10.8	15.9	-	53.2
36	43.2	17.8	8.9	-	39.1
39	17.7	11.8	8.2	-	62.4
Mean	27.0	13.4	10.9	-	49.6
Std. Dev.	10.0	3.5	3.5	-	9.0
1-Hour Post-Exposure					
1	41.2	21.9	11.2	3.1	22.5
21	42.0	24.0	-	-	34.0
39	37.8	16.0	9.2	10.1	26.9
Mean	40.3	20.6	10.2	6.6	27.8
Std. Dev.	2.2	4.1	1.4	-	5.8
2-Hour Post-Exposure					
8	40.3	24.6	10.9	-	50.0
39	38.2	26.5	11.8	2.9	20.6
Mean	39.3	25.6	11.4	2.9	35.3
Std. Dev.	1.5	1.3	0.6	-	20.8

TABLE 6 Continued

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
4-Hour Post-Exposure					
1	27.7	17.6	10.6	-	44.0
21	36.0	15.3	18.0		30.6
39	25.4	15.9	11.6	-	47.1
Mean	29.7	16.3	11.1	-	40.6
Std. Dev.	5.6	1.2	0.7	-	8.8
8-Hour Post-Exposure					
1	40.1	19.1	-	-	40.8
8	45.6	12.6	-	-	41.7
21	38.2	20.2	11.2	3.4	26.9
36	32.6	13.0	-	-	54.4
39	48.0	17.6	3.9	-	30.2
Mean	40.9	16.5	7.6	-	38.8
Std. Dev.	6.1	3.5	5.2	-	10.9
16-Hour Post-Exposure					
1	54.0	16.9	5.6	-	23.6
8	52.8	13.2	3.3	1.1	29.7
21	50.0	7.1	-	-	42.9
36	50.7	13.8	7.7	-	27.7
39	55.0	7.0	5.0		33.0
Mean	52.5	11.6	5.5	-	31.4
Std. Dev.	2.1	4.4	2.2	-	7.3
24-Hour Post-Exposure					
1	42.9	-	-	-	57.2
8	10.9*	-	-	-	89.2*
21	34.6	18.5	9.9	-	37.1
36	39.8	6.8	3.4	-	50.0
39	32.3	10.5	5.7	-	50.5
Mean	37.4	11.9	6.3	-	48.7
Std. Dev.	4.8	6.0	3.3	-	8.4

*not included in statistical analysis

TABLE 6 Continued

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
48-Hour Post Exposure					
1	11.7	3.2	2.1	-	83.0
8	3.9*	-	-	-	96.2*
21	24.0	-	-	-	76.0
36	21.9	19.1	10.8	1.3	47.1
Mean	19.1	11.2	6.5	-	68.7
Std. Dev.	6.5	11.2	6.2	-	19.0
88-Hour Post-Exposure					
1	20.6	4.1	2.7	-	72.7
8	26.8	7.2	7.1	7.1	51.8
21	25.4	15.9	12.7	-	46.0
36	20.6	15.5	10.3	6.2	47.4
39	27.5	3.5	-	-	69.0
Mean	24.2	9.2	8.2	-	57.4
Std. Dev.	3.4	6.1	4.3	-	12.6
	Levels of Significance (Student's "t" Test) (x = $P<0.05$)				
Post-Exposure					
"0"-Time	x	-	-	-	x
1-Hour	x	-	-	-	x
2-Hour	x	x	x	-	-
4-Hour	-	-	-	-	x
8-Hour	x	x	-	-	x
16-Hour	x	-	-	-	x
24-Hour	x	-	-	-	x
48-Hour	-	-	-	-	-
88-Hour	-	-	-	-	-

*not included in statistical analysis

TABLE 7

PLASMA LDH ISOENZYME DISTRIBUTION IN RATS EXPOSED TO A NORMAL AIR ENVIRONMENT FOR 5 HOURS AND IN ANIMAL ROOM CONTROL RATS

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Air Controls (5-Hour)					
4	17.5	11.0	10.4	6.5	54.5
15	19.1	10.5	8.6	5.6	56.2
29	17.2	1.3	-	1.3	90.4
41	11.3	4.7	5.7	3.8	74.5
45	19.1	11.3	6.9	3.5	59.1
Mean	16.8	7.8	7.9	4.1	66.9
Std. Dev.	3.2	4.5	2.0	2.0	15.3
Animal Room Controls					
13	15.6	-	-	-	84.3
33	13.3	6.7	3.6	1.3	75.2
35	18.9	5.8	1.2	-	74.1
42	21.7	3.2	-	-	75.1
34	17.6	4.8	2.7	1.1	73.7
Mean	17.7	5.1	2.5	1.2	76.5
Std. Dev.	3.2	1.5	1.2	0.1	4.4
Exposure	Levels of Significance (Student's "t" Test) (x = $P<0.05$)				
Air - 5-Hour	-	-	-	-	-
Air - 4-Hour	-	-	-	-	-
Animal Room	-	-	-	-	-
Air - 5-Hour	-	-	-	-	-

TABLE 8

PLASMA LDH ISOENZYME DISTRIBUTION IN RATS EXPOSED TO A NORMAL AIR ENVIRONMENT FOR 4 HOURS (EXPRESSED AS PERCENTAGE OF TOTAL LDH)

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
"0"-Time Post-Exposure					
2	14.9	-	-	-	85.0
3	17.0	3.5		1.4	78.0
6	18.8	-	2.6	-	78.6
9	19.8	-	-	-	80.2
18	17.9	8.7		13.6	59.8
Mean	17.7	6.1	-	-	76.3
Std. Dev.	1.9	3.7	-	-	9.6
1-Hour Post-Exposure					
2	20.0	-		15.0	65.0
3	19.0	6.4		22.2	52.4
18	34.2	17.2	-	-	48.4
Mean	24.4	11.8	-	-	55.3
Std. Dev.	8.5	7.7	-	-	8.7
2-Hour Post-Exposure					
6	26.4	5.7	5.7	4.6	57.5
9	32.4	8.8	4.4	1.8	52.6
Mean	29.4	7.3	5.1	3.2	55.1
Std. Dev.	4.2	2.2	0.9	2.0	3.5
4-Hour Post-Exposure					
2	26.8	-	-	-	73.3
3	31.2	8.3		3.2	58.3
18	26.3	21.1	-	-	52.7
Mean	28.1	14.7	-	-	61.4
Std. Dev.	2.7	9.1	-	-	10.7

TABLE 8 Continued

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
8-Hour Post-Exposure					
2	30.3	7.9	6.6	6.6	48.7
3	37.5	10.0	-	-	52.5
6			(Hemolyzed)		
9	28.0	10.3	6.5	0.9	54.2
18	35.5	8.3	-	-	56.2
Mean	32.8	9.1	6.6	3.8	52.9
Std. Dev.	4.4	1.2	0.1	4.0	3.2
16-Hour Post-Exposure					
2	30.2	-	-	-	69.7
3	21.8	-	-	-	78.2
6	29.0	6.3	-	-	63.7
9	23.7	-	-	-	76.4
18	39.6	13.2	-	-	47.2
Mean	28.9	9.8	-	-	67.0
Std. Dev.	7.0	4.9	-	-	12.5
24-Hour Post-Exposure					
2	26.3	-	-	-	73.7
3	27.6	7.3	-	-	65.2
6	24.1	3.1	-	-	72.9
9	22.8	11.4	8.1	-	57.7
18	26.3	9.5	3.6	-	60.6
Mean	25.4	9.4	5.9	-	66.0
Std.Dev.	1.9	2.1	3.2	-	7.2
48-Hour Post-Exposure					
2	11.5	4.9	3.3	-	80.3
3	26.2	16.7	11.9	-	45.3
6	24.8	3.1	1.6	-	70.7
9	27.3	-	-	-	72.6
18	17.5	5.3	3.5	1.8	72.0
Mean	21.5	7.5	5.1	-	68.2
Std. Dev.	6/8	6/2	4/6	-	13.3

TABLE 8 Continued

Rat No. (SD-♂)	LDH Isoenzyme Distribution				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
88-Hour Post-Exposure					
2	21.8	-	-	-	78.2
3	25.9	10.6	8.3	1.2	54.2
6	12.3	-	-	-	86.7
9			(Hemolyzed)		
18	23.8	4.8	4.8	-	66.7
Mean	21.0	7.7	6.6	-	71.5
Std. Dev.	6.0	4.1	2.5	-	14.1
	Levels of Significance (Student's "t" Test) (x = $P<0.05$)				
Post-Exposure					
"0"-Time	-	-	-	-	x
1-Hour	-	-	-	-	-
2-Hour	-	-	-	-	-
4-Hour	-	-	-	-	-
8-Hour	-	-	-	-	x
16-Hour	-	-	-	-	-
24-Hour	-	-	-	-	-
48-Hour	-	-	-	-	-
88-Hour					

TABLE 9

DISTRIBUTION OF LDH ISOENZYMES IN VARIOUS SPECIES AND TISSUES [After Wilkinson (84).]

Tissue	LDH Isoenzyme Distribution (%)				
	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Human:					
Heart	67	39	4	1	1
Kidney	30	50	15	5	-
Brain	25	25	34	15	1
Liver	2	2	3	12	80
Rat:					
Heart	50	30	12	6	2
Kidney	20	15	10	20	35
Brain	40	20	15	20	5
Liver	1	1	3	10	85
Rabbit:					
Heart	94	2	1	3	1
Kidney	63	10	8	9	9
Brain	43	20	27	6	4
Liver	1	3	17	39	40