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THE EFFECTS OF HYPOXIA AND HYPOXIA ON
SPONTANEOUS AVIAN ATHEROSCLEROSIS

A DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By

Keith E. Meske, B.A., M.A.

The Ohio State University
1971

Approved by

Harold S. Weis
Advisor
Department of Physiology
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VITA

Identity: KEITH MESKE
Born January 6, 1939
in Lorain, Ohio.
Married

Education: Primary and secondary education
in Lorain, Ohio

1957 - 1959 College of Wooster
1961 - 1963 M. A. Kent State Univ. (Cytology)
1966 - 1971 Ph. D. Ohio State Univ. (Physiology)

Special Distinctions

1969 - 1971 NIH Traineeship

Organizations:

1960 Phi Gamma Delta
1971 Sigma Xi

Experience

1961 - 1963 Dean of Men's Staff (KSU)
1963 - 1966 Instructor in Biology - Rio Grande College
1963 - 1964 Asst. Dean of Men - (RGC)
1964 - 1965 President, Faculty Assoc. (RGC)
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<th>Position</th>
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<td>1964 - 1965</td>
<td>Member President's Executive Council (RGC)</td>
</tr>
<tr>
<td>1966 - 1970</td>
<td>Graduate Teaching Asst. (OSU)</td>
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INTRODUCTION AND REVIEW OF LITERATURE

According to Robbins\textsuperscript{100} atherosclerosis is a common disease characterized by lipid deposition in the form of elevated fatty-fibrous plaques called atheromas within the intima and inner media of arteries. These lesions can decrease the cross sectional area of the lumen vessels as well as disrupt laminar flow. Certain stages of the lesion development may predispose to thrombosis.

Atherosclerosis is increasingly being referred to as a multifactorial disease because many factors appear to be involved but none shows high enough correlation with the course of the disease to be classified as the main cause. Among the implicated factors are clotting mechanisms\textsuperscript{104, 137}, blood pressure\textsuperscript{27, 64, 130}, blood and tissue cholesterol levels\textsuperscript{28, 115, 128, 129, 137}, peroxides\textsuperscript{6, 43, 44, 51}, hormone effects\textsuperscript{76, 80, 81}, enzyme levels\textsuperscript{84, 141, 148-151}, and various dietary factors\textsuperscript{32, 33}.

It has been known for some time that arterial tissue is relatively avascular\textsuperscript{89-91, 144} and exhibits a relatively low QO$_2$ when compared to the activity of liver\textsuperscript{55}. The normally low QO$_2$ in arteries may be due to some extent to cardiac work supplying a portion of the energy for electro-osmosis\textsuperscript{104}. The lack of
vascularization especially in the larger vessels such as the aorta, might be expected under a variety of conditions to result in hypoxia.

A considerable number of workers (6, 28, 59, 60, 106, 117, 141, 148-151) have suggested an involvement of hypoxia with many of the factors associated with the development of atherosclerosis. Kjeldsen et al. (62), Altschul (3) and Maximova (85) have shown a decreased development of atherosclerosis in cholesterol-fed rabbits resulting from exposure to hyperoxia. On the basis of this work it seemed logical to pursue a research program whereby an experimental animal that characteristically develops spontaneous type lesions be used to determine the effects of hypoxic and hyperoxic environments on atherosclerosis.

The chicken was a logical choice for an experimental animal because it develops spontaneous type lesions, thus avoiding the necessity of cholesterol feeding or other methods used to induce atherosclerosis. The lesions also have some similarity to the human form of the disease (91). The hen was chosen because its size and temperament enables it to tolerate confinement in an environmental chamber and it has been shown to be relatively resistant to oxygen toxicity (135, 136, 149). Adults were chosen because their well-developed lesions would allow both a reduction and an increase in severity to be measured. Finally there is a large amount of
information available on the chicken as an experimental animal especially in the areas related to atherosclerosis such as blood pressure, clotting mechanisms, hormones, plasma and tissue lipid concentrations, aortic metabolism and dietary induced changes (7, 8, 19, 20, 29, 31, 32, 35, 42, 70, 71, 101, 128-138).

Morphological considerations.

Recently Moss and Benditt (89-91) undertook an extensive ultrastructural survey of chicken aortae in which they compared normal areas with those having spontaneous and cholesterol-induced lesions. The spontaneous plaques are composed of slender and tapering cells which are smaller than the smooth muscle cells (SMC) of the media. These are found in abundant interstitial material. The SMC of the plaque differ from medial SMC only by lacking the characteristic peripheral vesicles and having a thickened basement membrane. The SMC of the abdominal segment differs from those of the arch by the presence of enlarged Golgi bodies, free ribosomes, and infrequent marked distention of the rough endoplasmic reticulum. The plaques range from 3-60 cells in thickness. It is especially interesting that there were no areas with an excessive accumulation of lipid which suggests that lipid deposition does not precede the spontaneous plaque. This is in agreement with Maier and Haimovici (78).
Aoyama and Iwakami(6) noted several changes in the intima prior to sudanophilia. An edematous intimal thickening was followed by an accumulation of metachromatic mucoid substance. After a fragmentation of elastic fibers occurs lipid deposition begins in these metachromatic areas.

With cholesterol feeding Moss and Benditt(91) found microscopic pinpoints of pale yellow in the aortic bifurcation and in thicker areas as early as the first month. None of the controls showed similar changes. Cellular alterations in the cholesterol-fed groups consisted of the presence of large round vacuoles in the cytoplasm of endothelial and medial cells. The sparsely distributed cells displaying prominent Golgi bodies, endoplasmic reticulum distention and increased numbers of ribosomes in the spontaneous lesion became more frequent in the cholesterol induced plaque. Similar changes were noted by Thomas et al. (123) in swine cells showing increased size and vacuolation (were referred to as foam cells) and were especially prominent directly adjacent to the endothelium. With cholesterol feeding many cells of the spontaneous plaques become vacuolated and the proportion of non-SMC increased. On the basis of cholesterol feeding for one and two month periods, they concluded that both the magnitude and duration of elevated plasma cholesterol levels influence the plaque with duration being the more
important. They compared the spontaneous lesion in the chicken to the intimal thickening stage in humans as described by Geer\(^{38}\). They also suggested similarities between the cholesterol-modified spontaneous plaque and the well developed human lesion.

Fritz et al.\(^{(36)}\) maintains that the undifferentiated cells in lesions which arise through de-differentiation of mature SMC are the cells showing the greatest incorporation of tritiated thymidine which may explain why so few SMC are seen in mitosis in these areas. The presence of varying degrees of de-differentiation in SMC of the plaque is also verified by Moss and Benditt\(^{(89-91)}\). Hassler\(^{(45)}\) suggests, however, that intimal thickening in rats caused by injury does not differ from pre-atherosclerotic changes and seems to be composed of SMC of the media and some endothelium.

Thomas et al.\(^{(123)}\) suggest that ultrastructural lesions are representative of the pre-proliferative phase of atherosclerosis and are capable of proliferating or healing. As a result of feeding swine cholesterol rich diets for periods up to six months, this group proposed three stages of atheroma development. The first or pre-proliferative phase is characterized by imbibition of cholesterol rich fluid into the intra- and extra-cellular compartments, the presence of formed and non-formed blood elements in the intimal intracellular
space and an increased turnover of endothelium and SMC as a result of damage to these cell types. A final or atheromatous phase would embody larger foci of necroses. Both the work of Thomas et al.\(^{123}\) and Fritz et al.\(^{36}\) would appear to disagree with Whereat\(^{141}\) who reports no DNA increase in atherosclerosis. In aortic tissue culture hyperlipemic serum has been shown to cause cell proliferation due to the globular fraction and cell degeneration due to the lipid fraction\(^{94}\).

Focal areas of altered cytoplasmic composition by which endothelial cells attach to each other and their basement membranes with fibrillar components are called dense zones. These have been shown to modify the stability and tenacity of the endothelial to endothelial and endothelial to basement-membrane bonds which could relate to thrombogenesis and intimal proliferation when such a bond is disrupted\(^{124}\).

**Metabolic considerations**

With the development of new techniques in evaluating tissue metabolism and the use of radioisotopes, an increasing emphasis is being placed on metabolic changes in the vessel wall that may predispose to or accompany the development of atherosclerosis\(^{6, 52, 56, 63, 103, 111, 141, 148-151}\). There is not much change in rat
(atherosclerosis resistant species) aortic $QO_2$ with age according to Sasaki et al.\(^{(103)}\). However, there was a progressively decreased incorporation of $P^{32}$ into ATP. This seeming contradiction may be a result of decreased oxidation-phosphorylation efficiency. Kirk\(^{(56)}\) has indicated a decrease in certain of the aerobic oxidative enzymes with atherosclerosis and age. He presents results which suggest a reduced aerobic and increased anaerobic metabolism in pathogenic compared to normal human peripheral arteries. Zemplenyi\(^{(151)}\) has also found decreased Krebs' enzyme activity accompanied by increased lactic acid dehydrogenase with age and in lesions.

Munro et al.\(^{(92)}\) have shown that atherosclerosis resistant species (rats) respond to increased dietary cholesterol by an increase $QO_2$ in the aorta while the atherosclerosis prone animals (cockerals) have shown the reverse. They also point out that the cockeral's thoracic $QO_2$ was lower regardless of diet and suggest that it is these areas of the vessel with higher initial $QO_2$ that are most susceptible to lesions. Christie and Dahl\(^{(23)}\) found that the thoracic $QO_2$ in the rat was greater than that of the abdominal. In this species atherosclerosis develops to a greater extent in the thoracic aorta. The rabbit aortic arch has a higher $QO_2$ and this is the area most severely affected in this animal. They also suggest that three factors associated with atherosclerosis in rats (age, sex
and site of lesions) appear to be correlated with \( \text{QO}_2 \). Further evidence\(^{108}\) for an early aortic metabolic effect in atherosclerosis is tissue culture work on swine intimacytes which showed increased \( \text{QO}_2 \) consumption in samples taken from atheromatous areas. This increase in \( \text{QO}_2 \) was also found to exist in the early stages of lipid accumulation by these same type cells. It should be pointed out that grossly normal areas containing these cells have been shown by electron microscopy to be pre-proliferative sites of lesions.

Maier and Haimovici\(^{78}\) from their study of succinic dehydrogenase and cytochrome oxidase in rabbits and dogs proposed that pre-atherosclerotic conditions may be associated with a decrease in aerobic enzyme activity followed by an increased activity in the early stages of atherosclerosis. Later stages of lesion development associated with fibrosis and necrosis are accompanied by a decrease in the enzyme activity. In Moss\(^1\) and Benditt\(^1\)'s study\(^{90}\) of the spontaneous plaque in chickens an increase or at least a change in cellular metabolic activity was indicated by the presence of enlarged Golgi bodies, swollen endoplasmic reticulum, and free ribosomes in intimal cells of lesions. They consider the spontaneous lesion to be an early phase.

Work with cholesterol-fed rabbits has shown an increased fatty acid synthesis by mitochondrial chain elongation which is associated
with an increased $O_2$ consumption. Cholesterol feeding has also been associated with an oxidative-phosphorylation uncoupling due to damage of the respiratory assembly by cholesterol accumulation on the mitochondrial membrane. Lofland et al.\textsuperscript{(72)} have emphasized the importance of the ability of aortic tissue to synthesize fatty acids in relation to their ability to remove cholesterol and other lipids from the tissue. It has also been shown that serum lipids and lipoproteins enter the aortic wall but are also synthesized endogenously\textsuperscript{(73)}. Analysis of intracellular lipid droplets taken from fatty streak lesions suggest that this is not unaltered plasma lipid infiltrate\textsuperscript{(66)}.

Zemplenyi and coworkers\textsuperscript{(148-151)} have shown that atherosclerosis resistant animals (rats) have a higher lipoproteolytic activity increased in atherosclerosis but is still less than that of normal rat aorta. The results of an inadequate level of activity is lipid deposition in the intima\textsuperscript{(68)}. If sufficient phospholipids are present in aortic tissue, cholesterol is reported to not be deposited because of their mutual solubility. It is only when the phospholipid:cholesterol ratio is decreased that deposition of cholesterol occurs \textsuperscript{(84)}.

An increased esterase activity has been measured according to Maier\textsuperscript{(79)} in early phases of plaque development and has been postulated to be due to an increased phospholipid synthesis. She also
suggests that phospholipids play a role in solubilizing the cholesterol in preparation for removal. A second possibility to the increased esterase activity would be cell proliferation. The amount of lipid diffusing into the arterial wall can be correlated with serum levels of cholesterol. Astrup et al.\(^9\) have measured a 2:1 ratio of serum cholesterol to aortic tissue cholesterol in rabbits on atherogenic diets. They have also shown that this ratio changes to 1:1 when the animals are exposed to CO.

A number of workers who have studied atherosclerosis from a variety of approaches have suggested a relationship between the disease process and hypoxia. Some workers\(^{59, 68, 69, 150}\) have discussed the effects of hypoxia on the lipemia-clearing response. It has been proposed that hypoxia causes the inhibition of vascular endothelial lipoprotein lipases which result in increased lipid deposition in the intima. Hypoxia is also reportedly responsible for an increased fibroplasia\(^{91}\).

A number of papers\(^{6, 9, 112}\) have indicated one effect of hypoxia to be an alteration of membrane permeability and this in turn would accelerate the above process with not only increased serum levels but also increased passage through the aortic wall. The normal ultraporous aortic wall is involved in the preservation of the blood-intimal interface which would permit selective ion
diffusion across the wall under normal blood pressure. There are changes in electro-osmosis associated with atherosclerosis and flux studies relate the absence of normal membrane chemistry to the absence of normal membrane architecture\(^{(104)}\).

Simard-Duquesne and Allard\(^{(111)}\) mentioned a coating action of lipids on the endothelium which might decrease the diffusion of \(O_2\). Scott et al.\(^{(106)}\) have mentioned the possibility of such a lipoprotein coating increasing aortic wall permeability. Others\(^{(51, 150)}\) have proposed the existence of an intimal metabolic barrier in various species. As lipid and other material accumulates, the diffusion distance increases to decrease the availability of \(O_2\). Sasaki et al.\(^{(103)}\) believe that an increased wall thickness and increased extracellular materials could possibly decrease the incorporation of \(P ^{32}\) into ATP in the aorta as they have shown with increasing age in rats. This could affect the lipid metabolism in the form of a decreased ability to synthesize phospholipids. Mauclesley-Thomas\(^{(84)}\) points out that this may result in increased deposition of cholesterol and thus perpetuate a vicious cycle. Astrup and co-workers\(^{(9)}\) have shown very clearly the effects of hypoxia on the development of atherosclerosis in cholesterol-fed rabbits. Their work demonstrates that aortic lesions are more severe as a result of prolonged hypoxia. In CO-induced atherosclerosis Webster\(^{(127)}\)
has shown a stimulation of the rate of lesion development but not its induction.

A scheme proposed by Zempleyni (151) could be used for purposes of summarization. He believes progressive intimal thickening is normal but a prerequisite for atherosclerosis. This would cause an impaired diffusion of O₂ which could cause metabolic damage in the form of decreased Krebs' cycle enzymes. Decreased ATP availability would reduce the synthesis of enzymes necessary for phospholipid synthesis which would lead to a decreased solubilization of hydrophobic lipids in the arterial wall. A decreased removal of sclerogenic lipids leads to further thickening and decreased defense mechanisms in the wall which would accelerate the atherogenic process.
METHODS

Animals and oxygen exposure

The following list is an outline of the procedure used in sequence:

1. Initial body weights
2. Distribution of animals into 3 treatments
3. Initial blood samples for lipid analysis and hematocrit
4. Exposure to hyperoxia and hypoxia
5. Pair-feeding during exposure
6. Weekly blood samples
7. Final body weights
8. Final blood samples for lipid analysis and hematocrit
9. Arterial blood pressure
10. Each bird killed (Steps 11-15 performed individually)
11. Remove, clean, cut aorta
12. Visual scoring
13. Area determination
14. Wet weight of tissue
15. Aortic metabolism
16. Lipid analysis
17. DNA analysis
18. Data reduction
   a) atherosclerotic index
   b) QO2
   c) analysis of variance
   d) correlations

The primary data of this study is based on 3 separate runs of 22-28 month old White Leghorn hens (Carey's Poultry Farm, Marion, Ohio) which were exposed in environmental chambers to hyperoxic (60% O2 at 1 atm.) and hypoxic (12% O2 at 1 atm.) environments continuously for a period of 4 weeks. With each run
a control group was maintained in room air adjacent to the environmental chambers. Temperature and humidity were similar to that in the chambers. Over a period of 2 years approximately 6 preliminary runs involving over 60 chickens of both sexes and ages from 12 - 24 months were utilized to establish techniques and improve equipment reliability. Each run was composed of a random selection of 12 animals, 4 to a treatment, from an initial pool of 45 hens. This group of 12 was divided into 2 weight classes (light and heavy) and then randomly distributed so that each treatment group would have two light and two heavy birds. There were 2 cages per treatment and 2 birds per cage. In earlier runs we had found that when two birds, unmatched for size, were housed in the same cage, the larger bird tended to dominate the smaller to the extent of causing injury and preventing it from feeding. For this reason an attempt was made to match the sizes of cagemates.

All subjects were housed in commercial plastic chicken cages (12'' wide, 14'' deep, and 14'' high) that were collapsible so they could be removed or replaced in the isolators when necessary. Environments were maintained in sealed isolators similar to the type used in germ-free work as indicated in Figure 1 and described in previous publications\(^{134}\). The bases of the chambers used for the hyperoxic- and hypoxic-treatment groups consisted of stainless
Figure 1: Environmental Chamber
steel tubs (4' long, 2' wide, 2' high) which were surmounted by transparent flexible polyethylene bags (OSU Gnotobiotic laboratory) of approximately the same dimensions as the tubs. Each bag had rubber gloves, ports and air locks to facilitate maintaining the animals and performing various sampling procedures.

The artificial atmospheres were maintained within reasonably close limits by electronic gas controllers: for example, a Servomex OA150 Oxygen Analyzer, as modified by Grimard\(^{(40)}\), operated a 3-way solenoid valve connected to O\(_2\) and N\(_2\) gas cylinders. The O\(_2\) concentration in each isolator was sampled continuously by the electronic controller which determined which gas should be delivered to the chamber. (Figure 2) In some trials, an O\(_2\) controller (Versatronics) or a 2-gas controller (Space-Defense Corp.) was utilized in place of the modified Servomex O\(_2\) analyzer.

The pressure within the chamber was controlled by an electronic relay which was operated by a pressure sensing device (Fairchild) to control a second solenoid valve downstream from the O\(_2\) - N\(_2\) valve. This device admitted the appropriate gas as determined by the gas controller whenever the isolator pressure fell below a certain level (1 - 2 mm H\(_2\)O above atmospheric pressure). This system is diagrammed in Figure 3.
Figure 2: Electronic Gas Controller
Figure 3. Diagram of apparatus used to control $O_2$ concentration and inflation levels in the environmental chambers.
Gas was recirculated by a pressure-vacuum pump (Thomas Industries) which forced it through a refrigerated-dryer (Kahn and Co., Hardford, Conn.) at a rate of about 4-8 cu. ft. per minute. In the gas line was a charcoal absorber, fiberglass filter and a cannister of soda lime for CO₂ absorption. (Figure 4) The result was that the gas within the chamber was maintained within a temperature range of 70-80°F, relative humidity range of 30-50% and a CO₂ level of less than 0.5%. The turnover rate of the 40 cu. ft. chamber was a complete cycle of air in less than ten minutes. The leakage rate of each system insured a complete change of gas every 36-48 hours. The gas concentrations in each chamber were checked daily with a Beckman CO₂ analyzer (LB - 1) and a Beckman Oxygen Analyzer (E-2).

Each chamber was equipped with a tap water supply, food storage containers, maximum-minimum thermometers (Taylor) relative humidity indicators (Lufft) and a spring platform balance (Hanson). Other material could be transferred to and from the chambers through air locks as needed. One of the problems common to this type of system is the gases given off by the urine and fecal wastes which accumulate between the weekly removals. In addition to charcoal, boric acid was used to alleviate the ammonia problem by adding it directly to the litter. Standard laboratory animal bedding (Paxton
Figure 4. Diagram of the environmental system used in maintaining proper levels of CO$_2$, humidity, temperature and various contaminants.
Proc. Co.) was used in each isolator below the cages.

Both hyperoxia and hypoxia can depress the food intake, and reduced food intake may decrease atherosclerosis (137). For this reason the treatments were pair-fed daily based on the group which had the lowest intake. The diet for all animals was a standard commercial laying ration (Purina Layena-G - Ralston Purina Co.). Water was supplied ad libitum.

Blood gas measurements

Arterial O₂ and CO₂ tensions and arterial pH were measured on a separate run of birds exposed to hyperoxia (60% O₂), hypoxia (12% O₂) and room air (21% O₂) to determine whether or not the environments were causing changes at the arterial level. After 30 days exposure the treatment groups were subjected to an arterial catheterization. The chickens were secured to a stand designed for restraining chickens and were administered a local anesthetic (2% Novacain) subcutaneously. An incision approximately 2" long was made on the ventral-medial surface of the neck beginning about 1" caudal to the greater angle of the lower mandible. The skin was reflected and the right common carotid artery isolated. The cranial end of the vessel was tied, the caudal end was clamped with a serrifine and a small incision was made in the cranial end in pre-
poration for the catheter insertion. Catheters made from polyethylene tubing (PE 160, Clay-Adams) coupled to a 3-way stopcock (Tomac) were inserted centrally into the artery and tied in place. The birds were allowed to recover in the isolators for 30 - 60 minutes before blood samples were taken for measurement. Arterial blood samples were taken into heparinized plastic syringes (2 1/2 ml, B-D) from the hyperoxic and hypoxic birds while they were still in the isolators and immediately used for a blood gas and pH determination on a Beckman 160 Physiological Gas Analyzer. The temperature in the measurement chambers was maintained at 41°C with a circulating water bath (Haake type F). The entire apparatus is pictured in Figure 5.

Blood gas determination from the hyperoxic birds, however, had to be accomplished through a direct catheter coupling with the analyzer. This connection was made through one of the isolator ports with a 10" length of catheter tubing at the ends of which were appropriate Luer-type connectors for the 3-way stopcock of the catheter and the analyzer. In earlier runs hyperoxic blood samples when taken in a syringe were found to vary widely and usually showed considerably lower O₂ tension levels than those obtained through a direct catheter coupling as suggested by Fletcher and Barber[35]. The results of some of these preliminary tests along with data on losses of O₂ tension related to time are included in the Appendix. These
Figure 5: The Beckman 160 Physiological Gas Analyzer and Circulating Temperature Bath used for Blood Gas Analysis.
losses are thought to be dependent on syringe leakage, presence of bubbles, and the metabolism of the nucleated red blood cells of chickens(35).

**Plasma lipid analysis**

Body weights and blood samples were taken at the beginning of each run and then weekly thereafter. Two ml of blood were taken into heparinized plastic syringes (2 1/2 ml, B-D), a microhematocrit was performed, and the remaining sample centrifuged to obtain the cell free plasma for lipid analysis. A 0.5 ml sample of plasma was removed and mixed with 6 ml of a 2:1 chloroform-methanol mixture which was allowed to stand for a minimum of 24 hours before the extraction of the total plasma lipid according to the Folch procedure. (34). The initial lipid separation and subsequent H₂O washes (2) were accomplished in separatory funnels equipped with teflon stop-cocks. The extract was then dried under N₂ in a heat block at 40°C and total lipid was determined gravimetrically.

**Final Blood pressure**

At the end of the 4 week exposure, blood pressure was determined directly by carotid catheterization. The catheterization procedure was identical to that used in the blood gas determinations.
The catheters were connected to a Statham pressure transducer (model P23AA) which was used in conjunction with a Grass polygraph (model 7). This method of measurement would seem particularly expedient due to the lack of a carotid sinus reflex in birds. Indirect measurements in animals have been less reliable than direct according to Weiss and others. Mean blood pressure was determined by the sum of diastolic pressure and one-third the pulse pressure.

**Preparation of aorta**

After the blood pressure determination, each animal was killed by decapitation and its aorta from the heart to the bifurcation quickly removed. Each aorta was cleaned of adhering tissue, opened longitudinally and divided into an arch and an abdominal portion. In previous work the aorta had been divided into the thoracic and abdominal segments. However, there is a short segment of tissue between the thickened aortic arch and the relatively thinner abdominal segments which is relatively free from gross atherosclerotic involvement. The author prefers to call this the thoracic segment and it will not be considered in this paper. The arch then is demarcated by the left ventricle and a line formed by a sharp change in thickness and texture between the thicker arch and the thinner thoracic segment. The abdominal segment is composed of the tissue
between the celiac artery and about 1 cm beyond the bifurcation of
the sciatics. Figure 6 shows a chicken aorta preparation and indi-
cates the approximate boundaries for the arch and abdominal segments.
The segments were then outlined in pencil on a 4" x 6" note card for
an area determination by planimetry, weighted to the nearest 0.1 mg,
and scored according to Weiss and others\(^{133}\). The whole aortic
segment was then placed in the biological monitor's incubation vials.

**\( \text{O}_2 \text{ measurements} \)**

\( \text{O}_2 \) uptake measurement on segments of the aorta were made
with a Clark-type polarographic electrode which was used in con-
junction with a d-c chopper-stabilized amplifier made by Yellow
Springs Instruments (YSI) as modified by Grimard\(^{40}\) to accommo-
date four electrodes. Temperature was maintained with a water
bath and circulator (Forma Scientific model 2095) which supplied
constant temperature water to the chamber that housed the incubation
vials. This chamber was equipped with a magnetic stirrer that
rotated magnetic stirring discs in the vials and resulted in a more
even distribution of \( \text{O}_2 \) throughout the medium and prevented temper-
ature layering effects. Output from the amplifier was recorded on
a servo-recorder (Heathe EV-20B). The entire setup is pictured
in Figure 7.
Figure 6: Chicken aorta Preparation
Figure 7: The YSI Biological Monitor and Circulating Temperature Bath used for Measuring Tissue Respiration.
The biological monitor's incubation vials each contained 10 ml of an unsupplemented Krebs-Ringer phosphate buffer solution that had equilibrated with room air a minimum of 10 minutes at 37°C.

The electrodes were preheated in a water bath at 37°C in order that temperature equilibration of the system would be as rapid as possible. The electrodes were then calibrated on the assumption that the incubation solutions were 100% equilibrated with room air at the prescribed temperature and ambient pressure.

Room air was used to avoid a number of problems associated with the use of 100 percent O₂, including lipid and enzyme peroxidation and deviations from normal tissue O₂ tensions. The time from death to this point in the procedure was normally 20 minutes. It was during the first 10 minutes of measurement (time period 20 to 30 minutes after death) that the curves showed the least change in slope. It was for this reason that the QO₂ values were determined by the O₂ uptakes averaged over this first 10 minute period.

Scott et al. (109) found a falloff in aortic tissue respiration in unsupplemented Krebs-Ringer buffers beyond a thirty minute period after death. We found similar falloffs in both supplemented and unsupplemented solutions (see Appendix for results). They also showed lowered QO₂ at 37°C when tissue has previously been cooled.
in an ice bath following removal from the animal. It was for this reason that the aortae in this study were not iced after removal as had been done in earlier Warburg studies (138).

The pH of the incubation solutions were found to be in the 7.15 to 7.20 range at 37°C. Ten ml of buffer solution was used in each incubation vial. This volume was large enough in relation to tissue QO₂ so that the O₂ tension of the solution would not change markedly in the measurement period and thus re-equilibration could be avoided.

Generally speaking, the QO₂ could be defined as the quantity of O₂ consumed per unit weight per unit time. We have chosen to use ul of O₂ per mg wet weight tissue per hr, ul of O₂ per mg lipid-free dry weight of tissue per hr and ul O₂ per mg DNA per hr for QO₂(WW), QO₂(LFD) and QO₂(DNA), respectively. Tissue respiration can be expressed as per cent change of O₂ content of incubation media over the 10 minute period of measurement. There are approximately 50 ul of O₂ dissolved in 10 ml of incubation solution which has been equilibrated with room air at 37°C (40). The produce of the change in O₂ content (expressed as a decimal for the 10 minute period), the factor 6 (to connect the 10 minute measurement to one based on an hour), 50 ul O₂ (to obtain the actual
amount of O$_2$ consumed per hour) and the reciprocal of the tissue weight results in a QO$_2$ value.

Tissue analyses

Subsequent to the respiration studies, the tissue was removed, shaken for a minimum of 20 hours in 6 ml of 2:1 chloroform-methanol mixture for lipid extraction. Total tissue lipid was determined gravimetrically according to the Folch procedure$^{(36)}$ as described under blood lipid analysis, page 24. The lipid-free tissue was then dried at 105°C for 24 hours and weighed to obtain a value for lipid-free dry weight. This figure when combined with the amount of lipid extracted gives the total dry weight. The per cent total tissue lipid (TTL) was determined by dividing the weight of lipid extracted by the total dry weight of the tissue and multiplying by 100.

DNA content was determined colorimetrically by diphenylamine according to Schneider$^{(105)}$. The lipid-free dry tissue is incubated for 20 hours at 37°C in 1N KOH and followed by a precipitation of proteins and DNA with 6N HCl and 5% tri-chloro-acetic acid (TCA). This precipitate is removed by centrifugation and the DNA suspended in hot (90°C) TCA for 15 minutes. Diphenylamine reagent is then used with the DNA extract and the intensity of the colorimetric
reaction measured with a spectrophotometer (Bausch and Lomb; Spectronic 20). The DNA determinations allowed a calculation of $QO_2$ to be based on a measure related to the number of cells present (141) as well as providing a DNA per unit area value which could be useful as an indication of cellular proliferation. $QO_2$ was also based on lipid free dry weight and wet weight of tissue so that comparisons could be made with earlier work. As Maier and Haimovici (78) point out, however, these are less valid when used for calculating $QO_2$ because of the presence of inert components.

Several measurements which have been used in previous work (137, 138) to evaluate atherosclerotic development were used in this study as a basis for relating the effects of treatments to lesion severity. Visual Scoring (VS) is based on a 1-10 scale that is primarily weighted by the area and degree of plaque protrusion into the lumen. Secondary weight is given to gross lipid deposition, hardness, and roughness of the intimal surface. The normal range encountered in our work with chickens has been 1-4. Wet weight in mg per unit area in cm$^2$ (WW/area) was used as an indication of the thickness of the tissue, i.e., overall swelling due to edema, tissue proliferation and non-cellular component accumulation. The lipid free dry weight (LFD) added to the amount of lipid extracted gives the total tissue dry weight (TDW). The per cent total tissue
lipid (TTL) bears some relationship to the relative amounts of both endogenous and infiltrated lipid.

Finally an attempt was made to combine these atherosclerotic measurements into a single figure (Index) which would give equal weight to tissue thickness (WW/area), presence of lipid (TTL) and a subjective evaluation of atherosclerotic involvement (VS). The index is a summation of the 3 factors after WW/area and TTL have been scaled to the score within each treatment for each aortic segment. Score is used as a base because of its widespread use as a measure of lesion severity. In addition each measurement category is further weighted by a factor which is determined by the ratio of the respective treatment mean to the hyperoxic group mean. This was done to maintain the differences which existed between treatments. The product of these two factors and the individual TTL and WW/area measurements results in 3 scaled figures for each bird and for each aortic segment. The sum of these figures and the score results in the individual subject's index. These indices are then averaged for each treatment.

In addition to the three measurements commonly used to assess atherosclerotic development in this laboratory further measurements
were used in an attempt to evaluate tissue proliferation and non-cellular component concentration. Lipid-free dry weight in mg per unit area in square cm (LFD/area) was employed in the latter case while de-oxyribonucleic acid in mg per unit area in square cm (DNA/area) served as a proposed basis for the former situation. These measurements were also related to each other for further assessment of their interrelationships.

**Statistical analysis**

A one-way analysis of variance program for the Olivetti Programma 101 Computer was used to determine if a significant difference existed between treatment means in all of the measurement categories. When such an analysis showed significance at the $P = 0.05$ level or less, Tukey's test as given in Snedecor$^{(114)}$ was used to obtain a $D$-value which determined which treatments differed significantly at the 0.05 level. While $P = 0.05$ was considered the cut-off point for significance some attention was given to results in which $0.1 > P > 0.05$ as possibly indicating near significance. Selected variables were also subjected to a correlation analysis. Correlation coefficients were obtained within the control, hyperoxic and hypoxic treatments and compared to each other according to a procedure given in Snedecor$^{(114)}$ which tests the possibility that several $r$-values
are from the same population. The test results in a P-value obtained from a Chi-square distribution. If no treatment effect existed, an r-value for the entire set of data was obtained.
RESULTS

Introduction - Table Format

In general the tables of results given in this chapter will have two basic formats which involve mean values and correlation r-values. In the former, the results of the treatments and the entire population means will be presented as \((n) - \text{mean} \pm S.E.\) which represents the number of animals involved \((n)\) and the group mean plus or minus the standard error \((S.E.)\). The correlation tables are presented in the same manner with the correlation coefficient \((r)\) replacing the mean value and the S.E. is deleted. The first three rows of results in each table represent the three treatment groups listed as control, hyperoxia and hypoxia. The next row down lists the P-values from an analysis of variance (ANOVA). This row is followed by Tukey's D-value (significant at the 0.05 level) which is included only if the ANOVA shows significant differences. The last row indicates the population means which are listed only when the ANOVA does not show significant differences between treatment means. In certain tables where both aortic segments are involved, this sequence of rows will be repeated for each segment.

The correlation tables have a similar format to the aforementioned.
tioned with a few exceptions. Following the hypoxic group, the fourth row of results (for each aortic segment) refers to a test given in Snedecor\(^{(114)}\) which results in a \(P\)-value from a Chi-square distribution. As mentioned in Methods page 34, this procedure tests the possibility that several \(r\)-values are from the same population. If no treatment effect exists, an \(r\)-value for all three treatments is given as the common \(r\) in the last row (for each aortic segment).

**Blood gas, pH and hematocrit**

The blood gas analysis showed the expected changes as a result of exposure to the hypoxic and hyperoxic environments (Table 1.) The hyperoxic group (Hyper) showed a significant increase (253\%) in arterial \(PO_2\) over the control values while the hypoxic group (Hypo) showed a significant decrease (58\%) according to a one way analysis of variance. Carotid blood samples were taken at the end of a thirty day exposure period from a separate run of the three treatment groups. The results represent the means of six animals per treatment.

Fletcher and Barber\(^{(34)}\) have suggested correction factors be applied at higher \(PO_2\) when glass and plastic syringes are used and when samples are stored in ice for periods of time. Our preliminary tests showed in part a large \(P_{A02}\) variation in the 60\% hyperoxic
### TABLE 1

**BLOOD GASES, pH AND HEMATOCRITS AFTER 30 DAY EXPOSURE TO HYPEROXIA AND HYPOXIA**

<table>
<thead>
<tr>
<th></th>
<th>Arterial O₂</th>
<th>Arterial CO₂</th>
<th>Arterial pH</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tension</td>
<td>Tension</td>
<td>pH</td>
<td>Initial</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(21% O₂)</td>
<td>(6) 92± 6.5</td>
<td>(6) 24±2.2</td>
<td>(6) 7.6±0.02</td>
<td>(12) 31±0.09</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(60% O₂)</td>
<td>(6) 323±16.3</td>
<td>(6) 22±3.9</td>
<td>(6) 7.8±0.03</td>
<td>(11) 31±0.6</td>
</tr>
<tr>
<td>Hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12% O₂)</td>
<td>(6) 41±2.1</td>
<td>(6) 16±1.9</td>
<td>(6) 7.6±0.03</td>
<td>(12) 29±0.4</td>
</tr>
<tr>
<td>ANOVA</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Tukey's D</td>
<td>77.1</td>
<td>4.64</td>
<td>.06</td>
<td>---</td>
</tr>
<tr>
<td>Population Mean</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

*Note: Values are given in (mm Hg) for Tension, (mm Hg) for Arterial O₂ and Arterial CO₂, and pH for Arterial pH and Hematocrit.*
group when syringes were employed for sampling. The use of a direct
catheter coupling between the carotid and the Beckman 160 physiological
gas analyser produced much more consistent results, and eliminated
the need for correction factors. The control and hypoxic group samples
were obtained with syringes but were used immediately for measure-
ment, thus eliminating problems due to RBC respiration, syringe leak-
age, and temperature differences when samples are stored in ice for
periods of time. The control mean value of 92 mm Hg. for arterial
PO$_2$ agrees with the 90 mm Hg. value given in Sturkie$^{118}$. Weiss et al.
$^{134}$ have shown slightly higher values for young birds in room air.

The arterial CO$_2$ tension of the Hypo showed a significant re-
duction (16 mm Hg) compared to the control (24 mm Hg) and Hyper
(22 mm Hg) levels as determined by ANOVA and Tukey's test. In
the third column of Table 1 the Hyper pH of 7.8 was significantly ele-
vated in relation to the other treatments. The last two columns repre-
sent the initial and final hematocrits for each group. While there is
no significant difference in the initial means, the final means do show
significant changes in both experimental groups as compared to the
control. It is apparent that the final control mean is the same as the
initial population mean of 31. The hyperoxic exposure resulted in a
depression of the hematocrit from an initial mean of 31 to a final
value of 25. Hypoxia elevated the blood cell concentration from a
mean value of 29 to one of 43. The initial and final control means are found to agree closely with the value (31) given in Sturkie\(^{(118)}\) for adult female chickens. Weiss et al.\(^{(135)}\) reported increased \(P_{\text{ACO}_2}\) and decreased hematocrits similar to the hyperoxic group here when young chicks were exposed to 100% oxygen at atmospheric pressure for several weeks. Blood pH was lower and \(P_{\text{ACO}_2}\) higher in their work compared to the results in this study, however.

**Body weight**

On the basis that there were no significant differences in initial body weights among the treatments, the three groups could be considered as coming from a common population with a mean initial weight of 2361 grams (Table 2). Body weight change over the thirty day exposure period resulted in a mean loss of 7%. While there is no significant difference in body weight loss among the groups, the hyperoxic birds demonstrated a slightly greater loss in spite of pair feeding based on this group's food consumption. Other work has shown similar declines in body weight upon exposure to hyperoxic environments\(^{(134)}\).

**Blood pressure and heart rate**

The blood pressures and heart rates were determined by carotid catheterization at the end of the thirty day exposure. Although
TABLE 2

BODY WEIGHT CHANGES OVER THE 30 DAY PERIOD OF
EXPOSURE TO HYPOXIA AND HYPOXIA (GRAMS)

\[
(n) \text{ Mean} \pm S.E.
\]

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(12) 2404 ± 111</td>
<td>(12) - 86 ± 78</td>
</tr>
<tr>
<td>(21% O₂)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>(11) 2353 ± 83</td>
<td>(11) - 311 ± 107</td>
</tr>
<tr>
<td>(60% O₂)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(12) 2343 ± 99</td>
<td>(12) - 118 ± 57</td>
</tr>
<tr>
<td>(12% O₂)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANOVA</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Population</td>
<td>(35) 2361 ± 59</td>
<td>(35) - 161 ± 49</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
there were no significant differences in either measurement between treatments, the hypoxic group tended toward lower blood pressure and higher heart rate (Table 3). Since no differences were shown in either measurement according to an ANOVA the birds could be considered a common population with a mean blood pressure of 134 mm Hg and a mean value of 286 for heart rate. This is in contrast to human subjects in hypoxia but agrees with other work on chickens\(^{(119)}\).

Generally, short term studies with hyperoxia have shown increased blood pressure\(^{(134)}\). All these heart rates are low compared to the 336 value given in Sturkie\(^{(118)}\). The mean blood pressure of the control group does, however, approximate Sturkie's\(^{(118)}\) value of 143.

**Plasma lipid**

Venous blood samples were taken initially and then weekly throughout the exposure period to obtain the total plasma lipid (TPL) results given in Table 4. These results are given in three columns to represent the initial values, the average of those values obtained throughout the exposure period, and the difference between the initial and average exposure TPL (ie, change).

The initial TPL means are unusual in that the hypoxic group shows a much higher value (significant). This failure in randomization could be partially explained by the presence of two birds with
### TABLE 3

**BLOOD PRESSURE AND HEART RATE AFTER 30 DAY EXPOSURE TO HYPOXIA AND HYPOXIA**

<table>
<thead>
<tr>
<th></th>
<th>Mean Arterial Pressure (mm Hg)</th>
<th>Heart Rate (Beats/min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong> (21% O₂)</td>
<td>(12) 139±4.5</td>
<td>(12) 270±12</td>
</tr>
<tr>
<td><strong>Hyperoxia</strong> (60% O₂)</td>
<td>(10) 136±5.3</td>
<td>(10) 280±19</td>
</tr>
<tr>
<td><strong>Hypoxia</strong> (12% O₂)</td>
<td>(12) 127±3.5</td>
<td>(12) 303±11</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Population Mean</strong></td>
<td>(34) 134±2.7</td>
<td>(34) 286±8</td>
</tr>
</tbody>
</table>
TABLE 4

TOTAL PLASMA LIPID (mg%)

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Exposure mean&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Change&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(21% O₂)</td>
<td>(12) 1015±124</td>
<td>(12) 933±151</td>
<td>(12) - 82±118</td>
</tr>
<tr>
<td><strong>Hyperoxia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(60% O₂)</td>
<td>(11) 1107±138</td>
<td>(10) 1226±</td>
<td>(11) 119±212</td>
</tr>
<tr>
<td><strong>Hypoxia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12% O₂)</td>
<td>(12) 1967±410</td>
<td>(12) 1506±142</td>
<td>(12) -461±373</td>
</tr>
</tbody>
</table>

*ANOVA*<sup>c</sup> [ (10) 1416±198 ] [ (10) 1391±142 ] [ (10) - 25±232 ]

*Tukey's D* 964  521

---

<sup>a</sup> Mean of four weekly samples.

<sup>b</sup> Initial minus average.

<sup>c</sup> Refers to the hypoxic group with two aberrant birds excluded.
abnormally high initial TPL (5840 and 3600 mg%) which were more than
twice as high as the mean of the remaining ten birds. For this reason,
these two birds have been omitted in the row indicated by the $[-2]$.
The exclusion of the two birds brings the Hypo values in all three
columns closer to that of the other two treatments and results in no
significance when a one way analysis of variance is applied. Whether
or not the two aberrant birds are included in the analysis, there is no
significant difference among the groups with respect to change in TPL
which is a highly variable measurement. Nevertheless, it may be of
interest to point out that whereas controls and Hypo tended to decrease,
the Hyper tended to increase.

Egg production in hens has been shown to influence TPL and de-
pends not only on whether the hen is laying but also on the particular
stage of egg development or yolk reabsorption at the time the sample
is taken\(^{(129)}\). According to Sturkie\(^{(118)}\) the adult non-laying hen has a
TPL of 550 mg% while the laying hen has a range of 1200-1700 mg%.
The variability in TPL of the treatment groups is therefore not un-
expected in hens of this age when variable stages in egg development
are taken into consideration.

**Atherosclerosis**

Those factors which have traditionally been used to assess the
degree of atherosclerotic involvement, percent total tissue lipid (TTL), wet weight per area (WW/area) and visual score (VS) are listed separately by aortic segment in Tables 5 and 6. TTL showed no significant difference among treatment groups in either the arch or the abdominal aortic portion. If any trend existed, it was in the direction of slightly higher values for the hyperoxic group and lower for the hypoxic group. The standard errors in both aortic segments demonstrate that greater variability existed in the Hyper. The abdominal aortic segment of the female chicken in Table 6 shows an almost 3-fold greater lipid content (TTL) than the arch (Table 5).

No significance was seen between treatments in either arch or abdominal segments with respect to wet weight per area (WW/area), but, as with TTL a trend toward higher values in the hyperoxic group may be detected. As expected the arch was found to have a greater WW/area than the abdominal segment. The third measurement used to determine atherosclerotic involvement, visual scoring (VS) was also not statistically different among treatment groups, but as with TTL and WW/area there was a slight tendency toward higher values in the abdominal segment of the Hyper.

In the last column of Tables 5 and 6 entitled atherosclerotic index we have included calculations based on the previous three measurements in an attempt to summarize the atherosclerotic involvement.
### TABLE 5

**ATHEROSCLEROSIS MEASUREMENTS IN THE AORTIC ARCH OF ANIMALS EXPOSED TO HYPEROXIA AND HYPOXIA**

<table>
<thead>
<tr>
<th></th>
<th>Tissue lipid</th>
<th>Wet Weight/area</th>
<th>Atherosclerotic Score</th>
<th>Atherosclerotic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>(12) 11.3±0.8</td>
<td>(12) 63.1±2.5</td>
<td>(9) 1.6±0.05</td>
<td>(12) 4.8±0.09</td>
</tr>
<tr>
<td>(21% O₂)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hyperoxia</strong></td>
<td>(11) 11.7±1.6</td>
<td>(11) 68.1±2.7</td>
<td>(11) 1.6±0.05</td>
<td>(11) 5.0±0.23</td>
</tr>
<tr>
<td>(60% O₂)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Hypoxia</strong></td>
<td>(12) 11.7±0.7</td>
<td>(12) 66.8±2.1</td>
<td>(11) 1.6±0.08</td>
<td>(12) 4.9±0.16</td>
</tr>
<tr>
<td>(12% O₂)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td>(35) 11.6±0.6</td>
<td>(35) 65.8±1.3</td>
<td>(31) 1.6±0.03</td>
<td>(35) 4.9±0.10</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a) Total tissue lipid given as per cent of total dry weight of tissue.
b) Wet weight of tissue per unit area.
c) Atherosclerotic index calculated from TTL, WW/area, and score.*
### TABLE 6

**ATHEROSCLEROSIS MEASUREMENTS IN THE AORTIC ABDOMINAL SEGMENT OF ANIMALS EXPOSED TO HYPOXIA AND HYPOXIA**

<table>
<thead>
<tr>
<th></th>
<th>Control (21% O₂)</th>
<th>Hyperoxia (60% O₂)</th>
<th>Hypoxia (12% O₂)</th>
<th>ANOVA</th>
<th>Population Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TTL a</strong> (%) TDW</td>
<td>(12) 34.5±2.2</td>
<td>(11) 35.0±3.0</td>
<td>(10) 31.6±1.8</td>
<td>NS</td>
<td>(33) 33.8±1.4</td>
</tr>
<tr>
<td></td>
<td>(12) 47.1±2.3</td>
<td>(11) 47.9±1.8</td>
<td>(12) 47.5±2.6</td>
<td></td>
<td>(35) 47.5±1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(31) 2.8±0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(35) 8.4±0.21</td>
</tr>
<tr>
<td><strong>WW/Area b</strong>(mg/cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(12) 47.1±2.3</td>
<td>(11) 47.9±1.8</td>
<td>(12) 47.5±2.6</td>
<td></td>
<td>(35) 47.5±1.3</td>
</tr>
<tr>
<td><strong>Atherosclerotic Score (units)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(9) 2.8±0.21</td>
<td>(11) 2.9±0.13</td>
<td>(11) 2.8±0.11</td>
<td></td>
<td>(31) 2.8±0.08</td>
</tr>
<tr>
<td><strong>Atherosclerotic Index c</strong>(units)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(35) 8.4±0.21</td>
</tr>
</tbody>
</table>

- **TTL a** = Total tissue lipid given as per cent of total dry weight of tissue.
- **WW/Area b** = Wet weight of tissue per unit area.
- **Atherosclerotic Index c** = Atherosclerotic index calculated from %TTL, WW/Area, and Score.
Although no significant difference exists among the groups in either segment, the same trends found in the individual measurements are evident in the index. The hyperoxic group demonstrates a slightly greater involvement than the other treatments as indicated by the higher index for both segments.

In addition to the conventional methods for assessing atherosclerosis, lipid free dry weight per unit area (LFD/area), and DNA content, expressed either in relation to tissue area (DNA/area), to lipid free dry weight of tissue (DNA/LFD), to total dry weight (DNA/TDW), or to tissue lipid content (DNA/TTL) was used to determine if tissue proliferation might be related to the environmental treatment. Tables 7 and 8 which represent results for the arch and abdominal segments, respectively, show no significant differences among the treatment categories according to a one way analysis of variance (ANOVA). It may also be seen, however, that the trend toward higher values in the hyperoxic group, as seen with the conventional atherosclerosis measurements from the previous two tables continues in the aortic arch only. In the abdominal segment it is the hypoxic group which consistently shows higher values.

There is almost twice as much non-lipid material per unit area (LFD/area) in the arch than in the abdominal. The aortic arch also shows larger DNA/area and DNA/TTL values than the abdominal
<table>
<thead>
<tr>
<th></th>
<th>LFD/Area a (mg/cm)</th>
<th>DNA/Area b (ug/cm)</th>
<th>% DNA/LFD</th>
<th>% DNA/TDW c (ug/mg)</th>
<th>DNA/TTL d (ug/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ARCH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (21% O₂)</td>
<td>(12) 15.1±0.8</td>
<td>(11) 52.5±3.1</td>
<td>(11) 0.34±0.01</td>
<td>(11) 0.30±0.01</td>
<td>(11) 29.1±2.2</td>
</tr>
<tr>
<td>Hyperoxia (60% O₂)</td>
<td>(11) 15.6±0.7</td>
<td>(11) 60.8±5.5</td>
<td>(11) 0.37±0.02</td>
<td>(11) 0.33±0.02</td>
<td>(11) 35.6±7.5</td>
</tr>
<tr>
<td>Hypoxia (12% O₂)</td>
<td>(12) 14.8±0.5</td>
<td>(12) 52.9±5.3</td>
<td>(12) 0.35±0.03</td>
<td>(12) 0.30±0.02</td>
<td>(12) 26.8±2.0</td>
</tr>
<tr>
<td>ANOVA</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Population Mean</td>
<td>(35) 15.2±0.4</td>
<td>(34) 55.3±2.8</td>
<td>(34) 0.35±0.01</td>
<td>(34) 0.31±0.01</td>
<td>(34) 30.4±2.6</td>
</tr>
</tbody>
</table>

a) Lipid free dry weight of tissue per unit area.

b) Deoxyribonucleic acid content of tissue per unit area.

c) Per cent DNA based on total dry weight of tissue.

d) Ratio of DNA to total tissue lipid given as per cent of total dry weight.
## TABLE 8
AORTIC TISSUE PROLIFERATION AND CHANGES IN NON CELLULAR COMPONENTS IN HYPEROXIA AND HYPOXIA IN THE ABDOMINAL SEGMENT

<table>
<thead>
<tr>
<th></th>
<th>LFD/Area $^a$ (mg/cm)</th>
<th>DNA/Area $^b$ (ug/cm)</th>
<th>% DNA/LFD (ug/mg)</th>
<th>% DNA/TDW $^c$ (ug/mg)</th>
<th>DNA/TTL (ug/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABDOM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (21% O$_2$)</td>
<td>(12) 8.2±0.50</td>
<td>(11) 39.1±2.2</td>
<td>(11) 0.50±0.02</td>
<td>(11) 0.32±0.01</td>
<td>(11) 9.9±0.8</td>
</tr>
<tr>
<td>Hyperoxia (60% O$_2$)</td>
<td>(12) 8.2±0.4</td>
<td>(11) 41.0±1.6</td>
<td>(9) 0.51±0.03</td>
<td>(11) 0.33±0.01</td>
<td>(11) 10.1±1.1</td>
</tr>
<tr>
<td>Hypoxia (12% O$_2$)</td>
<td>(12) 8.3±0.4</td>
<td>(12) 42.3±2.7</td>
<td>(12) 0.51±0.02</td>
<td>(10) 0.35±0.03</td>
<td>(11) 11.0±1.5</td>
</tr>
<tr>
<td><strong>ANOVA</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Population Mean</strong></td>
<td>(35) 8.2±0.2</td>
<td>(34) 40.9±1.3</td>
<td>(32) 0.51±0.01</td>
<td>(32) 0.33±0.01</td>
<td>(33) 10.4±0.7</td>
</tr>
</tbody>
</table>

- **a)** Lipid free dry weight of tissue per unit area.
- **b)** Deoxyribonucleic acid content of tissue per unit area.
- **c)** Percent DNA based on total dry weight of tissue.
- **d)** Ratio of DNA to total tissue lipid given as percent of total dry weight.
segment in all treatments. The DNA/LFD and DNA/TDW values are, however, higher in the abdominal segment. It should be mentioned that even though these trends are evident, no statistically significant differences exist among treatments in any of the measurements.

**Aortic O₂ uptake**

As shown in Table 9, oxygen uptake was based on wet weight (WW), lipid free dry weight (LFD) and DNA content. There are some generalities that can be found in the different expressions of QO₂. Regardless of the basis for the calculations, QO₂ was higher in the Hyper and lower in the Hypo when compared to the controls. While these differences occur in both arch and abdominal segments, it is more apparent in the arch. In two cases, QO₂ based on wet weight and lipid free dry weight in the arch, analysis of variance indicates that the differences are highly statistically significant. When QO₂'s are based on LFD, it can be seen in the center column of Table 9 that each group's arch value is less than the corresponding abdominal value. This trend is lost or even reversed to a slight extent when the QO₂'s are based on DNA. The abdominal segment shows no significant difference among the groups in any of the QO₂'s.

Recently, we have found in separate studies that chicken aortic tissue undergoes a steady decline in respiration whether or not the
<table>
<thead>
<tr>
<th></th>
<th>QO2(WW)</th>
<th>QO2 (LFD)</th>
<th>QO2 (DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ul O2/mg wet weight/hour</td>
<td>ul O2/mg lipid free dry weight/hour</td>
<td>ul O2/ug DNA/hour</td>
</tr>
<tr>
<td><strong>ARCH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(12) 0.24±0.01</td>
<td>(12) 1.02±0.06</td>
<td>(11) 0.31±0.02</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(11) 0.27±0.01</td>
<td>(11) 1.21±0.05</td>
<td>(11) 0.34±0.03</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(11) 0.19±0.01</td>
<td>(11) 0.87±0.05</td>
<td>(11) 0.28±0.03</td>
</tr>
<tr>
<td>ANOVA</td>
<td>0.01</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Tukey's D</td>
<td>0.04</td>
<td>0.22</td>
<td>___</td>
</tr>
<tr>
<td>Population</td>
<td>(34)0.24±0.01</td>
<td>(34)1.03±0.04</td>
<td>(33)0.31±0.02</td>
</tr>
<tr>
<td><strong>ABDOM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(12) 0.23±0.01</td>
<td>(12) 1.35±0.10</td>
<td>(11) 0.28±0.02</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(11) 0.25±0.01</td>
<td>(11) 1.51±0.10</td>
<td>(11) 0.30±0.01</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(12) 0.22±0.01</td>
<td>(12) 1.23±0.03</td>
<td>(12) 0.25±0.01</td>
</tr>
<tr>
<td>ANOVA</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Population</td>
<td>(35)0.23±0.01</td>
<td>(35)1.36±0.55</td>
<td>(34)0.27±0.01</td>
</tr>
</tbody>
</table>
incubation medium is supplemented. (Appendix) This was especially true, moreover, with the abdominal segment which showed a greater loss of activity with time than did the arch (which would correspond to the thoracic segment of Weiss et al.\(^{138}\)). Such a differential decline in \(QO_2\) would help to explain the relative differences in arch and abdominal segments between the earlier work in this laboratory and this present study.

**Correlation: visual scores**

A significant positive \(r\)-value, close to 0.7, was found in the abdominal segment of the control and hypoxic groups in the VS versus WW/area correlation, as shown in Table 10. The Hyper, by contrast, showed a correlation of only 0.2. In the relation of VS to TPL which showed no differences between groups according to Snedecor's test the common \(r\)-value of the abdominal segment was significant at 0.05. In spite of the other relatively low correlations, some interesting trends may be seen. VS relative to WW/area showed a generally negative trend in the arch while the relationship was positive in the abdominal. There is a positive correlation between VS and WW/area in the abdominal. In the arch, however, score (VS) is a poorer measure of tissue proliferation, although it is not clear why the relationship is negative. These results do agree, however, with previous data\(^{133}\).
### TABLE 10

CORRELATION ANALYSIS BETWEEN VISUAL SCORES AND OTHER FACTORS AFTER EXPOSURES TO HYPEROXIA AND HYPOXIA

\[
[(n) - r \text{ value}]\]

<table>
<thead>
<tr>
<th>Wet Weight/Area</th>
<th>(\text{O}_2) (LFD)</th>
<th>Plasma Lipid</th>
<th>Tissue Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ARCH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (21% (\text{O}_2))</td>
<td>(9) -0.39</td>
<td>(9) -0.56</td>
<td>(9) 0.15</td>
</tr>
<tr>
<td>Hyperoxia (60% (\text{O}_2))</td>
<td>(11) -0.45</td>
<td>(11) 0.13</td>
<td>(11) 0.56</td>
</tr>
<tr>
<td>Hypoxia (12% (\text{O}_2))</td>
<td>(11) -0.12</td>
<td>(10) -0.42</td>
<td>(11) 0.15</td>
</tr>
<tr>
<td>(P^b)</td>
<td>0.70</td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td>Common (r^c)</td>
<td>(31) -0.26</td>
<td>(30) -0.28</td>
<td>(31) 0.29</td>
</tr>
</tbody>
</table>

| **ABDOM**       |                      |              |              |
| Control (21% \(\text{O}_2\)) | (9) 0.68\(^a\) | (9) -0.42 | (9) 0.13 | (9) 0.46 |
| Hyperoxia (60% \(\text{O}_2\)) | (11) 0.17 | (11) 0.14 | (11) 0.52 | (11) 0.22 |
| Hypoxia (12% \(\text{O}_2\)) | (11) 0.66\(^a\) | (11) -0.47 | (11) 0.43 | (9) 0.30 |
| \(P^b\)         | 0.30                | 0.40         | 0.70         | 0.80        |
| Common \(r^c\)  | (31) 0.53\(^a\)    | (31) -0.12   | (31) 0.39\(^a\) | (29) 0.35 |

\(^a\) Individually significant correlation.

\(^b\) \(P\) value from chi square test in Snedecor\(^{114}\) to determine if treatment \(r\)'s differ from one another.

\(^c\) Average \(r\) for category where no significant difference exists due to treatment.
A consistent trend appears in the correlation of VS versus $QO_2$
LFD in that a slight positive $r$-value for the Hyper (arch and abdominal
segments) contrasts with a relatively negative $r$-value for the control
and Hypo. The negative correlation between $QO_2$ and VS in the abdomi-
nal control is contrary to the Warburg studies in this laboratory\(^{(138)}\). The differences in technique mentioned earlier may be responsible for
this difference.

In relating VS to TPL the correlations are all positive, with
a relatively high positive $r$-value noted for the Hyper in both arch and
abdominal, compared to the Hypo or controls. The common $r$-value
of 0.39 for the abdominal segment is significant. In correlating VS
with TTL one notices in the arch negative correlations for the control
and hyperoxic groups in contrast to a larger positive $r$ in the Hypo.
The differences are apparently close to significance as indicated by a
chi-square $P$-value of 0.1. Positive correlations are characteristic of
VS vs. TTL in the abdominal for all treatments while the common
$r$-value barely misses significance at the .05 level.

Table 11 further relates VS to measurements which may repre-
sent to some degree the proliferation of cellular-(DNA/area) and non
cellular-(LFD/area) tissue components, in addition to a correlation
between the two factors. In the arch, the hyperoxic group is char-
<table>
<thead>
<tr>
<th></th>
<th>LFD&lt;sup&gt;b&lt;/sup&gt;/area</th>
<th>DNA&lt;sup&gt;c&lt;/sup&gt;/area</th>
<th>LFD/area vs. DNA/area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg cm&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>ug cm&lt;sup&gt;-2&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>ARCH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(9) 0.10</td>
<td>(8) -0.02</td>
<td>(11) 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(11) -0.43</td>
<td>(11) -0.19</td>
<td>(11) 0.37</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(11) 0.34</td>
<td>(11) 0.49</td>
<td>(12) 0.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>0.25</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Common r</td>
<td>(31) 0.01</td>
<td>(30) 0.21</td>
<td>(34) 0.51</td>
</tr>
<tr>
<td><strong>ABDOM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(9) 0.13</td>
<td>(8) 0.03</td>
<td>(11) 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(11) 0.15</td>
<td>(11) 0.13</td>
<td>(11) 0.22</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(11) 0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(11) 0.34</td>
<td>(12) 0.60&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>P</td>
<td>0.25</td>
<td>0.75</td>
<td>0.25</td>
</tr>
<tr>
<td>Common r</td>
<td>(31) 0.26</td>
<td>(30) 0.16</td>
<td>(34) 0.55</td>
</tr>
</tbody>
</table>

a) Individually significant correlations
b) LFD/area = lipid free dry weight of aortic tissue per unit area
c) DNA/area = deoxyribonucleic acid of aortic tissue per unit area
acterized by a negative correlation between VS and LFD/area while the Hypo and controls tend toward positive relationships. The differences are not significant, however. In the abdominal, the hypoxic group demonstrates a significant positive r-value for this same correlation, compared to positive but very low values in the control and Hyper groups. The hypoxic birds again show positive trends in both arch and abdominal segments with regard to VS vs. DNA/area, in contrast with very low positive or slightly negative values in the control and Hyper. In the correlation between LFD/area and DNA/area the hyperoxic group has much lower positive r-values in both segments compared to the individually significant positive r-values of the control and hypoxic chickens.

Correlations: blood pressure

In Table 12 high correlations are shown only for the Hyper in both arch and abdominal with regard to BP versus VS. For the abdominal segment, the differences among the treatments are close to significant (P = 0.1).

A significant (P = .05) r-value of 0.69 between BP and TPL exists for the Hyper while the Hypo and controls show a much smaller positive relationship.
### TABLE 12

**CORRELATION ANALYSIS BETWEEN MEAN BLOOD PRESSURE AND OTHER FACTORS AFTER EXPOSURES TO HYPEROXIA AND HYPOXIA**

<table>
<thead>
<tr>
<th></th>
<th>Visual Score</th>
<th>Plasma Lipid</th>
<th>Tissue Lipid</th>
<th>Lipid Free Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ARCH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (21% O₂)</td>
<td>(8) -0.19</td>
<td>(12) 0.05</td>
<td>(12) -0.14</td>
<td>(12) -0.35</td>
</tr>
<tr>
<td>Hyperoxia (60% O₂)</td>
<td>(9) 0.50</td>
<td>(9) 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(9) -0.25</td>
<td>(8) -0.02</td>
</tr>
<tr>
<td>Hypoxia (12% O₂)</td>
<td>(10) 0.03</td>
<td>(12) 0.39</td>
<td>(12) 0.15</td>
<td>(12) 0.33</td>
</tr>
<tr>
<td>p&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.25</td>
<td>0.25</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td>Common r&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(27) 0.14</td>
<td>(33) 0.27</td>
<td>(33) -0.13</td>
<td>(32) -0.03</td>
</tr>
<tr>
<td><strong>ABDOM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (21% O₂)</td>
<td>(9) -0.34</td>
<td></td>
<td>(12) -0.39</td>
<td>(12) -0.12</td>
</tr>
<tr>
<td>Hyperoxia (60% O₂)</td>
<td>(9) 0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(9) -0.04</td>
<td>(8) 0.37</td>
<td></td>
</tr>
<tr>
<td>Hypoxia (12% O₂)</td>
<td>(11) 0.26</td>
<td>(10) 0.14</td>
<td>(12) 0.28</td>
<td></td>
</tr>
<tr>
<td>p&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10</td>
<td>0.25</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Common r&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(29) 0.11</td>
<td></td>
<td>(31) -0.08</td>
<td>(32) 0.20</td>
</tr>
</tbody>
</table>

<sup>a</sup> Individually significant correlation.

<sup>b</sup> P-Value from chi-square test in Snedecor (114) to determine if treatment r's differ from one another.

<sup>c</sup> Average r for category where no significant difference exists due to treatment.
These results are in general agreement with Weiss\textsuperscript{(137)} where low and sometimes negative correlations between these factors were also encountered. Blood pressure does not show much of a relationship with either tissue lipid or non-lipid components.

The correlations between TPL and TTL in Table 13 show some interesting contrasts which may have implications to a differing response of aortic tissue to plasma lipid levels. In agreement with Astrup\textsuperscript{(9)} high total plasma lipid is related to a low tissue lipid in the aortic arch of the hyperoxic group while the hypoxic arch demonstrates a positive relationship. Both the difference between the \( r \)-values and the Hypo \( r \)-value itself are significant at the 0.05 level. In the abdominal segment both Hyper and Hypo show a high positive correlation. In the control arch and abdominal there is essentially no correlation.

In Table 13 \( QO_2 \) (DNA) vs. TPL indicates a difference in the abdominal between the Hypo on the one hand and the Hyper and controls on the other (\( P \) value of 0.10 among \( r \)-values). Thus the higher the TPL the higher the \( QO_2 \) in the hypo group, opposed to a slight inverse relationship in the Hyper and controls. The \( r \)-value of 0.63 in the Hypo group is significant in itself at the 0.05 level.

The results of the correlation between \( QO_2 \) (DNA) and \% TTL in
### TABLE 13

## CORRELATION ANALYSIS BETWEEN PLASMA LIPIDS AND OTHER FACTORS AFTER EXPOSURE TO HYPEROXIA AND HYPOXIA

<table>
<thead>
<tr>
<th></th>
<th>( \text{( Q\O_2 )} \text{ (DNA)} )</th>
<th>( \text{( \text{Tissue lipid} )} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ARCH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(21% ( O_2 ))</td>
<td></td>
</tr>
<tr>
<td>(11)</td>
<td>-0.37</td>
<td>(12) 0.04</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>(60% ( O_2 ))</td>
<td></td>
</tr>
<tr>
<td>(11)</td>
<td>0.10</td>
<td>(11) -0.44</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(12% ( O_2 ))</td>
<td></td>
</tr>
<tr>
<td>(11)</td>
<td>0.37</td>
<td>(12) 0.67 (^a)</td>
</tr>
<tr>
<td>( P^b )</td>
<td>0.30</td>
<td>0.05</td>
</tr>
<tr>
<td>Common ( r^c )</td>
<td>(33) 0.16</td>
<td></td>
</tr>
<tr>
<td><strong>ABDOM</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(21% ( O_2 ))</td>
<td></td>
</tr>
<tr>
<td>(11)</td>
<td>-0.02</td>
<td>(12) 0.06</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>(60% ( C_2 ))</td>
<td></td>
</tr>
<tr>
<td>(11)</td>
<td>-0.19</td>
<td>(11) 0.60</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>(12% ( O_2 ))</td>
<td></td>
</tr>
<tr>
<td>(12)</td>
<td>0.63(^a)</td>
<td>(10) 0.53</td>
</tr>
<tr>
<td>( P^b )</td>
<td>0.10</td>
<td>0.30</td>
</tr>
<tr>
<td>Common ( r^c )</td>
<td>(34) 0.19</td>
<td>(33) 0.29</td>
</tr>
</tbody>
</table>

\(^a\) Individually significant correlation.

\(^b\) \( P \)-value from chi-square test in Snedecor\(^{114}\) to determine if treatment \( r \)'s differ from one another.

\(^c\) Average \( r \) for category where no significant difference exists due to treatment.
the arch (Table 14) are a larger positive $r$ for the Hyper in contrast to a negative value in the Hypo. The population correlation is positive.

The $QO_2$ vs. WW/area correlation was calculated to see if thickness of the tissue might be influencing the $O_2$ uptake. Although there is no relationship in the relatively thin abdominal segments, in the thicker arch there is a slight indication of a decreased $O_2$ uptake relative to an increase in WW/area (thickness).
TABLE 14
CORRELATION ANALYSIS BETWEEN $QO_2$ (DNA)
AND OTHER FACTORS
AFTER EXPOSURES TO HYPEROXIA AND HYPOXIA

<table>
<thead>
<tr>
<th>(n) r value</th>
<th>Plasma Lipid</th>
<th>Tissue Lipid</th>
<th>Wet Weight/Area</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ARCH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (21% $O_2$)</td>
<td>(11) -0.37</td>
<td>(11) 0.21</td>
<td>(11) -0.32</td>
</tr>
<tr>
<td>Hyperoxia (60% $O_2$)</td>
<td>(11) 0.10</td>
<td>(11) 0.59</td>
<td>(11) -0.26</td>
</tr>
<tr>
<td>Hypoxia (12% $O_2$)</td>
<td>(11) 0.37</td>
<td>(11) -0.36</td>
<td>(11) -0.02</td>
</tr>
<tr>
<td>$P^b$</td>
<td>0.30</td>
<td>0.10</td>
<td>0.80</td>
</tr>
<tr>
<td>Common $r^c$</td>
<td>(33) 0.16</td>
<td>(33) 0.31</td>
<td>(33) -0.16</td>
</tr>
<tr>
<td><strong>ABDOM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (21% $O_2$)</td>
<td>(11) -0.02</td>
<td>(11) -0.02</td>
<td>(11) -0.02</td>
</tr>
<tr>
<td>Hyperoxia (60% $O_2$)</td>
<td>(11) -0.19</td>
<td>(11) -0.10</td>
<td>(11) -0.05</td>
</tr>
<tr>
<td>Hypoxia (12% $O_2$)</td>
<td>(11) 0.63$^a$</td>
<td>(10) 0.31</td>
<td>(12) 0.01</td>
</tr>
<tr>
<td>$P^b$</td>
<td>0.10</td>
<td>0.80</td>
<td>0.95</td>
</tr>
<tr>
<td>Common $r^c$</td>
<td>(33) 0.19</td>
<td>(32) 0.12</td>
<td>(34) -0.06</td>
</tr>
</tbody>
</table>

a) Individually significant correlation.

b) $P$ value from chi-square test in Snedecor\((114)\) to determine if treatment $r$'s differ from one another.

c) Average $r$ for category where no significant difference exists due to treatment.
INTRODUCTION

The purpose of exposing chickens to hyperoxia and hypoxia was to explore the effects of the environments on spontaneous avian atherosclerosis and on related factors such as blood pressure, plasma and aortic tissue lipids, and aortic metabolism through significant increases and decreases, respectively, in the blood $O_2$ tensions. The hypothesis under examination was that altered aortic tissue $O_2$ tensions could lead to metabolic derangements which would affect atherogenesis. According to Whereat (141) and other (6, 9, 28, 59, 151, 108, 11) the aortic media is already hypoxic due to its avascularity. Previous work (9, 37, 47, 61, 62) in the area would indicate that lowered tissue $O_2$ tensions would predispose to atherosclerosis while elevated $O_2$ tensions should at least inhibit such atherosclerotic development.

The results of the blood gas analysis in Table 1 where arterial $O_2$ tension was elevated (251%) and lowered (56%) in the hyperoxic and hypoxic groups, respectively, would seem to indicate that we were indeed producing significant hyperoxia and hypoxia at the arterial level. Furthermore, the decrease (20%) in the Hyper and
increase (39%) in the Hypo in hematocrit compared to initial and final
control values (both 31.0) was taken as evidence that sustained hyper-
oxia and hypoxia at the arterial level had respectively been achieved.

Atherosclerosis

On the basis that there were no significant differences among
treatments with respect to the three atherosclerotic measurements
(TTL, VS, and WW/area), either individually or combined into an
index, we must conclude that spontaneous atherosclerosis in the adult
hen is little affected by the thirty day exposures to hyperoxia and
hypoxia. Nevertheless, both segments of the hyperoxic group do
show a trend in all the individual measures and in the index toward
more extensive atherosclerotic involvement. It is possible that small
focal lesions of the spontaneous type mentioned by Moss and Benditt
(90) may have been altered to a greater extend during the exposure
period, but were not clearly detected by our macroscopic scoring or
chemical analyses.

Additional evidence of more active atherogenesis in the Hyper
may be derived from the measurements given in Table 7. Trends,
though slight, may be seen toward increased proliferation in the
cellular and extracellular component of the arch, judging from the
slightly greater DNA/area and LFD/area, respectively. It is unfor-
tunate that in whole tissue preparations the adventitial layers may mask changes in the intima and inner media, especially in the abdominal segment, where the adventitial layer constitutes a major portion of the wall\(^{(89)}\). Mandel\(^{(82)}\) based his conclusions of increased proliferation of non-lipid material in rabbit lesions on increases in the LFD/area measurement. Lofland et al.\(^{(73)}\) mention tissue weight increases in pigeons due to fatty acids rather than increases in cell numbers, however, cholesterol-fed rabbits showed considerable cellular proliferation.

**Circulatory factors**

The results from Table 3 would seem to indicate that mean arterial blood pressure in both experimental groups was depressed as a result of the thirty day exposures. In Table 3 the lowered blood pressure (8%) in the Hypo could account to some extent for the failure of the hypoxic environments to encourage atherogenesis. The five percent loss in body weight (Table 2) in addition to the decrease in plasma lipids (-23%) during the exposure could also have contributed to the lack of effect, although the hypoxic group had higher plasma lipid levels throughout the thirty day period (Table 4). In contrast, the Hyper demonstrated a slight trend toward greater lesion severity in spite of an even greater body weight loss (-13%) and lower plasma
lipid values during the exposure period. The hyperoxic group did indicate slight increases (11%) in plasma lipid during this time.

In previous work\(^\text{137}\) atherosclerotic development has shown low positive correlations with blood pressure, plasma cholesterol, and body weight.

\(\text{QO}_2\)

\(O_2\) uptake of the aortic segments is one area of measurement in this study that shows consistent trends and statistically significant differences between treatments as indicated in Table 9. The depressed metabolism in the hypoxic group might be indicative of a shift in the aerobic-anaerobic metabolic balance toward the anaerobic side. Zemplenyi\(^\text{151}\) has indicated a metabolic damage due to impaired diffusion of oxygen which resulted in a decrease of certain aerobic enzymes and an increase in lactic dehydrogenase. He suggests that the more hypoxic an area becomes, the more it depends on anaerobic glycolysis. In the hyperoxic group a significant increase in oxygen uptake was noted. Some workers\(^\text{46, 141, 143}\) have indicated that hyperoxia results in elevated \(\text{QO}_2\)'s because of lipid peroxidation and a decrease in oxidative-phosphorylative efficiency. The former can be ruled out in this study because the incubation media were equilibrated with room air. The latter proposal would
be a strong possibility, however. Bannister et al.\(^{10}\) has suggested that hyperoxia may cause a decreased anaerobiasis as determined by a decreased blood lactate production which they attributed to a decreased availability of NADH. Williams and Haugaard\(^{143}\), however, found glycolysis more resistant to \(O_2\) toxicity than was oxidative-phosphorylation in brain tissue.

The elevated \(QO_2\) in the Hypoxic group could be related to their slight increase in atherosclerotic involvement if this stage of lesion development is considered to be an active process. Such a possibility could also explain the lack of effect of the hypoxic environments on atherosclerosis. Maier\(^{79}\) has found an early stage of lesion development where \(O_2\) uptake is elevated. Previous work in this laboratory has shown significant positive correlations between \(QO_2\) and atherosclerosis in adult chickens\(^{138}\).

The previously mentioned work and those studies\(^{103, 141}\) where decreased ATP has been associated with increased \(QO_2\)'s further suggest a need for a more comprehensive approach to the investigation of the metabolic changes. In addition to oxygen uptake and lactate production an attempt should be made to concomitantly measure substrate utilization, ATP production and the fate of the various substrate metabolites.
Lofland et al. (73) used glucose 1-\textsuperscript{14}C and glucose 6-\textsuperscript{14}C to distinguish between glycolysis related to the hexose monophosphate shunt and that related to Krebs' Cycle, respectively. They could not find differences in the ratios of these pathways in normal and atherosclerotic areas.

Correlations

In the correlations, the hyperoxic group stands out in a number of instances. In Table 10 the relation of score to wet weight/area in the aortic abdominal segment reveals a significant positive relationship with respect to the control and hypoxic groups while the hyperoxic group has a very slight positive correlation. Thickness (i.e. WW/area) of the tissue is one of the important criteria for visual scoring of abdominal atherosclerosis aortic and was higher in the Hyper, but would not seem to be important in differentiating among members of this group. Other criteria in the scoring such as texture, color and hardness apparently play a greater role within this group.

The low correlation between visual scoring and TTL would seem to indicate that lipid content is a small factor in the spontaneous atherosclerosis score. This interpretation would be in agreement with Moss and Benditt\textsuperscript{(90)}, Maier and Haimovici\textsuperscript{(78)}, Aoyama and
Iwakami(6) and Weiss et al.(137). Weiss et al.(133) have determined that weight per unit area with body weight held constant accounts for forty-six percent of the variation in abdominal visual scores. They also found low positive correlations in the arch portion but not the negative values which our study produced. One difference between the results may be the decreased food intakes in the present study.

In the control and hypoxia there is a consistent negative relationship between \( QO_2 \) (LFD) and visual score in both arch and abdominal segments. The hyperoxic group does not show this depression in \( QO_2 \) with higher scores. This then would be in agreement with the idea that the elevated \( QO_2 \) in the hyperoxic group was associated with increased lesion severity. Maier(79) indicated a decrease in cytochrome oxidase activity with pre-atherosclerotic tissue of the dog. This was followed by an increase in oxidative enzyme activity in the earlier stages of lesion development. Final stages are accompanied by a decrease in these enzymes which is believed to be associated with fibrosis and necrosis.

In the light of this work the negative correlations in the control and hypoxic groups might be interpreted to relate to the latter stages of atherosclerosis, as described by Maier(79), and the positive
r-values in the Hyper correspond to the early stage of lesion development.

To compare the hyperoxic group to an intermediate phase according to Maier(79) may be questionable, however, because of the rather strong possibility that the elevated $QO_2$ of the hyperoxic group is due to a decrease in oxidative-phosphorylating efficiency associated with oxygen toxicity. Furthermore, the hyperoxic group does show a trend in the direction of greater cellular and non-cellular component proliferation as indicated by relatively elevated DNA/area and LFD/area measurements, respectively. Such an increased activity would produce an increased $O_2$ uptake and could relate to elevated atherosclerotic measurements.

It is difficult to interpret the meaning of the slight positive relationship between visual score and plasma lipid in the hyperoxic arch and the hyperoxic- and hypoxic-abdominal and the absence of such a relationship in the control. These apparent discrepancies may indicate that because of feed restrictions, the control lesions were inhibited and the normal relations between score, plasma lipid and tissue lipid are nullified. Tissue lipid relates positively to score in the arch which would be expected considering the criteria for scoring but the explanation for the negative relationship in the
control arch is not readily apparent.

In Table II it appears that visual scoring in the hypoxic abdominal segments is highly dependent on LFD/area or extracellular component proliferation as used by Mandel\(^{82}\). Chvapil et al.\(^{24}\) have suggested that hypoxia induces fibroplasia which would be in agreement with these results. Cellular proliferation shows a very slight positive relationship to atherosclerosis in both segments of the hypoxic group as measured by visual score versus DNA/area. The positive relationship in the hypoxic group between atherosclerosis and cellular- and non-cellular component proliferation should be reflected in increased metabolic activity. This is not supported by the Hypo \(\text{QO}_2\) measurements even if a shift in the aerobic-anaerobic ratio is taken into consideration. A negative correlation exists between both types of proliferation and atherosclerotic development in the hyperoxic group which have significant elevations in \(\text{O}_2\) uptake. This would seem to cast some doubt on the possibility that lesion development in these adult chickens is an active process.

The fairly consistent deviation of the Hyper from the controls and Hypo is encountered again in the correlation between LFD/area and DNA/area (Table II), where non cellular component proliferation is less related to cellular proliferation. This could possibly mean that
hyperoxia stimulates one type of proliferation in advance of or in-
dependently of the other. The mean values of LFD/area and DNA/area
in Tables 7 and 8 do not shed much light on the problem except per-
haps in the arch where the hyperoxic group has a relatively higher
DNA/area.

The hyperoxic group continues to distinguish itself from the other
treatments in the correlation of blood pressure with visual score. In
both arch and abdominal segments of the Hyper the correlation is
strongly positive while in the other groups less of a relationship exists.
This could mean that this group is particularly susceptible to athero-
genesis induced by hemodynamic factors. In addition high blood pres-
sure is correlated strongly with high TPL only in the Hyper. This
could indicate that the hyperoxic birds with high blood pressure also
had high TPL, and thus presented a situation predisposing to increased
atherosclerosis. Still(116) reports increased blood pressures coincide
with increased tissue lipid deposition and are accompanied by increases
in foam cells which is in accord with the filtration theory of lipid de-
position. The poor relationship between blood pressure and tissue
lipid in our study does not support this contention.

Koletsky et al.(64) suggest that higher blood pressures may cause
stretching and focal injury which leads to increased intimal permea-
bility. They stress the need for both elevated blood pressures and
plasma lipids in promoting lesions and found increased blood pressures resulted in presence of increased polymerized acid mucopolysaccharides in intima and media. Aoyama and Iwakami\(^6\) found similar increases in acid mucopolysaccharide under decreased \(O_2\) tension and submitted this as a cause of edema and permeability changes. Wolinsky\(^{145}\) has indicated an increase in the absolute amounts of collagen and elastin due to elevated blood pressure with no change in the relative amounts. Koletsky et al.\(^{64}\) found the presence of subendothelial fat-laden macrophages in hypertension in contrast to their absence in normotensive rats. The results of the correlation between blood pressure and LFD did not show any definitive relationships in this work.

Whereat\(^{141}\) found marked increases in intimal \(QO_2\) with atherosclerosis and suggests that this increase is due to increased lipid synthesis in atherosclerosis. Zilversmit and Newman\(^{152}\) demonstrated increased production of phospholipids in rabbit atherosclerosis which they suggest as necessary to solubilize cholesterol. We have found high TTL associated with high \(QO_2\) in the hyperoxic arch while the hypoxic group reveals a slight inverse trend (Table 14). Interpretation of this depends on which variable is selected as the independent one. It TTL is the independent variable then tissue lipid is stimulating \(O_2\) uptake in the hyperoxic and is inhibiting it in
the hypoxic. Whereat(141) relates increased $QO_2$ due to increased work pumping out excess tissue lipid and suggests that this is due to fatty acid synthesis which may play a role in solubilizing the cholesterol. Lipid droplets from fatty streak lesions are primarily cholesterol esters and fatty acids which represent lipid synthesized from precursors in the lesion(66). Thus increased $QO_2$ may be in response to increased removal and/or increased synthetic activity.

If $QO_2$ is taken as the dependent variable then a possible interpretation would be that tissue having high atherosclerosis potentials react to elevated plasma lipids by increasing endogenous lipid synthesis possibly for purposes of lipid removal. The same trends are not seen in the abdominal segment which is considered to be more susceptible to atherosclerosis in chickens. TPL does not seem to have much of a relationship to $QO_2$ in the arch. However in the abdominal segment in contrast to a very slight negative r-value in the Hyper there is significant positive correlation in the hypoxic. This would suggest that the hypoxic group with its relatively low $QO_2$ has an increased $O_2$ uptake in response to high TPL as has been suggested by some workers (108, 73, 140, 141). Mandel(82) suggests the the $QO_2$ of normal rabbit aortic tissue was not affected by the amount of lipid in the incubation medium while the involved aortae responded to increased lipid by an increased $QO_2$. What is confusing is that Munro(92) found decreased
$QO_2$ in response to cholesterol feeding in chickens. A possible interpretation is that the hyperoxic group is less susceptible to lipid infiltration from the plasma.

Table 13 shows the relationship of TPL to both $QO_2$ and TTL. In the hyperoxic arch an increased plasma lipid is negatively related to tissue lipid while the hypoxic group responds to increased TPL by a statistically significant increased in TTL. This agrees with Astrup et al. (9) The differences between treatments are significant at the 0.05 level. An interpretation of the relationship to TTL, TPL, and $QO_2$ in the correlations of the hyperoxic arch might be that increased TPL results in decreased TTL because of the elevated $QO_2$ supposedly induced by the increased tissue lipid as related to $QO_2$. In the hypoxic arch there is a strong increase in tissue lipid and a slight increase in $QO_2$ in response to increased TPL possibly because of the inability of the tissue to respond positively in terms of $QO_2$ to lipid entering the tissue. This is also in agreement with positive correlations between VS and TTL in the Hypo arch. It is interesting that score seems to depend more on TPL in the Hyper arch than in the other treatments.

TPL is positively related to TTL in the abdominal segment of both Hyper and Hypo whereas there is a significant positive relationship between TPL and $QO_2$ only in the Hypo. $QO_2$ does seem to be very re-
sponsive to TTL in the abdominal segment of the Hyper group. In spite of the greater positive relationship of TPL to TTL in the Hypo arch these aortic segments had about the same TTL as the Hyper and control. The Hypo's TPL changed in a negative direction throughout the exposure, however, they had higher absolute values than the other treatments during this time.

Several general explanations may be advanced for the small effects of O₂ treatment and the failure to bear out our expectations in terms of reduced O₂ tensions accelerating spontaneous atherosclerosis. It is possible that the 12% O₂ level in the hypoxic treatment was not severe enough to produce changes detectable by our measurements over a one month period of exposure. Astrup et al.⁹ has exposed cholesterol-fed rabbits to 10% O₂ for similar periods resulting in increased lesion severity and tissue lipid. Garbarsch et al.³⁷ achieved similar results by brief daily exposures to 5% O₂. A second possibility is that the duration of the exposure was not adequate to produce grossly measurable changes in the slowly developing spontaneous lesions. It is also possible that the lesion in this advanced stage is not very responsive to decreased O₂ levels. Acclimatization to our continuous levels of hypoxia could have occurred in contrast to the acute exposures in Garbarsch et al.³⁷.

Hyperoxia (60% O₂ at one atmosphere) if anything showed slight trends toward increased severity in terms of the atherosclerotic index and related measurements. Kjeldsen et al.⁶² and others (3, 85)
have decreased cholesterol-induced atherogenesis as a result of hyperoxia (Kjeldsen used 28% O\textsubscript{2} at one atm.). Haugaard\textsuperscript{(46)} notes, however, that the activity of enzymes having active SH groups can decrease within 10 min. in 100% oxygen at one atmosphere and peroxidation of some lipids and enzymes may occur. Aoyama and Iwakami\textsuperscript{(6)} have pointed out that peroxidation of lipids and enzymes may lead to a deactivation of SH enzymes which could lead to decreased oxidative-phosphorylation. The resulting decrease in availability of ATP could parallel the proposed effects of hypoxia\textsuperscript{(141)}.

**Summary**

Three separate groups of two year old white leghorn hens were continuously exposed to hyperoxic (60% O\textsubscript{2} at one atm.) and hypoxic (12% O\textsubscript{2} at one atm.) environments for thirty day periods which resulted in significant increases and decreases, respectively, in the \(P_{A\text{O}_2}\). There were no significant differences shown between the treatments with regard to body weight changes, total plasma lipid changes, or mean blood pressure. Atherosclerotic measurements, likewise, showed no significant differences among the treatments but there seemed to be a trend toward greater involvement in the hyperoxic group. The aortic segments of the hyperoxic and the hypoxic groups had significantly higher and lower \(Q_{O_2}\)'s respectively, compared to the controls. The hyperoxic group rather consistently differed from
the other treatments in the correlations of the various factors involved in atherosclerosis. These differences were especially prominent in the correlation between plasma lipid and tissue lipid in the aortic arch where the hypoxic group had a significantly positive r-value in contrast to the negative r-value of the hyperoxic group. The hyperoxic group showed a strongly positive relationship between \( QO_2 \) and TTL in the arch while the hypoxic group responded negatively.

The hypoxic group did not demonstrate an increased lesion severity as had been expected from other work while the hyperoxic treatment indicated, if anything, a slight trend towards increased lesion severity. These treatments caused basic changes in the metabolic picture in aortic tissue as judged by the \( QO_2 \). The laying hen however, is not the animal of choice when relating plasma lipid changes to hyperoxia and hypoxia due to the extreme variability in this measurement depending on egg development. The hyperoxic levels used in this study may have resulted in \( O_2 \) toxicity and masked any beneficial effect of an increased tissue \( O_2 \) tension. Depressed blood pressure and body weight losses in the hypoxic group could have antagonized any trend toward increased atheromatosis as result of lowered \( O_2 \) tensions. The possibility that lesion development in these birds was dependant on active processes would explain the relation-
ship of the slight increase in atherosclerosis and elevated $QO_2$'s in the hyperoxic group and lack of change in atherogenesis coupled with decreased $QO_2$'s in the hypoxic group. This possibility was not supported by the negative correlation in the hyperoxic group contrasted with a positive correlation in the hypoxic group between atherosclerosis and tissue proliferation.
APPENDIX - TABLE 15

$P_{AO_2}$ Measurements (mm Hg): A Comparison Between methods using syringe vs. direct Catheter Coupling for Transfer of hyperoxic samples to Measurement Chamber.

<table>
<thead>
<tr>
<th>Bird</th>
<th>$P_{AO_2}$</th>
<th>$P_{AO_2}$ (direct-catheter)</th>
<th>Chamber $O_2$ conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>378</td>
<td>467</td>
<td>100%</td>
</tr>
<tr>
<td>#2</td>
<td>300</td>
<td>314</td>
<td>59%</td>
</tr>
<tr>
<td>Mean (6 birds)$^a$</td>
<td>323</td>
<td>60%</td>
<td></td>
</tr>
</tbody>
</table>

$a$) This was the separate group used (Results - Table 1) in determinations for effects of hyperoxic environment on blood gases. All samples were taken by direct catheter method.
### APPENDIX - TABLE 16

**$P_{AO2}$ Measurements (mm Hg): The Changes in $O_2$ Tensions in Blood Samples as a Function of Time.**

<table>
<thead>
<tr>
<th>Bird</th>
<th>$P_{AO2}$ - initial</th>
<th>$P_{AO2}$ - after specified interval (min)</th>
<th>Chamber $O_2$ conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>319</td>
<td>130 (13)</td>
<td>62%</td>
</tr>
<tr>
<td>#2</td>
<td>330</td>
<td>97 (13)</td>
<td>61%</td>
</tr>
<tr>
<td>#3</td>
<td>330</td>
<td>118 (17)</td>
<td>75%</td>
</tr>
<tr>
<td>#4</td>
<td>93</td>
<td>77 (20)</td>
<td>21% (Room air)</td>
</tr>
<tr>
<td>#5</td>
<td>41</td>
<td>41 (16)</td>
<td>12%</td>
</tr>
<tr>
<td>#6</td>
<td>45</td>
<td>43 (10)</td>
<td>12%</td>
</tr>
<tr>
<td>#7</td>
<td>40</td>
<td>27 (20)</td>
<td>10%</td>
</tr>
</tbody>
</table>
### APPENDIX - TABLE 17

Supplemented and Unsupplemented Buffers on O$_2$ Uptake by Aortic Tissue

<table>
<thead>
<tr>
<th>Bird$^a$</th>
<th>0 - 10 min</th>
<th>arch/abdom</th>
<th>0 - 90 min</th>
<th>arch/abdom</th>
<th>% change$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 arch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supplemented$^c$</td>
<td>1.31</td>
<td>1.09</td>
<td>0.71</td>
<td>1.16</td>
<td>46</td>
</tr>
<tr>
<td>unsupplemented</td>
<td>1.64</td>
<td>1.33</td>
<td>0.99</td>
<td>1.60</td>
<td>40</td>
</tr>
<tr>
<td>abdominal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supplemented</td>
<td>1.20</td>
<td>0.61</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unsupplemented</td>
<td>1.23</td>
<td>0.62</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>#2 arch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supplemented</td>
<td>1.48</td>
<td>1.33</td>
<td>0.82</td>
<td>1.74</td>
<td>45</td>
</tr>
<tr>
<td>unsupplemented</td>
<td>1.51</td>
<td>1.22</td>
<td>0.85</td>
<td>1.57</td>
<td>44</td>
</tr>
<tr>
<td>abdominal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supplemented</td>
<td>1.11</td>
<td>0.47</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unsupplemented</td>
<td>1.24</td>
<td>0.54</td>
<td>56</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Young (12 week) White Leghorn cockerals

$^b$ This the percent change (decrease in QO$_2$) of the 0-90 min. period relative to the 0-10 min period

$^c$ Krebs-Ringer phosphate buffer supplemented with 200 mg % glucose
LIST OF REFERENCES


41. Griffel, W. Biochem. 2. 220:290, 1930 Quoted by Astrup (9)


50. Hollis, T. Cholesterosis on Oxidative Metabolism of Rabbit Renocortices; Analysis of Lactate Dehydrogenase Activity.


