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STUDIES ON EICOSANOID METABOLISM: RESPONSES OF THE CARDIOVASCULAR SYSTEM TO AEROBIC TRAINING AND ISCHEMIA

The Ohio State University

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STUDIES ON EICOSANOID METABOLISM: RESPONSES OF THE CARDIOVASCULAR SYSTEM TO AEROBIC TRAINING AND ISCHEMIA

DISSERTATION

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

BY
Harold Wayne Davis, B.S., M.S.

*****

The Ohio State University
1984

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A. J. Merola
Adviser
Dedicated to the memory
of Dr. Edward L. Fox,
advisor, teacher, friend
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By the early 1930's, Burr and Burr (1, 2) had discovered that certain unsaturated fatty acids, particularly linoleic acid, were necessary in the diets of rats to maintain their proper growth and development. At approximately the same time in unrelated work, Kurzrok and Leib (3) noticed that human seminal fluid caused contractions of uterine strips in vitro. Shortly thereafter, Goldblatt (4) and von Euler (5) independently demonstrated that extracts of semen and of the vesicular gland could lower blood pressure in vivo.

After it was established that the hypotensive effect was not due to any known substance (4, 5) von Euler (6) discovered the compound to be a lipid soluble, acidic material and coined the term "prostaglandin" because he believed that the compound originated from the prostate gland.

For nearly a quarter of a century, the prostaglandins were ignored until, in 1960, Bergström and Sjovall isolated prostaglandin F (PGF; 7) and prostaglandin E (PGE; 8) from sheep prostate glands. These prostaglandins were extracted with phosphate (fosphate) buffer and ether, respectively, and were named accordingly. Prostaglandins discovered later were named alphabetically. The subscript number designates the number of double bonds in the side chains (see Figure 1).

In 1964, van Dorp, et al. (9) and Bergström, et al. (10), independently, demonstrated that arachidonic acid was a precursor of
Figure 1. Arachidonic Acid Cascade. Reproduced from Nelson, et. al. (80)
prostaglandins. Arachidonic acid is an elongation and desaturation product of linoleic acid. Therefore, this research provided a link between the essential fatty acids and the prostaglandins.

Several years later, Piper and Vane (11) noticed that a novel prostaglandin-like compound was released during anaphylaxis. This compound was called rabbit aorta-contracting substance (RCS) for one of its actions. In 1974, Hamberg and Samuelsson found a new prostaglandin-like substance produced by blood platelets (12). The following year it was determined that this recently found compound was identical to RCS and was named thromboxane A₂ (TxA₂) since it was involved in thrombosis (13). Thromboxane A₂ was found to be derived from arachidonic acid through the prostaglandin endoperoxides (PGG₂, PGH₂) and was shown to be a very potent vasoconstrictor and platelet aggregator (13). It is quickly degraded to a stable metabolite, thromboxane B₂ (TxB₂).

In 1976 another novel prostaglandin was discovered by Moncada, et al. (14) and was temporarily named prostaglandin X. This prostaglandin was produced by the endothelial cells of the blood vessel walls and was shown to be a potent inhibitor of platelet aggregation and a vasodilator (15, 16). As with TxA₂, the activity had been demonstrated several years before but the compound had not been isolated (17). Prostaglandin X was later renamed prostaglandin I₂ (PGI₂) or the more trivial "prostacyclin". Like TxA₂, prostacyclin is a very unstable compound and is rapidly broken down to its stable metabolite, 6-keto prostaglandin F₁ alpha.
Originally it was thought that PG1<sub>2</sub> was produced from prostaglandin endoperoxides derived from platelets ("endoperoxide steal") and that this occurred only when the vessel wall was injured. It was postulated that platelets would adhere to the damaged area and donate endoperoxides to the PG1<sub>2</sub> synthase (18, 19). Researchers (20, 21) later demonstrated that PG1<sub>2</sub> could be synthesized from endoperoxides made from endothelial arachidonic acid. Prostacyclin was also originally considered to be a circulating hormone-like compound since it was not metabolized in the lungs as are other prostaglandins (22, 23) but further research has indicated that PG1<sub>2</sub> acts locally at the site of production (24, 25).

Prostaglandins are ubiquitous in multicellular animals and are involved in many biochemical processes but this thesis will be restricted to only the prostaglandins produced within or that act upon the cardiovascular system and how these prostaglandins may influence the genesis of atherosclerosis or acute myocardial infarction. Cardiovascular disease is the primary cause of death in western society. In fact, more than half of all deaths in the United States are due to some type of cardiovascular disease and, while the major lipids such as cholesterol and triacylglycerols play the principal role in atherogenesis, the disease has a multifaceted etiology. Prostaglandins, as well as several other substances have important roles in the development of this malady and these compounds may possess significant interrelationships with the major lipids.

Endothelial injury and the resultant platelet aggregation have been implicated in the initiation of atherosclerosis (26). Blood
platelets release TxA$_2$ during aggregation and this compound causes vasoconstriction and more aggregation resulting in thrombus formation. If an adequate amount of PGI$_2$ is released at this time the thrombus will be limited to the damaged area but a high level of TxA$_2$ or a low level of PGI$_2$ may result in uncontrolled platelet aggregation which could lead to excessive thrombosis and myocardial ischemia and may enhance atherosclerotic progression (26).

Ischemia has repeatedly been shown to cause extensive damage to the myocardium as well as to other tissues (27, 28, 29, 30). The exact mechanisms responsible for this damage are not known but researchers in this area have proposed several hypotheses. Many of these hypotheses deal with the phenomenon of the "oxygen paradox".

The proponents of the oxygen paradox theory suggest that most of the tissue damage due to ischemia is incurred when the tissue is reoxygenated and not during the period of hypoxia (31, 32, 33). The current understanding of the oxygen paradox is as follows. During hypoxia, adenosine triphosphate (ATP) levels become depleted and reducing equivalents (i.e., nicotinamide adenine dinucleotide, reduced; NADH) cannot be oxidized so the concentrations of these products become elevated. Calcium leaks into the cytoplasm but cannot be pumped out because of the ATP reduction. The elevation of calcium can also activate phospholipases, which release fatty acids from the phospholipids (34).

When oxygen is reintroduced to the tissue some of the reducing equivalents donate their electrons directly to oxygen instead of giving them to the electron transport system components, thereby forming
reactive oxygen species (i.e., superoxide, $\text{O}_2^-$; hydrogen peroxide, $\text{HOOH}$; singlet oxygen, $\text{O}_2$; or hydroxyl radical, $\cdot\text{OH}$)(35). These reactive oxygen species, especially $\text{O}_2$ and $\cdot\text{OH}$ can then react with polyunsaturated fatty acids, esterified in phospholipids and triacylglycerols, to produce lipid peroxides (35, 36). The peroxidation of lipids esterified to phospholipids causes an activation of phospholipase $\text{A}_2$ and thus the peroxidized lipid is removed (37).

Lipid peroxides can crosslink with other lipids and with proteins and thus, may destroy the membranes of the cell (35). Furthermore, lipid peroxides may conjugate to form "lipid peroxide clusters" which produce pores in the membrane (38). These pores allow calcium, as well as other ions, to enter the cells in massive quantities. The calcium can then bind to troponin and, with a little ATP which is produced upon reoxygenation, may cause irreversible contracture of the myofilaments (32).

The calcium also activates the phospholipases and the resulting fatty acids and lysophospholipids can serve as detergents and further damage the membrane, thereby setting up a vicious cycle (39, 40).

The most common fatty acid released by the phospholipases is arachidonic acid (34) and during the reoxygenation period prostaglandins may be formed (41, 42, 43). These prostaglandins may enhance myocardial survival (44, 45) or exacerbate the damage (46).

Along with its effects on platelet aggregation and vessel diameter, $\text{PGI}_2$ has been shown to have anti-arrhythmic (47, 48) and fibrinolytic (49, 50) properties and has recently been shown to inhibit metastasis by inhibiting platelet aggregation (51). Thromboxane $\text{A}_2$, 
on the other hand, has been shown to enhance arrhythmia (48) and metastasis (52).

From the discussion above, it could be deduced that a decrease in the TxA₂: PG₁₂ ratio would be desirable to protect against cardiovascular disease. Two major types of therapeutics have been used to favorably alter this ratio. They are vitamin E supplementation (53, 54) and aspirin ingestion (55). Although the former is probably safe and somewhat effective, the latter may cause even more problems later on (56).

Aerobic training has been shown to have many beneficial effects on risk reductions of atherosclerosis including increased concentration of high-density lipoproteins (HDL; 57, 58) and decreased concentrations of low-density (LDL) and very low density (VLDL) lipoproteins (59, 60). Therefore, it was of interest to investigate the effects of aerobic training on prostaglandin synthesis from platelets and the blood vessel wall.

There are some reports that aerobic training decreases platelet aggregation and adhesion (61, 62) but these results are not unequivocal (63). Several studies have been conducted to ascertain the effects of exercise on prostaglandin concentrations (64, 65, 66, 67), however, there has been no research directed at establishing the effects of training on these parameters. A favorable alteration of the TxA₂: PG₁₂ ratio by aerobic training would provide further evidence as to why this type of activity reduces the risk of developing cardiovascular disease.
**Purpose of the Study**

This study was conducted to establish the changes in prostaglandin synthesis from platelets and vascular endothelium that occur with training and to see if this training can alter the effects of an atherogenic diet on these parameters. It was also of interest to determine what happens to eicosanoid metabolism during ischemia of the heart since prostaglandins may protect the myocardium from irreversible damage. In a separate experiment isolated cardiomyocytes were exposed to anoxia and subsequent reoxygenation and prostaglandin synthesis and lipid peroxidation were ascertained.
Prostaglandin Formation and Actions

Dihomogamma linolenic acid, arachidonic acid and eicosa 5, 8, 11, 14, 17-pentaenoic acid can all be used as precursors of prostaglandins. They give rise to the one, two and three series of prostaglandins, respectively. These fatty acids are esterified, primarily, to phospholipids in cellular membranes (68) but can also be esterified to triacylglycerols and cholesterol. Although the prostaglandin synthase enzyme is membrane bound (69, 70) the fatty acids must be released from the phospholipid before they can be converted into prostaglandins (71).

Arachidonic acid is almost exclusively esterified in the second position of the phospholipid (72) and is removed by either phospholipase A$_2$ or the combination of phospholipase C and diacylglycerol lipase (73, 74). The resulting free fatty acid can then be reesterified, undergo beta oxidation, be converted to a hydroperoxy fatty acid (HPETE) by lipoxygenase (75) or can be acted upon by cyclooxygenase (a part of the prostaglandin synthase enzyme), with the addition of oxygen (76, 77 78) to form prostaglandin G$_2$ (PGG$_2$) and then by peroxidase (another part of prostaglandin synthase) to form prostaglandin H$_2$ (PGH$_2$; 75, 79). Prostaglandin G$_2$ and PGH$_2$ are the prostaglandin endoperoxides; the latter is converted enzymatically or non-enzymatically to the other prostaglandins (79, 80).

Endothelial PG1$_2$ can be synthesized from membrane-derived arachidonic acid or from platelet-derived PGH$_2$ through the action
of prostacyclin synthase (20, 80, 81). Besides being produced in the endothelial cells of the blood vessel walls, PGI$_2$ is produced in the lung (22, 82), gastric mucosa (83), kidney (84), macrophages (85), leukocytes (86) and cardiomyocytes (87, 88, 89). Thromboxane A$_2$ is produced, primarily in platelets by thromboxane synthase (75) but is also synthesized in the lung (80), spleen (82), kidney (90) and the blood vessel wall (91, 92, 93). Upon synthesis of TxA$_2$, 12 hydroxy 5, 8, 10 heptadecatrienoic acid (HHT) and malondialdehyde (MDA) are formed in equimolar quantities by the thromboxane synthase (94).

Oxygen-centered free radicals greatly affect prostaglandin synthesis. Phospholipase A$_2$ is activated by free radicals (95) but the resulting free fatty acids are already peroxidized and are, therefore, unsuitable as substrate for cyclooxygenase. The activation of phospholipase by the lipid peroxides is probably a protective mechanism and is used to remove peroxidized lipids from the cell membrane. Egan, et al. (96) have shown that as prostaglandins are formed, free radicals are released from the prostaglandin synthase and that these free radicals consequently destroy the enzyme. Therefore, the prostaglandin synthase is self-regulating.

Prostacyclin synthase is also inactivated by lipid peroxides from the lipoxygenase pathway (14) or from other sources (79) or by oxygen-centered free radicals generated by the prostaglandin synthase or by a peroxidase in the lipoxygenase pathway (97). Thromboxane synthase does not seem to be affected by the free radicals (14, 97).

Essentially all of the prostaglandins alter adenylate cyclase activity, generally by increasing it, but this discussion will be
limited to the effects of PGI$_2$ and TxA$_2$. Prostacyclin is a very potent activator of adenylate cyclase in platelets (98, 99). The resulting elevation of cyclic 3', 5' adenosine monophosphate (cAMP) inhibits TxA$_2$ synthesis (77). Cyclic AMP stimulates the uptake of calcium by membrane vesicles (100) and thereby lowers cellular free calcium which is required for activation of phospholipases A$_2$ and C (34). Since arachidonic acid cannot be released, TxA$_2$ is not synthesized (101). Cyclic AMP may also directly inhibit cyclooxygenase (100) and may decrease PGI$_2$ synthesis in the endothelium (102).

Thromboxane A$_2$, on the other hand, inhibits adenylate cyclase and increases mobilization of intracellular calcium (103). Thromboxane A$_2$ is a calcium ionophore and calcium inhibits adenylate cyclase so the effect of TxA$_2$ on cAMP levels may be via calcium (103). The increased concentration of intracellular calcium can also stimulate the phospholipase so that more TxA$_2$ can be formed, resulting in platelet aggregation.

**Platelets and Platelet Agonists**

Platelets, or thrombocytes, are elliptical, flat discs of approximately two to three microns in diameter. They originate from much larger blood cells called megakaryocytes and lack a nucleus but contain mitochondria, granules, microtubules and contractile proteins (104, 105). Platelets possess two types of granules, alpha granules and dense granules. The former are more numerous and contain platelet factor four, beta thromboglobulin, platelet-derived growth factor and fibrinogen. The latter contain serotonin, calcium, adenosine triphosphate (ATP) and adenosine diphosphate (ADP) (106).
When platelets are stimulated \textit{in vivo}, usually by an injury to the endothelial wall, they round up, develop pseudopods, become sticky and adhere to the damaged area (104). The first phase of platelet aggregation is reversible and consists of a platelet shape change and adherence to the site of injury (104). Thromboxane A$_2$ is produced during this phase (107). The second phase of aggregation is irreversible and is accompanied by release of the granular content and the TxA$_2$. These compounds can then perpetuate aggregation and enhance hemostasis (104, 108). Thromboxane seems to be important in initiating the second stage of aggregation (107) presumably through the release of ADP from the dense granules (release I) (109). Alpha granule emptying is denoted as release II.

Several substances can trigger platelet aggregation including thrombin, collagen, ADP, epinephrine, arachidonic acid and TxA$_2$. Even excessive turbulence in the blood stream may cause platelets to aggregate (104). Born (110) was the first to describe aggregation due to a specific platelet agonist when he demonstrated that ADP could induce aggregation of platelets. Since then ADP has become a standard by which to measure agonist efficacy. Adenosine diphosphate bypasses TxA$_2$ synthesis while inducing aggregation (111). The aggregation seems to be the result of ADP exposing fibrinogen binding sites on the platelet membrane (105). Fibrinogen binding seems to be essential in ADP-induced platelet aggregation (108).

When the blood vessel wall is injured, collagen is exposed and this can initiate aggregation (104). Soluble collagen can also induce aggregation. Collagen probably exerts its effects by combination with
surface constituents of the plasma membrane. This results in a reduction of adenylate cyclase activity and, consequently, an increase in phospholipase action with subsequent TxA₂ synthesis and aggregation (111, 112).

Thrombin, a proteolytic enzyme involved in the blood clotting cascade, stimulates platelets by activating phospholipase C and eventually phospholipase A₂ thus providing free arachidonic acid for the platelet cyclooxygenase (113). The effects of epinephrine also seem to be through the activation of phospholipases (103), although, as with ADP, little TxA₂ is formed when platelets are stimulated with epinephrine (111).

Aggregation of platelets can also be induced by arachidonic acid, presumably via TxA₂ formation (111, 114). However, the arachidonic acid may also be converted into a compound that inhibits aggregation induced by other agonists (114). Thromboxane A₂ is a very important component in platelet aggregation but, as was mentioned earlier, aggregation can occur without significant TxA₂ accumulation (111). On the other hand, several groups of researchers have shown that TxA₂ can accumulate without aggregation (100, 115, 116), and Charo, et al. (117) have demonstrated that low levels of TxA₂ can cause aggregation without secretion while high levels of TxA₂ must cause aggregation through pathways other than just ADP release.

Low concentrations of thrombin, arachidonic acid and ADP give a biphasic type of aggregation with secretion occurring in the second phase. Collagen and higher concentrations of the aforementioned agonists, with the exception of ADP, give a monophasic response with
secretion occurring simultaneously with aggregation. Secretion due to ADP occurs after aggregation. Epinephrine always gives a biphasic response with secretion in the second phase (118, 119).

The Blood Vessel Wall

Prostacyclin inhibits aggregation and adhesion caused by collagen, ADP or epinephrine (121). This is presumably due to its tremendous ability to increase cAMP levels (99). Only a very small amount of PGI₂ is released from unstimulated endothelial cells (121) and circulating levels are generally low. However, several compounds have been described that stimulate PGI₂ synthesis. Some of these also enhance TxA₂ production so a similar mechanism is probably involved.

Thrombin increases PGI₂ formation (122) by activating phospholipases. Adenosine triphosphate and ADP have been shown to enhance prostacyclin synthesis in the rabbit pulmonary artery and the rat aorta (123). Prostacyclin production is also augmented by bradykinin which activates phospholipases A₂ and C via a calcium-calmodulin dependent pathway (74, 124). Low levels of arachidonic acid induce PGI₂ synthesis, however, high levels of arachidonate have been shown to promote TxA₂ production by the vessel walls (93).

Angiotension II, norepinephrine, platelet activating factor (PAF) and 5-hydroxytryptamine (serotonin) have all been shown to enhance PGI₂ production in the endothelial cells of humans, rats and rabbits (122, 125). Histamine stimulates PGI₂ formation in rabbit tissue and lysophosphatidate slightly enhances prostacyclin production in rabbit
and human endothelium (122). Beta-thromboglobulin, a platelet release product, inhibits prostacyclin synthesis (122).

**Lipoproteins and Prostaglandins: Interrelationships**

Several comprehensive reviews on lipoprotein structure and function have been written within the last few years (see 126, 127, 128, 129) so the following discussion will be only a brief introduction to this expansive area of research.

Chylomicrons, the largest and least dense of the lipoproteins, carry dietary triacylglycerols from the intestinal mucosa to adipocytes and muscle. These lipoproteins contain all of the apoproteins from the A series, the C series, apoprotein E and apoprotein B-48. The second class of lipoproteins, the very low density lipoproteins (VLDL), have a density similar to that of chylomicrons and carry triacylglycerols produced in the liver to extrahepatic sites. Chylomicrons and VLDL's have been implicated in atherogenesis but are not considered important risk factors (130, 131).

Very low density lipoproteins donate apoprotein B-100 and cholesterol to an intermediate density lipoprotein (IDL) which, in turn, donates these constituents to low density lipoproteins (LDL). The LDL's then pick up more cholesterol from the liver and from other lipoproteins and transport it to extrahepatic tissue including the blood vessel wall. Low density lipoproteins bind to a specific receptor on cells and are thereby incorporated. Their proteins are degraded and their lipid is used for maintenance of the cell membranes (126). However, if there is an excess of cholesterol, that cholesterol
is deposited in the cell as lipid droplets of cholesteryl ester. High levels of LDL are considered to be a risk factor in cardiovascular disease (131, 132).

High density lipoproteins (HDL) are derived from chylomicrons or VLDL's and contain all of the types of apoproteins except apo B. The HDL's also contain a substantial amount of phospholipid, primarily phosphatidylcholine. The principal function of the HDL's is to scavenge excess cholesterol from cells and to take it to the liver where it is converted to bile acids and excreted. As early as in 1951, Barr (133) suggested that the HDL may be protective against cardiovascular disease but it was not until 1975, after Miller and Miller (134) published their report on the risk reduction properties of these lipoproteins that they became a valuable predictor of potential cardiovascular disease.

The results of several studies have revealed that atherogenic diets increase TxA2 and decrease PGI2 production (135, 136, 137). These diets also increase LDL and reduce HDL levels so there seems to be some relationship between the lipoproteins and these prostaglandins. By 1969, Farbiszewski and Worowski (138, 139) had discovered that intravenous injections of cholesterol altered the composition of the low density lipoprotein in such a way as to enhance platelet aggregation and adhesion. Shattil, et al. (140) later found that liposomes containing high concentrations of cholesterol induced platelet hypersensitivity to epinephrine. Insel, et al. (141) have demonstrated that platelets incubated with cholesterol-rich liposomes acquire membrane cholesterol and this is associated with decreased
membrane fluidity and increased sensitivity to epinephrine. Alternatively, these researchers found that platelets incubated with cholesterol-free liposomes lost membrane cholesterol with a resultant increase in membrane fluidity and a decrease in the sensitivity to epinephrine.

Low density lipoproteins but not HDL or VLDL have been shown to be positively correlated with platelet aggregation by epinephrine (142) and Beitz, et al. (143) have demonstrated that LDL's stimulate, dose-dependently, MDA formation from human platelets. Furthermore, platelets from patients suffering from type IIA hyperlipoproteinemia seem to be more sensitive to aggregating agents and produce more TxA₂ than do platelets from normolipidemic persons (144). Treatment with the lipid-lowering drug, simvastatin, decreased platelet aggregation and MDA production in ischemic heart disease patients (145). It has been suggested that the high cholesterol concentrations (which parallel LDL concentrations) may actuate the release of arachidonic acid from platelet membrane phospholipids and then stimulate its conversion to TxA₂ (146).

On the other hand, an inverse relationship has been described between PGI₂ synthesis and plasma cholesterol concentrations (147). Nordoy, et al. (148) have shown that incubation of endothelial cells with LDL reduces their ability to inhibit platelet aggregation. Very low density lipoproteins had a moderate inhibitory effect on the synthesis of the platelet antagonist substance (presumably PGI₂) while HDL's were able to partially counteract the effects of LDL's. Beitz and Forster (149) found a negative correlation between LDL and
PGI₂ synthesis and further demonstrated that LDL from women inhibited PGI₂ synthesis less than LDL from men, possibly because of a higher content of polynonsaturated fatty acids in the female LDL (150).

Szczeklik and Gryglewski (151) have suggested that the inhibition of PGI₂ synthesis brought about by LDL's may be due to lipid peroxides carried in these lipoproteins, however, further research indicated that the peroxides were largely an artifact of the lipoprotein isolation procedure (152) and Beltz, et al. (153) have found that HDL's contain more lipid peroxides than do LDL's. While the lipid peroxide theory still has some merit, a more plausible explanation may be that lipoproteins which inhibit PGI₂ formation are deficient in polyunsaturated fats and, therefore, reduce the amount of substrate available for prostaglandin synthesis (153).

Beltz and Forster (149, 150) have revealed a positive correlation between HDL concentration and prostacyclin synthesis. This correlation is especially evident in the female where the HDL phospholipids and cholesteryl esters contain primarily polyunsaturated fats. Fleisher, et al. (154, 155) furthered this research by demonstrating that rat HDL, which contains more arachidonic acid than does human HDL, provided a greater stimulation of PGI₂ synthesis than did human HDL at equivalent doses. Rat and human HDL apoproteins induced equal but small increases in PGI₂ synthesis indicating that the stimulation of prostacyclin formation was due to the arachidonic acid carried by the HDL.

Atherosclerosis and Prostaglandins

Atherosclerosis is a disease of large and medium sized arteries
and is characterized by a focal thickening of the intimal lining. It may begin in early childhood as an accumulation of lipid along the arterial wall, known as a fatty streak, and is grossly apparent in most men by the end of the second decade of life (156).

The malady begins with damage to the endothelium and consequently, exposure of collagen (26). This damage may be caused by mechanical disruptions such as hypertension or by such chemicals as cholesterol, bradykinin and homocysteine. When the endothelium is damaged the permeability of the vessel wall is increased and this allows blood components such as monocyte-macrophages and LDL's to enter the site of injury. Macrophages can engulf the LDL's and convert the cholesterol to cholesteryl esters thus producing droplets of fat that make the cell look foamy, hence the name "foam cells" (157). These cells characterize the fatty streak.

Platelets adhere to the damaged area and as they aggregate they release numerous compounds including TXA₂, platelet-derived growth factor (PDGF; 26, 158) and 12-L-hydroxy 5, 8, 10, 14-eicosatetraenolic acid (12 HETE; 159). The latter two compounds stimulate smooth muscle cell migration through the internal elastic component of the blood vessel wall and into the intimia (158, 159). Smooth muscle cells produce collagen and elastin and these compounds along with sulphated glycosaminoglycans (GAG), which bind and help internalize LDL's (160), and calcium produce what is called the fibrous plaque. Smooth muscle cells can also incorporate cholesterol and become foam cells. Cholesteryl esters in the foam cells differ from those in the blood. The cellular cholesteryl ester is primarily cholesteryl oleate, whereas
linoleate is the major fat esterified to blood cholesterol (161). This would imply that the degradation of cholesteryl linoleate and the production of cholesteryl oleate are ongoing processes within the cell and they may play an important role in atherogenesis.

As the plaque progresses the center becomes hypoxic (162) so, to supply the core with oxygen and nutrients, capillarization occurs and this allows more macrophages and calcium to be brought in (163). Eventually, the plaque becomes calcified into the complicated lesion which is probably not reversible.

Prostaglandins seem to play key roles in the development of atherosclerosis both by modifying lipoprotein structure and by altering cellular metabolism. In 1952 Glavind, et al. (164) discovered that lipid peroxides were prevalent in atherosclerotic plaques and their concentration increased as the plaque progressed. These products were not detectable in normal arteries. Formazycek, et al. (165) have proposed that the lipid peroxides may modify the structure of the LDL so that its uptake by the LDL receptor is accelerated. Lipid peroxides from platelets may also enhance LDL uptake by smooth muscle cells or macrophages. Khalfen, et al. (166) have shown that activated platelets enhanced the penetration of LDL's into the arterial wall by increasing the permeability of the endothelium and by adsorbing the lipoproteins to their own surface. Furthermore, Fogelman, et al. (167, 168) have noticed that MDA from activated platelets alters the LDL in such a way as to allow non receptor-mediated uptake by monocyte-macrophages. The LDL is then degraded and the cholesterol and cholesteryl ester can be incorporated into the atherosclerotic lesion.
Once the cholesteryl ester (usually cholesteryl linoleate) has entered the cell it is hydrolysed and the cholesterol is reesterified to cholesteryl oleate by acylcoenzyme A: cholesterol acyltransferase (ACAT). Prostaglandin E$_2$ has been shown to inhibit ACAT in a dose dependent manner (56, 169) while PGI$_2$ significantly increases lysosomal acidic (ACEH) and cytosolic neutral cholesteryl hydrolases (NCEH) in smooth muscle cells (169, 170). The effects on the hydrolases are probably mediated through an elevation in cAMP. Together, the decrease in ACAT activity and the increases in ACEH and NCEH activities could possibly help control the excessive accumulation of smooth muscle cell cholesteryl esters observed in atherosclerosis.

Immediately following endothelial injury there is a temporary but considerable elevation of PGI$_2$ synthesis by subendothelial components of the vessel wall (171). This phenomenon may be due to the "endoperoxide steal" from platelets adhering to the damaged area (19). However, a few hours after the injury PGI$_2$ formation drops to around five to 25 percent of normal because of the loss of endothelial cells (171). If the damaged area is repeatedly irritated by hypercholesterolemia or some other factor, PGI$_2$ synthesis will remain low and atherogenesis may occur (172) but if the injured area is allowed to heal prostacyclin synthesis will return to normal.

As was mentioned previously, the concentration of lipid peroxides in the arterial wall increases with the degree of atherosclerosis (164) and this, as expected, is related to a decrease in prostacyclin synthesis (135). Gryglewski, et al. (136) showed that hearts and arteries of cholesterol-fed rabbits produced significantly less PGI$_2$
from exogenous arachidonic acid than did the hearts and arteries of control animals. At the end of three months on the diet of one percent cholesterol and three percent olive oil, PGI\(_2\) production was strongly suppressed but at five months prostacyclin formation started to return to normal. Voss, et al. (173) found that rabbits fed 0.3 percent cholesterol for four months produced more PGI\(_2\) in their aortas than did rabbits fed normal diets. Furthermore, these researchers demonstrated that regions of the aorta covered with plaque produced more PGI\(_2\) than did normal areas. The differences in these results may possibly be due to the age or strain of the rabbits. Gryglewski, et al. used 12 month old male albino rabbits while Voss, et al. used nine month old male Dutch rabbits. Also, the former group of researchers measured PGI\(_2\) synthesis from exogenous arachidonic acid while the latter group followed the synthesis from endogenous substrate. Saldeen and Saldeen (174) have found no difference in PGI\(_2\) production between normal and atherosclerotic areas of human vessels. These discrepancies clearly indicate the need for more research in this area.

Saldeen and Saldeen (174) have also shown that atheromatous regions of human arteries produce more TxA\(_2\) than do adjacent normal regions. However, there was no difference in TxA\(_2\) synthesis between early and advanced lesions, so it was suggested that the early lipid deposits were important in the enhancement of thromboxane production. There is also an increased synthesis of TxA\(_2\) from platelets of atherosclerotic rabbits as compared to those of normal rabbits (136, 175, 176). These platelets are hyperaggregable but they also seem to be more sensitive
to PGI₂ than are normal platelets. The latter phenomenon would be beneficial, especially if prostacyclin production was decreased with atherosclerosis. The results of these studies indicate that prostaglandins are involved in atherosclerosis and that if prostaglandin production in platelets and the blood vessel wall can be favorably altered the progression of the disease may be slowed or even totally suppressed.

Prostaglandins In Exercise and Training

Aerobic training has repeatedly been shown to reduce atherosclerosis associated with cholesterol feeding in rabbits (177, 178), cockrels (179) and in Macaca fascicularis monkeys (180), but the results of some other studies have demonstrated either no change in rabbits (181, 182, 183) or an acceleration of atherosclerosis in dogs (184) due to training. The contradictory findings may be due to the type of training or to the amount of stress encountered by the animals.

Assuming that aerobic training does inhibit atherogenesis, this occurrence is probably not solely due to changes lipid levels since Kobernick and Niwayama (178) found no correlation between blood lipids and exercise or the severity of atherosclerosis and Wong, et al. (179) saw no change in serum cholesterol levels with training even though there was a significant regression of the lesions. Although there are many reports of favorable alterations in the lipoprotein profile with chronic physical activity (57, 58, 59, 60), changes in platelet activation and prostaglandin synthesis may be very important in the inhibition of atherogenesis due to training.
The results of several studies indicate that there is a hyperactivity of platelets after a bout of exercise (185, 186, 187). However, a few researchers have reported a reduction in this exercise-induced hyperactivity following aerobic training (61, 62, 188).

Mehta, et al. (189) demonstrated increases in blood TxA$_2$ with mild to moderate intensity treadmill running while Risk, et al. (66) could only find significant elevations in TxA$_2$ levels after anaerobic running. Coronary artery disease patients had a much greater increase in TxA$_2$ concentration after exercise than did healthy controls although the resting values for the two groups were comparable (189). Sless, et al. (67) could not show a significant elevation of TxA$_2$ synthesis from platelet rich plasma even after rather severe bicycle riding. Norepinephrine, which is increased with exercise and is associated with platelet aggregation, seemed to have no effect on TxA$_2$ synthesis (67).

Mehta, et al. (189) and Demers, et al. (65) have reported increases in blood PGI$_2$ following exercise but Risk, et al. (66) saw no such increase. Cardiac patients had a much smaller elevation of PGI$_2$ than did healthy controls (189). The smaller elevation of PGI$_2$ together with the enhanced elevation of TxA$_2$ following exercise may be an important risk factor for experiencing exercise-induced myocardial infarction in these patients.

The sensitivity of platelets to PGI$_2$ was decreased following 20 minutes of jogging or playing squash (190). The decrease was greater, after squash, presumably because this activity is intermittently
anaerobic and therefore lactic acid is produced. Osterud and Brox (187), on the other hand, found no change in whole blood clotting time after 30 minutes of jogging if PG2 was present. Without PG2, however, the clotting time was significantly decreased after exercise.

These results provide some information as to why untrained persons occasionally suffer an exercise-induced myocardial infarction. The increase in TxA2 and the decrease in platelet sensitivity to prostacyclin may result in an acceleration of platelet aggregation and vasoconstriction and the coupling of these events could cause a thrombus to lodge in an artery and trigger a heart attack or stroke. Despite the interest in the effects of exercise on platelet aggregation and prostaglandin formation there have been no studies conducted on the consequences of training on these parameters. We, therefore, felt that the present studies would be of interest and may help explain why active populations have a reduced risk of cardiovascular disease.

Myocardial Ischemia

Lipids have long been known to play important roles in cell structure and function. Phospholipids are the predominant type of lipid in myocyte membranes and since they are involved in providing substrate for prostaglandin synthesis it is important to ascertain the phospholipid composition of these cells. This has not been an easy task as the myocardial phospholipid composition varies greatly from one preparation to another (191, 192, 193, 194, 195) as can be seen in Table I. Cardiolipin (CL), phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC) values are shown because, together, they make up approximately 65 to 90 percent of the total phospholipid content of
the heart (195) and because they are the only ones that all of the
groups measured.

TABLE I
MAJOR PHOSPHOLIPID COMPOSITION OF HEART TISSUE

<table>
<thead>
<tr>
<th>Reference</th>
<th>Source</th>
<th>Prep</th>
<th>Cl</th>
<th>PE</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>191</td>
<td>Adult rat</td>
<td>Whole heart homogenate</td>
<td>5.1</td>
<td>22.7</td>
<td>37.0</td>
</tr>
<tr>
<td>192</td>
<td>Human</td>
<td>Atrial mitochondria</td>
<td>17.0+</td>
<td>28.0</td>
<td>36.0</td>
</tr>
<tr>
<td>192</td>
<td>Human</td>
<td>Atrial microsomes</td>
<td>12.0+</td>
<td>26.0</td>
<td>35.0</td>
</tr>
<tr>
<td>193</td>
<td>Adult rat</td>
<td>Ventricle mitochondria</td>
<td>20.0+</td>
<td>36.7</td>
<td>34.0</td>
</tr>
<tr>
<td>193</td>
<td>Adult rat</td>
<td>Ventricle microsomes</td>
<td>15.2+</td>
<td>30.3</td>
<td>38.2</td>
</tr>
<tr>
<td>194</td>
<td>Adult rat</td>
<td>Whole heart homogenate</td>
<td>1.8+</td>
<td>33.7</td>
<td>37.5</td>
</tr>
<tr>
<td>194</td>
<td>Neonatal rat</td>
<td>Whole heart homogenate</td>
<td>2.0+</td>
<td>31.1</td>
<td>42.5</td>
</tr>
<tr>
<td>195</td>
<td>Adult rat</td>
<td>Atrial homogenate</td>
<td>5.9+</td>
<td>37.4</td>
<td>49.8</td>
</tr>
<tr>
<td>195</td>
<td>Adult rat</td>
<td>Ventricle homogenate</td>
<td>7.2+</td>
<td>38.1</td>
<td>49.4</td>
</tr>
</tbody>
</table>

* Cl, cardiolipin; PE, phosphatidyl ethanolamine; PC, phosphatidyl choline.

+ = Cardiolipin and phosphatidic acid were measured together.

These discrepancies may be due to differences in species, age or part of the heart or to treatment of the tissue or separation of the lipids. All of these hearts were extracted with chloroform and methanol and the lipids were all separated by thin layer chromatography with different solvent systems.

The results of studies of arachidonic acid incorporation into cardiac cells also show conflicting results. Isakson, et al. (196) have shown that approximately 17 percent of the radiolabeled arachidonic acid appeared in the neutral lipid fraction while 83 percent was in the phospholipid fraction. Of the arachidonate incorporated into phospholipids, 35.3 percent was in the PE fraction and 43.7 was in the PC fraction. Ahumada, et al. (197) found 70
percent of the label in neutral lipids and only 30 percent in the phospholipids. Eighty percent of the label incorporated in the phospholipid fraction was in PC and only 14 percent was found in PE.

The huge difference in these results may be due to the method of introduction of the arachidonic acid to the cells or to the quantity of arachidonate added. In the former study the fatty acid was perfused through an intact heart whereas, in the latter study the arachidonate was incubated with cultured heart cells. Furthermore, in the study conducted by Isakson, et al., 22.5 micromolar arachidonate was perfused while ten micromolar arachidonate was incubated with the cells in the study by Ahumada, et al.. Denning, et al. (198) have shown in endothelial cells that at 75 micromolar and higher more arachidonate is incorporated into triacylglycerols than into phospholipids while at arachidonate concentrations lower than this the opposite is true. Since the perfused arachidonate in the Isakson, et al. study was exposed mainly to endothelial cells in the heart the concentration may not have been great enough for the fatty acid to be esterified into the neutral lipids. The primary purpose of triacylglycerols seems to be to store excess fat until it is needed for energy production.

The arachidonate esterified in phospholipids is continuously turned over and the free fatty acid can be acted upon by cyclooxygenase or lipoxygenase to form prostaglandins or hydroperoxy fatty acids. The myocardium has an active prostaglandin synthase (199), however, under normal conditions heart cells synthesize only very small quantities of prostaglandins (197, 200, 201).
As with phospholipid composition and arachidonate incorporation, there are discrepancies in the literature as to which prostaglandins are produced by the heart. Ahumada, et al. (197) found that \( \text{PGE}_2 \) was the primary prostaglandin formed by rat myocytes with smaller amounts of \( \text{PGF}_2 \alpha \) produced. Their cells generated variable but small quantities of 6-keto \( \text{PGF}_1 \alpha \) and no \( \text{TxB}_2 \). Prostaglandin \( \text{E}_2 \) was also the major prostaglandin found by Bolton, et al. (200) in rat myocytes but dog myocytes produced more 6-keto \( \text{PGF}_1 \alpha \) and \( \text{PGD}_2 \) than anything else. Their rat myocytes synthesized significant amounts of both 6-keto \( \text{PGF}_1 \alpha \) and \( \text{TxB}_2 \). Vahouny, et al. (201) have shown that their rat cardiomyocytes produce mainly \( \text{PGE}_2 \) but also produce significant quantities of the other prostaglandins. All of these researchers followed the conversion of radiolabelled arachidonic acid to prostaglandins. In the intact heart the pericardium apparently is the major source of prostaglandins while significant amounts are also generated by the epicardium. Much smaller quantities are synthesized in the myocardium and the epicardium (202). Prostacyclin seems to be the major prostaglandin formed by the intact heart (202).

Sudden occlusion of a coronary artery due to thrombosis or an embolism results in a rapid conversion to anaerobic metabolism and, consequently, in a loss of high energy phosphates from the ischemic myocardium (28, 32, 203). The reduction in ATP allows calcium to accumulate in the cytosol and this free calcium can activate phospholipases with the resultant release of fatty acids. Since ATP is necessary for reacylation of phospholipids there is an elevation of
lysophospholipids (204, 205, 206) and unesterified fatty acids (27, 207) during ischemia.

Shalihk and Downar (206), Sobel, et al. (204) an Snyder, et al. (205) have demonstrated a substantial increase in lysophospholipids after as little as eight to ten minutes of myocardial ischemia, however, a drop in the total phospholipid content was not evident until after eight hours of ischemia (206). Chien, et al. (207) saw a decrease in total myocardial phospholipid after only three hours of ischemia and Smith, et al. (208) have shown that renal phospholipid content was decreased in as little as 30 minutes of ischemia. This decrease was primarily in the cardiolipin fraction (208).

The accumulation of unesterified arachidonate is minimal during the first 10 to 30 minutes of ischemia but there is a significant elevation of this fatty acid after one to three hours of coronary occlusion (207). However, the elevation of total unesterified fatty acids occurs much sooner than this, indicating a non specific degradation of cellular lipids (27, 207, 208). The fatty acids and lysophospholipids can act as detergents and further damage the membranes (39, 40). Furthermore, the lysophospholipids have been shown to be arrhythmogenic (204, 205).

During ischemia, pyridine nucleotides and cytochromes in the electron transport system become reduced. Upon reperfusion, these nucleophiles can donate their electrons directly to molecular oxygen thereby creating oxygen-centered free radicals (35). The oxygen radicals can then peroxidize either esterified or unesterified polyunsaturated lipids. The peroxidized lipids may be removed by
phospholipase A\textsubscript{2} which is activated in their presence (37) and this can cause further degradation of membrane lipids.

Rao, et al. (209) detected oxygen free radicals in blood after one minute and in tissue after 15 minutes of ischemia but no lipid peroxidation was evident until after 45 minutes of ischemia. Yamamoto, et al. (210), however, have shown that upon reperfusion of ischemic cerebrum there is a rapid elevation of lipid peroxides in both tissue and blood. Dickens, et al. (36) have shown that free radicals induced by ferric ions plus ADP cause peroxidation and subsequent degradation of phospholipids. The free radical most responsible for the damage is probably hydrogen peroxide (212) although there is some controversy about this (213).

One of the consequences of ischemia is the depression of antioxidant enzyme activities (i.e., superoxide dismutase, SOD; and glutathione peroxidase, GP) (31) and this may be one reason for the elevation of free radicals upon reoxygenation. The addition of SOD (33, 213) or catalase, an enzyme that breaks down hydrogen peroxide, (33, 212) has been shown to reduce the damage caused by or to enhance the recovery from ischemia-reperfusion.

As mentioned before, the intracellular accumulation of calcium may be very detrimental to the cell. Lipid peroxidation has repeatedly been alleged to alter calcium transport between subcellular compartments (38, 214, 215). Calcium accumulation in the cell due to lipid peroxidation seems to occur in two ways. First, the lipid peroxides can form "cluster pores" by which calcium can diffuse into the cytosol and secondly, the peroxides may damage the calcium ATPase
so that excessive calcium cannot be pumped out of the cytosol (38). The excessive calcium, along with a little ATP which may be formed upon reoxygenation of the cell may result in an irreversible, contracture of the myofilaments (32). Furthermore, phospholipases may be activated by the accumulated calcium (34) and, hence, substrate may be provided for prostaglandin synthesis.

McGiff, et al. (30) were the first to demonstrate prostaglandin synthesis in ischemic tissue. They found a release of mainly PGE$_2$ and PGF$_2$ alpha from canine kidney. Since then several researchers have detected prostaglandin synthesis during myocardial ischemia (41, 42, 43, 216). In the earlier studies (41, 42) PGE$_2$ and PGF$_2$ alpha were the main prostaglandins elevated, whereas, in the later studies prostacyclin predominated (43, 216). These differences may be due to the fact that PGI$_2$ was not isolated until 1976 (14) and methods for detection of 6-keto PGF$_1$ alpha may not have been available to the former researchers. These researchers conclude that the prostaglandins were produced during ischemia, hypoxia or even anoxia, however, some oxygen must have been present since prostaglandin synthesis has an absolute requirement for oxygen (76, 77, 78).

Carr and Goldfarb (46) have suggested that prostaglandins generated during ischemia may augment lysosomal enzyme release and, thereby, enhance myocardial damage. Araki and Lefer (45), on the other hand, have shown that PGI$_2$ infusion reduced ischemia-induced creatine kinase release and in earlier work Goldfarb and Glenn (44) found that the infusion of PGF$_2$ alpha increased the survival time of coronary artery-ligated cats by 353 percent. Since prostacyclin is a strong
vasodilator it may be released in an attempt to supply more blood to
the ischemic tissue.

Although the myocardial damage caused by ischemia-reperfusion is
probably multifaceted, the precise etiology of this damage must be
ascertained so that logical interventions can be adopted to combat this
rampant and sudden cause of death.
CHAPTER THREE
AEROBIC TRAINING AND PROSTAGLANDINS

Methodology

Materials

The following materials were purchased from Sigma Chemical Company (St. Louis, MO):

Dextran (average molecular weight = 80,000)
Bovine Thrombin
Bovine Serum Albumin (fraction V and essentially fatty acid free)
Cardiolipin (Bovine Heart)

The following compounds were obtained from either New England Nuclear Corporation (Boston, MA) or Amersham (Arlington Heights, IL):

1^-C arachidonic acid (55-60.1 mCi/mmol)
Thromboxane B$_2$, (5, 6, 8, 9, 11, 12, 14, 15 - $^3$H (n))
Prostaglandin E$_2$, (5, 6, 8, 11, 12, 14, 15 - $^3$H (n))
Prostaglandin F$_2$ alpha, (5, 6, 8, 11, 12, 14, 15 - $^3$H (n))
6-keto-prostaglandin F$_1$ alpha, 6-(5, 8, 9, 11, 12, 14, 15 - $^3$H (n))

The following materials were kindly supplied by Dr. John Pike (Upjohn Company, Kalamazoo, MI):

Thromboxane B$_2$
Prostaglandin E$_2$
Prostaglandin F$_2$ alpha
6-keto-prostaglandin F$_1$ alpha
Prostacyclin (sodium salt)

Antibodies to the prostaglandins were generously provided by Dr. L. Levine (Brandeis University) or Dr. R. Fertel (The Ohio State University).

The following were obtained from Avanti:

Phosphatidyl choline (egg)
Phosphatidyl ethanolamine (egg)
Phosphatidyl inositol (egg)
Phosphatidyl serine (egg)
Lysophosphatidyl choline (egg)
Lysophosphatidyl ethanolamine (egg)
Sphingomyelin (egg)

Collagen (Calfskin -- Chemalog (S. Plainfield, NJ)
Indomethacin -- Merck and Company, Inc. (Rathway, NJ)
Silicic acid (Unisil) -- Clarkson Chemical Co. (Williamsport, PA)
Sodium pentobarbital (1gm/ml) -- The Butler Co. (Columbus, OH)
Arachidonic acid (U-71-A) — Nu Chek Prep, Inc. (Elysian, MN)
Charcoal (Norit A) — Fisher Scientific Co. (Fairlawn, NJ)
Unopette platelet/leukocyte count dilutors — Benton Dickinson (Rutherford, NJ)
TLC plates (LK6D) — Whatman Co. (Clifton, NJ)
Thrift-Solve scintillation fluid — Kew Scientific Inc. (Columbus, OH)
Chloroform — Fisher Scientific Co. (Fairlawn, NJ)
HPLC grade hexane, Isopropanol and methanol — MCB Reagents (Gibbstown, NJ).

Methods

Animals, Diets and Training

Chow-fed rats — male Sprague Dawley rats were housed three or four to a cage in controlled environment of 12 hours of light and 12 hours of darkness. Food (Purina Rat Chow) and water were available ad libitum.

The animals were randomly assigned to an aerobic training (TR₁, n=9) or a sedentary (SR₁, n=11) group. The exercising group was trained on a modified human treadmill (Quinton #18-49-C; Quinton Instrument Co., Seattle, WA) geared for speeds of 11-135 meters per minute and elevations of 0 to 45 percent grade. The training protocol was progressive and by the end of 12 weeks the rats were running 60 minutes a day at 32 meters per minute and 12 percent elevation for 5 days a week. The program was maintained for the duration of the study. This training program has been shown to increase the oxidative capacity of the soleus and the vastus lateralis profundus muscles by more than 40 percent in rats (217). The sedentary controls were subjected to the same psychological stress as the trained rats by placing them on the treadmill for 15 minutes a day, however, the exercise training was kept to a minimum. Another set of rats (TR₂, n=7 and SR₂, n=7) were treated as described above but were used for different experiments.
Cholesterol-fed rats -- male Sprague Dawley rats were treated as above except that after an initial training period of 12 weeks some of these animals received a modified diet consisting of 10 percent fructose in their water. After 16 weeks it was determined that this modification did not significantly alter the blood lipid profile of the animals so a completely new diet was implemented. This diet consisted of: 48% dextrin, 18% casein, 11% sucrose, 10% coconut oil, 4% vitamin supplement, 1% cholesterol, 0.5% cholic acid, 0.4% liver concentrate powder, 0.2% choline chloride and 0.2% methionine. This diet was continued for an additional 16 weeks. Four rats served as controls (C) and five rats each were placed in a sedentary cholesterol (and fructose) fed groups (SC) or an aerobically trained cholesterol fed group (TC). Aerobic training was the same as described above.

Cholesterol-fed rabbits -- male New Zealand White rabbits were housed one to a cage under the same conditions as the rats. The animals were randomly assigned to a training (TB, n=6) or a sedentary (SB, n=5) group. Training consisted of hopping on a motor-driven treadmill at 26 meters per minute and 10 percent grade. The rabbits began training by running five minutes twice a day and gradually increased that time until, by 12 weeks, they were running 18 minutes twice a day. Sedentary controls were placed on the treadmill for equal periods of time but the motor was off and only occasionally turned on. After 12 to 15 weeks of training all of the rabbits were placed on a diet of 0.3 percent cholesterol and 5 percent cottonseed oil in Purina Rabbit Chow. The diet and training program were continued until the
conclusion of the study. One trained rabbit died a few days prior to the time he was to be sacrificed.

Lipid Measurements

After an overnight fast, rats were bled intraorbitally with a glass pipet and rabbits were gravity bled into a test tube with a 21 gauge needle from the central ear artery. The blood was allowed to clot and the serum was removed and stored at 4°C until use. All samples were assayed within five days.

Triglyceride concentration was determined by the colorimetric method of Soloni (218). High density lipoproteins were separated from other lipoproteins by a heparin-manganese chloride (MnCl₂) precipitation. Fifty microliters of a mixture of 2.5 parts MnCl₂ to two parts heparin (5,000 U/ml) were added to 500 microliters of serum. The mixture was incubated in an ice bath for 30 minutes then centrifuged at 5,000 x g for 30 minutes at 4°C. An aliquot of the supernatant was then used to measure cholesterol concentration. Total cholesterol and HDL-cholesterol were measured as described by Parekh and Jung (219).

Platelets were prepared as will be described later and the concentration was adjusted to 1 x 10⁹ platelets per milliliter. Platelet total cholesterol was then assayed according to the enzymatic, fluorometric method of Helder and Boyett (220).

Response of the Heart to PGI₂

The chow-fed rats (TR₁ and SR₁) were not exercised for 24 hours prior to sacrifice. One rat from each group was analyzed per day. The animals were anaesthetized intraperitoneally with 40 mg
sodium pentobarbital/kg body weight, their chests transected and their hearts removed and put in ice-cold Krebs-Henseleit bicarbonate buffer (0.7% NaCl, 0.035% KCl, 0.03% MgSO$_4$·7H$_2$O, 0.016% KH$_2$PO$_4$, 0.18% glucose, 0.21% NaHCO$_3$). The hearts were then cannulated via the aorta and the "Langendorff" perfusion was initiated. In the Langendorff procedure the perfusate flows retrograde through the aorta into the coronary arteries. The coronary circulation drains into the right ventricle, out of the pulmonary artery and drops off the heart into a drainage system. The left ventricle fills by way of the Thebesian circulation and beats isovolumetrically in the usual preparation (221). The perfusion medium was Krebs-Henseleit bicarbonate buffer aerated with 95% oxygen and 5% carbon dioxide to maintain the pH at 7.4 and the oxygen tension at 600 torr. The total time from transecting the chest to initiating the Langendorff perfusion was held to less than 30 seconds. Constant flow was maintained with a peristaltic pump and pressure was measured with a Statham Model P23AA pressure transducer (Statham, Hato Rey, Puerto Rico). The results were recorded on a Grass Model 5D polygraph (Grass Instrument Co., Quincy, MA).

At the end of a ten minute washout period the perfusion pressure was set at 70 torr and the heart rate was allowed to reach its intrinsic steady state. Prostacyclin was made into serial dilutions of 100 picograms per milliliter to ten nanograms per milliliter in the perfusion medium. Each concentration was made immediately prior to injection into the aortic cannula. After each bolus injection the perfusion pressure was allowed to return to the baseline control value.
A typical pressure reading of the Langendorff perfusion is shown in Figure 2.

**PGI\textsubscript{2} Synthesis from Aorta**

After an overnight fast and abstinence from exercise for 24 hours, the aortas from the second set of chow-fed rats (TR\textsubscript{2} and SR\textsubscript{2}), the cholesterol-fed rats (C, TC and SC) and from the rabbits (TB and SB) were removed and stripped of fat and adventitia while held on a block of frozen Tris-saline (0.01M Trizma base, 0.9% NaCl, pH 7.4). The rat aortas were cut into pieces of approximately ten milligrams while those from rabbits were cut into 30 milligram strips. The strips of rat aorta were rinsed for five minutes in Tris-saline (pH 8.0) then incubated in fresh buffer at 25°C for up to 120 minutes. Aliquots of the reaction mixture were taken and frozen at -70°C for later analysis of 6-keto-PGF\textsubscript{1} alpha. Strips of rabbit aorta were also rinsed for five minutes but they were then incubated for 90 minutes. The aorta strip was removed and the reaction mixture was stored at -70°C until it could be assayed for 6-keto-PGF\textsubscript{1} alpha.

**TxB\textsubscript{2} Synthesis from Washed Platelets**

When the animals were sacrificed they were bled via cardiac puncture with a 21 gauge needle into polyethylene tubes containing a one-tenth volume of 1.0 mM ethylene diamine tetraacetic acid (EDTA; pH 7.4). Platelets were obtained by centrifuging the blood at 200 x g for 20 minutes to separate the platelet rich plasma (PRP). The PRP was transferred to small plastic centrifuge tubes and these were spun at 4000 rpm at 49°C in a Sorvall Superspeed RC-2 centrifuge (Ivan Sorvall Inc., Norwalk CT) for 20 minutes. The supernatant was removed and the
Figure 2. Characteristic pressure recording after PGI$_2$ infusion into the Langendorff perfused heart.
platelets were resuspended in Tris buffer (0.006% EDTA, 0.61% Trizma base, 0.87% NaCl, pH 7.4). This was centrifuged at 4000 rpm at 4°C for 15 minutes. This procedure was performed once for rat platelets and twice for rabbit platelets. After the final wash the supernatant was removed and the platelets were resuspended in Krebs-Henseleit bicarbonate buffer. The platelets were counted on a phase contrast microscope and the concentration was adjusted to 1 x 10^8 platelets per milliliter. One-half milliliter aliquots of the platelet suspension were stimulated with agonists (thrombin, collagen, arachidonic acid) for various periods of time at 37°C. The reactions were terminated with 100 microliters of 1.0N HCl and the prostaglandins were extracted into three milliliters of diethyl ether. After solvent evaporation under nitrogen the residue was dissolved in Tris-albumin buffer (0.61% Trizma base, 0.1% Bovine serum albumin, 0.05% sodium azide) and then frozen at -70°C until analysed for TxB_2 and PGE_2. Protein concentration of the platelets was determined by the method of Lowry, et al. (222).

Radioimmunoassay of Prostaglandins

The radioimmunoassay of prostaglandins were conducted by using the method of Fertel, et al. (223). Briefly, an aliquot of the aorta incubation medium or of the platelet extract was placed in a test tube. An equal volume of prostaglandin antibody (diluted to give 25 to 40 percent binding) and finally 6.8 nanocuries of tritiated prostaglandin were then added. The mixture was refrigerated for five to 24 hours then dextran-charcoal (0.25% Norit A charcoal, 0.025% dextran in Tris-albumin buffer) was added to bind the free prostaglandins. After
refrigeration for another ten minutes the tubes were centrifuged and the supernatant was decanted into scintillation vials. Scintillation fluid was added and the samples were counted by a Beckman LS 8100 scintillation counter.

Prostaglandin analyses of rat tissue were conducted with antibody donated by Dr. L. Levine. The cross reactivities for these antibodies are as follows: 6-keto-PGF\(_1\) alpha antibody — PGF\(_1\) alpha, 0.15%; PGD\(_2\), 0.02%; PGF\(_2\) alpha, 0.10%; arachidonic acid, 0.005%. TxB\(_2\) antibody — PGF\(_2\) alpha, 0.26%; PGE\(_2\), 0.21%; PGD\(_2\), 1.6%; arachidonic acid, 0.001%.

Prostaglandin analyses of rabbit tissue and all subsequent prostaglandin assays were conducted with antibody supplied by Dr. R. Fertel. The cross reactivities for these antibodies are as follows: 6-keto-PGF\(_1\) alpha antibody — PGA\(_1\), 0.16%; PGE\(_2\), <0.03%; PGF\(_1\) alpha, <0.03%; PGF\(_2\) alpha, <0.03%; TxB\(_2\), <0.03%; arachidonic acid, <0.01%. TxB\(_2\) antibody — PGA\(_1\), <0.05%; PGD\(_2\), 1.0%; PGE\(_2\), 0.08%; PGF\(_1\) alpha, 0.1%; PGF\(_2\) alpha, 0.25%; 6-keto PGF\(_1\) alpha, <0.06%; arachidonic acid, <0.01%. PGE\(_2\) antibody — PGA\(_1\), 0.16%; PGD\(_2\), 1.6%; PGF\(_1\) alpha, 3.2%; PGF\(_2\) alpha, 0.75%; 6-keto PGF\(_1\) alpha, 0.05%; TxB\(_2\), 0.41%; arachidonic acid, 0.01%. PGF\(_2\) alpha antibody — PGA\(_1\), <0.01%; PGD\(_2\), <0.01%; PGE\(_2\), 0.02%; PGF\(_1\) alpha, 15.0%; 6-keto PGF\(_1\) alpha, 0.14%; TxB\(_2\), 0.06%; arachidonic acid, <0.01%.

Statistical Analysis

Statistical significance of differences between means for the chow-fed rat and for the rabbit data were evaluated using Student's two-tailed t-test. The cholesterol-fed rat and the Langendorff
perfusion data were analysed by independent simple analyses of variance (ANOVA). To ascertain where the differences were after a significant F value was found with the ANOVA, a Newman-Kuels Post Hoc test was used. The Pearson Product-Moment test was used to find correlations between different parameters. All data are presented as the mean ± the standard error of the mean (SEM).
Results

At the time of sacrifice, trained chow-fed rats weighed less than their controls (458.29±11.27g vs. 514.71±13.36g, p<0.01). Furthermore, trained cholesterol-fed rats weighed slightly, but not significantly less than their sedentary controls and there was a tendency for cholesterol feeding to lower body weight although this was also nonsignificant (C: 591.80±48.27g vs. SC: 538.60±5.35g vs. TC: 495.80±14.70g). The trained rabbits weighed significantly less than the sedentary rabbits for most of the study but by the time of sacrifice there was no weight difference between the two groups (TB: 3210.20±115.35g vs. SB: 3495.80±144.45g; Figure 3). This was due to a weight loss occurring in the sedentary group during the last few weeks of the experiment and was presumably caused because the cholesterol diet was making these animals ill. Besides being lighter, the aerobically trained, chow-fed rats had significantly lower intrinsic heart rates than did the sedentary rats (175±8.3 bpm vs. 195±9.1 bpm, p<0.05). Intrinsic heart rates for cholesterol-fed rats and rabbits were not measured.

Cholesterol feeding significantly elevated serum cholesterol in rats (see Table 2) and rabbits and serum triglycerides in rabbits. Rabbit serum cholesterol was increased after only two weeks on the cholesterol-supplemented diet (pre: 49.30±0.99 mg/ml vs. post: 627.09±79.87 mg/ml, p < 0.001) and triglycerides were elevated by eight weeks on the diet (pre: 41.85±4.53 mg/dl vs. post: 102.79±25.80 mg/dl, p<0.05). Both of these serum lipids continued to increase as
Figure 3. Body weights of rabbits. Time 0 is arrival date; rabbits were 6 to 8 weeks old upon arrival. ○ Sedentary (SB), ● Trained (TB). n is 6 for trained and 5 for control. At the time of sacrifice there were only 5 trained rabbits. *p<0.05 **p<0.01. Data are presented as mean ± SEM.
## TABLE 2

**Blood Lipid Levels in Cholesterol-Fed Rats and Rabbits at Sacrifice**

*Values are in mg/dl*

<table>
<thead>
<tr>
<th></th>
<th>TG++</th>
<th>CHOL</th>
<th>HDLc</th>
<th>HDLc/CHOL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rats</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>172.00±46.49</td>
<td>62.00±18.08</td>
<td>46.32±13.35</td>
<td>0.75±0.04</td>
</tr>
<tr>
<td>Sedentary (SC)</td>
<td>188.50±28.45</td>
<td>256.50±17.58*</td>
<td>39.00±8.40</td>
<td>0.16±0.04**</td>
</tr>
<tr>
<td>Trained (TC)</td>
<td>189.75±24.17</td>
<td>179.75±58.20</td>
<td>28.25±0.86</td>
<td>0.20±0.05**</td>
</tr>
<tr>
<td><strong>Rabbits</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary (SB)</td>
<td>146.25±31.28</td>
<td>1491.00±138.78</td>
<td>61.00±16.04</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Trained (TB)</td>
<td>165.25±78.38</td>
<td>1197.88±139.89</td>
<td>53.20±12.48</td>
<td>0.05±0.01</td>
</tr>
</tbody>
</table>

++ TG, triglyceride; CHOL, total cholesterol; HDLc, HDL-cholesterol; HDLc/CHOL, HDL-cholesterol to total cholesterol ratio.

* significantly different from control at p<0.05.

** significantly different from control at p<0.01.
the study progressed and at the time of sacrifice the cholesterol concentration was $1344.44\pm104.96$ mg/dl and the triglyceride level was $203.98\pm178.06$ mg/dl (see Figures 4 and 5). Cholesterol feeding significantly lowered the HDL-cholesterol to total cholesterol ratio ($\text{HDL}_c/\text{CHOL}$) in rats (Table 2). Training had no effect on any of the serum lipid parameters in rats or rabbits. Furthermore, rat platelet cholesterol was not altered by the cholesterol diet or training ($C:33.40\pm3.80$ mg/10$^9$ platelets vs. $SC:36.82\pm4.98$ mg/10$^9$ platelets vs. $TC:35.62\pm1.907$ mg/10$^9$ platelets). Visual inspection of the rabbits' thoracic and abdominal aortas revealed no gross morphological differences between the trained and sedentary animals.

By using the Langendorff perfusion system it was possible to demonstrate that the coronary vasculatures of the trained, chow-fed rats were more sensitive to the vasodilatory effects of PGI$_2$ than were those of the sedentary, chow-fed rats (Figure 6). At 1.0, 10.0 and 100.0 nanograms of PGI$_2$, trained hearts showed a greater responsiveness than did untrained hearts.

As can be seen in Figure 7 and Table 3 there were no significant differences in PGI$_2$ production from rat aortas due to training or cholesterol feeding although there was a slight tendency for training to reduce PGI$_2$ synthesis. On the other hand, there was a significant increase in PGI$_2$ synthesis from trained rabbit aortas compared to those from sedentary rabbits. It can also be seen that the rabbit aortas only produced about one-tenth as much prostacyclin as the rat aortas. This phenomenon corroborates the findings of other researchers (224).
Figure 4. Rabbit serum cholesterol concentrations. Time 0 is when cholesterol feeding was begun, ○ Sedentary (SB), ● Trained (TB). n is 6 for trained group and 5 for control group. At the time of sacrifice there were only 5 trained rabbits. Data are presented as mean ± SEM.
Figure 5. Rabbit serum triglycerides. Time 0 is when cholesterol feeding was begun. °Sedentary (SB), ●Trained (TB)n is 6 for trained group and 5 for control group. At the time of sacrifice there were only 5 trained rabbits. At 10 weeks only 4 trained and 3 control rabbits were used. Data are presented as mean ± SEM.
Figure 6. Percent change in pressure after infusion of prostacyclin. ◦ Sedentary (SR), ● Trained (TR), *p<0.05 **p<0.01. Data are presented as mean ± SEM.
Figure 7. 6-keto PGF₁α synthesis from chow-fed rat aortas. Aortas were treated as described in the text. ○ Sedentary (SR₂), ● Trained (TR₂). At 120 minutes n=5. Data are presented as mean ± SEM.
<table>
<thead>
<tr>
<th>time (min.)</th>
<th>arachidonic acid*</th>
<th>6-keto PGF₁ alpha (pmoles/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>-</td>
<td>64.35±3.94</td>
</tr>
<tr>
<td>60</td>
<td>+</td>
<td>278.75±8.22</td>
</tr>
<tr>
<td>rabbits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>-</td>
<td>4.52±0.28</td>
</tr>
</tbody>
</table>

* 27 nanomoles of arachidonic acid was added.
Training reduced soluble collagen- and thrombin (dissolved in Krebs-Henseleit buffer)-induced TxB₂ synthesis from platelets of chow-fed rats (Figure 8). There was also a tendency for training to reduce TxB₂ synthesis from platelets of cholesterol-fed rats stimulated by 0.25 units and 0.50 units of thrombin per milliliter of incubation medium while cholesterol feeding had a tendency to enhance thromboxane synthesis induced by these agents (Figure 9). However, platelets from trained rabbits actually produced more TxB₂ than did platelets from sedentary rabbits when they were stimulated by one nanomole of arachidonic acid (suspended in ethanol) and there was a tendency for platelets from trained rabbits to produce more TxB₂ in response to stimulation by other agonists as well (except with five nanomoles of arachidonic acid and one unit of thrombin per milliliter; see Figure 10). Prostaglandin E₂ synthesis from rabbit platelets roughly paralleled TxB₂ synthesis but there were no significant differences between the two groups (Figure 11).
Figure 8. TxB2 synthesis from washed rat platelets. Platelets were incubated with the agonist for 10 minutes. Sedentary (SR2), Trained (TR2). *p<0.05, **p<0.01. Data are presented as mean ± SEM.
Figure 9. Thrombin-induced TxA2 generation from rat platelets. Control (C), Cholesterol-fed Sedentary (SR), Cholesterol-fed trained (TC). Platelets were incubated with thrombin for 10 minutes. Data are presented as mean ± SEM.
Figure 10. Rabbit platelet TxB2 synthesis induced by collagen, thrombin or arachidonic acid (AA). Sedentary (SB), Trained (TB). Platelets were incubated with collagen and thrombin for 10 minutes and with AA for 5 minutes. * p<0.05. Data are presented as mean ± SEM.
Figure 11. Rabbit platelet PGE$_2$ synthesis induced by collagen, thrombin or arachidonic acid (AA). Sedentary (SB), Trained (TB). Platelets were incubated with collagen and thrombin for 10 minutes or with AA for 5 minutes. Data are presented as mean ± SEM.
Correlations were run between serum and platelet lipids and prostaglandins and between the levels of the various prostaglandins. Neither rat platelet cholesterol nor any of the serum lipids were correlated with TxB₂ synthesis however, there was a significant negative correlation between TxB₂ synthesis induced by one nanomole of arachidonic acid and serum cholesterol in rabbits (r=-0.804, p<0.05). There were no other significant correlations between serum lipids and platelet TxB₂ or PGE₂ production or aorta PG1₂ synthesis and there were no correlations between platelet prostaglandin synthesis and prostacyclin formation in the aorta strips. However, there were significant correlation coefficients between rabbit platelet TxB₂ and PGE₂ synthesis induced by one unit of thrombin per milliliter (r=0.825, p<0.05) and one nanomole of arachidonic acid (r=0.774, p<0.05). Two units of thrombin per milliliter and five nanomoles of arachidonic acid gave correlation coefficients between TxB₂ and PGE₂ of 0.675 and 0.619, respectively, but these were not significant.
Discussion

The lighter body weights of the trained animals as compared to the sedentary controls was expected and has been well documented (217, 225). It was probably due to a decreased percentage of body fat in the trained animals but this parameter was not measured.

The lower intrinsic heart rates of the exercised rats was also expected (226) as resting bradycardia has long been used as an indication of physical fitness. Furthermore, the training program for the rats has been shown to increase the oxidative capacity of the soleus and vastus lateralis profunders muscles; another indication of training (217). Various problems were experienced while trying to obtain the intrinsic heart rates of the rabbits so these data are not available, however, electrocardiograms performed on rabbits undergoing a similar training program have shown a training-induced resting bradycardia (personal communications with Dr. E. Kanabus and with B. Doerr).

Cholesterol feeding caused a substantial elevation of serum cholesterol in both rats and rabbits and a significant increase in rabbit triglycerides as was expected (228, 229, 230). Training had no significant effect on any of the measured lipid parameters in rats although there was a tendency for the exercised rats to have lower total cholesterol and HDL-cholesterol levels than did the sedentary controls. A training-induced cholesterol reduction in rats has been demonstrated by Ahrens and Broxton (227) but they did not measure HDL concentration. The HDL is usually considered to be protective against the development of cardiovascular disease and the slight decrease in
the HDL concentration in the trained animals may seem undesirable until it is realized that the decrease is due, primarily, to the drop in total cholesterol as the HDL-cholesterol to total cholesterol ratio is slightly, but not significantly, elevated in the trained animals.

Of the handful of studies dealing with trained rabbits, the results of one has shown no change in cholesterol levels (178), two have shown an increase (182, 183) and two have shown a decrease in serum cholesterol (177, 181). Brown, et al. (181) found an increased cholesterol concentration after four weeks in trained rabbits fed 0.5 percent cholesterol compared to sedentary rabbits fed the same diet. However, by the eighth week the situation had reversed. In both of the studies where the trained rabbits had higher cholesterol levels than the controls the animals were sacrificed after only two months on the cholesterol diet. If the study had been conducted for a longer period of time the sedentary rabbit cholesterol levels may have surpassed those of the trained rabbits. In the present study the trained rabbits showed a non-significant tendency for higher cholesterol levels than did the controls for the first 12 weeks, however, by the time of sacrifice, a few weeks later, this trend had been reversed as the exercised rabbits' cholesterol level dropped and the sedentary rabbits' cholesterol level continued to rise. Triglyceride and HDL concentrations were not measured in the early studies with rabbits but the results of studies on humans have repeatedly demonstrated a decrease in triglyceride and an increase in HDL levels with aerobic training (57, 58, 59, 60). The differences between the present results and those obtained from humans may be due to species variations, diet
or type of training. As with the rats, the trained rabbits had slightly lower HDL levels but also had a slightly higher HDL-cholesterol to total cholesterol ratio.

Isolated rat hearts perfused by the Langendorff procedure responded to bolus injections of as little as 0.1 ng PGI$_2$, however, there was no difference in the responses of the trained and sedentary hearts until a dose of 1.0 ng was added. Hearts from trained animals also showed greater responsiveness to 10.0 and 100.0 ng of PGI$_2$. These results suggest that aerobic training enhances the sensitivity of the vascular smooth muscle cells to prostacyclin. During ischemia prostaglandins, primarily PGI$_2$, are released from the heart (43, 216, 231). If the coronary vasculature of a trained animal was more sensitive to PGI$_2$ than that of a sedentary animal the trained animal's vessels might dilate further and allow more oxygen to reach the ischemic tissue and, thereby, reduce the extent of the damage.

Synthesis of 6-keto PGF$_1$ alpha by aorta strips from both chow-fed and cholesterol-fed trained rats was not significantly different from that from the sedentary rats, however, there was a tendency for the aortas from the sedentary rats to produce more 6-keto PGF$_1$ alpha. If this tendency was greater, it would seem to be an undesirable effect of training on the interactions of platelets and the endothelial wall but Panganamala, et al. (232) have shown that there is an increase in 6-keto PGF$_1$ alpha synthesis in rats with age and Pace-Asclak and Carraro (233) have demonstrated a pressure-related increase in prostacyclin synthesis in hypertensive rats. Therefore,
the effects of exercise may have been to keep the aortas more like those of young, nomotensive rats.

On the other hand, aortas from trained rabbits produced more PGI$_2$ than those from sedentary rabbits. The rat aortas produced approximately ten times more 6-keto PGF$_1$ alpha than did the rabbit aortas so there seems to be a species difference (224). Since the rat produces high levels of PGI$_2$ compared to the rabbit the slight decrease seen with training may be physiologically insignificant. However, since the rabbit produces such a small amount of PGI$_2$, the increase due to training may be tremendously important in the protection against cardiovascular disease.

Platelets from chow-fed, trained rats produced less TxB$_2$ than platelets from the sedentary, chow-fed rats and there was a tendency for training to reduce TxB$_2$ synthesis by platelets from cholesterol-fed trained rats. These results would be considered a positive finding on how training may reduce the risk of developing cardiovascular disease. However, platelets from trained rabbits actually generated more TxB$_2$ than those from sedentary rabbits when stimulated with one nanomole of arachidonic acid and there was a tendency for trained rabbit platelets to produce more TxB$_2$ in response to soluble collagen (both 0.17 and 0.34 mg/ml) also. These results suggest that training may enhance the activity of the cyclooxygenase in rabbit platelets while leaving phospholipase activity unaltered. The data from the rat platelets indicate that either the phospholipase or the cyclooxygenase activity, or both, is diminished with training. Since arachidonate is generally esterified in phospholipids and triglycerides (196, 207), the
addition of free arachidonic acid is untenable and, therefore, the
difference between TxB₂ synthesis in trained and untrained rabbits may
not be physiologically important. The levels of 6-keto PGF₁ alpha
produced by the aortas and the TxB₂ produced by collagen and thrombin
stimulated platelets were similar to those reported elsewhere (53, 54,
234) but the amount of TxB₂ synthesized by rabbit platelets in response
to arachidonic acid was much higher than reported previously (54).
This discrepancy is difficult to explain since the platelets were
handled in the same manner but it may have been partially due to the
cholesterol diet.

The addition of arachidonic acid greatly stimulated the synthesis
of prostaglandins from rat aortas and rabbit platelets. This was
expected since the release of arachidonate from phospholipids is
considered the rate limiting step in prostaglandin synthesis (34). On
the other hand, the results of cholesterol feeding were somewhat
surprising since it had no effect on 6-keto PGF₁ alpha synthesis from
the aorta and there even seemed to be a tendency for the diet to
decrease rat platelet TxB₂ synthesis. The cholesterol diet caused an
elevation of total cholesterol and the atherogenic lipoproteins (VLDL +
LDL; data presented elsewhere, 235) but lowered (non-significantly) HDL
levels in the rats. Fleisher, et al. (154, 155) have shown that HDL's
increase prostacyclin production from aortas and several researchers
have shown that LDL's decrease PGI₂ synthesis (148, 149, 150, 151,
152). Furthermore, Stuart, et al. (146) has shown that when platelets
are loaded with cholesterol they produce more TxB₂ upon stimulation.
Low density lipoproteins may be able to donate cholesterol to platelets
and, therefore, the increased LDL concentration seen in the cholesterol-fed rats should have resulted in an enrichment of platelet cholesterol. This was not the case and may partially explain why the platelets didn't become hypersensitive with cholesterol feeding. The negative correlation between rabbit serum cholesterol and the one nanomole arachidonic acid-induced TxB2 synthesis also contradicts the results of the other researchers. One reason for these differences may be that in the present study the aortas or platelets had been exposed to the cholesterol and lipoproteins in vivo, whereas, in the other studies cholesterol, HDL or LDL was added to an incubation mixture containing an aorta strip or platelets. In the absence of these exogenous lipids, aorta strips and platelets may react very differently than they would if the lipids were present.

Thromboxane A2 is released during platelet aggregation and this compound causes vasoconstriction and even more aggregation. Prostacyclin, on the other hand, relaxes vascular smooth muscle and inhibits platelet aggregation. The ratio of these two molecules may play an important role in the regulation of blood flow and thrombus formation. The results reported here can generally be viewed as favorable since training seemed to reduce the TxA2 to PGI2 ratio. The shift in the ratio of TxA2 synthesis to PGI2 synthesis taken together with the increased tissue sensitivity to prostacyclin may help to explain the observation that active populations have a reduced risk of cardiovascular disease.
Summary and Conclusions

1. Aerobic training had very little effect on blood lipid and lipoprotein levels in these rats and rabbits.

2. Hearts from trained rats perfused by the Langendorff method showed greater responsiveness to PGI$_2$ than did hearts from sedentary rats.

3. Training had no effect on PGI$_2$ synthesis from rat aortas but enhanced PGI$_2$ generation from rabbit aortas.

4. Rabbit aortas produced only about one-tenth the PGI$_2$ produced by rat aortas but platelet TxB$_2$ was similar for the two species.

5. Training reduced TxB$_2$ synthesis from chow-fed rat platelets but had no significant effect on cholesterol-fed rat platelets. Training increased TxB$_2$ synthesis from rabbit platelets simulated with one nanomole of arachidonic acid.

The rat is generally perceived as being immune to atherosclerosis while the rabbit is very susceptible to this malady. Humans lie somewhere between these two extremes. Leach and Thorburn (236) have shown that rabbit and human platelets respond similarly to collagen at different concentrations so the platelet data from the rabbits may more closely resemble what would happen with humans than would the data from the rat platelets.

Several studies have been conducted on prostaglandin synthesis during exercise but the present study is the only one, to our knowledge, to look at changes incurred by aerobic training. Mehta, et al. (189) and Risk, et al. (66) compared the exercise response of coronary artery disease (CAD) patients and diffuse pulmonary fibrosis (DPF) patients, respectively, with healthy controls. Resting values for 6-keto PGF$_1$ alpha and TxB$_2$ were comparable for all groups but during exercise Mehta, et al. saw an increase in both TxB$_2$ and 6-keto PGF$_1$ alpha with the CAD patients experiencing larger increases than
the controls. Risk, et al. saw an increase in only TxB\(_2\); the DPF patients experienced the increase with mild work while the healthy controls experienced the increase only after anaerobic work. These results suggest that people in better physical condition may have a smaller prostaglandin response to exercise. Demers, et al. (65) demonstrated an increase in 6-keto PGF\(_1\) alpha in well-trained runners after a marathon race but there were no comparisons between trained and untrained subjects. Vilnikka, et al. (237) have recently reported that exercise increased 6-keto PGF\(_1\) alpha levels but left TxB\(_2\) levels unaltered. These researchers compared athletes to non-athletes at rest and found no differences between the two groups. However, upon sampling for the TxB\(_2\) measurement they allowed the blood to clot and, therefore, they determined maximal TxB\(_2\) synthesis instead of that produced during rest or exercise. Prostacyclin does not circulate in the blood for very long (25) so it is not surprising that the TxB\(_2\) and 6-keto PGF\(_1\) alpha levels were not different for the two groups. It would be interesting to see if athletes had different prostaglandin responses to submaximal and maximal exercise than did non-athletes.

Burghuber, et al. (190) found that platelets became less sensitive to prostacyclin after jogging or playing squash. This could be one factor in exercise-associated myocardial infarction and it would be interesting to ascertain whether a trained person would have a smaller decrease in PG\(_{12}\) sensitivity than would an untrained person.

Much more research needs to be done in this area such as determining whether training increases platelet sensitivity to PG\(_{12}\) or whether the results acquired from rodents pertain to humans.
Although many questions remain unanswered, these findings show that aerobic training may, indeed, favorably alter prostaglandin synthesis in platelets and the blood vessel wall.
CHAPTER 4

HYPOXIA AND REOXYGENATION OF CARDIOMYOCYTES

Methodology

Materials

All of the materials used in these studies were listed earlier.

Methods

Preparation of Cardiomyocytes

Adult rat heart cells were prepared by a modification of the procedure of Kao, et al. (115) as described by Hohl, et al. (238). Male Charles River rats (usually 300-400g) were decapitated, the chest wall transected and an icy slush of Krebs-Ringer-phosphate was poured into the chest cavity to maintain adenine nucleotide levels. The heart was excised and the lungs removed. Excess fat and the pulmonary veins and arteries were trimmed and the aorta was cannulated with a blunt-end 15-gauge needle. The heart was then perfused at 37°C by the Langendorff method with oxygenated "high potassium (K)" buffer (90mM NaCl, 30mM KCl, 16mM Na$_2$HPO$_4$, 1.2mM KH$_2$PO$_4$, 1.2mM MgCl$_2$) containing 11mM glucose, 20mM creatine, 60mM taurine, a complete amino acid mixture and 0.1 percent bovine serum albumin (BSA). After a five minute washout period, collagenase (Worthington Class I or II, 1 mg/ml) was added to the buffer and the hearts were perfused until soft (45-65 minutes). The pH was adjusted to 7.2 throughout the perfusion and the flow rate increased from eight to 16 ml/minute over the perfusion period. The ventricles of the hearts were then minced with scissors and the cells were dispersed with a nalgene pipet and filtered through two layers of cheese cloth. The resulting myocytes were washed twice
with a "low K" Krebs-Ringer-phosphate buffer (120mM NaCl, 6mM KCl, 16mM Na₂HPO₄, 1.2mM KH₂PO₄, 1.2mM MgCl₂) containing 11mM glucose, 20mM creatine, 20mM taurine, the complete amino acid mixture, a vitamin supplement and 0.5 percent BSA at pH 7.2. Protein content was measured by the Biuret method after the cells had been washed free of the BSA (239). The cells were counted on a hemocytometer under a light microscope (200-300 cells/count) and viability was assessed by trypan blue (0.3%) exclusion and the percentage of the total cells with the rod shaped morphology, characteristic of heart cells in situ, as opposed to round cells in contracture (see Plate I). The cells used in these experiments were initially 94.6±0.67 percent viable and 87.0±1.11 percent rod shaped. Two to three milligrams of cell protein were used in prostaglandin experiments, five to ten milligrams were used in the lipid peroxidation (MDA) experiments and eight to ten milligrams were used in the lipid composition experiments. Glucose was excluded from the suspending medium during the anoxia, reoxygenation experiments since the presence of glucose protects the cells from rounding up (32).

Lipid Composition of the Myocytes and the Effects of Hypoxia and Reoxygenation

The cells were washed three times in high K Krebs-Ringer-phosphate buffer (without glucose, creatine, taurine, amino acids or BSA) to remove the BSA since bovine serum albumin interferes with the incorporation of arachidonic acid into the heart cells (24). After washing, the cells were resuspended in high K Krebs-Ringer-phosphate containing 5mM glucose, 5mM glutamate and 1mM EDTA. The glucose and
Plate I. Photomicrograph of isolated cardiomyocytes. These cells have the characteristic rod-shaped morphology with the exception of a rounded cell in the middle of the left border. Magnification is 100x.
glutamate were used as a fuel source for the cells so that they would not oxidize the arachidonic acid.

Radiolabelled (1-\textsuperscript{14}C) arachidonic acid (AA) was purchased in a toluene solution. This was evaporated under nitrogen then resuspended in ethanol. The purity of the AA was periodically evaluated by running it on a thin layer chromatographic (TLC) plate with a solvent system of hexane: diethyl ether: methanol: acetic acid (90:20:2:3) (241). Twenty nanomoles of the stock 1-\textsuperscript{14}C AA was transferred into each of six polypropylene tubes, the solvent was evaporated and the AA was resuspended in 10 ml ethanol and 50 ml 0.15M Na\textsubscript{2}CO\textsubscript{3}. Cells (in two milliliters of buffer) were added to this and incubated for 15 minutes so that AA could be incorporated into the cellular lipids. The supernatant was removed at the end of this period of time and the cells were washed with high K Krebs-Ringer-phosphate then resuspended in the same buffer. Oxygen from four of the tubes was evacuated by gassing with nitrogen for 1.5 to 2.0 minutes. The tubes were capped and wrapped in parafilm then all of the tubes were incubated in a 37°C water bath for one hour. During this time the cells were kept in suspension by stirring with a tiny stirring bar. At the end of the hour, two of the tubes were opened to the air and two of the tubes were immediately centrifuged at 3,000 rpm for two minutes. The latter tubes were opened, the supernatant was quickly removed and three milliliters of hexane: isopropanol (HIP, 3:2) were added to the cells. The other four tubes were removed from the water bath after an additional 15 minutes. The supernatant was removed and three milliliters of HIP (3:2) were added. The following extraction procedure is a modification
of that described by Hara and Radin (242).

The cells were vigorously extracted on a vortex mixer for one minute then centrifuged at 3,000 rpm. The supernatant was removed and the extraction procedure was repeated two more times. Anhydrous Na₂SO₄ was added to the pooled supernatant fractions to remove water and the solvent were transferred to another tube. The Na₂SO₄ was then washed with one milliliter of HIP (3:2) and this was removed and added to the other supernatant fractions. The solvent was evaporated under nitrogen and the lipids were resuspended in two milliliters of chloroform and stored overnight at -20°C.

A modification of the method described by R. Saunders and Dr. L. Horrocks (personal communication) was used to separate the major lipid classes. Cardiolipin (CL) was inseparable from the other phospholipids by our high performance liquid chromatographic (HPLC) technique so it was separated before HPLC. The method described below separates CL from the other phospholipids as qualitatively by the TLC method of Fine and Sprecher (243). Neutral lipids were eluted from a one gram silicic acid column with 100 milliliters of chloroform. Cardiolipin was eluted with 70 milliliters of chloroform: methanol (94:6) and phospholipids were eluted with 100 milliliters of methanol. The fractions (collected in evaporation flasks) were dried to one or two milliliters with a Buchi Rotovapor HB-140 evaporator then dried completely under nitrogen. The lipids were resuspended in HIP (3:2) and transferred to a ten micrometer fritted glass filter. The evaporation flask was rinsed twice and these rinses were added to the filter. The filtrate was collected in a small vial, dried under nitrogen and resuspended in a
known volume of HIP (3:2).

Fifty microliters of the CL or phospholipid sample were injected onto a Zorbax SI HPLC column (5μm, Dupont, Glasgow, DE). A Beckman-Altek HPLC system was used to mix and pump the solvents through the column and the absorbance at 206 nanometers was read on a Hitachi spectrophotometer Model 100-10. Solvent A was HIP (3:2) and solvent B consisted of 5.5 percent water in solvent A. The HPLC program, modified from that of L. Dugan and Dr. L. Horrocks (personal communication), is shown in Table 4.

### TABLE 4

High Performance Liquid Chromatographic Program for the Separation of Phospholipid

<table>
<thead>
<tr>
<th>time (min)</th>
<th>variable</th>
<th>value</th>
<th>duration of change (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>flow rate</td>
<td>1.5ml/min.</td>
<td>---</td>
</tr>
<tr>
<td>0</td>
<td>% solvent B</td>
<td>50</td>
<td>---</td>
</tr>
<tr>
<td>10.0</td>
<td>% solvent B</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>21.0</td>
<td>% solvent B</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>30.0</td>
<td>end</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

At 30 minutes another sample can be added. Characteristic HPLC tracings for the CL fraction and for the phospholipid fraction are shown in Figures 12 and 13. Solvent elutions from the column that corresponded to peaks were recovered in acid-washed test tubes, dried under nitrogen and resuspended in 0.5 milliliters HIP (3:2). An aliquot of this was transferred to a scintillation vial, four milliliters of scintillation fluid were added and the vial was counted for radioactivity with a Beckman LS8100 scintillation counter. Counts per minute were converted to picomoles of arachidonic acid. The remaining portion of the sample fraction was analyzed for phosphorus
Figure 12. Characteristic HPLC tracing of phospholipids from cardiomyocytes, PC, phosphatidyl choline; PE, phosphatidyl ethanolamine; PI, phosphatidyl inositol; PS, phosphatidyl serine; SM, sphingomyelin; LPC, lysophosphatidyl choline; LPE, lysophosphatidyl ethanolamine.
Figure 13. Characteristic HPLC tracing of cardiolipin fraction of cardiomyocyte lipids. CL, cardiolipin; PC, phosphatidyl choline; PE, phosphatidyl ethanolamine.
(244) after evaporation of the solvent.

An aliquot of the neutral lipid fraction was spotted on a TLC plate and the plate was run in a solvent system containing petroleum ether (bp, 30-60°C): diethyl ether: acetic acid (40:10:1) (245). After drying, radioactivity was located by scanning with a Packard Radio-chromatograph Scanner Model 7220/21 (Figure 14) and the bands corresponding to peak and non-peak areas were scraped into scintillation vials. Scintillation fluid was added and the vials were counted. Counts per minute were again converted to picomoles of arachidonic acid.

The recovery of radioactivity when the supernatant was considered was 68.58±2.72 percent for the cells incubated in air for one hour and 15 minutes, 76.50±1.86 percent for the anoxic cells and 82.08±1.31 percent for the anoxic, reoxygenated cells. These differences may be due to oxidation of arachidonic acid by the cells while they are exposed to oxygen (240) but Chien, et al. (207) have stated that arachidonic acid is a poor substrate for beta oxidation. When only the lipid fractions were taken into account 40.36±2.95 percent recovery of radioactivity was found in the control cells, 48.63±3.12 percent was found in the anoxic cells and 50.23±3.48 percent was found in the anoxic, reoxygenated cells. Results are given as the non-corrected values but correcting for recovery made no significant differences.

Malondialdehyde (MDA) Synthesis by Myocytes

Lipid peroxidation has been associated with ischemia and subsequent reperfusion in intact hearts (33, 209) so it was of interest to see if lipid peroxidation would occur in myocytes under these
Figure 14. Characteristic radiographic TLC scan of neutral lipids from cardiomycyes. FA, fatty acid; TG, triacylglycerol.
conditions. Myocytes were washed as described above. At the end of the incubation period three milliliters of ice cold, one percent phosphoric acid was added to the cell suspension and the mixture was placed in ice. The thiobarbituric acid assay described by Uchiyama and Mihara (246) was used to determine MDA concentration. To determine whether the capped polypropylene tubes could keep the cells anoxic for one hour, myocytes were put in 25 milliliter siliconized glass Erlenmeyer flasks, closed to air with thick rubber stoppers and wrapped in parafilm. Malondialdehyde production was the same for the two procedures but the glass flasks were used for all subsequent anoxia, reoxygenation experiments. For the anoxic experiments, acid was injected through the rubber stopper so that the reactions were stopped before the cells were exposed to oxygen. The introduction of 95 percent oxygen and five percent carbon dioxide during the control incubation and upon reoxygenation seemed to increase MDA production slightly so all prostaglandin reoxygenation experiments were conducted with the high oxygen concentration.

Ferrous-ADP with or without antimycin A was also added to the myocytes in some experiments as a comparison to the anoxia-reoxygenation-induced lipid peroxidation. Ferrous-ADP is a potent inducer of lipid peroxidation in some systems and Antimycin A is a respiratory chain inhibitor so it may mimic the effects of anoxia-reoxygenation on lipid peroxidation.

Prostaglandin Synthesis from Myocytes

There is a controversy as to whether cardiomyocytes produce prostaglandins (196, 197, 199, 200, 201, 240, 247) so prostaglandin
synthesis from heart cells was evaluated and compared to that of other systems. Rat hearts were perfused by the Langendorff procedure as described above (221). The perfusate of low K Krebs-Ringer-phosphate (containing 0.1% BSA and 0.2% glucose and aerated with 95% oxygen and 5% carbon dioxide) was recirculated for 15 minutes. An aliquot of this was then procured for prostaglandin analysis. Rat thoracic aortas were removed, stripped of fat and adventitia while held on a block of frozen Tris-saline (0.01M Trizma base, 0.9% NaCl, pH 7.4). The aortas were cut into pieces of approximately ten milligrams and were incubated at 37°C for one hour in Krebs-Ringer-phosphate. In two experiments the aortas were incubated in the presence or absence of myocytes. After a one hour incubation the supernatant was removed and analyzed for prostaglandins.

A heart mince was obtained by removing excess fat and blood vessels from an intact heart then mincing with scissors in Krebs-Ringer-phosphate until a fine homogenate was formed. The mince was incubated at 37°C for one hour and the reactions were terminated by the addition of two milliliters of isopropanol. The prostaglandins were extracted by mixing on a vortex for one minute. An aliquot of the supernatant was removed and the isopropanol was evaporated under nitrogen. The remaining buffer was diluted with the radioimmunoassay buffer. Some myocytes were also extracted with isopropanol and treated as were the heart minces. Other myocyte suspensions were acidified to pH 3.0 with 2N formic acid then extracted three times with three milliliters of ethyl acetate (197). The extracts were pooled and anhydrous Na2SO4 was added. The supernatant was removed and the
Na$_2$SO$_4$ was washed with one milliliter of ethyl acetate. This was removed and added to the pooled extracts. The combined extracts were dried under nitrogen and the prostaglandins were resuspended in the radioimmunoassay buffer. This extraction procedure recovered approximately 80 percent of 6-keto PGF$_1$ alpha, PGF$_2$ alpha and TxB$_2$ and 90 percent of PGE$_2$. The reported values were not corrected for extraction efficiency.

Unlabelled arachidonic acid, prepared in the same manner as was the radioactive arachidonic acid, was added to the heart minces and to some of the aortas and myocytes (those extracted with isopropanol). In the experiments where arachidonate was added to the cells, the buffer contained 5mM glucose and 5mM glutamate but no BSA. In the experiments where exogenous arachidonate was not present, the buffer did contain BSA. Bradykinin (in 70% ethanol) or reduced glutathione (in water) plus hematin (in 10% NaOH) were added to cells in some experiments. Indomethacin was dissolved in ethanol as a stock solution and an aliquot of this was diluted in the incubation buffer and then added to cells or tissue in some experiments.

Myocytes in Krebs-Ringer-phosphate containing BSA but no glucose, were exposed to anoxia and reoxygenation as described before. In these experiments the cells were aerated with 95 percent oxygen and five percent carbon dioxide during the control incubations and during reoxygenation. Prostaglandin concentrations were analyzed with antibodies supplied by Dr. R. Fertel, as described previously.
Results

As was mentioned previously, the myocytes were initially 94.60±0.67 percent viable and 87.04±1.11 percent rod-shaped after suspension in the low K Krebs-Ringer-phosphate buffer containing 0.5 percent BSA. After washing the cells with high K Krebs-Ringer-phosphate without BSA, the viability decreased to approximately 70 percent and the portion of the cells that were rod-shaped declined to 58 percent. Cells suspended in buffer with BSA but without glucose show a decrease in viability to approximately 70 percent after 45 minutes of anoxia and to 55 percent after 15 minutes of reoxygenation. The values for rod-shaped cells are 60 percent and 20 percent, respectively (32). The exclusion of BSA seemed to have very little affect on MDA production or prostaglandin synthesis.

The phospholipid composition of these cardiomyocytes is shown in Table 5.

TABLE 5
The Phospholipid Composition of Isolated Rat Cardiomyocytes

<table>
<thead>
<tr>
<th>Percent of Total Phospholipid</th>
<th>PE*</th>
<th>PC</th>
<th>CL</th>
<th>SM</th>
<th>LPC</th>
<th>Total PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.93±2.56</td>
<td>48.10±3.63</td>
<td>6.58±0.43</td>
<td>4.41±1.67</td>
<td>4.27±1.62</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nanomoles PL/mg Protein</th>
<th>PE</th>
<th>PC</th>
<th>CL</th>
<th>SM</th>
<th>LPC</th>
<th>Total PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.81±0.95</td>
<td>21.90±2.28</td>
<td>3.00±0.28</td>
<td>2.19±0.93</td>
<td>2.29±0.83</td>
<td>45.93±3.97</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as the mean ± SEM. *PE, phosphatidyl ethanolamine; PC, phosphatidyl choline; CL, cardiolipin; SM, sphingomyelin; LPC, lysophosphatidyl choline; PL, phospholipid.
The other phospholipids, phosphatidyl inositol (PI), phosphatidyl serine (PS) and lysophosphatidyl ethanolamine (LPE) could not be routinely separated so their values are not reported.

Forty to fifty percent of the added radiolabelled arachidonate was incorporated into the cellular lipids within 15 minutes. Most of this was found in the triglyceride fraction while the major portion of the arachidonate incorporated in the phospholipids fraction was esterfied to phosphatidyl choline (Table 6).

### TABLE 6

Arachidonic Acid Incorporation Into Myocyte Lipids

<table>
<thead>
<tr>
<th></th>
<th>PE*</th>
<th>PC</th>
<th>CL</th>
<th>SM</th>
<th>LPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent</td>
<td>2.37±0.37</td>
<td>12.44±3.08</td>
<td>0.44±0.08</td>
<td>0.19±0.08</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>FA**</td>
<td>14.38±0.92</td>
<td>61.90±3.21</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percent of Arachidonate Incorporated Into Phospholipids

<table>
<thead>
<tr>
<th></th>
<th>PE</th>
<th>PC</th>
<th>CL</th>
<th>SM</th>
<th>LPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.84±1.47</td>
<td>70.17±2.56</td>
<td>2.62±0.32</td>
<td>0.99±0.36</td>
<td>0.12±0.04</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as the mean ± SEM. *Abbreviations are the same as those used in Table 5. **FA, fatty acid; TG, triacylglycerol.

Malondialdehyde production is commonly used as an indication of lipid peroxidation (246) and prostaglandin synthesis (143, 249, 250). Ferrous iron (Fe2+) bound to a chelator such as ADP or ethylene diamine tetraacetic acid (EDTA) is a potent inducer of lipid peroxidation (248). To determine whether the myocyte lipids undergo peroxidation, cells were incubated with ferrous-ADP in the presence or
absence of antimycin A, an electron transport inhibitor. As can be seen in Figure 15, ferrous-ADP greatly enhanced lipid peroxidation while antimycin A actually seemed to decrease the iron-induced peroxidation.

Prostaglandin synthesis in the whole heart has been shown repeatedly (196, 231, 251, 252) but prostaglandin generation in isolated myocytes is equivalent (197, 200, 201, 247). The results displayed in Figures 16 and 17 show that, when compared to other systems, myocytes produce very little prostaglandin. When arachidonic acid was added to the incubation mixtures, prostaglandin synthesis increased tremendously in the aorta but only slightly in the heart cells. Furthermore, when indomethacin, an inhibitor of cyclooxygenase, was added, prostaglandin synthesis in the aorta was largely inhibited while that in the myocytes was virtually unaffected. Heart minces, containing coronary vasculature as well as myocytes, showed only a slightly higher synthesis of prostaglandins than did the isolated myocytes. Since the coronary vasculature should produce substantial quantities of prostaglandins (231, 251, 252, 253), the possibility that the myocytes may inhibit prostaglandin synthesis was investigated. The results of this experiment (shown in Figure 18) demonstrate that the myocytes did, indeed, inhibit prostaglandin synthesis by aorta strips.

To further demonstrate that prostaglandin synthesis by the myocytes was insignificant, cells were incubated for 0, 20 and 60 minutes with or without indomethacin. As can be seen in Figure 19, prostaglandin concentration did not increase with time and was not affected by indomethacin. These results suggest that the
Figure 15. The effects of ferrous-ADP and antimycin A on MDA production from cardiomyocytes. Cells were incubated for 15 minutes with or without the agents. Values represent the mean ± SEM of two experiments. Control, 0.4mM FeSO₄ + 0.1mM ADP, 20 mg antimycin A, 0.4mM FeSO₄ + 0.1mM ADP + 20 mg antimycin A.
Figure 16. Comparison of prostaglandin synthesis by the intact heart, isolated aorta and cardiomyocytes. Values are represented as the mean ± SEM from two experiments. 6-keto PGF₁α, PGF₂α, PGF₂β, TxB₂.
Figure 17. Prostaglandin synthesis from isolated aorta, heart mince and cardiomyocytes incubated for one hour with 10 nanomoles of arachidonic acid. Values are the mean ± SEM of two experiments for 6-keto PGF$_1\alpha$ and PGE$_2$, but of only one experiment for PGF$_2\alpha$ and PGF$_2\beta$. Indomethacin (1 ng/ml) was added to some cells.
Figure 18. The effect of myocytes on prostaglandin synthesis from aorta strips. Aorta strips were incubated for one hour with or without two milligrams of myocytes. Values represent the mean ± SEM of two experiments. □ 6-keto PGF$_1$α, ▪ PGE$_2$

= PGF$_2$, □ TxB$_2$. 
Prostaglandin synthesis in cardiomyocytes with time. Values represent the mean ± SEM for two experiments. 6-keto PGF₁α, PGE₂, PGF₂α, TxB₂. Indomethacin (2 ng/ml) was added to some cells.
prostaglandins extracted from the myocytes were already present upon isolation and may have been bound to some intracellular protein.

To determine whether these heart cells could produce prostaglandins upon stimulation with agents known to induce prostaglandin synthesis in other systems, bradykinin (254, 255) or reduced glutathione plus hematin (250) were added to the incubation buffer. The results shown in Figure 20 demonstrate that these agents did not alter prostaglandin generation.

The effects of hypoxia and reoxygenation on the lipid composition of the heart cells are shown in Tables 7 and 8. The concentration of the major phospholipids and the specific activity of arachidonate were not appreciably altered by these perturbations. However, there was an increase in the specific activity of arachidonate in the lysophosphatidyl choline (LPC) fraction during hypoxia but, this returned to control levels during reoxygenation. The increased specific activity of the LPC fraction seems to have been the result of a slight (but nonsignificant) decrease in LPC concentration during hypoxia. This was also returned to normal after reoxygenation. The most striking change seen with hypoxia was an increase in the free fatty acid level. This increased further, but not significantly, during reoxygenation. Malondialdehyde production and prostaglandin (Figures 21 and 22) syntheses were unaltered by anoxia-reoxygenation so, even though arachidonate was released during hypoxia, the present results indicate that this fatty acid was not peroxidized or converted to prostaglandins.
Prostaglandins (p mol/mg protein)

Figure 20. The effect of bradykinin and reduced glutathione plus hematin on prostaglandin synthesis in cardiomyocytes. Cells were incubated for one hour with either bradykinin (3.75 mg/ml) or hematin (0.1mM) plus reduced glutathione (GSH) (0.1mM). Values represent the mean ± SEM of two experiments. 6-keto PGF1α, PGE2, PGF2α, TxB2.
### TABLE 7

Effects of Anoxia and Reoxygenation on the Phospholipid Composition of Myocytes

<table>
<thead>
<tr>
<th></th>
<th>PE*</th>
<th>PC</th>
<th>OL</th>
<th>SM</th>
<th>LPG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.81±0.95</td>
<td>21.90±2.28</td>
<td>3.00±0.28</td>
<td>2.19±0.93</td>
<td>2.13±0.93</td>
</tr>
<tr>
<td>Anoxic</td>
<td>14.28±1.51</td>
<td>20.52±2.40</td>
<td>3.10±0.42</td>
<td>1.92±0.79</td>
<td>1.00±0.50</td>
</tr>
<tr>
<td>Reoxygenated</td>
<td>12.16±1.00</td>
<td>26.27±5.04</td>
<td>3.43±0.68</td>
<td>3.31±0.38</td>
<td>2.70±0.08</td>
</tr>
</tbody>
</table>

Values are given as the mean ± SEM. Control cells were incubated in the presence of air for 1 hour and 15 minutes, anoxic cells were gassed with nitrogen and incubated in the absence of oxygen for 1 hour, reoxygenated cells were anoxic for 1 hour the exposed to air for 15 minutes. *Abbreviations are the same as those used in Tables 5 and 6.
### TABLE 8

**Release of Arachidonic Acid After Anoxia-Reoxygenation**

<table>
<thead>
<tr>
<th></th>
<th>PE*</th>
<th>PC</th>
<th>CL</th>
<th>SM</th>
<th>LPC</th>
<th>FA</th>
<th>TG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.41±3.19</td>
<td>116.34±25.96</td>
<td>4.15±0.82</td>
<td>1.79±0.72</td>
<td>0.25±0.11</td>
<td>138.59±10.93</td>
<td>597.09±43.48</td>
</tr>
<tr>
<td>Anoxic</td>
<td>20.64±3.50</td>
<td>101.13±18.74</td>
<td>4.46±0.87</td>
<td>1.48±0.71</td>
<td>0.22±0.11</td>
<td>292.03±29.59++</td>
<td>670.75±34.76</td>
</tr>
<tr>
<td>Reoxygenated</td>
<td>20.33±1.44</td>
<td>100.46±7.49</td>
<td>4.59±0.49</td>
<td>1.56±0.88</td>
<td>0.20±0.07</td>
<td>339.14±37.05++</td>
<td>681.76±9.71</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>PE*</th>
<th>PC</th>
<th>CL</th>
<th>SM</th>
<th>LPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.59±0.18</td>
<td>5.00±0.68</td>
<td>1.37±0.25</td>
<td>0.57±0.23</td>
<td>0.08±0.03</td>
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<tr>
<td>Anoxic</td>
<td>1.41±0.16</td>
<td>4.70±0.48</td>
<td>1.49±0.30</td>
<td>0.70±0.22</td>
<td>0.27±0.05++</td>
</tr>
<tr>
<td>Reoxygenated</td>
<td>1.70±0.15</td>
<td>4.15±0.61</td>
<td>1.54±0.35</td>
<td>0.70±0.44</td>
<td>0.08±0.03</td>
</tr>
</tbody>
</table>

Values are given as the mean ± SEM. Conditions are the same as those described in Table 7. *Abbreviations are the same as those used in Tables 5 and 6. +Significantly different control p < 0.05, ++p < 0.005.
Figure 21. MDA production from cardiomyocytes before and after anoxia. Values represent the mean ± SEM for three experiments. 
- Time 0 control, 
- exposed to air for one hour and 15 minutes, 
- anoxic for 1 hour, 
- anoxic for one hour then reoxygenated for 15 minutes.
Figure 22. The effects of anoxia and reoxygenation on prostaglandin synthesis from cardiomyocytes. Values represent the mean ± SEM of two experiments. Control I, time 0 control; Control II, 1 hour, 15 minutes in air; Anoxic, 1 hour anoxia; Reoxygenated, 1 hour anoxia plus 15 minutes reoxygenation. 6-keto PGF₁α, PGE₂, PGF₂α, TxB₂.
Discussion

Several researchers have investigated the phospholipid composition of the heart (191, 194, 195, 206), heart cells (194) and subcellular components of heart cells (192, 193). Values ranged from 22.7 to 38.1 percent of the total phospholipid as phosphatidyl ethanolamine (PE), 34.0 to 49.8 percent as phosphatidyl choline (PC), 1.8 to 20.0 percent as cardiolipin (CL), 2.2 to 12.2 percent as sphingomyelin (SM) and 1.0 to 3.3 percent as lysophosphatidyl choline (LPC). All of the values obtained in this study fall into these ranges except those of lysophosphatidyl choline which are slightly higher than others have reported. The present results most closely parallel those obtained from rat, whole heart homogenates by Charnock, et al. (195) who have stated that PE, PC and CL comprise approximately 90 percent of the total phospholipids in heart tissue.

Incorporation of radiolabelled arachidonic acid into cellular lipids has also received a great deal of attention (196, 197, 254, 255). The reports of arachidonate uptake by heart lipids vary greatly with values of 0.5 to 33.0 percent of arachidonate incorporated into PE, 24.7 to 68.5 percent in PC, 9.5 to 69.8 percent in neutral lipids (triacylglycerols, TG and cholesteryl esters, CE) and 0.6 to 7.3 percent remaining as free fatty acid (FA) within the cell. These differences are probably due to labelling or incubation procedures or to the different tissue preparations. The results of the present study fall into these ranges with the exception of incorporation into PC, which is a little lower than the previously reported values and FA,
which is higher. Previous data on the incorporation of arachidonate into CL or LPC were not available but one study (196) showed that 2.0 percent of the radioactivity was in sphingomyelin. The large amount of arachidonate found esterified in triacylglycerols is probably due to storage of excess fat (198). After incubating cells with a similar amount of arachidonate, Ahumada, et al. (197) recovered a percentage of radioactivity in triacylglycerols that was similar to that recovered in this study.

The most active phospholipid, in terms of uptake of fatty acid, seemed to be PC since this species incorporated approximately 70 percent of all the arachidonate found in the phospholipid fraction. This was not simply due to the higher concentration of PC and is in agreement with the results obtained by several other researchers (196, 197, 254, 255).

The deacylation of the phospholipids and the subsequent conversion of the radiolabeled arachidonic acid into prostaglandins was not followed in the present investigations but Hsueh, et al. (254) and Isakson, et al. (196) have suggested that, at least with bradykinin stimulation, the arachidonic acid is released, primarily, from phosphatidyl choline. Several researchers have shown that the intact heart can produce prostaglandins (196, 231, 251, 252) and that the major prostaglandin generated is 6-keto PGF₁α (251, 252). Prostacyclin is the predominant prostaglandin produced by the blood vessel walls so prostaglandin synthesis by the intact heart may, simply, be a reflection of that in the coronary vasculature. However, within the last few years, prostaglandin synthesis has also been
demonstrated in isolated cardiomyocyte preparations (197, 200, 201, 247). In all of these experiments, radiolabelled arachidonic acid was added to the cells. Arachidonic acid is a potent inducer of prostaglandin synthesis in many tissues but only very small amounts of prostaglandins were formed in the myocytes even though exogenous arachidonate was present. Conversions of arachidonate to prostaglandins was 0.2 percent and 0.33 percent for cells isolated by Brandt, et al. (247) and Ahumada, et al. (197), respectively. however, the cells used by Bolton, et al. (200) converted approximately 1.8 percent of the added arachidonate to prostaglandins. The greater conversion exhibited in the latter cells may have been due to the much greater quantity of arachidonate used. Furthermore, trypsin was used in the preparation of these cells and this enzyme may have destroyed a protein that regulated prostaglandin synthesis.

Many researchers are of the opinion that all cells produce prostaglandins but in comparison to other cells and tissues, cardiomyocytes convert very little arachidonic acid to these compounds. Panganamala, et al. (257) have demonstrated a 15 percent conversion of arachidonate to 6-keto PGF\(_1\) alpha by rat aorta and Stuart, et al. (146) have shown that human platelets convert as much as 30 percent of exogenous arachidonate to TxB\(_2\). Furthermore, Gerritsen and Printz (253) have found that coronary arteries and veins convert 3.6 and 3.3 percent of radiolabelled arachidonate to prostaglandins, respectively.

In the present study the quantities of prostaglandins present in heart cells were very small compared to those present in the Intact
heart or isolated aorta. Furthermore, the concentrations did not change appreciably upon incubation with arachidonic acid or indomethacin. These results suggest that the myocytes used in the present study do not synthesize prostaglandins but may incorporate small amounts of prostaglandins produced by other cells. Bolton, et al. (200) have shown a time-dependent conversion of radiolabelled arachidonate to prostaglandins which was inhibited by Indomethacin. The discrepancies between these results are difficult to reconcile but may be due to the large amounts of arachidonate used by Bolton, et al. or to contamination by other cells which actively produce prostaglandins.

The inability of arachidonic acid or Indomethacin to alter prostaglandin synthesis in these heart cells suggests that cyclooxygenase may be absent. Limas and Cohn (199) have demonstrated prostaglandin synthase activity in heart cells, however, these cells were contaminated with other cells. Gerritsen and Printz (253) have shown that, while almost no exogenous arachidonate was converted to prostaglandins in isolated myofibrils, nearly all exogenous PGH₂ was converted to PGI₂, PGE₂ and PGD₂. This would indicate that the myofibrils are deficient in cyclooxygenase but do exhibit prostaglandin isomerase and prostacyclin synthase activities.

Bradykinin has been shown to induce prostaglandin synthesis in intact hearts (196, 255), however, the myocytes used by Ahumada, et al. (197) did not respond to this hormone. Hematin, in the presence of reduced glutathione, has also been shown to stimulate prostaglandin
synthesis in some systems (250) but the cells used in the present experiments showed no response to either of these agents.

The inhibition of prostaglandin synthesis by the myocytes is an interesting phenomenon and should be further investigated. Lipid peroxides can inhibit PGI₂ synthesis in aorta (14) but these myocytes generated very little lipid peroxide (measured as MDA) so some other factor must be involved. The cells are probably not oxidizing the prostaglandins either, since there is no decline in myocyte prostaglandin concentration over time.

Ahumada, et al. (197), Bolton, et al. (200) and Vahouny, et al. (201) found that PGE₂ was the major prostaglandin generated by their respective myocytes while Brandt, et al. (247) found 6-keto PGF₁ alpha in the highest concentration. In the present Investigation PGF₂ alpha was the primary prostaglandin isolated from the heart cells but, as mentioned previously, this probably was not produced by the myocytes.

Very little TxB₂ was found in the myocytes used in this study and Ahumada, et al. (197) and Brandt, et al. (247) found no trace of TxB₂ in the cells they used. However, Bolton, et al. (200) and Vahouny, et al. (201) recovered 18 and 10 percent of the total prostaglandins as TxB₂, respectively. The conflicting results of these researchers further suggest that if any prostaglandins are produced in myocytes they have an insignificant role in maintaining vascular integrity or cardiac contractility and rhythm.

During ischemia of the intact heart, prostaglandins are released (41, 42, 43, 216, 254) and may help protect against arrhythmia and
irreversible damage (44, 257, 258). These prostaglandins are probably produced by the blood vessels or the pericardium (202) since the myocytes produce such small quantities of these compounds. In the present study anoxia and reoxygenation had no affect on prostaglandin synthesis in the myocytes. This is not surprising since the cyclooxygenase seems to be deficient in these cells and anoxia tends to activate the phospholipases (207).

Lipid peroxides also accumulate in the intact myocardium after ischemia and reperfusion (33, 209) and may cause extensive damage to the cellular membranes (35, 259). During ischemia, reducing equivalents (i.e. NADH, reduced cytochromes) accumulate. Upon reperfusion, the reducing equivalents can donate electrons directly to molecular oxygen and thus produce oxygen-centered free radicals (33). The free radicals can then induce lipid peroxidation. Antioxidant enzyme activity is reduced during ischemia (31, 259) and this could exacerbate the ischemia-reperfusion damage.

The production of MDA by the heart cells used in the present study was not enhanced by anoxia or reoxygenation. Furthermore, the addition of antimycin A inhibited the MDA formation induced by Fe^{2+}-ADP. Antimycin A is an electron transport chain inhibitor and causes a reduction of all the components proximal to cytochrome b (Figure 23). One to two percent of all electrons passing down the electron transport chain leak off in the ubiquinone-cytochrome b region. During anoxia and subsequent reoxygenation, or in the presence of an inhibitor (such as antimycin A) there is an increased production of free radicals from the chain. Under normal conditions the intramitochondrial antioxidant
Figure 23. Free radical generation from the mitochondrial electron transport chain. Reproduced from Freeman and Crapo (35).
enzymes can degrade the free radicals but after anoxia, when these enzymes have been decreased, the free radicals may accumulate and cause lipid peroxidation (35). The reason for the lack of effect of anoxia-reoxygenation on MDA production is not clear but these hearts may have had enough antioxidant enzyme activity even after one hour of anoxia to protect against free radical damage (259). A longer period of anoxia prior to reoxygenation may be required for MDA production to be enhanced. However, one hour of reoxygenation after the one hour anoxic incubation did not enhance MDA production in these cells. The explanation for the decrease in MDA production caused by antimycin A is more complex and requires further study.

There were no significant changes in the phospholipid composition of the myocytes following anoxia and reoxygenation but there was a tendency for the concentration of LPC to decrease during anoxia and then return to normal upon reoxygenation. The specific activity of LPC increased during anoxia and returned to control values after reoxygenation. These results suggest that lysophospholipases and phospholipase A₁ are activated during anoxia (260). Phospholipase A₁ removes the fatty acid from the one position of the phospholipid leaving a lysophospholipid that co-chromatographs with lysophospholipids missing the fatty acid from the two position. Arachidonate is incorporated in the two position of phospholipids (196) so removal of the one position could lead to an enrichment of arachidonate-containing LPC. On the other hand, selective degradation of lysophospholipids with fatty acids in the one position could also lead to an accumulation of arachidonate-containing LPC with a reduction
of total LPC. The increase in the LPC concentration and the decrease in the specific activity upon reoxygenation are more difficult to explain. Upon reoxygenation, PC may be degraded to LPC by phospholipase A2, thus causing an increase in LPC concentration with a concomitant decrease in specific activity. In this study, however, PC concentration was not decreased. Phosphatidyl ethanolamine can be converted to PC by the action of cholinephosphotransferase (261). If this occurred during reoxygenation it could partially explain how LPC concentration could increase without a net loss of phosphatidyl choline.

While the source remains unknown, there was a substantial elevation of free arachidonate following anoxia and subsequent reoxygenation. Other researchers have demonstrated increases in free fatty acids during ischemia and reperfusion of the intact heart (39, 40, 207, 254) and, as in this study, the exact source of these fatty acids is not known (207). Calcium accumulates in the cell during anoxia (32) and this can activate phospholipases (34) and cause a nonspecific release of fatty acids (254). Since the fatty acids are probably released from all of the phospholipids and neutral lipids, small decreases in the concentration of any of these fractions may not be detectable.

The results of these studies indicate that, although a large amount of arachidonate was released during anoxia, this fatty acid was not peroxidized or converted to prostaglandins. These results are in agreement with those of Paul and Kinsella (255) and Hsueh, et al.
who demonstrated that arachidonic acid could be released without subsequent formation of prostaglandins.

Prostaglandin release during cardiac ischemia may play an important role in the preservation of contractility and the reintroduction of oxygen (through vasodilation) to the ischemic area. Prostaglandins are not stored in cells and do not remain in blood for long periods of time so they must be produced as they are required. The primary sources of prostaglandins during myocardial ischemia are the coronary vasculature and the pericardium. Other tissues may also contribute but prostaglandin synthesis from the myocyte seems to have an insignificant role in this scenario.
Summary and Conclusions

1. The phospholipid composition of the myocyte was approximately 48 percent PC, 31 percent PE, 6.5 percent CL, 4.5 percent SM and 4.3 percent LPC.

2. Radiolabelled arachidonate was incorporated mainly into the neutral lipid fraction (76 percent) of cellular lipids. Triglyceride, itself, esterified over 60 percent of the fatty acid. Phosphatidyl choline incorporated most of the arachidonate recovered from the phospholipid fraction (70 percent).

3. Unstimulated heart cells generate very little MDA but when they are incubated with Fe²⁺-ADP, their MDA production is greatly enhanced. Antimycin A, an inhibitor of the electron transport chain, seems to reduce Fe²⁺-ADP-induced MDA production by the myocytes.

4. Myocytes produce insignificant quantities of prostaglandins when compared to other cardiovascular tissues. The addition of arachidonate or indomethacin does not seem to affect myocyte prostaglandin synthesis so the cells may be deficient in cyclooxygenase. Furthermore, myocytes exhibit a tendency to inhibit prostaglandin formation by aorta.

5. The addition of bradykinin or reduced glutathione plus hematin also had no affect on prostaglandin synthesis by myocytes.

6. Anoxia and reoxygenation had very little affect on the phospholipid composition of the heart cells, however, there was a significant release of fatty acid during anoxia. The source of this free arachidonate is not known but it is clear that this fatty acid was not peroxidized or converted to prostaglandins.

Lipid peroxides can cause extensive damage to cellular membranes while prostaglandins may protect an ischemic myocardium from irreversible injury. The balance of these two compounds may determine whether or not the tissue infarcts. The cells used in the present study did not synthesize either of these products even though their production has been repeatedly demonstrated in the intact heart. The results reported here suggest that the lipid peroxidation and
prostaglandin synthesis occur in areas of the heart other than the myocytes and that the widely held belief that all cells produce prostaglandins should be reevaluated.
CHAPTER FIVE

CONCLUDING REMARKS

Blood platelets release thromboxane A₂ (TxA₂) during aggregation and this compound causes vasoconstriction and further aggregation. Prostacyclin (PGI₂) produced in the arterial wall inhibits platelet aggregation and causes vasodilation. The ratio of these two molecules may play an important role in the regulation of blood flow and hemostasis.

Platelets from aerobically trained rats produced less TxA₂ when stimulated with thrombin or collagen than did those from sedentary rats. Training also showed a tendency to reduce thrombin-induced TxA₂ synthesis in cholesterol-fed rats. However, platelets from trained rabbits produced more TxA₂ than did platelets from sedentary rabbits after stimulation with arachidonic acid but not with other agonists.

Prostacyclin production from trained rat aorta strips was slightly (but not significantly) less than that from aorta strips from sedentary rats, however, the sensitivity of the coronary vasculature to PGI₂ was significantly elevated in the trained rats. Training enhanced PGI₂ synthesis from rabbit aortas. The increased ratio of PGI₂/TxA₂ and the increased responsiveness of the coronary vasculature to PGI₂ seen in the trained animals may provide protection from cardiovascular disease.

Isolated myocytes prepared from adult rat hearts were incubated with radiolabelled arachidonic acid. Most of the arachidonate was incorporated into the neutral lipid fraction of the cellular lipids.
while the arachidionate incorporated into the phospholipid fraction was found, primarily, in phosphatidyl choline.

The heart cells contained very little prostaglandin and stimulation by arachidionate, bradykinin or hematln did not increase the prostaglandin concentration. Furthermore, inhibition of cyclooxygenase with Indomethacin had no affect on the content of prostaglandins. These results suggest that the heart cells used in this study do not produce prostaglandins.

After hypoxia and subsequent reoxygenation there were no changes in the major phospholipids of the myocytes, however, there was an elevation of fatty acid. Prostaglandin and malondialdehyde concentrations were not altered during these perturbations suggesting that even though fatty acids may be released they are not peroxidized or converted to prostaglandins. Therefore, the prostaglandin synthesis and lipid peroxidation seen during ischemia of the intact heart must originate in cells other than the myocytes.


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