MYOCARDIAL ADAPTATION TO HYPOXIA

DURING NUTRITIONAL ANEMIA

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# MYOCARDIAL ADAPTATION TO HYPOXIA

DURING NUTRITIONAL ANEMIA

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SUMMARY

The effects of nutritional anemia stress induced by a diet of milk were monitored as a function of hemoglobin and body weight in rats, and compared with myocardial parameters. Myocardial glycogen, ATP, and lactate dehydrogenase isozymes were changed as a result of the anemic stress.

The animals fed the milk diet exhibited a large decrease in blood hemoglobin concentration relative to the control animals. A severe retardation of body weight gain was observed in the experimental animals. The ratio of heart weight to total body weight was significantly higher in the anemic animals.

An electrophoretic analysis of myocardial tissue homogenates indicated a significantly lower H/M sub-unit ratio for lactate dehydrogenase isozymes in the anemic animals. The anemic animals also had higher myocardial glycogen stores and ATP stores than did the control animals.

The observed data may be indicative of an increased rate of anaerobic glycolysis in the anemic animals and thus may have involved an increased rate of anaerobic ATP production. A tissue level adaptation to hypoxia may have occurred since the ATP levels of the control animals were not substantially different from the experimental animals. The possibility that the tissue level changes may be related to mitotic activity, cellular differentiation, or cardiac hypertrophy is not excluded.

CHAPTER I

INTRODUCTION

General Adaptation

Adaptation in the broadest sense, refers to differences within individual

organisms as they respond to variations in the environment. Adaptation holds

different meanings for those in their respective fields of biology. For the popu-

lation biologist adaptation may mean genetically determined morphological char-

acteristics that enable an organism to maintain a certain degree of fitness with

respect to the environment. For the physiologist adaptation includes the environ-

mentally determined factors that enable the plant or animal to survive in a par-

ticular environment. Adaptation allows the continuation of vital physiological

activities when environmental factors begin to change. However, such a broad

definition does not apply to sensory adaptation unless this activity is included in

the overall reaction of the organism to the environmental stimuli.

The broad definition of adaptation requires other definitions. (In order

to work within such a broad framework certain key terms must be clearly defined.)

Response refers to a direct reaction to a stimulus from the environment, whether

the response involves adaptation or not, or whether it is physiological or morpho-

logical in nature. Genetically determined adaptive variations can be distinguished

from those such as acclimation and acclimatization which are environmentally

induced. Compensatory alternations that occur in a laboratory animal when one

stressful parameter is altered come under the category of acclimation. When multiple parameters vary under natural conditions, it is referred to as acclimatization. Parameters of acclimatization may include, but are not limited to, climatic, seasonal, and geographic differences. Physiological adaptation may occur in one or several functional systems of an organism. Therefore, the study of adaptation is important in all areas of physiology (Prosser, 1964).

This importance is evidenced by the fact that it has been mentioned in the literature for hundreds of years. Adaptation was called "assuefaction" in the seventeenth century (Boyle, 1670). In the nineteenth century much of the early literature on the effects of high altitude was reviewed (Bert, 1878). In this same century the differences between genetic and physiological adaptations were recognized. This difference has more recently been better defined as a result of studies directed toward microbiological and biochemical adaptations (Adolph, 1956).

Adaptation to Hypoxia

Probably one of the most avidly explored areas of adaptation has been that of adaptation to hypoxia. Hypoxia is an abnormally low oxygen concentration. Hypoxia can be caused by a number of conditions including pathologies in the respiratory or cardiovascular systems, extreme exercise, and a low partial pressure of oxygen in inhaled air (Barbashova, 1964). This adaptation can occur at either the systemic or the tissue level, and has been said to involve a "struggle for oxygen" and an increased tolerance to tissue hypoxia.

At the systemic level the struggle for oxygen includes adjustments in respiration (such as hyperaeration), changes in the acid-base balance, and a group

of interrelated adaptations in the cardiovascular system that help to maintain adequate tissue oxygenation (Barbashova, 1960). An increase in the oxygen-carrying pigments of the blood is one of the most readily apparent aspects of adaptations of this nature. At 15,000 feet altitude the hemoglobin in the blood is only partially saturated with oxygen. However, resting oxygen requirements at high altitudes are met by an increased hematocrit (volume of red blood cells/total blood volume) with its attendant increase in hemoglobin concentration. The increased level of red blood cells is called polycythemia.

Polycythemia at high altitudes is due to an erythropoietic stimulus to bone marrow activity. The resulting increase in the ratio of red cells to plasma tends to open unused capillaries, and therefore improves oxygen delivery to the tissues. A preferential routing of blood to the lungs has been noted, as well as a change in muscle tissue vascularization. However, the increased blood viscosity may counteract any such advantages by placing an extra burden on the heart (Horvath and Howell, 1964). Conceivably the heart might eventually increase its size in order to compensate for this additional burden.

Cardiac hypertrophy is a common occurrence in all species, and is found to occur in animals living at both low and high altitudes. The fact that other organs, for example, the adrenal glands, show signs of hypertrophy when placed under a hypoxic stress, indicates that hypertrophy may be a more general response to stress rather than a specific reaction to hypoxia. The hypertrophy occurs primarily in the right ventricle and thus may be a response to pulmonary congestion rather than a general reaction to a hypoxic stress (Horvath and Howell, 1964).

Nevertheless, cardiac hypertrophy probably does account for some degree of adaptation at the systemic level.

Studies of adaptation at the tissue level are relatively recent and require additional study (Barbashova, 1964). In tissues the struggle for oxygen encompasses an increase in concentrations of myoglobin, which is a respiratory pigment with greater affinity for oxygen than hemoglobin, a change in enzyme activity, and an increase in the capacity to utilize oxygen even when the partial pressure of oxygen is low.

The struggle for oxygen at the tissue level, and at the systemic level, comprises only one of two mechanisms proposed for acclimatization to hypoxia. The other is called "adaptation to hypoxia" and is believed to occur exclusively at the tissue level. Adaptation to hypoxia includes a decrease in oxygen consumption, an intensification of anaerobic glycolysis, and an increase in the "resistance" of tissues. Resistance refers to the general or non-specific resistance of organisms not only to an oxygen deficiency, but also to other undesirable factors in their external environment (Barbashova, 1960).

Adaptive changes to hypoxia at the tissue level have been examined via two separate approaches. One includes the study of changes in enzyme activities and of cellular respiration. The general consensus of opinion is that such changes will allow the maintenance of a sufficiently high level of oxidative metabolism regardless of the low oxygen levels in the capillary blood. Also, studies have been made of anaerobic processes that allow metabolism to continue when there is an oxygen deficiency. Adaptation at the tissue level must be considered

as one method that organisms have for coping with a hypoxic stress (Barbashova, 1964).

Thus, the compensatory actions of the body systems, such as the circulatory and blood-forming systems, in response to hypoxia do not necessarily indicate true adaptation. Adaptation in an organism is more realistically achieved through biochemical and physical changes at the tissue level (Barbashova, 1960). In this view the more readily apparent mechanisms do not appear to be as representative of adaptation as those at the tissue level.

Tissue level adaptation of the myocardium to hypoxia has been the subject of many studies involving biochemical changes that enable the heart muscle to function better under hypoxic conditions (Anderson and Bullard, 1971; Ballo and Messer, 1968; Blatt et al., 1966; Cryer and Bartley, 1973; Mager et al., 1968; Miller and Hale, 1968; Olson et al., 1972; Penny, 1974; Poupa, 1972). Some of the biochemical parameters considered by these authors were concentration levels of adenine nucleotides, creatine phosphate, cytochrome oxidase, glycogen, lactate dehydrogenase, lactic acid, and succinic dehydrogenase under conditions of hypoxia.

Of all these substances related to oxidative metabolic pathways, LDH (lactate dehydrogenase) is probably the most frequently mentioned because LDH is an integral part of the glycolytic pathway. LDH is a tetrameric molecule made up of four subunit polypeptides. These subunits have been termed heart (H) and muscle (M) because they are readily observed in these tissues. The MMMM isozyme is composed of four M subunits while the HHHH isozyme is composed of

four H subunits. Other isozymes that exist are MMMH, MMHH, and MHHH. The rates of synthesis of the two subunits have been found to vary with oxygen tension. Low oxygen tension favors the synthesis of the M subunit of LDH which is the most suited one for anaerobic metabolism. The H subunit synthesis predominates when tissue oxygen tension is higher and is most suited for aerobic metabolism. Thus, changes in the H to M ratio are indicative of differential protein synthesis (Dawson et al., 1964).

Anemia as a Hypoxic Stress

A large portion of the studies dealing with myocardial hypoxia at the tissue level have been related to hypobaric stresses (those associated with a low partial pressure of oxygen in the inhaled air). The use of an anemic stress in rats and mice for the study of iron in nutrition was quite prevalent in the first half of this century (Scott, 1923 a and b; Mitchell, 1926; Waddell et al., 1928; Elvehjem and Kemmerer, 1931; Stucky, 1932; Mitchell, 1932; Fitz-Hugh et al., 1933; Smith and Medlicott, 1944). However, comparatively few studies have been concerned with the myocardial hypoxia that may be attendant with sideropenic (iron-deficient) anemia. Poupa (1964) found both absolute and relative cardiac hypertrophy in the rats exposed to an anemic stress. He also found that the right ventricles of these animals showed an increased resistance to anoxia when using isolated, perfused preparations. Nevertheless, his study was not concerned with biochemical adaptation to hypoxia (in the strictest sense) at the tissue level.

There have been two studies relating the effects of sideropenic anemia to changes in the isozyme patterns of lactate dehydrogenase (Penny et al., 1974;

Jelińkova' et al., 1968). These authors indicated a shift in the isozyme pattern
of LDH to the more anaerobic M subunit. Penny et al. also described elevations
in pyruvate kinase (the enzyme just prior to lactate dehydrogenase in the glycolytic
pathway) activity in the anemic animals. However, these changes in enzymatic
activity may have been related to mitotic activity and/or cellular differentiation,
or possibly to changes in tissue growth patterns during cardiac hypertrophy
(Penny, 1974). Nevertheless, both studies pointed out a possible increase in the
anaerobic glycolytic capacity of the anemically stressed rats, and thus indicated
the possibility of a tissue level adaptation to hypoxia.

It is the purpose of this study to look at myocardial energy metabolism in
anemically stressed rats. The biochemical parameters to be measured include
LDH isozymes, glycogen stores, and levels of adenosine-5'-triphosphate. These
parameters were chosen since they could potentially be used to reflect tissue level
changes due to the presumed hypoxic stress. Also, blood hemoglobin levels,
body weight, and the ratio of heart weight to total body weight will be measured.
The experimental procedures to be employed do not eliminate the possibility that
any tissue level changes may be related to mitotic activity, cellular differentiation,
or cardiac hypertropy.

CHAPTER II

METHODS AND MATERIALS

Induction of Anemia

Sprague-Dawley Holtzman rats (both males and females) from the stock colony at the Georgia Institute of Technology, School of Biology, were used. The animals were weaned at approximately one month after birth and transferred to stainless steel cages with screen bottoms (to prevent coprophagy). They were separated by sex in order to prevent mating.

Anemia was induced in 54 animals by weaning to a milk diet (Elvehjem and Kemmerer, 1931). The experimental animals were fed raw cow's milk ad libitum for 107 days (R. L. Mathis Dairy, Decatur, Georgia). Forty-five control animals were fed the normal laboratory diet of Purina rat chow and water ad libitum.

Hemoglobin Determination

The hemoglobin concentration in the blood was determined periodically during the course of the study. Blood samples were always obtained from superficial vessels of the lower leg in order to avoid differences in hematocrit seen between superficial and deep vessels.

Initially the hemoglobin was determined using the Haden-Hausser hemoglobinometer (Haden, 1935) through day 48 of exposure to the iron-deficient diet.

All subsequent determinations were made with a Bausch and Lomb Spectronic 20

using a cyanmethemoglobin standard (Cartwright, 1966). The spectrophotometric

method was not used initially, because the method was not available at that time.

Preparation of Tissue

The animals were anesthetized with diethyl ether in a large dessicator

jar. A thoracotomy was performed on each one, and the beating heart was quickly

excised. The heart was then quick-frozen between two aluminum blocks that had

been attached to a pair of metal tongs and submerged in liquid nitrogen. The

frozen hearts were broken into small pieces and stored in individual vials in a

liquid nitrogen refrigerator (Linde type LR-10). The storage procedure mini-

mized degradation of lactate dehydrogenase isozymes (Zonday, 1963).

Quantitation of Lactate Dehydrogenase Isozymes

Tissue homogenates were prepared by grinding a small sample of each

heart in 10 volumes (volume/volume) of pH 8.2 Michaelis buffer (Blatt et al., 1966)

for three minutes in a ground glass homogenizing tube. A motor-driven Teflon

pestle was used and the process was carried out in an ice bath to minimize

denaturation. The homogenate was then centrifuged for 30 minutes at 4,500g

at 5°Celcius. The supernatant liquid was immediately subjected to electrophoresis.

The electrophoretic separation of the lactate dehydrogenase isozymes was

performed at pH 8.8 (barbital buffer, $\mu = 0.05$) on one by six inch cellulose acetate

(Sepraphore III, Gelman Instrument Company, Ann Arbor, Michigan) at a potential

of 300 volts (1 to 2 ma/strip) for 35 to 40 minutes. Six to nine microliter samples

were applied in a thin line onto test strips that had previously been allowed to

soak overnight in the electrophoresis buffer (Gelman manual, 1970; Blatt et al.,

1965).

The cellulose acetate strips were developed by superimposing upon them a

substrate strip of the same variety that had been impregnated with substrate and

staining reagents prepared as follows:

(1) Substrate: 4.0 ml of distilled water plus 1.0 ml of 60% sodium
        lactate (Sigma Chemical Co.)

(2) Nitroblue tetrazolium: 1 mg/ml made up in distilled water
        (Sigma Chemical Co.)

(3) Phenazine methosulfate: 1 mg/ml made up in distilled water
        (Sigma Chemical Co.)

(4) Nicotinamide adenine dinucleotide, oxidized form: (Sigma
        Chemical Co.)

The final reaction mixture was prepared by combining one ml of substrate, three

ml of nitroblue tetrazolium, 0.3 ml of phenazine methosulfate, one ml of distilled

water, and ten mg of nicotinamide adenine dinucleotide added to the final solution

dry (Gelman manual, 1965; Blatt et al., 1965). The final concentrations of sodium

lactate, nitroblue tetrazolium, phenazine methosulfate, and nicotinamide adenine

dinucleotide in the reaction mixture were 1.9×10^{-1}, 6.8×10^{-4}, 1.7×10^{-4}, and

2.8×10^{-3} molar respectively.

The substrate strips were saturated by floating them in the substrate mixture

on a glass plate. The strips were then superimposed on the test strips and sand-

wiched between two glass slides. The ends of the strips were tucked under the

sandwiches, wrapped in Saran wrap, and incubated in the dark at 37$^{\text{o}}$ Celcius for

30 to 45 minutes.

After incubation the strips were separated and their backgrounds cleared

in 5% acetic acid. The strips were then blotted between absorbent sheets (Gelman

Instrument Company). The isozyme bands were quantitated by cutting out the

respective bands and dissolving each in three ml of a 50% glacial acetic acid-50%

acetone mixture. The absorbance values were obtained in round cuvettes on a

Spectronic 20 (Bausch and Lomb Company) at 570 nm (Gelman Manual, 1970).

Translation of the electrophoretic bands into the respective subunit per-

centages was made according to a formula based on the subunit theory (Thorling

and Jensen, 1966). The level of significance between control and experimental

H/M subunit ratios was calculated by using the t-test (Croxton, 1959; Goldstein,

1967).

Glycogen Assay

The anthrone reagent was used to determine the glycogen levels in the

myocardial tissue. The glycogen was extracted by boiling with 30% potassium

hydroxide solution. It was then precipitated from the extract with 95% ethanol.

After centrifugation the precipitates were dissolved in distilled water and allowed

to react with the anthrone reagent (Carroll et al., 1956).

Frozen samples weighing approximately 10 to 60 mg were each dissolved

in one ml of 30% potassium hydroxide and placed in a boiling water bath for 45 min.

The samples were then removed and 1.2 ml of 95% ethanol were added to each with

vigorous mixing. The samples were allowed to stand overnight at room temperature.

The following day the tubes were centrifuged at 1,600g for 15 minutes. The supernatant was discarded and two ml of distilled water were added to each tube.

A reagent blank was prepared containing two ml of distilled water. At the same time two ml of a glucose standard solution was prepared that contained 0.1 mg of glucose. These two tubes, along with all of the sample tubes, were placed in a cold water bath and 10 ml of the anthrone reagent (Carroll et al., 1956) were added to each tube. After the tubes had reached the temperature of the water, they were immersed in a boiling water bath for 15 min. They were then allowed to cool to room temperature in another cold water bath.

At least three ml of each tube was transferred to a cuvette (100 x 13 mm) and read at 620 nm. The reagent blank was used to zero the Spectronic 20. The glycogen levels were calculated by a modified formula as follows:

$$\frac{\text{mg glycogen}}{\text{gm of tissue (wet weight)}} = \frac{DU}{DS} \times \frac{1}{\text{gm of tissue (wet weight)}} \times 0.09$$

DU = Absorbance of unknown

DS = Absorbance of standard

0.09 = (0.1 mg of glucose in 2 ml of standard) x (0.9 = factor for converting glucose value to glycogen value)

The level of significance between control and experimental means was determined using the t-test (Croxton, 1959; Goldstein, 1967).

Adenosine Triphosphate Assay

The method of assay used was a modification of the luciferase (firefly enzyme) procedure (Strehler et al., 1957). Tissue samples were kept frozen in an acetone-dry ice bath and freeze-dried on a lyophilizing apparatus (Virtis Company). A vacuum of 100 microns of mercury was achieved initially. Then the samples were put on three at a time and kept cold with the freezing mixture until the 100 micron vacuum was attained again. The lyophilization was then allowed to proceed for three hours.

Two to six mg portions of freeze-dried tissue were weighed. Each portion was homogenized for three min in 0.5 ml of 0.3 molar perchloric acid and allowed to extract for 30 min in an ice bath. Following extraction 4.5 ml of 0.02 molar Tris (Sigma Chemical Company) buffer (pH 7.4) was added to each sample with thorough mixing. The samples were centrifuged for 30 minutes at 4,500g at 5° Celcius. Each sample was diluted to 50 ml with 0.02 molar Tris buffer.

A standard solution of adenosine-5'-triphosphate (Sigma Chemical Company) was prepared (10^{-6} molar) in 0.02 molar Tris buffer. Luciferase was prepared from a DuPont Luminescence Biometer Reagent Kit. One buffer-salt tablet from the kit was dissolved in 3 ml of deionized distilled water. To this solution was added one vial of enzyme-substrate powder (from the kit) with gentle mixing. This reaction mixture was allowed to stand about 30 min at ambient temperature.

Exactly 0.1 ml of the reaction mixture was added to each reaction cuvette (Dupont Luminescence Biometer Reaction Cuvette). The cuvette was then placed in a Chem-Glow Photometer (American Instrument Company) and 30 microliters of

the sample was injected with a constant rate syringe. The resulting readings for

the samples were converted to adenosine-5'-triphosphate concentrations by com-

puting linear percentages of the reading observed for the standard. Testing for a

significant difference between controls and experimentals was done with the t-test

(Croxton, 1959; Goldstein, 1967).

CHAPTER III

RESULTS

Anemic Stress

The animals fed the raw cow's milk diet for 107 days exhibited a very large decrease in blood hemoglobin concentration relative to the control animals (down to a level 38% that of the controls). The hemoglobin concentration of these animals was 10.9 gm/100 ml less than that of the controls. In the animals on the control diet the hemoglobin rose from 14 gm/100 ml at 16 days after weaning to 17.5 gm/ 100 ml at 107 days after weaning (Figure 1).

A severe retardation of body weight gain was observed for both male (165% weight increase) and female (203% weight increase) experimental animals, compared to male controls exhibiting a 342% increase and female controls a 244% increase in weight respectively (Figure 2).

Comparison of the ratio of heart weight to total body weight between controls and experimentals showed a highly significant difference ($p < 0.001$) between the two groups. The ratio of heart weight to total body weight for experimental animals was 153% that of the control animals (Table 1).

Lactate Dehydrogenase Isozyme Patterns

The milk diet also resulted in a decrease in the H/M sub-unit ratio for LDH in the myocardial tissue. Elution and quantitation of the bands indicated the H/M

Figure 1. Hemoglobin vs. Time

Control animals were fed the normal laboratory diet of Purina rat chow and water. Experimental animals were fed raw cow's milk only (an iron-deficient diet). Numbers adjacent to points indicate the sample sizes at the respective points. Error bar indicates mean ± std. error of the mean.

- - - - - Male Control

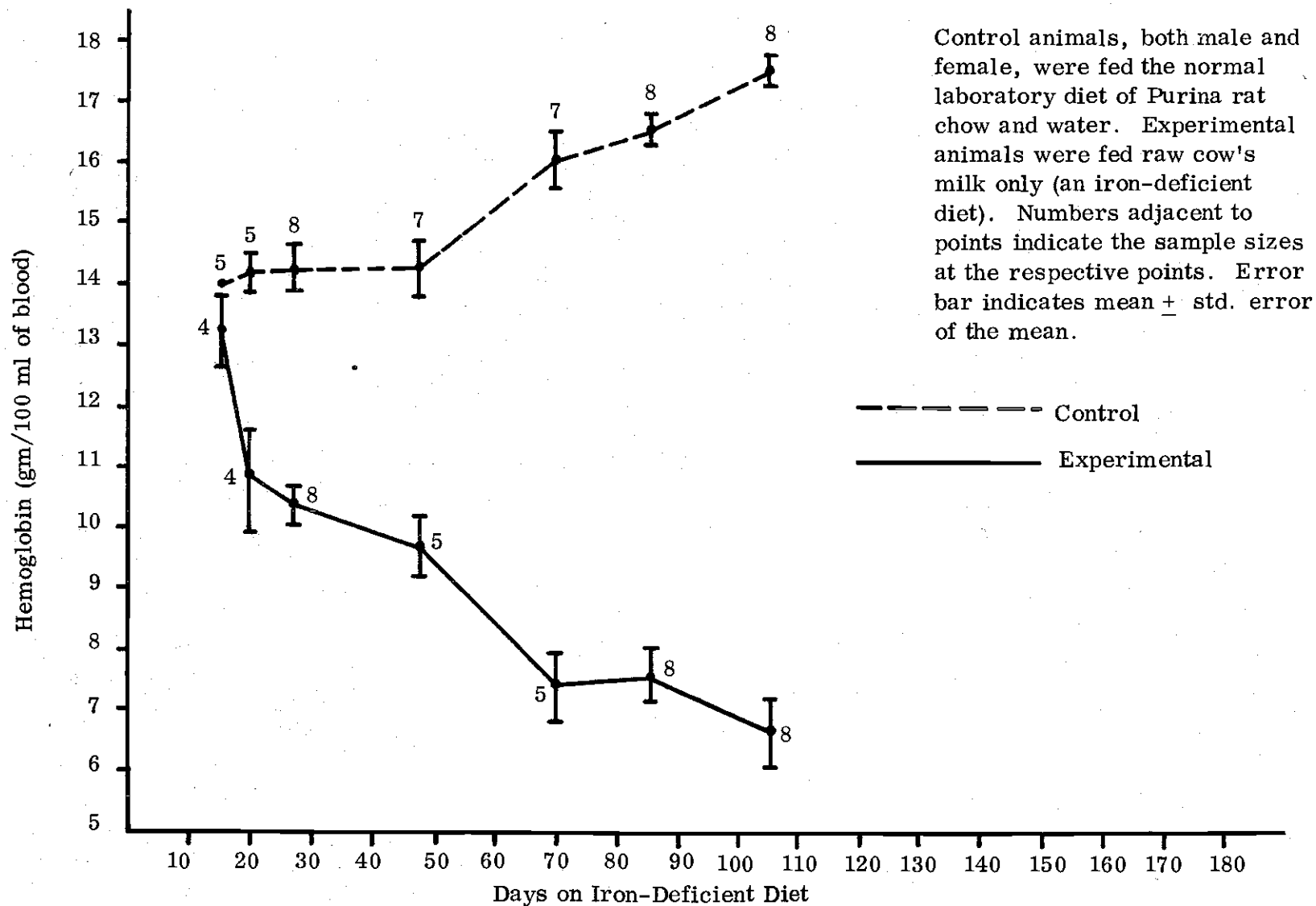- · - · - · - Female Control

——— Male Experimental
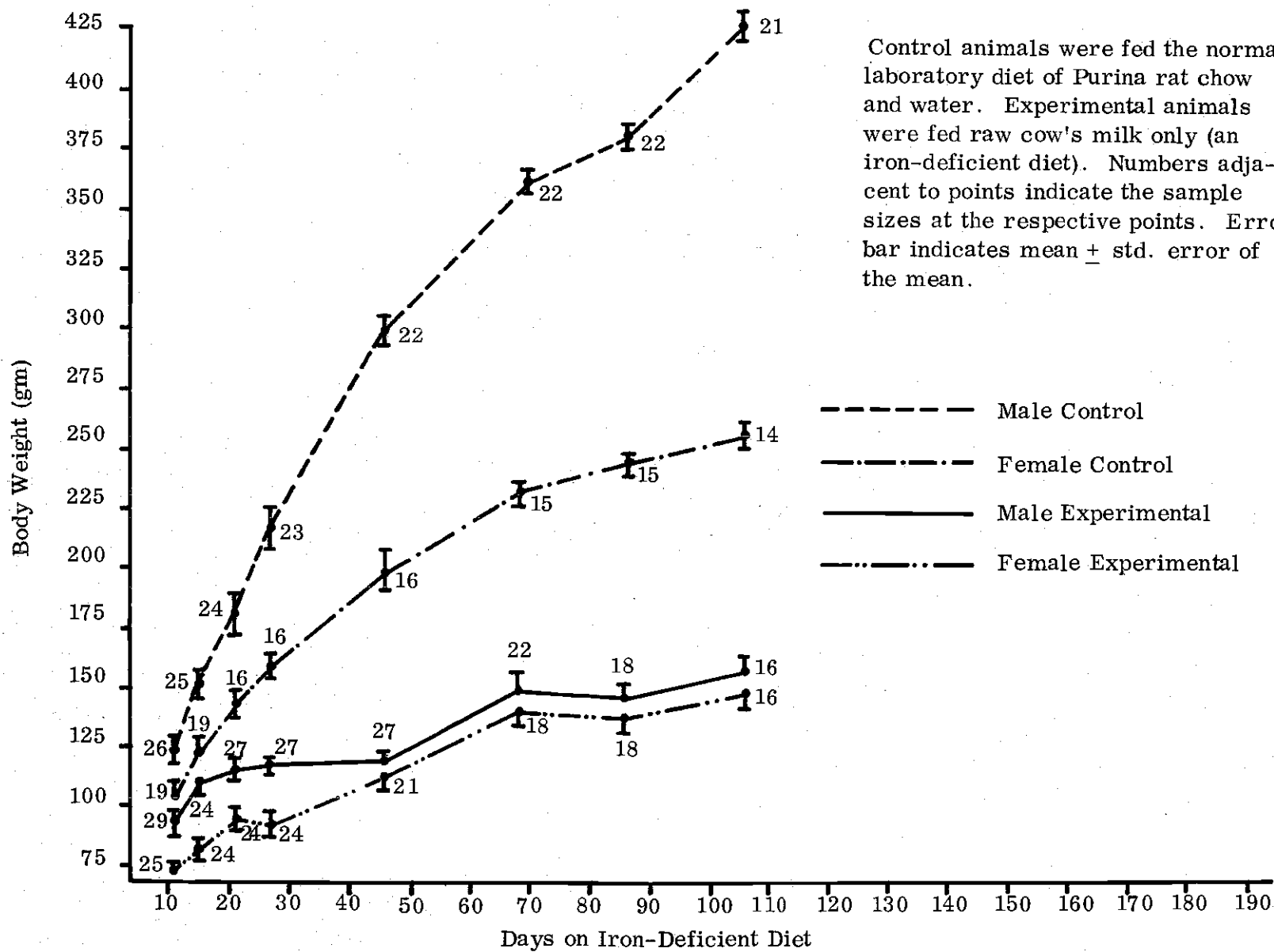
- ·· - ·· - Female Experimental

Figure 2. Weight vs. Time

Table 1. Comparison of Myocardial Parameters[1]

	Myocardial H/M[2] Ratio	Myocardial Glycogen (mg/gm, wet wt.)	Myocardial ATP (10^{-6} moles/gm, dry wt.)	Total Heart Weight/ Total Body Weight (gm/kg)
Control[3]	1.57 ± 0.07 (N = 20)	3.3 ± 0.6 (N = 12)	5.08 ± 0.53 (N = 12)	3.41 ± 0.09 (N = 14)
Experimental[3]	1.20 ± 0.04 (N = 23)	6.0 ± 0.5 (N = 14)	7.37 ± 0.71 (N = 13)	5.23 ± 0.03 (N = 14)
	$p < .001$[4]	$p < .005$[4]	$p < .05$[4]	$p < .001$[4]

[1] The subject animals were weighed, sacrificed, and their hearts were quick-frozen in liquid nitrogen. The above data were obtained from these frozen hearts at later dates.

[2] Lactate dehydrogenase % H sub-units/% M sub-units.

[3] All data above listed as mean $\pm$ std. error of the mean.

[4] Significance determined using Student's t-test between two means.

ratio to be significantly (p < 0.001) different from the controls. The H/M ratio of the experimental animals was 76% of that of the control animals (Table 1).

Tissue Energy Stores

Myocardial glycogen stores were found to be significantly (p < 0.005) greater in the experimental animals. The level found in the experimentals was 180% of that found in the controls (Table 1).

Also, adenosine-5'-triphosphate levels were significantly (p < 0.05) greater than in the controls. The experimental animal level was 145% of that found in the controls (Table 1).

CHAPTER IV

DISCUSSION OF RESULTS

Comparison with Similar Studies

When animals are fed an iron-deficient diet for a considerable time period, the characteristic responses include a highly depressed blood hemoglobin level and an increase in the ratio of heart weight to total body weight (Penny et al., 1974; Korecky et al., 1964; Poupa et al., 1964). Both of these responses were noted in the animals weaned to the raw cow's milk diet in the foregoing data. As in these same studies, the anemically stressed rats also showed a decreased rate of weight gain as compared to the control animals on the standard laboratory diet.

The observed changes in lactate dehydrogenase isozyme patterns are typical of those found in earlier studies (Penny et al., 1974; Jelihkova et al., 1968). The decreased H/M ratio which was observed for LDH may be indicative of increased anaerobic glycolytic capacity in the experimental animals (Dawson et al., 1964).

Significance of Changes in Myocardial Energy Stores

An increased rate of glycolysis occurs during myocardial hypoxia as a compensatory mechanism (Brachfeld and Scheuer, 1967). It has also been observed that glycogenolysis accompanies myocardial hypoxia (Evans, 1934; Klarwein et al., 1961). The increased glycogenolysis suggests that stores of glycogen may be important in the myocardial response to hypoxia (Scheuer and Stezoski, 1970).

Studies that show a protective effect of increased glycogen stores in the

anoxic rabbit heart (Gelli et al., 1968) and in the dog heart (Hewitt and Lolley,

1972) help to substantiate this concept of an increased rate of glycogenolysis.

Glycogen has also been shown to be an important energy substrate in the turtle

heart that has been subjected to anoxia (Reeves, 1963). However, absolute levels

of glycogen have been shown to decrease with time in the hypoxic rat heart (Olson

et al., 1972; Dhalla et al., 1972; Scheuer and Stezoski, 1970). (This decrease is

in agreement with the attendant increase in glycogenolysis during hypoxia). But,

these authors used hypobaric hypoxia in isolated, perfused heart preparations as

opposed to an anemic stress which is reported here.

There are other factors that must be considered when looking at glycogen

levels. There has been shown to be a 24-hour rhythm of glucose-6-phosphate

in the rat myocardium (Bockman et al., 1970). Glucose-6-phosphate is the common

point of entry for both glucose and glycogen in the glycolytic pathway. Also, gly-

cogen levels are affected by hormonal factors such as corticosteroids (Daw et al.,

1968) and thyroid hormone (Bray and Goodman, 1967), dietary factors (Opie et al.,

1963), changes in glycogenolysis induced by catecholamines (Mayer et al., 1967),

and exercise (Scheuer et al., 1970).

In any case the levels of myocardial glycogen observed by experiments

reported here (Table 1) are in agreement with those published in the literature.

One author reported, using hypobaric hypoxia in the isolated, perfused rat heart,

levels ranging from 12 mg/gm of dry weight at the onset of hypoxia down to 4 mg

after seven minutes (Dhalla et al., 1972). The observed values of 6 mg for the

experimentals (per gm of wet tissue) and 3.3 mg for the controls agrees quite well with the published data. The agreement is especially close when one considers that the glycogen levels per unit dry weight would be less if converted to levels per unit wet weight.

The observation that the experimental animals had more myocardial glycogen stores than the controls (Table 1) is in agreement with the literature cited. The animals potentially had an increased tolerance to hypoxia, and may have had an increased ability to carry on the production of high-energy phosphate compounds. One group of authors found that "anaerobic ATP production per mole of hexose was greater in hearts with higher glycogen stores" (Scheuer and Stezoski, 1970).

The maintenance of high-energy phosphate production during hypoxia is not just a simple matter of maximizing anaerobic glycolysis, but entails a consideration of the complex interactions relative to the balance between ATP supply and demand. The rate of ATP generation in the cytoplasm is stimulated by several factors. These include a high circulating concentration of glucose, an alkaline pH, a high glycogen content (important when the glucose level is low), and increased insulin levels. ATP generation in mitochondria is stimulated by an increase in intermediates of the Kreb's cycle. Myocardial ATP demand is decreased by a depressed level of contractile activity, low temperatures, and depressed levels of inotropic agents such as catecholamines. The coronary circulation also plays an important part in myocardial ATP levels. A high coronary flow rate makes possible a rapid delivery of glucose and insulin, a rapid removal of lactate and hydrogen ions from hypoxic tissue, and a proportionately greater oxygen delivery rate (Opie, 1972).

A number of studies in the literature have quantified high-energy phosphates in the hypoxic rat and dog myocardium. Several of these authors reported a decrease in ATP levels during hypobaric hypoxia (Olson et al., 1972; Lang and Saborowski, 1972; Scheuer, 1972; Dhalla et al., 1972). The levels reported varied from 6.7 to 25.1 x 10^{-6} moles per gm of dry weight for the control animals and from 5.5 to 21.5 x 10^{-6} moles per gm for the experimentals. This agrees quite well with the experimentally observed values of 7.3 x 10^{-6} moles per gm of dry weight for the experimental animals and 5.1 x 10^{-6} moles per gm for the controls. However, it should be noted that the experimental animals had the highest ATP levels. Obviously this trend is not consistent with the literature in that it indicates the opposite effect.

The ATP levels in the anemic animals may have a significance. In anemia it is well known that the cardiac output and the energy expenditure of the heart are both elevated (Gregg and Fisher, 1963). The energy supply of the anemically stressed hearts seemed to be adequate since the difference in ATP levels between control and experimental animals was not that substantial. This is presumably due in part to the observed changes in LDH and glycogen levels that were previously described. The likely increase in anaerobic glycolysis, as indicated by shifts in the LDH isozyme patterns, and the higher myocardial glycogen stores in the experimental animals could contribute to maintenance of ATP levels. Therefore, it may be said that there was no evidence of an inability of the anemically stressed animals to maintain a sufficient energy supply. Tissue level adaptation to hypoxia may well have occurred.

Comparison of Anemic and Hypobaric Hypoxia

This study and others like it on anemic hypoxia derive primarily from those conducted in the area of hypobaric hypoxia. There are subtle differences between anemic and hypobaric hypoxia that should be known when considering the results of a study such as this.

In hypobaric hypoxia blood hemoglobin levels increase with exposure to the stress. The cardiac hypertrophy is less in hypobaric animals. The myocardium of hypobaric animals is supplied by a denser capillary network than is the heart of anemic animals. In anemic hearts the muscle fibers grow at a rate comparable with the capillaries. Thus there is no net increase in perfusion capacity in anemic animals.

In hypobaric hearts there is an increase in myoglobin while in anemic hearts there is a drop in its level. This gives the hypobaric heart an increased oxygen storage capacity and facilitates the transport of oxygen into the tissue. But, both hypobaric and anemically stressed hearts do have the capacity for anaerobic glycolysis.

Generally speaking, the heart of a hypobaric animal has a better capacity to endure a hypoxic stress than does the heart of an anemic animal. This is due primarily to the higher capillary density and to the increased myoglobin levels in the hypobaric heart (Poupa, 1972).

However, the response of the anemic myocardium, presumably to hypoxia, at the tissue level is quite like that of the hypobaric myocardium. Similar changes in lactate dehydrogenase isozyme patterns have been observed in animals subjected

to hypobaric stresses (Anderson and Bullard, 1971; Penny, 1974; Mager et al.,

1968; Miller and Hale, 1968). Also, as previously discussed, ATP and glycogen

data collected from studies on both types of stresses are reasonably consistent.

CHAPTER V

CONCLUSIONS

1. Myocardial lactate dehydrogenase isozyme patterns and glycogen levels may have been indicative of an increased anaerobic glycolytic capacity in the anemic animals.

2. The myocardial tissue of the anemic animals may have had an increased ability for anaerobic ATP production.

3. There was no apparent reduction of ATP levels in the hearts of the anemic animals. (In fact, ATP levels in the anemic animals were significantly higher at the 0.05 level than those for the controls.)

4. There appears to have been a tissue level adaptation to hypoxia in the anemic animals.

5. The observed tissue level responses, presumably to anemic hypoxia, were similar to those expected from a hypobaric stress (Anderson and Bullard, 1971; Penny, 1974; Mager et al., 1968; Miller and Hale, 1968).

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