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The interacting effects of tetraiodothyronine and epinephrine on the activities of Na+ + K+ -dependent adenosine triphosphatase, hexokinase and pyruvate kinase in the cardiac tissue of white rats

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ABSTRACT

The interacting effects of tetraiodothyronine (T4) and epinephrine on Na^++K^+-dependent ATPase, hexokinase, and pyruvate kinase in cardiac tissue of white rats were investigated in an attempt to elucidate the hormonal regulation of heart metabolism. Previous evidence has shown that 90% of the increase in oxygen consumption exhibited by hyperthyroid rats was due to an activation of Na^++K^+-dependent ATPase. In addition, hypothyroidism in sheep was reported to lower cardiac Na^++K^+-dependent ATPase activity as well as β-adrenergic binding sites for epinephrine. As T4 and epinephrine enhance carbohydrate metabolism, hexokinase and pyruvate kinase, two essentially irreversible glycolytic enzymes, were also assayed.

All animals were initially made hypothyroid by the addition of propylthiouracil to their drinking water. Hyperthyroidism was achieved through daily injections of T4 and epinephrine action on the heart was blocked by dl-timolol maleate.

No significant interacting effects of T4 and epinephrine were demonstrated on the activity of Na^++K^+-dependent ATPase. However, significant interacting effects of the two hormones were exhibited on hexokinase and pyruvate kinase activities. The simultaneous deficiency of T4 and epinephrine appeared to result in enhanced activities of both hexokinase and pyruvate kinase. The high activities of hexokinase and pyruvate kinase in these epinephrine-blocked hypothyroid rats may be attributed to low blood glucose levels which forced the combustion of lipids and amino acids or stimulated the release of glucocorticoids.

INTRODUCTION

The purpose of this study was to determine the interacting effects of tetraiodothyronine and epinephrine on the activities of Na^++K^+-dependent ATPase, hexokinase, and pyruvate kinase in the cardiac tissue of white rats (Rattus norvegicus).

Increased levels of the thyroid hormones tetraiodothyronine (T4) and triiodothyronine (T3) have varied effects on specific tissues in mammals. Some of these are: excessive elimination of water and various cations (K^+, Na^+, Ca^{++}), increased oxygen consumption and heat production, increased carbohydrate metabolism, and an overall increase in basal metabolic rate (Turner and Bagnara, 1976). The modes of action of thyroid hormones are not entirely known. It was noted by Babior et al. (1973) that, in rat liver, thyroid hormones (T4 and T3) increased the rate of mitochondrial oxidation of various Krebs cycle intermediates and the uptake of ADP. They presumed the increased ADP uptake was accompanied by the discharge of an equivalent amount of ATP from the mitochondrial matrix.

In studying liver subcellular compartments, Sterling et al. (1977, 1978) discovered specific binding sites for thyroid hormones (T4 and T3) in cytosol, nuclei, and mitochondria. The binding protein from the inner mitochondrial membrane had the highest association constant for thyroid hormone (T3). The inner membrane of the mitochondrion is the site of oxidative phosphorylation (Lehninger, 1975). Oppenheimer (1979), suggested that a T3 nuclear receptor complex directly or indirectly stimulates synthesis of a variety of messenger RNA indicating thyroid hormones

may enhance the production of various enzymes located in the cytosol, cell membrane, and mitochondrion.

The enzymes examined in the present study included a plasma membrane-bound enzyme, Na^++K^+-dependent ATPase, and two cytosolic enzymes, hexokinase and pyruvate kinase. Na^++K^+-Dependent ATPase was assayed because an increase heart rate would require more rapid depolarization and repolarization of the pacemaker and cardiac muscle fibers. Restoration of ionic (Na^+, K^+) gradients would be accomplished by increased activity of Na^++K^+-dependent ATPase, the enzymatic equivalent of the sodium pump (Keeton, 1972). Ismail-Beigi and Edelman (1970) found that over 90% of the increase in oxygen consumption produced by injections of T4 in euthyroid (normal levels of thyroid hormones) rats was due to an increased energy (ATP) utilization by the sodium pump in the liver. The pump was probably activated at the cell membrane. Smith et al. (1978) showed a 60% fall in cardiac Na^++K^+-dependent ATPase activity in hypothyroid sheep. Increases in Na^++K^+-dependent ATPase concentration in liver, kidney, and skeletal muscle of hyperthyroid rats were reported by Lin and Akera (1978).

Ismail-Beigi et al. (1973) demonstrated a decrease in ATP and an increase in ADP levels of T3-injected euthyroid rats, indicating a high utilization of energy. A continued high level of energy consumption, as in the case of a hyperthyroid heart, would result in a corresponding increase in production of the major energy source, ATP, probably through increased combustion of glucose (Prosser, 1973; Geise, 1973). Hyperthyroidism causes

the onset of a heavy work load on the heart which in turn would enhance the rate of glycolysis in cardiac tissue (Lehninger, 1975). The steady-state heart predominantly uses free fatty acids from blood as fuel but, after the onset of extra work, the major fuel is glucose, mainly from glycogen (Shipp, 1964; Shipp et al., 1964; Shipp et al., 1967; Mayer, 1967).

Hexokinase and pyruvate kinase were the glycolytic enzymes chosen for this study. Hexokinase catalyzes the initial step of glycolysis in which glucose is phosphorylated to form glucose-6-phosphate. Pyruvate kinase catalyzes the dephosphorylation of phosphoenol pyruvate thus forming pyruvate which is then catabolized through the Krebs cycle (Dixon and Webb, 1964). These two enzymes were chosen for examination because the ΔG values are -4.0 kcal and -7.5 kcal for the hexokinase and pyruvate kinase reactions respectively (Lehninger, 1975). Reactions with ΔG values more negative than -1 kcal are essentially irreversible and will lean heavily toward the formation of end product (Crockford and Knight, 1959). Measurement of such irreversible reactions is desirable so that recorded activity of hexokinase or pyruvate kinase can be interpreted as combustion (towards pyruvate) of glucose.

The effects of epinephrine were included in this study because of the stimulatory action of this hormone on the mammalian heart and because of evidence showing a regulating ability of thyroid hormones on the number of epinephrine binding sites in the heart. In the heart, epinephrine binds with β-adrenergic

sites and evokes three distinct responses: 1) cardiac acceleration through the sinoatrial node, 2) increased force of contraction, and 3) altered rhythmic function of the ventricle (ventricular extrasystole, tachycardia, and potential fibrillation) (Di Palma, 1965). The same cardiac abnormalities are exhibited by hyperthyroid mammals (Levey, 1975; Waldstein, 1966). Levey (1971) reported increased heart rates in rats injected with thyroid hormones and epinephrine. Concurring data were presented by Dietrick and McClure (1978) on heart rates from T4-injected rats and Buccino et al. (1967) on heart rates of hyperthyroid cats.

Some of the symptoms of hyperthyroidism such as tachycardia, tremor, restlessness, and anxiety are reduced through the administration of various β-adrenergic blocking drugs. These drugs successfully compete for β-adrenergic sites in the heart thus blocking the effects of epinephrine in the heart (Levey, 1975; Tachikawa and Takenaka, 1973; Goulding et al., 1976; Das and Krieger, 1969; Buckle, 1968; Poticha et al., 1968). Although some results of hyperthyroidism on the heart are lessened by these drugs, they are not totally abolished and some are not affected. For example, cardiac muscle dilation, hypertrophy, increased oxygen consumption, accelerated pulse rate, and to some extent tachycardia are not substantially affected by β-adrenergic blockade (Levey, 1971). Through the use of reserpine (a drug which lowers plasma catecholamine levels), Buccino et al. (1967) demonstrated that changes in the accelerated con-

tractile state of the cat myocardium induced by hyperthyroidism were not dependent on catecholamine stores. This and previous information indicates a possible independent action of thyroid hormones on hyperthyroid hearts.

Epinephrine alone has been shown to have a high glycogenolytic activity through the direct stimulation of cyclic AMP synthesis (Murad et al., 1962; Mayer, 1967). Cyclic AMP activates enzymes which hydrolyze one glucose molecule from glycogen resulting in an increase in blood glucose (Robison et al., 1965; Villar-Palasi and Larner, 1970).

Thyroid hormones are suspected to have a similar activating effect on the synthesis of cyclic AMP (Ciaraldi and Marinetti, 1978). Aside from having similar accelerating effects on mammalian cardiac tissue, thyroid hormones and epinephrine both cause increases in glycogenolysis and carbohydrate metabolism (Waldstein, 1966).

Evidence has been supplied suggesting a definite interaction between thyroid hormones and epinephrine in the mammalian heart. Ciaraldi and Marinetti (1977, 1978) found an increase in the number of β-adrenergic receptor sites (epinephrine binding sites) in the hearts of hyperthyroid rats. The converse was true for hypothyroid (lower than normal levels of T4 and T3) conditions. Concurrent data were provided by Williams et al. (1977), who observed an increase in the binding of radioactive β-adrenergic agonists in the myocardium of hyperthyroid rats. Therefore, heart was chosen as the subject tissue in this study because of

similar effects of the two hormones on carbohydrate metabolism which is increased in the hyperthyroid heart (Waldstein, 1966).

Even though there seems to be a definite interaction between thyroid hormones and epinephrine in the hyperthyroid heart, there is some question as to the extent of the interaction. In other words, it is not entirely known which effects are due to thyroid hormones and epinephrine individually and which are the results of an interaction of the two.

In order to observe interacting or independent effects of the two hormones on cardiac tissue, four groups of experimental animals were arranged which were (1) hypothyroid, (2) hyperthyroid, (3) hypothyroid with epinephrine blocked, and (4) hyperthyroid with epinephrine blocked. Epinephrine was blocked through the use of dl-timolol maleate (Hall et al., 1975). Hyperthyroidism was induced by treatment with T4. Tetraiodothyronine was used for the thyroid hormone because, in mammals, it normally circulates in higher titres than triiodothyronine and has a longer lasting effect in comparison to T3 (Turner and Bagnara, 1976).

Cardiac tissue preparations from treated rats were assayed for activity of the appropriate enzymes in an effort (1) to determine if the reported increases in $Na^+ + K^+$-dependent ATPase in hyperthyroid rat hearts is due to an interaction of T4 and epinephrine or to an independent action of either hormone, and (2) to determine if the activity of two glycolytic enzymes, hexokinase and pyruvate kinase, is responsive to treatment with

thyroid hormone and/or epinephrine.

MATERIALS AND METHOD

Male adult rats (Rattus norvegicus) of the Sprague-Dawley strain were fed Purina Lab Chow ad libitum and were maintained in an 8 hour light - 16 hour dark photoperiod for 10 days. Rats weighed 230-350 grams at the termination of the experiment with the exception of one animal which weighed 167 grams displaying an abnormal weight loss. Data from this animal, which belonged to the unblocked hyperthyroid group were not included in the following results. Four groups with 7 rats in each group were arranged as follows: unblocked hypothyroid, unblocked hyperthyroid, epinephrine-blocked hypothyroid, and epinephrine-blocked hyperthyroid.

To provide a base line, all animals were initially made hypothyroid by adding 0.1% (w/v) propylthiouracil (Sigma Chemical Co.) to their drinking water (Ciaraldi and Marinetti, 1978). This treatment was continued throughout the injection period. Each animal received the appropriate treatment daily for a period of 10 days. Hyperthyroidism was induced by subcutaneous (S.C.) injections of L-thyroxine (T4) (Sigma Chemical Co.) at a dosage of 75 µg/100 g body weight (Ciaraldi and Marinetti, 1978). β-Adrenergic blockade was achieved through intraperitoneal (I.P.) injections of dl-timolol maleate (A.H. Robins Laboratories) at a dosage of 1 mg/kg body weight (Hall et al., 1975). L-Thyroxine and dl-timolol maleate were dissolved in

saline (0.9% NaCl) and distilled water respectively.

The unblocked groups received I.P. injections of distilled water whereas the blocked groups received I.P. injections of dl-timolol maleate. The hyperthyroid group received S.C. injections of L-thyroxine and the hypothyroid groups received S.C. injections of saline (Table 1).

Homogenate preparation

Rats were killed by cervical dislocation on day 11, following the initial injection. All subsequent homogenization steps took place in a cold room maintained at 0-4°C. Each ventricle was excised and placed in homogenate buffer consisting of 0.25M sucrose, 6mM disodium ethylenediamine tetraacetic acit (EDTA), and 20mM imidazole (pH 6.8) (Hendler, 1972). Tissue was blotted dry on Kimwipes, weighed to the nearest 01 mg and placed into a Teflon-glass Potter-Elvehjem homogenizer which contained 40 volumes of homogenate buffer and 0.1% sodium deoxycholate. The tissue was homogenized with 18 complete strokes at 1,725 rpm by a motor driven homogenizer. The resulting homogenate was strained through two layers of cheesecloth into a plastic test tube and kept on ice in the cold room until needed. All enzyme assays were performed on the same day.

Na^++K^+-Dependent ATPase assay

Na^++K^+-Dependent ATPase activity was assayed according to Towle et al. (1976). The homogenate was diluted to a final dilution of 100x by combining 1 ml of the crude homogenate with

2.5 ml of homogenate buffer and mixing thoroughly. Total ATPase activity was assayed in triplicate tubes containing 200mM imidazole-HCL (pH 7.2), 0.9M NaCl, 0.2M KCl, 50mM $MgCl_2$, and 0.1 ml homogenate (final concentration). Ouabain-insensitive ATPase activity was measured in triplicate tubes containing 200mM imidazole-HCL (pH 7.2), 1.1M NaCl, 20mM ouabain, 50mM $MgCl_2$, and 0.1 ml homogenate (final concentrations). Following preincubation at 37°C for 12 minutes, the reaction was started by adding 0.2 ml of 50mM disodium ATP (adjusted to pH 7 with 10% imidazole) to each tube giving a final volume of 2 ml. After a 30 minute incubation period at 37°C, the reaction was stopped by adding 2 ml of ice-cold 10% (w/v) trichloroacetic acid to each tube. The tubes were placed on ice for 10 minutes, then centrifuged at 11,000 x g max for 10 minutes in a Beckman model L3-50 ultracentrifuge. The resulting supernatant was measured for free inorganic phosphate according to Fiske and SubbaRow (1925).

Na^++K^+-Dependent ATPase specific activity was calculated by subtracting the ouabain-insensitive activity from the total ATPase activity. Specific activity was expressed as μmoles Pi/min/mg protein. Protein concentration for all enzyme assays was determined by the coomassie blue dye-binding method (Bradford, 1976).

Hexokinase assay

The homogenate was diluted 400x by combining 1 ml of the crude homogenate with 10 ml of buffer and mixing thoroughly.

The homogenate was then centrifuged at 100,000 x g max for 15 minutes. The resulting supernatant was used for the hexokinase and pyruvate kinase assays. Hexokinase (EC 2.7.1.1) specific activity was assayed according to Easterby and O'Brien (1973). In this procedure hexokinase is coupled to glucose-6-phosphate dehydrogenase (G-6-PDH) (EC 1.1.1.49). The reaction is as follows:

$$\text{D-glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NADP} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-phosphate} + \text{NADPH} + \text{H}^+.$$

Total hexokinase activity was measured in duplicate tubes containing 10mM ATP (pH 7), 50mM Tris-HCl buffer (pH 7.6), 3mM glucose, 20mM $MgCl_2$, 0.1mM NADP, and 0.4 units of glucose-6-phosphate dehydrogenase (final concentrations). Non-specific kinase activity was measured in duplicate tubes containing the same reagents with the substitution of distilled water for glucose. All solutions were maintained on ice. Assay tubes were incubated in a 37°C water bath for at least 5 minutes prior to the addition of supernatant. The reaction was started with the addition of 0.1ml of the supernatant to the assay tube, giving a final volume of 2.02ml. The tube was immediately vortexed and a portion of the contents was drawn up by a sipper attached to a Bausch and Lomb Spectronic 700 spectrophotometer set at a wavelength of 340nm. The change in absorbance per minute was recorded on a Houston Instruments Omniscribe recorder.

Hexokinase specific activity was calculated as the difference between total activity and non-specific kinase activity and is expressed as units/mg protein. One unit catalyzes the reduction

of one µmole of NADP/minute.

Pyruvate kinase assay

Pyruvate kinase (EC 2.7.1.40) specific activity was assayed according to Decker (1977). In this procedure pyruvate kinase is coupled to lactate dehydrogenase (LDH) (EC 1.1.1.27). The reaction is as follows:

$$\text{Phosphoenol-pyruvate} + \text{ADP} \xrightarrow{\text{Pyruvate kinase}} \text{pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LDH}} \text{lactate} + \text{NAD}.$$

Duplicate samples were run on each animal using the same tube arrangements as in the hexokinase assay. Total activity tubes contained an ADP/Mg^{++} mixture consisting of 1.5mM ADP + 60mM $MgSO_4$ (adjusted to pH 7 with 10% imidazole), assay buffer consisting of 20mM imidazole + 1mM EDTA + 1mM $MgSO_4$ (adjusted to pH 7.6 with HCl), 2.5mM phosphoenol-pyruvate, 0.1mM NADH, 15mM KCl, and 34 units of LDH (final concentrations). Non-specific kinase activity tubes contained the same reagents with distilled water substituted for phosphoenol-pyruvate. Assay tubes were incubated for at least 5 minutes in a 37°C water bath prior to the addition of supernatant. The reaction was started by the addition of 0.05ml supernatant to the assay tube giving a final volume of 2.02ml. Change in absorbance per minute at 340nm was measured using the same technique as in the hexokinase assay. Pyruvate kinase specific activity was calculated in the same way as hexokinase and expressed as units/mg protein. One unit catalyzes the oxidation of one µmole of

NADH/minute.

Statistics were performed on all activity values to determine mean, variance, and standard error. Students t-test was used to detect significant differences between groups at the 0.05 confidence level.

RESULTS

Preliminary determinations of all enzymes tested demonstrated that the assays used exhibited linearity of activity with respect to the amount of enzyme added and thus were valid measurements of enzyme specific activity (Figures 1-3). A coupled Na^++K^+-dependent ATPase assay (Saintsing and Towle, 1978) was not applicable to this study as rat tissues are not totally sensitive to ouabain (a cardiac glycoside which inhibits Na^++K^+-dependent ATPase activity). An assay which required the absence of K^+ (K^+ is needed for Na^++K^+-dependent ATPase activity) plus the addition of ouabain was needed to doubly block the enzyme and give reproducible activity readings. The initial hexokinase assay, a modification of Saintsing and Towle (1978), was not reproducible or linear. The assays for hexokinase and pyruvate kinase finally employed in this study were found to be reproducible and linear only when a cytosol fraction was used as opposed to a crude hemogenate. Centrifugation of the homogenates eliminated substantial background kinase activity.

Although the group mean specific activity values for Na^++K^+-dependent ATPase were not significantly different, observed

trends did coincide with expected results (Figure 4). The mean specific activities of unblocked hyperthyroid and blocked hyperthyroid groups were 5.8% and 6.1% greater than the unblocked hypothyroid and blocked hypothyroid groups respectively. Also, the mean specific activities of unblocked hypothyroid and unblocked hyperthyroid rats were 3.5% and 2.9% greater than the blocked hypothyroid and blocked hyperthyroid rats respectively (Table 2). These data possibly indicate an interacting effect between thyroxine (T4) and epinephrine with the former having a higher excitatory effect than the latter on $Na^{+}+K^{+}$-dependent ATPase activity in the hearts of hyperthyroid rats. However, this cannot be determined because the differences between the mean values among the four groups were not significant ($p>0.25$) (Table 2).

Results of hexokinase assay did not totally support the original hypothesis (Figure 5). The unblocked hyperthyroid group had the highest mean activity value followed by the blocked hyperthyroid group. The mean activity for the unblocked hypothyroid group was lower than that of the blocked hypothyroid group. A significant difference ($p<0.05$) was observed between the two unblocked groups. The hyperthyroid group mean was 18% higher than that of the hypothyroid group (Table 3) demonstrating a probable interaction of thyroxine and epinephrine on hexokinase activity in the hyperthyroid rat heart. The unblocked hyperthyroid mean value was 8.6% higher than the blocked hyperthyroid value and the blocked hypothyroids had 6.5% more hexokinase activity than the unblocked hypothyroids, but these differences

were not significant ($p>0.25$) (Table 3).

The highest mean value for pyruvate kinase activity was found in the unblocked hyperthyroid group. The blocked hypothyroid group had a higher pyruvate kinase activity than the unblocked hypothyroid and blocked hyperthyroid groups (Figure 6). The high pyruvate kinase activity exhibited by the blocked hypothyroid group was not expected because the rats in that group had low levels of both thyroid hormones and epinephrine. The unblocked hyperthyroids had 23.3% and 15.4% ($p<0.01$) more pyruvate kinase activity than the unblocked hypothyroid and blocked hyperthyroid groups respectively (Table 4). The blocked hypothyroid rats had 17.8% ($p<0.05$) more pyruvate kinase activity than the unblocked hypothyroid rats and 9.4% ($p>0.05$) more than the blocked hyperthyroid rats (Table 4).

The unblocked hyperthyroid groups exhibited the highest mean specific enzyme activity in all the assays. Significant differences in mean activity values were present only in the hexokinase and pyruvate kinase assays.

DISSCUSSION

Tetraiodothyronine and epinephrine have calorigenic effects on various target tissues in mammals. The calorigenic effects of T4 are, however, higher than those of epinephrine (Turner and Bagnara, 1976). Therefore, it was expected that the unblocked hyperthyroid group would have the highest specific activity for all three enzymes assayed, followed in descending order by the

blocked hyperthyroid, unblocked hypothyroid, and blocked hypothyroid groups.

Although there were no significant differences between mean Na^++K^+-dependent ATPase values in the four animal groups, the trends found should be discussed. The data did lend some support to the above hypothesis in that the unblocked hyperthyroid activity value was highest, followed by the blocked hyperthyroid, unblocked hypothyroid, and blocked hypothyroid activity values., Smith et al. (1978) demonstrated a 60% decrease in Na^+ +K^+-dependent ATPase activity along with a 90% decrease in β-adrenergic receptor sites in hearts of thyroidectomized sheep. Ciaraldi and Marinetti (1977) found increases in the number of β-adrenergic receptor sites in the hearts of T4-treated rats when compared to propylthiouracil-treated (hypothyroid) rats suggesting a direct interaction of T4 on the number of available epinephrine binding sites in the heart. Therefore, it can be inferred that some Na^++K^+-dependent ATPase activity may be directly dependent on the number of accessible β-adrenergic sites which in turn may be manipulated by varying levels of T4. This type of activity would be expected in a hyperthyroid heart in which an increase in epinephrine binding, brought about by T4, would enhance heart rate and cause a corresponding increase in Na^+ +K^+-dependent ATPase activity that would permit maintenance of increased influx and eflux of Na^+ and K^+ in the neurons and muscle fibers of the heart.

Grossman et al. (1971) found in humans that tachycardia,

shortened circulation time, and widened pulse pressure of thyrotoxicosis (hyperthyroidism) were somewhat alleviated by β-adrenergic blocking treatment, but the heightened ventricular activity was unaffected. Levey (1971, 1975) and Waldstein (1966) noted that the use of β-adrenergic blocking drugs did not significantly inhibit the increases in oxygen consumption, cardiac muscle dilation, hypertrophy, and tachycardia observed in hyperthyroid dogs and rats. These observations lend credence to the assumption that some effects of T4 on cardiac tissue are independent of β-adrenergic (epinephrine) activity. It could not be determined from the present study if there were any effects of T4 on Na^++K^+-dependent ATPase activity independent of epinephrine.

Possible independent effects of thyroid hormones (T4 and T3) on Na^++K^+-dependent ATPase have been proposed. Philipson and Edelman (1977a) reported a 50% increase in ventricular Na^++K^+-dependent ATPase activity in T3-treated hypothyroid rats. Ismail-Beigi and Edelman (1970, 1971) showed a correlation between increased Na^++K^+-dependent ATPase activity and a decrease of the Na^+/K^+ ratio in the liver cells of T3-injected rats. They hypothesized that T3 was activating the sodium pump. In a later study, Philipson and Edelman (1977b) demonstrated a significant increase in cardiac cellular K^+ in T3-injected hypothyroid rats, concurrent with a significant increase in Na^++K^+-dependent ATPase activity. They attributed the increased K^+ concentrations to an activation of the sodium pump by T3.

Lin and Akera (1978) supplied supportive evidence reporting an increase in concentration of the Na^++K^+-dependent ATPase enzyme in liver, kidney, and skeletal muscle of T3-injected rats. They believed that the increase in enzyme concentration was due to a stimulation of enzyme synthesis. Sterling et al. (1977) noted T3 binding sites in nuclei of rat liver and kidney which indicates that T3 may indeed be involved in enzyme synthesis.

Although it cannot be determined, the small increases in cardiac Na^++K^+-dependent ATPase in the present study were probably the result of an increased number of available β-adrenergic sites in the heart augmented by T4 and an independent action of T4 on enzyme activity and concentration. Philipson and Edelman (1977a) found significant Na^++K^+-dependent ATPase activity in both crude homogenates and microsomal fractions from ventricles of T3-treated hypothyroid rats, but the activity was much higher in the microsomal fraction. Both T4 and T3 elicited increases in cardiac Na^++K^+-dependent ATPase activity in previous research with T3 having a higher stimulatory effect than T4 (Smith et al., 1978). Tetraiodothyronine binds more strongly to serum proteins than T3 making it less available to cells. Also, T4 has a longer lasting effect than T3, but the effects of T3 are stronger than T4 (Turner and Bagnara, 1976). Possible reasons for the conflict of the Na^++K^+-dependent ATPase data in this study with those of previous studies are that a crude homogenate was used in the present study instead of a microsomal fraction and that T4 was used in place of T3.

In the hexokinase assay, the unblocked hyperthyroid rats showed the highest specific activity followed by the blocked hyperthyroid, blocked hypothyroid, and unblocked hypothyroid groups. The unblocked hypothyroid group was expected to have a higher hexokinase activity value than the blocked hypothyroid group because the former had lower levels of thyroid hormones and in the latter epinephrine was blocked in the heart.

A significant difference in specific hexokinase activity was exhibited only in comparing the two unblocked groups in which the hyperthyroid group had a higher mean hexokinase activity than the hypothyroid group. This shows that an interaction of T4 and epinephrine has a direct or indirect effect on hexokinase activity in rat heart because either hormone alone did not elicit any significant effect on hexokinase activity. Robison et al. (1965) demonstrated increased levels of cyclic AMP in isolated rat hearts perfused with epinephrine. Mayer et al. (1965) and Mayer et al. (1967) also showed that epinephrine is directly involved with the activation of cyclic AMP which in turn activates enzymes which hydrolyze a single glucose molecule from glycogen. Thyroid hormones also activate cyclic AMP by enhancing the concentration of adenyl cyclase (Villar-Palasi and Larner, 1970). Therefore epinephrine and T4 may indirectly effect hexokinase activity by increasing glucose which is the substrate for hexokinase, through an enhancement of glycogenolysis.

Kubista et al. (1971) reported marked increases in hexokinase activity in cardiac and skeletal muscle of T4 and T3

injected (hyperthyroid) rats. Because epinephrine was not blocked, the observed effects may have been due to a combination of thyroid hormones and epinephrine. Tata et al. (1963) performed in vitro studies on liver slices from T3 injected rats in which hexokinase concentrations were mechanically varied. Results showed that increases in QO_2 (oxygen consumption) were regulated in direct proportion to the amount of hexokinase present, thus implying that there is an increase in hepatic hexokinase activity in hyperthyroid rats, in addition to increased O_2 consumption.

In view of the aforementioned information and the fact that there were no significant differences in hexokinase activity between all groups except in comparison to the unblocked hypothyroids ($p<0.05$) which had the highest levels of T4 and epinephrine, it was evident that an interaction of the two hormones was required to significantly enhance cardiac hexokinase activity in rats.

The highest pyruvate kinase activity was exhibited by the unblocked hyperthyroid group as was the case for the hexokinase assay. The blocked hypothyroid was not expected to have such a high pyruvate kinase activity because that group supposedly had the lowest levels of T4 and T3 and epinephrine action in the heart was blocked.

The unblocked hyperthyroid pyruvate kinase specific activity value was higher than the activity value for the blocked hyperthyroids and unblocked hypothyroids. The former comparison shows the effects of epinephrine because both groups are hyper-

thyroid but epinephrine is blocked in one. The latter comparison shows a combined effect of T4 and epinephrine on pyruvate kinase activity because the unblocked hyperthyroids received T4 and did not receive β-adrenergic blocking treatment. A probable interaction of T4 and epinephrine on pyruvate kinase activity was indicated by the highest activity value seen in the unblocked hyperthyroid group which had the highest levels of T4 and epinephrine.

As mentioned earlier, it was not expected that the blocked hypothyroid pyruvate kinase activity value would exceed that of the unblocked hypothyroid group. The blocked hypothyroid group demonstrated slightly higher hexokinase activity than the unblocked hypothyroid group but the difference was not significant. Most likely, the continued low levels of epinephrine in the heart and overall low levels of thyroid hormones (T4 and T3) in the blocked hypothyroid rats caused low levels of glucose as both hormones are glycogenolytic agents. (Villar-Palasi and Larner, 1970; Mayer et al., 1967). If the rate of glycogenolysis was decreased in the blocked hypothyroid rats then cells would have to use fuels other than glucose, namely amino acids and lipids, which would either be combusted via glycolysis or the Krebs cycle (Williamson and Krebs, 1961; Stumpf, 1969). The amino acids and lipids which are combusted through glycolysis enter after the hexokinase stage but before the pyruvate kinase step (Geise, 1973) which may explain why the blocked hypothyroid pyruvate kinase value was significantly high and why the hexokinase value

in blocked hypothyroid rats was not quite significantly high.

Fatty acids were probably not being combusted to any great extent in the blocked hypothyroids because epinephrine deficiency would decrease circulating titres of free fatty acids (Ashmore et al., 1962; Love et al., 1963; Kvam et al., 1965; Mayer et al., 1965). Therefore, the combustion of amino acids could have been partially responsible for the heightened pyruvate kinase activity observed in the blocked hypothyroid rats. This hypothesis could be tested by studying a group of rats maintained on a low protein diet and closely monitoring body weights. There was no significant change in the body weights of the blocked hypothyroid animals, but they were fed a diet ad libitum which consisted of a mixture of lipids, carbohydrates, and proteins. If amino acids were combusted they could have been replaced through the diet.

Another possible explanation for the observed pyruvate kinase activity in the blocked hypothyroids is that the inability to break down glycogen created a stressful situation in the animal. Such a stress would stimulate the release of ACTH (adrenal corticotrophic hormone) from the pituitary gland. The circulating ACTH would stimulate the adrenal cortices to release glucocorticoids. Glucocorticoids enhance gluconeogenesis (production of new glucose from amino acids, lipids, and sugars) which would increase the levels of glucose (Turner and Bagnara, 1976). This explanation could be investigated by establishing a bilaterally adrenalectomized group thus restricting the release of glu-

cocorticoids.

It is also possible that low levels of T4, T3 and epinephrine in the blocked hypothyroid animals indirectly stimulated the endocrine pancreas to release glucagon in response to low blood sugar levels. Glucagon stimulates glycogenolysis in the liver and has an overall hyperglycemic effect on the blood (Levey and Epstein, 1969). If blood sugar levels did rise, then insulin was probably released by the endocrine pancreas facilitating the movement of glucose into cells (Turner and Bagnara, 1976). If this did occur then it would be expected that the hexokinase value for the blocked hypothyroids would be significantly higher along with the pyruvate kinase value for that group. Hexokinase activity in blocked hypothyroid animals was not significantly higher than any of the other groups. Therefore, the endocrine pancreas was probably not stimulated to any great extent in the blocked hypothyroid rats. This could be checked by establishing a group of rats in which glucagon and insulin levels were chemically or surgically lowered.

In summary, significant interacting effects of T4 and epinephrine on hexokinase and pyruvate kinase activities of rat myocardium were exhibited, implying direct or indirect action of T4 and epinephrine on glycolysis. More than likely, the actions were indirect because a hyperthyroid state causes an increase in glycogenolysis thus elevating glucose levels which would stimulate the various energy producing pathways including glycolysis. Further studies are needed to provide information on

possible interactions of T4 and epinephrine with various other hormones such as glucagon, insulin, and glucocorticoids on metabolism in cardiac tissues of hyperthyroid mammals.

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Table 1. Group arrangement showing the number of rats per group, type of injection, and injectable.

Group Name	No. of Rats	Injection Type and Injectable
Unblocked hypothyroid	7	*S.C. saline and **I.P.distilled water
Unblocked hyperthyroid	6	S.C. L-thyroxine and I.P. distilled water
Blocked hypothyroid	7	S.C. saline and I.P. dl-timolol maleate
Blocked hyperthyroid	7	S.C. L-thyroxine and dl-timolol maleate

* S.C. = subcutaneous

**I.P. = intraperitoneal

L-thyroxine dosage was 75µg/100g body weight

dl-timolol maleate dosage was 1mg/kg body weight

Distilled water and saline dosages were the same as dl-timolol maleate and L-thyroxine dosages respectively.

Table 2. Per cent difference, T-values, and P-values of mean activity values for $Na^+ + K^+$-dependent ATPase between unblocked, blocked, hypothyroid, and hyperthyroid groups.

Groups Compared	Per Cent Difference	T-Value	P-Value
Unblocked hypothyroid - Unblocked hyperthyroid	+5.8	0.201	>0.25
Blocked hypothyroid - Blocked hyperthyroid	+6.1	0.428	>0.25
Unblocked hypothyroid - Blocked hypothyroid	-3.5	0.127	>0.25
Unblocked hyperthyroid - Blocked hyperthyroid	-2.9	0.168	>0.25

Table 3. Per cent difference, T-values, and P-values of mean activity values for Hexokinase between unblocked, blocked, hypothyroid, and hyperthyroid groups.

Groups Compared	Per Cent Difference	T-Value	P-Value
Unblocked hypothyroid - Unblocked hyperthyroid	+18	2.24	<0.05
Blocked hypothyroid - Blocked hyperthyroid	+6.5	0.87	>0.25
Unblocked hypothyroid - Blocked hypothyroid	+4	0.56	>0.25
Unblocked hyperthyroid - Blocked hyperthyroid	-8.6	0.99	>0.25

Table 4. Per cent difference, T-values, P-values of mean activity values for Pyruvate Kinase between unblocked, blocked, hypothyroid, and hyperthyroid groups.

Groups Compared	Per Cent Difference	T-Value	P-Value
Unblocked hypothyroid - Unblocked hyperthyroid	+23.3	3.36	<0.01
Blocked hypothyroid - Blocked hyperthyroid	-9.4	1.6	>0.05
Unblocked hypothyroid - Blocked hypothyroid	+17.8	2.18	<0.05
Unblocked hyperthyroid - Blocked hyperthyroid	-15.4	3.34	<0.01

Figure 1. Preliminary data for rat heart $Na^+ + K^+$-dependent ATPase activity showing μmoles Pi/min. versus various volumes homogenate. Values are averages of two samples.

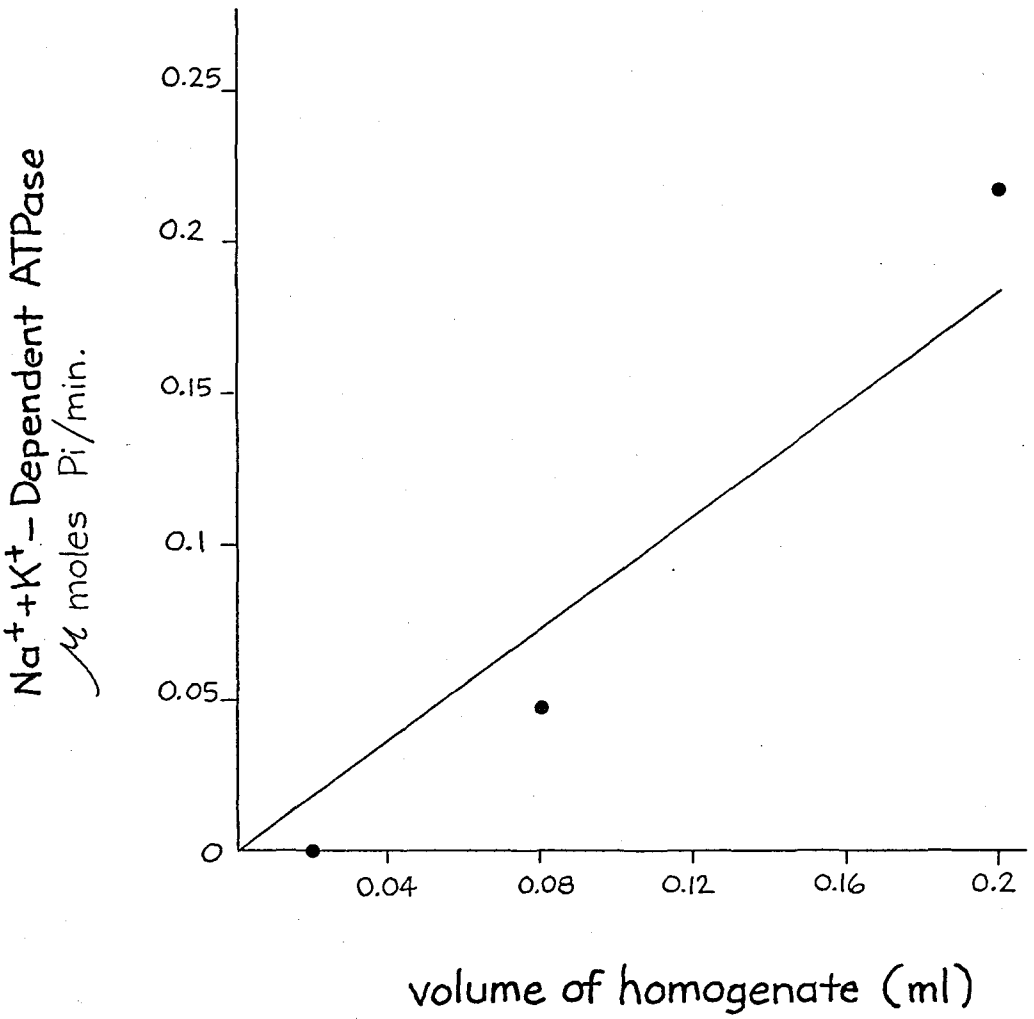

$Na^+ + K^+$ – Dependent ATPase
μ moles Pi/min.
0.25
0.2
0.15
0.1
0.05
0
0.04
0.08
0.12
0.16
0.2
volume of homogenate (ml)

Figure 2. Preliminary data for rat heart hexokinase showing ΔA/min. versus varied volumes of a 400 times diluted supernatant. Values are averages of two samples.

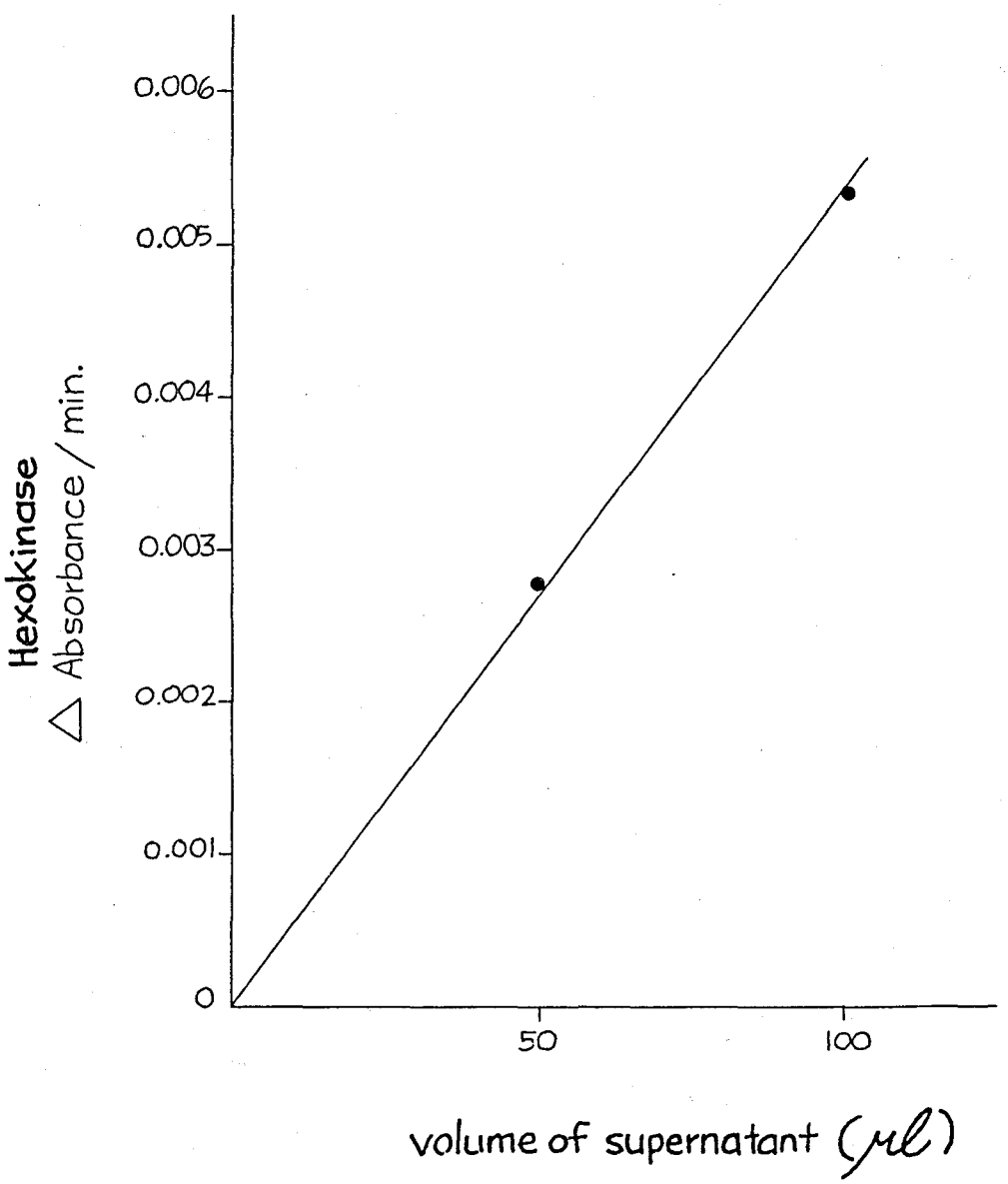

Hexokinase
△ Absorbance / min.
0.006
0.005
0.004
0.003
0.002
0.001
0
50
100
volume of supernatant (μl)

Figure 3. Preliminary data for rat heart pyruvate kinase showing ΔA/min. versus varied volumes of a 400 times diluted supernatant. Values are averages of two samples.

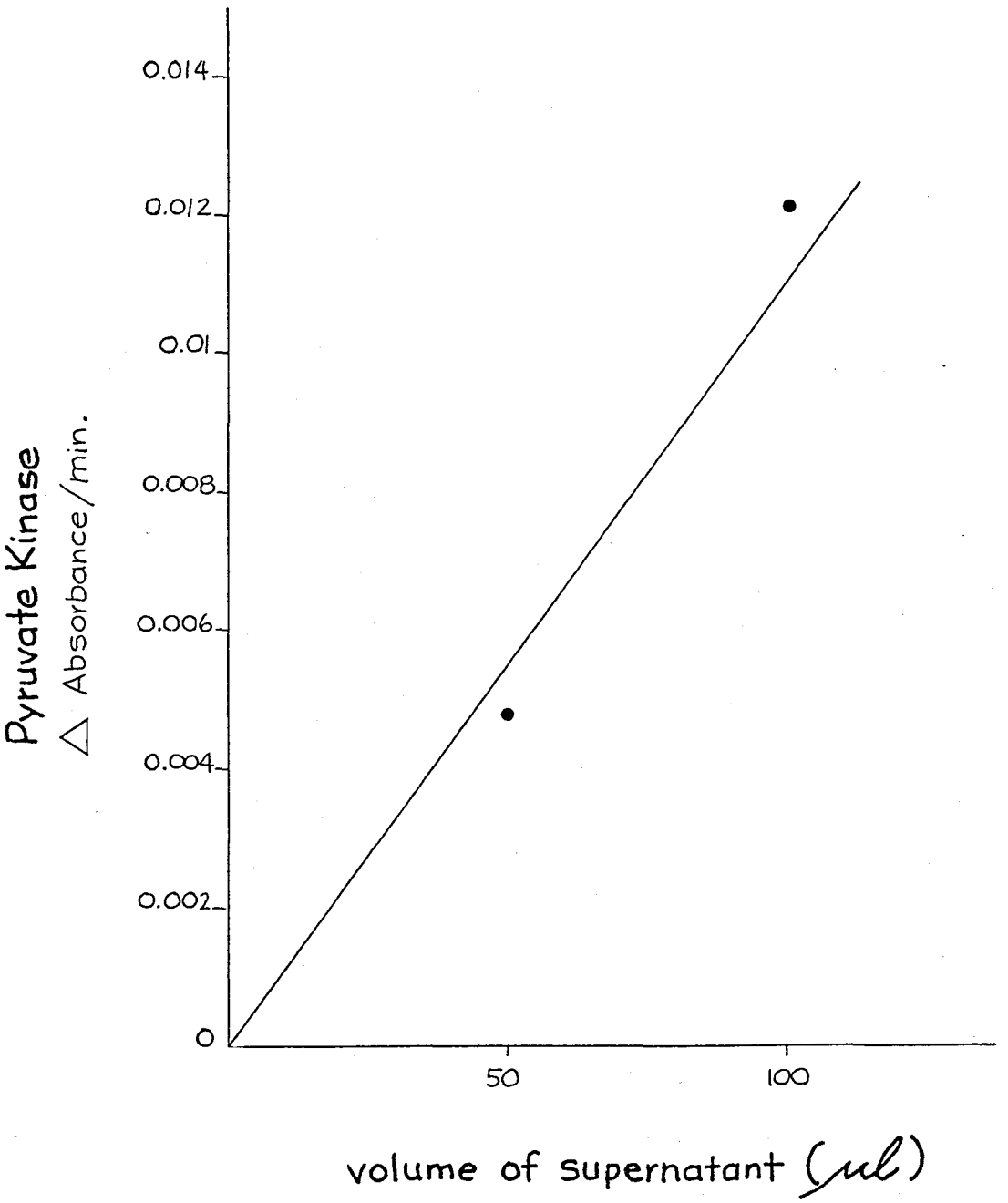

Pyruvate Kinase
△ Absorbance/min.
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
50
100
volume of supernatant (µl)

Figure 4. Comparison of group mean specific activity values (± S.E.) for rat heart Na^++K^+-dependent ATPase.

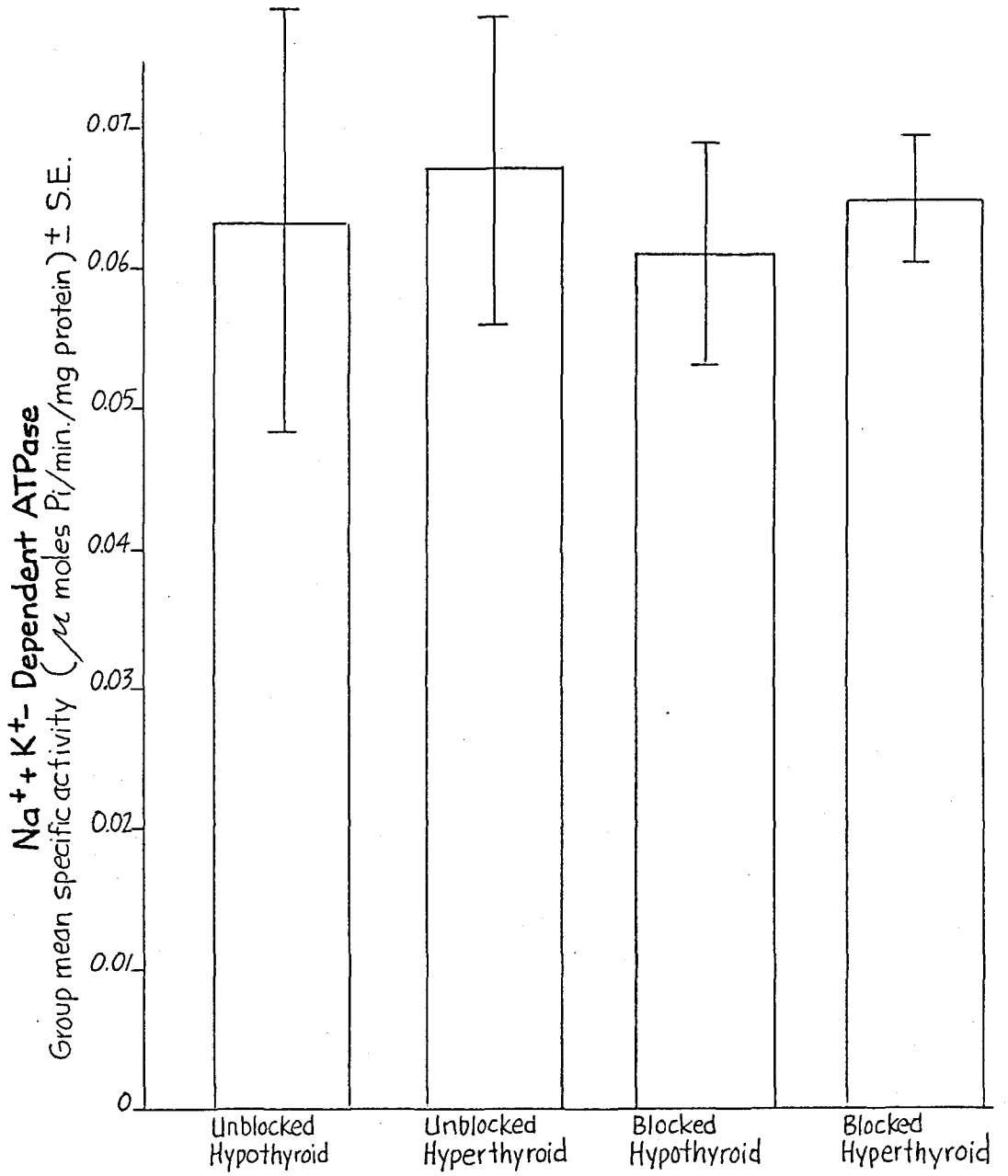

Na+ + K+- Dependent ATPase
Group mean specific activity (μ moles Pi/min./mg protein) ± S.E.
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Unblocked Hypothyroid
Unblocked Hyperthyroid
Blocked Hypothyroid
Blocked Hyperthyroid

Figure 5. Comparison of group mean specific activity values (± S.E.) for rat heart hexokinase.

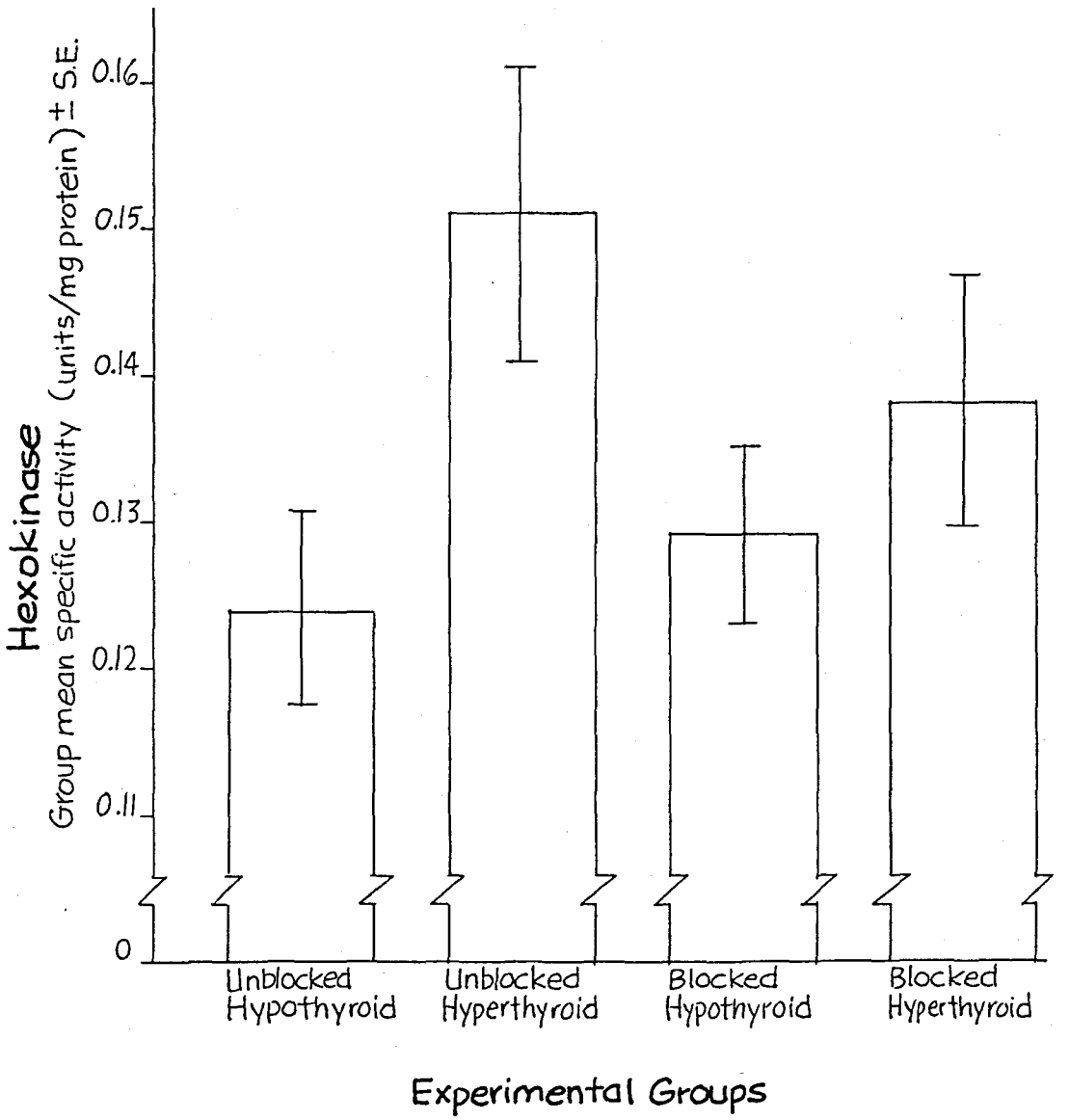
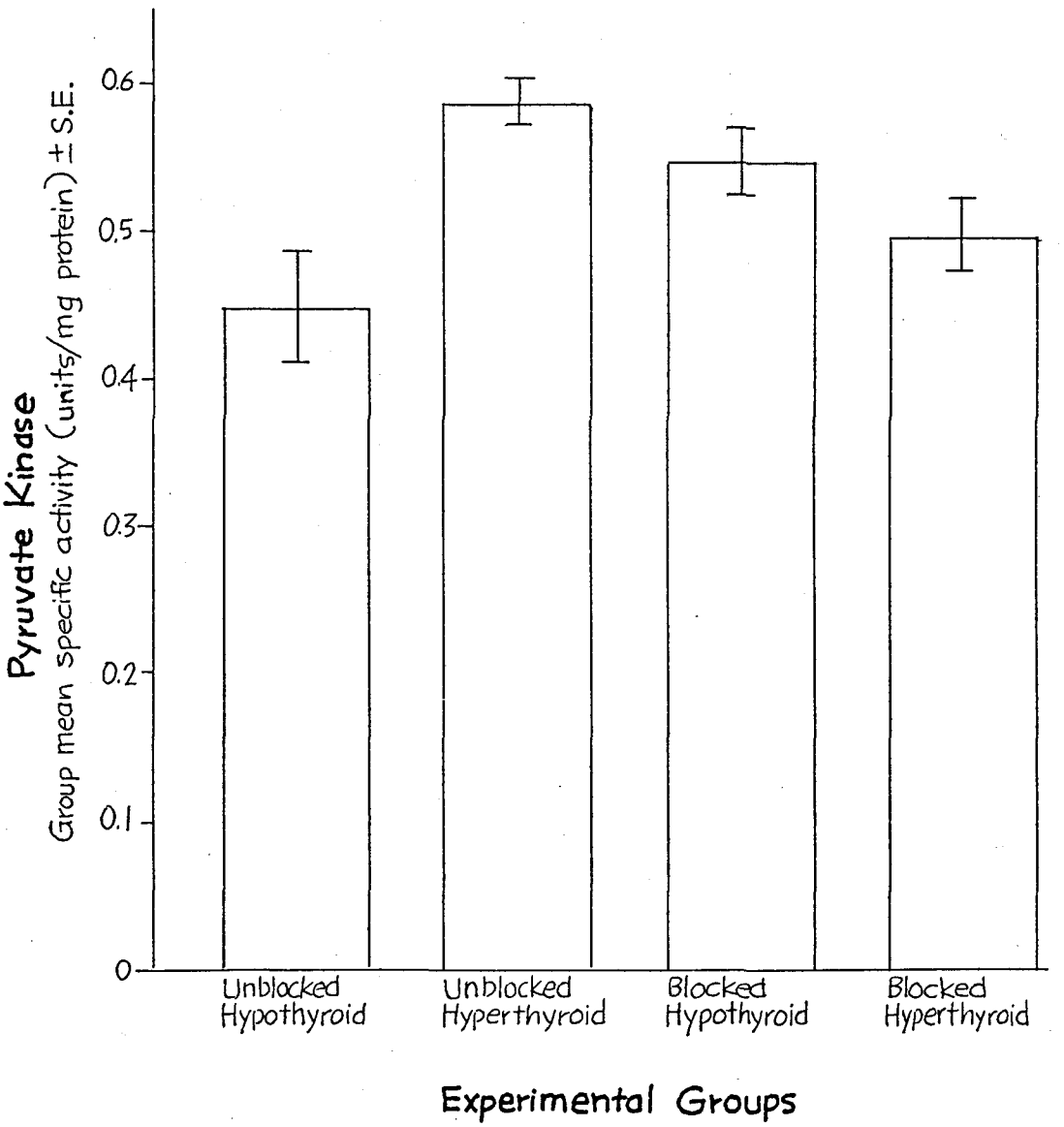

Hexokinase
Group mean specific activity (units/mg protein) ± S.E.
0.16
0.15
0.14
0.13
0.12
0.11
0
Unblocked Hypothyroid
Unblocked Hyperthyroid
Blocked Hypothyroid
Blocked Hyperthyroid
Experimental Groups

Figure 6. Comparison of group mean specific activity values (± S.E.) for rat heart pyruvate kinase.


Pyruvate Kinase
Group mean specific activity (units/mg protein) ± S.E.
0.6
0.5
0.4
0.3
0.2
0.1
0
Unblocked Hypothyroid
Unblocked Hyperthyroid
Blocked Hypothyroid
Blocked Hyperthyroid
Experimental Groups

VITA

Timothy Joseph Dietrick was born on August 4, 1955, in Philadelphia, Pennsylvania. He received his primary education in the Richmond Parochial Schools System and graduated from Benedictine High School in June, 1973. He then attended the University of Richmond, Richmond, Virginia, where he majored in Biology and received a degree of Bachelor of Arts in May, 1977. While enrolled at the University of Richmond he was elected into Beta Beta Beta Honorary Biological Society. Upon graduation he began graduate studies in Biology at the University of Richmond and received a Master of Science degree in August, 1979. A career in Veterinary Medicine is planned for the future.