

Thyroidal Influence on Cardiac Carbohydrate

Metabolism

A Thesis

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To My Parents

## TABLE OF CONTENTS

|  | Page |
|--|------|
| ACKNOWLEDGEMENTS.....                                | I    |
| List of Tables.....                                  | II   |
| List of Abbreviations.....                           | IV   |
| Diagram of the Main Pathways of Glucose Metabolism.. | VI   |
| ABSTRACT.....  | VII  |

### HISTORICAL REVIEW

|  |    |
|--|----|
| Thyroid hormones and the calorigenic effect....  | 1  |
| Effect of thyroid hormones on oxygen consumption.....  | 1  |
| Mitochondrial studies.....   | 2  |
| Hyperthyroidism and oxidative phosphorylation in mitochondria.....                                   | 2  |
| Hypothyroidism and oxidative phosphorylation in mitochondria.....                                    | 4  |
| In vitro effects of thyroid hormones on oxidative phosphorylation in whole mitochondria.....         | 6  |
| In vitro effects of thyroid hormones on oxidative phosphorylation in submitochondrial particles..... | 7  |
| Mitochondrial swelling.....  | 9  |
| Regulation of oxidative processes by thyroid hormones.....   | 12 |
| In vitro effects of thyroid hormones on enzymes  |    |
| (a) Citric acid cycle.....   | 13 |
| (b) Carbohydrate metabolism.....   | 15 |
| In vivo effects of thyroid hormones on enzymes   |    |
| (a) Citric acid cycle.....   | 15 |

## TABLE OF CONTENTS

|  | Page |
|--|------|
| ACKNOWLEDGEMENTS.....                                | I    |
| List of Tables.....                                  | II   |
| List of Abbreviations.....                           | IV   |
| Diagram of the Main Pathways of Glucose Metabolism.. | VI   |
| ABSTRACT.....  | VII  |

### HISTORICAL REVIEW

|  |    |
|--|----|
| Thyroid hormones and the calorogenic effect....  | 1  |
| Effect of thyroid hormones on oxygen consumption.....  | 1  |
| Mitochondrial studies.....   | 2  |
| Hyperthyroidism and oxidative phosphorylation in mitochondria.....                                   | 2  |
| Hypothyroidism and oxidative phosphorylation in mitochondria.....                                    | 4  |
| In vitro effects of thyroid hormones on oxidative phosphorylation in whole mitochondria.....         | 6  |
| In vitro effects of thyroid hormones on oxidative phosphorylation in submitochondrial particles..... | 7  |
| Mitochondrial swelling.....  | 9  |
| Regulation of oxidative processes by thyroid hormones.....   | 12 |
| In vitro effects of thyroid hormones on enzymes  |    |
| (a) Citric acid cycle.....   | 13 |
| (b) Carbohydrate metabolism.....   | 15 |
| In vivo effects of thyroid hormones on enzymes   |    |
| (a) Citric acid cycle.....   | 15 |

|   |            |
|---|------------|
| (b) Carbohydrate metabolism .....                           | Page<br>17 |
| Thyroid hormones and protein synthesis .....                | 21         |
| Stimulation of the nuclear matrix by thyroid hormones ..... | 21         |
| Introduction .....  | 29         |

## MATERIALS AND METHODS

|   |    |
|---|----|
| Chemicals and doses .....   | 32 |
| Experimental procedure .....  | 34 |
| Effect of radiothyroidectomy .....  | 34 |
| Effect of a single injection of triiodothyronine .....                                  | 34 |
| Actinomycin and cycloheximide inhibition of triiodothyromine-induced enzyme responses.. | 34 |
| Dose response studies with triiodothyronine .....                                       | 35 |
| Effects of hyperthyroidism .....  | 35 |
| Effects of cycloheximide, puromycin and ethionine .....                                 | 36 |
| Preparation of homogenates and supernatant fluids .....                                 | 36 |
| Measurement of basal metabolic rate .....   | 37 |
| Biochemical Procedure .....   | 38 |
| Enzyme assays .....   | 38 |
| Hexokinase .....  | 39 |
| Pyruvate kinase .....   | 39 |
| Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase .....            | 40 |
| $\alpha$ -glycerophosphate dehydrogenase .....  | 42 |
| Glyceraldehyde-3-phosphate dehydrogenase .....  | 43 |

| RESULTS  | Page |
|--|------|
| Influence of inhibitors of RNA and protein synthesis on basal metabolic rate of T <sub>3</sub> -treated rats .....             | 45   |
| Effects of inhibitors of RNA and protein synthesis on heart and body weights in T <sub>3</sub> -treated hypothyroid rats ..... | 45   |
| Influence of T <sub>3</sub> , cycloheximide and actinomycin D on cardiac enzyme activities in hypothyroid rats .....           | 48   |
| Duration of radiothyroidectomy on heart and body weights .....   | 54   |
| Prolongation of hypothyroidism on cardiac enzyme activities .....  | 54   |
| Effects of different doses of T <sub>3</sub> on heart and body weights as well as cardiac enzymes of hypothyroid rats .....    | 60   |
| Effects of inhibitors of RNA and protein synthesis on heart and body weights of hyperthyroid rats .....                        | 66   |
| Influence of inhibitors of RNA and protein synthesis on cardiac enzyme activities of hyperthyroid rats ,.....                  | 69   |
| Effects of inhibitors of RNA and protein synthesis on heart and body weights of euthyroid rats .....                           | 74   |
| Influence of inhibitors of RNA and protein synthesis on basal cardiac enzyme activities in euthyroid animals.....              | 76   |
| DISCUSSION .....   | 82   |
| SUMMARY .....  | 113  |
| REFERENCES .....   | 117  |

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## LIST OF TABLES

| Table  | Page |
|--|------|
| 1. Influence of inhibitors of RNA and protein synthesis on basal metabolic rate of triiodothyronine-treated rats .....   | 46   |
| 2. Effects of inhibitors of RNA and protein synthesis on heart and body weights in triiodothyronine-treated hypothyroid rats...  | 47   |
| 3. Influence of triiodothyronine, cycloheximide and actinomycin D on the activities of cardiac hexokinase and pyruvate kinase of hypothyroid rats .....  | 50   |
| 4. Effects of cycloheximide and actinomycin D on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in the myocardium of triiodothyronine injected hypothyroid rats .....                         | 51   |
| 5. Influence of inhibitors of RNA and protein synthesis on the activities of cardiac $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase in triiodothyronine-treated hypothyroid animals ..... | 52   |
| 6. Duration of effects of radiothyroidectomy on heart and body weights .....   | 55   |
| 7. Effects of prolonged duration of hypothyroidism on cardiac hexokinase and pyruvate kinase activities .....  | 57   |
| 8. Effects of prolonged duration of radiothyroidectomy on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in the myocardium of rats .....  | 58   |
| 9. Effects of prolonged duration of hypothyroidism on myocardial $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities .....  | 59   |
| 10. Effects of different doses of triiodothyronine on heart and body weights of hypothyroid rats .....   | 61   |

### III

|  | Page |
|--|------|
| 11. Influence of various doses of triiodothyronine on the activities of cardiac hexokinase and pyruvate kinase of hypothyroid rats .....   | 63   |
| 12. Effects of varying doses of triiodothyronine on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in the myocardium of hypothyroid rats .....                    | 64   |
| 13. Influence of different doses of triiodothyronine on the activities of myocardial $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase in hypothyroid rats ..... | 65   |
| 14. Effects of inhibitors of RNA and protein synthesis on heart and body weights of hyperthyroid rats .....  | 67   |
| 15. Influence of inhibitors of RNA and protein synthesis on cardiac hexokinase and pyruvate kinase activities in hyperthyroid rats .....   | 70   |
| 16. Effects of inhibitors of RNA and protein synthesis on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in the myocardium of hyperthyroid rats ..                | 71   |
| 17. Influence of inhibitors of RNA and protein synthesis on myocardial $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities in hyperthyroid rats.        | 72   |
| 18. Effects of inhibitors of RNA and protein synthesis on heart and body weights of euthyroid rats .....   | 75   |
| 19. Influence of inhibitors of RNA and protein synthesis on the activities of cardiac hexokinase and pyruvate kinase of euthyroid rats .....   | 78   |
| 20. Effects of inhibitors of RNA and protein synthesis on basal enzyme activities of myocardial glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase .....                         | 79   |
| 21. Influence of inhibitors of RNA and protein synthesis on basal enzyme activities of cardiac $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase .....           | 80   |

## LIST OF ABBREVIATIONS

|                   |   |
|-------------------|---|
| ADP               | - adenosine-5'-diphosphate                            |
| $\alpha$ -GP      | - $\alpha$ -glycerophosphate                          |
| $\alpha$ -GPdh    | - $\alpha$ -glycerophosphate dehydrogenase            |
| ATP               | - adenosine-5'-triphosphate                           |
| BMR               | - basal metabolic rate                                |
| DHAP              | - dihydroxyacetone phosphate                          |
| DNA               | - deoxyribose nucleic acid                            |
| FDP               | - fructose-1-6-diphosphate                            |
| G3P               | - glyceraldehyde-3-phosphate                          |
| G6P               | - glucose-6-phosphate                                 |
| G-6Pdh            | - glucose-6-phosphate dehydrogenase                   |
| HK                | - hexokinase  |
| HMP               | - hexose monophosphate                                |
| LDH               | - lactate dehydrogenase                               |
| mRNA              | - messenger ribose nucleic acid                       |
| $\text{NAD}^+$    | - nicotinamide adenine dinucleotide                   |
| NADH              | - reduced nicotinamide adenine dinucleotide           |
| $\text{NADP}^+$   | - nicotinamide adenine dinucleotide phosphate         |
| NADPH             | - reduced nicotinamide adenine dinucleotide phosphate |
| PEP               | - phosphoenol pyruvate                                |
| 6-PGdh            | - 6-phosphogluconate dehydrogenase                    |
| 6-P-gluconic acid | - 6-phosphogluconic acid                              |

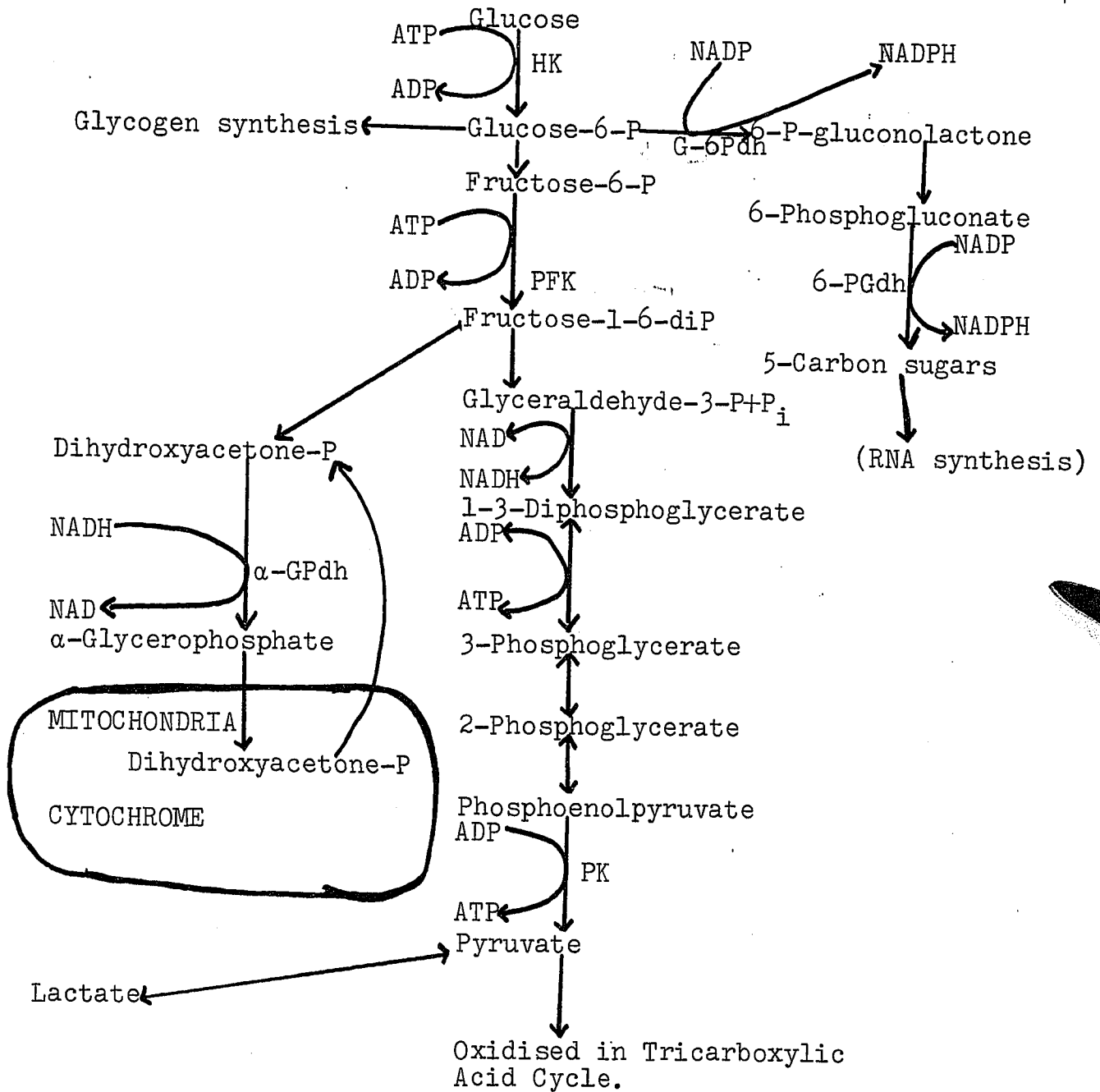
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6-P-gluconolactone - 6-phosphogluconolactone

PK - pyruvate kinase

T<sub>3</sub> - triiodothyronine

Diagram of the main pathways of glucose metabolism.



## ABSTRACT

Administration of thyroid hormones is known to result in marked structural and metabolic changes in myocardial tissue. The present study concerned the effects of altered thyroid states on representative rate-limiting enzymes of the glycolytic, hexose monophosphate and the  $H^+$  ion shuttle pathways in the myocardium of the male rat. In addition, the  $NAD^+$ -dependent glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase, has also been investigated.

Hypothyroidism, induced four weeks after a single injection of  $^{131}I$ , produced marked decreases in the activities of hexokinase to 20%, pyruvate kinase to 41%, glyceraldehyde-3-phosphate dehydrogenase to 43%, glucose-6-phosphate dehydrogenase to 42%, 6-phosphogluconate dehydrogenase to 43% and  $\alpha$ -glycerophosphate dehydrogenase to 42%. Forty-eight hours after administration of a single dose of triiodothyronine (34  $\mu g/100$  g body weight) to hypothyroid animals, the activities of all enzymes studied were significantly augmented. Concomitant administration of cycloheximide (70  $\mu g/100$  g) or actinomycin D (8  $\mu g/100$  g) with triiodothyronine to hypothyroid animals partially inhibited the triiodothyronine-induced increase in the activities of all cardiac enzymes studied.

## VIII

Hyperthyroidism, induced by administration of triiodothyronine (250  $\mu\text{g}/100\text{ g/day}$ ) to euthyroid animals for seven days produced significant increases in the activities of hexokinase to 175%, pyruvate kinase to 167%, glyceraldehyde-3-phosphate dehydrogenase to 147%, glucose-6-phosphate dehydrogenase to 204%, 6-phosphogluconate dehydrogenase to 437% and  $\alpha$ -glycerophosphate dehydrogenase to 182% of euthyroid values respectively. When cycloheximide (70  $\mu\text{g}/100\text{ g}$ ), puromycin (10  $\text{mg}/100\text{ g}$ ), or ethionine (100  $\text{mg}/100\text{ g}$ ), inhibitors of RNA and protein synthesis, were administered along with triiodothyronine for one week, the expected increases in enzyme activities were suppressed significantly indicating that the triiodothyronine-induced increases in the activities of these enzymes may have been the result of new enzyme protein formation.

Euthyroid animals, when treated with daily injections of cycloheximide (70  $\mu\text{g}/100\text{ g}$ ), puromycin (10  $\text{mg}/100\text{ g}$ ) or ethionine (100  $\text{mg}/100\text{ g}$ ), inhibitors of RNA and protein synthesis, for one week, exhibited marked decreases in basal activities of hexokinase, pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and  $\alpha$ -glycerophosphate dehydrogenase. The results suggest that these compounds

## IX

affect the rate of turnover of cardiac enzyme proteins of the animal in a steady state.

The data indicate that thyroid hormones are capable of eliciting profound changes in the protein synthesising ability of the heart.

## HISTORICAL REVIEW

### Thyroid hormones and the calorigenic effect

In 1895 Magnus Levy discovered that thyroid hormones increased overall oxygen consumption in hypothyroid and normal individuals and termed this action of thyroid hormones the "calorigenic effect". Until recently, the principal role of thyroid hormones was considered to be the control of basal metabolic rate, an index which is still used clinically as a measure of thyroid function. The evidence to be presented suggests that this may not be so.

### Effect of thyroid hormones on oxygen consumption

Gordon and Heming (1944), in experiments designed to show the effects of thyroxine on oxygen consumption of various tissues in the rat, demonstrated that slices of liver, kidney, diaphragm and heart from thyrotoxic animals consumed oxygen at a faster rate than normal, while those of spleen, brain and testis did not.

Barker and Klitgaard (1952) reported decreased rates of oxygen consumption in liver and kidney of rats as well as cardiac, skeletal and gastric mucosal muscles following thyroidectomy, which were reversed to various extents after injection of a single dose of thyroxine. In addition, the effect of thyroxine was greatest on the heart. In contrast, the oxygen consumption of brain, spleen and testis remained unchanged under these experimental conditions. These results

suggested, that in spite of the increased metabolic rate observed in the whole animal after thyroxine administration in vivo, not all tissues respond to the same degree.

### Mitochondrial studies

Based on the experiments of Lehninger (1959), it is now well known that mitochondria incorporate a solid state energy-transforming macromolecular mechanism which oxidises substrates and produces the high-energy bonds of ATP. Hoch (1962) noted that, since the mitochondria were the principal loci of oxidative activity, this process of oxidative phosphorylation was considered as a target both for the regulating activities and for the action of excess amounts of thyroid hormones. Thus, the effects of various thyroid states, including hyper- and hypothyroidism as well as the addition of thyroid hormone to mitochondria in vitro, on mitochondrial respiration and oxidative phosphorylation were extensively studied by a number of investigators.

### Hyperthyroidism and oxidative phosphorylation in mitochondria

In 1951, Niemeyer et al, Martius and Hess, as well as Lardy and Feldott, reported that mitochondria from

hepatic tissue of hyperthyroid rats had an enhanced oxygen consumption. In agreement with Niemeyer et al (1951), Fairhurst et al (1959) reported, that well washed mitochondria extracted from liver tissues of hyperthyroid rats exhibited an increased rate of oxygen consumption without any concomitant alteration in the efficiency of oxidative phosphorylation, in terms of an unchanged P/O ratio. Zaimis et al (1969) demonstrated that mitochondria from cardiac tissue of thyroxine-treated guinea pigs and cats showed increases in both ADP-stimulated and basal rates of respiration, although the efficiency of oxidative phosphorylation remained unaffected. Tata et al (1963) failed to demonstrate any alteration in the P/O ratio or in respiratory rate for either liver or muscle mitochondria when a change in basal metabolic rate was effected by doses of triiodothyronine small enough to have no effect on growth rate. Dow (1967) reported no changes in respiratory rates or oxidative phosphorylation in muscle mitochondria from thyrotoxic rats.

However, Martius and Hess (1951) as well as Lardy and Feldott (1951) found decreases in the efficiency of oxidative phosphorylation which were concomitant with an increase in oxygen consumption in hepatic mitochondria from hyperthyroid rats. Maley and Lardy (1955) also

demonstrated that mitochondria from thyrotoxic rat livers exhibited P/O ratios that were 50 percent lower than those of controls. Heninger et al (1970) observed that hepatic mitochondria from rats that were made hyperthyroid by acclimatization to extreme cold demonstrated an increase in oxygen consumption as well as a decrease in the P/O ratio in comparison to controls.

In the light of the evidence presently available, no definitive conclusions can be reached in regard to the effects of hyperthyroidism on both mitochondrial respiration and oxidative phosphorylation.

#### Hypothyroidism and oxidative phosphorylation in mitochondria

Maley and Lardy (1955), in attempting to elucidate the effects of hypothyroidism on mitochondrial respiration and oxidative phosphorylation, used mitochondria isolated from livers of thyroidectomized rats. No change in P/O ratios were observed although the rate of oxygen consumption was markedly decreased. Bronk and Bronk (1962) confirmed the results obtained by Maley and Lardy (1955) on mitochondrial respiration and oxidative phosphorylation in hepatic tissues of hypothyroid rats. In addition, Bronk and Bronk (1962) demonstrated increases

in the rates of oxidation and phosphorylation with no change in P/O ratio when hypothyroid rats were given physiological doses of thyroxine. Heninger et al (1970), working with rats subjected to thyroidectomy of varying completeness, were able to measure the thyroid hormone content of mitochondria prepared from hepatic tissues. This was accomplished by placing rats on an iodine free diet for four weeks followed by a period of thirty days in which these animals were fed a diet containing  $^{125}\text{I}$ . The amounts of  $^{125}\text{I}$  present as iodide, triiodothyronine and thyroxine in liver mitochondria were determined radiochromatographically. In addition, the rate of oxygen consumption and the P/O ratios were measured simultaneously under different thyroidal states in order to test the correlation of these three parameters. These workers found that mitochondria from liver of hypothyroid rats exhibited a decreased rate of oxygen consumption, an increased P/O ratio and a decreased thyroid hormone content when compared to controls. As the P/O ratio approached a value of 3, there was a progressive decrease in thyroid hormone content of hepatic mitochondria, i.e. an inverse linear relationship existed between mitochondrial thyroid hormone content and P/O ratio. They interpreted these results to mean that thyroid hormones

produce a "loosening" of the coupling of oxidation to phosphorylation in mitochondria.

Although hypothyroidism appears to reduce the rate of oxygen consumption by hepatic mitochondria, its effect on oxidative phosphorylation still needs further clarification.

In vitro effects of thyroid hormones on oxidative phosphorylation in whole mitochondria

Martius and Hess (1951), in experiments designed to show the in vitro effects of thyroxine on mitochondrial respiration and oxidative phosphorylation, demonstrated the uncoupling effect of thyroxine on rat liver mitochondria at concentrations of  $5 \times 10^{-5}$  and  $5 \times 10^{-4}$ M. Maley and Lardy (1953) successfully demonstrated the uncoupling of oxidative phosphorylation in both hepatic and kidney mitochondria by thyroxine at a concentration of  $2.6 \times 10^{-5}$ M. In addition, the rate of oxygen consumption was significantly lowered. Klemperer (1955b) confirmed the results of Maley and Lardy (1953) using hepatic mitochondria and thyroxine at a concentration of  $10^{-4}$ M. He also found a decreased rate of oxygen consumption by these organelles.

However, not all investigators have been successful

in demonstrating the uncoupling of oxidative phosphorylation by thyroid hormones in vitro.

Hoch and Lipmann (1954), using concentrations of thyroxine, ranging from  $10^{-5}$  to  $10^{-4}$ M, were unable to demonstrate any uncoupling of oxidative phosphorylation in rat liver mitochondria. Tapley and Cooper (1956b), using rat liver mitochondria incubated with thyroxine ( $10^{-4}$  M) in isotonic sucrose, reported no change in the P/O ratio, although an uncoupling of oxidative phosphorylation and a slight fall in oxygen consumption occurred when mitochondria were incubated with thyroxine and magnesium in hypotonic sucrose solution.

Based on this evidence no real conclusion concerning the uncoupling effect of thyroid hormones on oxidative phosphorylation in mitochondria in vitro can be made.

#### In vitro effects of thyroid hormones on oxidative phosphorylation in submitochondrial particles

The in vitro effects of thyroid hormones on submitochondrial particles with respect to oxidative phosphorylation and rate of respiration have also been investigated. Tapley and Cooper (1956b) showed that digitonin-prepared submitochondrial particles from rat liver were capable of catalyzing oxidative phosphorylation. When

these submitochondrial particles were incubated in hypotonic sucrose solution containing magnesium and thyroxine ( $10^{-4}$  M) no uncoupling of oxidative phosphorylation was observed.

However, Bronk (1960) reported an improved P/O ratio when smaller concentrations of thyroxine ( $2.5 \times 10^{-5}$  M) were added in vitro to rat liver submitochondrial particles obtained by sonication. At higher concentrations ( $2.5 \times 10^{-4}$  M), a decreased P/O ratio, which is indicative of uncoupling of oxidative phosphorylation, was observed with a concomitant decrease in oxygen consumption. The results obtained from such experiments would seem to indicate that submitochondrial particles obtained by sonication retain their integrity better than those prepared by digitonin.

Hoch (1962) and Tata (1964) have reviewed in detail the effects of thyroid hormones on mitochondria and concluded that the inconsistencies observed in earlier work may have resulted from factors which include the dose or concentration of the hormone, substrate used, cofactors added, preincubation procedure or method of preparation of mitochondria.

### Mitochondrial swelling

Tapley (1956) while studying the in vitro effects of thyroid hormones on mitochondrial respiration and oxidative phosphorylation, observed a decrease in optical density from 0.5 to 0.2 at 520 m $\mu$  of a suspension of mitochondria isolated from hepatic tissue of normal rats. This change in optical density was interpreted as a swelling of the mitochondria and suggested to Tapley (1956) that this swelling, which might cause alterations in enzyme-enzyme organization, resulted in the uncoupling of oxidative phosphorylation. Klemperer (1955a) also worked with rat liver mitochondria and found that after the first minute of incubation in serum medium, thyroxine was taken up before any change in mitochondrial structure was observed. The first sign of mitochondrial swelling was observed only after three minutes. Thus, mitochondrial swelling was probably not a direct effect of the hormone, but mediated by the release of some endogenous substance from within the organelle. In fact, Wojtczak and Lehninger (1961) demonstrated the release of a factor U, probably a fatty acid, from liver mitochondria incubated with calcium or thyroxine. These workers observed that the mitochondria from which factor U was released, were also swollen. Addition of ATP or serum albumin (which acts as a trap for

factor U by virtue of its ability to bind fatty acids) to swollen mitochondria, produced a shrinkage of the mitochondria and disappearance of factor U. Wojtczak and Lehninger (1961) concluded that both calcium and thyroxine, when added to hepatic mitochondria in vitro, caused the release of factor U, which in turn produced mitochondrial swelling. Wolff and Wolff (1964) in reviewing the biochemical effects of thyroid hormones have questioned the physiological significance of the factor and its release in mitochondria by thyroid hormones.

Tapley (1956) also investigated the swelling of mitochondria from livers of hyper- and hypothyroid rats and reported that the former swell more readily and the latter less readily than preparations from normal rats.

However, Tapley and Cooper (1956a) demonstrated that mitochondria from rat cardiac and skeletal muscles were less sensitive than liver to both thyroxine and triiodothyronine-induced swelling in vitro, although the former tissues and especially cardiac muscle, when isolated from thyroxine-treated rats, were responsive in terms of oxygen consumption (Barker and Klitgaard, 1952). In accordance with the failure of slices of spleen, brain and testis to show any change in oxygen consumption in thyrotoxic rats, essentially no swelling was produced by

thyroxine or its analogues with the mitochondria from these tissues. Zaimis et al (1969) confirming Tapley and Cooper's work (1956a) on cardiac muscle, reported that thyroxine treatment of heart muscle mitochondria in vitro had little effect on the swelling of these organelles. Only mitochondria isolated from hearts of thyroxine-treated cats showed small degrees of swelling.

The data presently available and the finding of Tapley and Cooper (1956a), that thyroxine was capable of inducing the in vitro swelling of hepatic but not cardiac mitochondria at a concentration ten times lower than that used to demonstrate uncoupling of oxidative phosphorylation in both tissues, cast doubts about the relationship between the two phenomena.

Since uncoupling of oxidative phosphorylation in vitro is induced by relatively large concentrations of thyroxine or its analogues (ranging from  $10^{-6}$  to  $5 \times 10^{-4}$  M) such a phenomenon could more rightly be termed a pharmacological response rather than a physiological mechanism of thyroid hormone action. Tata (1964) demonstrated that physiological doses of thyroid hormones were actually anabolic and energy-conserving while pharmacological doses produced uncoupling, which is a catabolic and energy-wasting process.

In addition, the conflicting evidence presented above on mitochondrial uncoupling from both in vivo (Martius and Hess, 1951; Zaimis et al, 1969) and in vitro (Maley and Lardy, 1955; Hoch and Lipmann, 1954) studies as well as the fact that other agents such as 2-4-dinitrophenol, which uncouple oxidative phosphorylation but fail to mimic fully the metabolic and physiological effects of the thyroid hormones, discredit uncoupling as the mode of physiological action of the thyroid hormones.

Hoch's findings (1967), that stimulation of mitochondrial respiration by small doses of thyroxine (5.2  $\mu\text{g/g}$ ) in vivo occurred in the absence of demonstrable uncoupling of oxidative phosphorylation or loosening of respiratory control, confirm that some additional mechanism of action of thyroid hormones must account for the physiological effects observed.

#### Regulation of oxidative processes by thyroid hormones

Hoch (1962) proposed that thyroid hormones affect the supply of biologically utilisable energy by altering the rates of oxidative processes in the particulate or soluble enzyme systems of the cell. He further stated that the activity of oxidative enzymes could be changed either by a direct chemical interaction between hormones

and active loci on enzymes and this could explain the in vitro effects of thyroid hormones on enzymes, or by alterations in enzyme concentration through increased or decreased rates of synthesis of specific catalyzing proteins, which could be the mechanism of the in vivo effect of thyroid hormones.

In vitro effects of thyroid hormones on enzymes

(a) Citric acid cycle

Gemmill (1952) reported that addition of thyroxine to an incubation mixture of rat kidney homogenate containing cytochrome C and succinate, augmented the rate of succinate oxidation. Clarke and Ball (1955) as well as Barker and Lewis (1956) also demonstrated an increase in succinate oxidation with the addition of either thyroxine or triiodothyronine to rat heart homogenates. Simultaneous inhibition of malate dehydrogenase activity was observed in these homogenates.

In mitochondria, succinate is metabolized to oxalacetate, via a portion of the citric acid cycle (succinate  $\rightarrow$  fumarate  $\rightarrow$  malate  $\xrightarrow{\text{NAD}^+}$  oxalacetate), while oxalacetate acts as a potent inhibitor of succinic dehydrogenase (Wolff and Wolff 1964). Wolff and Ball (1957) were able to show that sufficient oxalacetate accumulates

in the control experiment to account for inhibition of succinic dehydrogenase and that thyroxine prevents this by inhibition of malic dehydrogenase. Removal of oxalacetate, e.g. by promoting transamination with glutamate to form aspartate or by omission of  $\text{NAD}^+$  required for malate dehydrogenase, can also relieve the inhibition and thus eliminate the thyroxine effect.

Wolff and Wolff (1964) further demonstrated that fumarase activity was not altered by thyroid hormones in vitro. Hellerman et al (1960), working with the soluble succinic dehydrogenase prepared from hog sarcosomes, found that thyroxine was inhibitory with respect to the dehydrogenase itself. This was in contrast to the action of thyroxine as observed by Clarke and Ball (1955), Barker and Lewis (1956) as well as Wolff and Ball (1957) in studies of homogenates containing, in addition to succinate dehydrogenase, malate dehydrogenase, fumarase and DPN. Wolff and Wolff (1957) also demonstrated that thyroxine, when added in vitro to purified malic dehydrogenase, produced an inhibition of this enzyme which is in agreement with the studies using homogenates and mitochondria (Clarke and Ball, 1955; Barker and Lewis, 1956).

(b) Carbohydrate metabolism

Wolff and Wolff (1957) studied the in vitro effects of thyroxine on a number of dehydrogenases obtained from various sources. Crystalline preparations of muscle glyceraldehyde-3-phosphate dehydrogenase and lactic dehydrogenase as well as purified glucose-6-phosphate dehydrogenase prepared from yeast were all inhibited by thyroxine addition in vitro.

The presently available evidence indicates that in vitro thyroid hormones are primarily capable of inhibiting enzymes of the glycolytic, hexose monophosphate shunt and citric acid cycle pathways.

In vivo effects of thyroid hormones on enzymes

(a) Citric acid cycle

Maley (1957), in attempts to correlate the in vitro and in vivo effects of thyroid hormones on succinoxidase, reported increases and decreases of hepatic succinoxidase activity under conditions of hyper- and hypothyroidism respectively. Tipton et al (1959) confirmed Maley's work in regard to the effects of thyroxine on liver succinoxidase activity. In experiments in which rats were fed propylthiouracil, an inhibitor of iodide oxidation to iodine and iodination of monoiodotyrosine

in the thyroid gland (Ganong, 1967), they demonstrated a decrease in hepatic succinoxidase activity. When partially hepatectomized propylthiouracil-fed rats were administered various doses of thyroxine, increases in succinoxidase activity were found in the regenerating liver, demonstrating that thyroxine was responsible for the maintenance of this enzyme in the liver of the rat. Gangloff et al (1960), in attempting to elucidate whether the increases in hepatic succinoxidase activity following thyroxine treatment were due to release of product inhibition by decreased levels of oxalacetate as reported for in vitro studies (Wolff and Ball, 1957), found elevated oxalacetate levels in liver and skeletal muscle of hypothyroid rats while a depression was observed in the hyperthyroid animal. These results suggested that the in vitro mechanism of thyroxine stimulation of succinoxidase could also account for the in vivo effects.

Wolff and Wolff (1964) demonstrated that liver and kidney slices obtained from thyrotoxic rats were capable of enhancing the metabolism of citrate and fumarate when added in vitro. Fumarate oxidation was also greatly enhanced by diaphragms from hyperthyroid rats while mitochondrial preparations obtained from livers of thyrotoxic rats rapidly oxidized exogenously added

$\alpha$ -ketoglutarate. This evidence suggests an enhancement of the activities of enzymes involved in the citric acid cycle, in direct contrast to the in vitro effects of thyroxine on these enzymes, which have all been inhibitory.

(b) Carbohydrate metabolism

Dow and Allen (1961) studied the in vivo effects of thyroid hormones on glycolysis. They found an increase in glucose oxidation, by glycolysis rather than the pentose phosphate pathway, in hyperthyroid rats subjected to a labelled glucose load. Such an action of thyroid hormones could be effected by altering the activity of key enzymes of the glycolytic pathway.

Bargoni and Grillo (1967) demonstrated increased activities of hexokinase, phosphofructokinase, aldolase and phosphoglyceromutase in skeletal muscle and kidney tissue of hyperthyroid rats.

Tata et al (1963) reported increased lactate dehydrogenase activity in hepatic tissues of hyperthyroid rats.

Furthermore, Bargoni and Grillo (1967) showed that hypothyroidism caused a decrease in muscle pyruvate kinase activity.

Krause et al (1967) also reported markedly depressed activities of enzymes of the "phospho-trioseglycerate

group", namely glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, glycerate phosphomutase and enolase as well as the glycogen cycle enzymes, UDPG pyrophosphorylase, glycogen phosphorylase and glycogen synthetase, from skeletal muscle of hypothyroid rats.

Triiodothyronine treatment to hypothyroid animals increased enzyme activities of both the "phosphotrioseglycerate group" and the glycogen cycle. These workers concluded that extramitochondrial enzyme pattern in muscle was at least to some extent related to thyroid state. Furthermore, the disappearance and reappearance of the enzymes studied under conditions of radiothyroidectomy and triiodothyronine replacement was possibly the result of hormone action at the genetic level.

Additionally, Glock and McLean (1955) demonstrated that hyperthyroidism was associated with increases in both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in hepatic tissue. This is in direct contrast to the in vitro effects of thyroxine observed on glucose-6-phosphate dehydrogenase activity (Wolff and Wolff, 1957).

It also appears as though mitochondrial  $\alpha$ -glycerophosphate dehydrogenase activity is under thyroidal control. Lardy et al (1960) and Lee and Lardy (1965) reported

that the activity of mitochondrial  $\alpha$ -glycerophosphate dehydrogenase, which was markedly depressed in cardiac and hepatic tissues of hypothyroid rats, was significantly elevated following administration of a physiological dose of triiodothyronine and rose to even greater heights (2200 percent of control) in the thyrotoxic condition. The increase in enzyme activity was most likely due to the de novo synthesis of new dehydrogenase protein, for administration of ethionine, an inhibitor of protein biosynthesis blocked the triiodothyronine-induced increases. Sellinger and Lee (1964) further showed that, when puromycin or actinomycin D were administered along with triiodothyronine, hepatic mitochondrial  $\alpha$ -glycerophosphate dehydrogenase activity remained at basal levels, i.e. induction of this enzyme by triiodothyronine was completely blocked.

Lardy et al (1960) postulated that regeneration of cytoplasmic  $\text{NAD}^+$  in hepatic tissue is dependent upon the  $\alpha$ -glycerophosphate shunt pathway, in which dihydroxyacetone phosphate is reduced to  $\alpha$ -glycerophosphate by  $\alpha$ -glycerophosphate dehydrogenase, with NADH acting as a hydrogen ion donor in the cytoplasm. The  $\alpha$ -glycerophosphate formed is reoxidized intramitochondrially to dihydroxyacetone phosphate by mitochondrial  $\alpha$ -glycerophosphate

dehydrogenase.  $H^+$  ions are thus funnelled from the cytoplasm into the mitochondria and then through the electron transport chain. Since  $NAD^+$  is required as a coenzyme for the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase in the Embden-Meyerhof pathway, under conditions in which  $\alpha$ -glycerophosphate dehydrogenase activity remains unchanged but glycolysis is accelerated, the  $\alpha$ -glycerophosphate cycle would limit the rate of substrate oxidation at the glyceraldehyde-3-phosphate dehydrogenase step.

The available evidence indicates that experiments in which thyroid hormones were added in vitro to either tissue preparations or purified oxidative enzymes such as glucose-6-phosphate dehydrogenase and lactate dehydrogenase conflict with the results obtained when the activities of these enzymes were assayed from thyroid-treated animals. This implies that a direct reaction between the active sites of individual oxidative enzymes and the thyroid hormones does not occur in vivo. The available data suggest that the finding of increased enzyme activity in vivo following thyroid hormone treatment appears to be indirect i.e. by the induction of enzyme synthesis rather than by activation of pre-existing protein.

### Thyroid hormones and protein synthesis

Sokoloff and Kaufmann (1961) showed increased rates of incorporation of labelled leucine-1-C<sup>14</sup> into proteins of cell-free hepatic homogenates following incubation with thyroxine. Hypo- and hyperthyroidism were associated with decreased and increased rates of leucine incorporation into proteins of rat liver respectively.

Additionally, Michels et al (1963) demonstrated that kidney and heart tissues of hyperthyroid rats displayed elevated rates of amino acid incorporation while spleen, brain and testis did not. In these experiments the mitochondrial fraction as well as a substrate for oxidation by the mitochondria were considered necessary for increased incorporation. These investigators suggested that thyroid hormones probably interact with mitochondria to release some factor that initiates an increase in protein synthesis, since it had previously been shown that even in the presence of an ATP-generating system no significant change in amino acid incorporation was observed in a cell-free preparation from hyperthyroid rats.

Bronk (1963) proposed that ATP was not required for mitochondrial protein synthesis but that high energy intermediates in the phosphorylation process might serve

as, at least a partial energy source for the incorporation process. His conclusions were based on experiments in which rat liver mitochondria incorporated amino acids into proteins either in the absence of ATP or upon addition of inhibitors, which block terminal phosphate addition during ATP synthesis.

Weiss and Sokoloff (1963) measured basal metabolic rate and demonstrated that the hypermetabolic effect in rats treated with thyroxine for six to thirteen days could be reversed two hours after puromycin was administered.

Tata (1963) demonstrated that both the calorigenic effect (BMR) and increase in body weight induced by small physiological doses of triiodothyronine to hypothyroid rats, were greatly suppressed by actinomycin or puromycin treatment. Since actinomycin D inhibits the DNA-directed synthesis of mRNA (Reich, 1963) and puromycin inhibits protein synthesis by forming a peptide bond between the terminal carboxyl group of the growing peptide, thus causing the release of the unfinished peptide chain from the ribosome template (Yarmolinsky and De La Haba, 1959), the results obtained with these antibiotics suggest that thyroid hormones influence both the rate of protein synthesis as well as oxygen consumption (BMR)

and that the latter phenomenon is in some way associated with the former.

Sokoloff et al (1964) in trying to elucidate whether the thyroid hormone-induced increase in rat liver protein synthesis was the result of nuclear stimulation, found that there was no enhancement in labelled AMP incorporation into RNA although accelerated rates of amino acid incorporation into proteins were observed. Addition of either RNA polymerase or actinomycin D to the incubation medium was without any effect on the thyroxine-induced amino acid incorporation into proteins. The results of these studies suggested to Sokoloff et al (1964) that the mechanism by which the thyroid hormones regulate the rate of protein synthesis was independent of any action at the gene level.

Hoch (1967) working with hepatic tissue, reported that two minutes after injection of a physiological dose of thyroxine to thyroidectomized rats, an increase in state four respiration to normal levels occurred, while state three respiration remained unchanged. The latter respiratory state has been defined by Chance & Williams (1956) as the active state of rapid respiration and phosphorylation, with adequate supplies of substrate and phosphate acceptor being present. In this state the level of cytochrome is maintained constant while ADP levels change over a wide range. The former respiratory state (State 4) may be considered as a resting aerobic state, which is characterized by a low

respiration rate even though substrate is present. In this state the concentrations of both  $\text{NAD}^+$  and phosphate acceptors are greatly reduced. Furthermore, thyroxine content in hepatic tissue as determined by the butanol-extractable and butanol-insoluble iodine, was increased 550-fold, three hours after thyroxine injection. Hoch (1967) was unable to demonstrate that the increased oxygen consumption was the result of protein synthesis. He therefore concluded that the primary action of thyroxine was on the mitochondrion but failed to explain how such an action mediates the other biochemical effects observed, such as increased protein synthesis. It is possible that the interaction between thyroxine and mitochondria causes the release of some factor from the organelle, which could then stimulate protein synthesis. It would be of interest to study the nature of such a factor and the mechanism of its release.

#### Stimulation of the nuclear matrix by thyroid hormones

In their experiments with thyroidectomized rats, Tata and Widnell (1966) demonstrated the sequential changes in hepatic tissue resulting from a single injection of triiodothyronine and  $\text{C}^{14}$  orotic acid. The first phenomenon observed was an increase in the specific activity of rapidly labelled nuclear RNA beginning three to four hours after hormone administration and reaching a peak value of 300 percent of control at about sixteen hours. During this time interval, the second phenomenon, viz. stimulation of the magnesium-activated RNA-polymerase

reaction, was observed and this activity continued to rise until forty-two hours after triiodothyronine was administered. The third phenomenon reported was the rise in the amount of cytoplasmic ribosomal RNA and an increase in microsomal RNA/protein ratio at about twenty-four to thirty hours after the hormone was given, indicative of an elevation in hepatic ribosomal RNA content. In view of the sustained increase in the synthesis of nuclear RNA and the net accumulation of RNA in the ribosomal fraction, Tata concluded that administration of thyroid hormones accelerates nuclear RNA synthesis as well as RNA transport into the cytoplasm. It is interesting to note that an enhanced incorporation of amino acids into hepatic proteins was observed only thirty hours after injection of the hormone which is the time at which the BMR begins to rise.

Indeed, the triiodothyronine-induced stimulation of nuclear material is reminiscent of similar actions produced by other hormones in liver as well as accessory sex organs. Ui and Mueller (1963) reported increased incorporation of uridine  $H^3$  into uterine RNA two to three hours after injection of estradiol-17 $\beta$  to ovariectomized rats.

Williams-Ashman (1965) demonstrated that prostatic and seminal vesicular tissues exhibit an increased

synthesis of transfer, ribosomal and messenger RNAs following testosterone injection. These phenomena were the earliest detectable metabolic events observed in the intact male. Early increases in RNA polymerase activity of crude nuclear extracts of the prostate were also observed after testosterone administration in vivo.

The glucocorticoids also appear to act via stimulation of the nuclear matrix. Fiegelson et al (1962) presented evidence that administration of cortisone acetate produced a significant increase in  $P^{32}$  incorporation into hepatic RNA four hours after the hormone was administered to rats. Their experiments further demonstrated that cortisone induced the de novo synthesis of purine as well as accelerated nucleotide condensation into RNA.

From the available evidence, it appears as though hormones such as the estrogens, androgens and glucocorticoids cause primary changes in RNA metabolism which then result in secondary changes such as an increase in functional protein synthesis, viz. synthesis of rate-limiting enzymes from different metabolic pathways.

Singhal et al (1967), Valadares et al (1968) and Singhal and Valadares (1970) reported that the activities of the key glycolytic enzymes, hexokinase,

phosphofructokinase and pyruvate kinase were modulated by estradiol-17 $\beta$  in the uterus of the ovariectomized rat.

Additionally, Weber et al (1965a) reported significantly augmented activities of two rate-limiting enzymes of gluconeogenesis, namely glucose-6-phosphatase and fructose-1-6-diphosphatase in the liver of the rat following administration of cortisone acetate, for three days. These workers have proposed that the mechanism of cortisone action involves a "turning on of the functional genome unit" and this was responsible for the enhanced activities of the key gluconeogenic enzymes.

It is conceivable that thyroid hormones may stimulate certain genome units when administered in physiological doses. By regulating the rates of synthesis of specific functional proteins, viz. enzymes, these hormones may be capable of controlling the rate of oxidation of substrates along certain metabolic pathways. The increased rate of glycolysis observed when thyroid hormones are administered to hypothyroid or normal animals is most likely due to the increased synthesis of enzymes of the Embden-Meyerhof pathway. Such a mechanism could also explain the paradoxical effects observed when hormones are treated with enzymes in vitro and those observed in vivo.

The results of Krause et al (1967) on the triiodothyronine-induced increases in enzyme activities of the "phosphotriose glycerate group" and glycogen cycle as well as Lardy et al (1960) on the activity of mitochondrial  $\alpha$ -glycerophosphate dehydrogenase suggest a primary action of thyroid hormones on the nuclear matrix with subsequent increases in enzyme protein synthesis.

Since Glock and McLean (1955) as well as Bargoni and Grillo (1967) demonstrated that the activities of enzymes associated with the hexose monophosphate shunt and glycolysis were increased in hyperthyroidism, it is quite possible that the increases observed were also the result of new enzyme protein synthesis.

## INTRODUCTION

Thyrotoxicosis is associated with a considerable loss of body weight which is largely due to the protein catabolic effect of thyroid hormones on tissues such as liver and skeletal muscle (Tata, 1964).

One organ that does not appear to be thus affected is the heart, since cardiac hypertrophy results under conditions of hyperthyroidism in rats and mice (Gemmill, 1958; Sandler and Wilson, 1959). Furthermore, Bressler and Wittels (1966) demonstrated that cardiac hypertrophy also occurs in thyroxine-treated guinea pigs and that these increases in heart weight were not the result of tissue edema but an increased rate of amino acid incorporation into proteins. Cohen et al (1966) after administration of thyroxine orally to mice for ten days, demonstrated an increase in heart to body weight ratio as well as an absolute increase in heart weight and reported that dry heart weight to wet heart weight ratios were virtually identical in control mice and in those fed thyroxine. In addition, these workers found that the thyroxine-induced changes in cardiac protein synthesis and heart weight appeared to be a direct effect of the hormone on cardiac metabolism and not the result of cardiovascular changes, since an increased incorporation of labelled leucine into myocardial proteins occurred before any change in either

heart rate or in the product of heart rate times arterial pulse pressure was observed. In their experiments with thyrotoxic guinea pigs Bressler and Wittels (1966) reported elevated levels of myocardial proteins, increase in total cardiac RNA content and unchanged DNA levels in the heart. The increase in RNA/DNA ratio above that observed for controls and the changes in protein levels are indicative of "true" hypertrophy of the organ in the hyperthyroid state, thus confirming the results of Cohen et al (1966).

In the light of this information, thyroid hormones may act primarily on the nuclear matrix of the myocardial cell stimulating increased RNA synthesis, which then brings about an increase in cytoplasmic, structural and functional proteins as proposed by Tata (1964).

The purpose of the present investigation was to establish whether triiodothyronine was capable of altering the activities of certain key rate-limiting enzymes of the glycolytic, hexose monophosphate shunt and the  $H^+$  ion shuttle pathways in heart muscle of the rat, since such alterations could influence the rate of oxidation of extramitochondrial substrates. In an attempt to elucidate such a mechanism the activities of two of the key glycolytic enzymes, hexokinase and pyruvate kinase, as well as

glyceraldehyde-3-phosphate dehydrogenase; glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities, both rate-limiting enzymes of the hexose monophosphate pathway and the activity of the  $H^+$  ion shuttle enzyme, cytoplasmic  $\alpha$ -glycerophosphate dehydrogenase were investigated under conditions of hypo- and hyperthyroidism in the rat. In addition, the responses of cardiac enzymes to varying doses of triiodothyronine, when administered to hypothyroid animals were also studied. The nature of these changes in myocardial enzyme activities induced by altering the thyroid status of the animal was also investigated by the use of inhibitors of RNA and protein synthesis.

## MATERIALS AND METHODS

Seven-week-old male rats of the Wistar strain weighing initially between 125 and 150 g, maintained on Master Laboratory chow and water ad libitum, were used throughout this study.

Chemicals and doses. 3, 3', 5 triiodo L thyronine was dissolved in 1.0 cc of 0.1 N NaOH and diluted with 0.9% NaCl solution to result in solutions of 34, 68, 136, 200 and 250  $\mu\text{g}$  per 0.1 cc and injected in doses of 34, 68, 136, 200 or 250  $\mu\text{g}$  per 100 g body weight intraperitoneally.

Radioactive, carrier-free iodine,  $^{131}\text{I}$ , was obtained from Atomic Energy of Canada and administered in a single intraperitoneal injection of 800  $\mu\text{c}$ /100 g body weight. Control rats received injections of an equal volume of the vehicle solution, 0.9% NaCl alone.

Cycloheximide was dissolved in 0.9% NaCl solution and injected intraperitoneally in a dose of 70  $\mu\text{g}$ /100 g body weight.

Actinomycin D was dissolved in 0.9% NaCl solution and administered as a single intraperitoneal injection of 8  $\mu\text{g}$ /100 g body weight.

Ethionine was first dissolved in warm physiological saline solution and adjusted to pH 7.4 with 0.1 N NaOH. The solution was injected intraperitoneally in two

equally divided doses of 50 mg/100 g body weight at 8 hour intervals per day.

Puromycin was dissolved in 0.9% NaCl solution and administered intraperitoneally in 4 equally divided doses of 2.5 mg/100 g body weight at 4 hour intervals per day.

When both inhibitor and triiodothyronine were administered to the same animal, the inhibitor was injected one hour prior to hormone treatment. In the case of puromycin and ethionine which were injected more than once per day,  $T_3$  was also administered in a similar sequence with these two inhibitors.

Sources of chemicals were as follows:

Cycloheximide (Upjohn Company of Toronto, Canada),  
Actinomycin D and puromycin hydrochloride (Nutritional Biochemicals of Ohio, U.S.A.),  
D-ethionine, 3, 3', 5 triiodothyronine and all other cofactors (Sigma Chemical Company, of St. Louis, U.S.A.)  
and  
Glucose-6-phosphate dehydrogenase, lactate dehydrogenase and aldolase (Boeringer Mannheim Corporation of New York, U.S.A.).

### Experimental procedure

On arrival animals were divided into two groups, A and B.

Group A. In this group of rats, hypothyroidism was induced by means of a single, intraperitoneal injection of  $^{131}\text{I}$ , 800  $\mu\text{c}$ /100 g body weight. Eight to ten animals were then housed in a single large cage and 4 to 6 weeks later were treated as described below.

Effect of radiothyroidectomy. To study the effect of radiothyroidectomy on cardiac hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase,  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities, rats were killed 4 to 6 weeks after  $^{131}\text{I}$  injection.

Effect of a single injection of triiodothyronine. In an attempt to reverse the changes found in hypothyroid rats, triiodothyronine was administered in a dose of 34  $\mu\text{g}$ /100 g body weight and the animals were killed 48 hours later.

Actinomycin and cycloheximide inhibition of triiodothyronine-induced enzyme responses. Since hormones appear to affect enzyme activity at the level of protein synthesis (Weber et al, 1965), the influence of two inhibitors of protein biosynthesis, actinomycin D and

cycloheximide was studied. Three groups of 4 to 7 hypothyroid animals were used: (a) rats treated with a single injection of triiodothyronine ( $34 \mu\text{g}/100 \text{ g}$ ); (b) triiodothyronine-injected animals given cycloheximide ( $70 \mu\text{g}/100 \text{ g}$ ); (c) rats treated with triiodothyronine and actinomycin D ( $8 \mu\text{g}/100 \text{ g}$ ). Cycloheximide or actinomycin D was administered one hour before triiodothyronine injection and animals from respective groups were killed 48 hours following injection of the hormone.

Dose response studies with triiodothyronine. In an attempt to investigate the effects of varying doses of triiodothyronine, groups of radiothyroidectomized rats (6 weeks after  $^{131}\text{I}$  injection) were given single intraperitoneal injections of triiodothyronine (68, 136,  $200 \mu\text{g}/100 \text{ g}$  body weight) and killed after 48 hours.

Group B. Group B animals were permitted a period of 3 weeks for equilibration and then subjected to the following experimental procedures.

Effects of hyperthyroidism. The effects of hyperthyroidism on cardiac enzyme activities measured, were studied by injecting rats with triiodothyronine ( $250 \mu\text{g}/100 \text{ g}$  body weight) daily for 7 days. The animals were killed on the following day.

Effects of cycloheximide, puromycin and ethionine.

In order to examine the effects of inhibitors of protein and RNA synthesis on the triiodothyronine-induced changes of cardiac enzyme activities in hyperthyroid rats, 7 groups of animals were used: (a) euthyroid-control rats; (b, c, and d) animals treated with cycloheximide (70  $\mu$ g/100 g/day), puromycin (10 mg/100 g/day), and ethionine (100 mg/100 g/day) respectively; (e) rats treated with triiodothyronine (250  $\mu$ g/100 g/day); (f, g, and h) triiodothyronine-treated animals (250  $\mu$ g/100 g/day) given injections of cycloheximide (70  $\mu$ g/100 g/day), puromycin (10 mg/100 g/day) and ethionine (100 mg/100 g/day) respectively. All animals were treated with the above drugs for 7 days and killed on the 8th day.

Preparation of homogenates and supernatant fluids.

Animals were stunned, decapitated and exsanguinated. The beating heart was excised immediately, cleaned of all extraneous tissue, including the aorta, the atria and pericardium and these were discarded. Both right and left ventricles were blotted on filter paper, weighed quickly on a Roller-Smith torsion balance, placed in beakers chilled in crushed ice and minced finely with scissors. The ventricles were homogenized in ice cold

0.14 M KCl, pH 7.4, (5% w/v) for 90 seconds with a Teflon pestle turning at 700 rpm. The supernatant was obtained by centrifuging the homogenate for 30 minutes at 100,000 x g at 0°C using a refrigerated IEC model B-60 centrifuge. The activities of hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase,  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase were all determined in the supernatant fraction as described below.

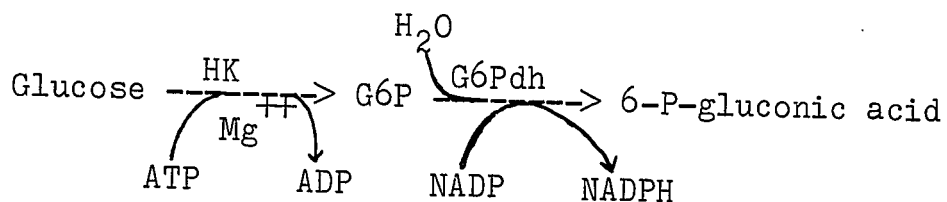
Measurement of basal metabolic rate (BMR). In order to establish the degree of hypo- or hyperthyroidism as well as the effects of various inhibitors of protein and RNA synthesis on T<sub>3</sub>-induced thyroxicosis, basal metabolic rates were determined by the method of Watts and Gourley (1953). The apparatus used consisted of an air tight cylindrical vessel 23 cms long and 9 cms in diameter made of plexi-glass, open at one end and divided into 2 compartments by a wire mesh, which prevented a small animal from coming in contact with soda lime located below. The respiration chamber was closed by means of a rubber cork in which was located a 10 cc pipette with the tapered end removed. To determine the oxygen consumption of a rat, the animal was first anesthetized by an intraperitoneal injection of Na pentobarbital (20 mg/kg)

and then placed in the respiration chamber, making sure that the animal did not come in contact with the soda lime. The chamber was flushed with oxygen and after wetting the walls of the pipette with soap solution, it was fitted to the vessel by means of the rubber cork. After waiting 5 minutes for temperature equilibration, a film of soap was wiped across the end of the pipette. As the rat utilized oxygen and the carbon dioxide was absorbed by soda lime, the soapy film moved up the pipette in proportion to the rate of oxygen consumption and the time taken for the bubble to traverse a 5 ml volume was measured with a stop watch. Five determinations were made with the animal resting quietly in the cage and the mean was used for calculation. BMR was expressed as ccs of  $O_2$  consumed/h/g animal.

#### Biochemical Procedure.

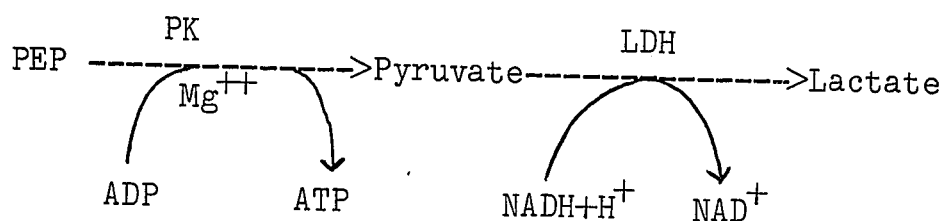
Enzyme assays. Enzyme activities were assayed under strictly linear kinetic conditions. The rate of formation of reduced coenzyme or of its reoxidation was measured at 340 m $\mu$  in a Unicam model SP800 constant recording spectrophotometer at 37°C. The components for the reaction mixtures are all given in the order of their addition.

Hexokinase activity was assayed by a modification of the method described by Di Pietro and Weinhouse (1960). The principle behind this reaction is as follows:-



The rate of formation of reduced NADP indicates the activity of hexokinase. The assay medium contained the following in a total volume of 2.5 ml at the designated final concentrations: 50mM glycyl-glycine buffer, pH 7.5; 5mM magnesium chloride; 5mM ATP; 0.75mM NADP; 2.0mM cysteine; 100mM glucose and 1.43  $\mu$ g protein of glucose-6-phosphate dehydrogenase. This was preincubated for 3 minutes and the reaction was initiated by the addition of supernatant fluid corresponding to 5.0 mg wet weight of the heart. Changes in optical density were read against a blank which contained neither ATP nor glucose and water was added to substitute for these two reagents.

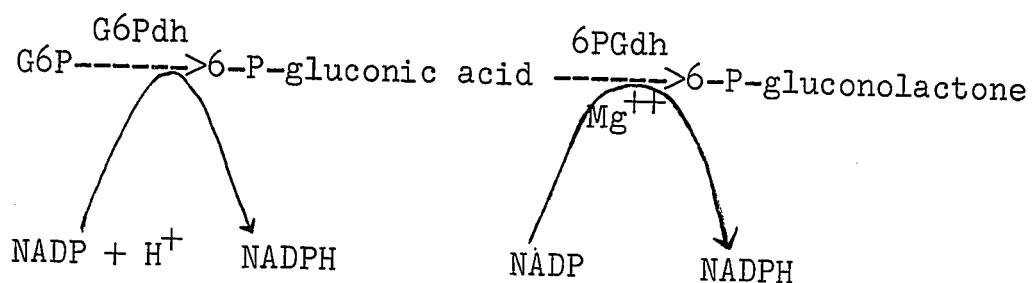
Pyruvate kinase activity was assayed by a modified procedure of Weber et al (1965). The principle behind this reaction is as follows:



The rate of formation of  $\text{NAD}^+$  from  $\text{NADH}$  is an indication of pyruvate kinase activity. The reaction mixture contained the following components at the given concentrations in a final volume of 3.0 ml:

Tris buffer, pH 7.4, 41.7mM; magnesium sulphate, 6.3 mM; potassium chloride, 25mM; phosphoenol pyruvate, 5.3mM; ADP, 1.3mM; NADH, 0.22mM; and lactate dehydrogenase, 0.1 mg protein. After 3 minutes of incubation the reaction was started by adding supernatant fluid corresponding to 0.5 mg wet weight of cardiac tissue. Changes in optical density were recorded against a blank in which water was substituted for phosphoenol pyruvate. The reaction rate was found to be linear for a period of at least 5 minutes.

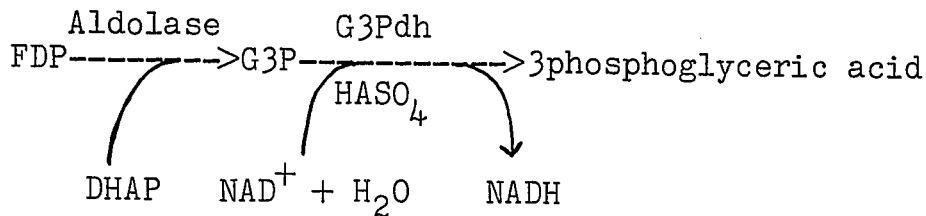
Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were assayed by a modification of the method originally described by Glock and McLean (1953). The principle behind this assay method is as follows:



The assay medium consisted of the following in a total volume of 2.5 ml at the designated final concentrations: glycyl-glycine buffer, pH 7.5, 50mM;  $\text{MgCl}_2$ , 7.5mM; glucose-6-phosphate, 2.0mM; 6-phosphogluconic acid, 2.0 mM and NADP, 0.75mM. This mixture was incubated for 3 minutes and the reaction was initiated by the addition of the supernatant fluid corresponding to 20 mg wet weight of the heart. The rate of formation of NADPH, in the presence of both glucose-6-phosphate and 6-phosphogluconate, measures the activities of both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. However, in the presence of 6-phosphogluconate alone, the rate of formation of NADPH indicates the activity of 6-phosphogluconate dehydrogenase only. Glucose-6-phosphate dehydrogenase activity was calculated by difference. Changes in optical density were read against blanks which contained neither glucose-6-phosphate nor 6-phosphogluconate for measuring both enzyme activities together as well as 6-phosphogluconate dehydrogenase. The reaction rate was found to be linear



Glyceraldehyde-3-phosphate dehydrogenase activity was assayed by a modified procedure of Freedland (1967) and Freedland et al (1968). The principle behind this assay method is as follows:-



In this reaction, the conversion of glyceraldehyde-3-phosphate to 3-phosphoglycerate is non-reversible in the presence of arsenic salt. The rate of formation of reduced  $\text{NAD}^+$  indicates the activity of this enzyme. The assay medium contained the following components at the given concentrations in a final volume of 3.0 ml: glycine buffer, pH 9.0, 27mM; magnesium sulphate 3.5mM; sodium arsenate, 17.0mM; NAD, 4.2mM; cysteine, 20mM; aldolase, 50  $\mu\text{g}$  of protein; supernatant fluid corresponding to 5 mg of fresh tissue; and fructose-1-6-diphosphate, 36mM. The reaction mixture was incubated for 3 minutes before the glyceraldehyde-3-phosphate dehydrogenase reaction was initiated by the addition of fructose-1-6-diphosphate. Changes in optical density were recorded against blanks which contained no fructose-1-6-diphosphate. The reaction rate was linear after the first 2

minutes for at least 5 minutes.

All enzyme activities were expressed in two ways: as  $\mu$  moles of substrate converted per hour per gram fresh weight of tissue at  $37^{\circ}\text{C}$ ; and as  $\mu$  moles of substrate metabolized per hour per gram fresh weight of tissue at  $37^{\circ}\text{C}$  times the weight of the ventricles. The data were analyzed statistically and significant differences between the means were calculated as P values. No statistical difference is indicated when the P value was  $<0.05$ .

## RESULTS

### Influence of inhibitors of RNA and protein synthesis on basal metabolic rates of T<sub>3</sub>-treated rats.

The data presented in Table 1 shows the effects of altered thyroid states and inhibitors of RNA and protein synthesis on the basal metabolic rate of male rats. Hypothyroidism, induced by a single injection of <sup>131</sup>I, produced a significant decrease in BMR after 4 weeks to 46% and hyperthyroidism, induced by daily injections of 250 µg/100g of T<sub>3</sub> for one week, caused an elevation to 159% of that observed for euthyroid animals. Administration of puromycin or ethionine to triiodothyronine-treated rats completely blocked the hypermetabolic effect of T<sub>3</sub>. Cycloheximide did not completely block hyperthyroidism induced by T<sub>3</sub> (135%), possibly due to the low dose used.

### Effects of inhibitors of RNA and protein synthesis on heart and body weights in T<sub>3</sub>-treated hypothyroid rats.

The heart and body weights of radiothyroidectomized rats, hypothyroid rats injected with a 34 µg dose of T<sub>3</sub>, and T<sub>3</sub>-treated rats injected with the protein inhibitors, actinomycin D and cycloheximide, are

TABLE 1

The results represent means  $\pm$  SE. Hypo- and hyperthyroidism were induced by a single intraperitoneal injection of  $^{131}\text{I}$  (800  $\mu\text{c}$ /100 g body weight) and administration of triiodothyronine, 250  $\mu\text{g}$ /100 g body weight per day for seven days respectively. Cycloheximide was administered in a single dose of 70  $\mu\text{g}$ /100 g/day; puromycin (10 mg/100 g) was injected in 4 equally divided doses at 4 hour intervals each day; ethionine (100 mg/100 g) was given twice daily at 8 hour intervals. Each inhibitor was injected intraperitoneally one hour prior to triiodothyronine injection. Animals of groups III, IV, V and VI were treated for seven days and killed on the day following. Euthyroid rats received an equal volume of the vehicle solution. The basal metabolic rate is expressed as the volume of  $\text{O}_2$  (cc) consumed per hour per gram under light pentobarbital anesthesia at 27 $^{\circ}$  C. Data are also given in percentages taking the values of euthyroid rats as 100%.

TABLE 1

Influence of inhibitors of RNA and protein synthesis on basal metabolic rate of tri-iodothyronine-treated rats.

| <u>Treatment</u>             | <u>No. of Animals</u> | <u>Basal Metabolic Rate</u> | <u>% of Control</u> |
|------------------------------|-----------------------|-----------------------------|---------------------|
| Euthyroid                    | 6                     | 1.08 ± 0.07                 | 100                 |
| Hypothyroid                  | 5                     | 0.50 ± 0.03                 | 46*                 |
| Hyperthyroid                 | 5                     | 1.73 ± 0.06                 | 159*                |
| Hyperthyroid + Puromycin     | 3                     | 1.05 ± 0.22                 | 96†                 |
| Hyperthyroid + Ethionine     | 3                     | 0.90 ± 0.03                 | 83†                 |
| Hyperthyroid + Cycloheximide | 4                     | 1.46 ± 0.04                 | 135*†               |

\*-Statistically significant difference as compared with the values of euthyroid rats ( $p < 0.05$ )

†-Statistically significant difference as compared with the values of hyperthyroid rats ( $p < 0.05$ )

## TABLE 2

Data represent means  $\pm$  SE. Cycloheximide (70  $\mu\text{g}/100$  g) and actinomycin D (8  $\mu\text{g}/100$  g) were injected one hour prior to triiodothyronine (34  $\mu\text{g}/100$  g) administration and animals were sacrificed 48 hours after hormone treatment. All drugs were injected intraperitoneally. Euthyroid rats received injections of an equal volume of physiological saline. Data are also given in percentages taking the values of euthyroid rats as 100%.

TABLE 2

Effects of inhibitors of RNA and protein synthesis on heart and body weights in triiodothyronine-treated hypothyroid rats.

| <u>Treatment</u>                                     | <u>No. of Animals</u> | <u>Heart Weight (mg)</u> | <u>Range of Body Weight (g)</u> | <u>Average Body Weight (g)</u> | <u>Heart Weight / Body Weight</u> |
|--|-----------------------|--------------------------|---------------------------------|--------------------------------|-----------------------------------|
| Euthyroid  | 6                     | 836.2±23.5<br>(100)      | 272-308                         | 288.5±5.0<br>(100)             | .0029                             |
| Hypothyroid  | 5                     | 412.7±11.5<br>(49)*      | 158-192                         | 176.2±6.1<br>(61)*             | .0023                             |
| Hypothyroid +<br>Triiodothyronine                    | 7                     | 514.0±15.8<br>(62)*†     | 160-190                         | 182.9±4.2<br>(63)*             | .0028                             |
| Hypothyroid +<br>Triiodothyronine +<br>Cycloheximide | 6                     | 490.5±9.0<br>(59)*†      | 160-177                         | 175.5±2.6<br>(61)*             | .0028                             |
| Hypothyroid +<br>Triiodothyronine +<br>Actinomycin D | 5                     | 409.2±21.8<br>(49)*§     | 150-162                         | 156.2±2.5<br>(54)*†§           | .0026                             |

\*-Statistically significant difference as compared with euthyroid rats (p<0.05)

†-Statistically significant difference as compared with hypothyroid rats (p<0.05)

§-Statistically significant difference as compared with T<sub>3</sub>-treated hypothyroid rats (p<0.05)

shown in Table 2. Both heart and body weights were significantly decreased after 4 weeks of hypothyroidism. However, a greater decrease in heart weight occurred and this was reflected in a lowering of the heart to body weight ratio. Administration of a single injection of  $T_3$  (34  $\mu$ g) to animals, which were radiothyroidectomized four weeks earlier, produced significant increases in heart but not body weights which was manifest by a rise in the heart to body weight ratio approximating the normal range. Whereas actinomycin D completely blocked the  $T_3$ -induced increase in heart weight of hypothyroid rats and also lowered significantly the body weight of these animals, cycloheximide was without any effect on these two parameters.

Influence of  $T_3$ , cycloheximide and actinomycin D on cardiac enzyme activities in hypothyroid rats.

The data on the effects of hypothyroidism,  $T_3$  replacement as well as actinomycin D and cycloheximide on cardiac hexokinase (HK), pyruvate kinase (PK), glucose-6-phosphate dehydrogenase (G-6Pdh), 6-phosphogluconate dehydrogenase (6-PGdh),  $\alpha$ -glycerophosphate dehydrogenase ( $\alpha$ -GPdh) and glyceraldehyde-3-

phosphate dehydrogenase (G-3Pdh) activities are presented in Tables 3, 4 and 5 respectively. Decreases in all cardiac enzyme activities, whether expressed per gram tissue or per total organ, were observed following radiothyroidectomy. With the exception of HK, which was decreased to 20% of control values when expressed per organ, all other enzymes, PK, G-6Pdh, 6-PGdh,  $\alpha$ -GPdh, and G-3Pdh were depleted to about 40% of euthyroid values. With a single injection of  $T_3$  (34  $\mu$ g), small but significant increases in the activities of the two glycolytic enzymes, HK and PK, to 33% and 53% respectively, the pentose phosphate enzymes, G-6Pdh to 76% and 6-PGdh to 70% as well as  $\alpha$ -GPdh to 82% of euthyroid values were observed 48 hours after hormone treatment.

When compared to hypothyroid values, HK activity was increased by 65%, PK by 29%, G-6-Pdh by 81%, 6-PGdh by 63%,  $\alpha$ -GPdh by 95% and G-3Pdh by 28%. G-3Pdh activity, although slightly increased by  $T_3$  to 55% of control values was not significantly different from hypothyroid values.

The effects of two antibiotics, actinomycin D and cycloheximide, on the  $T_3$ -induced increases in

TABLE 3

The results represent means  $\pm$  SE. Cycloheximide (70  $\mu\text{g}/100$  g) and actinomycin D (8  $\mu\text{g}/100$  g) were injected one hour prior to  $T_3$  (34  $\mu\text{g}/100$  g) administration and animals were killed 48 hours after hormone treatment. All drugs were injected intraperitoneally. Euthyroid rats received injections of an equal volume of 0.9% NaCl. Cardiac hexokinase and pyruvate kinase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolised per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of euthyroid rats as 100%.

TABLE 3

Influence of triiodothyronine, cycloheximide and actinomycin D on the activities of cardiac hexokinase and pyruvate kinase in hypothyroid animals.

| <u>Treatment</u>                                     | <u>No. of Deter-<br/>minations</u> | <u>Hexokinase<br/>Activity/g</u> | <u>Activity/organ<br/>(100)</u> | <u>Pyruvate kinase<br/>Activity/g</u> | <u>Activity/organ<br/>(100)</u> |
|--|------------------------------------|----------------------------------|---------------------------------|---------------------------------------|---------------------------------|
| Euthyroid  | 6                                  | 243.5±7.4<br>(100)               | 204.2±11.2<br>(100)             | 9684.9±391.5<br>(100)                 | 8072.0±265.8<br>(100)           |
| Hypothyroid  | 5                                  | 100.5±1.7<br>(41)*               | 41.4±1.0<br>(20)*               | 8052.7±203.1<br>(83)*                 | 3319.3±96.8<br>(41)*            |
| Hypothyroid +<br>Triiodothyronine                    | 7                                  | 131.1±3.9<br>(54)*†              | 66.8±2.7<br>(33)*†              | 8378.0±172.9<br>(87)*                 | 4283.0±124.8<br>(53)*†          |
| Hypothyroid +<br>Triiodothyronine +<br>Cycloheximide | 6                                  | 116.7±3.4<br>(48)*†§             | 56.6±2.0<br>(28)*†§             | 8696.5±167.9<br>(90)*†                | 4246.1±91.6<br>(53)*†           |
| Hypothyroid +<br>Triiodothyronine +<br>Actinomycin D | 5                                  | 40.4±1.1<br>(17)*†§              | 16.6±1.7<br>(8)*†§              | 7852.1±142.7<br>(81)*                 | 3218.4±208.9<br>(40)*§          |

\*-Statistically significant difference as compared with the values of euthyroid rats (p<0.05)

†-Statistically significant difference as compared with the values of hypothyroid rats (p<0.05)

§-Statistically significant difference as compared with the values of T<sub>3</sub>-treated hypothyroid rats (p<0.05)

TABLE 4

Data represent means  $\pm$  SE. Cycloheximide (70  $\mu\text{g}/100\text{ g}$ ) and actinomycin D (8  $\mu\text{g}/100\text{ g}$ ) were injected one hour prior to  $\text{T}_3$  (34  $\mu\text{g}/100\text{ g}$ ) administration and animals were killed 48 hours after hormone treatment. All drugs were injected intraperitoneally. Euthyroid animals received injections of an equal volume of physiological saline. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolised per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of euthyroid rats as 100%.

TABLE 4

Effects of cycloheximide and actinomycin D on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in the myocardium of triiodothyronine-injected hypothyroid rats.

| <u>Treatment</u>                                     | <u>No. of Deter-<br/>minations</u> | <u>Glucose-6-phosphate dehydrogenase<br/>Activity/g</u> | <u>6-Phosphogluconate dehydrogenase<br/>Activity/g</u> | <u>6-Phosphogluconate dehydrogenase<br/>Activity/organ</u> |
|--|------------------------------------|---|--|--|
| Euthyroid  | 6                                  | 16.3±0.4<br>(100)                                       | 13.3±0.4<br>(100)                                      | 58.8±0.7<br>(100)  |
| Hypothyroid  | 5                                  | 13.5±1.3<br>(83)*                                       | 5.6±0.5<br>(42)*                                       | 51.1±0.9<br>(87)*  |
| Hypothyroid +<br>Triiodothyronine                    | 7                                  | 19.2±1.0<br>(118)*†                                     | 10.1±0.6<br>(76)*†                                     | 67.0±4.8<br>(114)*†  |
| Hypothyroid +<br>Triiodothyronine +<br>Cycloheximide | 6                                  | 16.3±1.1<br>(100)                                       | 8.2±0.6<br>(62)*†                                      | 49.5±3.0<br>(84)*‡   |
| Hypothyroid +<br>Triiodothyronine +<br>Actinomycin D | 5                                  | 17.5±1.7<br>(107)                                       | 7.2±0.8<br>(54)*§                                      | 52.4±1.5<br>(89)*§   |

\*-Statistically significant difference as compared with the values of euthyroid rats ( $p < 0.05$ )

†-Statistically significant difference as compared with the values of hypothyroid rats ( $p < 0.05$ )

§-Statistically significant difference as compared with the values of  $T_3$ -treated hypothyroid rats ( $p < 0.05$ )

TABLE 5

Data represent means  $\pm$  SE. Cycloheximide (70  $\mu$ g/100 g) and actinomycin D (8  $\mu$ g/100 g) were injected one hour prior to T<sub>3</sub> (34  $\mu$ g/100 g) administration and animals were killed 48 hours after hormone treatment. All drugs were injected intraperitoneally. Euthyroid rats received injections of an equal volume of physiological saline. Cardiac  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolized per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of euthyroid rats as 100%.

TABLE 5

Influence of inhibitors of RNA and protein synthesis on the activities of cardiac  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase in triiodothyronine-treated hypothyroid animals.

| <u>Treatment</u>                                     | <u>No. of Deter-<br/>minations</u> | <u><math>\alpha</math>-glycerophosphate<br/>dehydrogenase<br/>Activity/g<br/>Activity/organ</u> | <u>Glyceraldehyde-3-phosphate<br/>dehydrogenase<br/>Activity/g<br/>Activity/organ</u> |
|--|------------------------------------|---|---|
| Euthyroid  | 6                                  | 420.7 $\pm$ 18.7<br>(100)   | 4254.0 $\pm$ 172.9<br>(100)   |
| Hypothyroid  | 5                                  | 352.7 $\pm$ 9.1<br>(84)*  | 3671.4 $\pm$ 133.3<br>(86)*   |
| Hypothyroid +<br>Triiodothyronine                    | 7                                  | 551.1 $\pm$ 5.1<br>(131)*†  | 3814.2 $\pm$ 627.7<br>(90)*   |
| Hypothyroid +<br>Triiodothyronine +<br>Cycloheximide | 6                                  | 423.1 $\pm$ 33.5<br>(101)§  | 4033.1 $\pm$ 139.0<br>(95)  |
| Hypothyroid +<br>Triiodothyronine +<br>Actinomycin D | 5                                  | 359.8 $\pm$ 6.2<br>(86)*§   | 2920.9 $\pm$ 86.9<br>(69)*†   |

\*-Statistically significant difference as compared with the values of euthyroid rats (p<0.05)

†-Statistically significant difference as compared with the values of hypothyroid rats (p<0.05)

§-Statistically significant difference as compared with the values of T<sub>3</sub>-treated hypothyroid rats (p<0.05)

enzyme activities of hypothyroid rats were also investigated. Whereas administration of 34  $\mu\text{g}$  of  $\text{T}_3$  enhanced the activities of HK from 20% to 33% and  $\alpha$ -GPdh from 42% to 82%, cycloheximide partially inhibited the  $\text{T}_3$ -induced increases in the former and latter enzyme activities to 28% and 60% of euthyroid values respectively. When compared to  $\text{T}_3$ -treated hypothyroid animals, HK and  $\alpha$ -GPdh activities were decreased by 15% and 27% respectively with this inhibitor. 6-PGdh activity was significantly lowered from 114% to 84% of euthyroid values when expressed per gram of heart but remained unaffected when expressed per organ. All other enzyme activities remained unaltered by this inhibitor. It is quite possible that the ineffectiveness of cycloheximide to block the  $\text{T}_3$ -observed increases was due to the dose of the inhibitor used. With actinomycin D, the  $\text{T}_3$ -induced increases in HK activity were blocked from 33% to 8%, PK activity from 53% to 40%, G-6Pdh activity from 76% to 54%, 6-PGdh activity from 70% to 44%,  $\alpha$ -GPdh activity from 131% to 42% and G-3Pdh activity from 55% to 34% of euthyroid values. Thus, actinomycin D had effectively lowered the activities of HK, PK,

G-6Pdh, 6-PGdh,  $\alpha$ -GPdh and G-3Pdh by 76%, 25%, 29%, 37%, 49%, and 38% of the values obtained from the  $T_3$ -treated hypothyroid group. It is of interest to note that the decrease in HK activity by actinomycin D was even lower than that observed for hypothyroid animals.

Duration of radiothyroidectomy on heart and body weights.

The effects of 4 and 6 weeks of radiothyroidectomy on heart and body weights are shown in Table 6. No further decreases in heart or body weights were observed in animals which were hypothyroid for 6 weeks compared to those that were hypothyroid for 4 weeks resulting in no appreciable change in the heart to body weight ratios. As expected, 13-week-old euthyroid rats had significantly higher heart and body weights than the 11-week-old controls, but since both were increased by about the same amount, no change in heart to body weight ratio was observed.

Prolongation of hypothyroidism on cardiac enzyme activities.

The duration of hypothyroidism on the activities of representative rate-limiting enzymes from the gly-

TABLE 6

Data represent means  $\pm$  SE.  $^{131}\text{I}$  was injected intraperitoneally in a dose of 800  $\mu\text{c}$ /100 g body weight. Euthyroid rats received injections of an equal volume of physiological saline. Data are also given in percentages (in parentheses) taking the values of euthyroid rats (11 weeks) as 100%.

TABLE 6

Duration of effects of radiothyroidectomy on heart and body weights.

| <u>Treatment</u> | <u>No. of Animals</u> | <u>Age (Weeks)</u> | <u>Time <sup>131</sup>I after Treatment (Weeks)</u> | <u>Heart Weight (mg)</u> | <u>Range of Body Weight (g)</u> | <u>Average Body Weight (g)</u> | <u>Heart Weight / Body Weight</u> |
|------------------|-----------------------|--------------------|---|--------------------------|---------------------------------|--------------------------------|-----------------------------------|
| Euthyroid        | 6                     | 11                 |   | 836.2±23.5<br>(100)      | 272-308                         | 288.5±5.0<br>(100)             | .0029                             |
| Euthyroid        | 4                     | 13                 |   | 939.7±20.1<br>(112)*     | 342-370                         | 355.8±5.7<br>(123)*            | .0026                             |
| Hypothyroid      | 5                     | 11                 | 4   | 412.7±11.5<br>(49)*†     | 158-192                         | 176.2±6.1<br>(61)*†            | .0023                             |
| Hypothyroid      | 4                     | 13                 | 6   | 374.1±20.7<br>(45)*†     | 159-197                         | 179.3±7.0<br>(62)*†            | .0021                             |

\*-Statistically significant difference as compared with the values of 11-week-old euthyroid rats ( $p < 0.05$ )

†-Statistically significant difference as compared with the values of 13-week-old euthyroid rats ( $p < 0.05$ )

colytic (HK and PK), pentose phosphate pathway (G-6Pdh and 6-PGdh) and  $\alpha$ -glycerophosphate shunt ( $\alpha$ -GPdh) as well as the  $\text{NAD}^+$ -dependent glycolytic enzyme, G-3Pdh, are presented in Tables 7, 8 and 9 respectively. All enzyme activities with the exception of G-6Pdh were further decreased in animals that were left for 6 weeks after radiothyroidectomy when compared to rats that were hypothyroid for 4 weeks. HK activity was reduced from 20% to 8%, PK activity from 41% to 32%, 6-PGdh activity from 43% to 35%,  $\alpha$ -GPdh activity from 42% to 34%, and G-3Pdh activity from 43% to 28% of values obtained from 11-week-old euthyroid rats. Thus, the activities of HK, PK, G-6Pdh, 6-PGdh,  $\alpha$ -GPdh and G-3Pdh were all lowered by 60%, 22%, 17%, 19%, 19% and 35% respectively of values obtained from 11-week-old hypothyroid rats. Although decreases from 42% to 35% in G-6Pdh activity were observed in 13-week-old hypothyroid animals, they were not significantly different from those of 11-week-old hypothyroid rats. The decreases in cardiac HK, PK, 6-PGdh,  $\alpha$ -GPdh and G-3Pdh activities from 13-week-old hypothyroid animals were significantly different

TABLE 7

Data represent means  $\pm$  SE.  $^{131}\text{I}$  was injected intraperitoneally in a dose of 800  $\mu\text{c}$ /100 g body weight. Euthyroid rats received injections of an equal volume of physiological saline. Hexokinase and pyruvate kinase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolised per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of 11-week-old euthyroid rats as 100%.

TABLE 7

Effect of prolonged duration of hypothyroidism on cardiac hexokinase and pyruvate kinase activities.

| <u>Treatment</u> | <u>No. of Determinations</u> | <u>Age (Weeks)</u> | <u>Time after Treatment (Weeks)</u> | <u>Hexokinase Activity/g Activity/organ</u> | <u>Pyruvate kinase Activity/g Activity/organ</u> |
|------------------|------------------------------|--------------------|-------------------------------------|---|--|
| Euthyroid        | 6                            | 11                 |                                     | 243.5±7.4<br>(100)                          | 204.2±11.2<br>(100)                              |
| Euthyroid        | 4                            | 13                 |                                     | 178.5±1.4<br>(73)*                          | 9684.9±391.5<br>(100)                            |
| Hypothyroid      | 5                            | 11                 | 4                                   | 167.7±2.9<br>(82)*                          | 7292.6±244.3<br>(75)*                            |
| Hypothyroid      | 4                            | 13                 | 6                                   | 41.4±1.7<br>(41)*†                          | 8052.7±203.1<br>(83)*†                           |
|                  |                              |                    |                                     | 41.8±4.1<br>(17)*†§                         | 6906.8±226.2<br>(71)*†§                          |
|                  |                              |                    |                                     | 15.7±1.7<br>(8)*†§                          | 2577.8±127.2<br>(32)*†§                          |

\*-Statistically significant difference as compared with the values of 11-week-old euthyroid animals ( $p < 0.05$ )

†-Statistically significant difference as compared with the values of 13-week-old euthyroid animals ( $p < 0.05$ )

§-Statistically significant difference as compared with the values of 11-week-old hypothyroid animals ( $p < 0.05$ )

TABLE 8

Data represent means  $\pm$  SE.  $^{131}\text{I}$  was injected intraperitoneally in a dose of 800  $\mu\text{c}$ /100 g body weight. Euthyroid rats received injections of an equal volume of physiological saline. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolised per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of 11-week-old euthyroid rats as 100%.

TABLE 8

Effects of prolonged duration of radiothyroidectomy on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in the myocardium of rats.

| <u>Treatment</u> | <u>No. of Determinations</u> | <u>Age (Weeks)</u> | <u>Time after Treatment (Weeks)</u> | <u>Glucose-6-phosphate dehydrogenase Activity/g Activity/organ</u> | <u>6-phosphogluconate dehydrogenase Activity/g Activity/organ</u> |
|------------------|------------------------------|--------------------|-------------------------------------|--|---|
| Euthyroid        | 6                            | 11                 |                                     | 16.3±0.4<br>(100)  | 58.8±0.7<br>(100)   |
| Euthyroid        | 4                            | 13                 |                                     | 14.2±2.0<br>(87)   | 54.9±1.5<br>(93)*   |
| Hypothyroid      | 5                            | 11                 | 4                                   | 13.5±0.5<br>(83)*  | 51.1±0.9<br>(87)*   |
| Hypothyroid      | 4                            | 13                 | 6                                   | 12.6±1.2<br>(77)*  | 46.3±1.9<br>(79)*+§   |
|                  |                              |                    |                                     | 13.3±0.4<br>(100)  | 49.2±1.5<br>(100)   |
|                  |                              |                    |                                     | 13.2±2.0<br>(99)   | 51.5±1.1<br>(105)   |
|                  |                              |                    |                                     | 5.6±0.5<br>(42)*†  | 21.1±0.6<br>(43)*†  |
|                  |                              |                    |                                     | 4.7±0.5<br>(35)*†  | 17.4±1.5<br>(35)*†§   |

\*-Statistically significant difference as compared with the values of 11-week-old euthyroid rats ( $p < 0.05$ )

†-Statistically significant difference as compared with the values of 13-week-old euthyroid rats ( $p < 0.05$ )

§-Statistically significant difference as compared with the values of 11-week-old hypothyroid rats ( $p < 0.05$ )

TABLE 9

Data represent means  $\pm$  SE.  $^{131}\text{I}$  was injected intraperitoneally in a dose of 800  $\mu\text{c}/100$  g body weight. Euthyroid rats received injections of an equal volume of physiological saline. Cardiac  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolised per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of 11-week-old euthyroid rats as 100%.

TABLE 9

Effects of prolonged duration of hypothyroidism on myocardial  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities.

| <u>Treatment</u> | <u>No. of Determinations</u> | <u>Age (Weeks)</u> | <u>Time after Treatment (Weeks)</u> | <u><math>\alpha</math>-glycerophosphate dehydrogenase Activity/g Organ</u> | <u>Glyceraldehyde-3-phosphate dehydrogenase Activity/g Organ</u> |
|------------------|------------------------------|--------------------|-------------------------------------|--|--|
| Euthyroid        | 6                            | 11                 |                                     | 420.7 $\pm$ 18.7<br>(100)  | 350.2 $\pm$ 11.4<br>(100)  |
| Euthyroid        | 4                            | 13                 |                                     | 442.0 $\pm$ 33.4<br>(105)  | 413.6 $\pm$ 23.8<br>(118)*                                       |
| Hypothyroid      | 5                            | 11                 | 4                                   | 352.7 $\pm$ 9.1<br>(84)*†  | 145.6 $\pm$ 6.1<br>(42)*†  |
| Hypothyroid      | 4                            | 13                 | 6                                   | 322.9 $\pm$ 11.1<br>(77)*†   | 120.6 $\pm$ 6.3<br>(34)*†§                                       |
|                  |                              |                    |                                     |  | 4254.0 $\pm$ 172.9<br>(100)                                      |
|                  |                              |                    |                                     |  | 3118.2 $\pm$ 8.2<br>(73)*  |
|                  |                              |                    |                                     |  | 3671.4 $\pm$ 133.3<br>(86)*†                                     |
|                  |                              |                    |                                     |  | 2660.0 $\pm$ 57.4<br>(63)*†§                                     |
|                  |                              |                    |                                     |  | 3557.1 $\pm$ 169.8<br>(100)                                      |
|                  |                              |                    |                                     |  | 2930.4 $\pm$ 69.0<br>(82)*                                       |
|                  |                              |                    |                                     |  | 1516.0 $\pm$ 72.0<br>(43)*†                                      |
|                  |                              |                    |                                     |  | 998.5 $\pm$ 76.9<br>(28)*†§                                      |

\*-Statistically significant difference as compared with the values of 11-week-old euthyroid rats ( $p < 0.05$ )

†-Statistically significant difference as compared with the values of 13-week-old euthyroid rats ( $p < 0.05$ )

§-Statistically significant difference as compared with the values of 11-week-old hypothyroid rats ( $p < 0.05$ )

when compared to the values obtained from the 11-week-old hypothyroid rats. However, 13-week-old euthyroid rats also exhibited significant decreases in HK activity to 82%, PK activity to 84% and G-3Pdh activity to 82% when these were compared to the values obtained for 11-week-old euthyroid rats. These results suggest decreased usage of the glycolytic pathway in the myocardium of older rats and could also account for the decreases in HK, G-3Pdh, and PK activities observed in 13-week-old hypothyroid rats. Conversely,  $\alpha$ -GPdh activity was significantly increased by 13% whilst 6-PGDh remained unchanged in the 13-week-old euthyroid animals, so that the reduction in both enzyme activities observed in the 13-week-old hypothyroid rats was probably not due to an aging effect.

Effects of different doses of  $T_3$  on heart and body weights as well as cardiac enzymes of hypothyroid rats.

The results obtained after administration of increasing doses of  $T_3$  on heart and body weights as well as on the heart to body weight ratios are shown in Table 10. With the lowest dose of  $T_3$  used (68  $\mu$ g/100 g body weight) neither heart nor body

### TABLE 10

Data represent means  $\pm$  SE. Groups of four to five 6-week-old hypothyroid animals were injected with varying amounts of triiodothyronine intraperitoneally and sacrificed 48 hours later. Data are also given in percentages (in parentheses) taking the values of hypothyroid rats as 100%.

TABLE 10

Effect of different doses of triiodothyronine on heart and body weights of hypothyroid rats.

| Triiodo-<br>thyronine<br>( $\mu\text{g}/100 \text{ g}$ ) | No. of<br>Animals | <u>Heart Weight</u><br>(mg) | <u>Range of</u><br><u>Body Weight</u><br>(g) | <u>Average</u><br><u>Body Weight</u><br>(g) | <u>Heart Weight</u><br><u>Body Weight</u> |
|--|-------------------|-----------------------------|--|---|---|
| control  | 4                 | 374.1 $\pm$ 20.7<br>(100)   | 154-197                                      | 179.3 $\pm$ 7.0<br>(100)                    | .0021                                     |
| 68   | 4                 | 431.6 $\pm$ 14.5<br>(115)   | 154-174                                      | 165.0 $\pm$ 4.8<br>(92)                     | .0026                                     |
| 136  | 4                 | 445.9 $\pm$ 17.7<br>(119)*  | 160-184                                      | 172.3 $\pm$ 4.9<br>(96)                     | .0026                                     |
| 200  | 5                 | 431.0 $\pm$ 16.2<br>(115)   | 146-169                                      | 158.4 $\pm$ 9.2<br>(88)*                    | .0027                                     |

\*-Statistically significant difference as compared with the values of hypothyroid rats (p<0.05)

weights were significantly altered although the former was increased to 115% and the latter decreased to 92% of control values respectively. These changes were reflected in the heart to body weight ratios which were increased from .0021 to .0026 for this group. A similar trend was seen in the other two groups treated with 136  $\mu\text{g}$  and 200  $\mu\text{g}$  of  $\text{T}_3$ . Body weights were decreased and heart weights were increased by approximately the same amount as that observed for 68  $\mu\text{g}$ -treated group of animals. Hence the heart to body weight ratios of the 3 groups were all about the same (.0026).

The data presented in Tables 11, 12 and 13 demonstrate the effects of different doses of  $\text{T}_3$  on activities of HK and PK, two key glycolytic enzymes; G-6Pdh and 6-PGdh, two rate-limiting enzymes from the HMP; and  $\alpha$ -GPdh, the representative enzyme involved in the  $\text{H}^+$  ion shuttle, as well as the glycolytic enzyme G-3Pdh, respectively, from rats that were hypothyroid for 6 weeks. Cardiac HK, PK, 6-PGdh, and  $\alpha$ -GPdh activities were significantly increased to 129%, 119%, 131% and 121% of control values respectively, with the smallest dose of  $\text{T}_3$  used (68  $\mu\text{g}$ ).

TABLE 11

Data represent means  $\pm$  SE. Six-week-old hypothyroid rats were treated with varying dosages of triiodothyronine intraperitoneally and killed after 48 hours. Hexokinase and pyruvate kinase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolised per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of hypothyroid rats as 100%.

TABLE 11

Influence of various doses of triiodothyronine on the activities of cardiac hexokinase and pyruvate kinase of hypothyroid rats.

| <u>Triiodo-<br/>thyronine<br/>(<math>\mu\text{g}/100\text{ g}</math>)</u> | <u>No. of<br/>Deter-<br/>minations</u> | <u>Hexokinase<br/>Activity/g</u> | <u>Hexokinase<br/>Activity/organ</u> | <u>Pyruvate kinase<br/>Activity/g</u> | <u>Pyruvate kinase<br/>Activity/organ</u> |
|---|--|----------------------------------|--------------------------------------|---------------------------------------|---|
| control   | 4                                      | 41.8 $\pm$ 4.1<br>(100)          | 15.7 $\pm$ 1.7<br>(100)              | 6906.8 $\pm$ 226.2<br>(100)           | 2577.8 $\pm$ 127.2<br>(100)               |
| 68  | 4                                      | 46.9 $\pm$ 0.2<br>(112)          | 20.3 $\pm$ 0.7<br>(129)*             | 7123.8 $\pm$ 163.8<br>(103)           | 3078.2 $\pm$ 151.1<br>(119)*              |
| 136   | 4                                      | 71.9 $\pm$ 1.9<br>(172)*         | 32.1 $\pm$ 1.8<br>(205)*             | 7557.9 $\pm$ 136.1<br>(109)*          | 3371.3 $\pm$ 156.3<br>(131)*              |
| 200   | 5                                      | 97.4 $\pm$ 2.5<br>(233)*         | 41.9 $\pm$ 1.7<br>(267)*             | 7786.0 $\pm$ 116.2<br>(113)*          | 3362.0 $\pm$ 166.0<br>(130)*              |

\*-Statistically significant difference as compared with the values of hypothyroid-control rats ( $p < 0.05$ )

TABLE 12

Data represent means  $\pm$  SE. Groups of four to five animals 6 weeks after radiothyroidectomy, were injected with different doses of triiodothyronine intraperitoneally and sacrificed after 48 hours. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolized per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of hypothyroid rats as 100%.

TABLE 12

Effects of varying doses of triiodothyronine on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in the myocardium of hypothyroid rats.

| Triiodo-<br>thyronine<br>( $\mu\text{g}/100\text{ g}$ ) | No. of<br>Deter-<br>minations | <u>Glucose-6-phosphate<br/>dehydrogenase<br/>Activity/g</u> | <u>6-Phosphogluconate<br/>dehydrogenase<br/>Activity/g</u> |
|---|-------------------------------|---|--|
| control   | 4                             | $12.6 \pm 1.2$<br>(100)                                     | $4.7 \pm 0.5$<br>(100)                                     |
| 68  | 4                             | $14.9 \pm 1.2$<br>(118)                                     | $46.3 \pm 1.9$<br>(100)                                    |
| 136   | 4                             | $17.5 \pm 1.3$<br>(139)*                                    | $53.0 \pm 1.6$<br>(115)*                                   |
| 200   | 5                             | $24.8 \pm 1.8$<br>(197)*                                    | $57.4 \pm 2.6$<br>(124)*                                   |
|   |                               | $10.8 \pm 1.0$<br>(230)*                                    | $22.8 \pm 0.5$<br>(131)*                                   |
|   |                               |   | $25.7 \pm 2.0$<br>(148)*                                   |
|   |                               |   | $25.1 \pm 0.9$<br>(144)*                                   |

\*-Statistically significant difference as compared with the values of hypothyroid rats ( $p < 0.05$ )

TABLE 13

Data represent means  $\pm$  SE. Six-week-old hypothyroid rats were treated with various doses of triiodothyronine intraperitoneally and killed 48 hours later. Cardiac  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolized per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of hypothyroid rats as 100%.

TABLE 13

Influence of different doses of triiodothyronine on the activities of myocardial  $\alpha$ -glycero-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase in hypothyroid rats.

| Triiodo-<br>thyronine<br>( $\mu\text{g}/100\text{ g}$ ) | No. of<br>Deter-<br>minations | $\alpha$ -Glycerophosphate<br>dehydrogenase<br>Activity/g organ | Glyceraldehyde-3-phosphate<br>dehydrogenase<br>Activity/g organ |
|---|-------------------------------|---|---|
| control   | 4                             | 322.9 $\pm$ 11.1<br>(100)                                       | 2660.0 $\pm$ 57.4<br>(100)                                      |
| 68  | 4                             | 336.9 $\pm$ 8.3<br>(104)  | 2626.2 $\pm$ 52.6<br>(99)                                       |
| 136   | 4                             | 389.0 $\pm$ 21.3<br>(121)*                                      | 2660.0 $\pm$ 55.5<br>(100)                                      |
| 200   | 5                             | 376.5 $\pm$ 8.7<br>(117)*                                       | 3088.8 $\pm$ 98.5<br>(116)*                                     |
|   |                               | 120.6 $\pm$ 6.3<br>(100)  | 998.5 $\pm$ 76.9<br>(100)                                       |
|   |                               | 145.3 $\pm$ 4.8<br>(121)*                                       | 1135.5 $\pm$ 59.3<br>(114)                                      |
|   |                               | 174.0 $\pm$ 13.9<br>(144)*                                      | 1185.8 $\pm$ 51.8<br>(119)                                      |
|   |                               | 162.3 $\pm$ 7.5<br>(135)*                                       | 1328.6 $\pm$ 50.5<br>(133)*                                     |

\*-Statistically significant difference as compared with the values of hypothyroid animals (p<0.05)

G-6Pdh activity, when expressed on the organ basis, was increased by 38% with the 68  $\mu$ g dose which was not significant because of large individual variation that was observed within this group. However, the 136  $\mu$ g dose of  $T_3$  significantly augmented G-6Pdh activity to 164% while the higher dose of 200  $\mu$ g enhanced G-3Pdh activity to 133% of control values respectively. It is interesting to note that when twice the dose of  $T_3$  was administered to hypothyroid rats the enzyme responses were not doubled. In fact, administration of 34  $\mu$ g of  $T_3$  in most cases evoked a greater response in enzyme activity than the 68  $\mu$ g dose although basal enzyme levels of rats treated with 68  $\mu$ g of  $T_3$  were significantly lower than that of the former group (Tables 3, 4, 5, 11, 12 and 13). It is possible that as the duration of hypothyroidism increases the sensitivity of cardiac enzymes to injected  $T_3$  simultaneously decreases. Of all enzymes studied, HK appeared to be most sensitive to  $T_3$  treatment.

Effects of inhibitors of RNA and protein synthesis on heart and body weights of hyperthyroid rats.

Since hypothyroidism produced marked decreases in heart and body weights, it was of interest to study the effects of hyperthyroidism on these parameters. Table 14 gives the data from these experiments. Whereas hyperthyroidism caused a significant rise in heart weight to 134% of euthyroid values the body weights of these rats were greatly decreased to 82% and this probably accounts for the elevation

TABLE 14

Data represent means  $\pm$  SE. Hyperthyroidism was induced by intraperitoneal injections of 250  $\mu$ g of triiodothyronine per 100 g body weight per day for seven days. Cycloheximide was administered in a single dose of 70  $\mu$ g/100 g per day; puromycin (10 mg/100 g) was injected in 4 equally divided doses at 4 hour intervals each day; ethionine (100 mg/100 g) was given twice daily at 8 hour intervals. Each inhibitor was injected intraperitoneally one hour prior to triiodothyronine injection. All animals were treated for seven days and killed on the day following. Euthyroid animals received an equal volume of the vehicle solution. Data are also given in percentages (in parentheses) taking the values of euthyroid rats as 100%.

TABLE 14

Effects of inhibitors of RNA and protein synthesis on heart and body weights of hyperthyroid rats.

| <u>Treatment</u>             | <u>No. of Animals</u> | <u>Heart Weight (mg)</u> | <u>Range of Body Weight (g)</u> | <u>Average Body Weight (g)</u> | <u>Heart Weight Body Weight</u> |
|------------------------------|-----------------------|--------------------------|---------------------------------|--------------------------------|---------------------------------|
| Euthyroid                    | 6                     | 836.2±23.5<br>(100)      | 272-308                         | 288.5±5.0<br>(100)             | .0029                           |
| Hyperthyroid                 | 7                     | 1121.0±47.7<br>(134)*    | 220-242                         | 236.2±5.7<br>(82)*             | .0047                           |
| Hyperthyroid + Cycloheximide | 9                     | 1029.4±25.7<br>(123)*    | 194-210                         | 207.1±3.4<br>(72)*†            | .0050                           |
| Hyperthyroid + Puromycin     | 3                     | 627.0±50.5<br>(75)*†     | 177-186                         | 181.7±2.6<br>(63)*†            | .0035                           |
| Hyperthyroid + Ethionine     | 8                     | 709.0±22.8<br>(85)*†     | 156-192                         | 174.1±5.1<br>(60)*†            | .0041                           |

\*-Statistically significant difference as compared with the values of euthyroid rats ( $p < 0.05$ )

†-Statistically significant difference as compared with the values of hyperthyroid rats ( $p < 0.05$ )

of the heart to body weight ratios from .0029 to .0047 of the animals in this group.

Cycloheximide was unable to block the  $T_3$ -induced increase in heart weight although a significant fall in body weight to 72% of control was observed. Puromycin and ethionine, however, not only blocked the  $T_3$ -induced increases in heart weight but significantly lowered the heart weights to 75% and 85% of control values respectively. Furthermore, body weights were markedly decreased to 63% and 60% of euthyroid values respectively by these two antibiotics. Whereas the heart to body weight ratios for hyperthyroid animals (0.0047) and cycloheximide-treated hyperthyroid rats (0.005) were essentially the same, both puromycin and ethionine-treated hyperthyroid rats exhibited decreases in this ratio to .0035 and .0041 respectively.

Actinomycin D was also administered to a sixth group of animals. However, no observations on these animals could be conducted since 100% mortality was obtained by the third day of treatment. In addition, a 50% mortality was observed for the puromycin-treated group of animals.

Influence of inhibitors of RNA and protein synthesis on cardiac enzyme activities of hyperthyroid rats.

Since hypothyroidism produced significantly lowered activities of cardiac enzymes, it was of interest to determine whether an opposite effect would occur in the thyrotoxic state of the animal. Hyperthyroidism augmented significantly the activities of HK and PK to 175% and 167% of control values respectively (Table 15), G-6Pdh to 204% and 6-PGdh to 437% (Table 16), and  $\alpha$ -GPdh and G-3Pdh to 182% and 147% of euthyroid values respectively (Table 17). The two pentose phosphate enzymes, G-6Pdh and 6-PGdh activities were most affected in the hyperthyroid animal when enzyme activities were expressed per organ.

The effects of cycloheximide, puromycin and ethionine on cardiac glycolytic, pentose phosphate and  $H^+$  ion shunt enzymes are also presented in Tables 15, 16 and 17 respectively. Cycloheximide administration to hyperthyroid animals significantly inhibited the  $T_3$ -induced increase in enzyme activities of G-6Pdh from 204% to 85%, 6-PGdh from

TABLE 15

Data represent means  $\pm$  SE. Hyperthyroidism was induced by intraperitoneal injections of 250  $\mu$ g of triiodothyronine per 100 g body weight for seven days. Cycloheximide was administered in a single dose of 70  $\mu$ g/100 g per day; puromycin (10 mg/100 g) was injected in 4 equally divided doses at 4 hour intervals each day; ethionine (100 mg/100 g) was given in two equally divided doses at 8 hour intervals. Each inhibitor was injected intraperitoneally one hour prior to triiodothyronine injection. All animals were treated for seven days and killed on the day following. Euthyroid animals received an equal volume of the vehicle solution. Hexokinase and pyruvate kinase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolized per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) with the values of euthyroid rats as 100%.

TABLE 15

Influence of inhibitors of RNA and protein synthesis on cardiac hexokinase and pyruvate kinase activities in hyperthyroid rats.

| <u>Treatment</u>                | <u>No. of<br/>Deter-<br/>minations</u> | <u>Hexokinase<br/>Activity/g</u> | <u>Activity/organ</u> | <u>Pyruvate kinase<br/>Activity/g</u> | <u>Activity/organ</u>   |
|---------------------------------|--|----------------------------------|-----------------------|---------------------------------------|-------------------------|
| Euthyroid                       | 6                                      | 243.5±7.4<br>(100)               | 204.2±11.2<br>(100)   | 9684.9±391.5<br>(100)                 | 8072.0±265.8<br>(100)   |
| Hyperthyroid                    | 7                                      | 318.3±8.9<br>(131)*              | 356.8±21.7<br>(175)*  | 12007.3±277.8<br>(124)*               | 13460.7±645.1<br>(167)* |
| Hyperthyroid +<br>Cycloheximide | 9                                      | 328.9±7.2<br>(135)*              | 338.5±12.6<br>(165)*  | 11614.1±335.6<br>(120)*               | 11951.4±658.6<br>(148)* |
| Hyperthyroid +<br>Puromycin     | 3                                      | 176.1±12.6<br>(72)*†             | 110.1±9.8<br>(54)*†   | 8688.1±473.7<br>(90)†                 | 5423.2±331.0<br>(67)*†  |
| Hyperthyroid +<br>Ethionine     | 8                                      | 239.7±5.4<br>(98)†               | 170.4±8.1<br>(84)*†   | 11722.7±160.6<br>(121)*               | 8315.3±305.7<br>(103)†  |

\*-Statistically significant difference as compared with the values of euthyroid rats (p<0.05)

†-Statistically significant difference as compared with the values of hyperthyroid rats (p<0.05)

TABLE 16

Data represent means  $\pm$  SE. Hyperthyroidism was induced by intraperitoneal injections of 250  $\mu$ g of triiodothyronine per 100 g body weight for seven days. Cycloheximide was administered in a single dose of 70  $\mu$ g/100 g per day; puromycin (10 mg/100 g) was injected in 4 equally divided doses at 4 hour intervals each day; ethionine (100 mg/100 g) was given twice daily at 8 hour intervals. Each inhibitor was injected intraperitoneally one hour prior to triiodothyronine injection. All animals were treated for seven days and killed on the following day. Euthyroid animals received an equal volume of the vehicle solution. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolized per hour per gram (fresh weight) of heart times the weight of the organ.

TABLE 16

Effects of inhibitors of RNA and protein synthesis on glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in the myocardium of hyperthyroid rats.

| <u>Treatment</u>                | <u>No. of<br/>Deter-<br/>minations</u> | <u>Glucose-6-phosphate dehydrogenase<br/>Activity/g</u> | <u>6-Phosphogluconate<br/>Activity/g</u> | <u>6-Phosphogluconate dehydrogenase<br/>Activity/organ</u> |
|---------------------------------|--|---|--|--|
| Euthyroid                       | 6                                      | 16.3±0.4<br>(100)                                       | 13.3±0.4<br>(100)                        | 58.8±0.7<br>(100)  |
| Hyperthyroid                    | 7                                      | 24.5±1.8<br>(150)*                                      | 27.1±2.9<br>(204)*                       | 192.4±9.9<br>(327)*  |
| Hyperthyroid +<br>Cycloheximide | 9                                      | 11.3±0.9<br>(69)*†                                      | 11.3±0.8<br>(85)†                        | 127.0±6.3<br>(216)*†                                       |
| Hyperthyroid +<br>Puromycin     | 3                                      | 32.3±7.8<br>(198)*                                      | 20.6±6.2<br>(155)                        | 65.5±1.5<br>(111)*†  |
| Hyperthyroid +<br>Ethionine     | 8                                      | 25.6±1.4<br>(157)*                                      | 18.2±1.3<br>(137)*†                      | 69.3±1.8<br>(118)*†  |

\*-Statistically significant difference as compared with the values of euthyroid rats ( $p < 0.05$ )

+Statistically significant difference as compared with the values of hyperthyroid rats ( $p < 0.05$ )

TABLE 17

Data represent means  $\pm$  SE. Hyperthyroidism was induced by intraperitoneal injections of 250  $\mu$ g of triiodothyronine per 100 g for seven days. Cycloheximide was administered in a single dose of 70  $\mu$ g/100 g; puromycin (10 mg/100 g) was injected in 4 equally divided doses at 4 hour intervals each day; ethionine (100 mg/100 g) was given twice daily at 8 hour intervals and each inhibitor was injected intraperitoneally one hour prior to triiodothyronine injection. All animals were treated for seven days and killed on the day following. Euthyroid animals received an equal volume of 0.9% NaCl solution. Myocardial  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolized per hour per gram (fresh weight) of heart times the weight of the organ.

TABLE 17

Influence of inhibitors of RNA and protein synthesis on myocardial  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities in hyperthyroid rats.

| <u>Treatment</u>                | <u>No. of Deter-<br/>minations</u> | <u><math>\alpha</math>-Glycerophosphate dehydrogenase<br/>Activity/g</u> | <u>Activity/organ</u>       | <u>Glyceraldehyde-3-phosphate dehydrogenase<br/>Activity/g</u> | <u>Activity/organ</u>        |
|---------------------------------|------------------------------------|--|-----------------------------|--|------------------------------|
| Euthyroid                       | 6                                  | 420.7 $\pm$ 18.7<br>(100)  | 350.2 $\pm$ 11.4<br>(100)   | 4254.0 $\pm$ 172.9<br>(100)                                    | 3557.1 $\pm$ 169.8<br>(100)  |
| Hyperthyroid                    | 7                                  | 568.7 $\pm$ 21.6<br>(135)*   | 637.5 $\pm$ 48.8<br>(182)*  | 4676.0 $\pm$ 66.1<br>(110)*                                    | 5242.0 $\pm$ 232.2<br>(147)* |
| Hyperthyroid +<br>Cycloheximide | 9                                  | 166.6 $\pm$ 20.4<br>(40)*†   | 169.0 $\pm$ 19.0<br>(48)*†  | 4578.3 $\pm$ 44.4<br>(108)*                                    | 4712.9 $\pm$ 241.6<br>(133)* |
| Hyperthyroid +<br>Puromycin     | 3                                  | 611.3 $\pm$ 23.9<br>(145)*   | 382.6 $\pm$ 27.7<br>(109)†  | 4070.7 $\pm$ 181.1<br>(96)                                     | 2574.3 $\pm$ 316.7<br>(72)*† |
| Hyperthyroid +<br>Ethionine     | 8                                  | 703.3 $\pm$ 23.5<br>(167)*†  | 497.8 $\pm$ 21.1<br>(142)*† | 4336.0 $\pm$ 121.2<br>(102)†                                   | 3080.8 $\pm$ 147.7<br>(87)†  |

\*-Statistically significant difference as compared with the values of euthyroid rats ( $p < 0.05$ )

†-Statistically significant difference as compared with the values of hyperthyroid rats ( $p < 0.05$ )

437% to 268% and  $\alpha$ -GPdh from 182% to 48% of euthyroid values. Thus, the activities of G-6Pdh, 6-PGdh and  $\alpha$ -GPdh were lowered by 58%, 39% and 74% respectively when compared to the values obtained from hyperthyroid animals. The reduction of  $\alpha$ -GPdh activity, the  $H^+$  ion shuttle enzyme, was even lower than that of euthyroid animals of the same age. Puromycin blocked significantly the increases in HK, PK, 6-PGdh,  $\alpha$ -GPdh and G-3Pdh activities of the hyperthyroid animal reducing them by 69%, 60%, 81%, 40% and 51% of hyperthyroid values respectively. Here again, HK, PK, 6-PGdh and G-3Pdh activities were significantly less than control values. Ethionine effectively inhibited the  $T_3$ -induced increases in the activities of all cardiac enzymes studied, HK being decreased by 52%, PK by 38%, G-6Pdh by 33%, 6-PGdh by 77%,  $\alpha$ -GPdh by 22% and G-3Pdh by 41% of hyperthyroid values respectively. As observed with the other inhibitors used, only HK activity, when expressed on the organ basis was significantly decreased below control values. However, certain enzyme activities (PK and G-6Pdh) from the ethionine-treated hyperthyroid group as

well as G-6Pdh and  $\alpha$ -GPdh (from the puromycin-treated hyperthyroid group) when expressed as per gram of tissue remained unaffected by inhibitor administration. Furthermore, the activity of  $\alpha$ -GPdh was significantly increased by 22% in rats that were treated with ethionine and  $T_3$ . A possible explanation for the discrepancy observed is that one gram of heart tissue from hyperthyroid animals contains fewer cells than a corresponding gram from the ethionine-treated hyperthyroid rats, since heart weights from the two groups of animals were significantly different (Table 14). Therefore, this way of expressing enzyme activity reflects the activity of unequal numbers of cells, a greater number being considered for the ethionine-treated hyperthyroid group than for the hyperthyroid animals alone. Thus, unaltered or increased enzyme activity per gram of heart is expected when the former group is compared to the latter.

Effects of inhibitors of RNA and protein synthesis on heart and body weights of euthyroid rats.  
Since the administration of puromycin and ethionine to hyperthyroid rats not only blocked the  $T_3$ -

TABLE 18

Data represent means  $\pm$  SE. Groups of euthyroid rats were injected intraperitoneally with: cycloheximide (70  $\mu$ g/100 g/day); puromycin (10 mg/100 g/day) in 4 equally divided doses at 4 hour intervals each day; and ethionine (100 mg/100 g) twice daily at 8 hour intervals. All animals were treated for seven days and sacrificed on the day following. Euthyroid animals received an equal volume of the vehicle solution. Data are also given in percentages (in parentheses) with the values of euthyroid rats as 100%.

TABLE 18

Effects of inhibitors of RNA and protein synthesis on heart and body weights of euthyroid rats.

| <u>Treatment</u>          | <u>No. of Animals</u> | <u>Heart Weight (mg)</u> | <u>Range of Body Weight (g)</u> | <u>Average Body Weight (g)</u> | <u>Heart Weight / Body Weight</u> |
|---------------------------|-----------------------|--------------------------|---------------------------------|--------------------------------|-----------------------------------|
| Euthyroid                 | 6                     | 836.2±23.5<br>(100)      | 272-308                         | 288.5±5.0<br>(100)             | .0029                             |
| Euthyroid + Cycloheximide | 6                     | 774.3±23.2<br>(93)       | 220-245                         | 237.8±4.4<br>(82)*             | .0033                             |
| Euthyroid + Puromycin     | 2                     | 535.1±30.9<br>(64)       | 196-218                         | 207.0±11.0<br>(72)             | .0026                             |
| Euthyroid + Ethionine     | 8                     | 597.7±18.1<br>(72)*      | 188-202                         | 193.6±1.7<br>(67)*             | .0031                             |

\*-Statistically significant difference as compared with the values of euthyroid rats (p<0.05)

induced increase in cardiac weight, but lowered it significantly below that of control values (Table 14), it was of interest to determine whether these inhibitors when administered to euthyroid animals would have any effect on heart and body weights (Table 18).

Cycloheximide had no effect on heart weight but significantly lowered the body weight of these animals to 82% of controls. A high mortality rate of 66% was recorded for the puromycin-treated group of animals and only two survived. It appears as though this inhibitor causes decreases in both heart and body weights, lowering these parameters to 64% and 72% respectively. Ethionine-treated animals had significantly reduced heart and body weights, both being decreased to 72% and 67% of control values respectively. The reduction in both heart and body weights to approximately the same level by the inhibitors used therefore accounts for the heart to body weight ratios being within the normal range.

Influence of inhibitors of RNA and protein synthesis on basal cardiac enzyme activities in

euthyroid animals.

Since puromycin, cycloheximide and ethionine were capable of markedly depressing  $T_3$ -induced increases in the activities of certain enzymes under study, experiments were designed to investigate whether these inhibitors by themselves were capable of exerting an effect on basal enzyme activities in the heart. Tables 19, 20 and 21 demonstrate the effects of these inhibitors of RNA and protein synthesis on the activities of HK, PK, G-6Pdh, 6-PGdh,  $\alpha$ -GPdh and G-3Pdh respectively. Cycloheximide produced significant decreases in all enzyme activities except 6-PGdh and G-3Pdh which values remained in the normal range. Of the enzymes affected by this inhibitor, G-6Pdh and  $\alpha$ -GPdh were lowered to 38% and 48%, while HK and PK were reduced to 75% and 77% of control values respectively. From the two determinations with puromycin, it appears as though this inhibitor causes a decrease in all enzymes studied, since the activity of HK was lowered to 40%, PK to 56%, G-6Pdh to 49%, 6-PGdh to 53%,  $\alpha$ -GPdh to 68% and G-3Pdh to 67% respectively of controls, when enzyme activity was expressed per

TABLE 19

Data represent means  $\pm$  SE. Groups of euthyroid rats were injected intraperitoneally with: cycloheximide (70  $\mu$ g/100 g/day); puromycin (10 mg/100 g/day) in 4 equally divided doses at 4 hour intervals each day; and ethionine (100 mg/100 g) twice daily at 8 hour intervals. All animals were treated for seven days and killed on the following day. Euthyroid rats received an equal volume of the vehicle solution. Hexokinase and pyruvate kinase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolized per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) taking the values of euthyroid rats as 100%.

TABLE 19

Influence of inhibitors of RNA and protein synthesis on the activities of cardiac hexokinase and pyruvate kinase of euthyroid rats.

| <u>Treatment</u>             | <u>No. of<br/>Deter-<br/>minations</u> | <u>Hexokinase<br/>Activity/g</u> | <u>Activity/organ</u> | <u>Pyruvate kinase<br/>Activity/g</u> | <u>Activity/organ</u> |
|------------------------------|--|----------------------------------|-----------------------|---------------------------------------|-----------------------|
| Euthyroid                    | 6                                      | 243.5±7.4<br>(100)               | 204.2±11.2<br>(100)   | 9684.9±391.5<br>(100)                 | 8072.0±265.8<br>(100) |
| Euthyroid +<br>Cycloheximide | 6                                      | 198.4±9.6<br>(82)*               | 153.0±5.7<br>(75)*    | 7998.4±384.5<br>(83)*                 | 6216.3±184.7<br>(77)* |
| Euthyroid +<br>Puromycin     | 2                                      | 152.0±7.2<br>(62)                | 81.5±8.6<br>(40)      | 8527.3±250.8<br>(88)                  | 4552.2±129.3<br>(56)  |
| Euthyroid +<br>Ethionine     | 8                                      | 191.6±2.5<br>(79)*               | 114.5±3.6<br>(56)*    | 10362.5±182.7<br>(107)                | 6184.3±175.0<br>(77)* |

\*-Statistically significant difference when compared with the values of euthyroid rats (p<0.05)

TABLE 20

Data represent means  $\pm$  SE. Groups of euthyroid rats were injected intraperitoneally with: cycloheximide (70  $\mu$ g/100 g/day); puromycin (10 mg/100 g/day) in 4 equally divided doses at 4 hour intervals each day; and ethionine (100 mg/100 g) given twice daily at 8 hour intervals. All animals were treated for seven days and killed on the day following. Euthyroid rats received an equal volume of physiological saline. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities are expressed as  $\mu$  moles of substrate converted per hour per gram as well as  $\mu$  moles of substrate metabolized per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) with the values of euthyroid rats as 100%.

TABLE 20

Effects of inhibitors of RNA and protein synthesis on basal enzyme activities of myocardial glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.

| <u>Treatment</u>             | <u>No. of<br/>Deter-<br/>minations</u> | <u>Glucose-6-phosphate dehydrogenase<br/>Activity/g</u> | <u>6-Phosphogluconate dehydrogenase<br/>Activity/g</u> | <u>6-Phosphogluconate dehydrogenase<br/>Activity/organ</u> |
|------------------------------|--|---|--|--|
| Euthyroid                    | 6                                      | 16.3±0.4<br>(100)                                       | 13.3±0.4<br>(100)                                      | 58.8±0.7<br>(100)  |
| Euthyroid +<br>Cycloheximide | 6                                      | 6.7±1.0<br>(41)*  | 5.1±0.8<br>(38)*                                       | 66.9±8.8<br>(114)  |
| Euthyroid +<br>Puromycin     | 2                                      | 12.1±0.4<br>(74)  | 6.5±0.6<br>(49)  | 48.4±2.2<br>(82)   |
| Euthyroid +<br>Ethionine     | 8                                      | 11.5±1.4<br>(71)*                                       | 6.9±0.8<br>(52)*                                       | 59.0±1.2<br>(100)  |
|                              |  |   |  | 49.2±1.5<br>(100)  |
|                              |  |   |  | 51.9±7.4<br>(105)  |
|                              |  |   |  | 25.9±0.3<br>(53)   |
|                              |  |   |  | 35.2±0.9<br>(72)*  |

\*--Statistically significant difference as compared with the values of euthyroid rats (p<0.05)

TABLE 21

Data represent means  $\pm$  SE. Groups of euthyroid rats were injected intraperitoneally with: cycloheximide (70  $\mu$ g/100 g/day); puromycin (10 mg/100 g/day) in 4 equally divided doses at 4 hour intervals each day; and ethionine (100 mg/100 g) given twice daily at 8 hour intervals. All animals were treated for seven days and killed the following day. Euthyroid rats received an equal volume of physiological saline. Myocardial  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activities are expressed as  $\mu$  moles of substrate metabolized per hour per gram as well as  $\mu$  moles of substrate converted per hour per gram (fresh weight) of heart times the weight of the organ. Data are also given in percentages (in parentheses) with the values of euthyroid rats as 100%.

TABLE 21

Influence of inhibitors of RNA and protein synthesis on basal enzyme activities of car-  
 diac  $\alpha$ -glycerophosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase.

| <u>Treatment</u>             | <u>No. of<br/>Deter-<br/>minations</u> | <u><math>\alpha</math>-Glycerophosphate<br/>dehydrogenase<br/>Activity/g</u> | <u>Glycerophosphate<br/>dehydrogenase<br/>Activity/organ</u> | <u>Glyceraldehyde-3-phosphate<br/>dehydrogenase<br/>Activity/g</u> | <u>Glyceraldehyde-3-phosphate<br/>dehydrogenase<br/>Activity/organ</u> |
|------------------------------|--|--|--|--|--|
| Euthyroid                    | 6                                      | 420.7 $\pm$ 18.7<br>(100)  | 350.2 $\pm$ 11.4<br>(100)                                    | 4254.0 $\pm$ 172.9<br>(100)  | 3557.1 $\pm$ 169.8<br>(100)  |
| Euthyroid +<br>Cycloheximide | 6                                      | 216.6 $\pm$ 16.1<br>(52)*  | 168.5 $\pm$ 15.4<br>(48)*                                    | 4171.8 $\pm$ 143.9<br>(98)   | 3277.8 $\pm$ 91.0<br>(92)  |
| Euthyroid +<br>Puromycin     | 2                                      | 448.1 $\pm$ 6.3<br>(107)   | 239.6 $\pm$ 10.5<br>(68)                                     | 4427.7 $\pm$ 173.6<br>(104)  | 2363.8 $\pm$ 43.9<br>(67)  |
| Euthyroid +<br>Ethionine     | 8                                      | 429.5 $\pm$ 10.8<br>(102)  | 256.7 $\pm$ 12.1<br>(73)*                                    | 3945.3 $\pm$ 65.9<br>(93)  | 2396.2 $\pm$ 73.8<br>(67)*   |

\*-Statistically significant difference as compared with the values of euthyroid rats  
 (p<0.05)

heart. Ethionine significantly decreased the activities of HK and G-6Pdh to 56% and 52% respectively, while PK, 6-PGdh,  $\alpha$ -GPdh, and G-3Pdh activities were also significantly lowered to 77%, 72%, 73%, and 67% respectively, when expressed per total heart.

## DISCUSSION

Dow and Allen (1961) in demonstrating the effects of hyperthyroidism on glucose metabolism in rats, found that the rate of expired  $\text{CO}_2$  was increased as a result of glucose catabolism through the Embden-Meyerhof pathway and Kreb's tricarboxylic acid cycle. Their experiments involved the measurement of  $\text{C}^{14}\text{O}_2$  produced in the steady state of the thyrotoxic animal when glucose-1- $\text{C}^{14}$  and glucose-6- $\text{C}^{14}$  were injected into these animals. The yields of  $\text{C}^{14}\text{O}_2$  obtained after a single injection of either glucose-1- $\text{C}^{14}$  or glucose-6- $\text{C}^{14}$  to hyperthyroid rats were about equal and indicated that the hexose monophosphate pathway was not involved in glucose metabolism under these conditions. These results led Dow and Allen (1961) to postulate that hyperthyroid animals preferentially metabolized glucose through the glycolytic pathway. Although such experiments provide useful information in corroborating results obtained from studies on the regulation of glycolytic enzyme activities by thyroid hormones, they furnish little information about either the organs affected or changes that occur at the cellular level.

Studies of hormonal regulation of enzyme activities provide valuable information which aids in elucidating the mechanism of hormone action on a tar-

get organ at the subcellular level. It would be completely erroneous though, to theorize about hormonal action in vivo without taking into consideration other factors such as alterations in substrate and coenzyme concentrations as well as the redox potential of the cell, all of which are crucial participants in the regulation of any given metabolic pathway. In this connection, Weber (1963) has proposed that substantial increases in the activities of some enzymes, particularly the rate-limiting enzymes of certain metabolic pathways, are strongly suggestive of an increased usage of that pathway.

The results of the present experiments indicate that hypothyroidism appears to be associated with a decrease in cardiac glucose oxidation, since depressed activities of 3 enzymes of the Embden-Meyerhof pathway, including 2 key glycolytic enzymes were observed in the heart. The activities of cardiac hexokinase, pyruvate kinase, and glyceraldehyde-3-phosphate dehydrogenase were significantly lowered to between 20% and 45% of control values and administration of a 34  $\mu\text{g}$  dose of  $\text{T}_3$  to hypothyroid animals significantly increased enzyme activities by about 15% above hypothyroid values (Tables 3 and 5). Since the

$T_3$ -induced increases were effectively inhibited by cycloheximide and actinomycin D, two inhibitors of RNA and protein synthesis, the results suggest that de novo synthesis of enzyme proteins may be one aspect of the mechanism of action of triiodothyronine.

In support of the present observations and suggestion are the experiments of Krause et al (1967) who proposed that the  $T_3$ -induced increases in the activities of triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerokinase, glycerophosphomutase and enolase in diaphragm muscle of the hypothyroid rat were due to new enzyme protein synthesis.

In addition, hypothyroidism resulted in lowered activities of lactate dehydrogenase and pyruvate kinase in both liver (Tata et al, 1963; Bottger et al, 1970) and adrenal gland (Freedland and Murad, 1969) while Bargoni and Grillo (1967) demonstrated reductions in the activities of glucose phosphate isomerase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase and pyruvate kinase from skeletal muscle of hypothyroid rats.

Although Tata et al (1963) neglected to

state the actual decrease in hepatic lactate dehydrogenase observed in hypothyroid rats, the activity of this enzyme was significantly increased by 42%, 88 hours after injection of a 30  $\mu\text{g}$  dose of  $\text{T}_3$ . Bottger et al (1970) reported significantly lowered activities of hepatic pyruvate kinase to 59% of control values in hypothyroid rats but were unable to demonstrate any significant increase 3 days after administration of a single 50  $\mu\text{g}$  dose of  $\text{T}_3$ .

Freedland and Murad (1969) showed decreases in adrenal lactate dehydrogenase and pyruvate kinase activities of 51% and 7% of control values respectively in hypothyroid rats. In these experiments, adrenal phosphohexose isomerase and glyceraldehyde-3-phosphate dehydrogenase remained unchanged. Treatment of hypothyroid animals with 1 mg of thyroxine for 5 days produced a significant increase in lactate dehydrogenase activity to 226% of control values although pyruvate kinase activity remained unaltered. Freedland and Murad (1969) concluded that exogenous thyroxine treatment to hypothyroid animals did not appear to increase the glycolytic potential of the adrenal in these animals. A similar conclusion could be derived from Bottger's experiments (1970) on hepatic pyruvate

kinase. However, the present results on pyruvate kinase activity are not suggestive of a similar lack of effect of thyroid hormones on the myocardium for significant increases in this enzyme activity were recorded 48 hours after a 34  $\mu\text{g}$  dose of  $\text{T}_3$ , was administered (Table 3). It would appear that the unaltered pyruvate kinase activities observed in the liver and adrenals and the enhanced activities presently observed in the heart after thyroid hormone treatment might be due to different responses by these organs to thyroid hormones.

Reductions in the activities of glucose phosphate isomerase to 23%, triose phosphate isomerase to 49%, glyceraldehyde-3-phosphate dehydrogenase to 20%, phosphoglycerate kinase to 31%, and pyruvate kinase to 33% have been reported by Bargoni and Grillo (1967) in skeletal muscle of propyl thiouracil-treated rats.

Conversely, hyperthyroidism produces an acceleration of glycolysis by augmenting the activities of enzymes associated with the Embden-Meyerhof pathway in cardiac muscle of hyperthyroid rats (Dow and Allen, 1961). In the present experiments, both rate-limiting enzymes of glycolysis, hexokinase and pyruvate kinase

activities were significantly enhanced to 175% and 167%, while glyceraldehyde-3-phosphate dehydrogenase activity was increased to 147% of control values respectively (Tables 15 and 17). The increases in cardiac hexokinase, pyruvate kinase and glyceraldehyde-3-phosphate dehydrogenase activities observed in the hyperthyroid animal were all suppressed by cycloheximide, puromycin and ethionine, inhibitors of protein synthesis, suggesting the involvement of RNA with subsequent synthesis of new enzyme protein as a possible sequelae to thyrotoxicosis.

Hyperthyroidism is also capable of inducing profound effects on glycolytic enzymes of the liver (Freedland, 1965), skeletal muscle (Bargoni and Grillo, 1967), kidney (Bargoni and Grillo, 1967) and adrenals (Freedland and Murad, 1969).

Freedland (1965), working with hepatic tissues of rats treated with 1 mg of thyroxine for 5 days, found that phosphohexose isomerase activity was elevated from 355 to 591, glyceraldehyde-3-phosphate dehydrogenase from 273 to 434 and lactate dehydrogenase from 1623 to 2894, when these changes were expressed as units of enzyme activity/100 g body weight. Hepatic aldolase activity remained unchanged by thyroxine

treatment. Since hepatic glucose-6-phosphatase activity was also significantly increased from 74.4 to 158 units/100 g body weight and liver glycogen stores were depleted to a non-measurable amount, this investigator suggested that gluconeogenesis might be accelerated in the liver of the hyperthyroid animal. However, there was insufficient evidence to come to this conclusion. Since only the "bifunctional" glycolytic enzymes (Weber et al, 1965) and glucose-6-phosphatase were studied by Freedland, an investigation into the effects of hyperthyroidism on the activities of key hepatic glycolytic enzymes as well as the other enzymes of gluconeogenesis would seem to be of great importance. The increased activities of the hepatic "bifunctional" enzymes observed in hyperthyroidism could have been indicative of an increased rate of glycolysis rather than gluconeogenesis and the hexose phosphates formed from increased glycogenolysis would then be metabolised very readily through the Embden-Meyerhof pathway. Also, it is generally known that glutamate-pyruvate transaminase activity increases during gluconeogenesis and Freedland reported no change in the activity of this enzyme under conditions of hyperthyroidism. This casts

additional doubt on Freedland's suggestion that thyroid hormones regulate gluconeogenesis in hepatic tissues.

The increases in the activities of hexokinase to 325%, glucose phosphate isomerase to 130%, phosphoglycerate kinase to 149% and lactic dehydrogenase to 115% from skeletal muscle of hyperthyroid rats as well as kidney fructose-1-6-diphosphate aldolase to 129%, phosphofructokinase to 172% and phosphoglyceromutase to 149% of control values obtained by Bargoni and Grillo (1967) indicate an acceleration of glycolysis in these organs.

Parallel effects also occur in the adrenals of hyperthyroid rats, since enhanced activities of phosphohexose isomerase to 190%, pyruvate kinase to 500% and lactate dehydrogenase to 134% of control values were observed (Freedland and Murad, 1969).

Although investigations into the hyperthyroid-induced increases in glycolytic enzyme activities were not undertaken by both Bargoni and Grillo (1967) as well as Freedland and Murad (1969), it is quite possible that the increases observed may be the result of new enzyme protein synthesis, since it has presently been demonstrated that inhibitors of protein biosynthesis

are capable of blocking effectively the  $T_3$ -induced increases in cardiac hexokinase, glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase activities.

Thyroid hormones also appear to regulate glucose catabolism via the pentose phosphate pathway, since Necheles et al (1962), in experiments with hypothyroid rats, found that less glucose was metabolized through the hexose monophosphate pathway when compared to euthyroid or hyperthyroid rats, as evidenced by the yield of expired  $C^{14}O_2$  after injection of labelled glucose-1- $C^{14}$ . In the present investigation, cardiac muscle from hypothyroid rats exhibited significant decreases in glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities of 42% and 45% of control values respectively (Table 4).

Similar effects of hypothyroidism on these two rate-limiting enzymes from the pentose phosphate pathway have also been reported for liver (Tata et al, 1963) and adrenals (Freedland and Murad, 1969).

Although Tata et al (1963) did not report the extent to which hepatic glucose-6-phosphate dehydrogenase activity was lowered during hypothyroidism, 7 days after a single injection of 30  $\mu$ g of  $T_3$ , glucose-6-phosphate dehydrogenase activity was augmented

significantly to 53% above that obtained for non-treated controls.

Freedland and Murad (1969) found that whereas 6-phosphogluconate dehydrogenase activity was decreased significantly to 85% of control values, glucose-6-phosphate dehydrogenase activity remained unchanged in the adrenals of hypothyroid rats. Administration of 1 mg of thyroxine for 5 days to hypothyroid animals elevated significantly the activities of both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase to 183% and 247% of control values respectively. Freedland and Murad (1969) concluded that the regulation of NADPH generating enzymes might be related to an increase in cortical steroid production in the adrenal glands of the rat.

In the present experiments the increases in cardiac glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities to 76% and 70% of euthyroid values respectively, observed after  $T_3$  treatment of hypothyroid rats, were significantly suppressed by inhibitors of RNA and protein synthesis (Table 4). Inhibition of the  $T_3$ -induced increases by actinomycin D strongly suggests that the observed hormonal induction of these enzymes may be dependent on

the stimulation of DNA-directed synthesis of mRNA.

Hyperthyroidism elevated significantly the activities of both glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase to 204% and 437% of control values respectively in the myocardium of the rat (Table 16). Furthermore, the hyperthyroid-induced increase in these enzyme activities was significantly blocked by cycloheximide, puromycin and ethionine, inhibitors of RNA and protein synthesis, and suggests that de novo synthesis of these functional proteins may have been the basis for the increases in enzyme activities observed.

Other organs which display an increase in pentose phosphate enzyme activities in hyperthyroidism are the liver (Glock and McLean, 1955; Murad and Freedland, 1965; Freedland, 1965), adrenals (Freedland and Murad, 1969) and kidney (Szepesi and Freedland, 1969).

Glock and McLean (1955) reported that glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities were increased significantly in the liver of thyrotoxic rats to 200% and 262% of control values respectively. Similar increases in both hepatic glucose-6-phosphate dehydrogenase and 6-phos-

phogluconate dehydrogenase activities to 209% and 221% of control values respectively were reported by Murad and Freedland (1965) and Freedland (1965).

The adrenals of hyperthyroid rats exhibited augmented activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, which were increased by 43% and 64% above euthyroid values (Freedland and Murad, 1969).

Rat kidney glucose-6-phosphate dehydrogenase activity was also significantly elevated by 24% (Szepesi and Freedland, 1969) under conditions of hyperthyroidism.

It would be of interest to determine if synthesis de novo could account for the hyperthyroid induced increases in pentose phosphate enzyme activities observed in tissues other than heart, i.e. liver, adrenal and kidney.

The rate of glycolysis is also dependent on adequate concentrations of coenzyme,  $\text{NAD}^+$ , and the  $\text{NADH}/\text{NAD}^+$  ratio. An increase in the  $\text{NADH}/\text{NAD}^+$  ratio would result in a decreased rate of glycolysis, an increased rate of lactate formation and increased glycogen accumulation (Williamson, 1962).

Williamson (1962) studied the removal of

lactate and glucose as well as changes in cardiac glycogen content when either lactate, glucose or insulin, alone or in combination, were added to the perfusate of a Langendorf rat heart preparation. When the heart was perfused with glucose and lactate in the absence of insulin, all the glucose removed from the perfusate was accounted for as glycogen, lactate being the principle source of energy. In the presence of insulin, glucose uptake was increased four-fold, glycogen levels were enhanced and lactate uptake was not only suppressed but reversed by the formation of additional lactate from glucose. These results were interpreted as being due to changes in the  $\text{NADH}/\text{NAD}^+$  ratio and explained as follows. In the absence of insulin, glucose oxidation was limited by glucose availability as well as lactate oxidation to pyruvate, since NADH is a byproduct of this reaction. The increase in NADH concentration would thus inhibit glycolysis and the hexose formed would then have to be shunted to the glycogen synthetic pathway or the hexose monophosphate shunt. In the presence of insulin, however, glucose penetration was no longer rate-limiting and the glucose uptake exceeded the capacity of the heart to oxidize it, thereby generating in-

creased concentrations of NADH. As a result of this increase in the  $\text{NADH/NAD}^+$  ratio, elevated levels of lactate appeared in the medium while the glycogen content of the heart increased. Consequently, the rate at which NADH was converted to  $\text{NAD}^+$  could be rate-limiting under anerobic oxidation. In addition to the pyruvate-lactate mechanism of reoxidizing NADH, Lardy et al (1960) have proposed that the  $\alpha$ -glycerophosphate cycle may act as a shuttle mechanism in heart and liver, for transferring electrons from extramitochondrial NADH through the mitochondrial barrier, thereby regenerating cytoplasmic  $\text{NAD}^+$ . Quite possibly the  $\alpha$ -glycerophosphate cycle regulates the  $\text{NADH/NAD}^+$  ratio and hence the rate of glycolysis, as well as other synthetic pathways requiring  $\text{NAD}^+$ . Furthermore, these workers have shown that the  $\alpha$ -glycerophosphate shunt, consists of two enzymes, mitochondrial and cytoplasmic  $\alpha$ -glycerophosphate dehydrogenases. Of interest is the observation of Lee and Lardy (1965) who found that hepatic mitochondrial  $\alpha$ -glycerophosphate dehydrogenase was extremely sensitive to the thyroid status of the rat, increasing to 2200% and decreasing to 20% of control under conditions of hyper- and hypothyroidism respectively. Similar effects have also been observed in

heart muscle of the rat by Isaacs et al (1969) who reported a three-fold elevation of mitochondrial  $\alpha$ -glycerophosphate dehydrogenase activity in the myocardium of hyperthyroid animals, while thyroid-ectomized rats exhibited a 75% decrease in the mitochondrial activity of this enzyme, leading these investigators to conclude that thyroid hormones could control carbohydrate metabolism through regulation of the  $\alpha$ -glycerophosphate cycle in cardiac tissue.

In the present study, cardiac cytoplasmic  $\alpha$ -glycerophosphate dehydrogenase activity was significantly influenced by the thyroidal status of the rat since this enzyme activity was lowered to 42% under conditions of hypothyroidism and rapidly restored to 82% of control values by a single dose (34  $\mu$ g/100 g) of triiodothyronine (Table 5). Inhibition of the  $T_3$ -induced increase in this enzyme activity occurred when either cycloheximide or actinomycin D was administered in conjunction with  $T_3$ . On the other hand, hyperthyroidism elevated significantly the activity of extra-mitochondrial  $\alpha$ -glycerophosphate dehydrogenase to 182% of control values. The hyperthyroid-induced increases in this enzyme activity were completely suppressed by administration of cycloheximide, puromycin and ethio-

nine, inhibitors of RNA and protein synthesis (Table 17). These results suggest that the increases in cytoplasmic  $\alpha$ -glycerophosphate dehydrogenase activity observed following  $T_3$  treatment of hypothyroid or normal animals most likely was the result of enzyme synthesis de novo.

It is of interest to note that Lee and Lardy (1965) were unable to demonstrate any changes in the activity of cardiac or hepatic cytoplasmic  $\alpha$ -glycerophosphate dehydrogenases under varying thyroid states, although alterations in the mitochondrial activity of this enzyme were demonstrated. A possible explanation for the discrepancy that exists between the present study and that of Lee and Lardy (1965) lies in the fact that the latter workers expressed their enzyme activity per mg protein of the heart. Since myocardial proteins do not remain constant but fluctuate concomitant with changes in enzyme activities under different thyroid states, it would appear that any change in enzyme activity expressed per mg protein would not be significantly different from control values. In hyperthyroidism, for example, myocardial proteins and RNA were both increased (Bressler and Wittels, 1966), however, when the RNA concentrations

were expressed per mg of cardiac protein, no change in RNA concentrations was evident in comparison to controls. Similarly, DNA concentrations, expressed per mg protein, appeared to be significantly lowered in the thyrotoxic animal heart, although cellular DNA content remained unaltered (Bressler and Wittels, 1966).

Freedland (1965) has provided information which supports such an explanation, since he obtained conflicting results from the same treated animals when enzyme activities were expressed per 100 g body weight, per liver or per unit protein. In experiments designed to show the effects of an iodinated casein diet on hepatic glycolytic enzyme activities, Freedland found that the activities of phosphoglucomutase, phosphohexose isomerase, aldolase, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase were significantly different from control values when enzyme activities were expressed per 100 g body weight. Since both liver/body weight ratio as well as total hepatic protein content were decreased in rats fed iodinated casein, the differences in phosphoglucomutase, phosphohexose isomerase, aldolase, glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase activities between treated and non-treated animals disap-

pared when enzyme activities were expressed per g of liver. If the activity per unit of liver protein was considered, the differences in the activities of phosphoglucomutase, phosphohexose isomerase and aldolase between treatments became negligible.

Since the cytoplasmic  $\text{NADH}/\text{NAD}^+$  ratio is regulated by extramitochondrial  $\alpha$ -glycerophosphate dehydrogenase, the activity of this enzyme should still be rate-limiting for glycolysis and other processes requiring  $\text{NAD}^+$  even in the presence of an increased activity of mitochondrial  $\alpha$ -glycerophosphate dehydrogenase. With both mitochondrial and extramitochondrial enzymes being controlled by thyroid hormones simultaneously, the  $\text{H}^+$  ion shuttle pathway would no longer limit the rate of glycolysis or reactions requiring the oxidized coenzyme,  $\text{NAD}^+$ . In fact, Isaacs et al (1969), using perfused rat hearts from hyper- and hypothyroid rats, showed that lactate uptake was actually increased by the hyperthyroid rat heart when compared to either control or hypothyroid hearts. This suggests that in hyperthyroidism, with an increased activity of the  $\alpha$ -glycerophosphate cycle,  $\text{NAD}^+$  becomes available for lactate oxidation at a greater rate, thereby enhancing its uptake. Although cytoplasmic

$\alpha$ -glycerophosphate dehydrogenase activity was not measured by Isaacs et al (1969), their results suggest that the cytoplasmic enzyme activity may also have increased. This would account for the fact that accumulation of either dihydroxyacetone phosphate or reduced  $\text{NAD}^+$  was not observed, as evidenced from the unaltered  $\text{NADH}/\text{NAD}^+$  and  $\alpha$ -glycerophosphate/dihydroxyacetone phosphate ratios in the hyperthyroid rat heart.

Dietrich and Smith (1960), in demonstrating the effects of different thyroid states on butyrate oxidation in rat heart, found that addition of nicotinamide, which maintains functional  $\text{NAD}^+$  concentrations, to heart homogenates of euthyroid animals enhanced butyrate oxidation, resulting in an increased uptake of oxygen. However in the presence of exogenously added nicotinamide, hyperthyroid rat heart homogenates failed to exhibit further increases in butyrate oxidation and oxygen uptake suggesting to these workers that functional  $\text{NAD}^+$  was not rate-limiting in heart homogenates from hyperthyroid rats as appeared to be the case with euthyroid animals. These results also indicate that adequate concentrations of  $\text{NAD}^+$  in the hyperthyroid rat heart must have been the result of increased activities of both mitochondrial and

cytoplasmic  $\alpha$ -glycerophosphate dehydrogenase activities.

The present study indicates that triiodothyronine greatly influences the behaviour of the two key glycolytic enzymes, hexokinase and pyruvate kinase, as well as the  $\text{NAD}^+$ -dependent glyceraldehyde-3-phosphate dehydrogenase in cardiac muscle of rats. In addition, the activities of the two rate-limiting enzymes from the pentose phosphate shunt, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase as well as  $\alpha$ -glycerophosphate dehydrogenase from the  $\text{H}^+$  ion shuttle would appear to be under the control of this hormone. The in vivo studies involving the use of a variety of inhibitors of RNA and protein synthesis raise the possibility that both new RNA and new protein synthesis may be involved in the observed triiodothyronine-induced increases in enzyme activities in myocardial tissue of the rat. In fact, Bressler and Wittels (1966) reported that thyrotoxic guinea pigs exhibited an increased cardiac content of both RNA and protein. Furthermore, Cohen et al (1966) showed that these increases were direct effects of thyroid hormones on the myocardium and not the result of cardiovascular changes. The present evidence suggests that the primary action of thyroid hormones on cardiac muscle might

be the nuclear stimulation of protein biosynthesis as observed for hepatic tissue by Tata and Widnell (1966). Such an action of triiodothyronine could account for augmented RNA synthesis and its consequent accumulation in the cytoplasm of the heart, which would then result in an increased incorporation of amino acids for the synthesis of functional proteins. It is conceivable that the ability of thyroid hormones to alter basal metabolic rate stems from their capacity to alter the activities of functionally-related enzymes in cardiac as well as other tissues of the rat.

Since increased activities of rate-limiting enzymes from the Embden-Meyerhof and pentose phosphate pathways were observed in the present investigation, glucose would consequently be metabolized at a faster rate through both the Embden-Meyerhof and pentose phosphate pathways in myocardial cells of hyperthyroid rats. Although cardiac hexokinase and pyruvate kinase activities were increased in thyrotoxicosis, glycolysis appears to be blocked at the phosphofructokinase step since an accumulation of glucose-6-phosphate and fructose-6-phosphate was observed in the heart of the thyrotoxic guinea pig (Bressler and Wittels, 1966).

These results were further supported by the finding that citrate, which is a potent inhibitor of cardiac phosphofructokinase activity (Newsholme et al, 1962) was also elevated in the hyperthyroid guinea pig heart (Bressler and Wittels, 1966). Under these conditions, glucose-6-phosphate and fructose-6-phosphate would have to be metabolized either via the glycogen synthetic or the pentose phosphate shunt pathways. Incorporation studies have indicated that glucose is rapidly synthesized into glycogen under conditions of hyperthyroidism (Bressler and Wittels, 1966), suggesting an enhanced activity of this pathway. Since increased activities of cardiac glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were observed in the present study, it is quite likely that some of the hexose phosphates must also be metabolized via the pentose phosphate pathway. Indeed, the importance of this pathway bears more significance in the hyperthyroid rat heart. Since the heart must require an increased amount of ATP under thyrotoxic conditions, the shunting of glucose-6-phosphate through the hexose monophosphate pathway is energetically more efficient. In so doing, an energy requiring step, conversion of fructose-6-phosphate to fructose-1-6-diphosphate by phosphofructokinase, is

minimized. In addition to the formation of pentoses, which are necessary for RNA synthesis (Meerson et al, 1967), the hexose monophosphate shunt also produces glyceraldehyde-3-phosphate, a substrate which can further be metabolized through the glycolytic pathway. The augmented activity of glyceraldehyde-3-phosphate dehydrogenase under conditions of hyperthyroidism is suggestive of an increased oxidation of glyceraldehyde-3-phosphate in this condition. Since an adequate supply of  $\text{NAD}^+$  (a necessary coenzyme for the conversion of glyceraldehyde-3-phosphate to 1-3-diphosphoglyceric acid by glyceraldehyde-3-phosphate dehydrogenase) results from an enhanced  $\alpha$ -glycerophosphate dehydrogenase activity, oxidation of glyceraldehyde-3-phosphate by glyceraldehyde-3-phosphate dehydrogenase proceeds unhindered in the myocardium of the hyperthyroid rat. The observation that cardiac pyruvate kinase activity was significantly augmented suggests that this rate-limiting reaction was increased under these conditions. Since the reaction catalyzed by this enzyme also generates ATP, an acceleration of this reaction would increase the rate at which cytoplasmic ATP is synthesized. It therefore appears as though glyceraldehyde-3-phosphate, on entering the glycolytic pathway is oxi-

dized unimpeded to pyruvate in hearts of thyrotoxic animals. Hence, it can be postulated that for every glucose molecule metabolized anerobically in the thyrotoxic rat heart 3 ATPs would be synthesized in comparison to hearts of euthyroid animals in which only 2 ATPs are produced. The heart of the hyperthyroid animal would therefore appear to be more efficient with respect to anerobic energy production (ATP synthesis) than that from a normal animal.

The increased oxidation of glucose via the hexose monophosphate shunt would by necessity give rise to an increased concentration of NADPH in the heart as noted for hepatic tissues (McGuire and Tomkins, 1959). However, the reduced co-enzyme can then be reoxidized by either an increase in fatty acid synthesis which occurs in the heart (Dietrich and Smith, 1960; Bressler and Wittels, 1966) and/or the NADPH cytochrome C reductase pathway. Since this enzyme does not appear to be attached to the particulate matter in hog myocardial cells (Ball and Cooper, 1957) and its activity as well as cytochrome C content are known to be elevated in hepatic tissue of hyperthyroid rats (Phillips and Langdon, 1956), the finding that this pathway is an energetically wasteful, heat producing process (Tata et al,

1963) suggested that oxidation of NADPH through this pathway could be the mechanism by which the uncoupling of oxidative phosphorylation is effected by thyroid hormones (Hoch, 1962).

In the light of the available evidence, a reversal of the above effects would be expected under conditions of hypothyroidism. In fact, concomitant with a depressed rate of glucose oxidation through the hexose monophosphate shunt (Necheles et al, 1962), decreases in the activities of enzymes associated with the glycolytic, hexose monophosphate shunt and  $\alpha$ -glycerophosphate shunt pathways have all been demonstrated in the hypothyroid rat heart. The increased accumulation of glycogen in cardiac muscle of hypothyroid rats suggests that glycogenolysis was significantly depressed in marked contrast to that observed from hearts of either euthyroid or hyperthyroid animals (Isaacs et al, 1969). The fact that the  $\text{NADH}/\text{NAD}^+$  ratio remains unaltered in the presence of depressed  $\alpha$ -glycerophosphate dehydrogenase activity (Isaacs et al, 1969) together with the results obtained in the present study support the theory of a decreased carbohydrate metabolism in cardiac muscle of hypothyroid rats.

The results obtained in the present study

indicate the importance of thyroid hormones in maintaining an adequate amount of functional enzyme proteins concerned with carbohydrate metabolism in the heart.

An interesting finding arising from the present study is the effect produced by inhibitors of RNA and protein synthesis on the basal activities of cardiac enzymes associated with the glycolytic, hexose monophosphate and  $\alpha$ -glycerophosphate shunt pathways.

Cycloheximide, an antibiotic that suppresses protein synthesis by inhibiting either the transfer of amino acyl transfer RNA to ribosomes or the formation of peptide bonds (Ennis and Lubin, 1964; Siegel and Sisler, 1964), caused a decrease in basal activities of cardiac hexokinase to 75%, pyruvate kinase to 77%, glucose-6-phosphate dehydrogenase to 38% and  $\alpha$ -glycerophosphate dehydrogenase to 48% of control values respectively (Tables 19, 20 and 21). Administration of puromycin, which inhibits protein synthesis by forming a peptide bond between the terminal carboxyl group of the growing peptide thus causing the release of an unfinished peptide chain from the ribosome template (Yarmolinsky and DeLaHaba, 1959), resulted in a lowering of the activities of hexokinase to 40%, pyru-

vate kinase to 56%, glyceraldehyde-3-phosphate dehydrogenase to 67%, glucose-6-phosphate dehydrogenase to 53%, 6-phosphogluconate dehydrogenase to 53% and  $\alpha$ -glycerophosphate dehydrogenase to 68% of basal values respectively. Ethionine, which is thought to inhibit the synthesis of proteins through an inhibition of RNA synthesis as well as by lowering ATP concentrations (Villa Trevino et al, 1966; Farber et al, 1963), caused significant decreases in the activities of hexokinase to 56%, pyruvate kinase to 73%, glyceraldehyde-3-phosphate dehydrogenase to 63%, glucose-6-phosphate dehydrogenase to 52%, 6-phosphogluconate dehydrogenase to 72%, and  $\alpha$ -glycerophosphate dehydrogenase to 73% of control values respectively.

The present results, in regard to a depression of basal enzyme activities following use of inhibitors, are in direct conflict with those obtained by other investigators. By virtue of their action on RNA and protein synthesis, these compounds have been used to supply information about the nature of the hormonal induction of enhanced enzyme levels. Although these inhibitors suppressed the cortisone-induced increases in the activities of hepatic glucose-6-phosphatase and fructose-1-6-diphosphatase (Weber et al, 1965), as

well as estradiol-induced increases in uterine phosphofructokinase and aldolase activities (Singhal et al, 1967; Schwark et al, 1969), administration of either ethionine, cycloheximide, actinomycin D or puromycin appeared to have no effect on enzyme levels in these tissues in the non-hormone treated animals.

Although the results obtained by these workers were unexpected, the present study indicates that in the steady state of the animal continuous turnover of functional proteins occurs in the heart. Since the basal enzyme activities were not all lowered to the same amount following inhibitor treatment, it would seem reasonable that different enzyme proteins exhibit different half lives in the resting state condition, a factor which could account for the observations of Weber et al (1965b) as well as Singhal et al (1967) and Schwark et al (1969). In fact, Kenney (1967) reported a half life of one and a half hours for hepatic tyrosine transaminase activity while Tschudy et al (1965) observed a half life of 67 - 72 minutes for rat liver amino levulinic acid synthetase. Bottger et al (1970) presented evidence that further corroborates the enzyme turnover rate as an important factor involved in the observed decreases in basal enzyme activities

after inhibitor treatment. They found that hepatic pyruvate kinase activity was significantly decreased to 38% of controls seventy hours after administration of 2 doses of 50  $\mu$ g of actinomycin, while the activities of phosphoenol pyruvate carboxykinase and pyruvate carboxylase remained unchanged.

The duration of treatment with these inhibitors of RNA and protein synthesis is also an additional factor, which might influence basal enzyme activities. In the present study, rats were injected with either cycloheximide, ethionine or puromycin for 7 days, while in the studies of Singhal et al (1967) and Schwark et al, (1969) inhibitor-treated rats were killed 16 hours after injection of antibiotics. It is conceivable that prolonged treatment with these compounds produces non-specific toxic effects, one of which could be a leakage of certain enzyme proteins from the myocardial cell into the serum. Such a mechanism of tissue enzyme depletion under pathologic conditions is not uncommon since glutamic-oxaloacetic transaminase activity increases within 24 to 28 hours after myocardial infarction (Wroblewski, 1959). Furthermore, the elevation of serum glutamic-oxaloacetic transaminase appears to be due to the release of intracellular enzymes into the blood

stream resulting from either death of the cell or loss of integrity of the cellular membrane. This was based on the findings that the activity of glutamic-oxaloacetic transaminase in infarcted muscle was appreciably less than that in the adjacent normal muscle of the same heart and this enzyme activity in infarcted muscle was diminished with the age of the infarct (Wroblewski, 1959). Similar observations have also been reported for hepatic tissues by Friend et al (1955) who found that viral hepatitis in mice was associated with increased activities of both serum glutamic-oxaloacetic transaminase and serum glutamic pyruvic transaminase. In addition, trauma to the liver following partial hepatectomy also resulted in an increased activity of glutamic-oxaloacetic transaminase in mice serum (Friend et al, 1955). In order to verify such a theory for inhibitor-action on the reduction of basal enzyme activities, further experiments on activities of these enzymes in serum after administration of these drugs would have to be carried out. Recently, Geleherter and Tomkins (1970) reported a lowering of tyrosine transaminase activity in rat hepatoma cells following addition of cycloheximide or actinomycin D to the incubation medium, but failed to comment about

these observations. It is possible that the decreases observed were due to the non-specific toxic effects of these compounds.

Since prolonged treatment of experimental animals with either cycloheximide (Grieg and Gibbons, 1959), ethionine (Farber et al, 1964) or puromycin (Wright et al, 1955) produces diarrhea and loss of body weight as well as lesions in the adrenals, stomach, pancreas, liver, testis and spleen, it is not surprising that basal enzyme values were lowered in the heart after administration of these compounds to rats for one week. It is therefore necessary to observe caution in the interpretation of results when working with inhibitors in cardiac tissue.

## SUMMARY

- (1) The effects of hypothyroidism on representative cardiac enzymes from the glycolytic, hexose monophosphate shunt and the  $\alpha$ -glycerophosphate shunt pathways were studied in rats 4 weeks after radiothyroidectomy. Hexokinase, pyruvate kinase, and glyceraldehyde-3-phosphate dehydrogenase activities were decreased to 20%, 41% and 43% of control values, while glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and  $\alpha$ -glycerophosphate dehydrogenase activities were reduced to about 40% of control values respectively.
- (2) Significant increases in the activities of myocardial hexokinase (33%), pyruvate kinase (53%), glyceraldehyde-3-phosphate dehydrogenase (55%), glucose-6-phosphate dehydrogenase (76%), 6-phosphogluconate dehydrogenase (70%), and  $\alpha$ -glycerophosphate dehydrogenase (82%) were observed 48 hours after injection of a single 34  $\mu$ g dose of triiodothyronine to hypothyroid rats. The triiodothyronine-induced increases were partially but effectively inhibited by concomitant administration of either actinomycin D or cycloheximide suggesting that stimulation of the synthesis of certain RNA species may be involved in the obser-

- ved enzymatic induction.
- (3) Cardiac enzyme activities of rats 4 and 6 weeks following radiothyroidectomy, were compared. The activities of hexokinase, pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and  $\alpha$ -glycerophosphate dehydrogenase were further decreased in animals 6 weeks after radiothyroidectomy. However, no further alteration in the activity of cardiac glucose-6-phosphate dehydrogenase activity was observed at 6 weeks.
  - (4) Dose-response studies in animals 6 weeks after  $^{131}\text{I}$  treatment indicated that significant elevations of hexokinase, pyruvate kinase, 6-phosphogluconate dehydrogenase and  $\alpha$ -glycerophosphate dehydrogenase activities were obtained by doses of 68  $\mu\text{g}/100$  g. The activities of glucose-6-phosphate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase were significantly increased only after injection of 136  $\mu\text{g}$  and 200  $\mu\text{g}$  of  $\text{T}_3/100$  g respectively.
  - (5) Hyperthyroidism, induced by administration of 250  $\mu\text{g}$  of triiodothyronine for one week to euthyroid animals, markedly enhanced the activities of car-

diac hexokinase (175%), pyruvate kinase (167%), glyceraldehyde-3-phosphate dehydrogenase (147%), glucose-6-phosphate dehydrogenase (204%), 6-phosphogluconate dehydrogenase (437%), and  $\alpha$ -glycerophosphate dehydrogenase (182%) respectively. The marked enhancement in the activities of the glycolytic, hexose monophosphate and  $\alpha$ -glycerophosphate shunt enzymes were effectively inhibited by ethionine, puromycin and cycloheximide and it was therefore concluded that these increases in the activities of the enzymes studied may have been due to de novo synthesis of new enzyme protein.

- (6) Treatment of euthyroid animals with cycloheximide, puromycin and ethionine produced significant decreases in basal levels of cardiac hexokinase, pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and  $\alpha$ -glycerophosphate dehydrogenase activities suggesting a possible toxic effect of these compounds on cardiac muscle cell.
- (7) The results suggest that stimulation of RNA synthesis is an early biochemical response elicited

by triiodothyronine resulting in the de novo synthesis of certain rate-limiting enzymes from the glycolytic, hexose monophosphate and  $\alpha$ -glycerophosphate shunt pathways in cardiac muscle of the rat.

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