MYOCARDIAL FUNCTION CHARACTERISTICS, RESPONSE TO ISOPROTERENOL, AND CALCIUM UPTAKE ACTIVITY IN RATS PRETREATED WITH THYROID HORMONES

by

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B.Sc., The University of Alberta, 1967

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE in

THE FACULTY OF GRADUATE STUDIES
Division of Pharmacology and Toxicology
of the Faculty of Pharmaceutical Sciences

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
October, 1982

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ABSTRACT

Thyroid hormone pretreatment altered rat myocardial function curve characteristics, showed no effect on the sensitivity of the heart to isoproterenol, and did not change the calcium uptake activity into sarcoplasmic reticulum vesicles.

Hearts from rats treated either for 3 days with L-triiodothyronine (T3) or for 7 days with L-thyroxine (T4) were subjected to changes in atrial filling pressure from 5 cm of water to 22.5 cm of water on the modified Neely working heart apparatus. Thyroid hormone pretreated rat hearts showed increased pressure and rate parameter measurements over their vehicle pretreated counterparts at all atrial filling pressures. Time to peak pressure was not significantly lowered, but relaxation time for the T3 treated rat hearts was significantly lower than that of control rat hearts at all atrial filling pressures. T4 hearts had shorter relaxation times than did control hearts at the lower filling pressures, but at filling pressures over 15 cm of water the T4 relaxation times approached those of control. At 17.5 cm of water filling pressure T3 hearts had significantly shorter relaxation times than did T4 hearts. Measurement of the total pulse period or total contraction time showed similar trends, however in this case T3 hearts had shorter total pulse periods than T4 hearts at all atrial filling pressures over 15 cm of water. Areas
were measured under the left ventricular pressure curve. None of the area measurements for thyroid hormone pretreated hearts were significantly different from control.

Isoproterenol dose response curves were also obtained from the working hearts of all experimental groups. T3 hearts consistently showed higher values in left ventricular developed pressure, rate of pressure development and rate of relaxation than did control and T4 hearts even before isoproterenol was administered. T4 hearts had values not different from control values at any dose of isoproterenol in the measurement of any parameter. Time to peak pressure and relaxation time did not vary between groups. At a sub-maximal dose of isoproterenol T3 hearts had a significantly decreased total time of contraction. Area under the curve from peak pressure back to baseline was significantly increased over control levels for T3 hearts at two low doses of isoproterenol. Total area under the curve was not changed in thyroid hormone treated hearts. In order to account for the wide difference in baseline values for all the parameters, the data were expressed in terms of percent maximum response, and the pD2 values (negative log ED50) were calculated. The pD2 values so obtained were not different between the groups.

Calcium uptake activity into cardiac sarcoplasmic reticulum was measured. No differences were seen between the experimental groups.

Thus, while performance of the rat heart in terms of left ventricular pressure, and rate of pressure rise and fall was
increased by thyroid hormone pretreatment, no change in sensitivity of the heart to isoproterenol could be shown. There was also no difference in calcium uptake activity. We speculate that changes in the calcium sensitivity of the contractile proteins in the rat heart may be responsible for the increased myocardial contractility seen after thyroid hormone pretreatment. The lack of change in sensitivity to isoproterenol of the thyroid pretreated hearts could then be explained by the steric hindrance of this enhanced calcium sensitivity by catecholamine-induced phosphorylation of the troponin I subunit by cyclic AMP dependent protein kinase. Calcium uptake activity was unchanged by thyroid hormone pretreatment. If the augmented contractility observed in thyroid hormone pretreated hearts over the function curve was due to increased sensitivity of these hearts to an unchanged concentration of calcium within the cell, this similarity of calcium uptake activity would be expected.

John H. McNeill
Thesis Supervisor
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I would like to thank Dr. John H. McNeill for his unstinting support and guidance throughout my years in his laboratory.

I am especially grateful to David P. Harris, without whose microcomputer expertise and generosity this project could not have been completed.

I would also like to acknowledge the help of Judy Wyne in preparing the graphs included in this thesis.
INTRODUCTION

Hyperthyroidism produces a variety of hemodynamic and physical changes in the mammalian cardiovascular system. Clinical investigations (Graettinger et al. 1959; Amidi et al. 1968) have shown elevations in cardiac index, increased coronary blood flow, decreased peripheral resistance, increased myocardial oxygen consumption and increased right and left ventricular work, associated with hyperthyroidism. These changes in the function of the heart and blood vessels can not only compromise individuals with pre-existing heart disease, but may also cause cardiac complications in the patient with an otherwise healthy heart (Sandler and Wilson, 1959). Although cardiovascular performance in hyperthyroidism and its accompanying acute state, thyrotoxicosis, have been studied in quite exhaustive detail in both clinic and laboratory, many questions remain unanswered regarding the dynamic mechanisms of thyroid hormone actions upon the heart and vasculature, and indeed, also regarding the precise nature of these actions.

The level of thyroid hormones within the body is regulated through the central nervous system. L-thyroxine (T4) is deiodinated after release from the thyroid gland to the active form of the hormone L-triiodothyronine (T3). The action of T3 at the cellular level in the myocardium is not understood. A theory put forward by Sterling (1979) proposes
that T3 may bind to the inner mitochondrial membrane within the myocardial cell. Binding is promptly followed by an increase in oxidative metabolism. This type of binding has been shown, however the concentrations of thyroid hormone necessary to produce the effect are well out of the physiological range. Thus the importance of mitochondrial binding of T3 in physiological situations is of questionable importance. Whatever the method of interaction of T3 at the cellular level, it has certain undeniable actions in the myocardium once it has arrived in situ.

A. Cardiac Hypertrophy and Protein Synthesis

Cardiac hypertrophy has been defined as an increase in the heart weight to total body weight ratio. The characteristic loss of body weight of the hyperthyroid patient, combined with the enlarged heart often seen in this condition, make cardiac hypertrophy so common a symptom in hyperthyroidism as to constitute one of the markers for the disease (McEachern and Rake, 1931).

Cardiac hypertrophy after thyroid hormone treatment has been demonstrated experimentally in rats (Gemmill, 1958; Cairoli and Crout, 1967; Frazer et al. 1968; Sandford et al, 1978), in guinea pigs (Muryama and Goodkind, 1968), in cats (Strauer and Scherpe, 1975), in mice (Gemmill, 1958), and in rabbits (Banerjee et al. 1976). This, of course, is only a partial list of the demonstrations of this phenomenon.

Cardiac hypertrophy in thyrotoxic patients had been suggested by aberrations in electrocardiograph tracings, such
as increased duration of the QRS complex and increased amplitude of the R peak (Sandler, 1959). Recent echocardiographic work by Nixon et al. (1979), has shown a 27% reduction in left ventricular mass in hyperthyroid patients after successful therapy had returned them to the euthyroid state.

Thyroid hormone-induced cardiac hypertrophy has been characterized by Gemmill (1958) as a "true" cardiac hypertrophy, i.e. it does not result from an increase in cardiac water content. Zaimis et al. (1969), in their electron microscopic work in guinea pig heart, saw myocardial hypertrophy, enlargement of the myocardial cells, and a dramatic increase in the number, size, and complexity of mitochondria inside the myocardial cell. A larger number of mitochondria and an increase in the amount of pleomorphism of these mitochondria, was noted by Nayler and co-workers (1971) in dog hearts subjected to electron microscopy. Callas and Hayes (1974) saw no differences from control in sarcoplasmic reticulum or sarcomere ultrastructure in hyperthyroid rat hearts. They did, however, notice marked hypertrophy of mitochondria without an increase in mitochondrial number, as well as disorientation in the cristae. These disarrangements appeared to be reversible, as the experimenters saw no ultrastructural changes in hearts of animals who were allowed to return to the euthyroid state. Bartosova et al. (1969) observed a proportional increases in the size of both right and left ventricles in rat hearts. They also found no
increase in the amount of collagenous material in the heart after thyroid hormone treatment, the increase in heart weight being wholly attributed to muscle cell augmentation. The latter observation has been reinforced by the work of Limas and Chan-Stier (1978), who saw no increase in either collagen or in interstitial tissue in their work in rat heart.

An increase in protein synthesis has been correlated with thyroid hormone-induced cardiac hypertrophy (Gemmill, 1958; Sandford et al. 1978). Limas and Chan-Stier (1978) noted an increase in RNA content of hearts from T3-treated rats, and decided to examine the mechanism responsible for the increase in RNA synthesis. Increased myocardial chromatin template activity was observed, together with an augmentation of the number of transcription initiation sites. These increased functions were further localized to the non-histone nuclear fraction of the myocardial preparation, while a significant rise in nuclear protein kinase activity was also noticed. In vitro, stimulation of RNA synthesis by non-histone proteins was enhanced by the addition of nuclear protein kinases and cyclic adenosine monophosphate (cAMP). These results led the authors to postulate that, in hyperthyroidism, increased protein (RNA) synthesis is mediated by the non-histone proteins of the nucleus, and is dependent on phosphorylation of these proteins by nuclear protein kinases.

Studies by Sandford et al. (1978) indicate that the increased growth of the myocardium in hyperthyroidism is due to increased protein synthesis, rather than decreased protein
degradation. T4 treatment of rats caused increased protein synthesis, with slight retardation of protein degradation. When T4 treatment ceased, protein synthesis decreased while no change was noted in the level of protein degradation.

Experiments by Gemmill (1958) and Sandford et al. (1978), as well as the clinical data of Nixon et al. (1979) suggest that hyperthyroid cardiac hypertrophy is a reversible phenomenon.

To summarize, cardiac hypertrophy in thyroid hormone excess involves the cardiac myocytes almost exclusively, is reversible, and is associated with increased protein (probably RNA) synthesis, increased RNA content, and with an augmentation of the transcription of DNA.

B. Hemodynamic Effects of Thyroid Hormones

1. Resting heart rate

Resting heart rate greater than that seen in euthyroid individuals is a continuing observation in experimental and clinical hyperthyroidism. Experimental evidence has been well established for this increase in heart rate at rest, in dog (Van der Schoot and Moran, 1965; Margolius and Gaffney, 1965; Brewster et al, 1956; Taylor et al, 1969), in cat (Strauer and Scherpe, 1975), in mouse (Gemmill, 1958), in rabbit (Lee et al, 1965; Anton and Gravenstein, 1970), in guinea pig (Zaimis et al, 1969; MacLeod amd McNeill, 1981), and in rat (Van der Schoot and Moran, 1965; Cairoli and Crout, 1967; Frazer et al,
Clinically, thyrotoxic tachycardia has also been exhaustively documented (McEachern and Rake, 1931; Graettinger et al, 1959; Sandler, 1959; Wilson et al, 1966; De Groot and Leonard, 1970).

The mechanism by which thyroid hormone excess produces an increased resting heart rate is not yet understood. Cairoli and Crout, in 1967, used propranolol in an attempt to block the enhanced initial heart rate in unanaesthetized hyperthyroid rats. The hyperthyroid group exhibited less cardiac slowing in response to this maneuver than did the euthyroid animals. If thyrotoxic tachycardia was due to increased adrenergic nerve activity or to enhanced responsiveness of the sino-atrial (SA) node to noradrenaline, it would be expected that propranolol would have shown more effect in the thyroxine treated animals. When atropine as well was administered to the rats to check for deficiency of cholinergic input, a sharp increase in heart rate was observed. The hyperthyroid state thus appeared to have little effect on vagal input to the SA node. Pretreatment with reserpine resulted in reduction of the hyperthyroid heart rate to control levels, an effect which could be reversed by the subsequent injection of atropine. Thyroid hormone induced tachycardia does not seem to be the result of an enhancement of adrenergic neural activity, a deficiency in cholinergic activity, nor an increase in sensitivity of cardiac beta receptors to endogenous noradrenaline. There is, however, no direct evidence in support of these authors' conclusion that
thyroid hormones exert a direct effect on cardiac pacemaker cells.

Extensive use of propranolol in clinical trials and recently in general practice has shown that administration of this drug will improve certain of the symptoms of hyperthyroidism in man. Tachycardia can be reduced, while tremor, restlessness, anxiety, myopathy, sweating and heat intolerance all are ameliorated. Propranolol treatment does not, however, improve goitre, exopthalmos, and thyroid function tests, nor does it reduce the augmented oxygen consumption of hyperthyroidism. It should be noted that while heart rate is reduced in human hyperthyroid subjects treated with propranolol, it is not necessarily reduced to normal levels. (McDevitt, 1976). In T3 treated human volunteers, Wilson et al (1966) were able to abolish isoproterenol-induced tachycardia through the use of propanolol. The beta-adrenergic blocker had no effect, however, on T3-induced augmented resting heart rate.

Data obtained from experimental animals in thyroid hormone excess, treated with propanolol also show only a partial reduction of the hyperthyroid heart rate towards control, in rats (Cairoli and Crout, 1967) and in guinea pigs (Goodkind, 1968). Thus, both clinical and experimental results suggest that part of the increased resting heart rate commonly seen in hyperthyroidism is a product of increased beta-adrenergic activity. An explanation for the remaining increase is not yet available.
2. Other hemodynamic changes

Increases in oxygen consumption in hyperthyroid animals have been demonstrated in rats (Van der Schoot and Moran, 1965), in cats (Buccino et al. 1967; Skelton et al. 1970), in dogs (Brewster et al. 1956; Van der Schoot and Moran, 1965), and in man (Graettinger et al. 1959; Aoki et al. 1967; Amidi et al. 1968).

Another common effect of thyroid hormone excess in man is decreased peripheral resistance, as shown by Graettinger et al. 1959 and by Amidi et al. 1968. Experimental demonstration of decreased peripheral resistance has also been accomplished in rats (Beznak, 1962).

Cardiac index is commonly increased in hyperthyroidism; this increase has been shown in rats (Beznak, 1962), and in cats (Buccino et al. 1967; Strauer and Scherpe, 1975), as well as in man (Graettinger et al. 1959; Amidi et al. 1968).

Amidi et al. (1968) also showed increases in mean arterial pressure in hyperthyroid patients, while similar increases have been shown in thyroid hormone treated rats (Frazer et al, 1969) and cats (Strauer and Scherpe, 1975).

In mild to moderate hyperthyroidism cardiac output is increased mainly through the mechanism of augmented heart rate. Only in severe hyperthyroidism is an increased stroke volume added to the chronotropic alteration (DeGroot and Leonard, 1970). The increase in cardiac output, however far outstrips the increase in oxygen consumption in the hyperthyroid heart (Graettinger et al. 1959).
Symptoms of hyperthyroidism, then, commonly involve increased resting heart rate, decreased peripheral resistance, increased oxygen consumption, increased mean arterial pressure, and increased cardiac index, all indicative of a hyperkinetic, hyperdynamic circulatory state.

C. Electrophysiological Changes

Cardiac rhythm disturbances, particularly an increased propensity for arrhythmias, have been appreciated as clinical complications of the hyperthyroid condition for many years. In the experimental laboratory, Zaimis et al. (1969) noted an increase in heart excitability in T4 treated cat and guinea pig hearts. Arnsdorf and Childers (1970) found decreases in effective refractory period in isolated perfused hearts of T4 treated rabbits, as well as lowered diastolic threshold for atrial responses, especially those evoked by multiple pulses. These workers also showed that no enhancement of the effect of T4 on conduction velocity or refractory period could be demonstrated with infusions of adrenaline, noradrenaline or atropine.

Johnson et al. (1973) saw an increased rate of diastolic depolarization and decreased duration of the action potential in sinoatrial node cells, in both the presence and absence of propranolol, in hyperthyroid rabbits.

Hyperthyroidism shortens the refractory period and increases conduction velocity in the heart. The mechanism
behind this action of thyroid hormones is unknown. An explanation for this action might conceivably arise from the ultrastructural changes in the myocardial cell in hyperthyroidism described by Callas and Hayes (1974) and Zaimis et al. (1969), and their possible effect on membrane transport.

D. Contractility Effects

Considerable controversy has developed in the study of the effects of thyroid hormone excess on the intrinsic contractile properties of the mammalian myocardium. The bulk of evidence concerning the effects of thyroid hormones on myocardial contractility has been obtained in in vitro studies of isolated cardiac tissue preparations. A brief statement of some of the data obtained in the resting state of hyperthyroid myocardial preparations, concerning basal tension and pressure levels, will serve to illustrate some of the ongoing problems involved in the experimental elucidation of the contractile properties of the heart in hyperthyroidism.

Levey et al. (1969) using papillary muscle preparations from cats treated with T4 observed basal developed tensions equal to those developed by euthyroid papillary muscles, but also observed increased rate of tension development and decreased time to peak tension in hyperthyroid as compared to control. MacLeod and McNeill (1981) observed equivalent basal tensions in guinea pig left atria and papillary muscle from
hyperthyroid (T4 treated) and euthyroid animals. Longhurst and McNeill (1978) working with rat left atria and right ventricle papillary muscle did not observe any differences in basal developed tension. However, in the same study basal developed tension in Langendorff hearts was significantly lower in hyperthyroid (T3 treated) animals. Nayler et al. (1971) in contractile studies with dog trabecular muscles and with open chest preparations, observed increased developed tension and rate of tension development in tissues from T4 treated animals as compared to controls. Thus, there are reports of decreased, increased and equivalent basal tension development in the results of these experimenters. A clear view of the contractile state of the hyperthyroid heart is difficult to achieve. Part of this difficulty arises from differing contributions to that contractile state from differing species, treatment differences and differences in the type of tissue preparation used. This is not to imply that species, treatment and preparation differences do not affect studies other than those into the mysteries of hyperthyroidism, but merely to make note of the wide variation possibly due to these inherent causes.

1. Isometric studies

Several studies have directly addressed the characterization of the contractile properties of the hyperthyroid myocardium in an isometric isolated tissue preparation, in vitro. Buccino et al. in 1967 assessed the isometric tension of papillary muscles from T4 treated cats
and their vehicle treated control counterparts. These experimenters saw no difference in maximum developed isometric tension. Significant increases in rate of tension development and significant decreases in time to peak tension were clearly shown. These experiments were repeated for a range of temperatures from 21 degrees to 37 degrees Centigrade, showing directionally similar changes in time to peak tension and rate of tension development for hyperthyroid and euthyroid tissues i.e. as temperature rose, tension fell, time to peak tension shortened and rate of tension development was augmented.

Parmley et al (1968) using virtually the same preparation of cat papillary muscle treated with the same dose of T4, demonstrated decreased time to peak tension in hyperthyroid muscles as compared to control, but also observed a significant increase in maximum isometric tension.

In 1970, Taylor examined cat papillary muscle preparations at a frequency of 12 contractions per minute, which was the frequency used by the previous two experimenters. His experiments were extended to determine papillary muscle behavior at frequencies of 30 and 60 contractions per minute as well. In hyperthyroid preparations, in agreement with the results of Parmley et al. and Buccino et al., Taylor observed increased developed tension, and increased rate of tension development, with decreased time to peak tension with frequency at 12 contractions per minute. At 30 contractions per minute these increases were still noted. When frequency of contraction was
increased to 60 per minute however, the developed tension and rate of tension development in tissues from hyperthyroid animals were similar to those seen in muscles from euthyroid animals. This result was accompanied by the observation that the inotropic effect of increasing frequency was less in the hyperthyroid than in the euthyroid tissues, and by the proposal that the increased oxygen consumption required by high contraction frequency may induce an hypoxic depressant effect upon the hyperthyroid myocardium.

In 1968, Muryama and Goodkind carried out a study in guinea pig left atria which examined the effects of differing frequencies of contraction and differing calcium concentrations on contractile properties in hyperthyroid and euthyroid animals. They found developed tension in the preparations from hyperthyroid animals to be significantly increased over control at all frequencies measured (from 0.5 to 200 contractions per minute), when the external calcium concentration was 0.625 millimolar. In 2.5 millimolar calcium at frequencies under 100 per minute, developed tension was greater in hyperthyroid than in euthyroid tissues; at frequencies above 100 per minute there was no significant difference between the two groups. When the external concentration of calcium was 5 millimolar, there a significant increase in developed tension in the hyperthyroid over the euthyroid preparations only at frequencies below 50 per minute.

Skelton et al. in 1970 showed decreased time to peak
tension, increased rate of tension development, and increased total tension in papillary muscles from hyperthyroid as compared with euthyroid cats, associated with a considerable increase in myocardial oxygen consumption. One year later, the same group of workers repeated these experiments with the results in the second case being similar to those of the first, except for the fact that total tension was no longer significantly different in papillary muscles from T4 treated animals.

The augmented contractility indices associated with hyperthyroidism were shown to be reduced to euthyroid levels in hypoxia (Palacios, 1979). In 1971 a similar result had been demonstrated by Skelton et al. who suggested that hyperthyroid cardiac muscle had an altered conversion of chemical energy to mechanical work, rendering the use of energy in the myocardium an inefficient process.

There has been one study of the effects of T3 on the rat myocardium employing the working heart preparation first described by Neely et al. in 1967. Brooks et al. (1981) measured cardiac output, coronary flow, and rate of left ventricular pressure development (dP/dt), at 10 centimeters of water atrial filling pressure. An increase in dP/dt was observed, but lower and higher atrial filling pressures were not examined.

Hearts from thyroid hormone treated animals have also been subjected to the scrutiny of in vivo experiments. Taylor et al. (1969) analysed the tension-velocity relationship in
closed chest dogs, measuring isovolumic contractions produced by sudden balloon occlusion of the aorta. Velocity of contraction was significantly increased in hyperthyroid animals, while total tension was slightly increased, leading to a displacement of the tension-velocity relationship of the left ventricle upwards and to the right. Time to peak tension was decreased in hyperthyroid animals, as were relaxation time and total time of contraction.

An increased rate of rise of systolic pressure and an increase in maximum left ventricular systolic pressure were shown in thyroxine guinea pigs which were subjected to aortic occlusion over the corresponding value in control animals (Goodkind, 1968).

Strauer and Scherpe (1975) in hyperthyroid cats also saw an increase in the rate of ventricular pressure development.

In man, indirect measurements of contractility have been used. Left ventricular ejection time (LVET) and pre-ejection period (PEP) are the most common systolic time intervals measured. Isovolumic contraction period (ICP), the time required in generation of pressure inside the ventricle to open the aortic valve is also commonly reported. PEP and ICP are thought to reflect velocity of shortening and time to peak pressure. In a typical study of this type (Amidi et al. 1968) decreased PEP, LVET, and ICP were reported in hyperthyroid humans. Lewis et al. (1979) also observed decreases in PEP and ICP. Furthermore, they demonstrated increased mean velocity of circumferential fibre shortening, using
echocardiographic methods. When hyperthyroid patients were returned to the euthyroid state, cardiac output and mean velocity of circumferential fibre shortening were returned to the euthyroid levels (Nixon et al. 1979).

2. Isotonic studies

Isotonic, in vitro studies have also been carried out, mainly in cat papillary muscle preparations. The maximum velocity of muscle fibre shortening (Vmax) has been measured by many workers (Buccino et al. 1967; Parmley et al. 1968; Taylor, 1970; Skelton et al. 1970; Strauer and Scherpe, 1975), all of whom found Vmax increased in the hyperthyroid state. Parmley et al. (1968) also conducted a study assessing the compliance of the series element involved in the contraction of papillary muscle from hyperthyroid and euthyroid cats. They obtained identical results for series element compliance for control and hyperthyroid animals, therefore concluding that the alteration in Vmax so readily demonstrable in papillary muscle from hyperthyroid cats must be due to some change in the contractile element of the cardiac muscle.

Thus, there is general agreement among experimenters that excess thyroid hormone does cause differences in at least some indices of heart performance. In animal studies, enhancement of maximum velocity of shortening, increased rate of tension development and decreased time to peak tension, have most often been demonstrated. Clinical studies appear to reflect similar changes in performance.
E. Catecholamine Interactions with Thyroid Hormones

There is striking similarity of the cardiovascular manifestations of hyperthyroidism to the symptoms of catecholamine excess. Many experimental attempts to improve the understanding of the interrelation of catecholamine and thyroid hormones have been undertaken. Investigation of the chronotropic and inotropic effects of catecholamines in thyroid hormone treated animals has been profuse. Catecholamine receptor studies and quantification of myocardial catecholamine content have also been explored with considerable diligence.

1. Chronotropic Interactions

Work by Brewster et al in 1956 found that increases in resting heart rate observed in hyperthyroid open chest dogs were abolished by epidural pre-ganglionic blockade. An increased change in heart rate in response to single dose infusions of adrenaline (ADR) and noradrenaline (NA) was also demonstrated in the thyrotoxic animals prior to the spinal procaine epidural blockade, while induction of the blockade lowered this response to euthyroid levels. This study provided an impetus toward productive investigation of the sympatholytic agents such as guanethidine, reserpine and propranolol in the treatment of the tachycardia of hyperthyroidism. However certain of the methods of the original work have been called into question. For instance, the method of blockade used is not specific for sympathetic impulses, leaving open the possibility of confusion of the
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results by influences from blockade of parasympathetic or spinal reflexes. In addition, the data were not subjected to statistical analysis. Despite some support from the work of Lee, Lee and Yoo (1965), who showed that atria from thyroxine treated rabbits had a greater increase in rate and amplitude in response to NA than did atria from control animals, most recent work has failed to show a potentiation of catecholamine induced chronotropic response due to thyroid hormone pretreatment.

Benfey and Varma (1963) saw equivalent chronotropic responses to intravenous ADR in spinal cats pretreated either with T3 or with vehicle. In open chest dogs, Van der Schoot and Moran (1965) showed that there was no difference in heart rate response to graded doses of NA and ADR between euthyroid and hyperthyroid groups. Corroboration of this result was provided by the work of Margolius and Gaffney (1965) in intact hyperthyroid and control dogs, who saw no difference between the two groups in measuring chronotropic response to NA. Van der Schoot and Moran (1965) showed no difference between T4 treated and control rats, in absolute increase in atrial rate as a response to NA. When expressed as a percent increase, this response was less in hyperthyroid rat atria than in control. In hyperthyroid rabbits no increased chronotropic response over control was observed to NA administered either with or without cocaine (Anton and Gravenstein, 1970). Goodkind in 1969 obtained no differences in chronotropic response to isoproterenol (ISO) and NA in open chest guinea
pigs treated with either T4 or vehicle.

On the other hand, Colville and Telford (1970) saw enhanced heart rate responses to NA, ADR, and ISO in Langendorf isolated heart preparations from T4 treated rats, compared to control. MacLeod and McNeill (1981) saw increased pD2 values for ISO-induced chronotropy in right atria from T4 treated guinea pigs, over those from euthyroid animals.

Evaluation of the thyrotoxic cardiac chronotropic response to catecholamines is further complicated by the report of Wildenthal (1972), who produced augmentation of NA-stimulated heart rate in spontaneously-beating fetal mouse atrial cultures exposed to T3 for 2 days. It must be noted, however, that the dose of NA used to obtain this effect was very large (5 x 10^{-10}).

Chronotropic responses to NA and A were measured in human volunteers treated with T3 by Aoki et al. (1967). They found no difference in response to these catecholamines between euthyroid and hyperthyroid groups.

While the majority of reports of catecholamine-thyroid hormone interaction with regard to chronotropic response appear to favor the lack of potentiation of response to catecholamines in thyroid hormone pretreated animals, the fact that several workers did see augmentation of response with this combination cannot be discounted.

2. Inotropic Interactions

Catecholamine-thyroid hormone interaction in augmentation
of inotropic responses is also controversial. Langendorf hearts from T3 treated rats in the hands of Young and McNeill (1974) showed no differences in intropic response to NA from that of their control litter-mates. Muryama and Goodkind showed a decreased developed tension in left atria from guinea pigs treated with T4 in response to NA compared with response from control atria. When both groups were pretreated with reserpine in order to deplete catecholamine reserves the NA responses were identical to those obtained prior to reserpine treatment. Goodkind in 1969 tested the inotropic response to NA and ISO in open chest T4 and vehicle treated guinea pigs. He found greater maximum developed left ventricular systolic pressure in response to both agonists in euthyroid over hyperthyroid animals.

Opposite trends to those obtained by Goodkind were seen by Hashimoto and Nakashima (1978) in their experiments on left atria from guinea pigs and papillary muscle from rabbits. They obtained a leftward shift of the ISO dose response curve for hyperthyroid tissues, indicating an increase in sensitivity to ISO in these tissues. MacLeod and McNeill (1981) saw increases in pD2 values for isoproterenol in left atria and papillary muscles from T4 treated guinea pigs, also indicating supersensitivity to catecholamines in the hyperthyroid tissues.

In rat, Van der Schoot and Moran (1965) saw less increase in hyperthyroid tissues in isometric tension developed in response to graded NA administration if the results were
expressed in absolute values. If percent change was the method of data presentation, no difference was seen.

Buccino et al. (1967) observed less inotropic response to NA in cat papillary muscles from hyperthyroid animals than from euthyroid. Similar results were obtained by Levey et al. (1969)

In contrast, Tse et al. (1980) showed an increased response to $1 \times 10^{-8}$ M ISO, with a decreased maximum tension development in hyperthyroid rat ventricle strips, as compared to control.

Obviously then, inotropic interrelated thyroid-catecholamine responses are not clearly defined. There is a wealth of conflicting evidence, the interpretation of which is extremely difficult.

3. Catecholamine Tissue Concentrations

The possibility that thyroid hormones might exert their effects upon the heart by altering tissue levels of free catecholamines has been considered. Release of NA from sympathetic nerve endings has been reported as slightly decreased in hyperthyroidism; decreased levels of dopamine beta-hydroxylase were also noted (Spaulding and Noth, 1975). Unaltered NA plasma levels in hyperthyroid animals have been documented (Nagel-Hiemke et al. 1981). Wurtman et al. 1963 have reported the activity of hepatic monoamine oxidase and cardiac catechol O-methyl transferase unchanged from control
in hyperthyroid rats. Normal ventricular NA content has been reported in cats (Buccino et al. 1967; Zaimis et al. 1969), in dogs (Nayler et al. 1971), in rats (Cairoli and Crout, 1967) and in guinea pigs (Zaimis et al. 1969). After reserpine administration, NA tissue levels were shown to be similarly reduced in control and hyperthyroid animals. There are reports of increased myocardial NA content, including Goodkind et al (1961) in T3 treated guinea pigs, and Lee et al (1965) in T4 treated rabbits, and of decreased concentrations of the neurotransmitter in rabbits (Anton and Gravenstein, 1970). Although no particular conclusions can be drawn from such varied data, certainly there is no clearcut correlation between changes in NA tissue levels and myocardial functional performance alterations in hyperthyroid animals.

4. Beta-Adrenergic Receptor Studies

If there is potentiation of catecholamine-induced responses in tissues from thyroid hormone treated animals, an increase in number or affinity of beta-adrenergic receptors would provide at least a partial explanation for this phenomenon. With the development of tritiated beta-adrenergic antagonist receptor labelling techniques in the late 1970's, it became possible for experimenters to directly assess the quantity and sensitivity of beta-adrenergic receptors present in myocardial tissues.

The effects of T3 and T4 pretreatment on beta-adrenergic
receptor characteristics in rats were first chronicled in the 1977 paper of Williams and Lefkowitz. Affinity of the binding sites for both the antagonist, dihydroalprenolol, and for the agonist, isoproterenol, were unchanged from control in both treatment groups. Up to $1 \times 10^{-6}$M thyroxine incubated with the control heart membranes did not increase binding of the labelled antagonist to the receptor, nor did thyroxine itself bind at the receptor site. There was, however a dramatic increase in the number of binding sites found in tissues from animals pretreated with thyroid hormones.

Ciaraldi and Marinetti (1977) provided almost immediate confirmation of this innovative finding with their work in thyroxine treated rats. They observed up to six-fold increases in beta-receptor number, with no change in binding affinity.

A less definite result was obtained by Banerjee and Kung (1977). They could show changes in beta-receptor number in thyroidectomized rats subsequently treated with thyroid hormone, but not in rats which were euthyroid at the initiation of thyroid hormone treatment. Dissociation constants were virtually identical in all groups. The lack of positive data concerning receptor number may have resulted from the treatment schedule used, which involved T3 injection only every other day for 3 doses.

Acute incubation of T3 and T4 with rat ventricle slices was shown by Kempson et al. (1978) to increase stereospecific dihydroalprenolol binding to beta-adrenergic receptors. This
action was not inhibited by cycloheximide, suggesting that it was a post-translational event. The increased binding produced by fifteen hours incubation with T3 could be inhibited by cycloheximide, however, indicating that translational or transcriptional synthesis may be involved.

In cultured heart cells from newborn rats, after 24 hours incubation with T3, Tsai and Chen (1978), noted an increase in beta-receptor number. It appears de novo protein synthesis is not required for beta-receptor number increase. Further effects are noted when synthesis is allowed to occur.

Ho et al (1980) confirmed the thyroid hormone-induced increase in beta-receptor number in dog heart. They also compared results of thyroxine treatment with those obtained from animals with left ventricular hypertrophy of other etiology. They concluded that beta-receptor increase was not a secondary effect of hypertrophy nor a result of hemodynamic changes.

Further corroborative evidence of increased beta-receptor number in hyperthyroid rats has been published by several workers (McConnaughey et al. 1979; Tse et al. 1980; Stiles and Lefkowitz, 1981).

A time course study for thyroid hormone induced effects on beta-receptor number has been provided by McConnaughey et al. (1979). Peak increases in receptor number occurred at 3 to 4 days of thyroxine treatment, then remained constant for the next 7 days.

Recently, binding study methods have been modified to
allow accurate measurement of binding and affinity of agonists as well as antagonists. The work of Stiles and Lefkowitz has shown that in hyperthyroid rat heart there are two affinity states for the binding of ISO to the receptor, having high and low dissociation constants. The ratio of the two constants is reported to indicate the efficacy of hormone stimulation of adenylate cyclase. In hyperthyroidism this ratio is increased, thus a more stabilized high affinity state is expected, leading to more efficient stimulation of adenylate cyclase.

Studies of beta-adrenoreceptors in hyperthyroidism have provided much information on possible mechanisms for the myocardial alterations of thyroid hormone excess. There is general agreement on thyroid hormone induced increase in beta-adrenergic receptor number, although there are wide differences in the interpretation of significance of this finding. The recent work of Stiles and Lefkowitz (1981) suggests another possible mechanism of thyroid hormone effect, that of increased efficacy of transmembrane coupling. Absolute proof of such a mechanism will await purification and characterization of the adenylate cyclase system.
F. Thyroid Hormone Effect on Myocardial Enzymes

1. Adenylate Cylase - Cyclic AMP

The similarity in appearance of the effects of thyroid hormones and catecholamines naturally focused attention on the myocardial adenylate cyclase system. After early work in hyperthyroid animals had indicated potentiation of cardiac catecholamine actions by thyroid hormone pretreatments, this interest was increased manyfold.

Levey and Epstein (1968) demonstrated a rise in cardiac adenylate cyclase activity when thyroxine was added to a cat left ventricular particulate preparation. They proposed that T4 might exert its inotropic and chronotropic effects through adenylate cyclase. In subsequent experiments it was found that propranolol could block catecholamine induced activation of adenylate cyclase, but had no effect on the thyroid hormone induced activation of the system. The existence of two adenylate cyclase systems was suggested as a possible explanation for this result. Concern about the physiological significance of these in vitro experiments was fostered because of the high concentration of T4 used to produce the effect. It was also considered suspicious that the dose of T4 used did not augment contractility, and that the D analogue of thyroxine was just as effective in producing the activation of adenylate cyclase, despite its much reduced physiological potency compared to the L form.

Continuing studies in cats (Levey et al. 1969; Sobel et al. 1969), gave further question to the earlier work. Basal
levels of adenylate cyclase were found to be unchanged in thyroid hormone pretreated animals, while NA stimulated cyclic AMP accumulation in euthyroid and hyperthyroid groups to the same degree.

Brus and Hess (1973) provided confirmation of the unchanged basal and NA stimulated adenylate cyclase levels in their work with T3 treated rats. McNeill et al. (1969) also demonstrated equal basal and NA stimulated cAMP levels and adenylate cyclase activity in T3 treated rats.

In 1978, McNeill showed no difference throughout the contractile response to calcium chloride in T3 and control Langendorf hearts, either in accumulation of cAMP or in adenylate cyclase activity.

An increased hyperthyroid response to isoproterenol induced maximum activity and sensitivity of adenylate cyclase has recently been reported by Tse et al. (1980). Basal and sodium fluoride stimulated adenylate cyclase activity, and cAMP tissue levels were not different from control.

The bulk of the evidence suggests that activation of the cyclic AMP system is not an important factor in mediating thyroid hormone cardiotonic effect.

2. Phosphorylase

Hyperthyroidism is a state of augmented energy consumption. The availability of energy from glycogenolysis is dependent on the conversion of the inactive phosphorylase b
enzyme to its active phosphorylase a form, which is capable of catalysing the breakdown of glycogen to glucose 1-phosphate. The energy provided by this route is not used in eliciting the initial response to a stimulus, as the peak of the inotropic response precedes increase in phosphorylase a levels, but glycogenolysis may provide the energy needed for sustained response.

Elevated tissue levels of phosphorylase a in hyperthyroidism have been well documented (Hornbrook and Brody, 1963; McNeill and Brody, 1968; Frazer et al. 1969; McNeill et al. 1969; McNeill, 1969; Longhurst and McNeill, 1979).

Catalysis of phosphorylase kinase activation which in turn activates phosphorylase b to the a form, can occur through a cAMP dependent protein kinase (Walsh et al. 1971). Pretreatment with propranolol has been shown to reduce basal and catecholamine stimulated phosphorylase a levels in hyperthyroid rats (McNeill and Brody, 1968; Frazer et al. 1969). When T3 treated rats are pretreated with reserpine as well, the increased phosphorylase a levels of hyperthyroidism are reduced to euthyroid levels, while phosphorylase a levels from euthyroid animals show no change with reserpine pretreatment. Thyroid hormone induced rise in phosphorylase a can be demonstrated, however, without increases in cAMP levels (McNeill et al. 1969) and without a rise in phosphorylase b kinase levels (Frazer et al. 1969). Thyroid hormone induced phosphorylase activation is not related to increased
sensitivity of cardiac adenylate cyclase (McNeill et al. 1969; Sobel et al. 1969). Phosphorylase activation has also been shown to be independent of the increased coronary blood flow of hyperthyroidism (Longhurst and McNeill, 1979). The phosphorylase activation is also not due to a reserpine-like supersensitivity (McNeill, 1969), nor to direct effects of thyroid hormones on cardiac phosphodiesterase in vivo (McNeill et al, 1971).

Dibutyryl cAMP was reported to have an increased effect on activation of phosphorylase in hyperthyroid animals as compared to control (McNeill, 1978). The possibility that this finding might be related to an increased sensitivity to cAMP at the protein kinase level is still under question as cAMP dependent protein kinase levels in hyperthyroid animals have been demonstrated as normal (Katz et al. 1977), elevated (Newcomb et al. 1978) or decreased (Tse et al. 1980).

The activation conversion reaction of phosphorylase is also markedly dependent on calcium (Friesen et al. 1969), but sensitivity to the phosphorylase activating effects of calcium was found to be not different throughout the calcium chloride dose response curve, in control and T3 treated guinea pigs (McNeill, 1978).

There is general acceptance of a thyroid hormone-induced increase in activation of phosphorylase. The pathway by which this increase occurs is still much in doubt. Most of the major influences on the phosphorylase activation system have been eliminated as prime causes in the increases seen in
thyroid hormone treated animals. Investigation of more subtle influences remains to be done.

3. Myocardial ATPases
   a. Myosin ATPase

   Energy for myocardial contraction is provided at the level of the myofibril by the splitting of ATP by myosin ATPase. Changes in myosin ATPase activity may be related to maximum velocity of shortening of the cardiac muscle.

   There is considerable species variation in myosin ATPase activity. The decreasing order of activity is rat, cat, guinea pig, dog, rabbit (Yazaki and Raben, 1975; Banerjee et al. 1976).

   Thyrum et al. (1970) measured myosin ATPase activity in T4 treated and control guinea pigs. They saw a 30% increase in calcium stimulated ATPase activity in guinea pigs treated with T4 over control. Increased helical content and an altered amino acid composition of the ATPase were also shown. It was thought that de novo synthesis of a new type of myosin, or synthesis of a new protein which became associated with the myosin, might then induce greater actin activated ATPase activity.

   In 1974, Goodkind et al. did time course studies of contractility and myosin ATPase activity in guinea pig papillary muscles following T4 injections. They saw increases in certain contractility indices, namely maximum developed
tension and rate of tension development (dT/dt), within 24 hours of the injection, with no change in the myosin ATPase activity. After 8 days of T4 treatment the myosin ATPase activity was increased, coinciding with interesting changes in the measurements of contractility. Increased dT/dt was still noted after 8 days treatment, but maximum developed tension had dropped to euthyroid levels, with a concomitant decrease in time to peak tension. In these experiments correlation of myosin ATPase activity could be shown only with the late effects of T4 on contractility, i.e. with shortening of time to peak tension. No acute effect of T4 on myosin ATPase was observed when the hormone was incubated with myocardial tissue.

Yazaki and Raben (1975) could not see an effect of T4 incubated in vitro with rabbit cardiac tissue. They did see increases in myosin ATPase activity after 3 days in rabbits treated with T4, an effect which continued through 14 days of treatment. Rats pretreated with thyroid hormone in the same manner did not show increased myosin ATPase levels in response to thyroid hormone. Basal levels of activity in rats were, however, four times higher than the corresponding values in rabbit. This study also demonstrated changes in the amino acid composition and enzymatic properties of the myosin, providing some reinforcing evidence for the theory of Thyrum et al. (1970) that T4 stimulates synthesis of a new myosin with altered enzymatic properties.

Sulfhydryl modification is known to augment myosin ATPase
activity. By blocking one thiol per myosin subunit, activity of the ATPase is elevated. If a second sulfhydryl group is blocked, however, there is a complete cessation of activity. Katagiri et al in 1975 explored this phenomenon in hyperthyroid rabbits. When basal levels of myosin ATPase activity were measured, hyperthyroid hearts had twice the activity of those from euthyroid animals. When N-ethylmaleimide (NEM) was added to the incubation mixture, euthyroid myosin ATPase activity increased by 177%. On the other hand, addition of NEM to hyperthyroid tissues had no effect on activity. This study suggested that stimulation of myosin ATPase by thyroxine might occur through a conformational modification of the myosin subunit around the thiol group. However T4 did not act by directly blocking the sulfhydryl group, as these groups were accessible to modification by NEM.

Rovetto et al (1972) could find no augmentation of myosin ATPase activity in hyperthyroid rat hearts, nor could Yazaki and Raben, in a later study (1975). Rat heart had the greatest basal myosin ATPase activity of the species studied. The extent of activation of myosin ATPase by thyroid hormone appears to vary inversely with the basal euthyroid myosin ATPase activity, which varies with the species. That is, guinea pig and rabbit hearts, with the lowest basal activity, will show the greatest increase in activity when treated with thyroid hormone. Rat heart myosin ATPase activity does not appear to be stimulated by thyroid hormones, possibly because
of its high activity in the euthyroid state.

b. Na⁺ K⁺ -ATPase

Yazaki and Raben (1975) and Katagiri et al (1975) reported no differences in Na⁺ K⁺ -ATPase activity from control values for rabbits treated with T4. These findings were confirmed in a recent study by McConnaughey et al. (1979), who saw no effect on Na⁺ K⁺ -ATPase activity in the T4 treated rat.

c. Ca²⁺ activated ATPase of Sarcoplasmic Reticulum

During the excitation-contraction cycle of cardiac muscle the intracellular organelle known as the sarcoplasmic reticulum (SR) accumulates and releases calcium. It may serve as the major intracellular calcium store. In the presence of magnesium ion and adenosine triphosphate (ATP), proteins embedded in the SR membrane and exposed to its external surface provide the agency for transmembrane calcium transport. Energy for calcium transport is obtained by hydrolysis of ATP. In the absence of ATP the SR membrane is quite impermeable to calcium. One of the major limiting factors of cardiac relaxation is thought to be the rate of calcium uptake into the SR from the sarcoplasm. Measurement of this uptake activity has attracted much interest as a possible indication of an explanation for the increased performance of the hyperthyroid heart.

Nayler et al. in 1971 were the first group to examine the
calcium accumulating ability of the hyperthyroid SR. Using
dogs treated with T4 for 10 days, these workers showed
increases in tension development, and rate of tension
development, as well as increased calcium accumulation and
exchange by SR enriched microsomes.

Similar results for calcium uptake activity were produced
by Suko in 1971 in hyperthyroid rabbits. Substantial
increases in total calcium uptake activity and in calcium
activated ATPase activity were observed in animals treated for
three to four weeks with T4.

Goodkind et al. (1974) were unable to see changes in
parameters of calcium uptake activity in guinea pigs treated
with thyroxine for 3 days.

Limas (1978a; 1978b), in rats treated for two weeks with
T4 saw increases in calcium uptake activity and calcium
activated ATPase activity. These increases could be prevented
through use of a protein synthesis blocker such as
cycloheximide. Suko (1971) had postulated the formation of a
phosphoprotein intermediate on the external surface of the SR
membrane is a necessary prelude to transmembrane calcium
transport. Limas (1978a) showed that increases in ATPase
activity were preceded temporally by formation of this
phosphoprotein intermediate, and that T4 induced higher steady
state levels of this intermediate than were achieved in
 euthyroid animals.

McConnaughey et al. (1979) in rats treated for 5 days
with T4, were unable to show any increase in calcium activated
ATPase activity over that seen in control animals.

 Increases in calcium uptake activity are well established in several species after at least 10 days treatment with thyroid hormone. However, increased calcium uptake activity has not been shown in studies of animals treated with T4 for 3 and 5 days.
PURPOSE OF THE INVESTIGATION

The puzzle of the heart in hyperthyroidism prompted this investigation. The literature of the hyperthyroid heart is a conglomerate of conflicting results, interspersed with the occasional generally accepted piece of evidence. Universally endorsed changes seen in hearts from animals treated with thyroid hormones include the increase in number of beta-adrenergic receptors, the increase in phosphorylase activation, the increased resting heart rate, and the increased protein synthesis associated with heart enlargement. Effects known to occur in certain species, or after a certain minimum treatment length are increased myosin ATPase activity, increased calcium uptake activity into sarcoplasmic reticulum, and increased rate of tension development of isolated tissue preparations. Areas of outright disagreement encompass the entire relation between thyroid hormone treatment and catecholamine elicited responses, as well as the existence of an intrinsically changed contractility in the hyperthyroid myocardium.

The relative dearth of information on hyperthyroidism obtained from the isolated working heart provided the initial direction for this study. By producing function curves from electrically paced working rat hearts, which could perfuse their own coronaries, and for which the peripheral resistance could be held constant it was hoped certain insights into intrinsic contractile state of the hyperthyroid heart could be
gained. With the advent of microcomputer technology to our pharmacological laboratory, measurement of parameters previously unavailable could be undertaken. Elucidation of the changes in myocardial performance seen by earlier workers was our goal.

Edifying perusal of the literature on the heart in hyperthyroidism is complicated by the variety of treatments and treatment schedules used to induce hyperthyroidism in experimental animals. An useful exercise then, would be the employment of two of the more common treatments to render separate groups of animals hyperthyroid, and then to compare them to each other as well as to euthyroid control animals.

Rats were chosen as the experimental species. This had been the species used in the only other study using the working heart preparation in examining effects of hyperthyroidism (Brooks et al. 1981). There was also much information on the hyperthyroid rat heart available from isolated tissue experiments.

It was deemed of interest as well to study the response to isoproterenol in the hyperthyroid rat working heart preparation. Many studies of this relation have been done; many are in sharp disagreement. While part of this divergence is undoubtedly due to differing interpretations of similar results, our study hoped to clarify the effects of different thyroid hormone treatments on the catecholamine-thyroid hormone interrelation.

Although many workers (Nayler et al. 1971; Suko, 1971;
Limas, 1978a) had previously shown increased calcium uptake activity in hyperthyroid cardiac sarcoplasmic reticulum, no one had ever shown such changes in rat hearts from the three day and seven day schedules of thyroid hormone treatment that we proposed to employ. It seemed of importance to measure calcium uptake activity in the animals of our study, so as to compare this biochemical index with the results obtained from the contractile and catecholamine dose response work.

In summary then, we proposed to examine two thyroid hormone treatment groups and a vehicle treated control group of rats with regard to their myocardial performance during function curve assessment of contractile state, and their response to graded doses of isoprote renol, on the working heart preparation. Calcium uptake activity into the cardiac sarcoplasmic reticulum would also be investigated.
A. Thyroid Hormone Treatments

Hearts used in working heart studies and in studies of calcium uptake activity into cardiac sarcoplasmic reticulum were obtained from male Wistar rats weighing 200 to 250 grams. Two different treatment schedules employing two different thyroid hormones were used to render the treated animals hyperthyroid.

One group of rats was given subcutaneous injections, once daily for three days, of 3,3',5' triiodo L-thyronine (T3) at a dose of 500 micrograms/kg administered in an alkaline saline vehicle. Control animals were given equal volume injections of vehicle alone.

A second group of rats was given subcutaneous injections of L-thyroxine (T4) 500 micrograms/kg in alkaline saline once daily for 7 days. Control animals were given equal volumes of vehicle on the same dosage schedule.

All animals received food and water ad libitum.
B. Contractile Studies

1. Preparation Protocol

Twenty-four hours after the last dose of thyroid hormone or vehicle, the rats were treated with heparin sodium (1000 U.S.P. units/kg) i.p. Ten minutes later the animals were stunned by a blow to the head and killed by cervical dislocation. The hearts were rapidly removed, and quickly attached to the stainless steel aortic cannula of a working heart apparatus (Neely et al. 1967) as modified by Rodgers et al. (1981). The hearts were maintained at 30 degrees Centigrade in Chenoweth-Koelle (CK) solution (Chenoweth and Koelle, 1946) of the following composition: 120 millimolar sodium chloride, 5.6 millimolar potassium chloride, 2.1 millimolar magnesium chloride, 1.8 millimolar calcium chloride, 19 millimolar sodium bicarbonate, and 10 millimolar glucose. The CK solution was aerated with a mixture of 95% oxygen and 5% carbon dioxide to maintain pH at 7.4.

After the pulmonary vein was cannulated, a PE 90 tubing attached to a 25 gauge stainless steel needle was used to cannulate the left ventricle. Left ventricular pressure and aortic pressure were monitored by means of Statham pressure transducers model P23 Db, connected to a Grass model 79D polygraph. The first derivative of left ventricular pressure (dP/dt) was also recorded on the polygraph. Heart rate was followed using a Grass tachograph. Spontaneous heart rate was
recorded during an equilibration period lasting fifteen minutes. The hearts were then electrically paced at twice threshold voltage at a rate of 300 beats per minute (5 milliseconds duration). A Mountain Hardware A/D and D/A converter was used to digitize the left ventricular pressure tracings from the Grass polygraph and to store them via an Apple II microcomputer on disk. See Appendix I for detailed descriptions of microcomputer methods used in data acquisition and analysis. See Figure 1 for a sample left ventricular pressure tracing from the Apple II microcomputer.

2. Function Curves

Left ventricular function was estimated by subjecting the hearts to a range of atrial filling pressures or pre-loads varying from 5 to 22.5 cm. of water. A series of function curves was generated by plotting atrial filling pressures against left ventricular developed pressure (LVDP), maximum rate of left ventricular pressure development (+ dP/dt), maximum rate of relaxation (- dP/dt), time required to reach peak left ventricular pressure development (TTP), left ventricular relaxation time from peak pressure back to baseline (TTB), total time of contraction (TTC), time between attainment of maximum + dP/dt and attainment of maximum - dP/dt (TPN), area under the contraction curve from baseline to peak left ventricular pressure (AP), area under the contraction curve from peak pressure back to baseline (AB),
SAMPLE LEFT VENTRICULAR PRESSURE TRACING AS STORED TO MICROCOMPUTER

SHOWING:

S - START OF PULSE \(i(\text{start})\)
E - END OF PULSE \(i(\text{end})\)
M - MAXIMUM PRESSURE \(i(\text{max})\)
P - MAXIMUM +dP/dt
N - MAXIMUM -dP/dt
and total area under the left ventricular contraction curve (AT).

3. Isoproterenol Dose Response Curves

After completion of the function curves, one dose response curve was obtained for each heart by the cumulative addition of dl-isoproterenol to the perfusion medium with the atrial filling pressure held constant at 15 cm. of water. The hearts were then carefully removed from the working heart apparatus, blotted dry and weighed.

4. pD2 Value Determination

Values for pD2 (negative log ED 50) of isoproterenol in L-triiodothyronine (T3) treated, L-thyroxine (T4) treated, and control rat working heart preparations were determined from graded log dose response curves through linear regression as described by Tallarida and Murray (1981) using an Apple II microcomputer.
C. Studies of Calcium Uptake Activity

1. Preparation of Rat Cardiac Microsomes Enriched in Sarcoplasmic Reticulum

The method employed for preparation of rat cardiac microsomes enriched in sarcoplasmic reticulum is that described by Sumida et al. (1978), modified in the following manner. Hearts were removed from hyperthyroid and control animals 24 hours after the last injection of thyroid hormone or vehicle and placed in 10 millimolar Tris maleate buffer. All buffer media used in this protocol were kept ice cold. Atrial tissue was trimmed from the hearts and discarded. After brief scissor chopping the ventricles were homogenized in 10 millimolar Tris maleate buffer (pH 6.8), by passing six times through a Dynamix Teflon homogenizer. The homogenized tissue was then centrifuged at 5000 rpm for 10 minutes. The resultant supernatant was then strained through four layers of cheesecloth. Centrifugation was again employed, this time at 11,000 rpm for 20 minutes. After further straining through four layers of cheesecloth, the supernatant was centrifuged at 20,000 rpm for 90 minutes.

The pellet obtained from this last centrifugation was then rinsed with 10 millimolar Tris maleate buffer (pH 6.8) which contained 0.6 molar potassium chloride. The pellet was resuspended using a Pasteur pipette. The resulting mixture of fine particles and buffer was then subjected to three gentle
passes in a glass homogenizer, and centrifuged at 20,000 rpm for 90 minutes. (See Figure 2 for centrifugation flow diagram.)

The supernatant obtained was discarded. The remaining pellet was rinsed with 10 millimolar Tris maleate buffer (pH 6.8) containing 40% by weight of sucrose. After the pellet had been resuspended, it was passed gently five times through a Teflon homogenizer. Aliquots of the resulting suspension were then frozen rapidly by immersion in methybutane, cooled in dry ice, and stored at in an ultralow temperature freezer at -70 degrees Centigrade.

2. Measurement of Calcium Uptake Activity

ATP-dependent calcium uptake activity was measured according to the method of Tada et al. (1974), with the modifications outlined below. Frozen aliquots of rat cardiac sarcoplasmic reticulum were rapidly thawed after a short period of storage, and preincubated at 30 degrees Centigrade for 7 minutes in 0.3 ml of a medium of the following composition: 0.11 molar histidine chloride, 2.0 molar potassium chloride, 1.0 molar magnesium chloride, 100 millimolar Tris oxalate, 50 millimolar sodium azide, 50 millimolar Tris adenosine triphosphate. Oxalate facilitated calcium uptake was then initiated with the addition of calcium buffer labelled with $^{45}$Ca++. Following a 5 minute incubation period, uptake activity was terminated by
PREPARATION OF RAT CARDIAC MICROSONES ENRICHED IN SARCOPLASMIC RETICULUM

FIGURE 2
filtration of a 0.41 ml aliquot through a 0.45 micron Millipore filter. A 0.04 molar Tris chloride solution (pH 7.4) was used to perform two 10 ml washes of the filter. After a 3 minute drying period in an oven at 40 degrees Centigrade, the filters were placed in vials containing 10 mls of Biofluor scintillation fluid, and analyzed for activity in a liquid scintillation counter (Isocap) over a 10 minute counting interval. Proteins were determined by the method of Lowry et al. (1951).

3. Calcium Buffer Solution Preparation

Ethylene glycol - bis (b - amino - ethyl ether) n, n - tetra - acetic acid (EGTA) - calcium solutions were prepared in which the amount of EGTA was varied in order to provide a range of free Ca\(^{2+}\) concentrations, using a modified equation according to Katz et al. (1970). Each solution contained a total concentration of 1.25 millimolar calcium chloride. A volume of \(^{45}\)Ca chloride solution sufficient to yield approximately 500,000 cpm / 50 ul was added to the solution for purposes of measuring extent of calcium uptake.

D. Statistical Analysis

Analysis of variance employing the Newman-Keuls multiple comparisons test was used to analyse the experimental data.
The criterion of significance was chosen to be a probability of $P < 0.05$. See Appendix II for detailed methods (Snedecor and Cochran, 1967).

E. Materials

The $^{45}$calcium chloride (40 mCi/mg) was supplied by Amersham Corporation. All other drugs and chemicals were obtained from Sigma Chemical Company.
RESULTS

A. Pre-experimental Treatment Effects

1. Body and Heart Weights

Table I shows rat total body weight preceding the first injection, weight at time of sacrifice, the change in body weight during the treatment period, and heart weight for T3 treated, T4 treated, and vehicle treated rats. Both T3 treated and T4 treated rats exhibited a significant decrease in body weight as compared to rats treated with alkaline saline vehicle. There was no significant difference in weight loss between T3 treated and T4 treated rats. Final body weight of the T4 treated rats was significantly different from the final body weight of control rats.

Hearts from both T4 treated and T3 treated rats were significantly different in weight from those from vehicle treated rats.

2. Heart Weight/Body Weight Ratio

Heart weight was divided by body weight and the resulting number multiplied by 100. Figure 3 represents a comparison of the values thus obtained for the experimental groups in question. The vehicle treated group had a heart weight/body weight ratio of \(0.33 \pm 0.01\), the T4 treated group \(0.43 \pm 0.01\),
and the T3 treated group a value of .42 ± .03. Both thyroid hormone treated groups of animals showed a significant difference from control heart weight/body weight ratio.

3. Spontaneous Heart Rate

Heart rate was recorded on the polygraph/tachograph prior to initiation of electrical pacing of the atria. Hearts from vehicle treated rats showed a spontaneous heart rate of 226 beats per minute ± 13. Heart rates for hearts from T4 treated and T3 treated rats were 294 beats per minute ± 23, and 336 beats per minute ± 27, respectively. These values were both significantly different from control values. There was no significant difference in spontaneous heart rates between the T4 and T3 groups. (Figure 4)
TABLE I

The Effect of Thyroid Hormone Pretreatment on Rat Body and Heart Weight

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>T4 Treated (n=7)</th>
<th>T3 Treated (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Body Weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>255 ±4 (#)</td>
<td>250 ±7</td>
<td>266 ±8</td>
</tr>
<tr>
<td>Final</td>
<td>265 ±6</td>
<td>235 ±5*</td>
<td>248 ±8</td>
</tr>
<tr>
<td>Δ in Body Weight</td>
<td>+10 ±2</td>
<td>-13 ±3*</td>
<td>-17 ±3*</td>
</tr>
<tr>
<td>Rat Heart Weight</td>
<td>0.87 ±.02</td>
<td>1.02 ±.04*</td>
<td>1.03 ±.06*</td>
</tr>
</tbody>
</table>

(#) Numbers indicate mean weight in grams, ±S.E.M.

* Indicates significant difference at P <0.05, compared to the control value
FIGURE 3.

Effect of triiodothyronine and thyroxine pretreatment on heart weight/body weight ratio in rat.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on a ratio which consists of rat heart wet weight, obtained immediately after contractile studies were completed, divided by rat total body weight obtained immediately prior to sacrifice, this number being multiplied by 100.

Each bar represents the mean heart weight/body weight ratio ± S.E.M of 5-12 observations.

* indicates significant difference from control at P<0.05.
HYPERTHYROID RAT HEART WEIGHT RATIO ANALYSIS

![Bar graph showing heart weight/total body weight ratio for control, T3, and T4 groups. The graph indicates a significant increase in the heart weight/total body weight ratio for T3 and T4 compared to the control group.]
FIGURE 4.

Effect of triiodothyronine and thyroxine pretreatment on spontaneous heart rate in rat.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control) on spontaneous heart rate measured on the polygraph/tachograph during equilibration prior to initiation of electrical pacing of the atria.

Each bar represents the mean spontaneous heart rate (beats/minute) ± S.E.M of 5-12 observations.

* indicates significant difference from control at P<0.05.
HYPERTHYROID RAT HEART
SPONTANEOUS HEART RATE

SPONTANEOUS HEART RATE (BEATS/MIN)

CONTROL  T3  T4

150  200  250  300  350  400
B. Contractile Studies

1. Function Curves

   a. Effect of T3 and T4 treatment on Left Ventricular Developed Pressure (LVDP):
      
      Hearts from animals subjected to T3 and T4 pretreatment showed increases over control in LVDP at all atrial filling pressures measured. These values were significantly different from control for T3 hearts at atrial filling pressures of 5cm, and 7.5cm of water. For T4 hearts only at 7.5 cm of water atrial filling pressure were the values obtained significantly different from control. (Figure 5)

   b. Effect of T3 and T4 pretreatment on maximum rate of left ventricular pressure development (+dP/dt):
      
      Hearts from T3 and T4 treated rats showed a significantly increased +dP/dt as compared to hearts from control animals at virtually all atrial filling pressures. All points on the graph for the thyroid hormone treated groups are significantly different from control except for the T4 treated group at 5 cm of water filling pressure. (Figure 6)

   c. Effect of T3 and T4 pretreatment on maximum rate of relaxation (-dP/dt):
      
      An increase in -dP/dt was evident at all atrial filling pressures in hearts from hyperthyroid animals as compared to
those from control animals. This difference was statistically significant for T3 hearts at 5cm, 15cm, 17.5cm, 20cm, and 22.5cm filling pressures. For T4 hearts the values obtained for this parameter at 5cm, 7.5cm, 15cm and 17.5cm of water filling pressures were significantly different from control values obtained at these settings. (Figure 7)

d. Effect of T3 and T4 pretreatment on the time required to reach peak left ventricular pressure development (TTP):

Although in general TTP for hyperthyroid rat hearts was numerically decreased from control heart values, only two points on the function curve were significantly different from control. At 7.5cm and at 10cm of water atrial filling pressure the value for TTP obtained for T3 hearts was significantly different from control. (Figure 8)

e. Effect of T3 and T4 pretreatment on left ventricular relaxation time from peak pressure back to baseline (TTB):

Relaxation time was decreased as a result of T3 and T4 pretreatment. Significantly different values from control were obtained for T3 treated hearts at all atrial filling pressures measured. T4 relaxation time values were significantly different from control at 5cm, 7.5cm, 10cm, 12.5cm and 15cm of water filling pressures. In addition, at 17.5cm of water filling pressure, values for relaxation time for T3 hearts were significantly lower than those for T4 hearts. (Figure 9)
f. Effect of T3 and T4 pretreatment on total time per contraction (TTC):

There was a decreased total time per contraction for hyperthyroid hearts as compared to control hearts. For T3 treated hearts all values obtained over the function curve were significantly different from control. In T4 treated hearts at 5cm, 7.5cm, 10cm, 12.5cm, 15cm, and 17.5cm, TTC was significantly different from control. Values for T3 hearts were significantly different from those for T4 hearts at 17.5cm, 20 cm, and 22.5cm. (Figure 10)

g. Effect of T3 and T4 pretreatment on time required from maximum positive dP/dt to maximum negative dP/dt (TPN):

Hearts from hyperthyroid animals showed a decreased time from maximum +dP/dt to maximum -dP/dt, as compared to hearts from control animals. Significant differences from control values occurred at 12.5cm, 15 cm, 17.5 cm, 20 cm, and 22.5 cm of water filling pressure for hearts from T3 treated animals. At 5cm, 12.5cm and 15cm, T4 hearts had TPN values which were significantly decreased from control times. (Figure 11)

h. Effect of T3 and T4 pretreatment on area under the contraction curve from baseline to the point of maximum LVDP (AP):

While area under the left ventricular pressure curve from initiation of contraction to peak pressure development was
numerically slightly increased in hearts from hyperthyroid animals, at no point on the function curve was this increased area significantly different from that seen in hearts from control animals. (Figure 12)

i. Effect of T3 and T4 pretreatment on area under the contraction curve from the point of maximum left ventricular pressure development to baseline (AB):

There were no statistical differences seen between hearts from control and from hyperthyroid animals as regards the area under the contraction curve from peak pressure back down to baseline. (Figure 13)

j. Effect of T3 and T4 pretreatment on total area under the pressure curve (AT):

When values for total pulse area obtained from hearts from hyperthyroid animals were compared to those obtained from control animals, no significant differences could be seen. (Figure 14)
FIGURE 5.

Effect of triiodothyronine and thyroxine pretreatment on left ventricular developed pressure in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on left ventricular developed pressure (LVDP) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean LVDP (mm Hg) \( \pm \) S.E.M of 5-12 observations.

* indicates significant difference from control at \( P<0.05 \).
(○) T3 treated (n=5)
(●) T4 treated (n=7)
(•) vehicle treated (n=12)

LVDP (mm Hg)

filling pressure (cm H2O)
FIGURE 6.

Effect of triiodothyronine and thyroxine pretreatment on rate of left ventricular pressure development in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on rate of left ventricular pressure development (+dP/dt) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean +dP/dt (mm Hg/sec) ± S.E.M of 5-12 observations.

* indicates T3 group significantly different from control at P<0.05

** indicates both hyperthyroid groups significantly different from control at P<0.05.
FIGURE 7.

Effect of triiodothyronine and thyroxine pretreatment on rate of relaxation from peak left ventricular pressure in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on rate of relaxation from left ventricular peak pressure (-dP/dt) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean -dP/dt (mm Hg/sec) ± S.E.M of 5-12 observations.

* indicates T3 group significantly different from control at P<0.05.

** indicates both hyperthyroid groups significantly different from control at P<0.05.

†* indicates T4 group significantly different from control at P<0.05.
FIGURE 8.

Effect of triiodothyronine and thyroxine pretreatment on time to peak left ventricular developed pressure in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on the time required to achieve peak left ventricular developed pressure (TTP) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean TTP (ms) ± S.E.M of 5-12 observations.

* indicates significant difference from control at P<0.05.
Effect of triiodothyronine and thyroxine pretreatment on relaxation time of the left ventricle in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on the time required for the left ventricle to relax from peak developed pressure back to baseline (RT) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean RT (ms) ± S.E.M of 5-12 observations.

* indicates significant difference from control at P<0.05.

** indicates significant difference from control and from T4 treated at P<0.05
(Δ) T3 treated (n=5) ——
(∞) T4 treated (n=7) ——
(○) vehicle treated (n=12) ——

relaxation time (MS)

filling pressure (cm H2O)
FIGURE 10.

Effect of triiodothyronine and thyroxine pretreatment on total pulse period in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on the time required for one complete left ventricular contraction (TTC) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean TTC (ms) ± S.E.M of 5-12 observations.

* indicates significant difference from control at P<0.05.

** indicates significant difference from control and from T4 treated at P<0.05.
FIGURE 11.

Effect of triiodothyronine and thyroxine pretreatment on time period from $+dP/dt$ to $-dP/dt$ in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on the time period in the left ventricular contraction cycle from maximum $+dP/dt$ to maximum $-dP/dt$ (TPN) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean TPN (ms) ± S.E.M of 5-12 observations.

* indicates significant difference from control at $P<0.05$. 

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![Graph showing period from +VE dP/dt to -VE dP/dt (MS) vs. filling pressure (cm H2O).]

- [▲] T3 treated (n=5)
- [●] T4 treated (n=7)
- [○] Vehicle treated (n=12)
FIGURE 12.

Effect of triiodothyronine and thyroxine pretreatment on area under the curve from baseline to left ventricular peak developed pressure in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on area under the left ventricular contraction curve from baseline to peak developed pressure (AP) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean AP (mm Hg)sec ± S.E.M of 5-12 observations.

There were no significant differences between the groups at any atrial filling pressure.
FIGURE 13.

Effect of triiodothyronine and thyroxine pretreatment on area under the curve from left ventricular peak developed pressure back to baseline in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on area under the left ventricular contraction curve from peak developed pressure back to baseline (AB) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean AB (mm Hg)sec ± S.E.M of 5-12 observations.

There were no significant differences between the groups at any atrial filling pressure.
FIGURE 14.

Effect of triiodothyronine and thyroxine pretreatment on total area under the curve for the entire left ventricular contraction curve in the rat working heart paced at 300 beats per minute.

The plot depicts the effects of the pretreatment of rats with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on total area under the left ventricular contraction curve for the entire pulse (AT) in rat working hearts at various atrial filling pressures, while being paced at 300 beats per minute.

Each point represents the mean AT (mm Hg)sec $\pm$ S.E.M of 5-12 observations.

There were no significant differences between the groups at any atrial filling pressure.
(>) T3 treated (n=5) ——
(∞) T4 treated (n=7) ———
(○) vehicle treated (n=12) ———

filling pressure (cm H2O)

total area of pulse (mm Hg ms)
B. Contractile Studies

2. Isoproterenol Dose Response Curves

a. Effect of isoproterenol on left ventricular developed pressure in the rat working heart preparation:

Throughout the isoproterenol dose response curve the LVDP response of hearts from animals treated with T3 was increased as compared to that of vehicle treated hearts. This increase was significant for all doses of isoproterenol given, from $1 \times 10^{-10}$ Molar (M) to $1 \times 10^{-7}$ M. Prior to the administration of isoproterenol there was also a significant difference in LVDP achieved by hearts from T3 treated as compared to vehicle treated animals. T4 heart response was at no point different from that of the vehicle treated hearts; it was, however, significantly different from the response of the T3 treated hearts at all points on the dose response curve. (Figure 15)

b. Effect of isoproterenol on the maximum rate of left ventricular pressure development:

Both thyroid hormone treatment groups had higher +dP/dt values than did the control group over the range of the dose response curve. Hearts from T4 treated animals did not show a significantly different response to isoproterenol at any dose of the drug as compared to hearts from vehicle treated animals. There was a significant difference in the +dP/dt exhibited by hearts obtained from T3 treated animals as
compared to vehicle treated animals at control levels as well as at doses of isoproterenol of $1 \times 10^{-10}$M, $3 \times 10^{-10}$M, and at $1 \times 10^{-9}$M. At a dose of $1 \times 10^{-10}$M, on the cumulative isoproterenol dose response curve, the T3 hearts also had a significantly greater rate of left ventricular pressure development than did T4 hearts. (Figure 16)

c. Effect of isoproterenol on maximum rate of relaxation:

While in general, values of $-\frac{dP}{dt}$ were greater in hearts from hyperthyroid animals than those from hearts of euthyroid animals, only at doses of isoproterenol of $1 \times 10^{-9}$M, $3 \times 10^{-9}$M, and $1 \times 10^{-8}$M were there significant differences when comparing T3 treated hearts to vehicle treated ones. At the last dose mentioned the response of T3 hearts was also statistically different from T4 hearts in response to isoproterenol as measured by the maximum rate of relaxation. (Figure 17)

d. Effect of isoproterenol on time to peak LVDP:

No statistically significant differences from euthyroid values of TTP were seen in hyperthyroid animals at any dose of isoproterenol. (Figure 18)

e. Effect of isoproterenol on relaxation time:

Although relaxation time in hearts from hyperthyroid animals was shorter than that of hearts from vehicle treated animals over the range of the isoproterenol dose-response
relationship, no significant differences between groups were seen at any dose. (Figure 19)

f. Effect of isoproterenol on total pulse period:

The dose response curve for isoproterenol in hearts from T3 treated animals showed a decrease from values for hearts from vehicle treated rats at all doses given. There is only one point of significant difference, however, at $1 \times 10^{-9}$M isoproterenol. No differences between T4 treatment and vehicle treatment in their effects on this parameter were seen to be statistically significant. (Figure 20)

g. Effect of isoproterenol on time required from maximum positive dP/dt to maximum negative dP/dt:

No significant differences between the treatment groups were observed in the measurement of this variable under these experimental conditions. (Figure 21)

h. Effect of isoproterenol on area to peak pressure:

Values obtained for this variable were not statistically different between groups. (Figure 22)

i. Effect of isoproterenol on area from peak pressure:

Over the entire range of the dose response curve, AB in hearts from T3 animals was greater than in hearts from T4 and vehicle treated animals. Significant differences were seen from vehicle treatment at control levels, at $1 \times 10^{-10}$M and at
1 x 10^{-9}\text{M} \text{ isoproterenol. At exactly the same doses hearts from T3 animals showed significant differences from those from T4 treated animals. (Figure 23)}

j. Effect of isoproterenol on total area of pulse:

No significant differences between the treatment groups were seen in the measurement of this parameter. (Figure 24)
FIGURE 15.

Effect of isoproterenol on left ventricular developed pressure in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on left ventricular developed pressure (LVDP) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean LVDP (mm Hg) ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

** indicates T3 treated group significantly different from vehicle treated and from T4 treated group at P<0.05.
The graph shows the effect of different treatments on LVDP (left ventricular developed pressure) at various negative log doses. The treatments include:

- (△) T3 treated (n=5)
- (Δ) T4 treated (n=5)
- (○) Vehicle treated (n=8)

The graph indicates a dose-response relationship with LVDP increasing as the negative log dose increases, with significant differences observed at certain doses for each treatment group.
FIGURE 16.

Effect of isoproterenol on rate of left ventricular pressure development in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on maximum rate of left ventricular pressure development (+dP/dt) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean +dP/dt (mm Hg/sec) ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

* indicates T3 group significantly different from control at P<0.05

** indicates T3 treated group significantly different from vehicle treated and from T4 treated group at P<0.05.
(△) T3 treated (n=5) ——
(∞) T4 treated (n=5) ——
(○) vehicle treated (n=8) ——

positive dP/dt (mm Hg/sec)

negative log dose
FIGURE 17.

Effect of isoproterenol on rate of relaxation from left ventricular peak pressure in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on maximum rate of relaxation from left ventricular maximum pressure (-dP/dt) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean -dP/dt (mm Hg/sec) ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

* indicates T3 group significantly different from control at P<0.05

** indicates T3 treated group significantly different from vehicle treated and from T4 treated group at P<0.05.
(>) T3 treated (n=5) ---
(∞) T4 treated (n=5) ----
(○) vehicle treated (n=8) ---

negative dP/dt (mm Hg/sec)

negative log dose
FIGURE 18.

Effect of isoproterenol on time to peak left ventricular pressure development in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on time required to achieve peak left ventricular pressure (TTP) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean TTP (ms) ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

There were no significant differences between groups at any dose of isoproterenol.
ISOPROTERENOL DRC - RAT WORKING HEART

(* T3 TREATED (N=5)
(0) T4 TREATED (N=5)
(O) VEHICLE TREATED (N=8)
FIGURE 19.

Effect of isoproterenol on relaxation time from peak left ventricular pressure development in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on time required for the left ventricle to relax from peak pressure (RT) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean RT (ms) ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

There were no significant differences between groups at any dose of isoproterenol.
ISOPROTERENOL DRC - RAT WORKING HEART

(•) T3 TREATED (N=5)
(M) T4 TREATED (N=5)
(o) VEHICLE TREATED (N=8)

RELAXATION TIME (MS)

NEGATIVE LOG DOSE
FIGURE 20.

Effect of isoproterenol on total pulse period in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on time required for one complete left ventricular contraction (TTC) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean TTC (ms) ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

* indicates significant difference from control at P<0.05.
FIGURE 21.

Effect of isoproterenol on time period from $+\frac{dP}{dt}$ to $-\frac{dP}{dt}$ in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on the time period in the left ventricular contraction cycle from maximum $+\frac{dP}{dt}$ to maximum $-\frac{dP}{dt}$ (TPN) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean TPN (ms) ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

There were no significant differences between groups at any dose of isoproterenol.
ISOPROTERENOL DRC - RAT WORKING HEART

(●) T3 TREATED (N=5)
(●) T4 TREATED (N=5)
(○) VEHICLE TREATED (N=8)

PERIOD FROM +VE DP/DT TO -VE DP/DT (MS)

NEGATIVE LOG DOSE
FIGURE 22.

Effect of isoproterenol on area under the curve from baseline to left ventricular peak developed pressure in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on area under the left ventricular contraction curve from baseline to peak developed pressure (AP) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean AP (mm Hg)sec ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

There were no significant differences between groups at any dose of isoproterenol.
ISOPROTERENOL DRC - RAT WORKING HEART

(•) T3 TREATED (N=5)
(△) T4 TREATED (N=5)
(○) VEHICLE TREATED (N=8)
FIGURE 23.

Effect of isoproterenol on area under the curve from left ventricular peak developed pressure back to baseline in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on area under the left ventricular contraction curve from peak developed pressure back to baseline (AB) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean AB (mm Hg) sec ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

** indicates T3 treated group significantly different from vehicle treated group and from T4 treated group at P<0.05.
FIGURE 24.

Effect of isoproterenol on total area under the curve for the entire left ventricular contraction curve in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on total area under the left ventricular contraction curve (AT) in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean AP (mm Hg)sec ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

There were no significant differences between groups at any dose of isoproterenol.
ISOPROTERENOL DRC - RAT WORKING HEART

(a) T3 TREATED (N=5)
(b) T4 TREATED (N=5)
(c) VEHICLE TREATED (N=8)

TOTAL AREA OF PULSE (mm Hg ms)

NEGATIVE LOG DOSE

6000 8000 10000 12000
B. Contractile Studies

3. Determination of pD 2 Values

The data plotted in Part B, 2, showed significant differences in the numerical values for the parameters measured at control levels i.e. before isoproterenol was added to the system. In order to partially correct for this difference, and in order to analyse the data with respect to pD2 values (- log ED 50), the data were replotted for each parameter in terms of percent maximum response to isoproterenol. No immediate clarification of the situation resulted. However, the difference in dose of isoproterenol at which the groups reached maximum $+dP/dt$ was clear enough to warrant calculation of pD2 values using maximum positive $dP/dt$ as the index. Hearts from vehicle treated animals showed a pD2 value for isoproterenol of $8.18 \pm .12$, those from T4 treated hearts had a pD2 value of $8.25 \pm .40$, and T3 treated hearts had a pD2 value of $8.80 \pm .15$. There was no significant difference in pD2 values for isoproterenol between experimental groups. As the parameter chosen on which to do this calculation had exhibited the largest differences in response to isoproterenol of all the parameters measured, it was assumed that no significant differences would be found in analysis of any of the other parameters for pD2 values. (Figure 25)
FIGURE 25.

Effect of isoproterenol as % maximum response of rate of left ventricular pressure development in working hearts from triiodothyronine, thyroxine and vehicle pretreated rats paced at 300 beats per minute.

The plot depicts the effect of various doses of isoproterenol on maximum rate of left ventricular pressure development (+dP/dt) expressed as % of maximum response in rat working hearts from animals pretreated with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (vehicle).

Each point represents the mean +dP/dt (% max. response) ± S.E.M of 5-8 observations.

Dose response curves were obtained at a constant atrial filling pressure of 15 cm of water on the working heart apparatus.

pD2 values were 8.8 ± .15 for T3, 8.25 ± .40 for T4, and 8.18 ± .12 for control hearts.

There were no significant differences in pD2 values for isoproterenol between the groups.
positive dp/dt (% maximum response)

- T3 treated (n=5)
- T4 treated (n=5)
- Control (n=8)

pD2 8.8 ± 0.15
pD2 8.25 ± 0.40
Euthyroid pD2 8.18 ± 0.12

negative log dose isoproterenol
C. Studies of Calcium Uptake Activity

1. Lowry Assay Standard Curve

The correlation coefficient obtained for the bovine serum albumin standard curve used in determining protein content in samples of rat cardiac sarcoplasmic reticulum was .9979. (Figure 26)

2. Calcium Uptake Activity

Sarcoplasmic reticulum (SR) prepared from hearts of T3 treated animals showed a trend towards higher calcium uptake activity than did SR from hearts of vehicle treated animals. Calcium uptake activity of hearts from T4 treated animals was virtually identical to that of hearts from control animals. There were no points of statistically significant divergence of hyperthyroid heart calcium uptake activity values from those of control hearts. (Figure 27)
FIGURE 26.

The standard curve of the Lowry protein assay using freshly prepared bovine standard albumin

The plot depicts the protein assay standard curve constructed from values of optical density or absorbance at 700nm (x 1000) of various concentrations of bovine serum albumin.

The correlation coefficient obtained from the plot was .9979.
STANDARD CURVE OF LOWRY PROTEIN ASSAY
- BSA STANDARD

optical density at 700 nm

0 25 50 75 100 125 150 175 200
micrograms BSA
FIGURE 27.

Effect of triiodothyronine and thyroxine pretreatment on calcium uptake activity in rat cardiac microsomes enriched in sarcoplasmic reticulum.

The plot depicts the effect of pretreatment with triiodothyronine (T3) for 3 days, with thyroxine (T4) for 7 days, and with vehicle (control), on calcium uptake activity in rat cardiac microsomes enriched in sarcoplasmic reticulum at various concentrations of free calcium.

Each point represents the mean calcium uptake activity (nmol/mg protein/minute) of 4-8 observations.

There were no significant differences between groups at any concentration of free calcium.
HYPERTHYROID RAT HEART

(Δ) T3 treated (n=4)
(Ξ) T4 treated (n=4)
(ο) control (n=8)
DISCUSSION

A. Pre-Experimental Treatment Effects

The treatment of rats with either T3 or T4 caused a significant loss of body weight. Vehicle treated rats gained in body weight over the course of the study. Heart weights of treated animals were greater than those of controls. The heart weight/body weight ratio was increased from control values in both T3 and T4 treated rats (Figure 3). These data are in agreement with previous reports regarding heart and body weight statistics in thyroid hormone treated rats (Gemmill, 1958; Cairoli and Crout, 1967; Frazer et al. 1969; McNeill et al. 1969; Sandford et al. 1978). The increased heart weight/body weight ratio is indicative of cardiac hypertrophy. This increase in the relative size of the heart is thought to be associated with increased protein synthesis in the hyperthyroid animal (Sandford et al. 1978). The results obtained in our study for changes in weight parameters are entirely in accordance with the assumption that the animals had indeed become hyperthyroid as a result of the thyroid hormone treatment schedules employed.

Spontaneous heart rate, measured prior to initiation of electrical pacing on the working heart apparatus, was found to be increased from control in both T3 and T4 hearts (Figure 4). This effect of thyroid hormone treatment in the rat has been reported many times previously (Gemmill, 1958; Van der Schoot and Moran, 1965; Cairoli and Crout, 1967; Frazer et al. 1969).
Increase in heart rate is an extremely important clinical consideration in thyrotoxicosis. In most hyperthyroid patients tachycardia is the major physiological mechanism employed by the heart to produce the increase in cardiac output required by the condition. In our experiments with hyperthyroid and control working hearts, heart rate was held constant through electrical pacing of the atria, and thus could not contribute to the changes in function seen as effects of thyroid hormone treatment. Spontaneous heart rates are reported here mainly as an index of thyrotoxicosis. Given the tachycardia of our treated animals, we are provided with further justification in believing them hyperthyroid after treatment.

It should be noted that the working hearts of thyroid hormone treated animals exhibited signs of electrophysiological changes. Tachycardia was accompanied by an increased tendency of the hyperthyroid hearts toward the development of arrhythmias. This propensity for arrhythmia is well noted in the clinical and experimental literature concerning hyperthyroidism (De Groot and Leonard, 1970; Freedberg et al. 1970; Johnson et al. 1973), and is thought to be associated with a lowered diastolic threshold for evocation of atrial responses especially those from multiple pulses (Arnsdorf and Childers, 1970).

Major delays in our attempts to characterize the function of the hyperthyroid myocardium were created by this tendency toward arrhythmia in the thyrotoxic animals. Among the
interim solutions employed to deal with the problem was an attempt to destroy the sino-atrial (SA) node of the heart with formalin after recording the spontaneous heart rate and before initiation of electrical pacing. This method was not successful. When enough formalin to destroy the SA node and slow the heart was given, the function of the heart was most often severely depressed as well, due to the non-specific nature of the maneuver.

Removal of the entire right atrium of each heart was also tried as a remedy for the tachycardia and arrhythmia of thyrotoxicosis. This method too, was unsatisfactory. The surgery was relatively crude and hard to control, also we found that removal of the entire atrium often did not slow the thyrotoxic hearts in the least. Many of them continued to beat rapidly due to some other, ectopic pacemaker. The control hearts, on the other hand, seemed often to be mechanically compromised by atrial excision.

A satisfactory conclusion to the dilemma was accomplished by reducing both the concentration of calcium in the buffer, and the temperature of the preparation. Originally we had used the standard Chenoweth-Koelle buffer containing 2.2mM calcium. By reducing the buffer concentration to 1.8mM calcium, still well within the physiological range, the tendency toward arrhythmia was diminished. By decreasing the temperature to 30 degrees Centigrade, arrhythmia was reduced to a minimum. Both control and hyperthyroid hearts, under these new experimental conditions, provided stable
preparations which could be electrically paced at 300 beats per minute. Stability of heart function was also preserved while the manipulations of the function curve were performed and while the isoproterenol dose response curve was completed.

Research of the literature produced the information that this problem had been faced by earlier workers using different tissue preparations. Buccino et al. (1967) had varied temperature from 21 degrees to 37 degrees Centigrade, eventually choosing 30 degrees as their experimental setpoint. Their temperature studies showed that while measurements of functional parameters might not remain numerically the same at different temperatures, the changes seen at different temperatures were directionally the same. Relative differences between treatment and control groups remained significant at lower temperatures. The effect of the temperature change was not different for hyperthyroid as compared to control hearts.

In the working heart preparation, Baisch et al. (1981), have shown that temperatures ranging from 29 through 37 degrees Centigrade provide reasonable and reproducible values for assessing myocardial function. We therefore felt quite confident in the validity of the measurements made under the experimental conditions of this study.
B. Contractility Studies

1. Function Curves

Hearts from both the T3 and T4 treatment groups developed more left ventricular pressure at faster rates of rise and fall for shorter periods of time than did controls (Figures 5 through 10). This data is consistent with certain of the reports of the literature (Amidi et al, 1968; Parmley et al, 1968; Muryama and Goodkind, 1968; Taylor, 1970; Goodkind et al. 1974; Brooks et al. 1981). Both hyperthyroid groups could develop a higher maximum ventricular pressure than could controls in response to the highest atrial filling pressure (22.5 cm of H2O) of the function curve (Figure 5). Over the range of the function curve hyperthyroid hearts consistently exhibited an increased contractile response over euthyroid hearts, measured in terms of rate of pressure development (+dP/dt, Figure 6) or rate of relaxation (-dP/dt, Figure 7).

Values for all parameters of the function curve experiments are expressed in terms of unit performance index per heart e.g. left ventricular pressure per heart. Since the hyperthyroid hearts were invariably larger than the control hearts (Table I), the possibility that the data might be biased by this fact was considered. Perhaps the ability of the hearts to contract was the same in hyperthyroid and control animals, and the differences seen due only to the increased weight of the hyperthyroid hearts. Analysis of our data in terms of grams wet weight of heart is somewhat spurious in our view, as prior to treatment all three groups
of rats had similar body and heart weights. Thus the enlarged heart of the hyperthyroid rat is part of the effect of treatment with thyroid hormone and the proper control from which to measure such an effect is a heart from an otherwise identical animal treated with vehicle. Nevertheless, in an effort to achieve completeness, the calculations were done to express the functional parameters in terms of unit weight. As might be expected, the values of LVDP, and positive and negative dP/dt's when corrected per unit weight were not different for hyperthyroid hearts compared to control. The differences in all time parameters, however, were magnified rather than diminished. Thus, every point on the time to peak tension curve would become significantly different from control for both hyperthyroid groups (Figure 8). In relaxation time and total contraction time, where significant differences had already been present, these differences would be exaggerated (Figures 9 and 10). Time to peak tension is at least as acceptable as a measure of the contractile response as is +dP/dt. Therefore, the statement that at each point on the working heart function curve a difference is seen between hyperthyroid and control heart function, does not seem unduly unwarranted, whether in terms of +dP/dt per heart or TTP per gram wet weight.

While certain of the parameters measured in this study are commonly reported, others are not as familiar, while still others are reported here for the first time. Some explanation of their possible significance is in order.
Measurements of developed force, whether pressure or tension, and measurements of the rate of development of that force have been commonly used to indicate the state of contractility of the experimental myocardium. The use of these parameters in assessing contractility is standard practice, but has been called into question by certain experimenters. (Abel, 1976). His contention, simply stated, is that assessment of contractility is often biased by the use of parameters and experimental procedures which involve dimensional change in response to Starling's law as an essential characteristic of their magnitude. That is, not only is the intrinsic contractility of the myocardial cell being measured by these parameters, but also included in the measurement is the response of the cell to stretch. In this regard, the working heart preparation is definitely guilty, as the very essence of the function curve is to subject the heart to varying filling pressures causing varying amounts of stretch of the muscle fibers, resulting in varying amounts of tension being generated in response. Abel (1976) advocated the use of parameters which throughout the course of such an experimental manipulation as the working heart function curve, maintain a relatively constant value, i.e. have relatively little of their value due to the contribution of the response to stretch. Time to peak tension is typical of this sort of measurement, while $+dP/dt$ can be used as an example of a parameter whose value increases considerably over the range of the function curve in response to stretch. With this
controversy in mind and with new measurements made possible through the use of microcomputer software technology, we decided to augment the three standard measurements of LVDP, +dP/dt, and - dP/dt, by seven more or less novel ones. Time to peak tension was an obvious choice. It has often been reported in the literature, and exhibits the characteristics considered necessary to evaluate the accurate measurement of contractility by Abel. We also examined relaxation time and total contraction time (very seldom measured in the literature) and time taken by the heart between the maximum +dP/dt and maximum -dP/dt (never before reported). Area under the left ventricular pressure curve was measured, and measurements of area under the curve to peak pressure, and from peak pressure back to baseline were also carried out. Area under the curve has been previously reported by Seigel and Sonnenblick (1963) as IIT or integrated isometric time tension index. This parameter varies directionally as end diastolic volume is changed, and is therefore not considered a contractility index of repute by Abel (1976). However, Seigel and Sonnenblick have reported IIT as a measure of the total impulsive force exerted by the muscle. Further, use of this index rather than either of its components (time or tension) provides an indication of the total mechanical energy available in each muscle contraction. If these assertions are correct, measurement of this index might prove useful in our study, so as to ascertain whether total mechanical energy per beat is increased in hyperthyroid hearts. In monitoring all
of these pressure, time and area indices, we hoped to be able to detect any subtle differences that might surface not only in comparing function of hyperthyroid hearts to controls, but also in comparison of the function of the two treatment groups.

This exercise had varied success. We found LVDP (Figure 5), +dP/dt (Figure 6), -dP/dt Figure 7) and TTP (Figure 8) to have almost identical values in the T3 and T4 treated groups. It was not expected that they be quite so similar, as the animals were treated with different hormones for different lengths of time. No effort had been made to achieve exactly the same level of hyperthyroidism, only to reproduce the most common treatments used.

While most of the parameters measured showed equal values for T3 and T4 rats, there were some interesting differences between the two groups when relaxation time was considered (Figure 9). Evaluation of this parameter showed that hyperthyroid working hearts in general relaxed much faster than their control counterparts. At high filling pressures, however, while T3 hearts continued to relax very quickly, T4 hearts behaved in a manner more similar to the control than to the T3 hearts, with their relaxation times approaching those of control. Relaxation in T3 hearts seems to have been expedited in some manner. T4 hearts show the same sort of enhanced relaxation at low to normal filling pressures, but at high filling pressures, this enhancement was no longer present. As the uptake of calcium into the sarcoplasmic
reticulum is thought to be a major determinant of relaxation efficiency in the myocardial cell, an obvious direction for further examination lay in measuring this uptake activity. This study was undertaken and will be discussed later.

Previous reports of relaxation time in hyperthyroidism include a study by Taylor et al. in 1969, in which isovolumic contractions produced by sudden balloon occlusion of the ascending aorta were measured in T4 treated dogs. Decreased relaxation times in comparison to control were seen in hyperthyroid animals, in agreement with our data. Guarnieri et al. (1980) also saw decreased relaxation times in their measurements in ventricle strips from hyperthyroid rats.

Trends seen in the measurement of total pulse period, or total time of contraction, mainly reflect the differences between hyperthyroid and euthyroid hearts seen in the relaxation time values (Figure 10). Thus, all T3 contraction times are reduced from control, as are all T4 times up to the two highest atrial filling pressures (20 and 22.5 cm. of H2O). For these two pressures and for 17.5 cm. of water, T3 values are different from T4 times. Taylor et al. (1969) and Guarnieri et al. (1980) also measured duration of contraction, finding it reduced in hyperthyroid preparations.

The measurement of time from $+\frac{dP}{dt}$ to $-\frac{dP}{dt}$ was inspired by the work of Allen and Blinks (1978) using aequorin in the measurement of calcium transients in myocardial cells. Their work appeared to indicate that cataloguing of this time value might approximate the time at which calcium was being
actively accumulated by the cardiac sarcoplasmic reticulum. The results obtained (Figure 11), however, did not particularly add to the information already gained from monitoring relaxation time, time to peak tension, and total pulse period. Further reinforcement of the differences between hyperthyroid and control heart function was obtained, but only at relatively high filling pressures. Differences between T3 and T4 function did not show up as clearly in this measurement as they did with relaxation time.

Area under the left ventricular pressure curve was measured (Figures 12, 13, 14). There were no differences observed between groups in the values obtained. This result suggests that the mechanical energy available during contraction was the same in euthyroid as it was in hyperthyroid myofibrils. This equivalence might be expected, as in essence, this parameter consists of time multiplied by tension. Thus, in hyperthyroid function curves, although developed pressure was increased, time of contraction was decreased from control, so the product of the two values will probably not be different from the product of the two control values.

The most important results of the function curve studies are the marked increases in rates of rise and fall of developed pressure in hyperthyroid hearts over control hearts (Figures 6, 7). Relaxation times and total contraction times were reduced from control values for both hyperthyroid groups, with the major differences in performance between the two
treatments being evident in the differences for these two parameters seen at high filling pressures on the working heart (Figures 9 and 10).

2. Isoproterenol Dose Response Curves

We had hoped the isoproterenol dose response curves obtained in this study might add to our understanding of myocardial function in hyperthyroidism. Variation within the groups was considerable, however, making analysis and interpretation difficult. The major difference between the groups seems to be a baseline difference between the T3 treatment group and the other two groups. In retrospect, although the outward performance of the two hyperthyroid groups through the function curve was very similar, perhaps the differences seen in relaxation time between T3 and T4 hearts was a presaging of the decline in function of the T4 hearts observed during the isoproterenol dose response curve. That the depression in function was a secondary change related to hypoxia is certainly a possibility in hyperthyroid tissue in which the oxygen consumption is markedly increased. However, in this case lack of oxygen seems unlikely as the cause of functional shortcomings. Although the two thyroid groups were treated in exactly the same fashion, the T3 hearts continued to demonstrate increased performance throughout the drug experiment, while T4 hearts exhibited declining performance. All groups did respond to isoproterenol, attesting to the
potency of the drug preparation.

Maximum response to isoproterenol expressed in absolute terms was very similar for the hyperthyroid and euthyroid groups. LVDP's of T3 hearts were significantly different from both control and T4 values at all doses of isoproterenol and also prior to the administration of the drug (Figure 15). At low doses of isoproterenol +dP/dt was also different from control; at the lowest dose of isoproterenol T3 heart response was different from that of T4 hearts as well (Figure 16). Guarnieri et al. (1980) reported supersensitivity to isoproterenol for T4 treated rats at low doses of isoproterenol. No absolute values for dT/dt were given, however the authors reported no baseline differences.

No differences from control were seen in measurements of time to peak tension (Figure 18) and relaxation time (Figure 19) in hyperthyroid hearts as compared to controls. At a dose of isoproterenol of 1 x 10E-9M in the measurement of total pulse period there was a significant decrease for the T3 treated animals as compared to control (Figure 20). The results obtained with this parameter are worthwhile examining in another regard. Control hearts in developing maximum response to isoproterenol showed a decrease in total pulse period of approximately 35 milliseconds, or a 20% reduction. The T3 group showed a decrease in total pulse period due to iso was 16 milliseconds or 10% reduced; T4 pulse period decreased about 14 milliseconds or an 8% decrease. All groups at maximum response had very similar total pulse period
values, despite the large spread of these values before drug treatment.

No differences were seen in measurements of area (Figures 22 and 24) except at low doses of ISO in area from peak pressure back to baseline (Figure 23). These differences appeared to be baseline derived.

Because of the major differences in baseline in all the measurements done in this part of the study, all parameters were further graphed in terms of percent maximum response. When analysed in this manner, the clearest differences between groups were seen in the measurements of +dP/dt. Accordingly +dP/dt was used to calculate pD2 values. No significant difference in this value was obtained in comparing controls and hyperthyroid animals. This result agrees with that shown by several experimenters (Margolius and Gaffney, 1965; Young and McNeill, 1974; Longhurst and McNeill 1979). Disagreement does occur with the results of others (Hashimoto and Nakashima, 1978; MacLeod and McNeill, 1981), who saw supersensitivity to catecholamines in guinea pigs and in rats (Tse et al. 1980; Guarnieri et al. 1980). Buccino et al. 1967 and Van der Schoot and Moran, 1965 on the other hand saw catecholamine subsensitivity in cats and rats respectively.

In this area of greatest controversy it is unfortunate that our own data are not more clear.
C. Studies of Calcium Uptake into Cardiac Sarcoplasmic Reticulum

Rate of calcium removal from the sarcoplasm is determined by the ATP dependent calcium transport mechanisms of the cardiac sarcoplasmic reticulum. This uptake activity can influence the rate of myocardial relaxation although a direct correlation between the two has not yet been shown. In various species, treatment with thyroid hormones for 10 days or more has been shown to increase the activity of calcium uptake into the SR (Nayler et al. 1971; Suko, 1971; Limas, 1978a).

In rats treated for 5 days with T4 however, McConnaughey et al. (1979) were not able to show calcium uptake activity increased over control. In addition Goodkind et al. (1974) saw no changes in activity of calcium uptake in guinea pigs treated with T4 for 3 days.

Our results agree with the results of the latter two groups. In our rats treated for 7 days with T4 or treated for 3 days with T3 we could see no differences from control animals in calcium uptake activity into cardiac sarcoplasmic reticulum (Figure 27).
D. Integration

Synthesis of the information gleaned from these experiments into one coherent picture is not a simple task. This study measured one biochemical parameter, calcium uptake activity of the sarcoplasmic reticulum. Dozens of other biochemical parameters may have had bearing on the functional changes seen in our studies of the hyperthyroid myocardium. As we have mentioned, the variety of treatments used makes extrapolation from one set of experiments to another set very arbitrary. It is also very risky to make the assumption based on observed results from one species that similar changes will occur in another species. Indeed, experiments in which more than one species was studied tend to indicate the opposite, as most often there are large species dependent differences.

Nevertheless some integration of our data with that collected by other laboratories is necessary, if only to provide direction to future studies.

Our studies in rat have shown an increased rate of left ventricular pressure development in T3 and T4 treated animals over controls (Figure 6). Maximum velocity of shortening of muscle may be affected by changes in myosin ATPase activity. Although in several other species myosin ATPase activity is known to increase as a result of hyperthyroidism, in the rat experimenters have not been able to demonstrate such a change (Rovetto et al. 1972; Yazaki and Raben, 1975). Even in species where changes in myosin ATPase have been shown, these biochemical changes were preceded temporally by changes in
contractility indices (Goodkind et al. 1974). These performance differences have been seen within 24 hours of the initiation of thyroid hormone treatment. Thus, later changes in contractility may involve myosin ATPase activity augmentation, but especially in the rat, some other mechanism must be responsible for the earlier function changes.

Thyroid hormones do not cause increases in adenylate cyclase activation, nor in tissue levels of cyclic adenosine monophosphate (McNeill et al. 1969; Brus and Hess, 1973) in rats. Tissue catecholamine levels have not been shown to be increased in T3 or T4 treated rats (Cairol and Crout, 1967). Activation of phosphorylase a has repeatedly shown to be increased in rats treated with thyroid hormones (McNeill and Brody, 1968; Frazer et al. 1969; McNeill et al. 1969; Longhurst and McNeill, 1979). Phosphorylase activation can occur through the indirect actions of cAMP dependent protein kinase. The suggestion has been made that in hyperthyroidism, sensitivity to cAMP at the protein kinase level is increased. There is no direct evidence for this hypothesis as yet. Consideration of the available reports make this an unlikely mechanism for the augmented performance of hyperthyroid rat hearts (Katz et al. 1977; Tse et al. 1980).

Calcium can directly activate phosphorylase in the myocardial cell. Perhaps the mechanism of enhanced contractility in hyperthyroidism relates to increased influx of calcium into the cell by means of the slow inward calcium current. However, Skelton et al (1976) have used cat
papillary muscles skinned with glycerol to demonstrate the preservation of the increased contractile performance of hyperthyroid tissues even in the absence of sarcolemmal influences. Furthermore, if the mechanism of increased contraction is increased concentrations of calcium within the cell, why is there no increase in calcium uptake activity to take this extra calcium back up out of the sarcoplasm? In the later stages of thyroid hormone treatment, perhaps, increases in the amount of calcium in the sarcoplasm are important to the functional changes seen, possibly due to perturbation of the sarcolemma. This would be consistent with the increased uptake of calcium into SR seen in animals treated for longer periods of time with thyroid hormones (Nayler et al. 1971; Suko, 1971; Limas 1978a).

Rate of force development depends on the shortening properties of the contractile element and the stiffness of the series elastic component. Early studies by Parmley et al (1967) eliminated changes in the series element or stiffness of the myocardium as a mechanism for the increased contractility of hyperthyroidism.

Very recent work by Allen and Kurihara (in print, as reported by Jewell, 1982) has used aequorin fluorescence to characterize the calcium transients or fluctuating calcium levels within the mammalian myocardial cell. One of these newly reported studies shows the tension response of skinned ventricular muscle to calcium with the muscle having different sarcomere lengths. The curve relating tension production to
calcium concentration can be shifted to the left, that is the sensitivity to calcium increased if the muscle is stretched.

Moving into the realm of speculation, perhaps the action of thyroid hormone within the myocardial cell is to produce changes in the contractile element so as to favor more tension development. This change need not be a stretched sarcomere, it could as easily be some change in binding affinity for calcium at troponin C. Enhanced contractile performance in hyperthyroidism would reflect an increase in calcium sensitivity at the contractile protein, generating more tension for the same concentration of calcium. This mechanism would be congruent with the lack of difference shown in our studies of the sarcoplasmic reticulum calcium uptake activity (Figure 27). The calcium concentration at the myofibril being the same in the euthyroid as the hyperthyroid, the need for increased calcium uptake activity would be obviated.

Several pieces of evidence are at odds with the mechanism of increased sensitivity to calcium at the contractile element. Firstly, McNeil (1978), did contractile studies in guinea pig Langendorf hearts which showed no increase in calcium sensitivity for hyperthyroid hearts over control hearts. Also, Goodkind (1969) showed a greater positive inotropy to calcium in euthyroid rather than hyperthyroid animals. Secondly, what of the increases in rate of relaxation seen in the hyperthyroid hearts? Increases in calcium sensitivity would not necessarily increase relaxation in conjunction with contraction.
However, in view of recent work by Ray and England (1976) and others (Solaro et al. 1976) on phosphorylation of troponin I by cAMP dependent protein kinase activated through catecholamines, some form of calcium supersensitivity at the contractile protein level remains an attractive theory. Inotropic intervention by catecholamines causes increased slow calcium current and cAMP production, leading to increased tension. The amount of tension produced however, is less than might be expected for the large calcium transients which have been seen after catecholamine administration (Jewell, 1982). The phosphorylation of troponin I by cAMP dependent protein kinase has been shown to decrease calcium sensitivity (Mope et al. 1980). The release of the bonds holding troponin I to actin and in steric hindrance of the myosin is the last step before crossbridge formation. With catecholamine administration phosphorylation of the troponin I by cAMP dependent protein kinase would tend to prolong this hindrance. Thus, if the contractility increases seen in hyperthyroidism were due to increased calcium sensitivity at the calcium binding troponin C, phosphorylation of troponin I would mask the increased calcium sensitivity. This would lead to no difference in the sensitivity of hyperthyroid rat hearts to isoproterenol over control rat hearts, just as seen in our results (Figure 25).

In practically any other receptor system the large increase in receptor number seen with beta-adrenergic receptors in hyperthyroidism would lead to greatly increased
sensitivity to the agonist. However with the large number of spare beta receptors in the heart the effects of number increase will not be great. By the law of mass action a certain increase in binding is predicted by the increase in receptor number. It may be a fairly subtle change however. This may explain to a certain extent the varied experimental results seen in the literature. The pD2 values seen by MacLeod and McNeill in 1981 in T4 treated guinea pigs are almost identical numerically to those obtained in this study for T3 treated rats. Values for control animals are also similar. Yet in our study there were no significant differences between groups, while in the earlier study supersensitivity to isoproterenol was shown. The variation in statistics used accounts for a certain part of the different conclusion. There was considerably more variation within the groups in our study as compared to the earlier one as well. Nevertheless, these results are indicative of the continuing problem. When the differences one is trying to examine are expected to be small, and the complications of differing treatments, species and experimental design are added to the picture, the chances of obtaining clearcut statistical differences are remote.

The differences in functional ability of hyperthyroid hearts and in reported sensitivity of these hearts to catecholamines are not found in this study to be due solely to treatment differences. If our experiments had been done recording only the parameters previously measured in our
laboratory (LVDP, +dP/dt, -dP/dt) the two thyroid hormone treatment groups would have been reported to have no difference in their performance throughout the function curve.

The large variability encountered in the isoproterenol studies cannot allow major decisions to be made concerning sensitivity of the treatment groups to ISO. If supersensitivity had been defined in this study as an increased response at a sub-maximal dose of isoproterenol, as it appears to have been defined by Guarnieri et al. (1980), we too would have supersensitivity by the T3 treated hearts at 1 x 10^{-9}M ISO in measurement of total pulse time. If only T3 treated hearts had been compared with control hearts in calculation of pD2 values, and therefore t-test statistics could have been used, we would have shown a leftward shift of the dose response curve in +dP/dt, a supersensitivity very similar to that seen by MacLeod and McNeill (1981).

Rather than continuing the age old battle over sensitivity to catecholamines in future studies, it might prove more edifying to examine the intrinsic contractile properties of the hyperthyroid myocardium. The obvious extension of the present studies is to involve other species. Guinea pigs, unlike rats, have been shown to develop increased myosin ATPase activity when treated with thyroid hormones. Would the functional differences in hyperthyroid hearts observed in this study in rats be magnified under the influence of this added biochemical parameter? Another obvious direction for future experimentation is the
determination of calcium sensitivity of the hyperthyroid rat working heart beginning with simple dose response curves to calcium chloride. Skinned muscle preparations could also be used to determine if calcium sensitivity remained the same in the absence of sarcolemmal influences.

Measurements of LVDP, +dP/dt, -dP/dt, time to peak tension, relaxation time and total contraction time, all provided valuable information and should be monitored in future studies of this type. The information provided by the recording of time from +dP/dt to -dP/dt appears to be redundant; continued measurement of this parameter seems unwarranted. The value of measurement of areas under the contraction curve is not yet established. It would probably be useful to continue to record these measurements for the next studies for the purpose of comparison especially if the measurements obtained in this manner were compared with work determinations such as stroke work.
SUMMARY

1. Thyroid hormone treatment increased rate of left ventricular pressure development and rate of relaxation in rat working hearts.

2. Hyperthyroid rat hearts had decreased relaxation times and total contraction times.

3. Hearts from T3 treated animals differed from those from T4 treated animals in relaxation time at high atrial filling pressures. T4 hearts at these high filling pressures had increased relaxation times, approaching control heart values.

4. No differences in sensitivity to isoproterenol were seen.

5. Calcium uptake activity in cardiac sarcoplasmic reticulum was unchanged from control in hyperthyroid hearts.
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TSE, J., R. W. WRENN, and J. F. KUO. 1980. Thyroxine-induced changes in characteristics and activities of beta-adrenergic receptors and adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate systems in the heart may be related to reputed catecholamine supersensitivity in hyperthyroidism. Endocrinol. 107: 6-16.


APPENDIX I

Microcomputer Methods

A. Data Acquisition

A simple operational amplifier circuit conditioned the left ventricular pressure signal from the Grass polygraph. The output from this circuit was connected to one channel of the Analog to Digital (A/D) converter.

A machine language routine was used to collect the data and to store it to disk. The pressure signal was sampled every 1.5 milliseconds for 1024 sample points, thus ensuring the capture of at least five complete pressure pulses at each collection time. An Applesoft Basic program provided a simple user interface to the machine language routine, calling the routine repeatedly and saving the raw data through the use of a sequential naming convention.

By means of another Applesoft Basic program, the stored raw data could be retrieved at will, read back, and plotted on the high-resolution graphics of the microcomputer for verification of the sampling process. This process allowed the user to consecutively store data throughout an experiment in which the apparatus and/or protocol demanded constant attention. Post-experimentally, the stored data could be recalled for perusal and analysis.
B. Data Analysis

A complete pressure pulse was identified in the data by means of yet another Applesoft Basic program. Maximum and minimum pulse pressures were found, and 10% and 90% pressure values calculated from these. For purposes of measuring the time parameters, the start and finish of a pulse were defined as the time at which the pressure was evaluated at 1% of the maximum pressure. These values were found by fitting the best fit exponential to twenty time points around the beginning of the curve. The time point of peak pressure was located by fitting a cubic equation to four points on the top 10% of the pressure curve, and then solving for the zero of the derivative of the resulting quadratic equation. Maximum positive and negative slopes of the pressure curve were determined by calculating areas of maximum change over points separated by several samples. Area under the curve was calculated by use of Simpson's Rule, that is by fitting quadratic equations to each set of three consecutive data points (Barrodale et al. 1971).

The above calculations were done on two more complete curves from each raw data file, and the averaged results stored to disk. Other programs written in Applesoft Basic allowed similar data from the same heart to be collected from separate disk files and averaged e.g. successive function curve determinations at a particular atrial filling pressure. Data from different trials of the same experiment could then
be collected and averaged into compiled files. These compiled files were used to compare differently treated groups, and to plot the values obtained. An ANOVA program, which included Newman-Keuls Studentized Range Statistics, was used for statistical comparison of the different treatments.

C. Mathematical Basis of Curve Analysis

The analysis of individual pulses began by isolating three pulses from each set of five digitized pulses sequentially into an array $f(i)$, with $1 \leq i \leq 200$. Then the following values and times were determined.

1. The maximum and minimum data values:
   
   $f(\text{max}) = \text{MAXIMUM} \left( f(i), 1 \leq i \leq 200 \right)$
   
   $f(\text{min}) = \text{MINIMUM} \left( f(i), 1 \leq i \leq 200 \right)$

2. The following values and their positions:
   
   $f(\text{range}) = f(\text{max}) - f(\text{min})$, $f(10%) = 0.1 \left( f(\text{range}) \right)$

   similarly for $f(50%), f(90%)$ and $f(1%)$.

   $i^+(10%) = (i, \text{such that } f(i) \leq f(10%) \text{ and } f(i+1) > f(10%)),$

   similarly for $i^+(90%), i^-(10%), \text{and } i^-(90%).$

3. To determine the start and finish of the pulse ($i(\text{start})$ and $i(\text{end})$), we assumed that the beginning of a pulse can be described by an exponential of the form: $y = ce^{dx}$. This equation can be linearized by taking the natural logarithm of both sides to give: $\ln(y) = dx + \ln(c)$. Linear regression was performed on $\ln(y)$ against $x$ to obtain the slope, $m$, and the
intercept, \( z(\text{int}) \), giving: \( \ln(y) = mx + z(\text{int}) \). Therefore we could solve for the value of \( x \) which would give any given \( y \). In this case we were interested in \( y = f(1\%) \), so solving the equation gave the time at which the curve was at 1\% of its maximum, or \( i^+(1\%) \).

4. Maximum positive and negative rates of change of pressure were determined as:

\[
+\frac{dP}{dt} = \text{MAXIMUM } ( (f(i+4) - f(i-4)) / 8, \text{ if } (10\%) < i < (90\%)),
\]

\[
i(+\frac{dP}{dt}) = (i' - i'') / 2, \text{ where } i' \text{ is the largest } i, \text{ and } i'' \text{ is the smallest } i, \text{ such that } f(i) = f(+\frac{dP}{dt}).
\]

Similarly for \(-\frac{dP}{dt}\) and \(i(-\frac{dP}{dt})\).

5. The time of maximum pressure, \( i(\text{max}) \), was determined by calculating the coefficients of the cubic equation passing through four points around the top of the pulse. More explicitly, find the equation \( g'(i) = qi^3 + ri^2 + si + t \), such that \( g(i) = f(i) \) at four equally spaced \( i \)'s in the interval \( (i^+(90\%) \text{ to } i^-(90\%)) \) The maximum of this cubic occurs at the zero of its derivative: \( g'(i) = 3qi^2 + 2ri + s \). By letting \( a=3q, b=2r, c=s, y=g'(i), \text{ and } x=i \) this becomes the familiar quadratic: \( y = ax^2 + bx + c \), and substituting the coefficients into the quadratic equation gave \( i(\text{max}) \).

6. The areas under the curve (AP, AB, and AT) were found using Simpson's rule. This method approximates the area under a curve by fitting a series of quadratic equations to succeeding sets of three consecutive points. Fortunately, this procedure reduces to the following formula:
\[ A = \left( \frac{h}{3} \right) (f(1) + 4f(2) + 2f(3) + 4f(4) + \ldots \]
\[ + 4f(n-3) + 2f(n-2) + 4f(n-1) + f(n)) \]

where \( n \) is odd and \( h \) is the time between samples; \( h = 1.5 \) milliseconds in our case.
Statistical Methods

A. Newman-Keuls Test for Multiple Comparisons
(Calculations for an example are shown in Part B)

1. One way ANOVA classification was carried out on the groups being tested.

2. The following data were tabulated:
   - Degrees of freedom between groups df(B)
   - Degrees of freedom within groups df(W)
   - Mean square within groups MS(W)
   - F value F

   The F value obtained was checked against the F value given in the P = 0.05, F-distribution tables (Table II). If the calculated F value was less than the tabulated F value, none of the treatment groups was significantly different from each other, and the test was terminated at this point.

3. If the calculated F value indicated that there was a difference between the groups the Newman-Keuls test was used to determine whether the differences between the group means were significant.

4. To this end, because the experimental groups were not of equal size, the harmonic mean of the group sizes was calculated for use in the test, through the use of the following formula:
HM = a/( 1/n(2) + ...1/n(a))

where a = number of experimental groups
n = number of subjects in each group

5. The harmonic mean was substituted into the following formula:

d = SQR (MS(W)/HM)

6. The Studentized Range Statistics (Q1, Q2, etc) were calculated from the Studentized Range Table (Table III) for number of comparisons made (e.g. a,a-1 etc.), versus the df(W) tabulated above. These statistics (Q1, Q2, etc) were multiplied by the value of d obtained in step 5, to obtain the critical values (C1, C2, etc), which the difference between each pair of means must exceed to differ significantly at P<0.05.

7. The group means were arranged in order from lowest to highest and the difference between each pair of means was calculated.

8. The largest difference between group means was compared with the largest critical value i.e. C1 obtained for "a" comparisons by multiplying Q1 x d. If this difference exceeded its corresponding critical value, the means were significantly different at P<0.05. The next largest difference between means was then compared to its corresponding critical value i.e. C2 obtained for "a-1" comparisons by multiplying Q2 x d, until all groups were compared. If the largest difference between group means was not larger than C1 the testing ceased at that point. An
example from the data follows.

B. Calculation Example: The Effect of Thyroid Hormone Pretreatment on Spontaneous Heart Rate in Rat.

<table>
<thead>
<tr>
<th>Control</th>
<th>T3 treated</th>
<th>T4 treated</th>
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<td>355</td>
</tr>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>215</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n   12   5   7
Mean 226 336 294
\[ \text{SEM} \] 13 27 23

# numbers indicate spontaneous heart rate in beats per minute from individual rat hearts.

1. From ANOVA:

\[ \text{df(B)} = 2 \]
\[ \text{df(W)} = 21 \]
\[ \text{MS(W)} = 2792 \]
\[ F_{\text{calc}} = 8.825 \]
\[ F_{\text{tab}} = 3.47 \]
Therefore there is a difference between the groups

2. Calculation of Harmonic Mean:
   \[ HM = \frac{3}{(1/12 + 1/5 + 1/7)} \]
   \[ = 7 \]

3. Calculation of \( d \):
   \[ d = \sqrt{2792/7} \]
   \[ = 19.97 \]

4. Determination of Studentized Range Statistic:
   \[ Q_1 = 3.58 \text{ (for } a = 3) \]
   \[ Q_2 = 2.95 \text{ (for } a-1 = 2) \]

5. Calculation of critical value:
   \[ C_1 = Q_1 \times d \quad C_2 = Q_2 \times d \]
   \[ = 3.58 \times 19.97 = 2.95 \times 19.97 \]
   \[ = 71.49 = 58.91 \]

6. Group Means
   Control = 226  T4 = 294  T3 = 336
   T3 - Control = 110  >  C1 *
   T4 - Control = 68  >  C2 *
   T3 - T4 = 42  <  C2

7. Significant differences at \( P<0.05 \) are indicated by an asterisk. In this example spontaneous heart rate in animals treated with T3 is significantly different from heart rate in control animals treated with vehicle. T4 treated animals also showed a significantly higher spontaneous heart rate than did control animals. There was no significant difference between heart rates of T3 and T4 treated animals.
Table II.

**TABLE A 15**

**UPPER 5% PERCENTAGE POINTS, Q, IN THE STUDENTIZED RANGE**

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<th>Degrees of Freedom, ( f )</th>
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<tr>
<td>3</td>
<td>3.46 4.34 4.90 5.31 5.63 5.89 6.12 6.32 6.49 6.65 6.79 6.92 7.04 7.14 7.24 7.34 7.43 7.51 7.59 7.64 7.68 7.71</td>
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